Abstracts of the 4th Annual Meeting of the German Society of Clinical Pharmacology and Therapy
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A 1

DEBRSOQUINE POLYMORPHISM IN PALESTINIANS

Abu Al-Kahia M, Roustom R, Siegmund W, Zschiesche M and
Franke G.

Thirty-three Palestinians healthy volunteers were phenotyped
with debrisoquine (DB). Urine was collected for 6 hours after DB
administration. DB and its metabolite 4-hydroxydebrisoquine 4-
OHDB was measured in urine by HPLC (2). The metabolic ratio
has been calculated (DB/OHDB). The antinode has been
excepted by 12.6. No, Poor metabolizer (PM) was found among
the phenotyped Palestinians. The commutative exertion of DB, 4-
OHDB and total DB was lower in the Palestinians compared with
that of the Syrians (1). No correlation was found between sex and
hydroxylation-phenotype. These results suggest that the DB-
hydroxylation may have other behavior in the Palestinians as in the
Syrians.

References
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A 2

CAN GRAPEFRUIT JUICE INFLUENCE THE BIOAVAILABILITY OF ETHINYLESTRADIOL ?

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Grapefruit juice may considerably increase the systemic bioavailability of
drugs as felodipine and nifedipine. This food-drug interaction has potential
practical importance because citrus juices are often consumed at breakfast-
time when drugs are often taken. It is likely that a plant flavonoid in
grapefruit juice, naringenin, is responsible for this effect (inhibition of
cytochrome P-450 enzymes in the liver or in the small intestinal wall).

Ethinylestradiol (EE2), the estrogen of oral contraceptive steroids, shows
a high first-pass-metabolism in vivo. Therefore, the purpose of this study is

to test the interaction between grapefruit juice and EE2. The area under the
serum concentration-time curve (AUC0-24h) of EE2 was determined in a
group of young healthy women (n = 13) on day 4 ± 1 of menstrual

cycle.

To compare intraindividually, the volunteers were randomly allocated to
two test days. The female volunteers took 50 µg EE2 together with either
200 ml of herb tea or with the same amount of grapefruit juice (content of
naringenin 887 mg/l). Furthermore, on the day of testing the women
drank 4 times 200 ml of the corresponding fluid every three hours up to four
times.

The AUC0-24h of EE2 amounts to 1110.5 ± 367.7 pg x ml⁻¹ x h after
the administration of the drug with grapefruit juice; that means 28 %
higher in comparison to 869.0 ± 490.0 pg x ml⁻¹ x h after concomitant
intake of tea. Also, the mean Cmax-value increases to 37 %, p ≤ 0.01
(117.5 ± 53.2 pg x ml⁻¹ and 85.5 ± 32.9 pg x ml⁻¹, respectively).

This result shows that the systemic bioavailability of EE2 increases after
intake of the drug with grapefruit juice.

The extent of this effect is lower than the extent of known interindividual

variability.

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A 3

KINETICS OF PROCARBAZINE AND AZOPROCARBAZINE IN PLASMA

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Procarbazine is a tumourstatic agent widely used in Hodgkin's disease,
non-Hodgkin's lymphomas and tumours of brain and lung. Procarbazine is an inactive prodrug which is converted by a
cytochrome P 450 mediated reaction to its active metabolites, in the
first step to azoprocarbazine. The kinetics of both procarbazine and
azoprocarbazine is not described in humans up to now.

On 10 tumour patients we have investigated the plasma kinetics of
both procarbazine and azoprocarbazine after oral administration of
300 mg procarbazine in form of capsules and drink solution,
respectively. A HPLC method with UV-detection (254 nm) and
detection limits of 50 and 150 ng/ml was developed for procarbazine
and azoprocarbazine respectively. After both the capsules and drink
solution the parent drug could be detected in plasma only for 1 h.
In contrast the t1/2 of terminal elimination of azoprocarbazine was
estimated in the range of 0.9 to 6.5 h with a mean of 2.96 h ± 1.46 h.
The AUC of procarbazine was less than 5 % of that of
azoprocarbazine. Cmax values of azoprocarbazine were determined in
the range of 1.3 to 6.1 µg/ml. In comparison to the drink solution we
determined on the basis of the plasma levels of azoprocarbazine a
bioavailability of the therapeutic used procarbazine capsules of 93.1 ±
26.3 %.

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A 4

TIRACIZINE DISPOSITION: DOES IT DEPEND ON URINARY PH ?

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The present investigation was carried out to obtain information on the circadian
variations of the pharmacokinetics of the antiarrhythmic agent tiracizine (AWD
Dresden GmbH) and its pharmacological active metabolites M1 and M2. During
a 7 day multiple dose study period (100 mg tiracizine b.i.d.) in eight healthy
volunteers a significant lower mean steady state total urinary recovery during
day-time compared to night-time (Aetot (1 = 42.0±15.7% vs.
Aetot n = 51.2±19.6%) had been observed. This difference was mainly due to a
substantial increase of M1 and a smaller increase of M2 urinary recovery by
night. Through serum levels of M1 and M2 tended to be higher at 7 p.m.
compared to 7 a.m. Circadian variations of drug absorption, metabolism as well
as glomerular filtration could not explain our observations.

The pH-dependence of the in vitro n-octanol/water partition coefficient of
tiracizine and metabolites (pKa about 8) was determined. It could be shown that
the ratio non-ionic/ionic form of M1 and M2 is highly dependent on pH in the
range of pH 5-pH 7. Therefore, the observed circadian variations might be
attributed to alterations of ionisation, i.e. non-ionic tubular reabsorption of the
metabolites due to the well known circadian differences in urinary pH.

Considering the narrow therapeutic index of class I antiarrhythmics the present
results indicate that the effect of alterations of urinary pH on the
pharmacokinetics of tiracizine and its metabolites should be investigated in vivo.

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**A 5**

**EXERCISE INCREASES SYSTEMIC NITRIC OXIDE PRODUCTION IN UNTRAINED AND ENDURANCE-TRAINED HEALTHY HUMAN SUBJECTS.**

Stefanie M. Bode-Böger, Rainer H. Böger, Jürgen C. Fröhlich.

The effects of submaximal exercise on systemic nitric oxide (NO) formation, as assessed by measuring the urinary excretion rates of NO−3 and of cyclic GMP, were compared in 10 endurance-trained and 6 untrained male subjects. Urinary excretion rates of NO−3 (by gas chromatography) and cyclic GMP (by radio-immunooassay) were assessed before and after a 30 min exercise test at 60% of each subject's individual maximal work capacity in hourly intervals. Urinary NO−3 excretion in untrained and trained subjects was comparable at rest (104 ± 35 vs. 110 ± 19 μmol/mmol creatinine, p = n.s.). It was more than doubled during exercise in both groups (to 236 ± 88 μmol/mmol creatinine in untrained and to 282 ± 39 μmol/mmol creatinine in trained subjects, each p<0.01) and rapidly decreased to baseline within 2 hours after the test. Resting urinary cyclic GMP excretion was fourfold higher in the athletes than in the untrained subjects (21 ± 5 vs. 6 ± 1 nmol/mmol creatinine, p<0.05). During exercise it increased about twofold in both groups (to 52 ± 12 and 10 ± 1 nmol/mmol creatinine, respectively, each p<0.01), and returned to baseline after its end. There was a correlation between urinary cyclic GMP and NO−3 excretion (p<0.05) in both groups. We conclude that submaximal exercise increases the excretion of NO in man, as reflected by the increased urinary excretion of NO−3 and cyclic GMP. This may contribute to the vasodilation during physical exercise, and at least partly explain the beneficial effects of physical training in patients with cardiovascular diseases.

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**A 7**

**SYSTEMIC AND PULMONARY HAEMODYNAMIC EFFECTS OF L-ARGININE IN PATIENTS WITH CORONARY HEART DISEASE AND PRIMARY PULMONARY HYPERTENSION.**

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It is well established that the endothelial EDRF/NO-mediated relaxing mechanism is impaired in atherosclerotic and in hypertensive arteries. Recently it was suggested that primary pulmonary hypertension might be another disease in which the endothelial EDRF/NO pathway is disturbed. We tested the hypothesis that intravenous administration of L-arginine (L-ARG), the physiological precursor of EDRF/NO, stimulates the production of NO, subsequently increasing plasma cGMP levels and reducing systemic and/or pulmonary vascular resistance, in patients with coronary heart disease (CHD; n = 15) and with primary pulmonary hypertension (PPH; n = 5). L-ARG (30g, 15 min) or placebo (NaCl) was infused in CHD patients, and L-ARG was infused in PPH patients undergoing cardiac catheterization. Mean aortic (Pao) and pulmonary (Ppul) arterial pressures were continuously monitored. Cardiac output (CO), by thermodilution, and total peripheral resistance (TPR) were measured before and during the infusions. Plasma cGMP was determined by RIA.

In CHD patients, Pao decreased from 87.2 ± 4.9 to 81.8 ± 5. mm Hg during L-ARG (p<0.05), whereas Ppul was unchanged. TPR decreased from 1090.9 ± 97.9 to 845.0 ± 84.7 dyn sec cm−5 during L-ARG administration (p<0.01). CO significantly increased during L-ARG (from 7.2 ± 2.8 to 8.1 ± 0.9 l/min, p<0.05). Placebo did not significantly influence any of the haemodynamic parameters. cGMP slightly increased by 12.2 ± 9.6% during L-ARG, but slightly decreased during placebo (-12.3 ± 9.2 %) (p<0.05 for L-ARG vs. placebo).

In PPH patients, L-ARG induced no significant change in Pao, TPR, and CO. Mean Ppul was 63.4 ± 8.8 mm Hg at the beginning of the study, but was only slightly reduced by L-ARG to 55.0 ± 12.5 mm Hg (p = n.s.). Plasma cGMP was not affected by L-ARG in these patients.

We conclude that L-ARG stimulates NO production and induces vasodilation in CHD patients, but not in patients with primary pulmonary hypertension. Thus, the molecular defects underlying the impaired NO formation may be different in both diseases.

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**A 6**

**HEMODYNAMIC AND BIOCHEMICAL EFFECTS OF A SINGLE INTRAVENOUS DOSE OF L-ARGININE OR PROSTAGLANDIN E1 IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE.**

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Prostaglandin E1 (PGE1) is used for the treatment of patients with peripheral arterial disease, and probably effective due to its vasodilator and antiplatelet effects. L-arginine is the precursor of endogenously synthesized nitric oxide (NO). In healthy human subjects, L-arginine also induces peripheral vasodilation and inhibits platelet aggregation due to an increased NO production. In the present study the influence of a single intravenous dose of L-arginine (30g, 60 min) or PGE1 (40μg, 60 min) on blood pressure, peripheral hemodynamics (femoral artery Duplex sonography), and urinary NO−3 and cGMP excretion rates was assessed in ten patients with peripheral arterial disease (Fontaine III - IV). Blood flow in the femoral artery was significantly increased by L-arginine by 68% (p<0.01), and by PGE1 by 25% (p<0.05). L-arginine more strongly decreased systolic and diastolic blood pressure than PGE1. Plasma arginine concentration was increased 4-fold by L-arginine, but unaffected by PGE1. Urinary NO−3 excretion was increased by 118% after L-arginine (p<0.05), and by 78% after PGE1 (p = n.s.). Urinary cGMP excretion increased by 76% after L-arginine and by 43% after PGE1 (each p = n.s.). We conclude that intravenous L-arginine decreases peripheral arterial resistance, resulting in enhanced blood flow and decreased blood pressure in patients with peripheral arterial disease. These effects were paralleled by increased urinary NO−3 excretion, indicating that systemic NO production was enhanced by the infusion. Increased NO−3 excretion may be a sum effect of NO synthase substrate provision (L-arginine) and increased shear stress (PGE1 and L-arginine).

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**A 8**

**INCREASED PROSTACYCLIN PRODUCTION DURING SUB-MAXIMAL EXERCISE IN TRAINED AND UNTRAINED SUBJECTS: EFFECT OF LOW-DOSE ASPRIN ADMINISTRATION.**

Rainer H. Böger, Stefanie M. Bode-Böger, Dimitrios Tsikas, Jürgen C. Fröhlich.

The influence of submaximal exercise on the urinary excretion of 2,3-dinor-PGF2a (the major urinary prostacyclin metabolite), 2,3-dinor-TXBl2 (the major urinary thromboxane A2 metabolite), and PGE2 (originating from the kidney), and on platelet aggregation was assessed in 6 untrained and 10 endurance-trained male subjects before and after 7 days of 50 mg/day of aspirin. Urinary 2,3-dinor-TXBl2 excretion was significantly higher in the athletes at rest (p < 0.05). Submaximal exercise increased urinary 2,3-dinor-6-keto-PGF2a excretion without affecting 2,3-dinor-TXBl2 or PGE2 excretion or platelet aggregation. Aspirin treatment induced an ~80% inhibition of platelet aggregation and 2,3-dinor-TXBl2 excretion in both groups. However, urinary 2,3-dinor-6-keto-PGF2a was inhibited by only 24% in the untrained, but by 51% in the trained group (p<0.05). Urinary PGE2 was unaffected by aspirin in both groups, indicating that cyclooxygenase activity was not impaired by a systemic aspirin effect. After low dose aspirin administration, the same selective stimulatory effect of submaximal exercise on urinary 2,3-dinor-6-keto-PGF2a excretion was noted in both groups as before. The ratio of 2,3-dinor-6-keto-PGF2a/2,3-dinor-TXBl2 was increased by exercise; this effect was potentiated by aspirin (p < 0.05). Our results suggest that the stimulatory effect of submaximal exercise on prostacyclin production is not due to an enhanced prostacyclin endoperoxide shift from activated platelets to the endothelium, but rather the result of increased prostacyclin synthesis activation from endogenous precursors. 50 mg/day of aspirin potentiates the favorable effect of submaximal exercise on endothelial prostacyclin production by selectively blocking platelet cyclooxygenase activity.

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EFFECT OF CONSENSUS CONFERENCES ON CLINICAL PRESCRIBING HABITS IN A GERMAN UNIVERSITY HOSPITAL.

Rainer H. Böger, Stefanie M. Bode-Böger, Jürgen C. Frölich.

Demand for cost-effectiveness in medical treatment in general, and especially in pharmacotherapy, has been increasing in recent years. During the same time period, an increasing number of new, expensive drugs has been introduced into clinical practice. Therefore, mechanisms need to be installed to maintain quality of treatment and, at the same time, to reduce costs produced by the inadequate use of drugs. Arriving at a consensus how to use drugs in different departments of a hospital may contribute to this process, by standardizing the way patients are treated and by placing clinical pharmacotherapy on a more rational basis. We have introduced a therapeutic consensus conferences concept into our hospital as a means of finding a consensus about pharmacotherapeutic concepts for our clinics, and of defining accepted indications and refuting misuses of certain drugs.

The first consensus conference focused on differential volume therapy with albumin or colloidal solutions. Consensus was recorded in a therapeutic guideline which was disseminated in our hospital. In the 12 months period following the consensus conference, savings of some 0.8 million DM (or 27% of the expenses for volume therapy in the previous year) were recorded. The second consensus conference established a therapeutic guideline for the prophylactic and therapeutic use of intravenous immunoglobulins, and resulted in a sharp reduction of the use of hyperimmunoglobulins, as well as in a ~24% reduction of costs in the following 6 months period.

These results show that consensus conferences are a useful means for reducing the inadequate use of drugs in a university hospital. This results in considerable reductions in the costs of drug treatment and may thus be an important tool for a more rational and a more cost-effective clinical pharmacotherapy.

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RECOMBINANT OVEREXPRESSION OF A FUNCTIONAL SOLUBLE GUANYLYL CYCLASE IN THE BACULOVIRUS / INSECT CELL SYSTEM

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Soluble guanylyl cyclases (GC-S) are heterodimeric hemeproteins consisting of two protein subunits (70 KDa, 82 KDa). The enzyme is activated by NO (120) and catalyzes the formation of the signal molecule "cGMP" (cyclic guanosine-3'5'-monophosphate) from GTP. Numerous physiological effects of cGMP are already very well characterized. However, detailed insights in the NO-activation mechanism of this enzyme have been described to date only in a hypothetical model (1). Recently, this concept was supported by experimental data using site-directed mutagenesis to create a NO-insensitive soluble guanylyl cyclase mutant (2). It is generally accepted that the prosthetic heme-group plays a crucial role in the activation mechanism of this protein. Nonetheless, some interesting questions with regard to structure and regulation of soluble guanylyl cyclases still need to be uncovered (e.g. activation with other free radicals, such as carbon monoxide). Since this kind of studies is limited so far by isolating large quantities of a biologically active enzyme with conventional purification techniques, the recombinant protein was expressed in the baculovirus / insect cell system. We describe here the construction and characterization of recombinant baculoviruses, harboring the genes that encode both protein subunits of the soluble guanylyl cyclase. Insect cells infected with these recombinant baculoviruses produce between 20-30% (as related to total cell protein) of functional soluble guanylyl cyclase. Positive infection was monitored as a change in morphology of the infected cell. After infection of the respective recombinant viruses detected by polymerase-chain-reaction (PCR). So far examined, the recombinant enzyme exhibits similar physicochemical characteristics as the "natural" protein. Exogenous addition of several heme analogues to the infected cells is able to stimulate or inhibit the enzymatic activity of GC-S. We are confident to purify milligram amounts of the recombinant protein in the near future.

PET studies of myocardial pharmacology have principally concerned the sympathetic nervous system and tracers have been developed to probe the integrality of both pre- and post-synaptic sites. The sympathetic nervous system plays a crucial role in the control of heart rate and myocardial contractility as well as in the control of the coronary circulation. Alterations of this system have been implicated in the pathophysiology of a number of cardiac disorders, in particular, heart failure, ventricular arrhythmogenesis, coronary artery disease, idiopathic dilated and hypertrophic cardiomyopathy.

Several beta blockers have been labelled with carbon-11 for imaging by PET. The most promising of these is CGP 12177 which is a non-selective beta adrenoceptor antagonist particularly suited for PET studies due to its high affinity and low lipophilicity, thus enabling the functional receptor pool on the cell surface to be studied. Studies in our institution in a group of young healthy subjects have yielded Bmax values of 10.4±1.7 pmol/g myocardium. These data are consistent with literature values of Bmax for beta adrenoceptors in human ventricular myocardium determined by a variety of in vitro assays. A recent study in patients with hypertrophic cardiomyopathy has shown that myocardial beta adrenoceptor density is decreased by approximately 25-30% relative to values in normal subjects. The decrease in receptor density occurs in both hypertrophied and non-hypertrophied portions of the left ventricle. These data are consistent with the hypothesis that sympathetic overdrive might be involved in the phenotypic expression of hypertrophic cardiomyopathy. A further decrease of myocardial beta adrenenceptor density to levels well below 76.5±7.7 pmol/g has been observed in those patients with hypertrophic cardiomyopathy who proceed to ventricular dilatation and heart failure.

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CYTOCHROME P4501A1 (CYPIA1) AND GLUTATHIONE-S-TRANSFERASE µ (GSTM1) type A and B: ASSOCIATION TO LUNG CANCER SUSCEPTIBILITY

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CYPIA1 hydroxylates polycyclic aromatic hydrocarbons such as benzo(a)pyrene occurring e.g. in cigarette smoke. Two hereditary mutations are discovered: m1; a T to C transition 1.194 bp downstream of exon 7; m2, located at position 4.889 in exon 7 representing an A to G transition resulting in a solecine to valine substitution in the heme-binding region. Recently we could demonstrate in Caucasians that carriers of the m2-mutation possess an increased risk to lung cancer (Draletz et al. Clin.Invest. 72:840,1994), whereas the m1-mutation shows no such association. The Phase-II enzyme GSTM1 catalyses the conjugation of glutathione to electrophilic compounds such as products of CYPIA1. GSTM1 is absent in 50.9% of the Caucasian population due to base deletions in exon 4 and 5 of the gene. We found no contrariety in the GSTM1 distribution, including frequencies of type A (µ) and type B (ν) among lung cancer patients (odds ratio = 1.01, n = 117; Cancer Res. 53:1004,1993).

149 lung cancer patients and 468 reference patients were investigated for mutations of CYPIA1 and GSTM1 by allele-specific PCR and RFLP. A statistically significant higher risk for lung cancer among carriers of the m2- and G-alleles was less often linked to m1 than in controls (odds ratio = 1.50 - 99.7, P = 0.0054). However, the frequency of CYPIA1 mutations did not differ among active and defective GSTM1 types. Consequently, we could not confirm in the Caucasian population the synergistic effects of CYPIA1 mutations (especially m2) and deficient GSTM1 as combined susceptibility factors for lung cancer as described among the Japanese (Cancer Res. 55:2994,1993).

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In healthy subjects the effect of gastrointestinal hormones like somatostatin and glucagon on splanchnic hemodynamics is not well defined due to the invasiveness of the direct measurement of e.g. portal vein (PV) wedged pressure.

Methods: Now, we applied Duplex sonography (3.5 MHz) and color coded flow mapping to compare the effects of ocreotide (100 µg sc), a long acting somatostatin agonist, and glucagon (1 mg iv) on the hemodynamics of the PV, superior mesenteric artery (SMA) and common hepatic artery (HA) in 14 healthy volunteers (13 α, 1 β; 25 ± 2y; x ± sem).

Results: Basal values of PV flow (12.9 ± 0.8 cm/s), PV flow volume (549 ± 50 ml/min), SMA systolic (sF: 195 ± 13 cm/s) and diastolic flow (DF: 28 ± 4 cm/s), SMA Pourcoulot index (FI) (0.86 ± 0.01), HA sF (80 ± 8 cm/s) and DF (19 ± 1 cm/s) and HA PI (0.75 ± 0.01) well agreed with previously reported results.

Within 15 min ocreotide resulted in a decrease of SMA sF (-32 ± 4%), SMA DF (-31 ± 4%), HA sF (-16 ± 6%) and HA DF (-24 ± 7%). Maximum drop of PV flow (-33 ± 8%) and flow volume (-34 ± 7%) occurred at 30 min. All effects diminished at 60 min. No significant change of vessel diameter and PI was seen.

The following use application glucagon caused a highly variable, only short lasting increase of PV flow (+51 ± 18%) and SMA DF (+49 ± 17%). HA F (+14 ± 4%) showed a tendency to rise (ns). We conclude that in clinical pharmacology: Duplex sonography is a valuable aid for measuring effects of hormones and drugs on splanchnic hemodynamics.

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EFFECTS OF THE PHOSPHODIESTERASE INHIBITOR PIROXIMONE ON PLATELET FUNCTION IN VITRO AND IN VIVO IN PATIENTS WITH HEART FAILURE

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Platelet inhibitors exert their positive inotropic effects by inhibiting cAMP degradation and increasing the intracellular calcium concentration in cardiac myocytes. An identical phosphodiesterase type III has been demonstrated in platelets and vascular smooth muscle cells. We studied the influence of piroximone on platelet function in vitro and ex vivo and the hemodynamic effects of a bolus application of piroximone in patients with severe heart failure (NYHA III-IV) using a Swan -Ganz-Catheter. In order to study the influence of piroximone on platelet function in vitro, platelet rich plasma from healthy volunteers was incubated with piroximone (10-100 µmol/l) from 1 minute to 2 hours and aggregation was induced by addition of ADP. For the ex vivo experiments platelet rich plasma was obtained from patients who received piroximone in doses of 0.25, 0.5, 1.0 or 2.0 mg/kg bw. Blood samples were drawn immediately before and 30, 60, 90, 120 and 240 minutes after bolus application. The ADP-induced platelet aggregation was inhibited time- and dose-dependently. The IC50 value for piroximone in vitro amounted to 67 ± 14 µmol/l. In the ex vivo experiments the maximal inhibition of ADP-induced aggregation was obtained in PRP from patients who had received 2 mg/kg bw piroximone 60 minutes before. The administration of piroximone resulted in a marked hemodynamic improvement with a dose-dependent increase in cardiac index and decreases in pulmonary artery pressure and resistance.

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EFFECTS OF OCREOTIDE AND GLUCAGON ON SPLANCHNIC HEMODYNAMICS IN HEALTHY VOLUNTEERS

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SPONTANEOUS CHANGES OF ST-SEGMENT DEPRESSION, HEART RATE AND BLOOD PRESSURE IN HYPERTENSIVES WITHOUT CORONARY ARTERY DISEASE: EFFECTS OF BEITA - BLOCKADE

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Pectanginal pain and signs of silent myocardial ischemia frequently occur in hypertensives, even in the absence of coronary artery disease (CAD) and/or left ventricular hypertrophy, probably due to a reduced coronary flow reserve. Since the oxygen extraction of the heart is nearly maximal during rest, increases of oxygen demand cannot be balanced by increases of myocardial perfusion. To assess the frequency of ischemic ST-segment depressions in this patient and to determine the influence of heart rate (HR) and blood pressure (BP), simultaneous 24 h Holter- and 24 h ambulatory BP monitoring were performed in 18 hypertensives (age 43 - 71 years, 9 f, 9 m) without CAD before and after four weeks on therapy with the 13 - blocker betaxolol for 4 weeks.

The extent of ST-segment depressions significantly correlated with HR and BP (p < 0.05). Drug therapy with 10 - 20 mg/d betaxolol for 4 weeks significantly decreased mean HR, systolic and diastolic BP (p < 0.05), 6 ischemic episodes of a total length of 38 min were recorded only in 4 of 15 hypertensives (26.7 %; p < 0.05; x²-test).

In conclusion, increases of HR and systolic BP seem to be the most important factors which induce myocardial ischemia in hypertensives without CAD. As silent ischemia is an independent risk factor for sudden cardiac death and other cardiac events, specific antihypertensive therapy should not only be aimed to normalize blood pressure, but should also address reduction of ischemic episodes as demonstrated here.

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PHARMACOECONOMIC ISSUES IN CRITICAL CARE. J.P. Dasta

Intensive care unit (ICU) patients often require many drugs to treat conditions associated with acute and chronic multi-organ dysfunction. Studies indicate patients receive approximately ten drugs, on average during their ICU stay, from several drug classes. Commonly prescribed drugs include narcotics, sedatives, antibiotics, antiarrhythmics, antihypertensives, drugs for stress ulcer prophylaxis, diuretics, vasoressors, and inotropes. Reports suggest surgical ICU patients cost the hospital an average of $18,000/patient in un-reimbursed costs under fixed-price reimbursement. Furthermore, patients with the greatest drain in revenue received catecholamines, triple antibiotics, or antifungal agents. Thrombolytics, antibiotics, plasma expanders, and benzodiazepines account for nearly two-thirds of the cost of drugs prescribed in medical and surgical ICUs. Agents with considerable economic impact include biotechnology drugs for sepsis. Pharmacoeconomic data in ICU patients suggest increased attention should be directed towards several areas, including patients with pneumonia, intraabdominal sepsis, nosocomial bloodstream infections, optimizing sedation and analgesic therapy, preventing persistent paralysis from neuromuscular blockers, preventing stress ulcers, treating hypotension, and providing optimal nutritional support. Studies are needed to assess the impact of strategies to improve ICU drug prescribing on length of stay and quality of life. If expensive drugs are shown to decrease the length of ICU stay, then their added costs can have positive economic benefits to the health care system.

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PULMONARY EXTRACTION OF DOBUTAMINE IN SURGICAL ICU PATIENTS. J. Dasta, C. Klem, T. Reilly, L. Flanebaum

The lung is known to be an organ for metabolism of several catecholamines. However, no data concerning pulmonary extraction of dobutamine (DBU) exist. Patients admitted to the surgical ICU who were receiving DBU and who had pulmonary and arterial catheters in place were eligible. A set of paired blood samples were obtained for DBU concentrations simultaneously from the arterial (Ca) and central venous (CV) catheters. Eleven patients (4M, 7F), 64 ± 16 years, 71 ± 24 kg were studied. Four patients had ARDS. DBU was administered at 7.8 ± 5.4 µg/kg/min for 8.5 ± 10.5 hrs before DBU concentrations were obtained. Ca averaged 172 ± 178 ng/mL, while Cv was 178 ± 187 ng/mL (ns by t-test). DBU total clearance averaged 82 ± 63 mL/kg/min. The absolute % extraction was 3.6 ± 23.0. Correlation between % extraction and Cv was r = 0.003. The % extraction for most patients could be explained by the coefficient of variation of the assay. A wide range of DBU concentrations were seen. The pulmonary extraction of DBU is low suggesting a minimal first-pass elimination of drug through the lung.

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β1- and β2-adrenergic contributions to β-adrenergic inodilatory responses in healthy man

C. de Mey, K. Eb, R. Buzer, G.G. Belz

The responses to 10 min iv. infusions of the β1- and β2-adrenoceptor agonist isoprenaline (ISO) and the β2- (and α1) adrenoceptor agonist adrenaline (ADR) at constant rates of 1 µg/min were evaluated noninvasively after pretreatment (pre-Tr) with placebo (PL), 100 mg of the β1-selective adrenoceptor antagonist talinolol (TAL) and 80 mg of the non-selective β- and α1-adrenergic agonist propranolol (PRO) in 12 healthy subjects. The following were assessed: heart rate (HR, bpm), pre-ejection period (PEP, ms), ejection time (VET, ms), HR-corrected electromechanical systole (QS2c, ms), impedance-cardiographic estimates of stroke volume (SV, ml), cardiac output (CO, l/min) and peripheral resistance (TPR, dyn.s.cm -5) calculated from CO and mean blood pressure (SBP and DBP according to auscultatory Korotkoff-l and -IV sounds). The average responses are detailed here below:

| Agonist | pre-Tr | TPR | HR | SV | CO | PEP | VET | QS2c |
|--------|--------|-----|----|----|----|-----|-----|------|
| ISO    | -34.5  | +28.5 | +239 | +45 | +47 | -34  | -40.6|
| ISO    | -46.9  | +39.3 | +239 | +2.6 | +25 | -4   | -10.6|
| ISO    | -99.9  | +2.8  | +57  | +0.6 | +9  | -1   | -4.1 |
| ADR    | -277.9 | +9.9  | +16.6| +23 | -29 | +8   | +8.1 |

This indicates that 1) about half the rise of HR and CO and half the shortening of PEP is β1- respectively β2-determined, 2) that predominant β2-adrenergic responses, whilst not affecting VET, take optimal benefit from the inodilatory enhancement of pump performance, 3) that an additional β1-adrenergic stimulation is proportionally less efficient, as VET is dramatically shortened, thus blunting the gain in SV so that the rise in CO relies substantially on the amplified increase of HR and 4) VET is more sensitive than QS2c in expressing additional β1-adrenoceptor agonism and 5) prime systolic time intervals provide a less speculative and physiologically more meaningful representation of cardiac pump dynamics than HR-corrected ones.

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A20

Regression between blunting of ergometric rise of heart rate and β1-adrenoceptor occupancies in healthy man

C. de Mey, D. Palm, K. Breithaupt-Gröger, G.G. Belz

The HR-responses to supine bicycle ergometry (4 min at appr. 200 Watt) were investigated at several time points after the administration of propranolol (δ-pro: 40, 80, 160 mg), carvedilol (CAR: 12.5, 25, 50, 100 mg), talinolol (TAL: 25, 50, 100, 400 mg), metoprolol (MET: 600 mg) and celiprolol (CEL: 1200 mg) to healthy man. The effects of the agents (= difference from resting values immediately before ergometry up to END. The effects were correlated with both the end values (END) and the increments (INC) from resting values to the active intercepts (I), slopes (S) and correlation coefficients (r) are detailed here below:

| END | CAR | MET | TAL | CEL |
|-----|-----|-----|-----|-----|
| 0.853 | -1.874 | -6.444 | -7.152 | -9.113 |
| 0.313 | -0.323 | -0.310 | -0.237 | -0.096 |
| 0.942 | 0.673 | 0.996 | 0.678 | 0.429 |
| 0.954 | 0.688 | 0.951 | 0.414 | 0.585 |

This indicates that 1) additional α-blockade does not distort the relationship (CAR vs. PRO), 2) MET and TAL had lower intercepts but similar slopes as PRO suggesting an "ergometric bonus", which could not be explained by the relative β1-adrenoceptor selectivity as it was maintained up to high doses where selectivity is impaired and 3) CEL's flat END-relationship gains its steepness when the slight increase of HR at rest is taken into account (as shown for INC). Mechanistically the INC-format might be more suitable for the analysis of complex effect constellations. Therapeutically though, the END-format might be more relevant.

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**A 21**

**INHIBITION OF LEUKOTRIENE PRODUCTION BY FISH OIL.**

W. Wilmanns, C. Denzlinger, T. Kliss, C. Haberl, C. Lemmen, O. Adam, and W. Willeiters.

Inhibition of leukotrienes is a promising approach to the treatment of several diseases because excess formation of these lipid mediators has been shown to play an important role in a wide range of pathophysiological conditions. Since recently we were not able to obtain specific drugs suppressing leukotriene biosynthesis or action for clinical practice, we started investigating the effects of putative natural modulators of leukotriene biosynthesis such as fish oil.

10 healthy male volunteers were supplemented for 7 days with fish oil providing 40 mg eicosapentaenoic and docosahexaenoic acid per kg body weight and day. The urinary concentration of leukotriene E4 plus N-acetyl leukotriene E4 served as a measure for the endogenous leukotriene production.

Treatment resulted in a significant increase in the eicosapentaenoic concentration in red blood cell membranes. Fish oil reduced the endogenous leukotriene generation in 8 of the 10 volunteers. The effect was associated with a decrease in urinary prostaglandin metabolites, determined as tetranor-prostaglandic acid. In contrast to what was expected from published in vitro and ex vivo experiments, no endogenously generated cysteinyl leukotrienes of the 5 series could be identified. The inhibitory effect of fish oil on the endogenous leukotriene generation was not synergistic to the effect of vitamin E, which also exhibited some suppressive activity.

Early clinical data on the effects of fish oil on leukotriene production in patients with allergy or rheumatoid arthritis are not yet conclusive.

**A 22**

**EFFECT OF ACETYLSALICYLIC ACID ON HUMAN THROMBOXANE AND PROSTACYCLIN RECEPTORS.**

M. Dobler, H. Bergmann, J. Michael-Hopp, J. Meyer, H. Darius.

The number and affinity of platelet thromboxane (TXA2) and prostacyclin (PGI2)-receptors are regulated by several factors. We studied the influence of oral intake of acetylsalicylic acid (ASA) on ex-vivo binding studies with human platelet membranes on the binding of the specific thromboxane A2 antagonist 1H-SQ-29548 and the PGI2 agonist 1H-Illoprost. The number of receptors (Bmax) and the binding affinity (Kd) were calculated using Scatchard’s plot analysis. In healthy male volunteers no significant difference was seen following intake of 500 mg/d of ASA for 14 days (mean ± SEM):

| drug | Bmax TXA2 pmol/mg prot. | Kd TXA2 nM | Bmax PGI2 pmol/mg prot. | Kd PGI2 nM |
|------|-------------------------|------------|-------------------------|------------|
| Placebo | 1.48 ± 0.17 | 46 ± 4.3 | 0.82 ± 0.12 | 31 ± 3.2 |
| 500 mg ASA | 1.69 ± 0.22 | 39.5 ± 4.2 | 0.98 ± 0.11 | 33.3 ± 3.1 |

In patients with stable coronary artery disease treatment with or without 40, 100 or 500 mg/d of ASA for 6 months showed now significant difference on PGI2 receptors (mean ± SEM):

| A2A | Bmax | n | Kd | Bmax | n |
|-----|------|---|----|------|---|
| 0 | 6 | 45 ± 3.6 | 1.7 ± 0.09 |
| 40 | 7 | 37 ± 6.1 | 1.6 ± 0.14 |
| 100 | 5 | 38 ± 4.6 | 1.6 ± 0.21 |
| 500 | 8 | 35 ± 3.3 | 1.4 ± 0.14 |

ASA at clinically relevant doses, did not show any significant influence on the number or affinity of thromboxane A2 or PGI2 receptors on platelets of human volunteers or patients with coronary artery disease.

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**A 23**

**MELOXICAM INHIBITS PREFERENTIALLY COX-2.**

G. Engelhardt.

The potency of Meloxicam (Mel), a new anti-inflammatory drug (NSAID), in the rat is higher than that of well-known NSAIDs. In adjuvant arthritis rats, Mel is a potent inhibitor of the local and the systemic signs of the disease. Mel is also a potent inhibitor of PG-biosynthesis by leukocytes found in rheumatic exudate in rats. Conversely, the effect of Mel on PG-biosynthesis in isolated enzyme preparations from bull seminal vesicle in vivo, the effect on intragastric and intrarenal PG-biosynthesis and the influence on the TXB2-level in rat serum is weak. In spite of the high anti-inflammatory potency in the rat, Mel shows a low gastro-intestinal toxicity and nephrotoxicity in rats.

Cyclooxygenase-2 (COX-2) has been recently identified as a lysosome of cyclooxygenase. NSAIDs are anti-inflammatory through inhibition of PG-biosynthesis by inducting COX-2 and are ulcerogenic and nephrotoxic through inhibition of the constitutive COX-1.

We have investigated the effects of Mel and other NSAIDs on COX-1 of non-stimulated and COX-2 of LPS-stimulated guine pig isolated macrophages. Cells were cultured with and without LPS for 6 hrs together with the NSAID. Arachidonic acid was then added for further 20 mins, the medium removed and PGE2 measured by RIA.

| variable | [K] | 95%-CI | [D] | 95%-CI | [Kd] | 95%-CI | [Kd] |
|----------|-----|--------|-----|--------|------|--------|------|
| SBP      | 1   | -4     | 7   | -1     | 4    | 3      |
| DBP      | 6   | -4     | 4   | -1     | 4    | 3      |
| HR       | 3.3 | 3.0    | 3.4 | 3.0    | 3.4  | 3.0    |
| PQ       | 2.2 | 2.0    | 2.2 | 1.9    | 2.2  | 1.9    |
| PEP      | 0.3 | 0.2    | 0.4 | 0.2    | 0.4  | 0.2    |
| GSPc     | 1.9 | 1.6    | 2.3 | 1.6    | 2.3  | 1.6    |
| LVEc     | 1.0 | 0.9    | 1.0 | 0.8    | 1.0  | 0.8    |
| CO       | 0.73| 0.70   | 0.74| 0.70   | 0.74 | 0.70   |
| TPR      | 1.42| 1.41   | 1.42| 1.41   | 1.42 | 1.41   |
| Hi       | 0.62| 0.61   | 0.63| 0.61   | 0.63 | 0.61   |

The high potency of Mel against the COX-2 explains the high anti-inflammatory activity of this compound in spite of the good gastrointestinal and renal tolerance.

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**A 24**

**CARDIOVASCULAR EFFECTS OF BIMAKALIM, DILTAZEM AND THEIR COMBINATION IN HEALTHY VOLUNTEERS.**

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Bimakalim, EMD 52692, is a new investigational K+-channel activator with vasodilating properties. Single peroral doses of 0.2 mg bimakalim, 60 mg diazepam, either alone or in combination, were investigated in 13 healthy male supine volunteers (20 to 28 years of age) in a placebo-controlled, period-balanced, randomised, double-blind, 4way cross-over design. Point estimates of the global effects of bimakalim (K), diltiazem (D) and their interaction [KxD, =0 in case of mere additivity] in 95% confidence intervals (CI) were analysed for systolic and diastolic blood pressure (SBP, DBP; minHg), heart rate (HR; bpm), PQ (ms), systolic time intervals (PEP, QRS, LVEc; ms), cardiac output (CO; 1.min⁻¹), total peripheral resistance (TPR; dyn.s.cm⁻⁵), Heather index (HI; 0.5.min⁻¹) and afterload and BP with little (reflectory) accompanying changes and had a negative dromotropic effect. The combination caused additive effects.

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| drug | Bmax TXA2 pmol/mg prot. | Kd TXA2 nM | Bmax PGI2 pmol/mg prot. | Kd PGI2 nM |
|------|-------------------------|------------|-------------------------|------------|
| Placebo | 1.48 ± 0.17 | 46 ± 4.3 | 0.82 ± 0.12 | 31 ± 3.2 |
| 500 mg ASA | 1.69 ± 0.22 | 39.5 ± 4.2 | 0.98 ± 0.11 | 33.3 ± 3.1 |

ASA at clinically relevant doses, did not show any significant influence on the number or affinity of thromboxane A2 or PGI2 receptors on platelets of human volunteers or patients with coronary artery disease.

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ENHANCED EXCRETION OF LEUKOTRIENE E4 IN PATIENTS WITH RHEUMATOID ARTHRITIS

J. Fauier, D. O. Stichtenoth, H. Zeidler and J.C. Frölich

Rheumatoid arthritis (RA) is characterized by an immunological mediated inflammatory reaction in affected joints. Infiltration of granulocytes and monocytes is the pathophysiological hallmark within the initial phase of inflammation. These cells are able to synthesize leukotrienes. LTE4 is a potent chemotactic factor and therefore could be responsible for the influx of granulocytes from the circulation. Cysteinyl leukotrienes LTC4, D4 and E4 augment vascular permeability and are potent vasocostrictors. LTC4 and cysteinyl leukotrienes have been detected in synovial fluid of patients with RA. However, these results are difficult to interpret, because the procedure is invasive and artificial synthesis cannot be excluded. We used a different, noninvasive approach by assessing the excretion of LTE4 into urine. Studies with 35S-LTC4 have demonstrated that LTE4 is unchanged excreted into urine and is the major urinary metabolite of cysteinyl leukotrienes in man. Urinary LTE4 was isolated from an aliquot of a 24 hour urine collection by solid phase extraction followed by HPLC and quantitated by RIA. Nine patients were enrolled in the present study. All met the American College of Rheumatology criteria for RA. Patients were treated with nonsteroidal inflammatory drugs and disease modifying drugs. Therapy with prednisolon was started after collection of the initial 24 hour urine sample. Disease activity was assessed by CRP (mean 59±22 mg/L) and ESR (mean 57±37/mm/hour). Patients with RA excreted 97±71 nmol/LTE4 into urine which was significantly (p<0.008) more than the 13.6 ± 3.1 nmol LTE4/mol creatinine excreted by comparable healthy volunteers. Prednisolon in a mean dose of 25 mg/day significantly (p<0.04) reduced LTE4 excretion within 7 days by more than 50%. The present data demonstrate an enhanced synthesis of cysteinyl leukotrienes in RA. Furthermore, we show an inhibition of this synthesis by prednisolon.

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A PHARMACOKINETIC DATABASE

T. Frankewitsch, D. Zeilner, T. Schromm, D. Nave, M. Giehl, F. Keller

Drug databases on computers are commonly textfiles or consist of tables of generic-names or prices for example. Until now pharmacokinetic data are not easily available for regular use, because searching parameters in a textfile is time consuming and personal intensive. On the other hand these pharmacokinetic data are the fundamental background of every dosage regimen and individual dosage adjustment. For many drugs elimination is dependent on the patients renal function. Renal failure leads to accumulation, possibly up to toxic plasma concentrations.

Therefore, the decision was to build up a pharmacokinetic database. The aim is to achieve simplicity and efficiency by using the basic rules. Only three parameters are needed to describe the pharmacokinetics: clearance (Cl), volume of distribution (Vd) and half-life (T1/2). Moreover, with two parameters the third can be calculated and controlled by the equation:

\[ Cl = \frac{Vd}{T_{1/2}} \]

According to the Detti-Equation and the Baye's theorem estimation of individual pharmacokinetic parameters will be done by a computer program.

The advantage is that the impact of therapeutic drug monitoring can be increased. Using the population data and the Bayesian approach, only one measurement of serum drug concentrations might be enough to achieve an individual dosage regimens (El Desoky et al., Ther Drug Monitor 1993, 15: 281).

Higher therapeutic, including those for the patient can be achieved. There is also a large pharmacoeconomic aspect: adapting drug dosage reduces costs (Susanka et al., Am J Hosp Pharm 1993, 50:909).

The basic database for future pharmacokinetic clinical decisions is going to be built up.

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A NEW APPROACHES TO THE INHIBITION OF PLATELET AGGREGATION (IIb/IIIa INHIBITORS). Desmond Fitzgerald.

Platelet aggregation is mediated by the binding of an adhesive protein, fibrinogen, to a surface inhibitor, the platelet glycoprotein IIb/IIIa. GPIIb/IIIa is one of a family of adhesion receptors, integrins, which consist of a Ca++-dependent complex of two distinct protein subunits. Under resting conditions, GPIIb/IIIa has a low affinity for fibrinogen in solution. However, activation of platelets by most agonists, including thrombin, ADP and thromboxane results in a conformational change in the receptor and the expression of a high affinity site for fibrinogen. Binding of fibrinogen to platelets is a common end-point for all agonists and therefore is a potential target for the development of antiplatelet drugs. These have included chimeric, partially humanised antibodies (7E3), peptides and peptidomimetics that bind to the receptor and prevent fibrinogen binding. The peptides often include the sequence RGD, a sequence that is present in fibrinogen and is one of the ligand's binding sites. When administered in vivo, antagonists of GPIIb/IIIa markedly suppress platelet aggregation in response to all known agonists, without altering platelet shape change, a marker of platelet activation. They also prolong the bleeding time in a dose and perhaps drug dependent manner, often to more than 30 min. In experimental models of arterial thrombosis, GPIIb/IIIa antagonists have proved highly effective and are more potent than aspirin. Studies in man have focused on coronary angioplasty, unstable angina and coronary thrombolysis and have given promising results. 7E3 given as a bolus and infusion combined with aspirin and heparin reduced the need for urgent revascularisation in patients undergoing high-risk angioplasty, although bleeding was more common. Some compounds have shown oral bioavailability raising the possibility that these agents could be administered chronically. Antagonists of the platelet GPIIb/IIIa provide a novel and potent approach to antithrombotic therapy.

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RENAI EXCRETION OF NARINGENIN AND ITS CONJUGATED METABOLITES FOLLOWING GRAPEFRUIT JUICE INTAKE.

U. Fuhr and A. Kummert.

The pharmacokinetics interactions with grapefruit juice reported for many drugs are attributed to the inhibition of cytochrome P450 enzymes by naringenin, which is the aglycone of the bitter juice component naringin. However, only circumstantial evidence exists that naringin is indeed formed when grapefruit juice is ingested, and the lack of drug interaction when naringin solution is given instead of the juice is still unexplained.

We investigated the pharmacokinetics of naringin, naringenin and its conjugated metabolites following ingestion of 20 ml grapefruit juice per kg body weight, containing 621 μM naringin, in 3 male and 3 female healthy adults. Urine was collected 0-2, 2-4, 4-6, 6-8, 8-10, 10-12, 12-16 and 16-24 hours after juice intake. Naringin and naringenin concentrations were measured by reversed phase HPLC following extraction using ethyl acetate, with a limit of quantitation of 300 nM. Conjugated metabolites in urine were transformed by incubation with glucuronidase (28000 U/ml) / sulfatase (733 U/ml) from abalone entails for 4 h at pH 3.8 and determined as parent compounds. Additionally, naringin and naringenin concentrations were measured in plasma samples from grapefruit juice interaction studies conducted previously.

Neither naringin nor its conjugated products were detected in any of the samples. Naringenin was not found in plasma. Small amounts of naringenin were found in urine after a median lag time of 2 hours and reached up to 0.365 % of the dose (measured as naringenin). After treatment with glucuronidase / sulfatase, up to 57 % of the dose was recovered in urine.

The absence of naringenin and its conjugates and the lag time observed for naringenin to appear in urine suggests that cleavage of the sugar moiety may be required before the flavonoid can be absorbed as the aglycone. Naringenin itself is a potent cytochrome P450 inhibitor, although inhibition of some of the enzymes is known but not probable. The pronounced variability of naringenin excretion provides a possible explanation for apparently contradictory results in grapefruit and/or naringin interaction studies.

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GRAPEFRUIT JUICE INCREASES ORAL NIMODIPINE BIOAVAILABILITY.
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Grapefruit juice increases the oral bioavailability of almost any dihydro-
pyridine tested, presumably due to inhibition of first-pass metabolism
mediated by the cytochrome P450 isoform CYP3A4. The mean extent of
increase was up to threefold, observed for felodipine, and more pronounced
drug effects were also reported. Thus, a such interaction may be of
considerable clinical relevance. No data are yet available for nimodipine.
We conducted a randomized cross-over interaction study on the effects of
concomitant intake of grapefruit juice on the pharmacokinetics of nimod-
pine and its metabolites M11 (pyridine analogue), M10 (demethylated) and
M9 (pyridine analogue, demethylated).
6 healthy young men (4 smokers / 4 nonsmokers) were included into the
investigation. Nimodipine was given as a single 30 mg tablet (Nimotop®) with
either 250 ml of water or 250 ml of grapefruit juice (Döhler GmbH, Darmstadt,
751 mlgrapefruit). Concentrations of nimodipine and its metabolites in plasma withdrawn up to 24 hours postdose were measured by GC-ECD,
and model independent pharmacokinetic parameters were estimated. The
study was handled as an equivalence problem, and ANOVA based 90 %
confidence intervals were calculated for the test (= grapefruit period) to
reference (= water period) ratios. The absence of a relevant interaction was
assumed if the CI were within the 0.67 to 1.50 range:

Thus, the null hypothesis "no relevant interaction" was rejected for AUC,
Cmax and the ratio AUCgrapefruit/AUCwater. Nimodipine effects should be
carefully monitored when the drug is taken concomitantly with grapefruit juice.

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RETRACTED ERYTHROMYCIN RELEASE AND BIOAVAILABILITY FROM FILM TABLETS STORED UNDER ARID CONDITIONS
M. Gerhardt1), A. P. Rump2), R. Sauerkrüppel1), M. Theisohn2)

The physical stability of erythromycin stearate film tablets was studied according to a 23 factorial design with experimental variables temperature,
relative humidity, and storage time. Within clinical trials the effects of poor compliance on the interpretation of study
results frequently leads to underestimating the efficacy of the treatment.
In the evaluation of the "Lipid Research Clinics Primary Coronary Prevention
Trial" and the "Helsinki Heart Study" special attention was focused on
compliance with medication. The strong influence of compliance on clinical outcome and the

dilutional effect of poor compliance on the efficacy of the respective
drug occurred in both these trials.
The indirect methods mentioned are commonly considered as unreliable. The direct methods can prove dose ingestion
a short time before the sample is taken, however, they cannot show the
time history of the drug use.
An advanced method of measuring compliance is to use electronic devices. The integration of time/date-recording microcircuitry into the medical
package, so as to compile a time history of package use, provides real-time data as
indicative of the time when dosing occurred. This method supports a precise,
quantiative definition of "patient compliance" as the extent to which the actual
time history of dosing corresponds to the prescribed drug regimen.
By taking real-time compliance data into account the results from clinical trials
show not only clearer evaluations of drug efficacy and dose-response-relationship
but also a better understanding of dose dependent adverse drug reactions.

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TREATMENT OF IMPOTENCE BY ERODERM CREAM

A.A. Gomaa, M.A. Shalaby, H.N. Anmed, M.E. Osman, A.E. Abdel-Menam and M.M. Abdelah

A new type of topical applied drugs (Eroderm creams) for impotence is presented. Eroderm creams contain vasoactive drugs. These drugs have ability to penetrate the penile cutaneous tissue and facilitate erection. Three different preparations (Eroderm 1, 2 and 3) were made in Pharmacology Depart. Fac. of Med. Assiut Univ. In the present study, we examined the usefulness of Eroderm-1 and Eroderm-2. Seventy five impotent men, 24 to 65 years old, participated in the present trial. The patients were classified into 3 groups, 25 patients each. The first group was treated by cream containing only co-dergocrine mesilate (Eroderm-1), the second received a cream containing isosorbide dinitrate, isoxsuprine HCL and co-dergocrine mesilate (Eroderm-2), while the third used a cream containing placebo. The cream was applied to penile shaft and gland 1/2-1 hr before sexual stimulation and intercourse. The patients were asked to report their experience via questionnaire after one week. The results of treatment are as follows: Seven patients (28%) who applied Eroderm-1 indicated a full erection and successful intercourse. The use of Eroderm-2 restored potency in 14 patients (56%) of the second group. Three men (12%) of psychogenic type reported a full erection and satisfactory intercourse. The patients reported their experience via questionnaire. Overall 70 percent of patients demonstrated a response with Eroderm-3. The other responders reported a partial erection and tumescence. Three men (8%) reported a full erection and satisfied intercourse with either cream. These patients were psychogenic impotence. Neither Eroderm-3 nor placebo cream produced marked response in 11 patients. Four patients were venous leakage which were advised to use tourniquet at the base of penis after 1/2 hr. of cream application. Only one of them indicated a good response. The highest activity proved to occur in psychogenic impotence. Less rate of success was observed in patients with minor to moderate neurological and/or arterial disorders. No marked side effects were recorded. For these reasons Eroderm-3 may be proposed as first line therapy of erectile dysfunction.

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TOPICAL THERAPY OF ERECTILE DYSFUNCTION

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A new type of topically applied drugs (Eroderm creams) for impotence is presented. Eroderm creams contain vasoactive drugs. These drugs have ability to penetrate the penile cutaneous tissue and facilitate erection. In the present study, we examine the usefulness of Eroderm-3 in the treatment of erectile dysfunction. Eroderm-3 contains tiemonium methylsulfate, A.F. piperazine and isosorbide dinitrate. A randomized, double blinded control trial on 36 patients was performed. The etiology of impotence was investigated. All patients received Eroderm-3 and placebo cream. The patients randomized into 2 groups of 18. The first group received Eroderm-3 on day 1 and placebo cream on day 2, however, group two received placebo on day 1. The patients were advised to apply the cream on the penile shaft 1/2 - 1 hr before sexual stimulation and intercourse. The patients reported their experience via questionnaire. Overall 70 percent of patients demonstrated a response with Eroderm-3. The other responders reported a partial erection and tumescence. Three men (8%) reported a full erection and satisfied intercourse with either cream. These patients were psychogenic impotence. Neither Eroderm-3 nor placebo cream produced marked response in 11 patients. Four patients were venous leakage which were advised to use tourniquet at the base of penis after 1/2 hr. of cream application. Only one of them indicated a good response. The highest activity proved to occur in psychogenic impotence. Less rate of success was observed in patients with minor to moderate neurological and/or arterial disorders. No marked side effects were recorded. For these reasons Eroderm-3 may be proposed as first line therapy of erectile dysfunction.

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COMPARATIVE BIOAVAILABILITY OF ACETYLSALICYLIC ACID FROM ACESAL®, ACESAL® EXTRA, MIRCISTIN® AND MINIASAL®
Claudia Hoffmann, M. Zschiesche, W. D. Kruger, Rita Sauter, G. Franke, W. Siegmund, and V. W. Steinijans

Bioavailability of Acesal®, Acesal® Extra, Mircistin® (all 500 mg acetylsalicylic acid - ASA), and Miniasal® (30 mg ASA), OPW Oranienburg, relative to respective listed references was studied in female and male healthy volunteers (age 18-35 y, weight 48-90 kg, height 161-198 cm). ASA, Acesal® and Acesal® Extra were administered using an HPLC method validated from 50 ng/ml to 60 µg/ml. Extent of absorption was assessed by AUC (bioequivalence range 0.8-1.25), rate by Cmax (bioequivalence range 0.7-1.43). Geometric means and 90%-confidence limits of the ratios Test/Reference (multivariate model) are shown in the Table.

Table: percent of the overall expenditures (100%) pharmaceticals in1993

| Agents       | A   | B   | C   | D   | E   | F   |
|--------------|-----|-----|-----|-----|-----|-----|
| Antibiotics  | 19.3| 18.3| 20.4| 20.7| 11.2| 20.0|
| Vincristine/Antimycotics | 7.6 | 6.1 | 5.7 | 7.5 | 3.1 | 1.3 |
| Immuno-globulins | 7.5 | 4.9 | 5.6 | 9.9 | 10.5| 2.8 |
| Cytokines/Immuno-suppr. | 7.1 | 6.7 | 3.6 | 7.5 | 5.2 | 5.2 |
| Infusion solutions | 6.9 | 6.5 | 10.1| 7.9 | 0.8 | 10.9|
| Cytostatics  | 4.5 | 5.5 | 5.6 | 6.2 | 4.3 | 7.9 |
| Hepatitis    | 2.5 | 2.5 | 2.9 | 2.7 | 1.5 | 6.0 |
| 5HT/5HTAntagonists | 1.4 | 1.8 | 2.2 | 4.4 | 3.1 | 0.8 |

Expenditures for group II products were about 20% up to 40% of group I and highest in hospitals A, B and E, but about 1/3 lower in hospitals C and D. These results suggest meaningful differences in the drug utilization between the old and new countries as well as between university institutions and community-based hospitals. However, although all hospitals provide oncology and traumatology services and all university hospitals offer NTX, differences in other subspecialties e.g. bone marrow and liver transplantation and treatment of patients with haemophilia must be considered, too.

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The cellular and biochemical mechanisms that lead to the long-term complications of diabetes mellitus are poorly understood.

Glycation is a multistep reaction that reduces sugars resulting in the formation of non-enzymatic protein glycation. The accumulation of glycosylated proteins may contribute to the development of the complications of diabetes and aging. In order to find suitable non-toxic glycation inhibitors we used rat tail collagen (proline, cysteine, and alpha-lipoic acid) and amino derivatives (amino-nitro-dine and amino-nitrate propionic acid) and postulated the mechanisms involved. The inhibitory effect of the drugs on the early and late Amadori products and advanced glycation products (AGEs), determined by affinity chromatography, deglycation method, EPLC and fluorescence spectroscopy, differs markedly depending on the drug used and is greatest (up to 80%) with aminoguanidine and penicillamine (20 mM) and smallest with alpha-lipoic acid. Aminoguanidine propionic acid had no inhibitory effect on the glycation process. The ability of penicillamine to inhibit both the formation of Amadori products and AGEs was demonstrated by isolation of the adducts of penicillamine with ribose, glucose, glutathione and p-oxo-glucosamine, a standard for glycosylated protein. Their structures were characterized using IR-, NMR- and 1H-NMR spectroscopy. The oxidative deglycation of glycosylated human serum albumin in the presence of copper (II) forming the carboxymethyllysine, was inhibited by the sulfhydryl derivatives. In the in vitro formation of pentosidine, a cross-linking by-product was not influenced by all drugs used.

However, captopril, alpha-lipoic acid and aminoguanidine propionic acid do not condense with sugar derivatives. The mechanism by which captopril and alpha-lipoic acid inhibit the formation of AGEs may be related to the formation, where a spontaneous thiol/disulfide interchange between the sulfhydryl and disulfide group takes place. Complications can appear at any time in diabetic patients. The cause of these complications, if not prevented, may lead to the development of complications. The clinical applications of substances which are able to prevent these events may have advantages. These results and the mechanism proposed show that the sulphydryl and amino groups of drugs reduce glycation and postglycation of proteins and thus have potential value in this regard.

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INHIBITION OF NON-ENZYMIC GLYCATION OF HUMAN SERUM ALBUMIN BY VARIOUS DRUGS

V. Jakus, U. Fuhr, N. Reitbrock

Non-enzymatic glycation starts by covalent binding of single sugars to α or ε amino groups of proteins. In subsequent reactions a number of early glycation products like Schiff bases or Amadori products, and of advanced glycation endproducts (AGEs) is formed. Glycation is increased in a variety of proteins in diabetic patients [Schleicher E (1992) Diabetes and Stoffwechsel 1: 274] including human serum albumin (HSA), the red cell membrane, hemoglobin, collagen, laminin, immunoglobulin G, low- and high-density lipoproteins. The process is supposed to be a key mechanism in the pathogenesis of diabetic complications and in tissue ageing [Vlassara H et al. (1994) Lab Invest 70: 138]. Inhibition of glycation may therefore be important for the prevention of late diabetic complications.

We investigated the in vitro glycation of human serum albumin (HSA) and its inhibition by aminoguanidine, penicillamine, captopril and α-lipoic acid (20 mM). HSA (40 g/l) was glyated by incubation with 20 mM glucose in 0.1 M phosphate buffered saline pH 7.4, at 37 °C for 28 days under sterile conditions in the presence of 0.1 % natrium azide. The glycation rate was determined by two different methods:

Deglycation - measures Amadori products based on the colorimetry of 2-keto-glucose which is released from the glycation product (ketoamine) on heating with hydrazine [Kobayashi K et al. (1993) Biol Pharm Bull 16: 195]

Fluorescence spectroscopy - measures advanced glycation endproducts.

All drugs tested reduced the glycation rate of HSA:

| Drug          | % Decrease HSA (20 g/l) | % Decrease HSA (40 g/l) |
|---------------|-------------------------|-------------------------|
| Aminoguanidine| 90%                     | 80%                     |
| Penicillamine  | 80%                     | 70%                     |
| Captopril     | 70%                     | 60%                     |
| α-Lipoic Acid | 60%                     | 50%                     |

The results provide evidence for a possible role of the investigated drugs in the prevention of the formation of early and late glucose-protein adducts in vivo.

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MODULATION OF BAY X 1005 MEDIATED 5-LOX INHIBITION IN HUMAN GRANULOCYTES BY ARACHIDONIC ACID.

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The quinoline derivative Bay X 1005 (OR=2-[4-(quinolin-2-yI-methylphenyl)-2-cyclopentyl] propanoic acid) is a selective inhibitor of leukocyte 5-LOX-biosynthesis in various in vitro and in vivo models. It acts via binding to the integral membrane protein Cysteinyl-leukotriene Receptor (FLAP), which has been shown to be an essential component of the cellular leukotriene synthesis machinery. The function of FLAP is only poorly understood, but recently it has been shown that a radiolabeled photoaffinity-analog of arachidonic acid binds specifically to FLAP. Consequently, a role of FLAP in the substrate transfer to 5-lipoxygenase (5-LOX) has been suggested.

Using intact and fractionated human polymorphonuclear leucocytes (PMNL), we present data showing that exogenously added arachidonic acid diminishes dose-dependently binding of [125I]-Bay X 1005 to its target. This effect correlates with the reduction of Bay X 1005 mediated inhibition of 5-LOX synthesis after stimulation of intact PMNL with the calcium ionophore A23187 in the presence of increasing extracellular arachidonic acid concentrations. In addition, enhancing the release of intracellular arachidonic acid from phosphatidic acid after activation of phospholipase A2 (PLA2) with increasing A23187 concentrations or after prolonged incubation of the cells with the ionophore results in a loss of Bay X 1005 potency. To further study the effect of arachidonic acid on the Bay X 1005 mediated LTβ1 synthesis inhibition, human PMNL were stimulated with PAF, C5a or FMLP in the presence of exogenous arachidonic acid. Under such conditions, LTβ1 synthesis is significantly enhanced in response to PAF, C5a or FMLP, which are only weak activators of cell-associated PLA2. The effect of extracellular arachidonic acid is dose-dependently inhibited by Bay X 1005. Coincubation of the cells with arachidonic acid and PAF, C5a or FMLP, respectively, enhances synergistically the translocation of 5-LOX from the cytoplasm to the membrane fraction. Again, this effect is inhibited by Bay X 1005. In summary, using Bay X 1005, we provide evidence, that FLAP is involved in the utilization of the 5-LOX substrate arachidonic acid from exogenous or endogenous sources for the agonist-induced leukotriene biosynthesis.

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THE INFLUENCE OF HEMATOPOIETIC GROWTH FACTORS ON VINCISTINE ACCUMULATION AND CYTOTOXICITY IN THE HL-60/AML CELL-LINE
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Hematopoietic growth factors have a yet to be established place in chemotherapy schedules of patients with acute myeloid leukemia (AML). These agents stimulate the incorporation of ara-C into DNA of AML blasts in vitro (C. Reuter et al. [1993] In: Drug resistance in leukemia and lymphoma. (Eds. G.J.K. Kaspers et al.), Harwood, Switzerland, pp. 215-226) but their effect on the expression and function of the multiple drug resistance gene product has not been studied in detail. We have therefore examined the effect of GM-CSF and G-CSF on the uptake and toxicity of vincristine and without the chemosensitizers R-verapamil and norverapamil in the P-glycoprotein-containing vincristine-resistant cell-line, HL-60/AML. Following a 48h pre-incubation with 10 ng/ml G-CSF or GM-CSF, cells were washed and reincubated in RPMI medium with 1H-vincristine and the modulators for 4 hours. The uptake of 1H-vincristine was increased up to 2-fold and this effect was equally marked when the vincristine-uptake was stimulated with the chemosensitizers in the range of 2.5 µg/ml to 100 µg/ml. Although cytokine-pretreatment increased the accumulation of vincristine, we could not show an increase in vincristine toxicity over 72h with the same concentrations of vincristine and chemosensitizers. It is concluded that the effects of cytokines on vincristine accumulation are compensated by a positive effect on cell-survival or cell-growth.

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COST-Benefit-Analysis of Drugs - An Example from Secondary Prevention of Stroke
A. Kempel, I. Kemper

From the economic point of view people often tend only to regard the price of a product. Savings for the health care system as a whole which could be gained by use of these drugs and which could more than compensate the high costs of medication are not considered. The cost-benefit-analysis enables a comprehensive analysis of the economic effectiveness of a pharmaceutical. The relevant cost factors such as the costs for in-hospital and for in-hospital out-patient treatment depending on the therapeutic success and the inability to work are compared to the costs of medications. Using the example of stroke prevention, it was possible to show that prophylaxis with an expensive but effective pharmaceutical is economically better than standard medication. According to the TASS study Ticlopidine can prevent at least 3 more strokes per 100 patients than ASA. On the basis of this study cost savings of up to DM 92,000 per 100 treated patients can be reached including higher costs of Ticlopidine. Projected onto 100,000 patients up to DM 92,000 per year can be saved for the German health care system by the use of Ticlopidine in secondary prophylaxis after TIA.

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Cost-Benefit-Analysis in the Healthcare System of the FRG - Cost Effectiveness of Prevention of NSAID-induced Ulcers and Prevention with Misoprostol
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The Healthcare Restructure Act (1993) changed the evaluation of drugs from efficacy to cost effectiveness: therapeutic necessity, utility, purpose; medical progress and economic utility have to be considered. Criteria and surroundings of pharmacoeconomic evaluations and studies according to the Healthcare Restructure act are demonstrated by an example: "Costs of NSAID-induced gastro-duodenal ulcers and prevention with misoprostol".

Methods: Cost-utility, Cost-benefit, legal requirements, decision-model and decision -tree, basic calculation and the transfer to the social system of the FRG. Previsions: rate of ulcers without prevention 21,7%, with prevention 5,6%; rate of compliance 70%, rate of hospitalisation 20% and duration of hospitalisation 16,6 days.

Results: basic calculation: Costs of prevention 62,77 DM / patient per 3 months. Transferred to the special circumstances in the FRG: rate of hospitalisation 30% and duration of hospitalisation 18,8 days for 50% of per 3 months cost-saving of 32,55 DM / patient per 3 months.

Literatur: C. Kori-Lindner, R. Eberhardt: Kosten-Nutzen-Bewertungen von Arzneimitteln und pharmakoeconomische Studien. Pharam.Ind. 56, Nr. 5 (1994) 419-424.

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THE CLINICAL PHARMACOKINETICS OF HYPERICIN AND PSEUDOHYPERICIN
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The naphthodianthrones Hypericin (H) and Pseudohypericin (PH) were proved to be effective in mild depressive disorders. Recently they were discovered to exhibit high in vitro activity against enveloped viruses fostering clinical trials in infections with HIV or herpes viruses. For studying the pharmacokinetics of H and PH a solidified extract from St. John's Wort (Hypericum perforatum L) was orally administered to 12 healthy male volunteers in three single doses of 250, 750, and 1500 µg of H and 526, 1578, and 3156 µg of PH, followed by a 14 days course of multiple dosing of 250 µg H and 526 µg PH. Plasma concentrations were determined by HPLC, the quantification limit was 0.2 µg/ml (Staffeld et al., Neuerheilverkurde 12/1993). Single-dose kinetics. H showed a median lagtime of 1.9 h (range: 1.4 - 2.3) h, which was significantly longer compared to 0.38 h (range: 0.5 - 1.0) h for PH. H was eliminated with a median half-life of 36 h (range: 12 - 56 h), and in case of PH the half-life ranged between 5.0 and 36 h (median: 16.5 h). Median Cmax levels were 15,7, 16, 16.8 µg/ml for H and 12, 11, 29.7 µg/ml for PH for the three single-dose levels given above, respectively. Lowest dose Cmax and AUC of H was lower than expected from medium and highest dose, which was statistically significant for Cmax (p<0.006). Anova; p=0.0156, Bonferroni/Dunn, indicating nonproportionally of pharmacokinetic response may exist in the lower dose range. Multiple-dose kinetics. During long-term dosing steady-state trough levels (Cmin) of 7.9 µg/ml (range: 3.4 - 13.6 µg/ml) for H and 4.9 µg/ml (range: 1.9 - 10.9 µg/ml) for PH were reached after 4 days for both. Cmax after 14 days of treatment were 8.9 µg/ml (range: 5.8 - 22.1) µg/ml for H and 8.5 µg/ml (range: 4.3 - 20.7) µg/ml for PH, this is about 2/3 of the Cmax reached after the corresponding single-dose of 250 µg H and 526 µg PH. The AUC for one dosing interval of 87 (range: 58.3 - 287) µg/ml for H and 121 (range: 41.7 - 244) µg/ml for PH is statistically not significant different to the corresponding single-dose AUC (interpolated to the same dosing interval) of 207 (range: 135 - 448) and 137 (range: 84.9 - 468) µg/ml for H and PH, respectively. Therefore acceleration of first-pass metabolism due to enzyme induction is unlikely during steady-state treatment in the dosing-range investigated. Plant extracted versus chemically synthesized H. To investigate the influence of galenics or other substances in the plant extract one single-dose of 750 µg of chemically synthesized H (in a 10% alcoholic solution) was given to one of the volunteers. Cmax and AUC were 2.6 µg/ml and 73.6 µg/ml, respectively, compared to 7.7 µg/ml and 145.6 µg/ml after the equivalent dose of a solidified extract. No difference was noticed in the lagtime (H in plant extract: 1.35 h versus chemically synthesized H: 1.5 h). Different sites of intestinal H and PH absorption may give an explanation, but this remains to be experimentally proven.

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Rheumatic disorders have become a frequent reason for services in ambulatory care. The use of drugs which are administered in rheumatic complaints is widespread. Data of the Scientific Institute of Statutory Health Insurances (Arzneimittelindex 1992) demonstrate that the group of antirheumatic/analgetics held the first rank due to the number of prescriptions. With regard to the costs this drug class was amongst the top three. Apart from antirheumatics and analgetics different other drug classes are used for the treatment of rheumatic disorders for example corticoids. To estimate the total expenditure for drug utilization in out-patients suffering from musculoskeletal disorders the data of the statutory health insurance Dortmund (AOK Dortmund) were investigated. A 5% random sample of insurees (n=478) was selected for diagnoses and drug therapy. The data were collected in a individual-related way for an observation period of one year (1988). The results demonstrate the high frequency of rheumatic disorders among out-patients: the 12-month prevalence of musculoskeletal disorders was about 40% in the studied population of whom one half suffered from back pain. Approximately 80% of the patients were prescribed any drugs for treatment of rheumatic disorders. As might be expected nonsteroidal antiinflammatory drugs were used most frequently. The extensive use of topical products was a considerable factor of costs. More than one half of the patients received drugs for topical application. Further details will be presented. Diachronous data of statutory health insurances that have been gathered in a person-related way permit the estimation of drug expenditure in out-patients with regard to the several musculoskeletal disorders.

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A 51
BIOAVAILABILITY OF TOPICAL ETOFENAMATE PRODUCTS IN RHEUMATOID ARTHRITIS PATIENTS
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Etofenamate is one of the most frequently applied topical NSAIDs in Germany. It can be regarded as a lipopholic prodrug of fnufenamic acid, which is liberated after transdermal absorption and cleavage of the ester. We compared several topical NSAIDs in patients suffering from osteoarthritis or rheumatoid arthritis with concomitant knee joint effusions. The products contained between 5 and 10% of etofenamate and were administered according to the recommended dosage schedule. Following multiple applications of defined doses we obtained synovial fluid and plasma samples in order to measure the concentrations and to compare the bioavailability of several products. The analysis of synovial fluid and plasma samples was achieved by HPLC. Following the addition of other N-phenyl-anthranilic acid derivatives as internal standards the standards were extracted twice with a 5-fold volume of acetone/methanol (1:1). The diluted extracts were concentrated with the use of preconditioned ODS-columns and subsequently eluted with methanol. The separation of the products was performed on a reversed phase column with an elution medium of methanol/water/acetic acid (72:28:0.1). The highest synovial fluid and plasma concentrations were measured after 3 hours following the final administration of etofenamate. The concentrations reached around average maximum values of 60.5 ng/ml of fnufenamic acid in plasma and 45.1 ng/ml in synovial fluid. The bioavailability could be estimated from the data. The concentration time interval between 0.5 and 3 hours, during which samples were obtained. They served for the comparison of the topical products. From in vitro data of cyclooxygenase or lipooxygenase inhibition with N-phenyl-anthranilic acid derivatives it can be concluded, that the synovial fluid levels of etofenamate and its metabolites are insufficient to produce effects relying on this mechanism. It can not be excluded, however, that other effects contribute to the clinical improvements observed by other investigators.

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A 52
A PLACEBO CONTROLLED DOUBLE BLIND CLINICAL TRIAL OF 7.5 MG AND 15 MG OF MELOXICAM IN SHORT TERM TREATMENT OF PATIENTS WITH RHEUMATOID ARTHRITIS (RA)
E-M Lemmel*

Introduction: Meloxicam (MEL) is a new non-steroidal antiinflammatory drug (NSAID) belonging to the enolic acid class. In animal studies it has been shown to have excellent anti-artritic efficacy and an improved tolerability profile over other NSAIDs. Early preclinical studies have shown that MEL preferentially inhibits cyclooxygenase-2 (COX-2).

The aim of this three-week, parallel, multicentre trial were to assess the efficacy and safety of two different doses of MEL in comparison to placebo (PLA) in patients with active RA. The specific question was, whether COX-2 inhibition relates to therapeutic efficacy and safety in the clinical setting.

Methods: 468 patients (377 F, 131 M, mean age 55 years) were randomised (MEL 7.5 mg =159, MEL 15 mg =162, PLA =147) and analysed by an intention-to-treat analysis. All trial drugs were given orally once daily.

Results: 31 patients (21%) in the PLA group, 11 patients (7%) in the 7.5 mg MEL group and 16 patients (10%) in the 15 mg MEL group discontinued the trial prematurely due to lack of efficacy or adverse events. 15 mg MEL was significantly superior (p<0.05) to PLA in 3 of the 4 primary endpoints: disease activity assessed by the investigator and by the patient and reduction of the tender/painful joint count. The difference between 7.5 mg MEL and PLA reached statistical significance in 2 of the 4 primary endpoints: disease activity assessed by the patient and reduction of the tender/painful joint count. In none of the primary endpoints a statistically significant difference between 15 mg and 7.5 mg MEL occurred. Global tolerability assessed by the patient and investigator at the last trial visit was similar in all three treatment groups. Adverse events were slightly more frequent with meloxicam than with placebo. In none of the patients gastric or duodenal ulcers, perforations or bleeds were observed.

Conclusion: The efficacy of meloxicam 7.5 mg and 15 mg per day was significantly superior to placebo in the treatment of patients with RA. There were no relevant differences regarding safety parameters between the 2 meloxicam doses and placebo. The preferential COX-2 inhibition may be the reason for the favorable efficacy and safety profile of meloxicam.

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MORPHINE GLUCURONIDES INTERACT DIRECTLY WITH THE CEREBRAL $\mu$-OPIOID-REZEPTOR

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Introduction: The major metabolites of morphine are morphine 3-0-β-D-glucuronide (M3G) and morphine 6-0-β-D-glucuronide (M6G). In humans plasma AUCs of M3G and M6G exceed those of morphine depending on form and route of application. M3G and M6G penetrate the blood-brain-barrier due to an unexpectedly high lipophilicity. M3G is an antagonist able to provoke withdraw symptoms even in opioid naive animals. M6G is an agonist markedly more potent than morphine.

Methods: Pig brains were homogenized and the homogenate centrifuged to give a microsomal preparation free of nuclei and coarse debris. Aliquots were incubated at 37°C in buffer (pH 7.4) containing 0.5 nM [3H]DAMGO.

Results: The IC₅₀ for morphine, M3G and M6G were 3.7 nM, 24 nM and 1.2 pM, respectively. M6G and M3G, respectively, shifted the IC₅₀ for morphine markedly to the right.

Discussion: Our results on the single substances confirm data related groups. The right we infer binding of M3G and M6G to the morphine binding site at the $\mu$-OR.

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DRUG USAGE PATTERNS AND COST ON SURGICAL INTENSITY CARE UNITS (SICU) OF THE UNIVERSITY HOSPITALS FRANKFURT/MAIN AND JENA

J.-P. Marschner*, J. Tauppi** and R. Schäfer**

Intensive care units are the most expensive wards consuming 15-20% of the hospital budget. Drug use reviews on SICU’s of the university hospitals Frankfurt (22 beds) and Jena (25 beds) were compared after recording the indication related administration of pharmaceuticals and blood products over a 4 months investigation period using a notebook-PC. The task of this group is to identify drug usage patterns, to record indication related drug administration including cost and to analyse these data. As a result recommendations concerning rational drug use and cost were recorded on a mixed Intensive care units are the most expensive wards consuming 15-20% of the hospital budget. Drug use reviews on SICU’s of the university hospitals Frankfurt (22 beds) and Jena (25 beds) were compared after recording the indication related administration of pharmaceuticals and blood products over a 4 months investigation period using a notebook-PC. The task of this group is to identify drug usage patterns, to record indication related drug administration including cost and to analyse these data. As a result recommendations concerning rational drug use and cost were recorded on a mixed

year | drugs | blood and | coagulation | total
--- | --- | --- | --- | ---
1982 | 15.7 | 6.1 | 4.6 | 26.4
1990 | 22.2 | 8.3 | 9.9 | 40.4
1991 | 24.1 | 10.0 | 9.9 | 44.0
1992 | 26.0 | 11.2 | 8.8 | 46.0
1993 | 23.6 | 4.8 | 3.9 | 32.3

The most expensive substances are blood-products (27%), antimicrobial agents (22%) and immunoglobulins (21%). In conclusion, at the University Hospital Frankfurt/Main the activities of the Drug-Utilisation-Review-Group seem to be successful and are at least in part responsible for the reduction of drug-cost of more than DM 10 Mio last year.

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According to a randomized double blind multiple cross over design, 6
the dose dependent hypokalemic effect of fenoterol required an negative
dose effect over 3 hours. The plasma fenoterol induced tachycardia and hypokalemia during and after a
observation period, the concentration response relationship remained
adequate described by an Emax model. Modelling of
central compartment. The relationship between heart rate and
did not reflect the in vivo in vitro dissolution profile determined as proposed by Brockmeier (I).
Additionally half value duration (HVD) and plateau time (PT) are reported.

Table: In vivo vs vitro pharmacokinetic parameters

| Parameter | A | B | C |
|-----------|---|---|---|
| T1/2 | 7.5 | 7.8 | 8.1 |
| Cmax(ng/ml) | 500 | 511 | 526 |
| tmax(h) | 5.0 | 4.9 | 7.1 |
| HVD(h) | 11.3 | 13.2 | 9.9 |
| PT(h) | 18.9 | 21.4 | 20.9 |
| AUC(ng/ml*h) | 6589 | 7493 | 7507 |

The difference of the dissolution profiles between the product is not reflected in the
parameters HVD and PT. Although preparation C has the longest PT and HVD, it has the largest Cmax value and shortest HVD. The origin of this apparent
discrepancy between in vivo and in vitro profiles deserves further elucidation

Calciumantagonists (CA) are lipophilic molecules which, however, have no
common structural similarities. Nevertheless, CA are characterized by the
ability to attach to various receptors and binding places in cardiac and
smooth muscle preventing calcium overload, necrosis and cellular death. In
order to study the physiological effects of CA on cerebral cortex of normal
and Vit D3 calcified rats, i.v. infusion of CA have been applied. Cerebral
blood flow (via hydrogen clearance) and oxygen supply (via surface oxygen
tension) have been measured in normal and sclerotic Albino rats in order to
differentiate cerebral and systemic effects. Miniaturized surface oxygen and
glucose electrodes were placed on top of the exposed cerebral cortex of
eketamine-xylazine anesthetized rats.

Nifedipine, verapamil, diltiazem and flunarizine induced hypotension in
normal and Monckeberg type sclerotic rats. Nifedipine and verapamil
showed comparable improvements in CBF and surface PO2, the initial
hypotension recovered completely under nifedipin but not under verapamil.

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Compared with verapamil, the hypotension was even more pronounced
under flunarizine (33.18%). Nevertheless, in response to both CA CBF was
improved to about 60%. However, diltiazem did not increase CBF due to
hypotension. Sclerotic rats showed a MAP decrease from 148 to 91 mm
Hg, the PO2 histogram was left-skewed. Diltiazem did not improve the
cortical supply of sclerotic animals.

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CARNITINE DEPLETION IN CHILDREN TREATED WITH PIVALOYL-SUBSTITUTED ß-LACTAM ANTIBIOTICS

J. Möller and M. Kurowski

Pivaloyl-substituted ß-lactam antibiotics belong to a new group of produgs with an improved bioavailability following oral administration. After metabolic cleavage the pivalic acid and the free ß-lactam are eliminated separately. Pivalic acid is subjected to enzymatic coupling with endogenous carnitine leading to carnitine-pivaloylester, which can be found in large amounts in the urine. Free carnitine is reduced under the treatment which may lead to neurological side effects in patients with metabolic predisposition. We showed, that the pattern of other acyl-carnitineesters excreted with the urine was not changed significantly after administration of a pivaloyl-substituted cephalosporine in healthy subjects.

In order to compare the pattern of excreted carnitineesters we analyzed urine samples of pediatric patients treated with cefetamet-pivoxil.

The analysis of carnitineesters in the urine was achieved by HPLC following pre-column derivatization. As an internal standard we used undecanoyl-carnitine ester, which does not occur in human plasma or urine. After the extraction using ion exchange columns the samples were incubated with p-bromo-phenacyl reagent. The mixture of derivatives was separated on a reversed phase column applying a gradient of different buffers. With this method the whole spectrum of carnitineesters can be quantitatively analyzed. The percentage of the pivalic acid ester eliminated with the urine in pediatric patients did not differ from that observed in healthy subjects, therefore an increased risk in this patient group cannot be inferred.

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Pharmacokinetics and bioavailability of Bupranolol-TTS and oral Bupranolol in patients with impaired liver function compared to healthy volunteers

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Bupranolol is nearly completely absorbed after oral administration, but more than 90% of the parent compound are metabolized by first pass through the liver. It can be expected that transdermal application of the substance will bypass the high first pass metabolism resulting in higher plasma concentrations or lower therapeutic doses, respectively. A reduced capacity of metabolizing liver enzymes might mimic this effect. The study was performed to investigate if metabolism by hepatic enzyme systems results in liver function-dependent pharmacokinetics. Special interest was to be given to possible differences between oral and transdermal application. In an open sequential single dose-study including 10 patients with impaired liver function and 10 age/sex/weight-matched healthy volunteers a bupranolol transdermal therapeutic system (containing 30 mg/24h) and bupranolol tablets (100 mg) were administered to each participant separated by a washout period of a week. Phenotyping of methylenedioxy and piperazine was done to exclude poor metabolizers. Blood samples for the quantification of bupranolol plasma concentrations were drawn before and 1,2,3,6,9,12,16,23,24 (before patch removal), 25,27,30,36,48,60 and 72 hours after patch application as well as before tablet administration and 0.5,1,1.5,2,3,4,6,6, 10,12,16, 24,30,36,48 and 60 hours post dose. Urine samples were collected in 5 (TTS) and 6 (tablets) intervals after dosing. Blood pressure and heart rate were measured before and 1,3,6,12 and 24 hours after dosing. ECG's were recorded before and 3.6 and 24 hours after dosing.

The pharmacokinetic results indicate only minor differences between patients and healthy controls after patch applications. For example the main parameter AUC(0-∞) was 66±28.3 ng·h/ml (mean±sd) in healthy controls and 57.8±18.1 ng·h/ml in patients. Completely other situation was given in comparison of data from oral administration: AUC(0-∞) decreased dramatically to 5.7±3.7 ng·h/ml in healthy controls and increased to 473.3±111.6 ng·h/ml in patients.

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Elevated Glucose Does Not Impair Endothelium-dependent Relaxation in Isolated Porcine Coronary Arteries

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Vascular complications are a common finding in patients with diabetes melititus. It has been reported that elevated glucose impairs acetylcholine- but not nitroprusside-mediated relaxation of rabbit aorta in vitro suggesting an abnormal endothelium-mediated vasodilator mechanism (Cohen, Circulation 1993; 87 (suppl. V): 67).

The purpose of the study was to investigate whether this phenomenon 1. implies general impairment of the nitric oxide-mediated relaxation (or only specific interference with muscarinic receptors), and 2. can be also demonstrated for coronary arteries. Porcine coronary arteries were cut in rings and were set up in organ baths for isometric tension recordings. Rings were randomized and incubated with different glucose concentrations (5.5, 22.0, or 44.0 mM) for 6 hours according to Cohen and coworkers. Rings were preconstricted with prostaglandin F2α (0.5 - 3 µM); the nitric oxide-mediated relaxation was induced by either substance P (0.01 - 10 nM), bradykinin (0.1 - 100 nM), or calciumionophor A23187 (1 - 1000 nM), the endothelium-independent relaxation by nitroglycerin (1 - 3000 nM). In addition, the effects of glucose were studied on acetylcholine- (0.01-10 µM) and serotonin- (0.001 - 10 µM) induced vascular responses. Results: Surprisingly, elevated glucose concentrations (22 and 44 mM) had neither an effect on the potency nor the efficacy of any of the agonists tested. It is concluded that this previously reported impairment of endothelium-mediated vasodilation of rabbit aorta by elevated glucose concentrations may not be important in other species or types of arteries.

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Urinary Nitrated Excretion in Cardiac Transplanted Patients: A Marker for Graft Rejection?

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Nitric oxide production may be induced by cytokine-mediated immune responses. We tested the hypothesis that graft rejection in cardiac transplanted patients is associated with an increased production of nitric oxide. The study group consisted of 83 patients in whom one or several myocardial biopsies (total 194 biopsies) were obtained to assess for graft rejection. At the day of biopsy, urine was collected for a time period of at least 4 hours (10% isopropanol was added for antimicrobial activity). Nitrate was measured in urine samples by gaschromatography. Nitrate excretion was expressed as the quotient of nitrate and creatinine content (µmol nitrate/mmol creatinine). Since nitric oxide is oxidized in biological systems to nitrite and finally nitrate, it was assumed that nitrate excretion reflects total nitric oxide release. Graft rejection was classified according to the Hannover classification system (A0: no/minor rejection, A2: A4: moderate/severe rejection). Results: Nitrate excretion showed a large interindividual variation (9 to 502 µmol nitrate/mmol creatinine). Although not significant, the nitrate excretion tended to increase with the degree of rejection (A0: 99±9, A1: 128±12; A2: 131±15; A3/4: 128±15; mean±SE). In a subgroup of patients the nitrate excretion was significantly associated with increased nitrate excretion (A0/A1: 111±13 vs. A2/A4: 161±30, p<0.05).

Conclusion: The urinary nitrate excretion is increased in patients with moderate/severe graft rejection. Because of large variation in excretion, this marker may be not suitable for rejection diagnosis.

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Platelets are widely used as an accepted model for the study of central neurotransmitter function in humans. However, only few comparative investigations on the influence of tricyclic antidepressants have been performed under in vivo conditions in patients. We analysed the changes of $V_{max}$ and $K_m$ as well as the uptake under half-saturation conditions in platelet rich plasma and washed platelets after 3 weeks of treatment with amitriptylin, nortripylin, maprotilin, and clomipramin in 50 depressed patients. The correlation of uptake-inhibition and amitriptylin/nortriptylin serum concentrations will be demonstrated. Potential effects of lithium augmentation in non-responders have also been studied.

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DT-TX 30, COMBINING THROMBOXANE-SYNTHETASE INHIBITION WITH -RECEPTOR ANTAGONISM, SELECTIVELY PREVENTS THE FORMATION OF LARGE THROMBI IN MAN EX VIVO. T.H. Müller, K. Rühr, H. Najjes, J. Krause.

The combination of thromboxane A2/prostaglandin endoperoxide (EPO) receptor antagonism with thromboxane synthetase inhibition (TRASI) is designed to both abolish the EPOs' prothrombotic role and direct EPOs from activated platelets to adjacent cells for the generation of antithrombotic prostaglandins. Thus, we have investigated the effect of the potenti TRASI DT-TX 30 on thrombus formation in models of vascular injury (of the media versus selective denoehelialization) in man ex vivo.

Healthy volunteers were treated with an oral dose of vehicle (placebo; n=10) or DT-TX 30 (25, 50, 100, 200, 400 mg; n=6 per dose). Blood was taken before and 1, 2, 4 and 24 h after the ingestion, anticoagulated and allowed to flow over a thrombogenic cell-free subendothelial matrix (CFM) or a layer of human venous smooth muscle cells (SMC) for 10 min. The "en face" area of platelets/thrombi deposited was determined by microscopic morphometry.

In the absence of cells of the vessel wall (CFM) the thrombus formation was inhibited by 32±15% (±SD) after 50mg, 43±26% after 100mg, 31±9% after 200mg and 57±29% after 400mg DT-TX 30 versus 5±21% after placebo. The subclass of the large thrombs (>7% of all platelets/thrombi but incorporating >90% of the platelet mass) was much stronger affected by the DT-TX 30 treatment: the mean area was reduced by 61±27% after 25mg, 69±20% after 50mg, 78±3% after 100mg, 53±25% after 200mg and 71±8% after 400mg DT-TX 30 versus -16±24% after placebo.

In the presence of cells of the vessel wall (SMC) the overall thrombus formation was reduced by up to 42±21% after only 25 mg, 36±31% after 50mg, 59±20% after 100mg, 72±5% after 200mg and 81±6% after 400 mg DT-TX 30 versus 2±12% after placebo.

In summary, DT-TX 30 showed a unique antithrombotic profile of selectively inhibiting the formation of large thrombi and cooperating with vascular smooth muscle cells in man ex vivo.

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INHIBITION OF COLLAGEN-INDUCED PLATELET AGGREGATION BY DT-TX 30, A COMBINED THROMBOXANE-SYNTHETASE INHIBITOR AND -RECEPTOR ANTAGONIST, IN MAN EX VIVO. T.H. Müller, H. Najjes, J. Krause.

DT-TX 30, a molecule combining potent and specific thromboxane synthetase inhibition with prostaglandin endoperoxide/thromboxane A2 receptor antagonism, has been examined in healthy male subjects. Collagen-induced platelet aggregation in platelet rich plasma prepared from venous blood was measured photometrically before and up to 24 hours after a single oral dose of 25, 50, 100, 200 or 400 mg DT-TX 30 in a placebo-controlled, double-blind study. Platelet aggregation was induced in the ex vivo samples by collagen in concentrations between 0.5 and 10 μg/ml to evaluate platelet aggregation in relation to the strength of the proaggregatory stimulus. The $E_{50}$, i.e. the concentration of collagen required for a half-maximal aggregatory response (defined as the maximal change of the optical density), was determined.

In the placebo-treated control group, the mean $E_{50}$ was 365±55 ng/ml collagen (+ SE; n=10) before treatment. It then varied between 362±41 and 417±83 ng/ml collagen after treatment. The ratio of the post- to the individual pre-treatment $E_{50}$ values was 1.08±0.10 (n=10) at 0.5 h, 1.05±0.11 at 1 h, 1.13±0.14 at 2 h, 1.15±0.20 at 4 h, 1.07±0.07 at 8 h and 1.02±0.06 at 24 h. This indicates that the sensitivity of the platelets to collagen was not affected by the placebo treatment.

Oral treatment with DT-TX 30, however, strongly inhibited the aggregatory response of the platelets to collagen stimulation. The $E_{50}$, ratio was increased to a maximum of 4.5±0.85 (1 h p.a.; n=8) by 25 mg, 8±1.9 (1 h p.a.; n=8) by 50 mg, 9±1.6 (0.5 h p.a.; n=6) by 100 mg, 11±1.9 (0.5 h p.a.; n=5) by 200 mg and 22±7.4 (2 h p.a.; n=6) by 400 mg DT-TX 30. A substantial inhibition of collagen-induced platelet aggregation was observed still 8 h after the administration ($E_{50}$, ratio of 3.8±0.6 (n=6) for 100 mg and 7.8±1.3 (n=6) for 400 mg DT-TX 30).

A single oral dose of DT-TX 30 inhibits collagen-induced platelet aggregation in both a dose-dependent and reversible manner in man ex vivo.

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ENDOGENOUS DRUG-LIKE FACTORS IN A POLISH POPULATION
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The detection of endogenous opioids suggested the opinion that in case of the presence in the organism of a receptor for an exogenous substance there is probably a similar endogenous substance. The occurrence in the blood of persons, who were not treated with cardiac glycosides, of endogenous digoxin-like or ouabain-like factors confirms that opinion. In our study we took up the research of drug-like factors in the blood serum of healthy people.

In two hundred and twenty-five healthy volunteers (110m,115f) non-smokers not receiving any treatment before or during the test and aged between 18 and 49 y(mean age 36y) the occurrence of drug-like factors in blood serum was studied. The examinations were carried out with the use of the fluorescence-polarization-immunoassay (FPISA)-TD Abbott. The presence of the following endogenous drug-like factors in the blood serum was evaluated: quinidine, phenytoin, carbamazepine, theophylline, cyclosporine and gentamicin. The occurrence of endogenous phenytoin-like, theophylline-like and cyclosporine-like factors has been demonstrated. The drug-like factors were not found in the case of quinidine, carbamazepine and gentamicin. The phenytoin-like factor was found in 91.4%, theophylline-like factor 39.1% and cyclosporine-like factor in 56.9% of examined volunteers. The mean value of the drug-like factors was as follows: phenytoin 0.18-0.05 μg/ml, theophylline 0.16-0.11 μg/ml and cyclosporine 12.41-4.24 ng/ml. The supposition may be proposed that organism produces drug-like substances according to its needs.

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POLYMORPHIC ARYLAMINE N-ACETYLTRANSFERASE (NAT2) GENOTYPES: CORRELATION WITH PHENOTYPIC ACTIVITY
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Recently the gene structure of NAT2 was elucidated and now the NAT2 genotyping methods may substitute the NAT2 phenotyping procedures in clinical and epidemiological investigations. The aim of this study was to compare various NAT2 genotypes with NAT2 phenotypic activity. NAT2 genotyping was performed in 176 children of Polish origin applying allele specific polymerase chain reaction (mutation at position 341 nt of the coding sequence) and restriction fragment length polymorphism with specific polymerase chain reaction (mutation at position 341 nt of the coding sequence). The genotype nomenclature of Grant (Pharmacogenetics 1993, 3, 45 - 50) was used. The genes coding for rapid acetylation were present in 37.5% of the cases, genes coding for slow acetylation were detected in 62.5%. The frequency of specific NAT2 alleles were: R1, 20.7%; R2, 1.2%; S1a, 30.4%; S1b, 31.1%; S1c, 6.3%; S2, 34.9%; S3, 1.4%; and S4, 2.0%. There was no mutation found at 191 nt. In 166 cases (94.3%) we have stated NAT2 geno- and phenotype concordance, but in 10 cases (5.7%) genotype and phenotype deviated from each other. Seven individuals were genotyped as S1aS2, one as S1aS1c, and one as S1aS4 but were phenotypically rapid acetylators. One child was genotyped as R1S1a but proved a slow acetylator.

We can confirm that in about 94% NAT2 genotyping allows to properly predict NAT2 phenotype. Discrepancies may derive, i. a., from yet unknown mutations which we try to detect by DNA-sequencing.

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THE ACETYLATION AND OXIDATION PHENOTYPES IN A POLISH POPULATION
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The acetylation and oxidation phenotypes were studied in 448 healthy volunteers (235m, 213f) aged between 18 and 46 years (mean 36y) in the Wielkopolska region in Poland. The acetylation phenotype was studied with the use of sulphadimidine which was given in a dose of 44 mg/kg b.w. per os. Sulphadimidine was determined by a spectrophotometric method. The border value of M.R. was 75% in urine.

The oxidation phenotype was studied with the use of sparteine which was given in a dose of 1.5 mg/kg b.w. per os. Sparteine was determined by the Gas Chromatographic method in urine. If M.R. was 20% the oxidation for intensive metabolism, if M.R. was 0% the oxidation for poor metabolism.

The determined phenotypes were bimodally distributed. Our studies have shown that 259 healthy volunteers (57.04%) were slow and 189 (42.96%) were fast metabolizers. Intensive oxidation phenotype could be ascertained in 423 volunteers (94.4%) and poor oxidation phenotype in 25 volunteers (5.6%). These findings are comparable with the values typical for the Caucasian population.

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NISOLDIPIN AND NITROGLYCERIN PREVENT DIGOXIN-INDUCED CONstriction OF EPICARDIAL CORONARY ARTERIES IN PATIENTS WITH CORONARY ARTERY DISEASE
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Several studies demonstrated that cardiac glycosides reduce diameter of epicardial coronary arteries. In patients (PTS) with coronary artery disease (CAD) this vasoconstrictive side effect of glycosides may cause ischaemic complications in the presence of high-grade stenoses. This study evaluated quantitatively the effect of an i.v. injection of digoxin on the diameter of epicardial coronary arteries in 20 PTS with CAD. Eleven PTS (group 1) were treated with aspirin 100mg/d, nine PTS (group 2) were treated with aspirin and 10mg nisoldipin 2 hours prior to angiography. Coronary angiograms were taken in identical projections before (0) and 5, 10, 15 and 30 minutes after i.v. injection of 0.8mg digoxin; at 35 min 0.1mg of nitroglycerin (NTG) was given. The mean diameters of normal segments and the minimum diameters of stenoses were analysed by CMS (Medis, Leiden). A total of 149 normal and 28 stenotic segments were analysed. Figure shows the change of coronary diameter (% prodrug value; mean ± SE) for group I (left) and II (right):

Conclusions: 1. Application of digoxin i.v. a in clinically common dosage induces constriction of normal and stenotic coronary arteries. 2. Nisoldipin prevents digoxin-induced vasoconstriction, specially in stenotic coronary arteries. 3. Digoxin-induced vasoconstriction is reversible with NTG. 4. PTS with CAD should be treated with NTG or nisoldipin before administration of digoxin in order to prevent vasoconstrictive side effects.

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Talinolol Metabolism in Human - Structure, Polarity and Urinary Recovery of the Metabolites

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Talinolol (1-4-cyclohexylurido-phenoxyl)-2-hydroxy-3-tet-butylamino-propan; AWD, Dresden) is frequently used as a cardioselective β-adrenoceptor antagonist. Talinolol metabolites in urine were detected by HPLC and GC/MS with previously characterized reference compounds and were quantified by HPLC with a normal phase silica column (Oettel et al., Be. 8 Cl. 8h 3 4, 37 (1994) 496). Less than one per cent of administered dose was recovered in urine as hydoxylated talinolol. Other metabolites could be excluded. In serum after therapeutic doses talinolol metabolites were not traceable. However, in a suicidal patient with a parent compound serum concentration of 1400 ng/ml (manifold higher as after usual therapeutic dose) two hydroxy metabolites at concentrations near the detection limit (10 ng/ml) were found. Obviously only a small amount of the talinolol dose is metabolized in human.

In this study the relation between structure, polarity and urinary recovery of the metabolites was examined. Four different isomers of mono-hydroxylated talinolol were identified in urine of volunteers after a single dose of talinolol. Eight isomers are possible: six hydroxyl-cyclohexyl-compounds and two hydroxy-phenoxo-compounds. There was a low degree of specificity of biotransformation seen with respect to the point of attack on the cyclohexyl ring. Hydroxylation of the phenyl ring could be excluded in human.

Using HPLC methods very small quantities of reference compounds are sufficient to estimate and compare the polarity and hydrophility of similar compounds. Long retention times in a normal phase system and short retention times in a reversed phase (RP) system are found for polar and hydrophilic compounds. The shortest retention times of all hydroxylated talinolol compounds in the normal phase system as well as the longest retention times in the RP system were found for the main metabolites 4-trans- and cis-hydroxy-cyclohexyl-talinolol. Accordingly, the main metabolites are the most polar and hydrophilic mono-hydroxylated talinolol isomers.

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Role of Protein Isoprenylation in Carcogenesis: Inhibitors of Farnesylation

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Increased cell proliferation induced by ras-oncogenes is mediated by growth factors. Growth factors bind to cell membrane surface receptors and activate tyrosine kinases leading to protein phosphorylation. This triggers posttranslational modification of the function of low molecular mass GTP binding Ras proteins, e.g. p21ras, including hetero-meric G proteins. These proteins carry a C-terminal amino acid sequence, CAAX where C is cysteine, which is the target of isoprenylation by farnesyl protein transferases (FPTases) and geranyl-geranyl protein transferases (GGPTases). The amino acids of this sequence determine whether the Ras protein is substrate for FPTases or GGPTases. This biochemical pathway is related to mevalonate metabolism, therefore, cholesterol biosynthesis shales several steps. The active compounds include a series of isoprenoid inter-mediates, such as geranyl pyrophosphate (C-10), farnesyl pyrophosphate (FPP, C-15) and geranyl-geranyl pyrophosphate (GGPP, C-20) which are critical for cell proliferation. Inhibitors of isoprenylation may (i) compete with FPP to its binding site on the alpha subunit of the enzyme, (ii) inhibit the Zn protease of FPTase beta subunit, and (iii) interfere with the binding of the CAAX peptide to its binding site on the beta subunit. The inhibitors include microbial products like manumycin, gliotoxin, acetylgliotoxin and pepticin-amines, FPP analogues, tetrapeptides and related pep tidomimetics that may compete for the CAAX sequence, products of plant origin like limonene as well as synthetic compounds. All these compounds have been known to retard cell proliferation, and their effect can be abolished by mevalonic acid. Inhibitors of HMG-CoA reductase, such as lovastatin, which have been developed as antihyperlipidemic drugs, may also effective as anticancer agents. The recognition of the importance of mevalonate metabolism in carcogenesis provides possibility to test simple chemical compounds against tumor growth, and gives promise for pharmacological exploitation of inhibitors of isoprenylation and HMG-CoA reductase inhibitors for cancer chemotherapy.

Effects on Platelet Functions of Single Oral Doses of Lysine Clonixinate and Acetylsalicylic Acid

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Lysine Clonixinate is a non-steroidal antiinflammatory drug with marked analgesic effects. It is used for treatment of acute pain such as postoperative, dental or menstrual pain. We have determined the biosynthesis of thrombocoye (TX)B2 and prostaglandin (PG)D2 clotting whole blood ex vivo as well as collagen-induced platelet aggregation before and 0.25, 0.5 (only TXB2; and PGD2 biosynthesis), 1, 2.5, 6, 24 and 48 hours after oral intake of 125 mg Lysine Clonixinate. The results were compared with data obtained after oral intake of 500 mg acetylsalicylic acid (ASA). While both TXB2 and PGD2 biosynthesis measured radioimmunologically were inhibited significantly (p<0.05 and p<0.01, respectively) 2.5 hours, but not 6 hours after Lysine Clonixinate, inhibition by ASA was much greater and still highly significant (p<0.01) after 48 hours. The mean concentrations of Lysine Clonixinate and of ASA inhibiting TXB2 biosynthesis in vitro by 50% (EC50) were 3.2±0.5 µM and 6.2±0.9 µM, respectively. Aggregation of 0.5 ml aliquots of platelet-rich plasma induced by 1 µg/ml collagen was inhibited by Lysine Clonixinate significantly (p<0.01), but not completely for up to 24 hours, while aggregation induced by 3 µg/ml collagen was inhibited for up to 6 hours only (p<0.01). Platelet aggregation induced by both collagen concentrations was inhibited by ASA almost completely (p<0.01) for at least 48 hours. The EC50 for in vitro inhibition of submaximal collagen-induced platelet aggregation was 59±16 µM for Lysine Clonixinate and 97±21 µM for ASA. It remains to be investigated, whether other mechanisms than inhibition of cyclooxgenase contribute to the analgesic efficacy of Lysine Clonixinate. The profile of side effects including only moderate inhibition of platelet function by Lysine Clonixinate as compared to ASA might be an advantage with regard to its use as an analgesic.

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The results of our study indicate that aspirin treatment slows carotid plaque growth in a dose-dependent fashion, with a dose of 900 mg daily more efficient than 50 mg daily.

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EFFECT OF LOW DOSE CICLETANIN ON PROSTACYCLIN SYNTHESIS AND BLOOD PRESSURE IN HYPERTENSIVE PATIENTS

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20 patients with essential hypertension were treated with cicletanin (100 mg/d) for 12 weeks. Compliance was verified by measuring urinary cicletanin excretion. Before entering the study antihypertensive medication had been withdrawn for at least 2 weeks (wash out phase). The study was preceded by a placebo phase of 1 week duration. Blood pressure measurements and urine collections for measurements of 2,3dionor-6-keto-Prostaglandin F2a (a stable prostacyclin metabolite) were performed at the start and end of placebo phase, after 1 week of treatment with cicletanin and in monthly intervals thereafter. Cicletanin decreased systolic and diastolic blood pressure, the effect becoming significant at 4 weeks.

| Wash out | Placebo | Cicletanin |
|----------|---------|------------|
| Systolic BP [mmHg] | 169±12.4 | 160,23±3.3** | 150,6±3.9 ** |
| Diastolic BP [mmHg] | 99±12.0 | 95±5.2 | 89±3.2 ** |
| Pulse [bpm] | 73±8.2 | 72±6.0 | 70±6.4 |
| Urine [m/d] | 150,6±169 | 132±120 | 138±64 |
| d-6keto PGF1α [pg/ml] | 11±13 | 11±8 | 13±15 * |

*p<0.05; **p<0.01; ***p<0.001 compared to the preceding phase; Cicletanin data are depicted as average of all treatment periods

Urinary excretion of the prostacyclin metabolite 2,3dionor-6-keto PGF1α increased by 21%. Hypertensive patients and control subjects (n=19; 2,3dionor-6-keto-PGF1α: 120±19 pg/ml) did not differ significantly in urinary excretion of 2,3dionor-6-keto-PGF1α. Neither systolic nor diastolic blood pressure showed a significant correlation with 2,3dionor-6-keto-PGF1α alpha excretion (systolic BP: R=0.12, p=0.21; diastolic BP: R=0.0007, p=1.0).

These data show that cicletanin is effective in reducing blood pressure. Increased prostacyclin production is probably not responsible for the observed reduction of blood pressure.

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DOSE-DEPENDENT EFFECT OF ASPIRIN ON CAROTID ATHEROSCLEROSIS

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Antiplatelet treatment with aspirin is well established as secondary prophylaxis after a transient ischemic attack or minor ischemic stroke, but the effect of aspirin treatment on the initial stages of carotid atherosclerosis is unknown. We tried to investigate the effect of aspirin on the initial stages of carotid atherosclerosis.

Patients were recruited from a prospective, randomized, double blind clinical trial to compare two doses of aspirin (900 mg vs 50 mg daily) with respect to restenoses after lower limb angioplasty. Of the 383 patients admitted to the angioplasty trial, 27 patients showing 104 small carotid atheroma (<50% lumen narrowing) were examined at entry and after 1 year of aspirin treatment using a high-resolution ultrasound duplex system. Disease progression and regression were defined by a change of maximal plaque area (as measured by longitudinal ultrasound sections) of more than 2 standard deviations of the method.

The change in plaque area was significantly different for the treatment groups: average plaque size remained unchanged after treatment with 900 mg aspirin daily but increased markedly after treatment with 50 mg aspirin daily (p = 0.011). There were significantly more lesions in the 50-mg group showing progression than in the 900-mg group (23 plaques [47%] vs 13 plaques [24%], p = 0.025). Ultrasonic disappearance of a lesion was observed only in the 900-mg group in 9 cases (7 soft plaques, 2 ulcerative plaques, p = 0.18). The 5 patients on 50 mg aspirin who continued smoking during the study showed significantly more progression as compared to the 7 non-smokers in the 50-mg group (17 plaques [59%] vs 6 plaques [30%], p = 0.036).

The results of our study indicate that aspirin treatment slows carotid plaque growth in a dose-dependent fashion, with a dose of 900 mg daily more efficient than 50 mg daily.

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THE APPLICATION OF POPULATION KINETICS IN THE TREATMENT OF NEONATAL PATIENTS WITH GENTAMICIN

U. Renz and M. Kurzawa

Gentamicin is frequently applied in neonatal patients for the treatment of severe infections. The use is associated with high incidences of ototoxic and nephrotoxicity. Ototoxicity occurs in 1-1.5 % of the treated patients. It can be avoided, if the trough levels remain under 2 µg/ml between the administered doses. If the nephrotoxic effect, which occurs in 2-10 % of the treated patients, can also be reduced under these circumstances is not yet clear. Recently it has been suggested to administer gentamicin only once daily in order reach the desired trough levels. This applies especially to children, in which the terminal half-life can be prolonged to approximately 9 hours. This dosage regimen assures a sufficient efficacy due to the marked postantibiotic effect, but should reduce the incidence of the toxic effects.

In order to test this hypothesis we perform a case-control study with neonatal children treated with gentamicin. The cases of nephrotoxicity during the previous 12 months are identified from the hospital records. For these patients the plasma level time curves are obtained with the use of a population based pharmacokinetics programme, which considers the measured plasma concentrations and the administered doses. Plasma concentrations were measured routinely in the process of therapeutic drug monitoring. From these curves the duration of times with concentrations lower than 2 µg/ml during the last 3 days prior to the diagnosis is estimated. For the cases matched controls were identified according to age, age of gestation and other factors affecting the renal function e.g. comedication. Different analyses are carried out according to the definition of exposure. The results will be discussed with regards to the modification of the dosage schedule for gentamicin and the application of population kinetics in order to reduce the adverse effects.

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ECONOMIC CONSEQUENCES OF THEOPHYLLINE TDM: PRELIMINARY RESULTS OF A ONE-YEAR OBSERVATION

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Economic evaluation of drug therapy is an important task of Clinical Pharmacology. Drug costs amount to a relatively small percentage of total health care costs. Some drugs may help to avoid more expensive therapeutic procedures. Drugs with small therapeutic ratio need a therapeutic drug monitoring (TDM) to optimize therapeutic results and to avoid side effects. TDM may enhance the costs of drug therapy and the question arises, whether the additional costs are justified from the economic point of view. The aim of the study was to investigate the effectiveness of therapeutic TDM in chronic obstructive pulmonary disease.

Methods: 30 outpatients suffering from chronic obstructive pulmonary disease and receiving theophylline as a basic medication were included in this study. During the one-year observation period the parameters of pulmonary function were examined four times (1st, 3rd, 6th and 12th month) by the same physician and theophylline trough levels were estimated to individualize the dosage. The clinical outcome was assessed by intra-individual comparison.

Results: Impaired parameters of pulmonary function were improved. Hospital admissions were reduced. During the one-year observation period 4 out of 30 patients had 6 hospital admissions with totally 64 days. Under the conditions of theophylline therapy without TDM during the year before 7 of them had 13 hospital admissions with 197 days of stay in hospital.

Discussion: The results agree with a previous study on inpatients. There it was observed, that parameters of pulmonary function significantly were improved due to TDM-supported theophylline therapy in comparison to a group receiving theophylline without TDM. Despite the additional costs theophylline TDM contributes not only to improved clinical outcome but also to decreased costs for the care of patients with chronic obstructive pulmonary disease.

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PHARMACOKINETICS OF MITOMYCIN-C IN THE CHEMOEMBOLISATION-TREATMENT OF LIVER METASTASES

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In cancer treatment arterial blood flow reduction by embolisation followed by intra-arterial chemotherapy may be advantageous by achieving high and prolonged drug concentrations in the tumor and lower systemic drug exposure. Pharmacokinetics of mitomycin-C (MMC) were investigated in 4 colorectal cancer patients (body weight: 55-92 kg) with liver metastases undergoing chemoembolisation. After hepatic artery branch catheterization and microspheres injection (Polyvinylalkohol, circumference: 150-250 μm, ITC-Contour Rehaforum Medical) 20 mg MMC dissolved in 20 ml physiological NaCl solution were infused in 5-8 min, followed by Echolock application. Serum MMC-concentrations were determined from peripheral venous blood samples by reverse-phase HPLC-system with ultraviolet detection (C18-column, Elution: phosphate-buffer (0.01M, pH:5.0) : methanol, V:V=70:30, 365 nm). Pharmacokinetic parameters were computed using non-compartmental or two-compartment model analysis assuming linear kinetics (Top Fit 2.0 Software). Results: On the average, maximum serum concentrations were observed at T_{max}=7.7 min following the beginning of MMC-infusion and amounted to c_{max}=1095 ng/ml (range: 562-1790 ng/ml). Mean steady state distribution volume was 0.56 l/kg with a central compartment volume of 0.22 l/kg. Concentration-time curves could be described by a distributional phase (T_{1/2}=8.5 min) followed by a terminal elimination phase (T_{1/2}=72.4 min). Mean total clearance amounted to 8.6 ml/min/kg. The area under the concentration time curve was 33 μg×min/ml.

Conclusion: Pharmacokinetic parameters for MMC-disposition in this study do not substantially differ from those reported for similar MMC-armounts administered as intralesional bolus. Systemic MMC exposure does not seem to be reduced following the embolisation procedure used compared to Intralesional application.

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STUDIES OF THE MECHANISMS INVOLVED IN PSEUDOCHOLINESTERASE ACTIVITY REDUCTION DURING CARDIOPULMONARY BYPASS

A.FE. Rump, M. Theisohn, M. Yilmaz, W. Biederbick, J. Schierholz, C. Diefenbach, M. Abel, U. Bömer, W. Buzello and W. Klaus.

Pseudocholinesterase-activity (PChE) is an important determinant of the elimination kinetics of drugs like mivacurium. Therefore, the time course of PChE was investigated in 16 patients undergoing cardiopulmonary bypass (CPB) in normo- or hypothermia. Anesthesia was induced and maintained with midazolam, propofol and fentanyl using mivacurium as a muscle relaxant.

...continued...
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Drug consumption review on surgical intensive care units

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Due to runaway costs of the national health service which are reflected as well in growing expenditures for drugs at the University Hospital of Jena, investigation of indication related drug administration patterns becomes more and more interesting. This holds especially true for intensive care units (ITU's) which are determined by similar high costs for technical equipment as for drugs (1) although any economical considerations seem to be questionable due to ethical reasons (2).

Over a 4 month period indication related drug administrations of 2 surgical ITU's of the University Hospital Jena have been recorded and analyzed by using a PC-notebook. Total expenditures for all 466 included patients add up to DM 1,444,773 regarding these drugs and blood products which caused 80% of total costs in 1993. The 10 leading substances (Anti-thrombin III, Human Albumin 20 %, Prothrombine complex, ...) represent 67 % of total costs including blood products, antibiotics and Ig M enriched intravenous immunoglobine. Therefore the indication of particular these drugs became more interesting for further investigations. Already during the study activation discussion in the treating medical staff has been made leading to new developed therapy recommendations. Providing same high standard of medical treatment a remarkable cost saving of some drugs by more critical and purposeful use could already be achieved as a first result. However, the results of the study underline impressively the benefit of such investigations for improvement of drug treatment. The simple replacement of expensive drugs (e.g. prothrombine complex) by higher quantities of cheaper ones of the same indication group (e.g. fresh frozen plasma (3)) does not necessarily mean less expenditures in all cases but may cause undesirable side effects.

(1) Mann HJ, Witzbrodt ET 1993 Identifying drug usage patterns in the intensive care unit. Pharmacoeconomics 4(4): 235-239
(2) Rothmann DJ, Shulken DJ 1993 The dsing cost of pharmaceuticals.
(3) Hiller E, Helm MU 1993 Indikationen for die Therapie mit frischgefrorenem

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COMPARISON OF MUSCULAR BLOOD FLOW OF THE LEG BETWEEN INTRAARTERIAL AND INTRAVENOUS PROSTA-GLANDIN E1 IN PERIPHERAL ARTERIAL DISEASE, ASSESSED BY [15-O]-H2O-PET. S. Schellong, W. Burchert, J.vd Hoff, C. Roth, H. Hundeshagen, K. Alexander.

Background: PGE1 has been shown to be efficacious in the treatment of critical leg ischemia. Despite of an almost complete first pass metabolism in the lung the clinical effects of intraarterial and intravenous PGE1 do not differ significantly. In addition, it is not fully understood which of the various pharmaceutical actions of PCE1 is the main factor; by most authors, however, it is thought to be the increase of cutaneous and muscular blood flow. By means of [15-O]-H2O-PET, we studied muscular blood flow (MBF) of the leg in patients with peripheral arterial disease comparing intraarterial and intravenous PGE1. Patients and methods: 8 patients (4 f, 4 m; mean age 59 y) with PAD were studied, (3 atherosclerosis, 3 thromboangiitis obliterans). At the first day, 50g PCE1 were infused intraarterially within 50 minutes; PET scanning was performed at minutes 0, 25 and 50. At the following day, 40ug PGE1 were infused intravenously within 2 hours; PET scanning was performed at minutes 0, 30, 60 and 120. Results: In the infused leg the increase of MBF caused by intraarterial PGE1 averaged 79±59% at minute 25 and 100±85% at minute 50; in the not infused leg there was no effect. The increase rate in the infused leg was highly variable but did not correlate with sex, age, disease or clinical outcome. For intravenous PGE1 the change of MBF at any time averaged almost 0%. Conclusion: Unlike intraarterial PGE1, intravenous PGE1 does not increase the muscular blood flow of the leg. A comparable clinical effect provided, increase of muscular blood flow may not be considered the main way of action of PGE1 in critical leg ischemia.

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RELEVANCE OF PS2-PROTEIN, EGFR AND CATHEPSIN D IN ASSOCIATION WITH ESTABLISHED PROGNOSTIC FACTORS IN BREAST CANCER

R. Schmitz, D. Sorger, F. Walter, M. Schönfelder, and R. Preis

Estrogen(ER) and Progesterone(PR) receptor status as well as lymph node involvement are important factors in predicting prognosis and sensitivity to hormone and chemotherapy in patients with breast cancer. Prognostic relevance of pS2 protein, EGF and Cathespin D is currently under debate. Especially pS2 and EGFR expression appears to provide additional information regarding the responsiveness of the tumour tissue to Tamoxifen. The aim of the present study was to investigate the relationships between these parameters and established prognostic factors in breast cancer. In a prospective study pS2 and Cathespin D were assayed immunoradiometrically in the tumour cytosol of 122 patients, EGFR was measured by ELISA. Relating the level of these factors to the lymph node involvement, metastasis status as well as tumour size, no significant association could be established. In our findings ER and PR are significantly correlated with the expression of pS2 but none is correlated with the cathespin D status. EGFR was shown to be inversely correlated with the content of ER. A significant association between Cathespin D and pS2 could be established in patients with early recurrence. At a median follow-up of 15-24 months, recurrence was more common in patients with tumours having negative status for pS2, independent of receptor status. In conclusion, because of the relative independence on the ER and PR status and other prognostic factors and the influence on the recurrence behaviour, demonstrated here, and their role in promoting tumour dissemination and changing hormone therapy sensitivity, all three factors represent markers of prognostic relevance.

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PHARMACOECONOMIC RESEARCH - ETHICAL ASPECTS
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Pharmacoconomic studies, conducted either separately from or together with clinical trials are increasing in both number and meaning. In a period of limited health care budgets, political and medical decision makers alike run the risk of accepting the results of such studies without critical reflection. Careful evaluation of those studies by state-of-the-art methods is one way out of the trap. Another could be to refer to ethical considerations. The problem in this context is, that the discussion concerning ethical aspects of pharmacoeconomic research, at least in Europe, is just in its beginning. Therefore, no widely accepted standards are available. But they are essential to answer four main questions: 1. Who should perform a pharmacoeconomic study? 2. Which objectives should be considered? 3. What kind of study should be performed (e.g. cost-effectiveness, cost-utility, cost-benefit analysis)? 4. Which consequences will be drawn from the results?

Based on the case study-oriented "moral cost-benefit model" (R. Wilson, Sci. Tech. Human Values 9: 11-22, 1984), a three-step decision and evaluation model is proposed to handle biethical problems in pharmacoeconomic studies: 1. Moral risk analysis. 2. Moral risk assessment. 3. Moral risk management. Possible practical consequences for decision making in research policy, study design and assessment of results are discussed.

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EFFECTS OF KETOCONAZOLE ON ACTH, CORTISOL AND 11-
DEOXYCORTISOL SECRETION IN CUSHING'S DISEASE.

T.H. Schürmeyer, C. Gutgesell and A. von zur Mühlen

Ketoconazole is known to decrease pituitary ACTH secretion in vitro and inhibits adrenal 11-hydroxy-
lase activity. To work out the clinical significance of both effects analysis of episodic secre-
tion of ACTH, cortisol (F) and 11-deoxycortisol (DF) was performed in patients with Cushing's syndrome (CS) requiring adrenocorticotherapy.

Methods: Ketoconazole was started in 11 patients with CS (9 ACTH-secreting pituitary adenomas [CD], 2 adrenal adenoma [AA]). In 9 of them (8 CD, 1 AA) blood samples were obtained for 24 hours at 10 min intervals (144 samples/patient) before and again under treatment (mean dose 1000 mg/d, >6 weeks). Hormone levels were measured by RIA and secretion patterns analysed by means of PULSAR, CLUSTER and DESADE. 2 patients were investigated only once because treatment was stopped due to side effects.

Results: The table shows the hormone levels of 8 patients with CD before (A) and under treatment (B) [x ± SEM] ACTH (pg/ml) F (pg/dl) DF (ng/ml)

|   | A       | B       |   | A       | B       |
|---|---------|---------|---|---------|---------|
| [A] | 89 ± 29 | 21.3 ± 2.0 | [A] | 9.6 ± 0.9 | 250 ± 56 |
| [B] | 139 ± 27 | 35.1 ± 1.7 | [B] | 16.0 ± 3.0 | 25.2 ± 4.2 |
| [A] | 171 ± 54 | 37.9 ± 5.5 | [A] | 15.1 ± 1.5 | 22.3 ± 4.2 |
| [B] | 250 ± 56 | 25.2 ± 4.2 | [B] | 28.3 ± 5.9 |

No change in the patterns of secretion occurred.

We conclude that the observed 34 % increase of plasma ACTH and the 58 % decrease of F/DF ratio de-

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ANTI-INFLAMMATORY TREATMENT CONCEPTS FOR
POSTOPERATIVE PAIN AND SWELLING PROPHYLAXIS FOLLOWING
DENTAL-ALVEOLAR SURGERY - RESULTS FROM 5 CLINICAL,
INTRAINDIVIDUAL DOUBLE-BLIND STUDIES

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The improvement of pain and swelling conditions by means of drugs is an important method of achieving an enhanced perioperative quality of life in cases of dento-

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SICCA SYNDROME FOLLOWING ORAL ADMINISTRATION OF A
BETA-BLOCKER

M Siepmann, J Barth, W Kirch

A sicca syndrome has been described very seldom after systemic administration of beta-blockers. We recently observed a 51 years old male patient who was treated with bisoprolol 5 mg once daily since 12 months. After the administration of the beta-

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PHARMAKOKINETICS OF IBOPAMINE - AN ORAL DOPAMINE
AGONIST- IN PATIENTS WITH NORMAL AND IMPAIRED RENAL
FUNCTION.

M Siepmann, U Ebert, W Kirch

Ibopamine is an orally active derivative of dopamine which is rapidly converted to epipamine (N-
methyldopamine) by esterasis hydrolysis. Pharmacokinetic data for ibopamine have hitherto been determined in patients with renal failure only following single oral dosing of this dopamine agonist. In a placebo controlled study 20 patients with congestive heart failure (NYHA class II) were treated orally for seven days with 100 mg ibopamine t.i.d. 10 subjects had a normal renal function (mean inulin clearance (GFR) 91 ± 3.4 ml/min), 10 patients suffered from chronic renal insufficiency (GFR 36 ± 3.9 ml/min; X ± SEM). Pharmacokinetic pa-

parameters of epipamine, the maximum plasma concen-
tration, the time to reach maximum plasma concen-
tration and the area under the curve from 0 to 6 hours were unaltered in impaired renal function when measured on the first or on the seventh treat-
ment day. However plasma concentrations in both groups were significantly higher on the first treat-
ment day than after one week of ibopamine ad-

ministration. In this context, antipyrene clearance as a parameter of oxidative liver metabolism which might have been induced by ibopamine revealed no differences between placebo and ibopamine values. In conclusion kinetic and dynamic behaviour of ibopamine was not altered by impaired renal function.
MONOCLONAL ANTIBODIES AND THEIR USE FOR MEASURING HUMAN PROTEIN C

E. Staboulidou, H.P. Metzger

Human Protein C (HPC) is a Vitamin K-dependent in the liver produced glycoprotein with anticoagulant properties. When active Protein C splits the coagulation factors Va and VIIIa by means of limited proteolysis (Kisiel et al 1977). Its concentration in normal plasma is 2.6 μg/ml. HPC’s biological importance became evident when a congenital Protein C deficiency, which results in difficult recurrent thromboembolic diseases was discovered (Griffin et al 1981).

The recognition of a congenital HPC deficiency, as well as the connection between acquired Protein C deficiency and the appearance of thromboembolic complications by means of highly accurate and sensitive ascertained methods is therefore of great practical importance for the clinic.

Murine monoclonal antibodies (moAbs) against HPC were formed. Antibody producing hybridomas were tested by an indirect ELISA against soluble antigens. The plates were coated with purified HPC up to 50 ng/100μl. The peroxidase-system was used to identify antibodies. The antibodies were tested with the remaining Vitamin K-dependent proteins for cross-reactivity, as well as with HPC deficiency plasma for disturbances by other plasma proteins. The above described experiment represents a sensitive and specific method for measuring the HPC concentration with moAbs.

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INDUCIBLE NITRIC OXIDE SYNTHASE IS EXPRESSED IN VENTRICULAR MYOCARDIUM FROM PATIENTS WITH SEPSIS

B. Stein, *U. Forstermann, H. Scholz, M. Thoenes

There is evidence that nitric oxide (NO) plays a role in cardiovascular disease like hypertension, myocardial ischemia and septic cardiomyopathy. NO stimulates the guanylyl cyclase leading to an increase in cGMP content. We investigated by immunoblotting the expression of the inducible nitric oxide synthase (iNOS) in left ventricular myocardium from failing human hearts due to idiopathic dilative cardiomyopathy (IDC, n=10), ischemic cardiomyopathy (ICM, n=7), Becker muscular dystrophy (n=2) and sepsis (SH, n=3) compared to non-failing human hearts (NF, n=4). Cytokine-stimulated mouse macrophages were used as positive controls. SDS-polyacrylamide gel electrophoresis (7.5 %) was performed with homogenates of left ventricular myocardium and mouse macrophages respectively. Proteins were detected by enhanced chemiluminescence using a mouse monoclonal antibody raised against iNOS. Furthermore, we measured the cGMP content in these hearts by radioimmunoassay. A band at about 130 kDa was observed in two out of three hearts from patients with sepsis and in stimulated mouse macrophages. No iNOS-protein expression was detected in either non-failing human hearts (n=4) or failing human hearts due to IDC, IHD or BMD. In ventricular tissue from patients with sepsis cGMP content was increased to 230 % (72 ± 17 fmol/mg ww, n=3) compared to non-failing hearts (100 % or 31 ± 6.9 fmol/mg ww, n=4). In left ventricular tissue from patients with heart failure due to IDC, IHD and BMD cGMP content did not differ from that in non-failing hearts. It is concluded that an enhanced iNOS protein expression may play a role in endotoxin shock, but is unlikely to be involved in the pathophysiology of end-stage heart failure due to IDC, IHD and BMD. (Supported by the DFG.)

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ENHANCED URINARY NITRATE EXCRETION IN RATS WITH ADJUVANT ARTHRITIS

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Nitric oxide (NO), synthesized by the inducible form of NO synthase, has been implicated as an important mediator of specific and non-specific immune response. Little is known about the in vivo synthesis of NO in inflammatory joint diseases. Therefore we have studied the excretion of the major urinary metabolite of NO, nitrate, in rats with adjuvant arthritis, a well established model of polyarthritis. In addition we assessed the urinary excretion of cyclic GMP, which is known to serve as second messenger for the vascular effects of NO, synthesized by the constitutive form of NO synthase, affecting blood vessels, platelet aggregation and neurotransmission.

In 24 h urines of 12 male Sprague Dawley rats at day 20 after induction of adjuvant arthritis we measured nitrate excretion by gas chromatography and cyclic GMP by radioimmunoassay. Control we determined the same parameters in 24 h urines of non-arthritis rats of same strain and age.

We found a significant (p <0.001, two-tailed, unpaired t-test), more than 3-fold increase of urinary nitrate excretion in arthritic rats (mean 54 ± SD 273 μmol/mmol creatinine) as compared to non-arthritic rats (169 ± 39 μmol/mmol creatinine). Urinary cyclic GMP excretion was slightly, but not significantly lower in arthritic rats (310 ± 44 μmol/mmol creatinine) than in controls (747 ± 33 μmol/mmol creatinine). There were no major differences in food or water intake which could account for these differences.

The increased urinary nitrate excretion accompanied by normal cyclic GMP excretion suggests that NO production by the inducible form of NO synthase is enhanced in rats with adjuvant arthritis.

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TABLETS WITH pH DEPENDENT DISSOLVING COATING AS A TOOL TO INVESTIGATE LOCAL DRUG ABSORPTION IN THE GUT

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Assessment of local drug absorption differences (“absorption window”) in the human gastrointestinal tract is relevant for the development of prolonged release preparations and for the prediction of possible absorption changes by modification of gastrointestinal motility. Current methods are either invasive and expensive (catheterization of the intestine, HF-capsule method) or do not deliver the drug to a precisely defined localization.

We evaluated the delay of drug release from tablets coated with methacrylic acid copolymer dissolving at different pH values as an alternative method. Three coated preparations of caffeine tablets (onset of drug release in vivo tests at pH 5.5, 6.0 and 7.0) and an uncoated tablet (control) were given to six healthy volunteers in a randomized order. Caffeine was used because of its rapid and complete absorption and good tolerability. Blood samples were drawn up to 24 h post dose (coating pH 7.0 up to 30 h postdose), and caffeine concentrations were measured by HPLC. AUC, time to reach measurable caffeine concentrations (Tmax), Tmax and mean absorption time (MAT) values for coated preparations were compared to the reference tablet (mean ± SD of n=6):

| Preparation | control | coating pH 5.5 | coating pH 6.0 | coating pH 7.0 |
|-------------|---------|----------------|----------------|----------------|
| Tmax (h)    | 3.5 ± 0.6| 1.3 ± 0.7      | 2.1 ± 0.2      | 3.3 ± 0.6      |
| Tmax (h)    | 1.0 ± 0.7| 2.0 ± 1.3      | 4.0 ± 0.3      | 5.6 ± 1.6      |
| Cmax (ng/ml)| 4.1 ± 0.4| 3.8 ± 0.6      | 3.4 ± 0.4      | 2.6 ± 0.8      |
| MAT (h)     |         | 1.4 ± 1.1      | 2.7 ± 0.4      | 4.4 ± 1.1      |

The relative bioavailability for the coated preparations did not differ from the reference, suggesting complete release of caffeine. All coatings delayed caffeine absorption onset. The Tmax for the pH 5.5 preparation suggests that release started immediately after the tablet had left the stomach. The mean delay of 2.1 h for the pH 6.0 coating was highly reproducible and should reflect small intestine release. The pH 7.0 coating delayed absorption to the highest extent, however the drug was probably released before the colon was reached.

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URINARY NITRATE EXCRETION IS INCREASED IN PATIENTS WITH RHEUMATOID ARTHRITIS AND REDUCED BY PREDNISOLONE
D.O. Schittenloeh, J. Fauler, H. Zedler and J.C. Frölich

Nitric oxide (NO) has been shown to be a major messenger molecule regulating blood vessel dilatation, platelet aggregation and serving as a central and peripheral neurotransmitter; furthermore NO is a crucial mediator of macrophage cytoxicity. NO production can be assessed reliably by determination of its main metabolites nitrite and nitrate in serum, reflecting NO synthesis at the time of sampling, or in 24 h urine, reflecting daily NO synthesis. Farrell et al. (Ann Rheum Dis 1992; 51: 1219) recently reported elevated serum levels of nitrite in patients with rheumatoid arthritis (RA). We report here total body nitrate production and the effect of prednisolone in patients with RA.

Nitrate excretion in 24 h urines of 10 patients with RA as defined by the 1987 revised criteria of the American Rheumatism Association was measured by gas chromatography at 2 times: First before start of an anti-inflammtory therapy with prednisolone, when the patients had high inflammatory activity as indicated by mean CRP serum concentrations of 71±36 SD mg/l and elevated ESR with a mean of 62±28 after 1 hour. Secondly 2-4 weeks after start of prednisolone therapy in a dosage of 0.5 mg/kg body weight, when the patients showed clinical and biochemical improvement (CRP ≤± 5 mg/l, p<0.05, ESG 32±17, p<0.001, two-tailed, paired t-test). For comparison 24 h urines from 18 healthy volunteers were obtained.

Before start of prednisolone therapy the urinary nitrate excretion in patients with RA (mean 223± SD 126 μmol/mmol creatinnine) was more than twofold higher (p<0.001, two-tailed unpaired t-test) than in healthy volunteers (83± 63 μmol/mmol creatinine). The urinary nitrate excretion decreased significantly (p<0.001, two-tailed, paired t-test) to 162± 83 μmol/mmol creatinine under therapy with prednisolone, when inflammatory activity was reduced considerably. Despite the decrease the urinary nitrate excretion was still twofold higher (p<0.05, two-tailed, unpaired t-test) in patients with RA than in the control group.

Our data suggest that the endogenous NO production is enhanced in patients with RA. Furthermore the results indicate that this elevated NO synthesis could be reduced in accordance with suppression of systemic inflammation by prednisolone therapy.

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DRUG BUDGET AT A UNIVERSITY HOSPITAL: COSTS DISTRIBUTION AND TRENDS.
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Drug budget considerably increased (100%) since 1986, more than the global expenses of the university hospital. This may be caused by the use of innova- tive drugs and therapies: ondansetron (antiemtic), ambisome (antimykotic), growth factors, immunosupressives in organ transplantations, blood products and drugs to influence coagulation (antithrombin III, factor VIII etc.). Trend analysis of the expenses at the various departments may be a basis for the economical use of the drug budget.

Total drug expenses amounted to 30 mill. DM in 1993: 10 mill DM (33%) were used in surgical departments with intensive care units (ICU) (general surgery, cardiovascualr surgery, neurosurgery, gynecology, anaesthesiology) of which 40% are needed by the ICU and 25% in the operating rooms. Surgical departments without ICU but similar patient numbers (ophthalmology, ENT, orthopedics and urology) get only 10% of the budget (50% needed for the operating rooms). The medical departments spent 10 mill. DM of which ICU needs only 10% whereas the oncology (OncU) and anti-infective units use more than 50%. Similar relation could be seen in the child hospital (2.4 mill DM, 8%) where 25% were spent for ICU and 40% for OncU. The departments of dermatology and neurology get 10%, the depart- ments of radiology, nuclear medicine and radiation therapy only 8% of the budget. Antiinfective drugs (antibiotics, antimykotics, virusinatics) are most expensive (21% of budget) followed by drugs used for radiological procedures (15%), heparin type anticoagulants (7.4%), narcotics (6.1%), cortistatins (5%), human albumin (3.2%), growth factors (3%), coagulation factors (2.5%), immunoglobulins (2.2%), immunosupressives (2%), antiemetics (1%) and tissue adhesives (2.3%).

Increasing the knowledge about the costs of medical items and the rational and economical use may stop the overproportional increase of the drug budget.

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PRESCRIPTIONS OF DRUGS PREPARED IN PHARMACIES IN AN EAST GERMAN REGION
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Most drugs used in therapy are finished products of the pharmaceutical industry. But now as ever the physicians are entitled to prescribe drugs which have to prepare in a pharmacy for a particular patient. Little information is available on the frequency and patterns of these prescriptions. We had occasion to analyse the prescriptions of drugs which were prepared in 6 pharmacies in North Thuringia (East Germany) from October to December 1993 at the expense of a large health insurance company (Allgemeine Ortskrankenkasse). The selected pharmacies are localised in 6 cities. We found 2172 prescriptions of drugs made up in pharmacies among a total number of 58472 reviewed drug prescriptions. This is 3.7% of the total. Most of these prescriptions were performed by dermatologists (56.6%), general practitioners (21.9%), paediatrists (7.9%) and otorharyngologists (4.1%).

According to this, the most frequently prescribed groups of drugs were dermatics (62.8%), followed by disinfectants (14.1%). Ointments (54.8%) and solutions (17.8%) were the most frequent drug forms. Prescriptions were written in either Latin (82.6%) or German (17.4%). Our results show that even now drugs prepared in pharmacies are used in the ambulatroy pharmacotherapy to a low degree, especially in the treatment of skin diseases.

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RELATIVE BIOAVAILABILITY OF ACETYL-SALICYLIC ACID (ASA) FROM ENTERIC COATED TABLETS (100 AND 300 MG) IN COMPARISON TO THE STANDARD PLAIN TABLET WITH 100 AND 300 MG ASA
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Introduction and Methods
Enteric coated tablets with 100 mg and 300 mg acetylsalicylic acid (ASA) have been developed which should avoid the known gastrointestinal adverse events by a controlled drug release mainly in the duodenum after having passed the stomach. A 4-way cross-over study in 24 healthy male subjects, aged from 19-39 years, was conducted to investigate the pharmacokinetics, bioavailability, safety, and tolerance of ASA and its metabolites salicylic acid and salicyluric acid following enteric coated tablets in comparison with plain tablets. ASA and its metabolites were determined by a sensitive, specific, and validated HPLC method. Pharmacokinetic parameters were determined by non-compartmental analysis. Bioequivalence was assessed by 90% confidence intervals.

Results and Conclusion

| relative bioavailability | point estimator [%] | 90% confidence interval |
|-------------------------|--------------------|------------------------|
| 100mg ASA               | salicylic acid     | 94.7                   | 79 - 113 |
|                          | salicyluric acid   | 96.9                   | 92 - 102 |
| 300mg ASA               | salicylic acid     | 84.7                   | 77 - 94  |
|                          | salicyluric acid   | 83.5                   | 84 - 104 |

Following the administration of enteric coated tablets, a delayed absorption can be observed for both the 100mg dose and the 300mg dose. This is likely due to a delayed release of the active substance from the enteric-coated tablets in the small intestine after gastric passage. Considering the mean residence times (MRT), there is a difference of at least 2.8 h following the enteric coated tablets compared to the plain tablets for ASA and the two metabolites measured. This difference represents the sum of residence time in the stomach plus the time needed to destroy the coating of the tablet when it left the stomach. In general, the maximum observed concentrations of both enteric coated formulations occurred 3-6 h post dose.
The pharmacokinetics of a novel immunoglobulin G (IgG) preparation (BT507, Biotest, Dreieich, FRG) have been determined in 12 healthy, male anti-HBs-negative volunteers. For this preparation only plasma from HIV-, HBV- and HCV-negative donors was used, the quality control for the product was in accordance with the EC-guideline for virus removal and inactivation procedures. Each volunteer received a single, intravenous infusion of 100 ml BT507 containing 5 g IgG and anti-HBs ≥ 5,000 IU. Anti-HBs were used as a newly measurable and representative marker for the IgG. Blood samples for determination of anti-HBs (AUSAB EIA, Abbott, FRG) were drawn before and directly after the infusion, after 1, 3, 6, 12 and 24 hours, on day 3, 5, 8, 15, 22, 29, 43, 57, 71, 85 and 99. Additionally, total protein, IgG, IgA, IgM and C3/C4 complement were measured and blood hematology and clinical chemistry parameters determined. The pharmacokinetic parameters of anti-HBs were calculated using the TOPFIT® PC program assuming a 2-compartment model.

\[
\begin{array}{|c|c|c|c|c|c|}
\hline
C_{\text{max}} & t_{\text{max}} & \text{AUC} & t_{1/2} & C_{\text{t}} & V_{\text{SS}} \\
(mg/ml) & \text{(h)} & (mg/ml*days) & (days) & (mg/ml) & (l) \\
\hline
\text{mean} & 1,778 & 1.4 & 28,867 & 22.1 & 0.130 & 5.4 \\
\text{sd} & 204 & 1.2 & 5,637 & 3.7 & 0.034 & 0.7 \\
\hline
\end{array}
\]

3 months after the application of BT507 all subjects were negative for HIV, HCV and HBV. No relevant changes occurred in total IgG, complement factors and safety laboratory variables. The calculated half-life of anti-HBs of 22 days correspond to that of natural IgG (about 21-23 days). Conclusion: Since pharmacokinetic characteristics of IgG represent both the entirety or a cleavage of the molecule, the results of this study suggest an almost completely preserved integrity of the IgG molecule in the preparation BT507.

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RENAI ELIMINATION OF TALINOLOL ENANTIOMERS
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Talinolol (Cordanum®), the mostly used β1-adrenoceptor antagonist in Eastern Germany, is a racemic drug [R/S-(+)-4-(Cyclohexyl-ureido-phenoxo)-2-hydroxy-3-tert-butyloxanopropane]. The S-enantiomer has a greater affinity to the β1-receptors (1:20) and a 100-fold higher efficiency than the R-form.

The elimination of the talinolol enantiomers was studied in 12 healthy volunteers (age: 23 - 32 years, body weight: 55 - 84 kg) given a single oral dose (50 mg) or an intravenous infusion (30 mg) of the racemic drug. Three volunteers were phenotypically poor metabolisers and nine were extensive metabolisers of the debrisoquine-type of hydroxylation. The R- and S-enantiomers of talinolol were analysed in urine by a HPLC method after enantioselective derivatisation. The concentrations of the enantiomers within every sampling period as well as the AUCs of S- and R-enantiomer eliminated within 36 h were compared with the t-test for paired samples (two-sided significance level: p < 0.05).

After intravenous infusion 7,516 ± 1,485 μg S-talinolol and 7,540 ± 1,492 μg R-talinolol were recovered unchanged in urine within the observation time. This corresponds to a S/R-ratio of 1.00 ± 0.02. The mean total amount of S-talinolol (S-enantiomer) eliminated was on average 50% of the administered dose. After oral administration 26 ± 7% of the dose were eliminated within 36 h. The amounts of talinolol enantiomers recovered were equally (S-enantiomer: 6416 ± 1,624 μg; R-enantiomer: 6,492 ± 1,674 μg; S/R-ratio: 0.99 ± 0.03). The ratios of S- to R-concentrations at every sampling interval and of every volunteer were assessed between 0.82 and 1,11 (mean: 1.00 after infusion and 1.01 after oral administration, respectively). There was no significant difference for any of the parameters calculated. No dependence on metaboliser phenotype was observed.

An enantioselective pharmacokinetics of talinolol can be excluded.

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PLATELET AGGREGATION AND SENSITIVITY TO PROSTAGLANDIN E1 IN RECOMBINANT HIRUDIN- AND HEPARIN- ANTICOAGULATED BLOOD.
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Hirudin is the most potent known natural inhibitor of thrombin and is presently gaining popularity as an anticoagulant since recombinant forms have become available. The aim of the present study was to compare platelet aggregation, sensitivity to prostaglandin E1 (PGE1) and thromboxane A2 (TXA2) release in r-hirudinized and heparinized blood. Platelet aggregation was measured turbidimetrically using a dual channel aggregometer (LAbor, Germany) in blood samples of healthy volunteers anticoagulated with r-hirudin W015 (Behring) and heparin (20 μg/ml blood each). Aggregation was induced by arachidonic acid (AA; 0.5, 1.0 and 2.0 mM) and ADP (1.0 μM). PGE1 in concentrations 10, 20 and 40 nM/ml was used. Plasma TXB2 content was measured by gas chromatography/mass spectrometry. This study showed a significantly lower AA-induced platelet aggregation in r-hirudinized plasma. Three minutes after the aggregation induction by 0.5 mM AA the plasma TXB2 concentration was 230 ± 45 μg/ml in blood anticoagulated with r-hirudin and 108 ± 41 μg/ml in heparin-anticoagulated blood. The extent of the ADP-induced aggregation was nearly the same in r-hirudinized and heparinized plasma. Platelet sensitivity to PGE1 was significantly higher in r-hirudinized blood. Thus, AA-induced platelet aggregation is significantly lower and sensitivity to PGE1 higher in r-hirudin-anticoagulated blood in comparison with heparin-anticoagulated blood.

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Sorbitol was used as a model substance to investigate the dynamics of the initial distribution process following bolus intravenous injection of drugs. To avoid a priori assumptions on the existence of well-mixed compartments data analysis was based upon the concept of residence time density in a recirculatory system regarding the pulmonary and systemic circulation as subsystems. The inverse Gaussian distribution was used as an empirical model for the transit time distribution of sorbitol across the subsystems. Distribution kinetics was evaluated by the relative dispersion of transit (circulation) times. The distribution volumes calculated from the mean transit times were compared with the model-independent estimate of the steady-state volume of distribution. Kinetic data and estimates of cardiac output were obtained from 10 patients after percutaneous transluminal coronary angioplasty. Each received a single 0.8 g iv bolus dose of sorbitol. Arterial blood samples were collected over 2 hours. While the disposition curve could be well fitted by a tri-exponential function the results indicate that distribution kinetics is also influenced by the transit time through the lungs, in contrast to the assumption of a well-mixed plasma pool underlying compartmental modelling. A significant correlation was found between estimated CI and cardiac output. This result reflects a linear dependence of hepatic perfusion on cardiac output in the observed range (CO between 4150 and 7248 ml/min).

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CHOLINERGIC TRANSMISSION IN HUMAN AIRWAYS AND NEURO-INAL EFFECTS OF 8-ADRENEROCEPTOR AGONISTS

1. Wessler, T. Reinheimer, K.D. Hohle* and K. Raedle**

Acetylcholine plays an important role in regulating various functions in the airways. In human lung less is known about regional differences in cholinergic innervation and about receptor-mediated regulation of acetylcholine release. In the present study the tissue content of endogenous acetylcholine and the release of newly-synthesized [3H]acetylcholine were measured in human lung. Tissue was obtained at thoracotomy from patients with lung cancer. Moreover, in isolated rat trachea with intact extrinsic vagal innervation possible effects of &beta;-adrenoceptor agonists on evoked [3H]acetylcholine release were studied. Endogenous acetylcholine was measured by HPLC with EC-detection, evoked [3H]acetylcholine release was measured after a preceding incubation of the tissue with [3H]choline. Human large (main bronchi) and small (subsegmental bronchi) airways contained similar amounts of acetylcholine (300 pmol/100 mg), whereas significantly less acetylcholine was found in lung parenchym (60 pmol/100 mg). Release of [3H]acetylcholine was evoked in human bronchi by transmural electrical stimulation (40-20 s at 15 Hz). Oxotremorine, an agonist at muscarinic receptors, inhibited evoked [3H]acetylcholine release indicating the existence of neuronal inhibitory receptors on pulmonary parasympathetic neurons. Scopolamine shifted the oxotremorine curve to the right suggesting a competitive interaction (pA2 value: 3.8, slope of the Schild plot not different from unity). However, a rather sluggish Schild plot was obtained for pirenzepine. Scopolamine but not pirenzepine enhanced evoked [3H]acetylcholine release. The present experiments indicate a dense cholinergic innervation in human bronchi. Release of acetylcholine appears to be controlled by facilitatory and inhibitory muscarinic receptors.

In isolated mucosa-intact rat trachea isoprenaline (100 nM) inhibited [3H]acetylcholine release evoked by preganglionic nerve stimulation Isoprenaline was ineffective in mucosa-denuded trachea or in the presence of indomethacin. Thus, &beta;-adrenoceptor agonists appear to inhibit acetylcholine release in the airways by the liberation of inhibitory prostanoids from the mucosa.

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A new African vegetable substance in the treatment of neurodermatitis and other skin diseases (Karité; Butyropermum Parkii)
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Karité "butter" is used traditionally in West African Healing culture as a cosmetic to protect the skin against the sun. Gas chromatography was used to analyze the ingredients of Karité butter from Guinea. We found 3% palmitic acid, 42% stearic acid, 42% oleic acid and 5% linoleic acid and 0.1% of other fatty acids with higher chain lengths like arachidonic acid. Some of these are essential fatty acids (vitamin F). Furthermore Karité contains vitamin A and D as well as sterigene alcohols and phytosteraines. An original extract was used to prepare a skin cream. This preparation was tested in 25 volunteers (18 women, 7 men; age 20-55 y.). The cream contained at least 50% Karité, glycerol, emulsifiers and no preservative agent except for sorbic acid. 24 of the volunteers very well tolerated the cream and thought it effective. The skin became more tender and elastic. Good results were obtained when the volunteers suffered from very dry skin. Two of them who were known to be allergic against the most available skin creams had no problems in using our Karité cream. Pure Karité butter was used for four months to treat an African infant with neurodermatitis. After this time the symptoms had markedly improved whereas previous therapy trials with other usual topical medicaments had been unsuccessful. These pre-studies had shown that dermatologic preparations containing Karité may be a good alternative in the treatment of therapy-resistant skin diseases and may in some cases be able to replace corticoid treatment.

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EFFECTS OF RECOMBINANT HIRUDIN (rh-HIRUDIN: HBW 023) ON COAGULATION AND PLATELET ACTIVATION IN VIVO: COMPARISON TO UNFRACTIONATED HEPARIN AND A LOW MOLECULAR WEIGHT HEPARIN PREPARATION (FRAGMIN®)
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In a double-blind, randomized cross-over study we have investigated in 15 healthy male volunteers the effects of recombinant hirudin (rh-hirudin, HBW 023, 0.35 mg/kg bodyweight s.c.), unfractionated heparin (UFH, 150 IU/kg bodyweight s.c.) and a low molecular weight heparin preparation (Fragmin®, 75 IU/kg bodyweight s.c.) on coagulation and platelet activation in vivo by measuring specific coagulation activation peptides (prothrombin fragment 1+2 (F1+2), thrombin antithrombin III complex (TAT), β-thromboglobulin (β-TG)) in bleeding time blood (activated state) and in venous blood (basal state). In bleeding time blood, rh-hirudin and the heparin preparations significantly inhibited the formation of both TAT and F1+2. However, the inhibitory effect of rh-hirudin on F1+2 generation was short-lived and weaker compared to UFH and LMWH and the TAT/F1+2 ratio was significantly lower after rh-hirudin than both UFH and LMWH. Thus, in vivo when the coagulation system is in an activated state rh-hirudin exerts its anticoagulant effects predominantly by inhibiting thrombin (β-TG), whereas UFH and LMWH are directed against both Xa and IIa. A different mode of action of UFH and LMWH was not detectable. In venous blood, rh-hirudin caused a moderate reduction of TAT formation and an increase (at 1 hour) rather than decrease of F1+2 generation. Formation of TAT and F1+2 was suppressed at various time points following both UFH and LMWH. There was no difference in the TAT/F1+2 ratio after rh-hirudin and heparin. Thus, a predominant effect of rh-hirudin on TAT and F1+2 was not detectable in venous blood. In bleeding time blood, rh-hirudin (but neither UFH nor LMWH) significantly inhibited β-TG release. In contrast, both UFH and LMWH caused an increase of β-TG 10 hours after heparin application. Our observation of reduction of platelet function after rh-hirudin compared to delayed platelet activation following UFH and LMWH suggests an advantage of rh-hirudin over heparin, especially in those clinical situations (such as arterial thromboembolism) where enhanced platelet activity has been shown to be of particular importance.

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IN VIVO VS. IN VITRO COMPARISON OF INHIBITORY DRUG EFFECTS ON THE HUMAN CYTOCHROM P450 ISOFORM CYP1A2.
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The human cytochrome P450 isozyme CYP1A2 determines the level of a variety of drugs metabolized by the enzyme, including caffeine (CA) and theophylline (TH). More than 50 compounds are potential or proven inhibitors of this enzyme. Some of them were reported to be substrates or inhibitors to CYP1A2 in vitro, others caused pharmacokinetic interactions with drugs metabolised by CYP1A2. We characterized a series of these compounds with respect to their effect on CYP1A2 in human liver microsomes in relation to published pharmacokinetic interactions in vivo. CYP1A2 activity in vitro was measured as CA 3-demethylation at the high affinity site in human liver microsomes, using 15 min incubation at 37 °C with 125 - 2000 μM caffeine, an NADPH generating system, and inhibitor concentrations covering 15 orders of magnitude. Apparent KI values were estimated using nonlinear regression analysis. Apparent KE values were estimated using nonlinear regression analysis. For inhibitory effects on CYP1A2 activity in vivo, the absorbed oral dose causing 50 % reduction in CA or TH clearance (ED50) was estimated from all published interaction studies using the Emax model. All drugs tested were competitive CYP1A2 inhibitors in vitro, suggesting dose dependent effects in vivo. A good agreement (table) was observed between CYP1A2 inhibition in vitro and in vivo effects on pharmacokinetics of both CA and TH. Inhibitors with KE values < 50 μM caused clinically relevant interactions (change 35 - 100 % for usual dosing schedules). Inhibitors with KE values > 1000 μM did not induce metabolic clearance dependency on CYP1A2 activity.

| inhibitor   | KE (μM) in vitro | ED50 (mmol) to CA | ED50 (mmol) to TH |
|-------------|-----------------|------------------|------------------|
| methoxsalen | 0.24            | 0.18             | -                |
| fluvoxamine | 0.47            | -                | 0.23             |
| propafenone | 42              | -                | 0.72             |
| mexiletine  | 63              | 1.46             | 1.22             |
| piperacillin | 130             | 1.36             | 1.78             |
| enoxacin    | 140             | 0.38             | 0.70             |
| ciprofloxacin| 200             | 2.78             | 2.82             |
| cimelagine  | 717             | 2.03             | 5.32             |
| lomefoxin   | 1100            | 22.6             | -                |
| oxiflavin   | 1500            | no effect        | 15.0             |

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