Possible biochemical and haematological adverse effects of new substances, potentially used as antitumour agents, may be a limiting factor of their therapeutic use. For instance, the influence of repeated i.v. administration of dimethoxybenfluron (NO-1-B) (12 or 24 mg base/kg once weekly) on biochemical and haematological parameters was studied in rabbits in vivo. No significant changes were mostly found in the serum ion levels between the dimethoxybenfluron and the control groups, as well as in most of other biochemical parameters (including total protein and albumin levels). Nevertheless, the lower dose of dimethoxybenfluron caused an increase in the glucose level. Furthermore, no significant changes were mostly present also in haematological parameters in the dimethoxybenfluron groups of rabbits (a mild decrease in thrombocytes and leucocytes). The results of our study support an assumption of good tolerance of dimethoxybenfluron from the viewpoint of its influence on biochemical and haematological parameters in rabbits and may be considered of importance for a possible therapeutic use of the derivatives.

Key words: Antineoplastic drugs, Benfluron, Dimethoxybenfluron, Rabbit, Biochemistry: Haematology

Introduction

Possible biochemical and haematological adverse effects of new substances, potentially used as antitumour agents, may be a limiting factor of their therapeutic use. For instance, the influence of repeated i.v. administration of dimethoxybenfluron (NO-1-B) (12 or 24 mg base/kg once weekly) on biochemical and haematological parameters was studied in rabbits in vivo. No significant changes were mostly found in the serum ion levels between the dimethoxybenfluron and the control groups, as well as in most of other biochemical parameters (including total protein and albumin levels). Nevertheless, the lower dose of dimethoxybenfluron caused an increase in the glucose level. Furthermore, no significant changes were mostly present also in haematological parameters in the dimethoxybenfluron groups of rabbits (a mild decrease in thrombocytes and leucocytes). The results of our study support an assumption of good tolerance of dimethoxybenfluron from the viewpoint of its influence on biochemical and haematological parameters in rabbits and may be considered of importance for a possible therapeutic use of the derivatives.

Material and methods

Small size Mchinchila males of an average weight of 3.2 ± 0.2 kg at the beginning of the experiment were used. All animals were fed dry pellets (formulated for rabbits) and water ad lib. The animals were maintained in an air-conditioned room. The experiment followed the Law of the Czech National Council for the protection of animals against cruelty and was under the supervision of the Ethics Committee of the Medical Faculty, Charles University, Hradec Králové. The animals were divided into three groups. The control group (fifteen rabbits) was given saline (1 ml/kg iv., twice). The lower dose of NO-1-B (2 mg base/kg iv.) was administered to seven animals (NO-1-B 12 group), six animals were administered a larger dose of the drug - 24 mg base/kg iv. (NO-1-B 24 group).

Drugs used in the study were administered once a week, intravenously (over 30 s) into the marginal ear vein for ten weeks. The period of the administration of drugs was selected on the basis of previous studies (4). Collection of the blood samples (from the ear artery) was performed during anaesthesia (ketamine 10 mg/kg i.m. in intervals: 1) (the control value at the beginning of the experiment; before the 1st administration of the drug), 2) (before the 5th admin-
nistration of the drug) and 3′ (at the end of experiment: one week after the last administration of the drug). At the end of the following week (5–7 days after the last administration of the drug), animals were killed with i.p. pentobarbital overdose. After the sacrifice of rabbits, gross autopsies were performed and the heart was excised.

Biochemical parameters were determined in arterial blood samples in plasma/serum with standard biochemical methods using an automatic analyzer HITACHI, 717. Japan. Haematological parameters were determined using an analyzer Coulter T890 (USA).

Non-invasive polygraphic recordings of the systolic time intervals in previously anaesthetized (ketamine in a dose of 50 mg/kg i.m.) and restrained rabbits were used to assess the cardiac function during the experiment as described previously (3). The biological and toxicological parameters (changes in the weight of rabbits and their survival) were followed up during the experiment.

Drugs used in the study:

- NO-1-B (3,9-dimethoxyflavonone) in the form of base, M.w. = 413.9 dissolved in Natrium chloratum sol. solutio
- Ketamin chlorohydrochloridum (Narkamon 5%, Lévia, Czech Republic), 50 mg/kg i.m.
- Natrium chloratum sol. solutio (Hoocht-Biota, Slovak Republic), 1.0 ml/kg
- Pentobarbital (NEMIBTAL, Abbott, USA), 30 mg/kg i.v.

Statistical analysis:

Statistical evaluation of values was performed using a paired t-test (within one group) and by means of an unpaired test ANOVA (comparison of different groups) for the level of significance p<0.05. Values are expressed as mean ± S.E.M. Significant differences of individual values are marked in the following manner: *~ significant differences to the initial value within a group, C~ significant differences between the value of the NO-1-B groups and the control group, B∗~ significant differences between NO-1-B groups.

Results

1. Biochemical parameters

No consistent differences were found between control and NO-1-B animals. Though significant, a mild decrease in the concentration of the K+, Ca2+, Cl- and phosphate was found in NO-1-B groups. Lower values of bilirubin and ALP were found in both NO-1-B groups at the end of experiment. No significant changes or a mild increase in protein and albumin levels were found after NO-1-B administration (Table 1).

2. Haematological parameters

There were mostly no significant differences present in haematological parameters between the NO-1-B and the control groups of rabbits. Only a mild decrease in the values of thrombocytes and leucocytes was found in NO-1-B groups. The changes were comparable in both groups, no significant changes between the groups were found in different bloodocyte images (Table 2).

3. Biological and toxicological parameters

There were no significant differences in the weight of animals between the groups present at the beginning of experiment (3.19 ± 0.06 kg in the control group, 3.19 ± 0.06 kg in the NO-1-B 12 group and 3.28 ± 0.06 kg in the NO-1-B 24 group). No significant differences were found in the weight gain of animals at the end of experiment (24.0 ± 2.4% in the control group, 17.1 ± 1.8% in the NO-1-B 12 group and 12.8 ± 2.9% of animals in the NO-1-B 24 group).

No premature deaths were found either in the control or in the NO-1-B groups.

4. Non-invasive polygraphic recordings of the systolic time intervals

No significant changes during the experiment were mostly found in the control group (the values oscillated between 0.30 (± 0.00) and 0.79 (± 0.01), i.e. 100.0 ± 111.7%). The dose of NO-1-B did not induce significant changes in the values of the systolic time intervals in rabbits during the whole experiment, either. The values of the PEP: LVET ratio in NO-1-B 12 group were 0.4077 ± 0.0286 at the beginning and 0.4135 ± 0.0286 at the end of experiment (i.e. 101.4%). The dose of NO-1-B 24 was 0.4077 ± 0.0286 in the NO-1-B 12 group and 12.8 ± 2.9% of animals in the NO-1-B 24 group).

Discussion

The derivatives of benfluron rank among prospective antimon antimug agents, which show a considerable cytolytic activity (11,12). Besides antitumour activity, these derivatives may also possess selective activity (Table 3) and was shown both in studies

Table 1: Changes in haematological parameters

| Parameter | Group | Time interval | 1 | 2 | 3 |
|-----------|-------|--------------|---|---|---|
| Erythrocytes (10³/µl) | NO-1-B 12 | 5.12 ± 0.03 | 4.28 ± 0.04 | 42.6 ± 0.05 | 45.2 ± 0.05 |
| | NO-1-B 24 | 5.48 ± 0.01 | 3.45 ± 0.02 | 46.2 ± 0.09 | 41.1 ± 0.10 |
| | NO-1-B 24 | 8.45 ± 0.18 | 6.32 ± 0.14 | 46.2 ± 0.16 | 41.1 ± 0.10 |
| | NO-1-B 24 | 12.09 ± 0.07 | 8.12 ± 0.13 | 52.8 ± 0.12 | 47.8 ± 0.15 |
| | NO-1-B 24 | 21.67 ± 0.17 | 7.53 ± 0.14 | 52.0 ± 0.12 | 47.8 ± 0.15 |

Table 2: Changes in haematological parameters

| Parameter | Group | Time interval | 1 | 2 | 3 |
|-----------|-------|--------------|---|---|---|
| Erythrocytes (10³/µl) | NO-1-B 12 | 5.12 ± 0.03 | 4.28 ± 0.04 | 42.6 ± 0.05 | 45.2 ± 0.05 |
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nistration of the drug) and 3 (at the end of experiment. one week after the last administration of the drug). At the end of the follow up interval (5-7 days after the last adminis-
tration of the drug), animals were killed with i.v. pentobar-
bital overdosing. After the sacrifice of rabbits, gross autopsies were performed and the heart was excised.
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lyser Coulter T8090 (USA).
Noninvasive polygraphic recordings of the systolic time intervals in previously anaesthetised (ketamine in a dose of 50 mg/kg i.m.) and restrained rabbits were used to assess the cardiac function during the experiment as described previously(3). The biological and toxicological parameters (changes in the weight of rabbits and their survival) were followed up during the experiment.

Drugs used in the study - NO-1-B (3,9-dimethoxybenflurone) in the form of base, M.w. = 413.9 dissolved in Natrium chloratum sol. soluttoni-
a (40°C, ultrasonic bath)
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Statistical analysis:
Statistical evaluation of values was performed using a pa-
tried test (within one group) and by means of an unpaired test ANOVA (comparison of different groups) for the level of significance p<0.05. Values are expressed as mean ± S.E.M. Significant differences of individual values are mar-
ed in the following manner: *= significant differences to the initial value within a group, ;**= significant differences between the value of the NO-1-B groups and the control va-
lue, B=* significant differences between NO-1-B groups.

Results
1. Biochemical parameters
No significant differences were found between control and NO-1-B animals. Though significant, a mild decrease in the concentration of the K+, Ca2+, Cl-, and phosphate was found in NO-1-B groups. Lower values of bilirubin and ALP were found in both NO-1-B groups at the end of experi-
ment. No significant changes or a mild increase in protein and albumin levels were found after NO-1-B administration (Table 1).

2. Haematological parameters
There were mostly no significant differences present in haematological parameters between the NO-1-B and the control groups of rabbits. Only a mild decrease in the valu-
es of thrombocytes and leucocytes was found in NO-1-B groups. The changes were comparable in both groups, no significant changes between the groups were found in the differential bloodocyte picture (Table 2).

3. Biological and toxicological parameters
There were no significant differences in the weight of animals between the groups present at the beginning of experi-
ment (3.19 ± 0.06 kg in the control group, 3.19 ± 0.06
kg in the NO-1-B 12 group and 3.28 ± 0.06 kg in the NO-
1-B 24 group). No significant differences were found in the weight gain of animals at the end of experiment (24.0 ±
2.4 % in the control group, 17.1 ± 1.8 % in the NO-1-B 12 group and 12.8 ± 2.9 % in the NO-1-B 24 group).
No premature deaths were found either in the control or in the NO-1-B groups.

4. Noninvasive polygraphic recordings of the systolic time intervals
No significant changes during the experiment were mostly found in the control group (the values oscillated bet-
ween 0.3030 and 0.3711, i.e. 100.0 - 111.7%). The dose of NO-1-B did not induce significant changes in the values of the systolic time intervals in rabbits during the whole expe-
riment, either. The values of the PEP: LVET ratio in NO-1-
B 12 group were 0.4077 ± 0.132* and 0.65 ± 0.17 in the control group and 12.8 ± 0.06 kg in the NO-1-B 24 group). No significant differences were found in the weight gain of animals at the end of experiment (24.0 ±
2.4 % in the control group, 17.1 ± 1.8 % in the NO-1-B 12 group and 12.8 ± 2.9 % in the NO-1-B 24 group).

Discussion
The derivatives of benfluron rank among prospective
- noninvasive polygraphic recordings of the systolic time intervals
- discussion

The derivatives of benfluron rank among prospective antimonat agents, which show a considerable cytotoxic ac-
tivity (11,12). Besides antimonat activity, these substances have been studied from other aspects, e.g. the influence of dimethbenuron on cell metabolic activity (6) or mor-
phological changes caused by the derivatives of benfluron (12). Mechanisms of the action of these substances include probably blocking cells in G1 and G2 phases of the cell cycle (6). Dimeth-
benuron was also reported to induce cell lysis. Cyto-
toxicity of benfluron is dose-related (5) and was shown to be similar in vivo and in vitro (11,12). This study was at-
temped to obtain a complex information about changes in some biochemical and haematological parameters follo-
wing repeated administration of NO-1-B in rabbits as the changes of these parameters can often limit possible thera-
peutic use of new derivatives with antimonat activity.

Our study has shown that mainly no significant changes
of haematological parameters occurred following repeated
administration of lower doses of dimethbenuron.
Furthermore, only mild (though in some parameters sig-
ificant)
IS FACTOR V LEIDEN A RISK FACTOR FOR FETAL LOSS?

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Summary: A successful pregnancy is dependent on the development of adequate placental circulation. The abnormalities of placental vasculature may result in a number of gestational pathologies, including fetal loss. The aim of our study was to determine whether women with FV Leiden are at an increased risk of pregnancy loss. For this purpose we assessed three groups of women. In a prospective group we examined 30 females with spontaneous abortions for FV Leiden. In a retrospective group we assessed the frequency of abortions in 80 women (372 pregnancies) with FV Leiden (72 heterozygous, 8 homozygous) from 57 unrelated families. In a control group we evaluated the frequency of abortions in 45 women without FV Leiden. Factor V Leiden was found in 3% of women in the 1st group. Fetal loss occurred in 10% of women in the 2nd group and in 9% in the 3rd group. Factor V Leiden was not found to be a risk factor for fetal loss in our study group.

Key words: Placental infarction, Hypercoagulable state, APC resistance, FV Leiden, Venous thromboembolism, Fetal loss

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