Comparative renal effects of metamizole (dipyrone) and naproxen in salt-depleted healthy subjects, a randomized controlled parallel group study

Supplementary Material

Supplementary Table 1 Inclusion / Exclusion criteria

| INCLUSION CRITERIA |
|--------------------|
| Healthy male subjects aged between 18 and 45 years (inclusive) at screening |
| BMI between 18 and 28 kg/m² (inclusive) and body weight at least 50 kg at screening. |
| SBP: 100-140 mmHg, DBP: 60-90 mmHg and HR: 45-90 bpm (inclusive), measured on the leading arm*, in the supine position at screening. |
| No clinically significant findings on the physical examination at screening. |
| 12-lead ECG without clinically relevant abnormalities at screening. |
| Signed informed consent prior to any study-mandated procedure. |
| Hematology and clinical chemistry results not deviating from the normal range to a clinically relevant extent at screening. |
| Ability to communicate well with the investigator and to understand and comply with the requirements of the study. |

| EXCLUSION CRITERIA |
|--------------------|
| Smoking > 5 cigarettes per day. |
| History or clinical evidence of alcoholism or drug abuse within the 3-year period prior to screening. |
| Loss of ≥ 250 ml of blood within 3 months prior to screening. |
| Treatment with an investigational drug within 30 days prior to screening. |
| Previous treatment with any prescribed or OTC medication (including herbal medicines such as St John’s Wort) within 2 weeks prior to the intended start of the study. |
| Legal incapacity or limited legal capacity at screening. |
| Positive results from urine drug screen at screening. |
| History or clinical evidence of any disease (e.g. GIT-disease: Morbus Crohn, Colitis Ulcerosa, anamnestic gastrointestinal bleeding) and/or existence of any surgical or medical condition, which might interfere with the absorption, distribution, metabolism or excretion of the study drugs, or which might increase the risk for toxicity. |
| Known hypersensitivity to Aspirin or other NSAIDs or any excipients of the drug formulations. |
| Known food allergy, especially intolerance to root vegetables (e.g. celeriac) |
| Any circumstances or conditions, which, in the opinion of the investigator, may affect full participation in the study or compliance with the protocol. |

*leading arm right = writing with right hand
## Supplementary Table 2 Visit and assessment schedule

| Study Day | Study Period | TREATMENT PERIOD |
|-----------|--------------|------------------|
|           |              | 2 (Week)         |
|           |              | 3a               |
| -2        | -1          | 0                |
| -2        | -1          | 0                |
| -2        | -1          | 0                |
| -2        | -1          | 0                |
| -2        | -1          | 0                |
| -2        | -1          | 0                |
| 1         | 2           | 3                |
| 1         | 2           | 3                |
| 1         | 2           | 3                |
| 1         | 2           | 3                |
| 1         | 2           | 3                |
| 1         | 2           | 3                |
| 2         | 3           | 4                |
| 2         | 3           | 4                |
| 2         | 3           | 4                |
| 2         | 3           | 4                |
| 2         | 3           | 4                |
| 2         | 3           | 4                |
| 3         | 4           | 5                |
| 3         | 4           | 5                |
| 3         | 4           | 5                |

**SCD**
- Day 
- Week 

**Visit and Assessment Schedule**

| Time | Activity |
|------|----------|
| 00:00 | Urine drug screen |
| 06:00 | BP, HR |
| 07:00 | BP, HR |
| 08:00 | BP, HR |
| 09:00 | BP, HR |
| 10:00 | BP, HR |
| 11:00 | BP, HR |
| 12:00 | BP, HR |
| 13:00 | BP, HR |
| 14:00 | BP, HR |
| 15:00 | BP, HR |
| 16:00 | BP, HR |
| 17:00 | BP, HR |
| 18:00 | BP, HR |
| 19:00 | BP, HR |
| 20:00 | BP, HR |
| 21:00 | BP, HR |

**Notes:**
- *X* indicates activity performed.
- *-* indicates activity not performed.
- **End** indicates the end of the study period.

*Provided free at the University Hospital*
**Supplementary Table 3** Mass transitions and compound specific settings

| ID          | Q1 Mass (Da) | Q3 Mass (Da) | Time (msec) | DP (V) | CE (V) | CXP (V) | EP (V) |
|-------------|--------------|--------------|-------------|--------|--------|---------|--------|
| 4-AA        | 204.2        | 56.2         | 30          | 71     | 39     | 8       | 10     |
| 4-AA-d3     | 207.2        | 59.1         | 30          | 46     | 39     | 10      | 10     |
| 4-AAA       | 246.2        | 83.1         | 30          | 46     | 43     | 14      | 10     |
| 4-AAA-d3    | 249.2        | 84.1         | 30          | 61     | 43     | 6       | 10     |
| 4-FAA       | 232.2        | 204.1        | 30          | 46     | 19     | 12      | 10     |
| 4-MAA       | 218.2        | 56.1         | 30          | 31     | 16     | 10      | 10     |
| 4-MAA-d3    | 221.2        | 56.1         | 30          | 46     | 37     | 8       | 10     |
| Naproxen    | 229.1        | 168.8        | 50          | -40    | -36    | -15     | -10    |
| Naproxen-d3 | 232.1        | 170.8        | 50          | -45    | -40    | -9      | -10    |
| 6-Keto PGF1α| 564.1        | 306.1        | 100         | -155   | -36    | -21     | -10    |
| 6-Keto PGF1α-d4 | 568.1  | 310.1        | 100         | -155   | -36    | -21     | -10    |
| 2,3-Dinor 6-Keto PGF1α-d9 | 545.2 | 259.0 | 100 | -210 | -28 | -17 | -10 |
**Supplementary Table 4**  Demographic and clinical data

|                           | Day 1*     | Day 7*     |
|---------------------------|------------|------------|
| Age (years)               | 25 (18 – 29)|            |
| Body weight (kg)          | 72.0 (57.4 – 97.1) |            |
| BMI (kg/m²)               | 22.2 (18.6 – 27.8) |            |
| Systolic blood pressure (mmHg) | 125 (110-146) | 125 (104 – 138) |
| Diastolic blood pressure (mmHg) | 76 (66 – 93) | 75 (60 – 87) |
| Serum creatinine concentration (µmol/L) | 75 (58 – 95) | 81 (62 – 100) |

Data are given as median (range). N=15 study subjects.

* before administration of inulin
**Supplementary Table 5** Creatinine excretion (µmol/h) by subjects treated with metamizole (n=8) or naproxen (n=7). Values represent means ± SEM.

| Collection period | Metamizole   | Naproxen   |
|-------------------|--------------|------------|
|                   | Day 1 | Day 7 | Day 1 | Day 7 |
| 08:00 - 09:00     | 686±64 | 545±63 | 496±39 | 628±58 |
| 09:00 - 10:00     | 649±54 | 544±51 | 551±31 | 536±117 |
| 10:00 - 11:00     | 622±68 | 652±82 | 591±84 | 578±65 |
| 11:00 - 12:00     | 671±80 | 601±71 | 536±50 | 566±100 |
| 12:00 - 13:00     | 625±63 | 593±48 | 510±42 | 601±48 |
| 13:00 - 14:00     | 632±91 | 631±39 | 487±29 | 511±72 |
**Supplementary Table 6** Pharmacokinetic parameters after a single oral dose of 500 mg naproxen (n=7) on day 1 and after multiple doses (500 mg BID) on day 7.

| Naproxen | Day 1 |  |  | Day 7 |  |  |
|----------|-------|---|---|-------|---|---|
|          |       |   |   |       |   |   |
|          |       |   |   |       |   |   |
| T1/2 [h] | 18.4  (12.8-21.8) |   |   |       |   |   |
| Tmax [h] | 2(2-5) |   |   |       |   |   |
| Cmax [mg/L] | 63.2 (51.7-76.0) |   |   |       |   |   |
| AUC0-inf observed [mg/L*h] | 1293 (889-1528) |   |   |       |   |   |
| AUC0-12h [mg/L*h] | 497 (398-560) |   |   |       |   |   |
|           |  |   |   | Tmax [h] | 2 (1-3) |   |
|           |  |   |   | Cmax [mg/L] | 85.7 (67-101) |   |
|           |  |   |   | Cmax ratio MD/SD | 1.37 |   |
|           |  |   |   | AUC0-12h [mg/L*h] | 754 (572-817) |   |
|           |  |   |   | AUC0-12h ratio MD/SD | 1.52 |   |

Data are given as median and range. AUC0-inf, area under the concentration-time curve from zero to infinity extrapolated based on the last observed concentration; AUC0-12h, partial area under concentration-time curve from 0 to 12 hours; Cmax, maximum plasma concentration; MD, multiple dose; SD, single dose; T1/2, half-life; BID, twice a day; Tmax, time to maximum plasma concentration.
Supplementary Figure 1 Study Design

Low-sodium diet
Day -7 till morning Day 8

Na⁺ depletion phase
Day -7 till Day -1

Day 1

Day 7

SD M MD

EOS

SD N MD

SV

Depletion control (24h urine) on Day -4 till Day -3

EOS  end of study visit
MD  multiple dose
M  metamizole
N  naproxen
SD  single dose
SV  screening visit
Supplementary Figure 2 Mean plasma concentration of naproxen vs. time in healthy volunteers (n= 7) following a single oral dose of 500 mg (A) and after seven days of continuous intake of 500 mg twice a day (B). Due to an additional naproxen dose 12 hours after the morning dose, the PK profile is only shown for the first 12 hours.
Individual pharmacokinetic and pharmacodynamic profiles Metamizole

Subject 1

Units for pharmacodynamic effect are urinary excretion of 6-keto-PGF1α in pg/mg creatinine
Subject 10

![Graph showing 4MAA and 4AAA levels over time for Subject 10.](image)

![Graph showing 6-keto-PGF1alpha/creatinine ratio over time for Subject 10.](image)
Individual pharmacokinetic and pharmacodynamic profiles Naproxen

Subject 2

Units for pharmacodynamic effect are urinary excretion of 6-keto-PGF1α in pg/mg creatinine

Subject 3
Subject 12

Subject 14
Subject 16

![Graph showing naproxen levels over time and 6-keto-PGF1alpha levels over time for Subject 16.](image-url)