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Study code: 3000-4208
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- **Study title:**

Effects on kidney function in rats.
Hydroxymatairesinol.

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Study Report

EFFECTS ON KIDNEY FUNCTION IN RATS

HYDROXYMATAIRESINOL

Study number: **P11.12-1999**

Date: 20.8.2002 (version 2)

Sponsor:

Hormos Medical Ltd.
Tykistökatu 6A
FIN-20520 Turku
FINLAND

Sponsor Study number: 1903008

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PreFa

Preclinical Pharmacology Research Unit
University of Turku

Key Words

Hydroxymatairesinol (HMR), safety pharmacology, kidney function, urine excretion, sodium, potassium, osmolality

1. GENERAL

1.1. SIGNATURES

Title Effects on kidney function in rats; Hydroxymatairesinol

PreFa study number: P11.12-1999

Sponsor study number: 1903008

Testi item: Hydroxymatairesinol

This Report version 2 replaces the 1st version dated 4.7.2000. Following changes have been made:

Section 2.3.3. Rationale for dose selection:

1. Reference to a study demonstrating the antitumor activity of HMR has been added.
2. Route of administration of the test item has been corrected (earlier: p.o.)

This report is a complete and accurate account of the methods employed and the data obtained


Aapo Honkanen
Study Director

20.8.2012
date

1.2. TABLE CONTENTS

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1.3. PURPOSE OF THE STUDY

The purpose of this study was to assess safety pharmacological properties of the compound Hydroxymatairesinol (HMR) by studying its effects on urinary excretion, urinary osmolality and urine sodium and potassium concentrations in rats.

In addition to HMR, the effects of another compound, HTS-101 was tested in the same experiment. Same control group (vehicle treatment) and reference compound-treated groups were used in the evaluation of these compounds. The results from HMR and HTS-101 are reported separately.

1.4. SUMMARY

12-weeks-old, male Sprague-Dawley rats were transferred to the metabolic cage 18 h before start the experiment and the food but not water was withdrawn. On experimental day, immediately after administration of vehicle, HMR (10, 30 or 100 mg/kg, s.c.), or reference compound (furosemide 50 mg/kg, s.c.), the rats were given orally tap water at the volume of 30 ml/kg, after which the animals were returned into the metabolic cages for 6 hours. During this time, the rats were without food and water. The urine samples were collected 6 hours after drug administration and water loading. The volume of urine samples were recorded and urine samples were frozen at -30 °C until the analyses for osmolality and K⁺ and Na⁺ concentrations.

In comparison with vehicle-treatment furosemide significantly increased urine-, Na⁺, and K⁺ excretion and Na⁺ and K⁺ concentrations in urine, but decreased urinary osmolality. Test compound HMR did not significantly alter excretion of urine or urinary osmolality or K⁺ or Na⁺ excretions.

1.5. GUIDELINES

The study procedures described were based on the guidelines listed below:

- Asetus Kokeellisiin ja muihin tieteellisiin tarkoituksiin käytettävien selkärankaisten eläinten suojelemiseksi tehdyn eurooppalaisen yleissopimuksen voimaansaattamisesta. Suomen säädöskokoelma n:o 1360/90. Helsinki, 21 joulukuuta 1990
- European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, European Treaty Series No. 123, (EU n:o 609/86) (Official Journal of the European Communities No L 358) Strasbourg 24th November 1986.

1.6. APPROVAL FROM THE ANIMAL CARE AND USE COMMITTEE

The study has a permission from the animal care and use committee of University of Turku n:o 922/99.

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University of Turku

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1.7. SPONSOR

Hormos Medical Ltd.
Tykistökatu 6A
FIN-20520 Turku
FINLAND

1.8. RESEARCH LABORATORIES

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Kiinamyllynkatu 10
FIN-20520 Turku

Yhtyneet Laboratoriot/Clinical chemistry
Höyläämöntie 14
FIN-00380 Helsinki
Finland

1.9. STUDY DIRECTOR

Aapo Honkanen M.Sc. (Pharm.), Project Manager

1.10. PERSONNEL INVOLVED IN THE STUDY

PreFa/Department of Pharmacology and Clinical Pharmacology
Esa Korpi, MD, Ph.D. Professor of Pharmacology
Aapo Honkanen, Project Manger
Elisa Riuttala, Laboratory Technician

CRST(Clinical Research Services Turku)/Biostatistics
Esa Wallius

Yhtyneet Laboratoriot
Arto Katajamäki, Chemist

1.11. TIME TABLE

Start of animal acclimatisation: 10.11.1999
 Experimental starting date: 15.11.1999
 Experimental completion date: 9.12.1999

2. MATERIALS AND METHODS

2.1. TEST SYSTEM

Experimental animals: Sprague-Dawley Hsd:SD

Age/weight: 12 weeks/247-297 g, 267 ± 11 g (Mean ± S.D.)

Source: Harlan Winkelmann GmbH, Germany

Number of animals in the study: 60

Number of animals/group: 12, samples from two rats were combined in each treatment group, so the final number of samples is 6/group.

Acclimatization period: 5 days before start of the experiment.

Principles for selection into test groups: Animals were selected randomly by hand into different test groups.

Identification of animals: The animals were marked on their tails with numbers in different colors.

Grounds for selection of species: Rats are commonly used in studies of this type.

2.2. ENVIRONMENTAL CONDITIONS

Animal care: The animals were cared and checked daily by the experimenters and/or personnel of the Central Animal Laboratory. The bedding of the animals was changed twice and water bottles once a week.

Number of animals/cage: 3 rats/cage. During the experiment, the rats were maintained in metabolic cages, 2 rats/cage

Cage Type: Polycarbonate Macrolon III (Scanbur AS, Denmark).
 Metabolic cage: Tecniplast, 3700MO (Buguggiate, Italy).

Bedding: Aspen chips (Tapvei Oy Kaavi, Finland). The results of the analysis for specified contaminants are attached.

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Water: Community tap water, *ad libitum*, except during the experiments. The results of the analysis for specified contaminants are attached.

Fodder: RM1 (E) SQC, Special Diet Service, Witham Essex, England. Certificate detailing nutritional composition and levels of specified contaminants is attached.

Ambient temperature: 21 ± 2.5 °C

Humidity: 50 % ± 15 %

Illumination: 12-h dark/light cycle; lights on from 7.00 to 19.00 and lights off from 19.00 to 7.00.

Room numbers: Colony room : BioCity C-department room 309
Experimental room: BioCity C-department room 311

2.3. REAGENTS

2.3.1. Test compounds

Hydroxymatairesinol (HMR, mw. 374)

Vehicle: PEG 300 Sigma (Chemicals Co, St Louis, MO, USA)
Batch: 00799
Storage: at 4 °C, desiccated, protected from direct light

2.3.2. Reference compound

Furosemide (mw. 330.7)

Manufacturer: Sigma Chemical Co, St Louis, MO, USA
Vehicle: PEG 300 Sigma
Lot: 53H0668
Storage: at room temperature protected from direct light

2.3.3. Rationale for dose selection

In the experiments assessing the pharmacodynamic efficacy of HMR ,e.g. antitumor activity (Saarinen et al. Nutrition and cancer 2000 (36):207-216) a dose 15 mg/kg, (p.o.) have been found to be effective. Thus, the doses selected for the present study (10, 30 and 100 mg/kg, s.c.) were within this therapeutic range or exceeded that.

2.3.4. Preparation and handling of test compound solutions

Fresh test compound and reference compound solutions were prepared on each experimental day. Both compounds were dissolved in Polyethylene glycol (PEG) 300. The solutions were sonicated at 40 °C for 8-15 min.

2.4. EXPERIMENT

2.4.1. Administration of compounds

Vehicle (PEG 300), reference compound furosemide and different doses of HMR were given s.c. (2 ml/kg).

2.4.2. Method

The animals were habituated to handling and oral administration for 3 days before start of testing. The animals were transferred to the metabolic cage (2 rats/cage) 18 h before start the experiment and the food but not water was withdrawn. On experimental day, immediately after administration of vehicle, test compound or reference compound, the rats were given orally tap water at the volume of 30 ml/kg, after which the animals were returned into the metabolic cages for 6 hours. During this time, the rats will be withdrawn from food and water.

Table 2.1. Treatments

Groups	Treatment	Dose
I	Vehicle (PEG 300)	-
II	Furosemide	50 mg/kg
III	HMR	10 mg/kg
IV	HMR	30 mg/kg
V	HMR	100 mg/kg

2.4.3. Data collection

The urine samples were collected 6 hours after drug administration and water loading. The volume of urine samples were recorded and manually entered in the spreadsheet. Urine samples were frozen at -30 °C until sent to Yhtyneet laboratoriot for analyses in dry ice. After the analyses, a report was received from the Yhtyneet laboratoriot, the data were entered to spreadsheet at PreFa and processed further. Excretion of K⁺ and Na⁺ was calculated with the following formula: concentration of ion in sample/volume of sample/weight of the animal.

2.4.4. Statistics

Means, standard deviations and standard errors for each group were calculated. The data were tested with analysis of variance for repeated measures (ANOVA) and between-group comparisons were made with Dunnett's post-hoc test. Vehicle-treated group was assigned as a control. Logarithmic transformation was used when the data departed markedly from parametric assumptions.

2.4.5. Termination of the experiments

At the end of the experiment, all surviving animals were sacrificed with CO₂.

3. ARCHIVING

Study plan, final report and original data from different experiments are retained in the archive of PreFa (Tykistökatu 6B) for 10 years. After that, the further treatment of the documentation is decided together with the Sponsor. The documentation or parts of it may be delivered to the Sponsor on request before the end of the 10-year term. No data or documentation will be destroyed without permission from the Sponsor.

4. DEVIATIONS FROM STUDY PLAN

The experiment was performed as described in the Study Plan.

5. RESULTS

5.1. BODY WEIGHTS

The average body weights of the animals in each treatment group are shown in the table 5.1. There was not significant differences in the average body weights of the animals in the different treatment groups ($F = 0.87$, $p = 0.49$). Sums of body weights of 2 rats/cage were used in the statistical analysis.

Table 5.1. Body weights of the animals in different treatment groups.

	Data calculated from 12 individual animals/group						Data calculated from sums of weights of 2 rats/cage					
	Mean	S.D.	S.E.M	MIN	MAX	N	Mean	S.D.	S.E.M	MIN	MAX	N
Veh	264	12	3	247	286	12	528	15	6	509	544	6
Furosemide	266	10	3	251	282	12	532	11	4	515	541	6
HMR 10	267	9	3	255	283	12	533	17	7	515	563	6
HMR 30	266	10	3	250	285	12	533	12	5	515	550	6
HMR 100	271	13	4	256	293	12	543	15	6	525	559	6

5.2. EFFECTS OF HYDROXYMATAIRESINOL ON URINE AND ELECTROLYTE EXCRETION

The ANOVA showed significant treatment effects for urine excretion; $F = 160$, $p < 0.0001$; urine osmolality; $F = 18$, $p < 0.0001$; $F = 77$, $p < 0.001$; K^+ concentration, 5.4 , $p < 0.01$ and K^+ excretion, $F = 91$, $p < 0.0001$. Data from Na^+ analysis were not normally distributed, so the Kruskal-Wallis test was applied. This test confirmed that also Na^+ concentration ($p < 0.01$ and Na^+ excretion ($p < 0.01$) differed between the groups.

In comparison with vehicle-treatment furosemide significantly increased urine-, Na^+ , and K^+ excretion and Na^+ and K^+ concentration in urine, but decreased urinary osmolality. Test compound HMR increased urinary osmolality at the dose of 10 mg/kg but not at higher doses. HMR did not alter urine, K^+ or Na^+ excretion.

Table 5.2. The effects of HMR and furosemide on urine excretion, urinary osmolality and sodium and potassium excretion and concentrations in the urine in rats.

	Urine excretion (ml/kg/6h)						U-Osmol (mosm/kg H ₂ O)					
	Mean	S.D.	S.E.M	MIN	MAX	N	Mean	S.D.	S.E.M	MIN	MAX	N
Veh	21	4	1	16	24	6	633	110	45	433	768	6
Furosemide	57*	2	1	56	60	6	387*	24	10	357	429	6
HMR 10	22	3	1	18	24	6	767*	45	19	731	855	6
HMR 30	23	2	1	19	25	6	689	86	35	572	813	6
HMR 100	23	4	2	18	29	6	707	116	47	557	903	6

	Na ⁺ concentration (mmol/l)						Na ⁺ -excretion (mmol/kg/6h)					
	Mean	S.D.	S.E.M	MIN	MAX	N	Mean	S.D.	S.E.M	MIN	MAX	N
Veh	5	3	1	2	10	6	0.10	0.06	0.02	0.0	0.2	6
Furosemide	62*	2	1	59	66	6	3.55*	0.17	0.07	3.3	3.7	6
HMR 10	4	1	1	3	6	6	0.08	0.02	0.01	0.1	0.1	6
HMR 30	7	9	4	3	25	6	0.18	0.23	0.09	0.1	0.6	6
HMR 100	3	1	0	3	4	6	0.08	0.02	0.01	0.1	0.1	6

	K ⁺ concentration (mmol/l)						K ⁺ excretion (mmol/kg/6h)					
	Mean	S.D.	S.E.M	MIN	MAX	N	Mean	S.D.	S.E.M	MIN	MAX	N
Veh	29	8	3	20	40	6	0.60	0.16	0.07	0.31	0.75	6
Furosemide	42*	2	1	40	45	6	2.42*	0.16	0.06	2.28	2.66	6
HMR 10	34	7	3	26	46	6	0.73	0.08	0.03	0.61	0.82	6
HMR 30	32	7	3	21	42	6	0.72	0.13	0.05	0.53	0.94	6
HMR 100	30	4	1	25	35	6	0.68	0.10	0.04	0.57	0.86	6

*p < 0.05, in comparison with vehicle (Veh) -treated control group (Dunnett's test)

6. CONCLUSIONS

These results demonstrate that in rats, HMR does not alter excretion urine or K⁺- or Na⁺-excretion at the doses used in the present study. HMR increased urine osmolality but this effect was found only at the smallest dose used (10 mg/kg).

7. DISTRIBUTION OF THE REPORT

The Report is written in duplicate, one original copy being retained in the Archives of PreFa and one delivered to the Sponsor.

Appendices

1. Values from the individual animals
2. Statistics
3. Report from analysis of bedding for contaminants
4. Report from analysis of water for contaminants
5. Report from analysis of fodder for nutritional composition and levels of specified contaminants.