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Study code: 3000-4204
(Old code 1903004)

● **Study title:**

Effects on rectal temperature in rats.
Hydroxymatairesinol.

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Study Report

EFFECTS ON RECTAL TEMPERATURE IN RATS

HYDROXYMATAIRESINOL

Study number: **P11.10-1999**

Date: 20.8.2002 (version 2)

Hormos Medical Ltd.
Tykistökatu 6A
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Sponsor Study number: 1903004

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PreFa

Preclinical Pharmacology Research Unit
University of Turku

Key Words: Hydroxymatairesinol (HMR), safety pharmacology, body temperature

1. GENERAL

1.1. SIGNATURES

Title Effects on rectal temperature in rats; hydroxymatairesinol

PreFa study number: P11.10-1999
Sponsor study number: 1903004
Testi item: Hydroxymatairesinol
Study Director Aapo Honkanen, Ph.D. (Pharm.)

This Report version 2 replaces the 1st version dated 20.4.2000. Following change has been made:

1. **Section 2.3.3. Rationale for dose selection:** Reference to a study demonstrating the antitumor activity of HMR has been added.

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


Aapo Honkanen
Study Director

20.8.2002
date

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1.3. OBJECTIVE / PURPOSE OF THE STUDY

The purpose of this study was to assess safety pharmacological properties of the compound Hydroxymatairesinol (HMR) by assessing its effect on rectal temperature in rats. In addition to HMR, the effects of another compound, HTS-101 were tested in the same experiment. Same control group (vehicle treatment) and reference compound-treated group were used in the evaluation of the effects of these compounds. The results from HTS-101 are reported separately.

1.4. SUMMARY

Male Sprague-Dawley rats (325 ± 20 , mean \pm S.D), withdrawn from 18 h from food but not water, were given vehicle (PEG 300), different doses of HMR (10, 30 or 100 mg/kg, p.o., 2 ml/kg), or reference compound, medetomidine (50 μ g/kg, s.c. 2 ml/kg in 0.9 % NaCl). The rectal temperature of the animals was measured 1 h, 2 h, 6 h and 24 h after the treatments with rectal thermal probe connected to a Physitemp BAT-12 thermometer. Medetomidine significantly decreased the body temperature of the animals, this effect being maximal following 2 h after the treatment. None of the tested doses of HMR altered the rectal temperature of the rats. These results indicate that HMR does not alter the body temperature of the rats with the doses studied.

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.

1.7. SPONSOR

Hormos Medical Ltd.
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1.8. RESEARCH LABORATORIES

University of Turku

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Central Animal Laboratory
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1.9. STUDY DIRECTOR

Aapo Honkanen, Ph.D. (Pharm.)

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/Biostastics
Esa Wallius

1.11. TIME TABLE

Start of animal acclimatisation:	20.10.1999
Experimental starting date:	7.11.1999
Experimental completion date:	10.11.1999

2. MATERIALS AND METHODS

2.1. TEST SYSTEM/SUBJECTS

Experimental animals:	Sprague-Dawley Hsd:SD
Age/weight:	12 weeks/318 ± 24 g (Mean ± S.D.)
Source:	Harlan Winkelman GmbH, Germany

Number of animals in the study:	30
Number of animals/group:	6
Acclimatisation period:	18 days
Principles for selection into test groups:	Animals were selected randomly by hand into various treatment groups.
Identification of animals:	The animals were marked on their tails with numbers in different colours.
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.
Cage Type:	Polycarbonate Macrolon III (Scanbur AS, Denmark).
Bedding:	Aspen chips (Tapvei Oy Kaavi, Finland). The results of the analysis for specified contaminants are attached (Appendix 3).
Water:	Community tap water, <i>ad libitum</i> , except during the experiments. The results of the analysis for specified contaminants are attached (Appendix 4.).
Fodder:	RM1 (E) SQC, Special Diet Service, Witham Essex, England. Certificate detailing nutritional composition and levels of specified contaminants is attached (Appendix 5.).
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:	Experimental room: 313 Colony room: 309

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, dessiccated, protected from direct light

2.3.2. Reference compound

Medetomidine (mw. 200.28, Domitor® 1 mg/ml, Orion Pharma)

Vehicle: 0.9 % NaCl (saline)
Lot: ZH 31-3
Batch: 11/98
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, p.o.) were within this therapeutic range or exceeded that.

2.3.4. Preparation and handling of test compound solutions

Fresh test compound solutions were prepared on each experimental day. HMR was dissolved in Polyethylene glycol (PEG 300). Reference compound medetomidine was diluted from Domitor® solution with 0.9 % NaCl. Test compound solutions were sonicated at 40 °C for 8-15 min. Medetomidine test solution was prepared once a week and stored at 4 °C between the experimental sessions.

2.4. EXPERIMENT

2.4.1. Procedure

The animals were habituated to handling, oral administration and measurement of rectal temperature before start of testing. The food but not water was withdrawn 18 h before start of the experiment and the animals were transferred to the cage with grid floor.

On the experimental day, the rectal temperature was measured by inserting thermal probe (RET-2, Physitemp, Clifton, NJ, USA) connected to Physitemp BAT-12 thermometer (Physitemp) to a depth of approximately 3 cm into the rectum. Body temperature of the animals was be measured 1 h, 2 h, 6 h and 24 h after the treatments.

2.4.2. Administration of compounds

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

Treatments

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

$n_i = 6, n = 30$

2.4.3. Data collection

Body temperature of rats were recorded and entered manually into the spreadsheet.

2.4.4. Statistics

Means, standard deviations and standard errors for each group are calculated. The data was tested with analysis of variance for repeated measures (ANOVA).

2.4.5. Termination of the experiments

At the end of the experiment, all 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 following approval of final report. 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 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. EFFECTS ON BODY TEMPERATURE

Average (\pm S.D.) body weights of the animals in different treatment groups are shown in table 5.1. and the effects of different treatments on body temperature are shown in table 5.2. There were no differences in the body weights of the animals between the groups ($F = 0.81$, $p = 0.53$, ANOVA).

Table 5.1. Average body weights (\pm S.D.) of the animals in different treatment groups

Group	Treatment	Dose	Mean	Body Weight (g)		n _i
				S.D.	Range	
I	Vehicle	-	333	25	292-356	6
II	Medetomidine	100 μ g/kg	312	18	283-328	6
III	HMR	10 mg/kg	312	21	281-342	6
IV	HMR	30 mg/kg	321	27	273-348	6
V	HMR	100 mg/kg	314	31	266-349	6

Effects of different treatments on the body temperature differed significantly ($F = 89$, $p < 0.0001$). There was also a significant time effect ($F = 70$, $p < 0.0001$) and time x treatment interaction ($F = 45$, $p < 0.0001$). However, only medetomidine significantly decreased the body temperature of the animals, this effect being maximal 2 h after the treatment. This was confirmed by ANOVA showing that when the medetomidine-treated group was omitted from the analysis, there was no treatment ($F = 0.56$, $p = 0.65$) or time x treatment interaction ($F = 0.29$, $p = 0.99$) any more, but the time effect remained ($F =$ between the groups ($F = 6.1$, $p < 0.001$). The time effect was due to a slight decrease of body temperature in the animals of all treatment groups at 2-h and 6-h measurement points relative to baseline.

Table 5.2. Effects of HMR or reference compound medetomidine on rectal temperature of the rats.

Treatment		Baseline	1 h	2 h	6 h	24 h
Vehicle	Mean	36.8	36.5	36.3	36.8	36.5
	S.D.	0.6	0.4	0.2	0.3	0.3
	S.E.M	0.2	0.1	0.1	0.1	0.1
	Range	36.1-37.5	36.0-37.0	36.1-36.6	36.5-37.4	36.0-36.9
	N	6	6	6	6	6
Medetomidine 100 µg/kg	Mean	36.4	32.1	29.7	33.8	36.6
	S.D.	0.3	0.7	0.9	1.0	0.6
	S.E.M	0.1	0.3	0.4	0.4	0.2
	Range	36.2-36.9	31.1-32.8	28.7-30.7	32.4-35.3	36.1-37.7
	N	6	6	6	6	6
HMR 10 mg/kg	Mean	37.0	36.6	36.4	37.0	36.7
	S.D.	0.6	0.3	0.3	0.6	0.3
	S.E.M	0.2	0.1	0.1	0.2	0.1
	Range	36.3-37.7	36.3-37.2	36.0-37.0	36.1-37.7	36.3-37.2
	N	6	6	6	6	6
HMR 30 mg/kg	Mean	36.8	36.5	36.5	36.8	36.5
	S.D.	0.5	0.4	0.5	0.3	0.5
	S.E.M	0.2	0.2	0.2	0.1	0.2
	Range	36.1-37.2	36.0-37.1	35.9-37.4	36.3-37.1	35.8-37.1
	N	6	6	6	6	6
HMR 100 mg/kg	Mean	36.5	36.5	36.2	36.7	36.6
	S.D.	0.4	0.5	0.6	0.7	0.5
	S.E.M	0.2	0.2	0.2	0.3	0.2
	Range	36.2-37.3	36.2-37.5	35.2-36.8	35.6-37.6	36.0-37.2
	N	6	6	6	6	6

6. CONCLUSION

These results indicate that orally administered HMR does not affect body temperature of the rats with the doses 10 –100 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.