

CONFIDENTIAL

Study code: 3000-4206

(Old code 1903006)

- **Study title:**

Effects on pain threshold in rats.
Hydroxymatairesinol.

- **CONFIDENTIAL**

CONFIDENTIAL

Study Report

EFFECTS ON PAIN THRESHOLD IN RATS

HYDROXYMATAIRESINOL

Study number: P11.16-1999

Date: 20.8.2002 (version 2)

Sponsor:

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

Sponsor Study number: 1903006

CONFIDENTIAL

PreFa

Preclinical Pharmacology Research Unit
University of Turku

Key Words: Hydroxymatairesinol (HMR), safety pharmacology, analgesia, tail-flick test, plantar test

1. GENERAL

1.1. SIGNATURES

Title Effects on pain threshold in rats; Hydroxymatairesinol

PreFa study number: P11.16-1999

Sponsor study number: 1903006

Testi item: Hydroxymatairesinol (HMR)

This Report version 2 replaces the 1st version dated 4.7.2000. 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

1.2. TABLE CONTENTS

	page
1. General	2
1.1. Signatures	2
1.2. Table Contents	3
1.3. Purpose of the study	4
1.4. Summary	4
1.5. Guidelines	4
1.6. Approval from the animal care and use committee	4
1.7. Sponsor	5
1.8. Research laboratories	5
1.9. Study Director	5
1.10. Personnel involved in the study	5
1.11. Time table	6
2. Materials and methods	6
2.1. Test system	6
2.2. Environmental conditions	6
2.3. Reagents	7
2.3.1. Test compound	7
2.3.2. Reference compound	7
2.3.3. Rationale for dose selection	7
2.3.4. Preparation and handling of test compound solutions	7
2.4. Experiments	8
2.4.1. Administration of compounds	8
2.4.2. Procedure/Method	8
2.4.3. Data collection	8
2.4.4. Statistics	8
2.4.5. Termination of the experiments	8
3. Archiving	9
4. Deviations from study plan	9
5. Results	9
5.1. Body weights	9
5.2. Effects of HMR on pain threshold	9
5.2.1. Plantar test	9
5.2.2. Tail flick test	10
6. Conclusions	11
7. References	11
8. Distribution of the Report	11

1.3. PURPOSE OF THE STUDY

The purpose of this study was assess general pharmacological properties of the compound hydroxymatairesinol (HMR) by studying its effect on nociception 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 these compounds. The results from HMR and HTS are reported separately.

1.4. SUMMARY

Male, 11-week-old Sprague-Dawley rats were habituated to handling and measurement of pain thresholds for 3 days before start of the testing. Food, but not water was withdrawn 18 h before start the experiment. Nociceptive responses were assessed with tail-flick test and plantar tests.

In the tail-flick test, noxious infra-red (IR) photo beam was focused on rat's tail and the latency to the flick of its tail away from photobeam-induced heat was recorded. In the plantar test, the animals were placed into a transparent enclosure, and a movable IR source was placed under hind paw of the animal and IR was activated. The latency until the animal moved its paw away from the photo beam was recorded. Vehicle (PEG 300), and HMR (10, 30 or 100 mg/kg) were given p.o. (2 ml/kg), while reference compound morphine HCl (10 mg/kg, in 0.9 % NaCl) was given s.c. The pain thresholds were measured before the drug administration and 1, 2 and 4 h after the drug administration.

Different treatments had no significant effect on the paw withdrawal latency in the plantar test. However, morphine increased the tail flick latency at 1 h after while HMR had no effect. These results demonstrate that HMR does not show central analgesic effect in the tail flick test at the doses used 10-100 mg/kg. Plantar test is designed for testing of cutaneous hyperalgesia. Therefore the results of the present study show that HMR neither increase nor decrease pain threshold at the doses used.

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.
Tykistökatu 6A
FIN-20520 Turku
FINLAND

1.8. RESEARCH LABORATORIES

University of Turku
PreFa/Preclinical Pharmacology Research Unit
Tykistökatu 6 B
FIN-20520 Turku
FINLAND

Central Animal Laboratory
BioCity
Tykistökatu 6B
FIN-20520 Turku
Finland

CRST/Biometrics
Kiinamylynkatu 10
FIN-20520 Turku

1.9. STUDY DIRECTOR

Aapo Honkanen Ph.D. (Pharm.)

Deputy Study Director
Kristiina Raatesalmi

1.10. PERSONNEL INVOLVED IN THE STUDY

PreFa/Department of Pharmacology and Clinical Pharmacology
Esa Korpi, Professor of Pharmacology
Aapo Honkanen, Study Director
Oxana Echenko, Researcher

CRST(Clinical Research Services Turku)/Biostatistics
Esa Wallius

1.11. TIME TABLE

Start of animal acclimatisation: 23.2.2000
Experimental starting date: 17.3.2000
Experimental completion date: 19.3.2000

2. MATERIALS AND METHODS

2.1. TEST SYSTEM

Experimental animals: Sprague-Dawley Hsd:SD

Age/weight: 11 weeks/261-322 g, 300 ± 14 g (mean ± S.D.)

Source: Harlan, France

Number of animals in the study: 40

Number of animals/group: 8

Acclimatisation period: 3 weeks before start of the experiment.

Principles for selection into test groups: Animals were randomly allotted into various test groups. Mean body weights of each group at randomization were not significantly different from each other (analysis of variance).

Identification of animals: The animals were marked on their tails with numbers in different colors with indelible felt-tip pen.

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.

PreFa/ Preclinical Pharmacology
Research Unit
University of Turku

P11.16-1999
REPORT
Version 2

non-GLP study
7(11)
CONFIDENTIAL

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: $50 \% \pm 15 \%$

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: YK 136, BioCity, B-department.

2.3. REAGENTS

2.3.1. Test compound

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

Morphine HCl (mw. 321.8, Ph. Eur. grade)

Source: University Pharmacy, Helsinki
Vehicle: 0.9 % NaCl
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 solutions were sonicated at 40 °C for 15 min.

2.4. EXPERIMENTS

2.4.1. Administration of compounds

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

2.4.2. Procedure/Method

The animals were habituated to handling, oral administration and measurement of pain thresholds for 3 days before start of the testing. Food, but not water were withdrawn 18 h before start the experiment and animals were transferred into the cages with a grid floor. Nociceptive responses were assessed with tail-flick test by using Ugo Basile tail flick unit (Ugo Basile, Comerio, Italy) and with Ugo Basile Plantar test (Ugo Basile).

In the tail-flick test, noxious infra-red (IR) photo beam was focused on rat's tail and the latency until the animal flicks its tail away from photo beam was automatically recorded by the equipment. In the Plantar test, the animals were placed into a transparent perspex enclosure, and a movable IR source was placed under hind paw of the animal and IR was activated. The latency until the animal moved its paw away from the photo beam was automatically recorded by the equipment. The cut-off times were 8 sec in tail flick test and 16 sec in planter test.

The pain thresholds were measured before the drug administration and 1, 2 and 4 h after the drug administration.

Table 2.1. Treatments

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

2.4.3. Data collection

The latency times recorded by the equipment were entered manually in the data collection form.

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).

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) at least 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 10-year term. No data or documentation will be destroyed without written permission from the Sponsor.

4. DEVIATIONS FROM STUDY PLAN

The used dose of morphine (reference compound) was 10 mg/kg instead of 3 mg/kg as stated in the Study Plan. This modification was made in order to extend the length of the morphine-induced analgesia. The Sponsor was informed about the modification before start of the experiment.

5. RESULTS

5.1. BODY WEIGHTS

The average body of weights of the animals in different groups are shown in the table 5.1. There were no significant differences in the body weights between the groups ($F = 0.05$, $p = 0.99$).

Table 5.1. Average weight of the animals in each treatment group.

Group	Treatment	Mean	S.D.	MIN	MAX	N
I	vehicle	300	13	283	320	8
II	Morphine	302	16	268	318	8
III	HMR 10	299	9	284	311	8
IV	HMR 30	302	19	281	333	8
V	HMR 100	300	13	278	321	8

5.2. EFFECTS OF HMR ON PAIN THRESHOLD

5.2.1. Plantar test

The effects of different treatments on the paw withdrawal latency in the Plantar test did not differ significantly (treatment effect: $F = 1.86$, $p = 0.14$). ANOVA showed a significant time effect ($F = 4.46$, $p < 0.01$) but no treatment x time interaction ($F = 1.81$, $p = 0.06$) indicating that paw withdrawal latency changed across the repeated testing, but this occurred similarly in all groups.

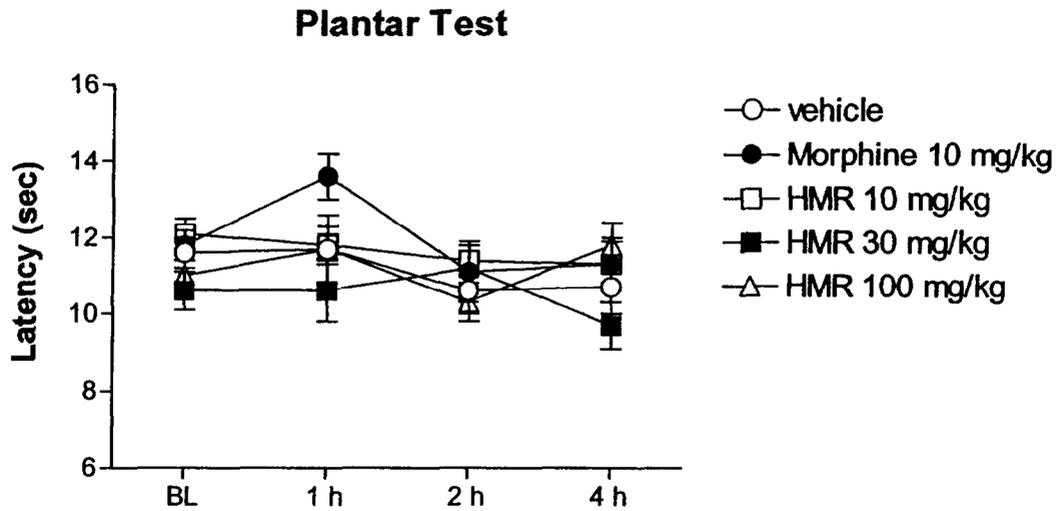


Figure 5.1. The effect of HMR and morphine on paw withdrawal latency in the Plantar test in rats (n = 8).

5.2.2. Tail flick test

For tail flick latency, ANOVA showed a significant treatment effect ($F = 7.77$, $p < 0.0001$), time effect ($F = 32.3$, $p < 0.0001$) and treatment x time interaction ($F = 9.11$, $p < 0.0001$). When morphine-treated group was excluded from the analysis, both treatment ($F = 0.68$, $p = 0.57$) and treatment x time interaction ($F = 0.91$, $p = 0.52$) disappeared, but time effect was still significant ($F = 8.03$, $p < 0.0001$) confirming that only morphine treatment induced analgesia.

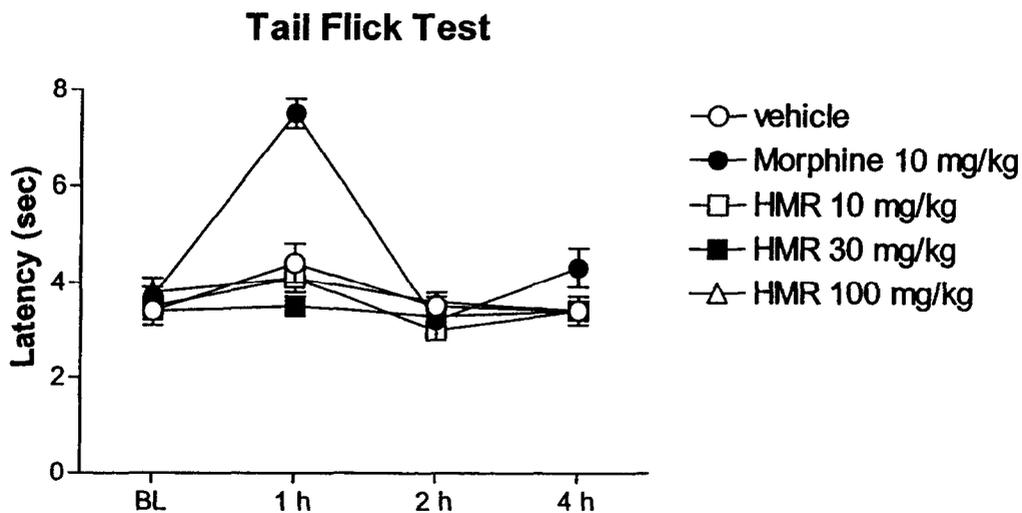


Figure 5.2. The effect of HMR and morphine on tail flick latencies in rats (n = 8).

6. CONCLUSIONS

These results demonstrate that HMR does not show central analgesic effect in the tail flick test at the doses used 10-100 mg/kg. Plantar test is designed for testing of nociceptive responses in cutaneous hyperalgesia (Hargreaves et al. Pain, 1988, 32:77-88). Therefore the results of the present study show that HMR neither increase nor decreases pain threshold at the doses used.

7. REFERENCES

Hargreaves K., Dubner R., Brown F., Flores C. and Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain (1988) 32:77-88.

8. 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.