2.6. Interpretation

The observed values were C_{max} and T_{max}.

The area under the curve (AUC) was calculated by the trapezoidal rule up to the last timepoint at which the concentration of RU 38 486 was measurable (—— $mg.l^{-1}$), i.e. 48 or 72 hours depending on the subject.

The ratio of the AUCs (oral/intravenous x 100) yielded the absolute bioavailability (F) as a percentage of the ingested dose.

The elimination t 1/2 was calculated from the concentrations measured from 8 to 48 or 72 hours depending on the case, i.e. 4 or 5 points assuming a mono-exponential decrease. A programme devised by ———— was used.

2 AIM OF STUDY

The aim of this study was to determine the absolute bioavailability of RU 38 486 in healthy women of childbearing age, administered intravenously and orally in a dose of 20 mg in the form of a solution.

3 MATERIAL AND METHODS

3.1. PROTOCOL

The protocol is given in full in Appendix VIII.

The clinical trial was performed in accordance with the Good Clinical Practice procedures in force in the Roussel Uclaf Medical Direction.

3.1.1 Study design

This was an open, randomised, cross-over study in healthy female volunteers, RU 38 486 being administered in a single dose.

3.1.2 Subjects

Ten subjects were included in the study.

Inclusion criteria

i) Exclusively female subjects.
ii) Subjects aged between 20 and 40 years.
iii) Subjects of childbearing age with a regular cycle.
iv) Subjects whose weight did not deviate by more than 10% from the mean weight for their age and height.
v) Subjects whose medical examination was normal or considered as such by the investigator.
This examination comprised the following:
a) a clinical examination,
b) laboratory investigations,
c) an ECG.

In addition the subjects had to have one of the following:

a) an IUD inserted at least 6 months previously,

b) or tubal ligation,

c) or a sterile partner,

d) or no partner during the study.

Exclusion criteria:

i) Subjects using hormonal contraception.

ii) Subjects regularly taking medication.

iii) Subjects having taken part in a clinical trial during the 4 weeks prior to this study.

iv) Subjects having received medication known to be potentially toxic in the 3 months prior to the study.

v) Subjects with a past or present history of gastro-intestinal, hepatic or renal disease which might interfere with the absorption, distribution, metabolism or excretion of drugs.

vi) Subjects consuming excessive quantities of alcohol or smoking excessively.

vii) Additionally, subjects with a positive pregnancy test during the 24 hours prior to administration of RU 38 486. The test was to be repeated before each dose of RU 38 486. This test, involving an assay of HCG (human chorionic gonadotropin) in the plasma or urine, was only to be done in subjects who had not undergone tubal ligation.

3.1.3 Dosage form

The dosage form of RU 38 486 was an injectable solution with the following composition:

RU 38 486	100 mg
0.1 N aqueous solution of hydrochloric acid ...	9 ml
Absolute ethanol to	10 ml

presented in an ampoule. Batch No. MMG 2096647.

3.1.4 Treatments

Each subject received 2 treatments:

20 mg of RU 38 486 administered intravenously, abbreviated to "IV 20 mg RU 38 486".

20 mg of RU 38 486 administered orally , abbreviated to "PO 20 mg RU 38 486".

Time 0 was taken as the beginning of infusion in the case of IV treatment and the time when the solution was drunk in that of PO treatment.

3.1.5 Conditions of administration

The treatments were allocated by a randomisation plan in a Latin square design (5 mixed 2 x 2 squares).

Each treatment was administered at the end of a cycle between the third day before the expected date of menstruation and the second day after the date of onset.
Before each treatment the pregnancy test was confirmed as negative.
The treatments were separated by an interval of one cycle.

The treatments were administered in the morning at about 8 a.m., the subjects having fasted overnight (about 10 hours fasting) and a light meal was served 4 hr after administration.

3.1.5.1 Intravenous administration

Ten ml of solution (1 ampoule) were to be diluted in 490 ml of injectable 0.9% sodium chloride solution. 100 ml of this solution containing 20 mg of RU 38 486 were to be injected.
The solution to be injected was infused for 1 hr at a constant rate by means of an electric infuser.

3.1.5.2 Oral administration

Two ml of solution, i.e. 20 mg of RU 38 486, were swallowed after being diluted in 150 ml of water. The subjects were in the upright position and remained in this position for 2 minutes.

3.1.6 Blood samples

Blood samples (5 ml) were taken by venipuncture and collected on dry lithium heparinate:

a) 0.25 hr before time 0,
b) 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 24, 30, 36, 48, 54, 60, 72 and 96 hr after time 0.

Time 0 was taken as the beginning of the infusion for IV treatment and the time at which the solution was drunk for PO treatment.

Each sample was immediately centrifuged cold, the plasma decanted and distributed into 2 dry tubes and frozen at -20°C until assay.

3.2 ASSAY METHODS

3.2.1 Assay of α_1-acid glycoprotein (AAG) in plasma

α_1-acid glycoprotein was assayed in the 0, 24 and 48 hour samples by ——— Five µl of plasma were applied to each well of an agarose gel plate containing anti-α_1-acid glycoprotein monospecific antiserum ——— batch no. 054329). Calibration was done with a range of standard sera ———) from ——— $g.l^{-1}$. All the assays were performed in duplicate on different plates with a diffusion time of 48 hr. The standards were applied to each plate used.

3.2.2 Assay of RU 38 486 and RU 42 633 in plasma

The assay method is given in full in Appendix I. RU 38 486 and one of its metabolites, RU 42 633, resulting from the loss of a methyl on the nitrogen, were assayed in all the samples.

An internal standard, RU 39 813, was added to the plasma, and RU 38 486, RU 42 633 and the internal standard were then extracted with ethyl acetate.
The organic extract was then subjected to ———— chromatography (HPLC) on a ———— 10 µm column with a mixture of acetonitrile and water supplemented with heptane sulphonic acid (PIC B7).
The separated products were detected and quantified at the column exit by U.V. densitometry at 304 nm.

A series of analyses was constituted from all the assays to be performed for both treatments in a single subject.
For each series two calibration curves were plotted, one for RU 38 486, the other for RU 42 633, by spiking control plasma with increasing, known quantities of each product, corresponding to concentrations ranging from 0.025 to 1.5 $mg.l^{-1}$.

Control plasma, spiked with known quantities of RU 38 486 and RU 42 633 and treated in exactly the same way as the samples, were included every 18 samples, giving 10 controls for each of the 3 concentrations chosen. The coefficients of variation were as follows:

RU 38 486: 3.3% for 0.075 $mg.l^{-1}$
4.8% for 0.300 $mg.l^{-1}$
2.6% for 1.600 $mg.l^{-1}$

RU 42 633: 5.3% for 0.076 $mg.l^{-1}$
3.7% for 0.305 $mg.l^{-1}$
2.8% for 1.626 $mg.l^{-1}$

The threshold of quantification was set at ——$mg.l^{-1}$. Concentrations below —— $mg.l^{-1}$ were considered to be zero, and concentrations equal to or greater than this were rounded off to the nearest 0.001.

APPENDIX VIII

Protocol

Laboratory norms

Curriculum vitae of the investigator

Agreement and composition of the Ethical Committee

Informed consents

Institut
ROUSSEL UCLAF

Medical Direction
Romainville

BL/SW/LB

September 1986

FINAL VERSION

PROTOCOL CH/86/486/06

ABSOLUTE BIOAVAILABILITY STUDY OF RU 38.486 ADMINISTERED IN A SINGLE 20 MG DOSE TO WOMEN

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1. INTRODUCTION:

RU 38.486 (MIFEPRISTONE) is an original compound synthesised by the ROUSSEL UCLAF Research Department, which has been shown in hormone receptor binding and animal pharmacology studies to be antiprogesterone, antiglucocorticoid and weakly anti-androgenic but to have no agonist properties.

A tolerance study of RU 38 486 (excipient: hydrochloric acid and alcohol) administered IV to men showed that the maximum tolerated IV dose (infusion for 1 hour) was 40 mg.

The pharmacokinetics of RU 38 486 have been studied in men and women. In women, a linearity study was performed at doses of 50 mg, 150 mg and 450 mg orally. The observed peak plasma concentrations (Cmax) were 1.2, 1.7 and 2.0 $mg.l^{-1}$ respectively, and the areas under the curve (AUC) 17.4, 28.8 and 63.6 $mg.l^{-1}.hr$ respectively. From these results it was concluded that the kinetics of the compound were nonlinear. Moreover, the calculated half-lives varied dose-dependently (19.7, 21.0 and 38.9 h).

A pilot absolute bioavailability study was performed in men at a dose of 40 mg. The bioavailability of orally administered RU 38.486 in solution was 70%.

As RU 38.486 is to be administered to women, it is therefore necessary to perform an absolute bioavailability study in women. In order to minimise a biased interpretation due to the nonlinearity of the pharmacokinetics, the dose selected on the basis of the previous pilot study will be 20 mg.

2. AIM OF TRIAL:

To determine the absolute bioavailability of RU 38.486 administered in a dose of 20 mg IV and orally (RU 38.486 in solution) to healthy women of childbearing age.

3 - MATERIAL AND METHODS

3.1 TRIAL DESIGN

This is an open, randomised, crossover study. The order of administration of the products will be determined by balanced randomisation.

3.2 SUBJECTS

3.2.1. Inclusion criteria:

i) Subjects aged from 20 to 40 years.
ii) Female subjects.
iii) Subjects of childbearing age with a regular cycle.
iv) Subjects whose weight does not deviate by more than 10% from the ideal weight for their age and height.

Subjects must also have one of the following:

a) an IUD in situ for at least 6 months;
b) or tubal ligation;
c) or a sterile partner;
d) or no partner during the study.

In the 10 days prior to the study, the subjects will undergo a clinical, electrocardiographic and laboratory examination, the results of which will be noted in the case record form. The results must be within normal limits, unless the investigator decides that any abnormalities found are without clinical significance.

3.2.2. Exclusion criteria:

i) Women with a positive pregnancy test in the 24 hours prior to administration of RU 38 486. This test, involving an assay of βHCG (human chorionic gonadotropin) in plasma or urine, will only be performed if the subject has not had a tubal ligation.

ii) Women with a history of allergy or hypersensitivity to drugs.

iii) Women using hormonal contraception.

iv) Women taking medication regularly.

v) Women who have occasionally taken medication in the week prior to the trial.

vi) Women with a severe acute disease in the month prior to the trial.

vii) Women with a current or previous history of gastro-intestinal, hepatic or renal disorders or any condition known to interfere with the absorption, distribution and elimination of drugs.

viii) Women smoking more than 10 cigarettes a day.

ix) Women consuming excessive amounts of alcohol.

3.2.3. Number of subjects:

The total number of subjects will be 10.

3.3. PRODUCT:

3.3.1. Presentation:

Active product: RU 38 486
10 ml ampoules containing 100 mg of product (10 mg/ml).
Batch No.:
Composition: RU 38 486 100 mg
0.1 N HCl 9 ml
Absolute ethanol to 10 ml

3.3.2. Dosage:

20 mg orally and IV.

3.3.3. Preparation and method of administration:

Orally: 2 ml of the contents of one ampoule (= 20 mg of RU 38 486) taken with 150 ml of water, the subject being in the upright position for administration and remaining so for the following 2 minutes.

IV: The solution of RU 38 486 contained in 1 ampoule will be diluted 1:50 as follows:

RU 38 486 solution: 10 ml
Phsyiological saline (0.9%): 490 ml

The volume to be injected per subject will be 100 ml (i.e. 20 mg).

The duration of infusion will be 1 hour. It will be performed at a constant rate by means of an electric infuser.

General conditions of administration for both routes:
Each dose will be administered on an empty stomach in a single dose in a randomised order.

Each treatment will be administered at the end of a cycle for two consecutive cycles, between the 3rd day before the expected date of menstruation and the second day after the expected date.

During the 24 hours prior to each dose, pregnancy will be excluded by an assay of plasma or urinary βHCG in subjects who have not undergone tubal ligation.

In the event of a positive pregnancy test RU 38 486 will not be administered and the subject will be excluded from the trial.

RU 38 486 will be administered in the morning at about 8 a.m. Subjects will have fasted overnight (minimum of 10 hours fasting).

Subjects may take a light meal 4 hours after administration of RU 38 486.

Subjects may only drink water.

Eight hours after administration they may resume their normal eating habits.

3.3.4 Concomitant treatments

No treatment may be taken during the study. If it is necessary to administer medication to a subject during the trial, the physician in charge will decide on whether or not to prescribe the medication and must enter the following information on the case record form:

a) the reason for treatment,
b) the name of the product and its presentation,
c) the dosage given,
d) the method and duration of administration.

4 - BLOOD SAMPLES

Blood samples (5 ml) will be taken by venipuncture at the following times (TO being the time of administration of the product or the beginning of infusion): -15 min, +15 min, +30 min, +45 min, 1 hr, 1.50 hr, 2 hr, 4 hr, 8 hr, 12 hr, 24 hr, 30 hr, 36 hr, 48 hr, 54 hr, 60 hr, 72 hr and 96 hr (total: 90 ml of blood per sequence).
The actual (observed) times of the samples will be recorded in the case record form.

The blood will be collected in dry heparinised tubes (lithium heparinate), centrifuged at 4°C and the plasma collected in two dry tubes. The plasma will be frozen immediately and stored at -20°C.

The tubes will be identified by labels bearing the following information:

i) the name and number of the study
ii) the date of sampling
iii) the subject's initials
iv) the sampling time.

At the end of the trial all the samples will be sent to [redacted] ROUSSEL UCLAF, 102 - 111 Route de Noisy, 93 230 ROMAINVILLE in an icebox containing dry ice.

5 - ASSAYS

RU 38 486 will be assayed by high performance liquid chromatography with detection by [redacted]

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ON ORIGINAL

6 - EXCLUSION FROM THE TRIAL

A positive pregnancy test.

Subjects may withdraw from the trial if they so wish, giving their reasons to the investigator.

The investigator may exclude a subject in the following cases:

i) the occurrence of severe unwanted effects,
ii) the failure of the subject to comply with the protocol,
iii) the impossibility of obtaining samples.

Subjects who withdraw from the trial must be replaced.

7 - SUBJECT MONITORING

The subjects will be under the supervision of the investigator from the time of administration of the product until 96 hours.

Any unusual incident or symptom occurring during this period will be recorded in the case record form, noting the date, type of incident, severity and outcome (duration, consequences).

During the week after the end of the trial, the subjects will undergo the same clinical, electrocardiographic and biological examination as on inclusion.

8 - PLANNING

8.1 CONSENT AND AGREEMENTS:

The investigator and the co-ordinator undertake to perform this study compliance with the rules of the Declaration of Helsinki (revised at Tokyo, 1975).

8.1.1 Ethical Council:

The investigator is free to submit this protocol to the Ethical Council of his choice. In the event of an objection by this Council, due note will be taken and the protocol amended accordingly.

8.1.2 Subjects' informed consent:

All subjects admitted to the trial will give their free and informed consent.

The subjects will be informed of the nature of the trial, its ai and its risks. They will be given a protocol which will be explained during a preparatory meeting prior to the trial. They will be informed that they may withdraw from the trial at any time.

The volunteers will give their written consent in the presence of a witness.

8.1.3 Confidentiality

All the results will be the property of Roussel Uclaf and may no be published until they have been forwarded for discussion and comments to the Roussel Uclaf Patents Department.

8.1.4 Protocol amendments:

Any modification to the protocol must receive the written agreement of the Roussel Uclaf co-ordinator. Any changes will b documented and submitted to the Ethical Council.

8.2 DOCUMENTATION:

The following documents will be provided:

i) investigator's brochure,
ii) protocol,
iii) case record forms,
iv) randomisation list.

8.3 FINANCING:

Roussel Uclaf will settle all costs related to the study. A financial protocol will be signed between Roussel Uclaf and the investigator.

8.4 TIMETABLE:

The principal scheduled dates are as follows:

- Beginning of study: OCTOBER 1986
- End of study: DECEMBER 1986

8.5 FOLLOW-UP OF STUDY BY ROUSSEL UCLAF

All the case record forms will be completed and signed by the investigator. Any missing or invalid data will be explained. This study will be monitored regularly by a member of the Medical Direction to ensure that the study is performed in compliance with the protocol adopted and according to Good Clinical Practice procedures.

8.6 DISCONTINUATION OF STUDY:

Roussel Uclaf reserves the right to discontinue the study at any time for medical or administrative reasons. Expenses incurred will be reimbursed.

8.7 INSURANCE:

The investigator is insured against civil liability for study CH/86/486/06.

9 - RESULTS

The pharmacokinetic and statistical analysis will be done by ROUSSEL UCLAF.

Any side-effects will be reported in detail.

The report will be produced jointly by the investigator and ROUSSEL UCLAF.

10 - SIGNATURES

"We totally accept this protocol which gives all the information necessary to perform this study.

We agree to perform this study".

NAME	SIGNATURE	DATE
Dr.		16.11.86
		16.11.86
		16/11/86

ADDENDUM

A third treatment sequence with an oral dose of 400 mg or 600 mg (therapeutic dose to be confirmed) is scheduled in the same volunteers after the 20 mg doses.

The purpose of this sequence, in view of the dose-dependent pharmacokinetics of RU 38 486, is to determine the pharmacokinetic parameters (after administration of the therapeutic dose) in subjects whose absolute bioavailability is known.

In addition a Latin square experimental design is difficult to implement given the possible disruption of the cycle with the 400 mg or 600 mg dose.

The protocol will be the same as that followed with the 20 mg doses.

Urine collections will also be done over the following time intervals:

0 - 2 hr; 2 - 4 hr; 4 - 6 hr; 6 - 8 hr; 8 - 12 hr; 12 - 24 hr; 24 - 36 hr; 36 - 48 hr; 48 - 60 hr; 60 - 72 hr; 72 - 96 hr.

The subjects will drink 100 ml of noncarbonated mineral water every hour for 4 hr after drug administration.

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A N N E X 1

MEAN AND IDEAL ADULT WEIGHT

Mean and ideal adult weights

Height in cm (with shoes)	Mean weight[1] (in kg, clothed) for age:								Ideal weight[2] (in kg, clothed) 25 +		
	15–16	17–19	20–24	25–29	30–39	40–49	50–59	60–69	Light build	Medium build	Heavy build
Men											
153	44.9	51.7	53.7	54.4	54.7	61.1	62.0	60.7			
154	45.6	52.1	56.2	56.9	58.3	61.6	62.5	61.2			
155	46.3	52.6	56.7	59.5	60.8	62.2	63.1	61.7			
156	47.2	53.2	57.2	60.0	61.3	62.7	63.6	62.2			
157	48.1	53.7	57.8	60.5	61.9	63.2	64.1	62.8	50.5–54.2	53.5–58.2	56.9–63.7
158	49.0	54.3	58.4	61.2	62.5	63.9	64.7	63.3	51.1–54.7	53.8–58.9	57.4–64.2
159	49.9	55.1	59.1	61.9	62.2	64.6	65.2	63.9	51.6–55.2	54.3–59.6	58.0–64.8
160	50.8	55.8	59.9	62.6	63.9	65.3	65.8	64.4	52.2–55.8	54.9–60.3	58.5–65.3
161	51.7	56.5	60.6	63.1	64.7	66.0	66.5	65.1	52.7–56.3	55.4–60.9	59.0–66.0
162	52.6	57.2	61.3	63.7	65.4	66.7	67.2	65.8	53.2–56.9	55.9–61.4	59.6–66.7
163	53.5	58.0	61.9	64.2	66.1	67.5	67.9	66.6	53.8–57.4	56.5–61.9	60.1–67.5
164	54.4	58.7	62.5	64.8	66.8	68.2	68.6	67.3	54.3–57.9	57.0–62.5	60.7–68.2
165	55.3	59.4	63.0	65.3	67.5	68.9	69.4	68.0	54.9–58.5	57.6–63.0	61.2–68.9
166	56.1	60.1	63.5	66.0	68.2	69.6	70.0	68.7	55.4–59.2	58.1–63.7	61.7–69.6
167	57.0	60.8	64.1	66.7	68.9	70.3	70.8	69.4	55.9–59.9	58.6–64.4	62.3–70.3
168	57.9	61.6	64.6	67.3	69.7	71.1	71.5	70.2	56.5–60.6	59.2–65.1	62.9–71.1
169	58.8	62.2	65.1	67.9	70.4	72.0	72.4	71.1	57.2–61.3	59.9–65.8	63.6–72.0
170	59.7	62.9	65.7	68.4	71.1	72.9	73.3	72.0	57.9–62.0	60.7–66.6	64.3–72.9
171	60.6	63.6	66.4	69.1	71.8	73.6	74.1	72.7	58.6–62.7	61.4–67.4	65.1–73.8
172	61.5	64.3	67.1	69.8	72.5	74.3	74.8	73.4	59.4–63.4	62.1–68.3	66.0–74.7
173	62.4	65.1	67.8	70.5	73.2	75.0	75.5	74.2	60.1–64.2	62.8–69.1	66.9–75.5
174	63.3	65.8	68.5	71.2	73.9	75.8	76.2	75.1	60.8–64.9	63.5–69.9	67.6–76.2
175	64.2	66.5	69.2	71.9	74.7	76.5	76.9	76.0	61.5–65.6	64.2–70.6	68.3–76.9
176	64.9	67.2	69.9	72.6	75.5	77.3	77.8	76.9	62.2–66.4	64.9–71.3	69.0–77.6
177	65.7	67.9	70.6	73.4	76.4	78.2	78.7	77.8	62.9–67.3	65.7–72.0	69.7–78.4
178	66.4	68.6	71.4	74.1	77.3	79.1	79.6	78.7	63.6–68.2	66.4–72.8	70.4–79.1
179	67.1	69.3	72.1	74.8	78.0	79.8	80.5	79.5	64.4–68.9	67.1–73.6	71.2–80.0
180	67.8	70.1	72.8	75.5	78.7	80.5	81.3	80.4	65.1–69.6	67.8–74.5	71.9–80.9
181	68.5	70.9	73.6	76.3	79.5	81.3	82.2	81.3	65.8–70.3	68.5–75.4	72.7–81.8
182	69.2	71.8	74.3	77.2	80.4	82.2	83.1	82.2	66.5–71.0	69.2–76.3	73.6–82.7
183	70.0	72.7	75.4	78.1	81.3	83.1	84.0	83.1	67.2–71.8	69.9–77.2	74.5–83.6
184	70.9	73.4	76.1	79.0	82.0	83.8	84.7	84.0	67.9–72.5	70.7–78.1	75.2–84.5
185	71.7	74.1	76.8	79.9	82.7	84.5	85.4	84.9	68.6–73.2	71.4–79.0	75.9–85.4
186	72.6	74.8	77.5	80.8	83.5	85.3	86.2	85.8	69.4–74.0	72.1–79.9	76.7–86.2
187	73.5	75.5	78.2	81.7	84.4	86.2	87.1	86.7	70.1–74.9	72.8–80.8	77.6–87.1
188	74.4	76.2	79.0	82.6	85.3	87.1	88.0	87.6	70.8–75.8	73.5–81.7	78.5–88.0
189	75.3	76.9	79.7	83.5	86.2	88.0	88.9	88.5	71.5–76.5	74.4–82.6	79.4–88.9
190	76.2	77.7	80.4	84.0	87.1	88.9	89.8	89.4	72.2–77.2	75.3–83.5	80.3–89.8
191	77.1	78.4	81.0	84.7	88.1	89.9	90.8	90.3	72.9–77.9	76.2–84.4	81.1–90.7
192	78.0	79.1	81.5	85.4	89.2	91.0	91.9	91.4	73.6–78.6	77.1–85.3	81.8–91.6
193	-	79.8	82.1	86.3	90.2	92.0	92.9	92.5	74.4–79.3	78.0–86.1	82.5–92.5
194	-	80.5	82.6	86.9	91.3	93.1	94.0	93.6	75.1–80.1	78.9–87.0	83.2–93.4
195	-	81.2	83.2	87.6	92.4	94.2	95.1	94.6	75.8–80.8	79.8–87.9	84.0–94.3
Women											
148	44.4	45.3	46.6	48.9	52.4	55.6	56.9	57.8	42.0–44.8	43.8–48.9	47.4–54.3
149	44.9	45.8	47.2	49.4	52.8	55.9	57.3	58.2	42.3–45.4	44.1–49.4	47.8–54.9
150	45.4	46.3	47.7	50.0	53.1	56.3	57.7	58.6	42.7–45.9	44.5–50.0	48.2–55.4
151	46.0	46.9	48.2	50.5	53.7	56.9	58.3	58.9	43.0–46.4	45.3–50.5	48.7–55.9
152	46.5	47.4	48.8	51.0	54.2	57.4	58.8	59.3	43.4–47.0	45.6–51.0	49.2–56.5
153	47.1	48.1	49.4	51.6	54.8	57.9	59.3	59.8	43.9–47.5	46.1–51.6	49.8–57.0
154	47.9	48.8	50.1	52.1	55.3	58.5	59.8	60.3	44.4–48.0	46.7–52.1	50.3–57.6
155	48.6	49.5	50.8	52.6	55.8	59.0	60.4	60.8	44.9–48.6	47.2–52.6	50.8–58.1
156	49.3	50.2	51.3	53.2	56.3	59.5	60.9	61.3	45.4–49.1	47.7–53.2	51.3–58.6
157	50.0	50.9	51.9	53.7	56.9	60.0	61.4	61.9	46.0–49.6	48.2–53.7	51.9–59.1
158	50.6	51.5	52.4	54.3	57.4	60.6	62.1	62.5	46.5–50.2	48.8–54.3	52.4–59.7
159	51.1	52.1	53.0	54.8	58.0	61.1	62.6	63.2	47.1–50.7	49.3–54.8	53.0–60.2
160	51.7	52.6	53.5	55.3	58.5	61.7	63.5	63.9	47.6–51.2	49.9–55.3	53.5–60.8
161	52.2	53.3	54.0	55.9	59.0	62.4	64.2	64.7	48.2–51.8	50.4–56.0	54.0–61.5
162	52.8	54.0	54.6	56.5	59.6	63.1	64.9	65.4	48.7–52.3	51.0–56.8	54.6–62.2
163	53.4	54.8	55.2	57.0	60.1	63.8	65.7	66.1	49.2–52.9	51.5–57.5	55.2–62.9
164	54.1	55.5	55.9	57.7	60.7	64.3	66.4	66.8	49.8–53.4	52.0–58.2	55.9–63.7
165	54.8	56.2	56.6	58.5	61.2	64.8	67.1	67.5	50.3–53.9	52.6–58.9	56.7–64.4
166	55.5	56.7	57.3	59.2	61.9	65.5	67.8	68.2	50.8–54.6	53.5–59.8	57.3–65.1
167	56.2	57.3	58.1	59.9	62.6	66.2	68.5	68.9	51.4–55.3	54.0–60.7	58.1–65.8
168	56.9	57.8	58.7	60.5	63.2	66.9	69.2	69.7	52.0–56.0	54.7–61.5	58.8–66.5
169	57.6	58.3	59.2	61.1	63.8	67.6	69.9	70.6	52.7–56.8	55.4–62.2	59.5–67.2
170	58.0	58.9	59.8	61.6	64.3	68.4	70.6	71.1	53.4–57.5	56.1–62.9	60.2–67.9
171	58.6	59.6	60.5	62.3	65.0	69.1	71.3	71.8	54.1–58.2	56.8–63.6	60.9–68.6
172	59.4	60.3	61.2	63.0	65.7	69.8	72.1	72.5	54.8–58.9	57.5–64.3	61.6–69.3
173	60.1	61.0	61.9	63.7	66.4	70.5	72.8	73.2	55.5–59.6	58.3–65.1	62.3–70.1
174	60.8	61.7	62.6	64.4	67.1	71.2	73.5	73.9	56.3–60.3	59.0–65.8	63.1–70.8
175	61.5	62.4	63.3	65.1	67.9	71.9	74.2	74.7	57.0–61.0	59.7–66.5	63.8–71.5
176	62.2	63.1	64.0	65.8	68.6	72.8	75.1	75.4	57.7–61.9	60.4–67.2	64.5–72.3
177	62.9	63.8	64.7	66.6	69.3	73.7	75.9	76.1	58.4–62.8	61.1–67.9	65.2–73.2
178	63.6	64.6	65.5	67.3	70.0	74.6	76.8	76.8	59.1–63.6	61.8–68.6	65.9–74.1
179	-	65.5	66.4	68.2	70.9	75.5	77.7	-	59.8–64.4	62.5–69.3	66.6–75.0
180	-	66.4	67.3	69.1	71.8	76.4	78.6	-	60.5–65.1	63.3–70.1	67.3–75.9
181	-	67.3	68.2	70.0	72.7	77.2	79.6	-	61.3–65.8	64.0–70.8	68.1–76.8
182	-	68.2	69.1	70.9	73.6	78.1	80.7	-	62.0–66.5	64.7–71.5	68.8–77.7
183	-	69.1	70.0	71.8	74.5	79.0	81.8	-	62.7–67.2	65.4–72.2	69.5–78.6
184	-	70.0	70.9	72.7	75.4	79.9	82.9	-	63.4–67.9	66.1–72.9	70.2–79.5
185	-	70.9	71.8	73.6	76.3	80.8	83.9	-	64.1–68.6	66.8–73.6	70.9–80.4

[1] After Society of Actuaries (ed.) Build and Blood Pressure Study, vol. 1, Chicago, 1959, p.lo, converted into metric units
[2] After Statistic. Bull. Metrop. Life Insur. Co., 40, Nov-Dec. (1959), converted into metric units. – Ideal weight: weight corresponding to longest life expectancy

A N N E X 2

DECLARATION OF HELSINKI

Declaration of Helsinki

Recommendations guiding medical doctors in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964
and As Revised by the 29th World Medical Assembly, Tokyo, Japan, 1975.

Introduction

It is the mission of the medical doctor to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the doctor with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "Any act or advice which could weaken physical or mental resistance of a human being may be used only in his interest."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies *a fortiori* to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for experiments must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every doctor in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Doctors are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. Basic Principles

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted to a specially appointed independent committee for consideration, comment and guidance.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interest of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Doctors should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Doctors should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the doctor is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participate at any time. The doctor should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the doctor should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a doctor who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. Medical Research Combined with Professional Care (Clinical Research)

1. In the treatment of the sick person, the doctor must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, re-establishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient—including those of a control group, if any—should be assured of the best proven diagnostic and therapeutic method.

4. The refusal of the patient to participate in a study must never interfere with the doctor-patient relationship.

5. If the doctor considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I,2).

6. The doctor can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. Non-therapeutic Biomedical Research Involving Human Subjects (Non-clinical biomedical research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the doctor to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subjects should be volunteers—either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

A N N E X 3

INFORMED CONSENT

APPEARS THIS WAY
ON ORIGINAL

INFORMED CONSENT

Study title: .....
.....
.....
.....

Protocol No.: .....

Investigator: Doctor

APPEARS THIS WAY
ON ORIGINAL

Subject's name: .....

Date of birth: ..... Age: ..... Sex: .....

Address: .....
.....
.....

Telephone: .....

I, the undersigned, ... certify that on my inclusion visit I received the protocol for study ....., that I have examined the information concerning the test compound, the aim of the trial and the conditions to be observed during the study, and that I have taken note of the risks commonly incurred in this type of study.

I undertake to comply strictly with this protocol.

As a volunteer, I enter this study of my own volition, with no moral or physical pressure and I may withdraw from it at any time. During this inclusion visit I have had the opportunity to ask the doctors present all the questions necessary for my own information.

Signatures:

Volunteer: ..... Date: .....

Investigator: ..... Date: .....

Enc.: study protocol.

00279

A N N E X 4

CASE RECORD FORMS

APPEARS THIS WAY
ON ORIGINAL

PROTOCOL CH/86/486/06

RU 38.486

ABSOLUTE BIOAVAILABILITY STUDY IN WOMEN

ALLOCATION OF TREATMENTS

SUBJECT a number will be allocated to each subject	D O S A G E: 20 MG	
	First dose	Second dose
1	IV	PO
2	IV	PO
3	PO	IV
4	PO	IV
5	IV	PO
6	PO	IV
7	PO	IV
8	IV	PO
9	IV	PO
10	PO	IV

PROTOCOL CH/86/486/06

RU 38.486

ABSOLUTE BIOAVAILABILITY STUDY IN WOMEN

SUBJECT'S NAME: _ _ _ No.:

PREGNANCY TEST

	before 1st sequence	before 2nd sequence
DATE		
βHCG		

ADMINISTRATION OF RU 38486

	1st sequence	2nd sequence
DATE		
ROUTE OF ADMINISTRATION		

BLOOD SAMPLES

THEORETICAL TIME hr. min.	1st sequence OBSERVED TIME hr.min.	2nd sequence OBSERVED TIME hr. min.
-15		
.15		
.30		
.45		
1		
1.50		
2		
4		
8		
12		
24		
30		
36		
48		
54		
60		
72		
96		

RU 38.486

ABSOLUTE BIOAVAILABILITY STUDY IN WOMEN

DATE:

IDENTIFICATION AND CHARACTERISTICS OF THE SUBJECT:

NAME: _ _ _ CHRISTIAN NAME:
SEX: WEIGHT:
HEIGHT: AGE:
OCCUPATION:

APPEARS THIS WAY ON ORIGINAL

HAS THE SUBJECT GIVEN HIS OR HER CONSENT TO PARTICIPATE IN THE STUDY?
YES [ ]

SMOKING
NONE [ ] CIGARETTE [ ] CIGAR [ ] PIPE [ ]
QUANTITY PER DAY: ....

CONSUMPTION OF ALCOHOL
NONE [ ] BEER [ ] WINE [ ] SPIRITS [ ]
QUANTITY PER DAY: ....

DRUGS TAKEN REGULARLY
NONE [ ] NAME OF DRUGS: .....
DOSAGE: .....

DRUGS TAKEN OCCASIONALLY
NONE [ ] NAME OF DRUGS: .....
DOSAGE: .....
DATE OF FIRST DOSE: .....

00283

PROTOCOL CH/86/486/06

RU 38.486

ABSOLUTE BIOAVAILABILITY STUDY IN WOMEN

SUBJECT'S NAME: _ _ _

GYNAECOLOGICAL AND OBSTETRIC HISTORY

AGE OF FIRST MENSTRUAL PERIOD: .....
CURRENT DURATION OF CYCLE (days): .....
CURRENT DURATION OF MENSTRUATION (days):.....
PAIN AT TIME OF MENSTRUATION: .....
DATE OF EXPECTED MENSTRUATION: .....
NUMBER OF PREGNANCIES: .....

CONTRACEPTION

ORAL	Date commenced:		Date stopped:	
IUD	Date commenced:		Date stopped:	
LIGATION	YES [ ]	Date:	NO [ ]	
STERILE PARTNER	YES [ ]		NO [ ]	
NO PARTNER	YES [ ]		NO [ ]	

COMMENTS:

NONE [ ]

APPEARS THIS WAY
ON ORIGINAL

INVESTIGATOR'S SIGNATURE:

00284

MIF 008431

PROTOCOL CH/86/486/06

RU 38.486

ABSOLUTE BIOAVAILABILITY STUDY IN WOMEN

SUBJECT'S NAME: _ _ _

PARTICIPATION IN ANOTHER DRUG TRIAL
NEVER [ ] DATE OF LAST TRIAL: .....

HISTORY OF ALLERGY
NONE [ ] IF YES, GIVE DETAILS: .....

ALLERGY TO DRUGS
NONE [ ] IF YES, WHICH DRUGS: .....

HISTORY OF DISEASE
NONE [ ] IF YES, GIVE DETAILS: .....

SURGICAL HISTORY
NONE [ ] IF YES, GIVE DETAILS: .....

COMMENTS

NONE [ ]

APPEARS THIS WAY
ON ORIGINAL

INVESTIGATOR'S SIGNATURE:

00285

PROTOCOL CH/86/486/06

RU 38.486

ABSOLUTE BIOAVAILABILITY STUDY IN WOMEN

SUBJECT'S NAME: _ _ _

DATE:

PRE-TRIAL CLINICAL EXAMINATION

HEART RATE (supine): .....
BLOOD PRESSURE (supine): systolic .....
diastolic .....

	NORMAL	ABNORMAL	NOT DONE	COMMENT
HEAD AND NECK	[ ]	[ ]	[ ]	
EYES	[ ]	[ ]	[ ]	
EARS	[ ]	[ ]	[ ]	
NOSE	[ ]	[ ]	[ ]	
THROAT	[ ]	[ ]	[ ]	
LUNG	[ ]	[ ]	[ ]	
HEART	[ ]	[ ]	[ ]	
ABDOMEN	[ ]	[ ]	[ ]	
LIMBS	[ ]	[ ]	[ ]	
LYMPH NODES	[ ]	[ ]	[ ]	
SKIN	[ ]	[ ]	[ ]	
BREASTS	[ ]	[ ]	[ ]	
ECG	[ ]	[ ]	[ ]	

APPEARS THIS WAY
ON ORIGINAL

COMMENTS:

NONE [ ]

INVESTIGATOR'S SIGNATURE:

! 00?86

PROTOCOL CH/86/486/06
87/592/CN

RU 38.486

ABSOLUTE BIOAVAILABILITY STUDY IN WOMEN

SUBJECT'S NAME: _ _ _

	Units	BEFORE FIRST TREATMENT Date:	AFTER LAST TREATMENT Date:
ESR	.....	.....	.....
ERYTHROCYTES	.....	.....	.....
LEUCOCYTES	.....	.....	.....
NEUTROPHILS	.....	.....	.....
EOSINOPHILS	.....	.....	.....
BASOPHILS	.....	.....	.....
LYMPHOCYTES	.....	.....	.....
MONOCYTES	.....	.....	.....
PLATELETS	.....	.....	.....
PROTHROMBIN	.....	.....	.....
BLOOD UREA	.....	.....	.....
BLOOD CREATININE	.....	.....	.....
TOTAL BILIRUBIN	.....	.....	.....
BLOOD GLUCOSE	.....	.....	.....
BLOOD URIC ACID	.....	.....	.....
BLOOD CALCIUM	.....	.....	.....
BLOOD PHORPHORUS	.....	.....	.....
TRIGLYCERIDES	.....	.....	.....
CHOLESTEROL	.....	.....	.....
SODIUM	.....	.....	.....
POTASSIUM	.....	.....	.....
CHLORIDE	.....	.....	.....
BICARBONATE	.....	.....	.....
TOTAL PROTEINS	.....	.....	.....
SGOT	.....	.....	.....
SGPT	.....	.....	.....
ALKALINE PHOSPHATASE	.....	.....	.....
PROTEINURIA	.....	.....	.....
GLYCOSURIA	.....	.....	.....

APPEARS THIS WAY ON ORIGINAL

OC

PROTOCOL CH/86/486/06

RU 38.486

ABSOLUTE BIOAVAILABILITY STUDY IN WOMEN

SUBJECT'S NAME: _ _ _

FIRST DOSE OF RU 38 486

DATE OF ONSET OF MENSTRUATION: .....
COMPARED WITH THE SUBJECT'S NORMAL PERIOD:
DURATION LESS [ ] THE SAME [ ] MORE [ ]
AMOUNT LESS [ ] THE SAME [ ] MORE [ ]
PAIN YES [ ] NO [ ]
IF YES LESS [ ] THE SAME [ ] MORE [ ]

SECOND DOSE OF RU 38 486

DATE OF ONSET OF MENSTRUATION: .....
COMPARED WITH THE SUBJECT'S NORMAL PERIOD:
DURATION LESS [ ] THE SAME [ ] MORE [ ]
AMOUNT LESS [ ] THE SAME [ ] MORE [ ]
PAIN YES [ ] NO [ ]
IF YES LESS [ ] THE SAME [ ] MORE [ ]

RU 38.486

ABSOLUTE BIOAVAILABILITY STUDY IN WOMEN

DATE:

POST-TRIAL CLINICAL EXAMINATION

HEART RATE (supine): .....
BLOOD PRESSURE (supine): systolic .....
diastolic .....

	NORMAL	ABNORMAL	NOT DONE	COMMENT
HEAD AND NECK	[ ]	[ ]	[ ]	
EYES	[ ]	[ ]	[ ]	
EARS	[ ]	[ ]	[ ]	
NOSE	[ ]	[ ]	[ ]	
THROAT	[ ]	[ ]	[ ]	
LUNG	[ ]	[ ]	[ ]	
HEART	[ ]	[ ]	[ ]	
ABDOMEN	[ ]	[ ]	[ ]	
LIMBS	[ ]	[ ]	[ ]	
LYMPH NODES	[ ]	[ ]	[ ]	
SKIN	[ ]	[ ]	[ ]	
BREASTS	[ ]	[ ]	[ ]	
VAGINAL EXAM.	[ ]	[ ]	[ ]	
ECG	[ ]	[ ]	[ ]	

APPEARS THIS WAY ON ORIGINAL

COMMENTS:

NONE [ ]

INVESTIGATOR'S SIGNATURE:

00789

A N N E X 5

CERTIFICATE OF INSURANCE

APPEARS THIS WAY
ON ORIGINAL

NORMAL VALUES - LABORATORY NORMS

ESR	M: 1-7 mm/hr	F: 1-12 mm/hr
Erythrocytes	M: 4.4 - 6.0 M/mm^3	F: 3.8 - 5.6 M/mm^3
Leucocytes	4 - 11,000 /mm^3	
Differential count		
Neutrophils	33 - 75%	
Eosinophils	0 - 0.5%	
Basophils	0 - 0.2%	
Lymphocytes	15 - 60%	
Monocytes	0 - 09%	
Platelets	150 - 350,000 /mm^3	
Prothrombin	70 - 100%	
Urea	2.8 - 8.6 mmol/l	
Creatinine	M: 56 - 120 µmol/l	
	F: 45 - 110 µmol/l	
Total bilirubin	5 - 17 µmol/l	
Blood glucose	4.2 - 6.0 mmol/l	
Urate	150 - 480 µmol/l	
Blood calcium	2.25 - 2.62 mmol/l	
Blood phosphorus	0.8 - 1.4 mmol/l	
Triglycerides	0.4 - 2.1 mmol/l	
Cholesterol	3.3 - 7.3 mmol/l	
Sodium	135 - 148 mmol/l	
Potassium	3.1 - 4.7 mmol/l	
Chloride	96 - 109 mmol/l	
Bicarbonate	23 - 30 mmol/l	
Total proteins	62 - 79 g/l	
SGOT	14 - 50 U/l	
SGPT	11 - 60 U/l	
Alk. phosphatase	30 - 125 U/l	
Proteinuria	neg.	
Glycosuria	neg.	

A P P E N D I X

Individual results

Protocol

Curriculum vitae of the investigator

Agreement and composition of the ethical committee

Informed consent

Quality assurance audit report

(clinical part of the study)

Medical Direction
Romainville

BL/LB - April 1987

PROTOCOL CH/87/486/10
PHARMACOKINETICS IN WOMEN
AFTR ORAL ADMINISTRATION OF 600 mg (50 µCi) OF ^{3}H-RU 38 486

INVESTIGATOR

Doctor ———
Medical Clinic
Department of Medicine
Cantonal Hospital
CH 1211 GENEVA 4
SWITZERLAND

ROUSSEL UCLAF CO-ORDINATOR

———
ROUSSEL UCLAF
Medical Direction
102,111 Route de Noisy
93 230 ROMAINVILLE tel. (1) 48.43.93.10

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1 - INTRODUCTION

RU 38 486 (MIFEPRISTONE) is an original product synthesised by the ROUSSEL UCLAF Research Department, which has been shown by hormone receptor binding and animal pharmacology studies to be anti-progesterone, antiglucocorticoid and weakly anti-androgenic without having any agonist properties.

Animal pharmacokinetics

The pharmacokinetics of RU 38 486 have been studied in starved male or female rats after intravenous or oral administration of a pharmacologically active dose (5 $mg.kg^{-1}$) in solution using a molecule tritiated in positions 6 and 7.

Intravenous administration (5 $mg.kg^{-1}$) showed that the product was rapidly eliminated (clearance 3 $l.hr^{-1}$ per kg of bodyweight). After oral administration (5 $mg.kg^{-1}$) absorption was early and rapid. It was satisfactory (almost 3/4 of the dose) but the bioavailability was reduced by a presystemic effect (40% of the ingested dose). Tissue distribution of radioactivity 0.5 and 24 hours after oral administration shows that elimination of the whole of the product and its metabolites was rapid in all the localisations studied, except for the erythrocytes. However, the concentration of the latter was weak, as the combined erythrocytes 24 hours after treatment accounted for only one thousandth of the ingested radioactivity. Excretion of radioactivity in the urine and faeces after intravenous administration (5 $mg.kg^{-1}$) was rapid and complete within 4 days (99% of the dose). The principal route of elimination was faecal (97% of the total excreted).

In the Cynomolgus monkey, the plasma kinetics of radioactivity of unchanged product after oral or intravenous administration of 3 $mg.kg^{-1}$ of product in solution showed slow and irregular absorption, although it was satisfactory in quantitative terms (3/4 of the administered dose). The bioavailability was reduced by a

presystemic effect (15% of the ingested dose). The total clearance was 1.5 $l.hr^{-1}$ per kg of bodyweight. The principal route of elimination of radioactivity after intravenous administration was faecal (92% of the total elimination within 7 days). Urinary and faecal elimination appeared practically complete after one week and the total amount eliminated by these two routes was equivalent to 85% of the administered dose.

Human pharmacokinetics

The pharmacokinetics of tritiated RU 38 486 were studied in man after intravenous administration of a tracer dose (280 ng. 25 µCi) and after oral administration of a pharmacologically active dose (100 mg, 50 µCi). In man, the principal route of elimination of total radioactivity is faecal (90% of total elimination over 6 days), irrespective of the route of administration. After 6 days, urinary and faecal elimination is practically complete: 92% of the total radioactivity administered orally is eliminated by these 2 routes during this period.

A linearity study was performed in women at doses of 50 mg, 150 mg and 450 mg, administered orally. The peak plasma concentrations (Cmax) observed were 1.2, 1.7 amd 2.0 $mg.l^{-1}$ respectively and the areas under the curve (AUC) 17.4, 28.8 and 63.6 $mg.l^{-1}.hr$ respectively. The conclusion drawn from these results was that the kinetics of the product were nonlinear. Furthermore, the calculated elimination t 1/2 varied with the dose (19.7, 21.0 and 38.9 hr).

A pilot absolute bioavailability study was performed in men at a dose of 40 mg. The absolute bioavailability of RU 38 486 in solution administered orally was 70%.

For its indication as an "alternative to early termination of pregnancy by aspiration" at a dosage of 600 mg (in a single oral dose), it is therefore necessary to study the pharmacokinetics and the metabolism of RU 38 486 at this dosage.

In view of the difficulties, and even the impossibility, of quantifying urinary and faecal excretion of the product and its metabolites by a "non-isotopic method," the pharmacokinetic study and the determination of the structures of the metabolites of RU 38 486 will be performed with the tritium-labelled product.

2 - AIM OF TRIAL

The aim of this trial is to study the pharmacokinetics and metabolism of 3H-RU 38 486 in healthy women of childbearing age.

3 - MATERIAL AND METHODS

3.1 TRIAL DESIGN

This is an open study in 4 subjects.

3.1.1 Inclusion criteria

i) Subjects aged from 20 to 40 years.
ii) Female subjects.
iii) Subjects of childbearing age with a regular cycle.
iv) Subjects whose weight does not deviate by more than 10% from the ideal weight for their age and height.

Subjects must also have one of the following:

a) an IUD in situ for at least 6 months;
b) or tubal ligation;
c) or a sterile partner;
d) or no partner during the study.

In the 10 days prior to the study, the subjects will undergo a clinical, electrocardiographic and laboratory examination, the results of which will be noted in the case record form. The results must be within normal limits, unless the investigator decides that any abnormalities found are without clinical significance.

3.1.2 Exclusion criteria:

i) A positive pregnancy test in the 24 hours prior to administration of RU 38 486.
This test, involving an assay of βHCG (human chorionic gonadotropin) in plasma or urine, will only be performed if the subject has not had a tubal ligation.

ii) A history of allergy or hypersensitivity to drugs.

iii) Regular use of drugs.

iv) Occasional use of drugs in the week prior to the trial.

v) A severe acute disease in the month prior to the trial.

vi) Gastro-intestinal, hepatic or renal disorders or any condition known to interfere with the absorption, distribution and elimination of drugs.

vii) Smoking more than 10 cigarettes a day.

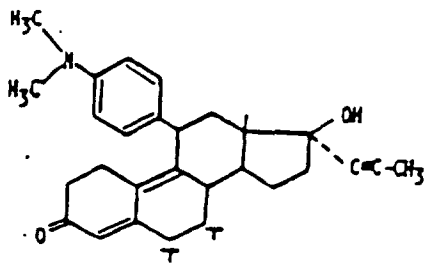
viii) Excessive consumption of alcohol.

3.2 PRODUCT

3.2.1 Presentation

Active product: RU 38 486 tritium labelled in positions 6 and 7.
Tablets containing 200 mg of 38 486 with an activity of 0.61 MBq (16.5 µCi).

Structural formula:



Composition:

3.2.2 Dosage:

600 mg (= 3 x 200 mg tablets of RU 38 486 (1.85 MBq - 50 µCi) in a single dose) orally.

3.2.3 Method of administration

The 3 tablets of RU 38 486 will be swallowed with 150 ml of noncarbonated water, the subject being in the upright position and remaining so for the next two minutes.

Conditions of administration:

The dose will be administered at the end of a cycle, between the 3rd day before the expected date of menstruation and the second day after the expected date.

During the 24 hours prior to administration, pregnancy will be excluded by an assay of plasma or urinary βHCG in subjects without a tubal ligation.

In the event of a positive pregnancy, RU 38 486 will not be administered and the subjects will be replaced.

RU 38 486 will be administered in the morning at about 8.00 a.m. Subjects will have fasted overnight (minimum ten hours).

Subjects may take a light meal 4 hours after administration of RU 38 486.

Subjects may only drink water.

Eight hours after administration they may resume their usual eating habits.

3.2.4. Concomitant treatments

No treatment may be taken during the study. If it is necessary to administer a drug to a subject during the trial, the physician in charge will decide on whether or not to prescribe the drug and must enter the following information on the case record form:

a) the reason for treatment,
b) the name of the product and its presentation,
c) the dosage given,
d) the method and duration of administration.

4 - SAMPLES

4.1 BLOOD

Blood samples (5 ml) will be taken by venipuncture at the following times (T0 being the time of administration of the product): - 15 min, 15 min, 30 min, 45 min, 1 hr, 1.30 hr, 2 hr, 4 hr, 8 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 96 hr, 120 hr, 144 hr, 168 hr, 192 hr, 216 hr and 240 hr (total volume of blood withdrawn: 105 ml).
The actual (observed) times of the samples will be noted in the case record form.

The blood will be collected in dry heparinated tubes (lithium heparinate) centrifuged at 4°C and the plasma collected in two dry tubes. The plasma will immediately be frozen and stored at - 20°C.

The tubes will be identified by labels bearing the following information:

a) the study number
b) the date of sampling
c) the subject's initials
d) the sampling time.

4.2 URINE

The subjects must empty their bladder during the 5 minutes before administration (sample 0).
The urine will then be collected over the following periods:
0 - 12 hr, 12 - 24 hr, 24 - 48 hr, 48 - 72 hr, 72 - 96 hr, 96 - 120 hr, 120 - 144 hr, 144 - 168 hr, 168 - 192 hr, 192 - 216 hr, 216 - 240 hr.

Subjects must empty their bladder at the end of each collection period.

In practice, the urine will be stored at 4°C during each collection period, the subjects returning their specimens to the hospital at the time of each blood sample. After measurement of the pH, all the urine will be stored at - 20°C.
The samples will be identified by labels bearing the following information:

a) the study number,
b) the date of sampling,
c) the subject's initials,
d) the sampling time
   beginning - end.

4.3 STOOLS

The stools will be collected every day until 24 hours after the end of the urine samples (i.e. up to 264 hr).
In practice, the stools will be stored at 4°C, the subjects returning their stools to the hospital at the time of each blood sample. The stools will be stored at - 20°C until assay.
They will be identified by labels bearing the following information:

a) the study number,
b) the date of collection
c) the subject's initials,
d) the collection time.

At the end of the trial all the specimens will be sent to ROUSSEL UCLAF, 102 - 11 Route de Noisy, 93 230 ROMAINVILLE in an ice box containing dry ice.

5 - EXCLUSION FROM THE TRIAL

- A positive pregnancy test.

Subjects may withdraw from the study at any time.

The investigator may exclude a subject in the event of :

i) noncompliance with the protocol by the subject;

ii) the impossibility of obtaining specimens.

Subjects withdrawing from the study must be replaced.

6 - SUBJECT MONITORING

The subjects will be under the supervision of the investigator from the time that the product is administered.

Any incident or any unusual symptom occuring during this period will be noted on the case record form. The following details will be recorded: date, type of incident, intensity, outcome (duration, consequences).

In the week after the end of the trial the subjects will undergo the same clinical, electrocardiographic and laboratory examination as on inclusion.

7 - PLANNING

7.1 CONSENT AND AGREEMENTS

The investigator and the co-ordinator undertake to perform this study in compliance with the rules of the Declaration of Helsinki (revised at Tokyo, 1975).

7.1.1 Ethical Council:

The investigator is free to submit this protocol to the Ethical Council of his choice. In the event of an objection by this Council, due note will be taken and the protocol amended accordingly.

7.1.2 Subjects' informed consent:

All subjects admitted to the trial will give their free and informed consent.

Subjects will be informed of the nature of the trial, its aim and its risks. They will be given a protocol which will be explained during a preparatory meeting prior to the trial. They will be informed that they may withdraw from the trial at any time.

The volunteers will give their written consent in the presence of a witness.

7.1.3 Confidentiality

All the results will be the property of Roussel Uclaf and may not be published until they have been forwarded for discussion and comments to the Roussel Uclaf Patents Department.

7.1.4 Protocol amendments:

Any modification to the protocol must receive the written agreement of the Roussel Uclaf co-ordinator. Any changes will be documented and submitted to the Ethical Council.

7.2 DOCUMENTATION

The following documents will be supplied:

- Investigator's brochure
- Protocol
- Case record forms

7.3 FINANCING

Roussel Uclaf will settle all costs related to the study. A financial protocol will be signed between Roussel Uclaf and the investigator.

7.4 TIMETABLE

The principal dates planned are as follows:

- Beginning of study: May 1987
- End of study: June 1987

7.5 FOLLOW-UP OF THE STUDY BY ROUSSEL UCLAF

All the case record forms will be completed and signed by the investigator. Any missing or invalid data will be explained. This study will be monitored regularly by a member of the Medical Direction to ensure that the study is performed in accordance with the protocol adopted and in compliance with the rules of Good Clinical Practice.

7.6 DISCONTINUATION OF THE STUDY:

Roussel Uclaf reserves the right to discontinue the study at any time for medical or administrative reasons. Expenses incurred will be reimbursed.

7.7 INSURANCE

The investigator is insured for civil liability for study CH/87/486/10 (cf. Appendix V).

8 - RESULTS

The pharmacokinetic and statistical analysis will be done by Roussel Uclaf.

Any side-effects will be reported in detail.

The report will be produced jointly by the investigator and Roussel Uclaf.

9 - SIGNATURES

"We totally accept this protocol which gives all the information necessary to perform this study.

We agree to perform this study".

NAME	SIGNATURE	DATE
[illegible]	[illegible]	26-5-87
[illegible]	[illegible]	26-5-87
[illegible]	[illegible]	26/5/87

2 - AIM OF STUDY

The aim of this study was to determine the plasma pharmacokinetics of RU 38 486 administered at the clinically used dose (600 mg orally) to healthy women of childbearing age.

3 - MATERIAL AND METHODS

3.1. Protocol

The protocol is given in full in Appendix VII.

The clinical trial was conducted in accordance with the Good Clinical Practice procedures in force in the Roussel UCLAF Medical Direction.

3.1.1 Study design

This was an open study in healthy female volunteers, RU 38 486 being administered in a single dose.

3.1.2 Subjects

Ten subjects were included in the study. These ten subjects had already participated in the absolute bioavailability study (Protocol CH/86/486/06, report 87/592/CN) for which the inclusion and exclusion criteria were as follows:

Inclusion criteria

i) Exclusively female subjects.
ii) Subjects aged from 20 to 40 years.
iii) Subjects of childbearing age with a regular cycle.
iv) Subjects whose weight did not deviate by more than 10% from the mean weight for their age and height.
v) Subjects whose medical examination was normal or considered as such by the investigator.

This examination comprised:

a) a clinical examination,
b) a laboratory examination,
c) an ECG.

In addition, the subjects had to have one of the following:

a) an IUD in situ for at least 6 months,
b) or tubal ligation,
c) or a sterile partner,
d) or no partner during the study.

Exclusion criteria

i) Subjects using hormonal contraception.
ii) Subjects regularly taking medication.
iii) Subjects having taken part in a clinical trial in the 4 weeks prior to the study.
iv) Subjects having received medication known to be potentially toxic in the 3 months prior to the study.
v) Subjects with a current or previous history of gastro-intestinal, hepatic or renal disease which might interfere with the absorption, distribution, metabolism or excretion of drugs.
vi) Subjects drinking excessive quantities of alcohol or smoking excessively.

The following subjects were also to be excluded:

Subjects with a positive pregnancy test in the 24 hours prior to administration of RU 38 486.

3.1.3 Dosage form

The dosage form of RU 38 486 was a tablet with the following composition:

RU 38 486	200 mg
Colloidal silica	3 mg
Maize starch	102 mg
Polyvidone excipient	12 mg
Microcristalline cellulose	30 mg
Magnesium stearate	3 mg
for a finished weight of	———

Batch RG 21236-12

3.1.4 Treatment

Each subject received one treatment:

3 x 200 mg tablets, i.e. 600 mg of RU 38 486.

3.1.5 Conditions of administration

Treatment was administered at the end of a cycle between the third day before the expected date of menstruation and the second day following the date of onset.

Before treatment the pregnancy test had to be confirmed as negative.

The dose was given in the morning at about 8 a.m., the subjects having fasted overnight (about 10 hours of fasting), and a light meal was served 4 hr after administration.

The 3 tablets were swallowed with 150 ml of water.

The subjects were in the upright position and remained in this position for 2 minutes.

3.1.6 Blood samples

Blood samples (3 ml) were taken by venipuncture and collected on dry lithium heparinate:

a) approximately 0.25 hr before time 0,
b) 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180, 192, 204, 216 and 228 hours after time 0.
Time 0 was taken as the time when the tablets were swallowed.

Each sample was immediately centrifuged cold, the plasma decanted, distributed into two dry tubes and frozen at -20°C until assay.

3.2. Assay methods

3.2.1 Assay of α_1-acid glycoprotein (AAG) in the plasma

α_1-acid glycoprotein was assayed in the 0, 24, 48, 96 and 144 hour samples by ——————— Five µl of plasma were applied to each well of an agarose gel plate containing anti-α_1-acid glycoprotein monospecific antiserum ——————— batch no. 054329). Calibration was done with a range of standard sera ——— from ——————— $g.l^{-1}$. All the assays were performed in duplicate on different plates with a diffusion time of 48 hr. The standards were applied to each plate used.

3.2.2 Assay of RU 38 486 and RU 42 633 in plasma

The assay method is given in full in Appendix I. RU 38 486 and one of its metabolites, RU 42 633, resulting from the loss of a methyl on the nitrogen, were assayed in all the samples.

An internal standard, RU 39 813, was added to the plasma, and RU 38 486, RU 42 633 and the internal standard were then extracted with ethyl acetate. The organic extract was then subjected to ——— chromatography (HPLC) on a ——— 10 µm column with a mixture of acetonitrile and water supplemented with heptane sulphonic acid (PIC B7). The separated products were detected and quantified at the column exit by U.V. densitometry at 304 nm.

A series of analyses was constituted of all the assays to be performed for one subject. For each series two calibration curves were plotted, one for RU 38 486, the other for RU 42 633, by spiking control plasma of increasing, known quantities of each product, corresponding to concentrations ranging from 0.025 to 1.5 $mg.l^{-1}$.

Control plasma, spiked with known quantities of RU 38 486 and RU 42 633 and treated in exactly the same way as the samples, was included, one after the range and two others after the 28 samples of one subject, giving 10 controls for each of the 3 concentrations chosen. The coefficients of variation were as follows:

RU 38 486: 7.1% for 0.082 $mg.l^{-1}$
2.5% for 0.816 $mg.l^{-1}$
2.7% for 1.326 $mg.l^{-1}$

RU 42 633: 6.8% for 0.083 $mg.l^{-1}$
3.0% for 0.832 $mg.l^{-1}$
2.1% for 1.352 $mg.l^{-1}$

The threshold of quantification was set at —— $mg.l^{-1}$. Concentrations below —— $mg.l^{-1}$ were considered to be zero, and concentrations equal to or greater than this were rounded off to the nearest 0.001.

3.3. Pharmacokinetic analysis

The following parameters were chosen for the pharmacokinetic analysis:

3.3.1 Peak plasma concentration, Cmax, in $mg.l^{-1}$

3.3.2 Time to peak plasma concentration, Tmax, in hr.

3.3.3 Area under the curve of the plasma concentrations, AUC, in $mg.l^{-1}.hr.$

Calculated by the trapezoidal rule:

$$AUC = 1/2 \sum (c_n + c_{n-1}) \times (t_n - t_{n-1})$$

3.3.4 Mean residence time, MRT, in hr

Calculated from the equation:

$$MRT = \frac{1/2 \sum (c_n \times t_n + c_{n-1} \times t_{n-1}) \times (t_n - t_{n-1})}{AUC}$$

3.3.5 Elimination half-life, t1/2 in hr

During the terminal elimination phase, the plasma concentrations were plotted semilogarithmically. A mono-exponential function was fitted to the concentrations of this phase from 120 hr onwards, or, if there were more than 6 measurable concentrations after 120 hr the function was fitted to the last 6 concentrations.

Fitting was done by iteration from an initial approximation taking as the criterion the minimisation of the sum of the squares of the weighted deviations between the observed concentrations and those calculated by the function (norm). The initial approximations were obtained by linear regression between the logarithm of the concentrations and the time.

Weighting was proportional to the observed concentration and the corresponding time.

The programme was written by [redacted]

The fitted function was of the form:

$$c = c_1 e^{-\lambda_1 t}$$

c	plasma concentration	$mg.l^{-1}$
c1	coefficient of the exponential term	$mg.l^{-1}$
λ1	apparent elimination rate constant	hr^{-1}
t	time	hr

The half-life was obtained from the equation:

$$t_{1/2} = \frac{\ln 2}{\lambda_1}$$

3.4. Statistical analysis

3.4.1 Plasma concentrations of α_1-acid glycoprotein

The plasma concentrations of α_1-acid glycoprotein were subjected to a 2-way analysis of variance (subject factor and time factor) to check that these concentrations remained constant throughout the study.

The variance was broken down as follows:

Origin	df
Time	4
Subject	9
Residual	36
Total	49

The mean plasma concentrations of α_1-acid glycoprotein measured at times 0, 24, 48, 96 and 144 hr were calculated by subject. These means were considered as individual values and used subsequently.

3.4.2 Comparison of the pharmacokinetic parameters between products

The pharmacokinetic parameters of RU 38 486 and RU 42 633 (Cmax, Tmax, AUC, MRT and t 1/2) were compared with one another and subjected to a 2-way analysis of variance (product effect and subject effect).

The variance was therefore broken down as follows:

Origin	df
Product	1
Subject	9
Residual	9
Total	19

3.4.3 Relationship between the pharmacokinetic parameters and the plasma concentration of α_1-acid glycoprotein

The coefficients of correlation between the concentration of α_1-acid glycoprotein and the pharmacokinetic parameters of RU 38 486 and RU 42 633 were calculated for each treatment.

When the correlation was significant ($p \leq 0.05$), each concentration of α_1-acid glycoprotein was recalculated in terms of the equation of the regression line.

A P P E N D I X I I

PROTOCOL
CASE RECORD FORM
METHOD OF ASSAY OF RU 38.486
LABORATORY DATA PARAMETERS

Protocol number: ZA/84/486/04

Title: STUDY OF TOLERABILITY OF SINGLE DOSES OF RU 486 IN HEALTHY MALE VOLUNTEERS

Investigator: Prof. B.H.MEYER
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2. INTRODUCTION

2.1. Product description

RU 486 is an anti-progesterone and anti-glucocorticoid steroid synthesized by ROUSSEL UCLAF. It is an 11-beta substituted 19-norsteroid.

Pharmacokinetics of tritiated RU 486 have been studied after I.V. administration of a tracer dose (280 mg, 25 uCI) and after oral administration of a pharmacological active dose (100 mg, 50 uCI). In both cases the plasma kinetic curves correspond to an open two-compartment model. After I.V. administration, t1/2 distribution = 1 hr, t1/2 elimination = 12 hrs; volumes of distribution are very low, Vc = 8 l and Vd ss = 26 l. After oral administration, t1/2 distribution = 1 hr and t1/2 elimination = 24 hrs volumes of distribution are higher than previously, Vc, = 45 l and Vd ss = 100 l. The maximum plasma concentration of RU 486, about 2% of the administered dose per liter, is observed one hour after intake of the tablets.

Urinary and fecal excretion reach completion in 6 days and 9% of the administered radioactivity is urinary excreted whatever the route of administration. RU 486 seems to be well absorbed (t max = 1 h and same urinary excretion of radioactivity after I.V. or oral route), however the absolute bicavailability calculated from AUCs is 30 to 50%. This appears to be due to a first past effect as the C max of RU 42633, the N monodemethyl metabolite of RU 486, is observed 1-2 hrs after oral administration and 9 h after I.V. administration. Moreover the AUCs of RU 42633 are higher after oral administration than after I.V. administration.

Further information is available in the Investigator's Brochure.

2.2. Aim of the study

To study the tolerability of the drug in healthy male subjects in doses ranging from 800 to 2.000 mg.

The trial will be performed at the [ ]

3. STUDY DESCRIPTION

- Open study
- Independant groups of 4 subjects for each dose
- Administration of increasing doses
- After administration of each dose, the occurence of unusual symptoms, the results of blood pressure and pulse measurements, Hematology, Clinical Chemistry and urinalysis will be taken into account when deciding whether to proceed to the next higher dose. Should any clinically significant effect(s) be noted, the next higher dose will not be administrated and the tolerability study terminated Hormone plasma levels will not serve as additional indication of whether to proceed with the next higher dose.
- If an undesirable effect appears which may be considered by the investigator as a chance occurrence, the same dose will be repeated in 4 new subjects in a cross-over randomized study versus placebo. If this effect is confirmed but not considered severe enough to stop the study, a smaller increment than what was originally planned may be used for the next dose. This increment will be defined jointly with ROUSSEL UCLAF.

4. SELECTION OF STUDY POPULATION

4.1. Inclusion criteria

Subjects must meet the following criteria:

a- Men between 18 and 45 years of age

b- Body weight not more than 10% above or below their ideal weights for heights and ages

c- Normal findings in the physical examination

d- Normal laboratory values (unless the investigator considers an abnormality clinically unimportant)

e- Normal ECG and vital signs

f- Normal chest x-ray

4.2. Exclusion criteria

a- Regular use of medication, abuse of alcoholic beverages, or participation in a trial with an investigational drug in the 4 weeks preceding the study

b- Treatment within the previous three months with any drug known to have a well defined potential for toxicity to a major organ (e.g. chloramphenicol)

c- A clinically important illness during the 4 weeks preceding the study

d- History of hypersensitivity to any drug

e- History or presence of gastrointestinal, liver or kidney disease, or other conditions known to interfere with the absorption, distribution, metabolism or excretion of drugs

4.3. Subject recruitment

Population from which sample is drawn: Healthy male volunteers recruited at the _____

4.4. Subject numbers

4.4.1. Number per treatment group: 4

4.4.2. total subject number: 16 subjects if all doses are well tolerated

5. DRUG ADMINISTRATION

5.1. Drug dosage

5.1.1. Test drug: RU 486 - Scored tablets of 50 mg

Increasing single doses of 800, 1200, 1600 and 2000 mg will be administered to four new volunteers for each dose, on a weekly basis, provided that the last dose has been tolerated

5.1.2. Placebo tablets (see # 3)

5.1.3. Dosage schedule and route of administration

- Each dose will be administered orally in one single intake, with 500 ml of water over 5 minutes, at 7:30 A.M., after an overnight fasting period of at least twelve hours

5.2. Drug Supplies

5.2.1. Source

RU 486 verum and placebo tablets will be prepared by the Pharmaceutical Department, at Roussel Uclaf

5.2.2. Packaging and labelling

Tablets will be packed in bottles of 200 tablets corresponding to — Tablets of RU 426 VERUM and — Tablets of RU 486 PLACEBO

Eventual randomisation (see # 3) and individual packaging will be performed at ————————

The bottles will carry the following information:

- Number of tablets

- Product identification

RU 486 50 mg tablets
or
RU 486 placebo tablets

- Batch number

5.3. Assignement of study medication

The investigator will be responsible for safe keeping of the study drug. It will be stored according to the prescribed conditions in the Pharmacology Unit, separate from other medicaments.

5.4. Concurrent treatments

5.4.1. Any treatment is forbidden during the study

5.4.2. Statement 5.4.1. is not valid if the use of drugs becomes necessary to protect the health of the subject, because of the occurrence of a pathological event whether this event is due to RU 486 or not

6. CRITERIA OF EVALUATION METHODS

6.1. Clinical criteria

- Medical history and physical examination
- Weight
- Vital signs (supine and standing radial pulse rate, respiratory rate, temperature and supine and standing blood pressure)

6.2. Laboratory examinations:

- Hematological status (hemoglobin, hematocrit, RBC, WBC and differential count, platelet count) and hemostasis parameters (fribrinogenemia, partial thromboplastin time, specific assay of factors X, VII, V, II, euglobulins lysis time)

- Clinical chemistry (glucose, total protein, albumin, globulin, A/G ratio, BUN, creatinine, total bilirubin, alkaline phosphatase, ASAT, ALAT, LDH, calcium, inorganic phosphorus, uric acid, sodium, potassium, chloride, cholesterol, triglycerides, CPK)

- Urinalysis (colour, pH of freshly voided specimens, specific gravity, protein, glucose, ketones, blood and microscopic sediment)

6.3. Hormone examinations

- ACTH
- Cortisol
- Testosterone

measured at 7:30 A.M.

6.4. Other parameters

6.4.1.: Before and 2 hours, 24 hours, 48 hours and 8 days after drug intake: electrocardiogram (standard 12-lead)

6.4.2.: Assay of RU 486 in plasma. 10 ml of blood will be taken 24 hours after drug intake. Frozen plasma will be kept in _____ then forwarded to Roussel Uclaf for assay of RU 486

6.5. Recording of side effects

Before commencement of each phase of the study, each subject will receive a form into which all side effects should be entered hourly up to 6 hours and thereafter 3-hourly up to 36 hours, after medication (except when asleep). As from 12 hours onwards, side effect forms may be completed at home by volunteers.

All adverse events occurring during the study must be reported in the Case Report Forms. A serious life threatening adverse event and/or death due to any cause occurring in a subject participating in this study should be immediately reported to Roussel Uclaf

7. COURSE OF THE STUDY

7.1. Pretreatment observations and investigations

The subject will be screened within two weeks before drug administration for their fitness to participate. This screening will include:

- Clinical examinations listed in # 6.1. and recording of height and weight
- Electrocardiogram (standard 12 lead)
- Chest X-ray if not taken within the last 6 months

7.2. Observations and investigations just before and after dosing

- Subjects will be under monitoring by the Pharmacology Unit for 36 hours. Subsequently, they will have to come for a morning visit at Day 3, 4, 6 and 8. Volunteers must be aware that any kind of stress must be prohibited before coming to the unit

- In each case the dose of RU 486 will be administered orally with 500 ml water over 5 minutes at 7:30 A.M.

- The day of administration is called day 1

- Before commencement of each phase of the study, each subject will receive a form into which all side-effects should be entered hourly up to 6 hours and thereafter 3-hourly up to 36 hours, after medication (except when asleep). As from 12 hours onwards, side-effect forms may be completed at home by volunteers.

- Blood pressure, respiratory rate and pulse rate (see # 6.1.) will be measured before medication and 1/2 hourly up to 3 hours post medication. Thereafter these parameters will be measured hourly up to 6 hours after medication and 12, 24, 48 and 72 hours after medication

- Body temperature will be recorded before medication, 4 and 12 hours after medication, then daily in the morning throughout the study

- Electrocardiogram will be recorded before and two hours, 24 hours, 48 hours and 8 days after medication

- Laboratory examination as listed under # 6.2. will be performed just before and 6 hours, 24 hours and 7 days after drug administration. If a laboratory parameter appears to be abnormal on the 7th-day examination, this parameter will be checked weekly until returned to normal

- ACTH, Cortisol and testosterone will be measured before dosing and at day 2, 3, 4, 6 and 8 at 7:30 A.M. Hormone assays will be performed altogether in one set at the end of the study

- All laboratory examinations including hormone assays will be performed in the

Blood sampling (10 ml) for assay of RU 486 will take place 24 hours after drug intake

9. PROTOCOL DEVIATIONS AND AMENDMENTS

Protocol deviations and amendments, if any, will be dated and described as an appendix to this protocol. See also # 3.

There will be no alteration of the protocol without the express written approval of Roussel Uclaf.

10. SUBJECT DROPOUTS AND WITHDRAWALS

All reasons for drop-outs and withdrawals will be carefully noted in the Case Report Forms.

These subjects will be replaced unless withdrawal is due to an event giving evidence of a major toxicity of the compound. Such an event would lead to stop the study.

11. BIOMETRICS

- Case Report Forms will be checked as soon as completed for correctness and completeness by the investigator

- Appropriate statistical analysis of the data will be performed in the Pharmacology Unit's Statistics Department

- Incomplete observations of drop-outs and withdrawals will be taken into account for the analysis

- A full report will be prepared at the Pharmacology Unit in and then forwarded to Roussel Uclaf

12. PLANNING

12.1. Agreements and consents

12.1.1. Ethical Committee

In accordance to Government regulations, the appropriate Ethical Committee or Institutional Review Board must review and approve this prototol

12.1.2. Informed consent of subjects

All subjects will give their written informed consent prior to commencement of the study. It will be made clear to the subjects that they have the right to discontinue their participation at any time and without explaining the reasons why.

12.1.3. Confidentiality

All data is the property of Roussel Uclaf and must not be communicated to third parties without the express written permission of Direction Médicale Roussel Uclaf.

12.1.4. Publication

The results of this study are not intended for publication

12.1.5. In performing this study, both the investigator and the sponsor endorse, as a minimum, the standards for conduct of Clinical Research activities as set forth in the Declaration of Helsinki

12.2. Time Table

12.2.1. Duration of study: Ca 3 months

12.2.2. Target dates: Start: Q4 1984
Finish: Q1 1985
Report: After statistical analysis is available

12.3. Study monitoring by Roussel Uclaf

This study will be monitored by Roussel Uclaf Clinical Research personnel at regular stages of its development by personal visits and telephone communications

12.4. Study termination

At the end of the study, the remaining unutilised tablets will be forwarded back to Roussel Uclaf

12.5. Signature of Chief Investigator

Professor B.H. MEYER

Date:

CASE RECORD FORM

Study number: ZA/84/486/04

TOLERANCE OF RU 486

Trial conducted in Clinical Pharmacology Research Unit,
Department of Pharmacology, _____

_____

APPEARS THIS WAY
ON ORIGINAL

Subject: Surname:

Name:

Initials:

Dose of RU 486 administered (mg) [ ]

Study number: ZA/84/486/04 Page: 1

Subject Number: [ ]

PATIENT IDENTIFICATION:

Surname: _____ Name: _____

Sex: _____ Weight (kg): _____

Height (cm): _____ Age (years): _____

Occupation: _____

CONSENT OBTAINED?

Yes [ ]

TOBACCO CONSUMPTION:

None [ ] Cigarettes [ ] Cigars [ ] Pipe [ ]

Quantity per day: ____ ____ ____

ALCOHOL CONSUMPTION:

None [ ] Beer [ ] Wine [ ] Hard liquor [ ]

Quantity per day: ____ ____ ____

DRUG CONSUMPTION (REGULAR)

None [ ]

Medication:	1.	2. .....
Daily dose:	.....	.....
Date started:	.....	.....
Date completed:	.....	.....

DRUG CONSUMPTION (OCCASIONAL)

None [ ]

Medication:	1.	2. .....
Daily dose:	.....	.....
Date started:	.....	.....
Date completed:	.....	.....

Surname of subject: Subject Number: [ ]

PARTICIPATION IN TRIAL WITH INVESTIGATIONAL DRUG:

Yes [ ] No [ ]

If yes, date of last trial:

drug involved:

HISTORY OF ALLERGY:

Yes [ ] No [ ]

If yes, details: .....

HISTORY OF HYPERSENSITIVITY TO DRUGS:

Yes [ ] No [ ]

If yes, details: .....

HISTORY OF DISEASE:

Yes [ ] No [ ]

If yes, details: .....

HISTORY OF SURGERY:

Yes [ ] No [ ]

If yes, details: .....

COMMENTS:

INVESTIGATOR'S SIGNATURE:

Surname of subject: _____ Subject Number: [ ]

Date of examination: _____

PHYSICAL EXAMINATION BEFORE TRIAL

Pulse rate (beats/minute) (supine): .....

Blood pressure (mmHg) (supine): systolic: .....

diastolic: .....

	Normal	Abnormal	Not done	Comments
Head + neck	[ ]	[ ]	[ ]	.....
Eyes	[ ]	[ ]	[ ]	.....
Ears	[ ]	[ ]	[ ]	.....
Nose	[ ]	[ ]	[ ]	.....
Throat	[ ]	[ ]	[ ]	.....
Lungs	[ ]	[ ]	[ ]	.....
Heart	[ ]	[ ]	[ ]	.....
Breasts	[ ]	[ ]	[ ]	.....
Abdomen	[ ]	[ ]	[ ]	.....
Extremities	[ ]	[ ]	[ ]	.....
Lymph nodes	[ ]	[ ]	[ ]	.....
Skin	[ ]	[ ]	[ ]	.....
ECG	[ ]	[ ]	[ ]	.....

ADDITIONAL COMMENTS:

INVESTIGATOR'S SIGNATURE:

Surname of subject: _____ Subject Number: [ ]

CLINICAL EXAMINATION

	Before medication	6 Hours after medication	24 Hours after medication
Head + neck	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Eyes	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Ears	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Nose	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Throat	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Lungs	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Heart	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Breasts	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Abdomen	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Extremities	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Lymph nodes	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Skin	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
ECG	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]	norm. [ ] abn. [ ]
Weight (kg):	.....	.....	

COMMENTS:

INVESTIGATOR'S SIGNATURE:

Study number: ZA/84/486/04 Page: 5

Surname of subject: _____ Subject Number: [ ]

HEMATOLOGY + URINALYSIS

	Before med.	6 hours after med.	24 hours after med.	168 hours after med.
Leucocytes				
R.B.C.				
Hemoglobin				
Hematocrit				
G.K.V.				
G.K.H.				
G.K.H.K.				
Platelets				
Sedimentation rate				
Reticulocytes				
Neutrophils				
Eosinophils				
Basophils				
Lymphocytes				
Monocytes				
Prothrombin time				
Fibrinogen				
Factor II				
Factor V				
Factor VII				
Factor X				
Euglobulin lysis time				
URINALYSIS:				
pH				
S.G.				
Abnormalities				

Surname of subject: _____ Subject Number: [ ]

CLINICAL CHEMISTRY

	Before med.	6 hours after med.	24 hours after med.	168 hours after med.
Sodium				
Potassium				
Chloride				
CO_2				
Urea				
Creatinine				
Urate				
Calcium				
Phosphates				
Proteins				
Albumin				
Tot. Bilirubin				
Conj. Bilirubin				
ALP				
G-GT				
AST				
ALT				
LD				
Cholesterol				
Triglycerides				
Glucose				
CPK				

Surname of subject: _____ Subject Number: [ ]

	ACTH (pg/mℓ)	CORTISOL (nmol/ℓ)	TESTOSTERONE (nmol/ℓ)
Before medication	_____	_____	_____
24 hrs. after medication	_____	_____	_____
48 hrs. after medication	_____	_____	_____
72 hrs. after medication	_____	_____	_____
120 hrs. after medication	_____	_____	_____
168 hrs. after medication	_____	_____	_____

ECG ABNORMALITIES

	NORMAL	ABNORMAL
Before medication	[ ]	[ ]
2 hrs. after medication	[ ]	[ ]
24 hrs. after medication	[ ]	[ ]
48 hrs. after medication	[ ]	[ ]
192 hrs. after medication	[ ]	[ ]

DESCRIPTION OF ABNORMALITIES:

INVESTIGATOR'S SIGNATURE:

Study number: ZA/84/486/04 Page: 8

Surname of subject: _____ Subject Number: [ ]

SIDE-EFFECTS

	NO	YES	IF YES, DETAILS
1 Hour after medication	[ ]	[ ]	_____
2 hours after medication	[ ]	[ ]	_____
3 hours after medication	[ ]	[ ]	_____
4 hours after medication	[ ]	[ ]	_____
5 hours after medication	[ ]	[ ]	_____
6 hours after medication	[ ]	[ ]	_____
9 hours after medication	[ ]	[ ]	_____
12 hours after medication	[ ]	[ ]	_____
15 hours after medication	[ ]	[ ]	_____
18 hours after medication	[ ]	[ ]	_____
21 hours after medication	[ ]	[ ]	_____
24 hours after medication	[ ]	[ ]	_____
27 hours after medication	[ ]	[ ]	_____
30 hours after medication	[ ]	[ ]	_____
33 hours after medication	[ ]	[ ]	_____
36 hours after medication	[ ]	[ ]	_____

COMMENTS:

INVESTIGATOR'S SIGNATURE:

Study number: ZA/84/486/04

Surname of subject: _____ Subject Number: [ ]

	Blood Pressure (mmHg)	Respiration rate/minute	Pulse rate beats/min.	Temperature (°C)
Before medication				
30 min. after med.				
1 hr. after med.				
1½ hr. after med.				
2 hrs. after med.				
2½ hrs. after med.				
3 hrs. after med.				
4 hrs. after med.				
5 hrs. after med.				
6 hrs. after med.				
12 hrs. after med.				
24 hrs. after med.				
48 hrs. after med.				
72 hrs. after med.				

COMMENTS:

INVESTIGATOR'S SIGNATURE:

RADIOIMMUNOASSAY OF RU 486

Centre de Recherches Roussel-Uclaf
93230 Romainville, France

SUMMARY

A rapid and sensitive radioimmunoassay for RU 486 has been developed. The straightforward assay procedure is described in detail.

An antigen was prepared by coupling bovine serum albumin with the 3-carboxymethyloxime of RU 486, and an anti RU 486 antiserum was produced in rabbits. Its specificity for 50% inhibition of maximum binding, is reported in Table I.

Sensitivity is about 10 pg/assay tube, and 100 pg of RU 486 reduce maximum binding by half its value. Non specific binding, measured with excess unlabelled RU 486 (100 ng), represents 4% of the added radioactivity. Using labelled RU 486, the recovery of radioactivity after diethyl ether extraction is 89.3 ± 1.3% (n=9).

REAGENTS

A phosphate buffer containing 0.1% gelatin was prepared by dissolving 9 g NaCl, 1 g sodium azide and 1 g gelatin in 1 liter of 0.1M phosphate buffer, pH 6.9. It was stored at +4°C.

The anti-RU 486-3carboxymethyloxime-BSA antiserum was raised in New Zealand rabbits using methods previously described by Raynaud et al. (1974). It had been stable for several months at 4°C when diluted 1/100 in phosphate-gelatin buffer.

A stock solution of RU 486 at 0.1 mg/ml ethanol was stored at 4°C. For the standard curve, solutions of RU 486 were prepared just before use by dilution in the phosphate-gelatin buffer supplemented with 0.025% Triton X 100. They contain, respectively, 0, 3.91, 7.81, 15.63, 32.25, 62.5, 125, 250, 500, 1000 and 2000 pg/0.1 ml.

Tritiated RU 486 (specific activity = 37 Ci/mmole), stored at 4°C in ethanol, was diluted at the time of use in the phosphate-gelatin-Triton X 100 buffer at a concentration of 25,000 cpm per ml.

The charcoal suspension was composed of 250 mg charcoal —— and 25 mg dextran T70 —— for 100 ml of phosphate buffer without gelatin. Scintillation fluid was ——

Table I - Percentage of Cross-Reaction of Various Ster
with the Anti-RU 486 Antiserum (in the RIA

RU 486	100
N-Didemethyl RU 486	84
N-Monodemethyl RU 486	60
Propargyl Alcohol RU 486	0.8
Progesterone	< 0.0
Testosterone	< 0.0
Cortisol	< 0.0
Desoxycorticosterone	< 0.0
17 β-Estradiol	< 0.0
Estrone	< 0.0
Estriol	< 0.0
Dexamethasone	< 0.0

ASSAY PROCEDURE

The standard curve ranged from ——— ng/ml (Fig. 1
samples had to be diluted 1/200 to 1/4000 when 100 mg of RU
administered to humans. Plasmas were diluted in phosphate-
supplemented with 0.025% Triton X 100. 0.1 ml of these dil
of standard solutions were added, in triplicate, to 0.4 ml
486 was extracted with 3.0 ml diethyl ether from a freshly
80 x 13 mm glass hemolysis tubes, using a ——— .

After four minutes of agitation, the aqueous phases were
methanol-dry ice bath. The ether phases were decanted in 6
hemolysis tubes and evaporated in a 40°C water bath. The re
taken up in 0.2 ml of the tritiated RU 486 solution (5,000
and allowed to stand 30 minutes at room temperature before
ml of antiserum (batch 655) diluted 1/100,000 in the phospha
gelatin buffer. After an overnight incubation at 4°C, 0.75
dextran-coated charcoal suspension was added to each tube.
minutes of incubation, the charcoal was pelleted at 3,000 rp

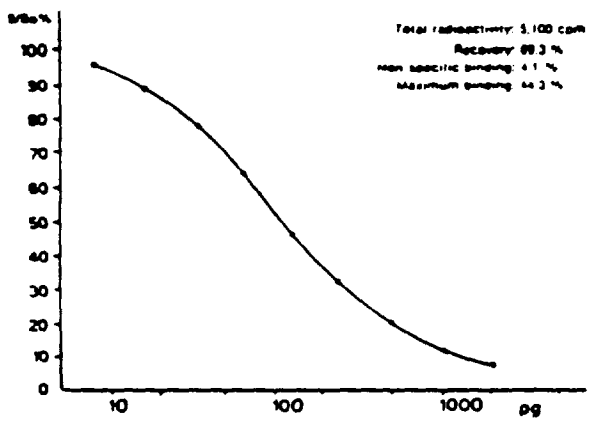

Fig. 1. RU 486 radioimmunoassay standard curve. RU 486 was
extracted, the final antiserum dilution was 1/125,0
separation of the unbound was obtained by dextran-co

minutes in a cooling centrifuge. Finally, supernatants were transferred to polyethylene counting vials and mixed with 10 ml of scintillation fluid for two minutes before they were counted.

REFERENCE

Raynaud, J. P., Azadian-Boulanger, G., and Bucourt, R., 1974, Anticorps spécifiques de l'estradiol, J. Pharmacol.(Paris), 5:27.

Laboratory investigations :

Definition of normal laboratory range (N.L.R.), predefined changes (P.D.C.) and extended range

HEMATOLOGY

	Units	N.L.R.	P.D.C.	Extended range
Erythrocytes	mill/cmm	4.5 - 6.5	decrease of 1 mill	3.85 - 7.15
Hemoglogin	g/dl	13.5 - 18.5	decrease of 2 g	11.65 - 20.35
Hematocrit	%	40 - 54	decrease of 5 %	34.6 - 59.4
Mean corpuscular volume (MVC)	fl	76 - 96	-	-
Mean corpuscular hemoglobin (MCH)	pg	27 - 32	-	-
Mean corpuscular hemoglobin concentration (MCHC)	%	31 - 35	-	-
Reticulocytes	mill/cmm	0.01 - 0.1	-	-
E.S.R.	mm 1st hour	0 - 5	increase of 10 mm	0 - 10
Leucocytes	thous/cmm	4 - 11	decrease of 2 thous	-
Neutrophils	thous/cmm	1.8 - 7.5	decrease or increase of 2 thous	1.05 - 8.25
Eosinophils	thous/cmm	0.04 - 0.45	decrease or increase of 0.25 thous	0 - 0.90
Basophils	thous/cmm	0.01 - 0.10	decrease or increase of 0.24 thous	0 - 0.2
Lymphocytes	thous/cmm	1.50 - 4.00	decrease or increase of 1 thous	0.5 - 5
Monocytes	thous/cmm	0.2 - 0.80	decrease or increase of 0.4 thous	0 - 1.6
Platelets	thous/cmm	150 - 400	decrease of 100 thous	50 - 500

Laboratory investigations :

Definition of normal laboratory range (N.L.R.), predefined changes (P.D.C.) and extended range

BIOCHEMISTRY

	Units	N.L.R.	P.D.C.	Extended rang
Sodium	mmol/l	136 - 147	increase or decrease of 8 mmol	-
Potassium	mmol/l	3.7 - 5.1	increase or decrease of 0.75 mmol	-
Chloride	mmol/l	98 - 108	increase or decrease of 5 mmol	-
Carbone dioxide	mmol/l	19 - 28	increase or decrease of 8 mmol	-
Urea	mmol/l	2.5 - 6.7	increase of 2.9 mmol	0.82 - 8.37
Creatinine	umol/l	60 - 110	increase of 40 umol	49 - 121
Urate	mmol/l	0.18 - 0.45	increase of 0.12 mmol	-
Calcium	mmol/l	2.20 - 2.60	increase or decrease of 0.5 mmol	1.9 - 2.9
Phosphate	mmol/l	0.80 - 1.45	increase or decrease of 0.43 mmol	0.66 - 1.59
Proteins	g/l	65 - 80	increase or decrease of 15 g	57 - 88
Albumin	g/l	38 - 52	increase or decrease of 7.5 g	33 - 57
Total bilirubin	umol/l	4 - 21	increase or decrease of 8 umol	0 - 31
Conjugated bilirubin	umol/l	1 - 4	-	-
Cholesterol	mmol/l	3.9 - 6.5	increase or decrease of 2 mmol	3.25 - 7.15
Glucose	mmol/l	3.6 - 5.8	increase or decrease of 1.5 mmol	2.7 - 7.2

Laboratory investigations :

Definition of normal laboratory range (N.L.R.), predefined changes (P.D.C.) and extended range

	Units	N.L.R.	P.D.C.	Extended range
COAGULATION TESTS				
Prothrombine time	%	80 - 100	decrease or increase of 20 %	70 - 110
Fibrinogen	mg/l	150 - 400	-	-
Factor II	%	50 - 150	-	-
Factor V	%	50 - 150	-	-
Factor VII	%	50 - 150	-	-
Factor X	%	50 - 150	-	-
Euglobulin lysis time	Sec.	$\geq$ 60	-	-
URINALYSIS				
pH	-	4.6 - 8	-	-
specific gravity	-	1005 - 1030	-	-
ENZYMOLOGY				
Alkaline phosphatase	IU/l	25 - 100	increase of 100 IU	0 - 125
GT	IU/l	5 - 65	increase of 65 IU	0 - 70
A.S.A.T.	IU/l	5 - 40	increase of 40 IU	0 - 80
A.L.A.T.	IU/l	5 - 35	increase of 35 IU	0 - 70
L.D.H.	IU/l	100 - 350	increase of 350 IU	65 - 385
C.P.K.	IU/l	15 - 130	increase of 130 IU	0 - 156

1 INTRODUCTION

RU 38 486 (INN: mifepristone) in pharmacological tests in vitro and in vivo has exhibited potent antiprogesterone and antiglucocorticoid activities, together with a not inconsiderable anti-androgenic activity.

Its relative binding affinity is 5 times greater than that of progesterone for the progestogen receptor and 3 times greater than that of dexamethasone for the glucocorticoid receptor. Its affinity for the androgen receptor is moderate, while at the same time it does not exhibit any progestomimetic, glucocorticoid or androgenic agonist activity.

Its tolerance has been studied in healthy male and female volunteers after single doses of up to 2000 mg. RU 38 486 was perfectly tolerated.

The variations in its plasma concentrations have been studied in healthy female volunteers after single doses of 50, 150 and 450 mg.

Its activity is currently under study in women wishing to have a termination of pregnancy, in single doses of up to 600 mg.

2 AIM OF STUDY

The aim of this study was to compare the bioavailability of RU 38 486 administered in the form of tablets containing 50 mg with the same formula but with a different raw material or manufacturing process.

3 MATERIAL AND METHOD

3.1 PROTOCOL

The complete protocol is given in Appendix V.

3.1.1 Study design

This was an open, randomised, crossover study in healthy male volunteers with RU 38 486 administered in a single dose.

3.1.2 Subjects

Twelve subjects were included in the study.

Inclusion criteria

The following subjects were included:

i) Exclusively male subjects.
ii) Subjects aged from 18 to 40 years.
iii) Subjects whose weight did not deviate by more than 10% from the average weight for their age and height.
iv) Subjects whose medical examination was normal or considered such by the investigator. This examination comprised:
    a) a clinical examination
    b) laboratory investigations
    c) an ECG.

Exclusion criteria

The following subjects were excluded:

i) Subjects taking a drug on a regular basis.
ii) Subjects who had taken part in a clinical trial in the 4 weeks prior to the study.
iii) Subjects who had been treated with a drug known to be potentially toxic in the 3 months prior to the study.
iv) Subjects with a history of allergy or hypersensitivity to drugs.
v) Subjects having presented or presenting with a gastro-intestinal, hepatic or renal disease which might interfere with the absorption, distribution, metabolism or excretion of drugs.
vi) Subjects who were heavy drinkers or smokers.

3.1.3 Dosage forms

RU 38 486 was administered in the form of tablets containing 50 mg of RU 38 486.

The formula of these tablets was as follows:

RU 38 486	50 mg
Polyvidone excipient	——
Lactose	——
Maize starch	——
Magnesium stearate	——

The difference between the tablets related to the source of the active ingredient and the wetting process:

Reference RG 20 780-152
Batch of active ingredient used: batch 36 non-micronised.
Wetting with purified water.

Reference RG 20 780-153
Batch of active ingredient used: batch 37 micronised.
Wetting with purified water.

Reference RG 20 780-140-1
Batch of active ingredient used: batch 31 micronised.
Wetting with purified water.

Reference RG 20 780-140-2
Batch of active ingredient used: batch 31 micronised.
Wetting with 50% V/V ethanol.

The dissolution rate of these tablets was measured in vitro at 37°C in 0.01 N hydrochloric acid by the method of the USP. The results are given in Appendix I.

3.1.4 **Treatments**

Each subject received 4 treatments:

A 1 x 50 mg tablet referenced RG 20 780-152
B 1 x 50 mg tablet referenced RG 20 780-153
C 1 x 50 mg tablet referenced RG 20 780-140-1
D 1 x 50 mg tablet referenced RG 20 780-140-2

3.1.5 Conditions of administration

The treatments were allocated by a randomisation plan.

The interval between 2 treatments was 1 week.

Treatments were administered in the morning, after an overnight fast of at least 10 h. The tablet was swallowed with 150 ml of water, with the subject in the upright position and remaining standing for at least 5 minutes.

The subjects also drank 200 ml of water in the 4 hours after administration. A meal was served after 4 hours.

3.1.6 Blood samples

Blood samples were taken by venipuncture and collected on dry lithium heparinate immediately after administration, and then 0.25, 0.50, 0.75, 1, 1.5, 2, 2.5, 3, 4, 8, 12, 24, 48 and 72 hours after administration.

Each sample was centrifuged cold immediately, the plasma decanted and distributed into 2 dry tubes and then frozen at -20°C until assay.

3.2 ASSAY METHOD OF RU 38 486 AND RU 42 633 IN PLASMA

The complete assay method is given in Appendix II. RU 38 486 and one of its metabolites, RU 42 633, resulting from the loss of a methyl on the nitrogen, were assayed in all the samples.

An internal standard, RU 39 813, was added to the plasma and then RU 38 486, RU 42 633 and the internal standard were extracted with ethyl acetate.

The organic extract was chromatographed by ——— high pressure liquid chromatography on a ——— 10 µm column, with a mixture of acetonitrile/water supplemented with heptane sulphonic acid (Pic B7).

The separated products were detected and quantified at the column exit by U.V. densitometry at 304 nm. A series of analyses was constituted from all the assays to be performed for 2 treatments of a single subject.

For each series of analyses, 2 calibration curves were established, 1 for RU 38 486, the other for RU 42 633, by spiking control plasma with increasing known quantities of each product, equivalent to concentrations ranging from 0.05 to 2 $mg.l^{-1}$.

Control plasma, spiked with known quantities of RU 38 486 and RU 42 633 and treated exactly like the samples, were included every 15 samples, i.e. 24 controls for each of the 3 concentrations chosen. The coefficients of variation were as follows:

RU 38 486: 15% for 0.082 $mg.l^{-1}$, 3.5% for 0.612 $mg.l^{-1}$ and 5% for 1.632 $mg.l^{-1}$.

RU 42 633: 14% for 0.086 $mg.l^{-1}$, 5% for 0.648 and 1.728 $mg.l^{-1}$.

The threshold of quantification was set at —— $mg.l^{-1}$. Concentrations of less than —— $mg.l^{-1}$ were considered to be zero and concentrations equal to or greater than 0.001 $mg.l^{-1}$ were rounded up to the nearest 0.001.

3.3 PHARMACOKINETIC ANALYSIS

The following parameters were adopted for the pharmacokinetic analysis of each product assayed:

3.3.1 Peak plasma concentration, C_{max}, in $mg.l^{-1}$

3.3.2 Time to peak plasma concentration, T_{max}, in h

3.3.3 Area under the curve of the plasma concentrations, AUC in $mg.l^{-1}.h$

Calculated by the trapezoidal rule

$$AUC = \sum \frac{1}{2} (c_n + c_{n-1}) \times (t_n - t_{n-1})$$

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3 - MATERIAL AND METHODS

3.1 PROTOCOL

The complete protocol is given in Appendix VI.

3.1.1 Study design

This was an open, randomised, cross-over study in a Latin square design in healthy male volunteers with RU 38486 administered in a single dose.

3.1.2 Subjects

Eight healthy subjects were included in the study.

Inclusion criteria: the following were eligible for inclusion:

i) Exclusively male subjects
ii) Subjects aged from 18 to 40 years
iii) Subjects whose weight did not deviate by more than 15% from the average weight for the subject's age and height
iv) Subjects whose medical examination was normal or considered such by the investigator. This examination involved a clinical examination, laboratory investigations, an ECG and a chest X-ray.

Exclusion criteria: the following were excluded:

i) Subjects who had suffered from an acute disease in the 3 months prior to the study
ii) Subjects regularly taking medication or having received medication in the 2 weeks prior to the study (3 months if this involved medication known to be potentially toxic)
iii) Subjects who had taken part in a clinical trial in the 3 months prior to the study
iv) Subjects with a history of allergy or hypersensitivity to drugs
v) Subjects with a current or previous history of gastro-intestinal, hepatic or renal disease which might interfere with the absorption, distribution, metabolism or excretion of drugs
vi) Heavy drinkers or smokers.

3.2 ASSAY METHOD OF RU 38486 AND RU 42633 IN PLASMA

The complete assay method is given in Appendix II. RU 38486 and one of its metabolites, RU 42633, resulting from the loss of a methyl on the nitrogen, were assayed in all the plasma samples.

An internal standard, RU 39813, was added to the plasma and RU 38486, RU 42633 and the internal standard were then extracted with ethyl acetate. The organic extract was chromatographed by reverse-phase high pressure liquid chromatography on a ——— 10 µm column with a mixture of acetonitrile/water, supplemented with heptane sulphonic acid (Pic B7).

The separated products were detected and quantified at the column exit by UV densitometry at 304 nm.

A series of analyses was constituted from all the assays to be performed for two treatments of a single subject.

For each series of analyses, calibration standards were obtained for RU 38486 and RU 42633 by spiking control plasma with known, increasing quantities of each compound, equivalent to concentrations ranging from ——— $mg.l^{-1}$.

Control plasma, spiked with known quantities of RU 38486 and RU 42633 and treated exactly like the samples, was included approximately every 10 samples, giving 18 controls for each of the 3 concentrations chosen. The coefficients of variation were as follows:

- RU 38486: 9.4% for 0.080 $mg.l^{-1}$, 6.3% for 0.318 $mg.l^{-1}$ and 8.4% for 1.696 $mg.l^{-1}$.
- RU 42633: 7.6% for 0.075 $mg.l^{-1}$, 5.5% for 0.300 $mg.l^{-1}$ and 6.1% for 1.600 $mg.l^{-1}$.

The threshold of quantification was set at —— $mg.l^{-1}$. Concentrations below —— $mg.l^{-1}$ were considered to be zero, and concentrations equal to or greater than this were rounded up to the nearest 0.001.

3.3 PHARMACOKINETIC ANALYSIS

The following parameters were adopted for the pharmacokinetic analysis for each product assayed:

3.3.1 Peak plasma concentration, Cmax, in $mg.l^{-1}$

3.3.2 Time to peak plasma concentration, Tmax, in h

3.3.3 Area under the curve of the plasma concentrations, AUC, in $mg.l^{-1}.h$

Calculated by the trapezoidal rule.

3.3.4 Mean residence time, MRT, in h, calculated from:

$$MRT = \frac{1/2\sum(c_n x t_n + c_{n-1} x t_{n-1}) x (t_n - t_{n-1})}{AUC}$$

3.3.5 Elimination half-life: t 1/2, in h

Semilogarithmic plot of plasma concentration against time showed a straight line during the terminal elimination phase. A mono-exponential function was fitted to the concentrations of this phase from 48 hours until the last measured (96 hours) or measurable concentration.

Fitting was done by computer by iteration from an initial approximation, taking as the criterion the minimisation of the sum of the squares of the weighted differences between the observed concentrations and those calculated by the function. The initial approximations were obtained by linear regression of the logarithm of the concentrations against time. Weighting was proportional to the observed concentration and the appropriate time.

The programme was written by [redacted] The fitted function was of the form:

$$c = c1\ e^{-\lambda 1 t}$$

c :	plasma concentration	$mg.l^{-1}$
c1:	coefficient of the exponential term	$mg.l^{-1}$
λ1:	apparent elimination rate constant	h^{-1}
t :	time	h

The half-life was obtained from the equation:

$$t_{\frac{1}{2}} = \frac{\ln 2}{\lambda 1}$$

3.4 STATISTICAL ANALYSIS

The mean and standard error of the mean were calculated for:
a) the subjects' physical characteristics (weight, height, age)
b) the plasma concentrations by time for each product assayed.

The pharmacokinetic parameters for each product were subjected to a 3-way analysis of variance with calculation of the mean, variance and standard error of the mean of the parameters for each factor.

The factors were the treatment effect, the subject effect and the period effect.

The variance was therefore broken down as follows:

Origin	df
Treatment	3
Subject	7
Period	3
Residual	18
Total	31

Where the analysis of variance showed a significant treatment effect, the means of the parameters for each treatment were compared with one another by Tukey's t test using the residual variance of the analysis of variance.

Westlake's confidence interval for the Cmax, AUC and MRT obtained after treatments B, C and D (tablets) was calculated using the residual variance of the analysis of variance and taking each tablet in turn as the reference.

A P P E N D I X V I

P R O T O C O L