(Official use only)

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14. **Pharmacological particulars:**

MIFEPRISTONE IS A SYNTHETIC STEROID WITH AN ANTIPROGESTATIONAL ACTION AS A RESULT OF COMPETITION WITH PROGESTERONE AT THE PROGESTERONE RECEPTORS.

AT DOSES RANGING FROM 3 TO 10 MG/KG ORALLY, IT INHIBITS THE ACTION OF ENDOGENOUS OR EXOGENOUS PROGESTERONE IN DIFFERENT ANIMAL SPECIES (RAT, MOUSE, RABBIT AND MONKEY). THIS ACTION IS MANIFESTED IN THE FORM OF PREGNANCY TERMINATION IN RODENTS.

IN WOMEN AT DOSES OF GREATER THAN OR EQUAL TO 1 MG/KG, MIFEPRISTONE ANTAGONISES THE ENDOMETRIAL AND MYOMETRIAL EFFECTS OF PROGESTERONE. DURING PREGNANCY IT SENSITISES THE MYOMETRIUM TO THE CONTRACTION-INDUCING ACTION OF PROSTAGLANDINS.

MIFEPRISTONE BINDS TO THE GLUCOCORTICOID RECEPTOR. IN ANIMALS AT DOSES OF 10 TO 25 MG/KG IT INHIBITS THE ACTION OF DEXAMETHASONE. IN MAN THE ANTIGLUCOCORTICOID ACTION IS MANIFESTED AT A DOSE EQUAL TO OR GREATER THAN 4.5 MG/KG BY A COMPENSATORY ELEVATION OF ACTH AND CORTISOL.

MIFEPRISTONE HAS A WEAK ANTI-ANDROGENIC ACTION WHICH ONLY APPEARS IN ANIMALS DURING PROLONGED ADMINISTRATION OF VERY HIGH DOSES.

15. **Pharmacokinetic particulars:**

AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 600 MG THE PEAK CONCENTRATION OF 1.98 MG/1 IS REACHED AFTER 1.30 HOURS (MEAN OF 10 SUBJECTS). THE ABSOLUTE BIOAVAILABILITY IS 69%.

IN PLASMA MIFEPRISTONE IS 98% BOUND TO PLASMA PROTEINS: ALBUMIN AND PRINCIPALLY ALPHA-1-ACID GLYCOPROTEIN, TO WHICH BINDING IS SATURABLE.

AFTER A DISTRIBUTION PHASE, ELIMINATION IS AT FIRST SLOW, THE CONCENTRATION DECREASING BY A HALF BETWEEN ABOUT 12 AND 72 HOURS, AND THEN MORE RAPID, GIVING AN ELIMINATION HALF-LIFE OF 18 HOURS.

TWO PRIMARY METABOLIC PATHWAYS HAVE BEEN DEMONSTRATED: N-DEMETHYLATION AND TERMINAL HYDROXYLATION OF THE 17-PROPYNYL CHAIN.

AFTER ADMINISTRATION OF THE SAME LABELLED DOSE, 10% OF THE TOTAL RADIOACTIVITY IS ELIMINATED IN THE URINE AND 90% IN THE FAECES.

DEPARTMENT OF HEALTH

Date: 25th SEPTEMBER 1990

(Official use only)

16. Name(s) of manufacturers and site(s) of manufacture of (a) the active substance(s) and (b) the dosage form.

(a) The active substance(s)	(b) The dosage form
ROUSSEL UCLAF 102 ROUTE DE NOISY 93230 ROMAINVILLE FRANCE	ROUSSEL LABORATORIES LTD SWINDON, WILTSHIRE AND ROUSSEL UCLAF, ROUTE DE CHOISY-AU-BAC, 60205 COMPIEGNE, FRANCE

17. Assembler(s):	18. Importer:
ROUSSEL LABORATORIES LTD SWINDON, UK AND ROUSSEL UCLAF, COMPIEGNE, FRANCE	(OF ACTIVE SUBSTANCE AND DOSAGE FORM) ROUSSEL LABORATORIES LTD, BROADWATER PARK, NORTH ORBITAL RD DENHAM, UXBRIDGE, MIDDX, UB9 5HP

19. Site and arrangements for quality control:

QUALITY CONTROL LABORATORIES AT:

ROUSSEL LABORATORIES LTD
KINGFISHER DRIVE, COVINGHAM
SWINDON, WILTSHIRE

AND

ROUSSEL UCLAF
COMPIEGNE, FRANCE

20. Distributor (where applicable)

ROUSSEL LABORATORIES LTD
BROADWATER PARK, NORTH ORBITAL ROAD
DENHAM, UXBRIDGE, MIDDLESEX, UB9 5HP

21. List other countries of registration:

FRANCE, CHINA.

Date: 25th SEPTEMBER 1990

DEPARTMENT OF HEALTH

DEPARTMENT OF HEALTH

MEDICINES ACT 1968

Product Licence No: 16152/0001

SCHEDULE

FURTHER PROVISIONS SUBJECT TO WHICH THE LICENCE HAS BEEN GRANTED

1. All the provisions of Part I of Schedule 1 of the Medicines (Standard Provisions for Licences and Certificates) Regulations 1971 (SI 1971 No 972) and any provision of those regulations as amended by the Medicines (Standard Provisions for Licences and Certificates) Amended Regulations 1972 (SI 1972 No 1226), the Medicines (Standard Provisions for Licences and Certificates) Amendment Regulations 1974 (SI 1974 No 1523) and the Medicines (Standard Provisions for Licences and Certificates) Amendment Regulations 1977 (SI 1977 No 1039) shall apply.

2. If the product consists wholly or partly of antigens, antitoxins, sera, antisera, toxins or vaccines within the meaning of the Medicines (Exportation of Specified Products for Human Use) Order 1971 (SI 1971 No 1198) then, in addition, the Medicines (Standard Provisions for Licences and Certificates) Regulations 1971 (SI No 972) as amended by the Medicines (Standard Provisions for Licences and Certificates) Amendment Regulations 1977 (SI 1977 No 675) shall also apply.

3. Leaflets issued with proprietary medicinal products shall comply with the requirements of the Medicines (Leaflets) Regulations 1977 (SI 1977 No 1055). Labels of medicinal products shall comply with the Medicines (Labelling) Regulations 1976 (SI 1976 No 1726) as amended by the Medicines (Labelling) Amendment Regulations 1977 (SI 1977 No 996), the Medicines (Labelling) Amendment Regulations 1981 (SI 1981 No 1791) and the Medicines (Labelling) Amendment Regulations 1985 (SI 1985 No 1558).

4. The product(s) shall not be recommended to be used for any purposes other than those specified in Part 1 of this Schedule as Clinical Indications.

5. The product(s) shall be manufactured only in accordance with the information submitted to the licensing authority in connection with the application for this product licence or any variation of this licence which is approved by the licensing authority: this applies in particular to information relating to -
   a. the specification of the constituents of the product
   b. the specification of the finished product
   c. the method of the manufacture of the product and
   d. the person(s) specified as the manufacturer of the product

DIRECTION AS TO REPORTING OF SUSPECTED ADVERSE REACTIONS

Marketing Authorisation Number	**16152/0001**
Authorised to	**Exelgyn**
In respect of	**Mifegyne** **Mifepristone Tablets 200mg**

1. In pursuance of the obligations laid down in the Medicines For Human Use (Marketing Authorisations, etc.) Regulations 1994 (SI 1994 No 3144) and the relevant community provisions as applied to the authorisation described above, the licensing authority directs the holder of the authorisation to furnish to the authority, except where the holder of the authorisation has already furnished the authority with the information and received an acknowledgement, copies of such reports originating in the United Kingdom or abroad, and of which he is aware, of adverse effects in human beings suspected of association with the use of the medicinal product to which the authorisation relates as indicated in the paragraphs 4-8 below.

2. This direction applies to reports which have been made by health care professionals. These include medically qualified doctors, coroners, dentists, pharmacists and nurses. When reports originate from pharmacists and nurses, there should be the capability to obtain further information about the case from a medically qualified doctor responsible for the patient.

3. Detailed guidance on requirements is available in the Notice to Applicants for marketing authorisations for medicinal products for human use in the European Union.

REPORTING REQUIREMENTS

4. All individual reports of **serious** suspected adverse reactions associated with the use of the product in the UK should be reported to the Medicines Control Agency **within 15 calendar days of receipt.**

5. Reports of **serious** suspected adverse reactions occurring elsewhere in the EC need not be reported to the Medicines Control Agency when they are associated with products authorised through the centralised licensing procedure. Individual reports of **serious** suspected adverse reactions occurring elsewhere in the EC which are associated with medicines authorised through other procedures, should be reported to the Medicines Control Agency **within 15 days of receipt.** For suspected adverse reactions occurring outside the EC individual reports of **serious and unexpected** adverse reactions, should be reported to the Medicines Control Agency **within 15 days of receipt.**

6. With regard to the reports required under paragraph 4, 5 and 8 the individual reports of adverse reactions association with the use of the product should be provided on a reporting form acceptable to the authority. For the purpose of this direction "serious reaction" is an adverse reaction which is fatal, life threatening, disabling, incapacitating or which results in or prolongs hospitalisation. An "unexpected adverse reaction" relates to an adverse reaction which is not mentioned in the authorised product information (data sheet or SPC).

7. All other suspected adverse reactions associated with the product are required to be reported periodically in a safety update report as follows:

- six monthly for the first two years following authorisation.
- annually for the subsequent three years.
- then five yearly at the time of renewal of the authorisation.

REPORTING REQUIREMENTS FOR POST-AUTHORISATION STUDIES

8. Copies of individual reports of serious suspected adverse reactions to the product which come to the marketing authorisation holder's attention during post authorisation studies conducted in the UK or abroad, are required to be reported to the Medicines Control Agency within 15 calendar days. Other suspected reactions and events not suspected to be due to the product should not be reported as individual cases but should be analysed in the final report provided to the Medicines Control Agency.

GENERAL

9. This direction is without prejudice to any specific direction made in connection with the particular product and remains in force until withdrawn or amended by a fresh notification in writing by the licensing authority.

Medicines Control Agency

2-4-2 Radioimmunological measurements

2-4-2-1 Reagents

A phosphate-gelatin buffer solution was prepared by dissolving 9 g sodium chloride, 1 g sodium azide and 1 g gelatin in 1 litre of 0.1 M sodium phosphate buffer previously adjusted to pH = 6.9. This solution was stored at 4°C.

Anti-RU 38486 antiserum was obtained by injecting the antigen (RU 38486 3-carboxy methyloxime conjugated to bovine serum albumin) in New Zealand rabbits according to the method described by J.P. Raynaud et al. (J. Pharmacol. (Paris), 5, 27 (1974)). Diluted 1:100 in phosphate-gelatin buffer, this antiserum was stable for several months at + 4°C.

A stock solution of 0.1 $mg.ml^{-1}$ RU 38486 in ethanol was stored at + 4°C. To establish the standard curve, solutions of RU 38486 were prepared immediately prior to use by dilution in phosphate-gelatin buffer containing 0.025% Triton X 100 so as to give concentrations of 0, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500, 1000 and 2000 pg for 0.1 ml.

Tritiated RU 38486 (37.5 Ci/mmol) was stored at +4°C in solution in ethanol. This solution was diluted extemporaneously with phosphate-gelatin-Triton X 100 buffer to give a concentration of 25,000 cpm per ml.

The charcoal-suspension was composed of 250 mg charcoal (Norit A) and 25 mg dextran T 70 (Pharmacia) per 100 ml phosphate buffer without gelatin.

The liquid scintillator, Dynagel(R), was supplied by Baker.

2-4-2-2 Assay procedure

With the standard curve established between 0.04 and 20 $ng.ml^{-1}$, the plasma samples of the subjects who had received 100 mg RU 38486 had to be diluted 200 to 4,000 times. This dilution was done with a phosphate-gelatin buffer containing 0.025% Triton X 100. Assays were done in triplicate : to 0.1 ml diluted plasma or 0.1 ml standard solution was added 0.4 ml water, then RU 38486 was extracted with 3.0 ml diethyl ether from a bottle opened the same day. After 10 minutes' shaking on the Vortex, the aqueous phase was frozen in a methanol-dry ice bath, and the organic phase decanted into a haemolysis tube and

The lot number of the radioactive tracer used in IV and oral administration in the preliminary study in healthy volunteers

Thin layer chromatography method:

Preparation of Samples:

Extraction of RU 38 486 from plasma (97.4 ± 0.3% for n=9):

1 mL of plasma + 1 mL distilled water
+ 10 mL ethyl acetate

Agitation in girotory for 15 minutes at 300 RPM
Centrifugation for 10 minutes at 4000 revolutions/min
Isolation of supernatant
Repetition of extraction a second time
Pooling of supernatants and evaporation
Capture of residue with 10 mL of methanol
Counting of radioactivity on a sample of 3 x 500 µL
Evaporation of the methanol solution
Capture of dry residue in 50 µL of ethyl acetate containing cold RU 38 486 (approximately 20 µg/mL in oder to protect the tracer from any auto-oxidation during chromatography)
Deposit of the extracted material on a Kieselgel 60F254 silicon plate, 20 x 20 cm 0.25 mm thick

Thin-layer chromatography procedure:

Migration in the system of the solvents Chloroform/Acetone 7/3 on a distace of 15 cm.
Scraping of the silicon plate in bands of 0.5 cm
Desorption of the adsorbed products in 2 mL of ethanol
Addition of 15 mL of Lumagel scintillating cocktail
Counting of the radioactivity in a β counter
Determination of the percentage of radioactivity detected at the Rf for RU 38 486 by adding the measures of the bands in question.
Quantification of RU 38 486 =

$$\frac{\text{Radioactivity extracted x rate of RU 38486 determined by thin-layer chromatography}}{\text{(Specific activity of product x yield of extraction)}}$$

In these chromatographic conditions, the references of RU 38 486 and its known main metabolites have been the following:

RU 38 486	:Rf 0.57
RU 42 633 (mono-N-desmethyated metabolite)	:Rf 0.51
RU 42 848 (di-N-desmethylated metabolite)	:Rf 0.37
RU 42 698 (metabolite hydroxylated at the CH_3 of the propargyle	:Rf 0.29

ASSAY TECHNIQUE OF RU 38 486 IN PLASMA

1 PRINCIPLE

After organic extraction of the plasma in the presence of an internal standard, RU 38 486 and its metabolite, RU 42 633, were assayed by ultraviolet densitometry after separation by high performance chromatography.

2 MATERIAL

2.1 CHROMATOGRAPHIC APPARATUS

- Pump SP 8700, Spectra-Physics France.
- SP 8780 SR automatic injector, Spectra-Physics France.
- Stainless steel Nucleosil C18 10 µ precolumn, 50 mm x 4.6 mm, Roussel-Uclaf.
- Stainless steel Nucleosil C18 10 µ analytical column, 250 mm x 4.6 mm, Société Française Chromato Colonne.
- Kratos Spectroflow 783 ultraviolet detector, circulation cell 12 µl, distance 10 mm, wavelength 304 nm, sensitivity 0.005 AUFS.
- SP 4200 recorder-integrator-calculator, Spectra-Physics France.
  The whole apparatus operates on the Labnet system, Spectra-Physics France.

2.2 MISCELLANEOUS APPARATUS

- Disposable 5 ml extraction tubes, Rossignol.
- Sampling vials for automatic injector, Spectra-Physics France.
- Girotory G2 horizontal shaker, New Brunswick Scientific Co. Inc.
- G110 SX refrigerated centrifuge, Jouan.
- Manual piston pump, Brand.
- Ultrapure Milli-Q Millipore water production apparatus with filtration on a Millistak GS filter 0.22 u.
- Brausonic 220 ultrasound bath.

2.3 REAGENTS

- Spectrographic grade acetonitrile, Riedel de Haën
- Spectrographic grade ethyl acetate, Riedel de Haën
- Spectrographic grade methyl alcohol, Riedel de Haën
- Ultrapure filtered demineralised water
- PIC B7 (heptane sulphonic acid), Waters S.A. no. 85103
- Plasma collected on EDTA, Blood Transfusion Centre, Hôpital René Dubos, Pontoise.

2.4 REFERENCE PRODUCT

- RU 38 486, batch 12 reference 4E 0344
- RU 42 633, batch 4 reference 5E 0734
- RU 39 813, batch 19 reference 5E 0743

3 EXTRACTION

3.1 OPERATING METHOD

To 0.3 ml of plasma to be assayed were added:

- 0.3 ml of solution of internal standard, RU 39 813, 375 $\mu g.ml^{-1}$ in demineralised water
  Homogenisation for 5 seconds in the Vortex.
- 3 ml of spectrographic grade ethyl acetate.

The tube was shaken mechanically for 10 minutes on the Girotory at 300 rpm and then centrifuged at 1700 g for 10 minutes. The organic phase was withdrawn and then evaporated to dryness at room temperature under a nitrogen current. The dry extract was dissolved in 0.05 ml of methyl alcohol, the solution decanted into a small volume cone flask for the SP 8780 SR automatic injector and 0.035 ml injected into the column.

3.2 EXTRACTION RECOVERY AND REPRODUCIBILITY

The extraction recoveries of RU 38 486, RU 42 633 and the internal standard, RU 39 813, were determined after spiking 3 series of

10 samples of human plasma with radioactive tracers (^{3}H - purity 96-98%) diluted with the radioinert compounds so as to obtain concentrations by weight of 0.22, 0.22 and 0.40 $mg.l^{-1}$ for the three compounds.

The extractions were done on 1 ml of plasma as in the protocol described except that the addition of the internal standard was omitted. The radioactivity was measured in the organic and aqueous phases and the extraction recoveries calculated. The results are given in Table 1 AII. The mean recovery yields were:

RU 38 486 96.7% (n = 9) C.V. 2.7%
RU 42 633 87.0% (n = 10) C.V. 3.3%
RU 39 813 93.1% (n = 10) C.V. 2.8%

4 CHROMATOGRAPHY AND DETECTION

- Eluant system:
  acetonitrile/ultrapure filtered demineralised water 41/59 V/V
  To 1 litre of this mixture were added 23 ml of PIC B7
- Flow rate: 1.5 $ml.min^{-1}$
- Room temperature
- Detection: 304 nm, 0.005 AUFS sensitivity

Under these conditions the retention times were:

RU 42 633 8.3 min
RU 38 486 10.2 min
RU 39 813 14.2 min

The control plasmas exhibit no peak at these times.
The retention times of the known metabolites are different:

RU 42 698 4.5 min
RU 42 848 6.7 min

5 CALIBRATION

The calibration ranges were established by spiking a pool of human plasma with RU 38 486 and RU 42 633 so as to obtain concentrations equivalent to about 0.05, 0.2, 0.50, 1.0, 1.5 and 2 mg, accurately determined, for each compound per litre of plasma.

The plasma for the calibration range was treated under the same conditions as those of the assays.

The calibration curves were obtained from the ratios of the peak heights, RH:

$$RH = \frac{\text{Peak height of compound to be assayed}}{\text{Peak height of internal standard}}$$

and the concentrations of the points on the corresponding range.

Over the range of concentrations used this curve is a straight line. In practice the calibration curves were fitted by a least squares linear regression to obtain the equations of the regression lines.

$$RH = \text{concentration} * \text{slope} + \text{origin}$$

This equation was rearranged to the equation:

$$\text{Concentration} = \frac{RH - \text{origin}}{\text{slope}}$$

from which the concentrations of RU 38 486 and RU 42 633 in the samples were deduced after measuring RH for the samples. The results were expressed in $mg.l^{-1}$. The limit of detection was 0.01 $mg.l^{-1}$.

6 IN-PROCESS CONTROLS

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

6.1 CALIBRATION

For each series of analyses, 2 calibration curves were established, one for RU 38 486, the other for RU 42 633. There were therefore 24 calibration curves for each compound. The parameters of the regression lines for each range (slope and origin) and the concentrations of the points on the range recalculated from the relevant equation are given in Tables 2 AII and 3 AII.

The means of the recalculated concentrations do not differ from the values of the spiked plasma, allowing for the confidence interval of these means, and the calibration is therefore without bias. The calibration curves of series 18 are illustrated in Figures 1 AII and 2 AII as examples.

6.2 QUALITY CONTROL

Control plasma was prepared by spiking control human plasma with RU 38 486 at 3 different concentrations: 0.08, 0.60 and 1.6 $mg.l^{-1}$.

The control plasma were frozen and stored at -20°C until assay.

For each series of analyses, control plasma for each concentration was included every 7 samples and treated under the same conditions.

The results of the assays of these controls are listed in Table 4 together with the means, the inter-assay coefficients of variation and the deviations from the theoretical values.

ASSAY TECHNIQUE OF RU 38486 IN PLASMA

1 PRINCIPLE

After organic extraction of the plasma in the presence of an internal standard, RU 38486 and its metabolite, RU 42633, were assayed by ultraviolet densitometry after separation by high performance liquid chromatography.

2 MATERIAL

2.1 CHROMATOGRAPHIC APPARATUS

- SP 8700 pump, Spectra-Physics France.
- SP 8780 R automatic injector, Spectra-Physics France.
- Stainless steel Nucleosil C18 10µ precolumn, 50 mm x 4.6 mm, Roussel-Uclaf.
- Stainless steel Nucleosil C18 10µ analytical column, 250 mm x 4.6 mm, Société Française Chromato Colonne.
- Kratos Spectroflow 783 ultraviolet detector, flow-cell 12 µl, light path 10 mm, wavelength 304 nm, sensitivity 0.005 AUFS.
- SP 4200 recorder-integrator-calculator, Spectra-Physics France.
  The whole apparatus operates on the Labnet system, Spectra-Physics France.

2.2 MISCELLANEOUS APPARATUS

- Disposable 5 ml extraction tubes, Rossignol.
- Sampling tubes for automatic injector, Spectra-Physics France.
- Girotory type G horizontal shaker, New Brunswick Scientific Co. Inc.
- G110 SX refrigerated centrifuge, Jouan.
- Manual piston pump, Brand.
- Milli-Q Millipore ultrapure water production apparatus with filtration on a Millistak GS filter 0.22 µ.
- Bransonic 220 ultrasound bath.

2.3 Reagents

- Spectrography grade acetonitrile, Riedel de Haën
- Spectrography grade ethyl acetate, Riedel de Haën
- Spectrography grade methyl alcohol, Riedel de Haën
- Filtered ultrapure demineralised water
- PIC B7 (heptane sulphonic acid), Waters S.A. no. 85103
- Plasma collected on EDTA, Blood Transfusion Centre, Hôpital René Dubos, Pontoise.

2.4 Reference product

- RU 38 486, micronised, reference 5E 0327
- RU 42 633, batch 4, reference 5E 0734
- RU 39 813, batch 18, reference 5E 0684

3 EXTRACTION

3.1 Operating method

To 0.3 ml of plasma to be assayed were added:
- 0.3 ml of solution of internal standard, RU 39 813, 433 $\mu g.ml^{-1}$ in demineralised water

Homogenisation for 5 seconds in the Vortex.
- 3 ml of spectrography grade ethyl acetate.

The tube was shaken mechanically for 10 minutes on the Girotory at 900 rpm and then centrifuged at 1700 g for 10 minutes. The organic phase was withdrawn and then evaporated to dryness at room temperature under a nitrogen current. The dry extract was dissolved in 0.05 ml of methyl alcohol, the solution decanted into a small volume cone flask for the SP 8780 R automatic injector and 0.035 ml injected into the column.

3.2 Extraction recovery and reproducibility

The extraction recoveries of RU 38 486, RU 42 633 and the internal standard, RU 39 813, were determined after spiking 3 series of 10 samples of human plasma with radioactive tracers (^{3}H - purity 96 to 98%) diluted with the radioinert compounds so as to obtain concentrations of 0.22, 0.22 and 0.40 $mg.l^{-1}$ for the three compounds.

The extractions were done on 1 ml of plasma as described in the protocol except that the addition of the internal standard was omitted. The radioactivity was measured in the organic and aqueous phases and the extraction recoveries calculated. The results are given in Table 1 AII. The mean recovery yields were:

RU 38 486	96.7% (n = 9)	C.V. 2.7%
RU 42 633	87.0% (n = 10)	C.V. 3.3%
RU 39 813	93.1% (n = 10)	C.V. 2.8%

4 CHROMATOGRAPHY AND DETECTION

- Eluant system: acetonitrile/filtered ultrapure demineralised water 41/59 V/V
  To 1 litre of this mixture were added 23 ml of PIC B7
- Flow rate: 1.5 $ml.min^{-1}$
- Ambient temperature
- Detection: 304 nm, sensitivity 0.005 AUFS

Under these conditions the retention times were:

RU 42 633	8.3 min
RU 38 486	10.2 min
RU 39 813	14.2 min

The control plasmas show no peak at these times.

The retention times of the known metabolites are different:

RU 42 698	4.5 min
RU 42 848	6.7 min

5. CALIBRATION

The calibration ranges were established by spiking pooled human plasma with RU 38 486 and RU 42 633 so as to obtain concentrations equivalent to about 0.025, 0.05, 0.10, 0.50, 1.0, 1.5 and 2.0 mg of each compound per litre of plasma.

The plasma for the calibration range was treated under the same conditions as that for the assays.

The calibration curves were obtained from the ratios of the peak heights, RH:

$$RH = \frac{\text{Peak height of compound to be assayed}}{\text{Peak height of internal standard}}$$

and the concentrations of the points on the corresponding range.

Because of the extent of their range the calibration curves were divided into two regions depending on the concentration with which the plasma was spiked:

a) a first region from 0.025 to 1 $ml.l^{-1}$

b) a second region from 1 to 2 $ml.l^{-1}$

In each region the calibration curve was a straight line, the equation of which was determined after fitting by linear regression using the least squares criterion:

$$RH = \text{concentration} \times \text{slope} + \text{origin}$$

This equation was rearranged to the equation:

$$\text{Concentration} = \frac{RH - \text{origin}}{\text{slope}}$$

from which the concentrations of RU 38 486 and RU 42 633 in the samples were deduced after measuring RH for these samples and choosing the region of the calibration curve according to the RH measured.

The results are expressed in $mg.l^{-1}$. The limit of detection is 0.01 $mg.l^{-1}$.

6 IN-PROCESS CONTROLS

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

6.1 Calibration

For each series of analyses, 2 calibration curves were established, one for RU 38 486, the other for RU 42 633. There were therefore 24 calibration curves for each compound. The parameters of the regression lines for each range (slope and origin) and the concentrations of the points on the range recalculated from the relevant equation are given in Tables 2AII to 5AII.

The means of the recalculated concentrations do not differ from the values of the spiked plasma, allowing for the confidence interval of these means, and the calibration is therefore without bias.

The regions of the calibration curves for series 19 are illustrated in Figures 1AII to 4AII as examples.

6.2 Quality control

Control plasma were prepared independently of the calibration ranges by spiking pooled human plasma with RU 38 486 and RU 42633 to obtain concentrations of about 0.08, 0.30 and 1.6 $mg.l^{-1}$, known exactly.

The control plasma were frozen and stored at -20°C until assay.

For each series of analyses, control plasma for each concentration were included every twenty samples and treated under the same conditions.

The results of the assays of these controls are listed in Table 6AII together with the means, the inter-assay coefficients of variation and the deviations from the theoretical values.

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 radial immunodiffusion. Five µl of plasma were applied to each well of an agarose gel plate containing anti-α_1-acid glycoprotein monospecific antiserum (Nor-Partigen Behring, batch no. 054329). Calibration was done with a range of standard sera (Behring) from 0.18 to 2.72 $g.l^{-1}$. 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 reverse phase chromatography (HPLC) on a Nucleosil C18 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 0.01 $mg.l^{-1}$. Concentrations below 0.01 $mg.l^{-1}$ were considered to be zero, and concentrations equal to or greater than this were rounded off to the nearest 0.001.

APPENDIX I

Assay technique

ASSAY TECHNIQUE OF RU 38 486 IN PLASMA

1 PRINCIPLE

After organic extraction of the plasma in the presence of an internal standard, RU 38 486 and its metabolite, RU 42 633, were assayed by ultraviolet densitometry after separation by high performance liquid chromatography.

2 MATERIAL

2.1 CHROMATOGRAPHIC APPARATUS

- SP 8700 pump, Spectra-Physics France.
- SP 8780 XR automatic injector, Spectra-Physics France.
- Stainless steel Nucleosil C18 10µ precolumn, 50 mm x 4.6 mm, ROUSSEL UCLAF.
- Stainless steel Nucleosil C18 10µ analytical column, 250 mm x 4.6 mm, Société Française Chromato Colonne.
- Kratos Spectroflow 783 ultraviolet detector, flow-cell 12 µl, light path 10 mm, wavelength 304 nm, sensitivity 0.005 AUFS.
- SP 4200 recorder-integrator-calculator, Spectra-Physics France.
  The whole apparatus operates on the Labnet system, Spectra-Physics France.

2.2 MISCELLANEOUS APPARATUS

- Disposable 5 ml extraction tubes, Rossignol.
- Sampling tubes for automatic injector, Spectra-Physics France.
- Gyrotory type G horizontal shaker, New Brunswick Scientific Co. Inc.
- G110 SX refrigerated centrifuge, Jouan.
- Manual piston pump, Brand.
- Milli-Q Millipore ultrapure water production apparatus with filtration on a Millistak CS filter 0.22 µ.
- Bransonic 220 ultrasonic bath.

2.3 REAGENTS

- Spectrography grade acetonitrile, Riedel de Haën
- Spectrography grade ethyl acetate, Riedel de Haën
- Spectrography grade methyl alcohol, Merck
- Filtered ultrapure demineralised water
- PIC B7 (heptane sulphonic acid), Waters S.A. no. 85103
- Plasma collected on EDTA, Blood Transfusion Centre, Hôpital René Dubos, Pontoise.

2.4 REFERENCE PRODUCTS

- RU 38 486, micronised, reference 6E0421
- RU 42 633, batch 1
- RU 39 813, batch 18, reference 5E 0684

3 EXTRACTION

3.1 OPERATING METHOD

To 0.3 ml of plasma to be assayed were added:

- 0.3 ml of solution of internal standard, RU 39 813, 433 $ng.ml^{-1}$ in demineralised water
  Homogenisation for 5 seconds on the Vortex.
- 3 ml of spectrography grade ethyl acetate.

The tube was shaken mechanically for 10 minutes on the Girotory at 300 rpm and then centrifuged at 1700 g for 10 minutes. The organic phase was withdrawn and then evaporated to dryness at room temperature under a nitrogen current. The dry extract was dissolved in 0.05 ml of methyl alcohol, the solution decanted into a small-volume cone flask for the SP 3730 XR automatic injector and 0.035 ml injected into the column.

3.2 EXTRACTION RECOVERY AND REPRODUCIBILITY

The extraction recoveries of RU 38 486, RU 42 633 and the internal standard, RU 39 813, were determined after spiking 3 series of 10 samples of human plasma with radioactive tracers (^3H - purity 96 to 98%) diluted with the radioinert compounds so as to obtain concentrations of 0.22, 0.22 and 0.40 $mg.l^{-1}$ for the three products.

The extractions were done on 1 ml of plasma as described in the protocol except that the addition of the internal standard was omitted. The radioactivity was measured in the organic and aqueous phases and the extraction recoveries calculated. The results are given in Table 1 AI. The mean recovery yields were:

RU 38 486	96.7% (n = 9)	C.V. 2.7%
RU 42 633	87.0% (n = 10)	C.V. 3.3%
RU 39 813	93.1% (n = 10)	C.V. 2.8%

4 **CHROMATOGRAPHY AND DETECTION**

- Eluant system: acetonitrile/ultrapure filtered demineralised water 41/59 V/V

To 1 litre of this mixture were added 23 ml of PIC B7

- Flow rate: 1.5 $ml.min^{-1}$
- Ambient temperature
- Detection: 304 nm, sensitivity 0.005 AUFS

Under these conditions the retention times are:

RU 42 633	9.6 min
RU 38 486	12.6 min
RU 39 813	16.3 min

The control plasmas show no peak at these times.

The retention times of the known metabolites are different:

RU 42 698	4.8 min
RU 42 348	7.2 min

5 CALIBRATION

The calibration ranges were established by spiking pooled human plasma with RU 38 486 and RU 42 633 so as to obtain exactly determined concentrations of each product, equivalent to about 0.025, 0.05, 0.1, 0.5, 1.0, 1.2 and 1.5 mg per litre of plasma.

The plasma for the calibration range was treated under the same conditions as that for the assays.

The calibration curves were obtained from the ratios of the peak heights, RH, and the concentrations of the corresponding points in the range:

$$RH = \frac{\text{Peak height of compound to be assayed}}{\text{Peak height of internal standard}}$$

Over the range of concentrations used, this curve was a straight line. In practice the calibration curves were fitted by linear regression using the least squares criterion to obtain the equations of the regression lines.

$$RH = (\text{concentration}) * \text{slope} + \text{origin}$$

This equation was rearranged to the equation:

$$\text{Concentration} = \frac{RH - \text{origin}}{\text{slope}}$$

from which the concentrations of RU 38 486 and RU 42 633 in the samples can be deduced after measuring RH for each of the products. The results are expressed in $mg.l^{-1}$. The limit of detection is 0.01 $mg.l^{-1}$.

6 IN-PROCESS CONTROLS

A series of analyses was constituted from all the assays performed for the treatment of a single subject.

6.1 CALIBRATION

For each series of analyses, 2 calibration curves were established, one for RU 38 486, the other for RU 42 633. There were therefore 10 calibration curves for each product. The parameters of the regression lines for each range (slope and origin) and the concentrations of the points in the range recalculated from the relevant equation are given in Tables 2AI and 3AI.

The means of the recalculated concentrations do not differ from the values of the spiked plasma, allowing for the confidence interval of these means, and the calibration is therefore unbiased. The calibration curves for series 1 are illustrated in Figures 1 AI and 2AI as examples.

6.2 QUALITY CONTROL

Control plasmas were prepared by spiking pooled human control plasma with RU 38 486 at three different concentrations: 0.08, 0.80 and 1.3 $mg.l^{-1}$.

The control plasma was frozen and stored at -20°C until assay.

Control plasma for each concentration was included in the series of analyses - one after the seven calibration points and then two at the end of the series, i.e. after 28 samples - and treated under the same conditions.

The results of the assays of these controls are listed in Table 4 AI together with the means, the inter-assay coefficients of variation and the deviations from the theoretical values.

Analysis of labeled proteins

Cells were plated out in 100 µl medium containing 3 % FCS/DCC in 0.8 cm diameter microwells (96 µ-well dishes, Nunclon Delk, Roskilde, Denmard). After steroid stimulation, the cells were labeled with 200 µCi/ml of ^{35}S-methionine (Radio-Chemical Center, Amersham, England ; SA > 1,000 Ci/mMole) in 60 µl of Eagle's minimum essential medium (MEM) containing one tenth the normal concentration of methionine for 6 h (released proteins) or 4 h (cellular proteins). Culture media were centrifuged at 700 g for 5 min and/or at 9,000 g for 2 min. Cells rinsed twice with phosphate-buffered saline were dissolved in 15 µl of lysis buffer A (17)(18) containing 0.5 % sodium dodecyl sulfate plus 40 µl H_2O and lysed by three freeze-thaw cycles (-80°C).

Total incorporation of ^{35}S-methionine in proteins was evaluated by precipitation with 15 % trichloroacetic acid (TCA) of 10 µl of medium on Whatman 3MM filters as previously described (4). ^{35}S-labeled proteins were analysed in one-dimensional gel electrophoresis (7). Gels were then processed for fluorography (19) and exposed for 7-10 days at -80°C to Kodak X-omat S films (preflashed to an absorbance of 0.2 OD). Films were scanned using a Vernon scanning densitometer and the amount of specific proteins was estimated from the traces.

APPENDIX A.1

PROTOCOL

APPEARS THIS WAY
ON ORIGINAL

LABORATOIRES ROUSSEL
DIRECTION MEDICALE

PROTOCOL FFR/91/486/14

EFFICACY AND TOLERANCE OF MIFEPRISTONE (RU 38486) IN A SINGLE DOSE OF 600 MG IN COMBINATION WITH MISOPROSTOL AS AN ALTERNATIVE TO UTERINE ASPIRATION FOR THE TERMINATION OF PREGNANCIES OF LESS THAN OR EQUAL TO 49 DAYS OF AMENORRHOEA

MAY 1991

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	Roussel Uclaf 102, route de Noisy 93230 **ROMAINVILLE**
Responsible for Information about Mifegyne:	
Pharmacovigilance:	Laboratoires ROUSSEL 97, rue de Vaugirard 75006 **PARIS**
Biometry:	
Good Clinical Practice and Quality Assurance:	Laboratoires ROUSSEL 97, rue de Vaugirard 75006 **PARIS**
<u>Principal investigator</u>	**Elisabeth AUBENY, M.D.** Centre d'Orthogénie Hôpital BROUSSAIS 96, rue Didot 75014 PARIS Tel. (1) 43 95 95 95

CONTENTS

1. INTRODUCTION

Mifepristone (RU 486, Mifegyne®) is an antiprogesterone compound synthesised by ROUSSEL UCLAF. Previous studies have shown that it is capable by itself to terminate about 80% of pregnancies of less than or equal to 41 days of amenorrhoea (DA) (1) when given in a single dose of 600 mg orally. After this date the efficacy of the compound alone decreases rapidly (a reduction of about 10% in the success rate for each additional week of amenorrhoea). Swedish (2), Scottish (3) and French (4-5) studies have shown that the combination of mifepristone with a synthetic prostaglandin analogue (sulprostone or gemeprost) achieves the complete termination of pregnancy in 95% of cases of amenorrhoea of up to 49 days. These studies also indicate that the combination of mifepristone + prostaglandin reduces the doses of prostaglandin necessary (0.25 mg for sulprostone, 0.5 or 1.0 mg for gemeprost), and hence their side-effects.

The optimum interval between administration of mifepristone and administration of the prostaglandin is 36 to 48 hours. In fact, the cervical dilatation induced by mifepristone is greater at 48 than at 24 hours and maximum sensitisation of the uterine muscle to the contracting action of prostaglandins occurs 36 to 48 hours after administration of mifepristone (6, 7).

Mifepristone has been registered in France as a medical alternative to uterine aspiration for pregnancies of less than 50 DA. It is prescribed in a single dose of 600 mg (3 tablets of 200 mg) in a single dose and is followed 36 to 48 hours later by administration of 1 mg of gemeprost or 0.25 mg of sulprostone

In a study in about 16,000 women (8), the tolerance of this method of pregnancy termination was good. In the 4 hours following prostaglandin administration painful uterine contractions occurred in about 80% of women. These contractions required treatment in 20 to 60% of patients depending on the dose of prostaglandin used (1 mg of gemeprost, 0.25 or 5 mg of sulprostone). During this period nausea (34% of women), vomiting (15% of cases) and diarrhoea (7.5% of cases) were observed. Malaise (lipothymia or hypotension), was also reported in about 1% of cases.

The uterine bleeding required a haemostatic endo-uterine procedure in 0.8% of cases and a transfusion in 0.1% of cases.

In all the women having recoursed to this method (about 60,000) 3 severe cardiac adverse events (myocardial infarction) have been reported, with a fatal outcome in one of the cases. These infarcts appear related to a coronary spasm and all occurred within 4 hours following the injection of sulprostone. The 3 patients concerned were all over the age of 30 years and smokers. These coronary spasms are very probably attributable to sulprostone and have also been described after isolated injection of sulprostone (9).

In view of these accidents, it was decided to investigate if other prostaglandins than those studied previously could be associated with mifepristone.

Misoprostol is a synthetic derivative of the PGE_1 series (15-desoxy-16-hydroxy-16-methyl analogue) administered orally at a dose of 4 tablets of 0.2 mg daily for gastric or duodenal ulcers (10).

The compound is very widely prescribed. At a dose of 4 tablets of 200 µg daily it does not cause hypotension and its cardiovascular tolerance appears to be good. No serious cardiovascular effect has been published to date and the pharmacovigilance data are favourable (11).

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A preliminary study in 100 women (12) showed that administration of 600 mg of mifepristone followed 48 hours later by 2 tablets of misoprostol achieved the termination and complete expulsion of 95% of pregnancies of not more than 49 days of amenorrhoea. The tolerance of the method was satisfactory. The main adverse effects were nausea (35 cases), vomiting (11 cases) and diarrhoea (7 cases), symptoms which did not require treatment. By contrast, however, the intensity of the uterine pain appeared to have been very markedly decreased in comparison with the prostaglandins used previously (sulprostone, gemeprost). The duration of bleeding was not affected.

It therefore appears of interest, in view of all the previous information, to confirm the efficacy and tolerance of this combination in a large-scale study.

2. STUDY OBJECTIVE

The aim of this study is to evaluate the efficacy and tolerance of the use of mifepristone (600 mg) in combination with 2 tablets of 0.2 mg of misoprostol administered 48 hours later in the termination of pregnancies of less than or equal to 49 days of amenorrhoea and under the law on abortion in France.

3. STUDY DESCRIPTION

It is a multicentre, open study assessing the following therapeutic regimen:

- Mifepristone will be administered at a dose of 600 mg (3 tablets of 200 mg) in the presence of the investigator on Day 1 after verification of the inclusion criteria.

- Misoprostol (2 tablets of 0.2 mg in a single dose) will be administered 48 hours later on the morning of Day 3, also in the presence of the investigator. The woman will be kept under observation at the hospital for 4 hours.

The efficacy and tolerance of the treatment will be assessed at a follow-up visit 8 to 15 days after administration of mifepristone.

4. SUBJECT SELECTION

4.1 Number

The scheduled number of patients is 500. These patients will be recruited in 24 centres.

4.2 Inclusion criteria

The following women will be included:

a) requesting a pregnancy termination,

b) having satisfied the compulsory legal requirements for abortion in France,

c) between the ages of 18 (legal majority; women below the age of majority may only be included with the consent of their legal guardian) and 35 years,

d) having agreed to comply with the restrictions of the study, particularly the follow-up visit after administration of the treatment,

e) informed of the normal procedure for an abortion,

f) agreeing to undergo an instrumental termination of pregnancy in the event of treatment failure,

g) informed of the study procedure and having given their written consent to participate in it (Appendix 1),

and whose pregnancy is:

- intra-uterine,

- on-going,

- of specified age and less than or equal to 49 days of amenorrhoea (calculated from the first day of the last menstrual period).

(A pregnancy with an IUD in situ is not a contra-indication if it is removed when mifepristone is taken).

4.3 Exclusion criteria

The following women will not be included:

a) with signs of a miscarriage,

b) with a suspected ectopic pregnancy,

c) with amenorrhoea of more than 49 days,

d) over the age of 35,

e) smokers, defined as smoking at least 10 cigarettes daily during the two years preceding the beginning of the study,

f) having one of the following disorders: a history of cardiovascular disease (angina pectoris, rhythm disorders, heart failure, severe hypertension, etc.), asthma, glaucoma or raised intra-ocular pressure, diabetes, hyperlipidaemia.

g) with a current or previous history of renal, adrenal or hepatic insufficiency,

h) having received chronic corticosteroid therapy within the previous 6 months,

i) having a known abnormality of haemostasis or receiving anticoagulant treatment,

j) having a known allergy to mifepristone,

k) with anaemia,

l) refusing to give their written consent to participate,

m) considered liable not to comply with the requirements of the protocol or who live too far away from the centre.

5. STUDY DRUGS

5.1 Mifepristone

Mifepristone will be supplied by Laboratoires Roussel in the form of tablets containing 200 mg of micronised active substance. The tablets will be packaged in blister packs containing 3 tablets.

The compound will be given in a single dose of 3 tablets in the presence of the investigator from a distance of a meal.

The boxes of mifepristone will be labelled:

. Protocol No. FFR 91/486/14
. Mifepristone - Misoprostol Study
. Laboratoires Roussel
. Batch number - Expiry date
. Patient number (from 0001 to 0500)

All the boxes of mifepristone necessary for one centre will be given to the responsible pharmacist within this centre who will distribute them to the investigator.

After the inclusion and exclusion criteria have been checked, the woman will be allocated a study entry number and given a box marked with this number. The numbers will be allocated in order.

A trial drug accountability form must be kept by the investigator.

At the end of the study all unused compounds and the drug accountability form must be recovered by the clinical research assistant.

5.2 Prostaglandin analogue

The prostaglandin analogue used will be misoprostol (Cytotec®). It will be administered 48 hours after administration of mifepristone in a single dose of 2 tablets of 0.2 mg in the presence of the investigator. The woman will then be kept under monitoring at the centre for 4 hours.

Misoprostol will be supplied to the responsible pharmacist of the centre by Laboratoires Roussel.

5.3 Concomitant medications

5.3.1 Permitted medications

As far as possible no other medications will be given concomitantly. Where a drug is prescribed, the type and dose of the medication will be entered in the case report form.

Current treatments will be reported in the case report form.

5.3.2 Prohibited medications

Acetylsalicylic acid and its derivatives, steroidal and non-steroidal anti-inflammatories, prostaglandin synthesis inhibitors (where necessary an analgesic belonging to another pharmacological class or an antispasmodic will be used in preference to one of these medications), enzyme-inducing medications.

Oxytocics or prostaglandins other than that used in the study.

The patient must refrain from self-medication.

The patient must refrain from smoking and drinking alcohol during the 48 hours between administration of mifepristone and misoprostol and on the day of administration of misoprostol.

6. ASSESSMENT CRITERIA

6.1 Efficacy

The efficacy will be assessed between 8 and 15 days after administration of mifepristone (Day 8 - Day 15) by the investigator on the basis of the clinical data (occurrence of bleeding, expulsion of the ovum, persistence of bleeding), laboratory data and/or ultrasound data.

A distinction will be made between the following:

1) The termination and complete expulsion of the pregnancy (disappearance of clinical signs, fall in beta HCG compared with Day 1 and/or an empty uterus on ultrasound) without the need for a supplementary surgical procedure (apart from the forceps extraction of ovular fragments protruding through the external os, where necessary). The date and if possible the time of expulsion will be noted. This possibility will be considered a success.

2) Pregnancy termination without complete expulsion.

3) On-going pregnancy.

4) The need for a haemostatic endo-uterine procedure.

Eventualities 2, 3 and 4 will be followed by a supplementary surgical procedure, the date of which will be noted. These cases will be considered failures.

6.2 Tolerance

6.2.1. At the time of administration of misoprostol (Day 3):

Tolerance will be evaluated on the basis of the following information:

Any adverse event occurring between Day 1 (administration of mifepristone) and Day 3.

The onset within 4 hours after administration of misoprostol of painful uterine contractions and gastro-intestinal disorders: nausea, vomiting, diarrhoea. The intensity of these symptoms will be noted and whether or not symptomatic treatment is required.

Hourly observation during the 4 hours following administration of misoprostol of blood pressure (systolic and diastolic) and heart rate.

The occurrence of an adverse effect other than those mentioned above.

6.2.2. At the follow-up visit (Day 8 - Day 15):

Tolerance will be evaluated on the basis of the following:

The duration of uterine bleeding and the need for specific measures: measurement of haemoglobin concentration, drug treatment, blood transfusion, haemostatic surgical procedure.

Any unusual clinical signs or symptoms occurring since Day 3.

6.2.3. Laboratory safety

This will be assessed on the haemoglobin level measured on Day 1 (before administration of mifepristone) and on Day 8 - Day 15 during the follow-up visit.

7. STUDY PROCEDURE

7.1 Initial assessment (Day 1)

Check that the patient has undertaken the necessary legal steps for a request for abortion and has fulfilled the conditions laid down by the law (reflection period):

a) Note:

- any previous history,
- any treatment in progress and its reason,
- the date of the last menstrual period.

b) Check that the gestational age is less than or equal to 49 days of amenorrhoea.

c) Perform an assay of βHCG or a uterine ultrasound.

d) Determine the Rhesus group if the patient does not have a blood group card and measure the haemoglobin level.

e) Give the patient an information sheet about the study and obtain her written consent to participate.

f) Allocate the woman a study enrolment number and give her the 3 tablets of mifepristone contained in the box bearing this number. Treatment will be taken immediately in the presence of the investigator. The number will be noted on the case report form.

g) Inform the woman that she must refrain from smoking and drinking alcohol during the following 48 hours and on Day 3.

h) Make an appointment for two days later in the morning (Day 3).

7.2 Day 3: Administration of misoprostol:

- Clinical examination

- Investigation of any adverse event.

- Give an injection of anti-D glamma-globulins if the patient is Rhesus negative.

- Administration of 2 tablets of 0.2 mg of misoprostol in a single dose (if expulsion has not already occurred) in the presence of the investigator.

- The patient must remain under observation in the centre during the following 4 hours.

- During these 4 hours of observation the following parameters will be assessed:

  - Painful uterine contractions, nausea, vomiting and diarrhoea, by means of the following scale:

  1: minimal
  2: moderate
  3: severe, not requiring treatment
  4: severe, requiring treatment

  * The overall intensity of the pain during this observation will also be evaluated on a visual analogue scale 4 hours after administration of misoprostol.

  * If premedication has been given, this will be noted in the case report form.

  * The drugs administered will be noted in the case report form.

- Heart rate, systolic and diastolic blood pressure measured hourly.

- The moment of ovular expulsion will be noted if it occurs during the time when the patient is under observation.

- If the patient experiments chest pain, a rhythm disorder or hypotension, an ECG must be performed. In the event of severe pain, fast-acting nitrate derivatives will be prescribed under the assumption of a coronary spasm.

- At the end of 4 hours the woman is allowed to leave the centre and given an appointment for Day 8 - Day 15 with a prescription for an assay of haemoglobin immediately before this visit.

- Oral contraception, to be started 24 to 48 hours later, can be prescribed at this visit.

7.3 Day 8 - Day 15: Follow-up visit:

- Further clinical examination and assessment of tolerance by the investigator.

- If possible, note the date of ovular expulsion and the interval between expulsion and prostaglandin administration.

- Final evaluation of the efficacy of treatment (by the data from the clinical examination, βHCG and/or ultrasound).

- If the patient has started oral contraception before this follow-up visit, note the name of the contraceptive prescribed.

- Evaluation of uterine bleeding:

a) duration,
b) was an emergency measurement of the haemoglobin concentration necessary (note the result)?

- Has treatment been necessary (drug, transfusion, haemostatic surgical procedure)?

- In the event of failure (on-going pregnancy, incomplete expulsion), recommend a supplementary surgical procedure.

- Note the results of the assay of haemoglobin.

8. COLLECTION AND ANALYSIS OF DATA

8.1. Collection of data:

A case report form will be completed for each patient admitted in the study. Only the investigator and his colleagues are qualified to complete the case report form and make any corrections on it.

Corrections of data on the case report form can be made only by crossing through the incorrect entry so that it remains visible and putting the correct figure by the side. The correction must be initialled and dated in the margin by the person making the correction. Each case report form must be signed and dated by the investigator.

8.2. Analysis of data:

The analysis of the data will be performed by the Biometry Department of Laboratoires Roussel. It will be primarily descriptive.

9. PROTOCOL AMENDMENTS

No changes may be made to a protocol without the written agreement of Roussel.

Any modification must be the subject of a documented amendment justified in writing. It must be signed by the investigator indicating his acceptance of the change to the study procedure.

This protocol amendment must be submitted to and approved by the Ethics Committee if it is likely to affect adversely the expected medical benefit/risk ratio for the patient.

If the protocol modification is necessary immediately in order to ensure the safety of the patients, those responsible for the study will submit the amendment to the Ethics Committee after its application as rapidly as possible.

10. SIDE-EFFECTS AND ADVERSE EVENTS

10.1. Serious adverse event:

A serious adverse event is defined as:

- Any event with a fatal outcome or which is life-threatening.
- Any event leaving sequelae or following a chronic course.
- Any event necessitating hospitalisation or the prolongation of hospitalisation.
- The discovery of a congenital abnormality or cancer.
- An overdosage.

All serious adverse events must be notified immediately to Laboratoires Roussel:

- Rémi PEYRON, M.D. — Tel. 1 40 62 41 40, Fax. 1 40 62 49 68

OR

- Louise SILVESTRE, M.D. — Tel. 1 49 91 46 60, Fax. 1 49 91 49 49, or 1 49 91 48 00

[redacted]

Written confirmation must be sent in the form of the adverse event record form (a copy of which is included in Appendix 2) either by fax or by urgent mail.

10.2. Mild adverse events:

These will simply be reported in the case report form.

11. WITHDRAWALS AND DEFAULTERS

Any patient enrolled in the study will be analysed for tolerance. Only women completing the study may be analysed for efficacy.

12. NOTIFICATION TO REGULATORY AUTHORITIES

The study will be declared to the Ministry of Health.

13. ETHICS

This study will be conducted in accordance with the principles of the Declaration of Helsinki (cf. Appendix 3) and French law on clinical trials.

13.1. Consent:

Prior to the patient's inclusion in the study her written consent will be obtained (signed by the patient and the signature preceded by "read and approved"). In order to obtain this consent, she will be given an information document on the study (Appendix 1).

In addition the investigator will sign an identification and consent obtained form "thereby attesting to the fact that the patient's consent has been obtained".

13.2. Ethics Committee:

The protocol will be submitted to an Ethics Committee.

The study may only begin after Laboratoires Roussel has received a copy of the written approval from this Committee.

In the event of a protocol amendment, this amendment must be submitted to and approved by the Ethics Committee if it is liable to affect adversely the medical benefit/risk ratio for the patients.

14. CONFIDENTIALITY

The data obtained during the study are considered to be confidential.

The information supplied by Laboratoires Roussel (product brochure, protocol, case report form) are also confidential.

For each patient the data will be identified by the patient's number in the study and by her initials and will be processed anonymously in the analyses.

All the data relating to the study must be accessible to other investigators participating in it, the Laboratoires Roussel Co-ordinator, the Head of Quality Assurance, the Ethics Committee and the Regulatory Authorities.

15. STUDY MONITORING AND QUALITY CONTROL

Members of Laboratoires Roussel will make regular contact with the investigator by means of on-site visits and telephone calls to monitor the procedure of the study and to ensure that it is conducted in compliance with the protocol.

The case report forms will be reviewed in detail at each visit.

The investigator and his team undertake to co-operate with the monitor and in particular to provide him with the documents and missing information whenever possible.

Each case report form will be signed by the investigator who must initial and date any corrections.

If there are any missing or unavailable data the reason for this must be stated.

Participation in the study implies that the investigator agrees to the possibility of a quality assurance audit to check that the procedures described in the protocol have been followed throughout the study.

16. STUDY DURATION

The study will begin in June 1991 and will last about 3 months.

17. INSURANCE

The investigator's civil liability in the context of this study is covered by an insurance policy taken out by Laboratoires Roussel (Appendix 4).

18. PUBLICATION

Any communication or publication of the results of this study will be the subject of a previous agreement between the investigators and Laboratoires Roussel.

19. DECLARATION AND RESPONSIBILITY OF INVESTIGATOR

All the information relating to the trial drug and the results of the study are considered to be confidential.

I have read the protocol and I consider that it contains all the information necessary for the conduct of the study.

I undertake to carry out this study in compliance with this protocol and will not modify it in any way without the written approval of Laboratoires Roussel.

I undertake not to begin the study before an Ethics Committee has given its approval.

I will conduct the study in accordance with the principles laid down in the Declaration of Helsinki and in compliance with Good Clinical Practice. In particular I will obtain the written informed consent of each patient before her admission to the study.

Furthermore, I also undertake to complete the case report forms carefully, to comply with the procedure in the event of a serious side-effect and to be responsible for the handling of the trial drug.

I agree to the monitoring of the study by a member of Laboratoires Roussel and to the possibility of a quality assurance audit.

I will make all data and information directly concerning the study available to Laboratoires Roussel and the Regulatory Authorities.

I will retain the raw data obtained during this study for a period of 10 years.

Drug name: MIFEPRISTONE
Protocol No.: FFR/91/486/14

Date — Signature of investigator

Date — Signature of the Laboratoires Roussel - Co-ordinators

APPEARS THIS WAY
ON ORIGINAL

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CHECK - LIST

DAY 1: INCLUSION:

- Confirmed pregnancy following a normal course.

- Unambiguous request for an abortion, legal procedures observed.

- Amenorrhoea of less than or equal to 49 days.

- Age more than 18 years (or consent of the legal guardian in the case of patients under the age of majority), and less than or equal to 35 years.

- No contra-indication to the method.

- Explain to the patient the procedure for an abortion and the details of the protocol, obtain her written consent.

- Assay of βHCG and/or ultrasound.

- Assay of haemoglobin, blood group.

- Have the patient take 600 mg (3 tablets of 200 mg) of mifepristone in a single dose in the presence of the investigator.

- Notify the patient of the requirement to refrain from smoking and drinking alcohol during the following 48 hours and on Day 3.

- Appointment for Day 3.

DAY 3: ADMINISTRATION OF MISOPROSTOL:

- Injection of anti-D gamma-globulins if the patient is Rhesus negative.

- Record any symptoms occurring after administration of mifepristone.

- Check that expulsion has not occurred between Day 1 and Day 3.

- In the absence of expulsion, administration of misoprostol: 2 tablets of 0.2 mg in a single dose.

- Monitoring during the 4 hours following this administration:

  . Measure heart rate, systolic and diastolic blood pressure hourly.

  . Investigation of painful uterine contractions, nausea, vomiting, diarrhoea; evaluate their intensity and note any treatment given.

- Appointment for Day 8 - Day 15 with a prescription for an assay of haemoglobin.

- Possible prescription of oral contraception to be instituted 24 to 48 hours later.

DAY 8 - DAY 15: FOLLOW-UP VISIT:

- Evaluation of the efficacy and tolerance of treatment.

- If possible, note the date and time of ovular expulsion.

- Note the results of the assay of haemoglobin.

- In the event of failure (on-going pregnancy or uterine retention), recommend a supplementary surgical procedure.

APPENDIX 1

- Patient information form

- Written consent record form.

APPEARS THIS WAY
ON ORIGINAL

READ THIS NOTICE CAREFULLY AND ASK YOUR DOCTOR TO EXPLAIN
ANY POINTS WHICH ARE NOT CLEAR TO YOU.

BEFORE TAKING MIFEGYNE, THE DOCTOR WILL ASK YOU TO SIGN A FORM
STATING THAT YOU HAVE READ AND UNDERSTOOD THIS NOTICE.

PATIENT INFORMATION

You have requested a termination of your pregnancy. It is proposed that you should participate in a study intended to evaluate on a large scale the efficacy of the combination of Mifegyne and an oral prostaglandin, misoprostol, in the termination of pregnancy.

This study complies with the law on clinical trials and the principles of the Declaration of Helsinki. It has been submitted to the Ethics Committee of the following Hospital
which granted its approval on

A preliminary study has been performed in 100 women and shows that this method appears as effective as that used currently, which combines Mifegyne with a prostaglandin ~~~~~ It is necessary to confirm these results on a larger scale and 500 women will participate in this study. They will be recruited in 24 public or private hospital centres.

Mifegyne is a drug which blocks the action of progesterone, a pregnancy maintenance hormone. Its action however requires to be completed 36 to 48 hours later by that of a prostaglandin, a substance which increases uterine contractions.

Mifegyne may only be used in accordance with the regulations in force on abortion (laws of 1975 and 1979).

The three tablets of Mifegyne must be taken within 49 days after the first day of your last menstrual period.

Mifegyne may not be used in the following cases:

- if the pregnancy is not confirmed,
- in the event of a suspected ectopic pregnancy,
- if the first day of your last menstrual period is more than 50 days
- if you are over the age of 35,
- in the event of one of the following diseases: renal failure, hepatic failure, adrenal insufficiency, abnormality of blood coagulation or administration of an anticoagulant medication, anaemia, asthma or a history of asthma, a history of cardiovascular disorders (angina pectoris, rhythm disorder, heart failure, severe hypertension), diabetes, hyperlipidaemia, glaucoma or raised intra-ocular pressure.
- in the event of prolonged treatment with corticosteroids,
- if you are a smoker (at least 10 cigarettes daily in the previous 2 years).

TERMINATION OF PREGNANCY WITH MIFEGYNE INVOLVES CONSTRAINTS AND IMPLIES RESTRICTIONS OF WHICH YOU MUST BE AWARE

1. It is mandatory that administration of Mifegyne is followed 36 to 48 hours later by the administration of a prostaglandin to obtain the maximum efficacy of the method.

2. Mifegyne is not 100 per cent effective and you cannot by yourself assess the efficacy of the method.
In fact, the uterine bleeding which occurs is not a proof of efficacy and the expulsion of the ovum which often occurs a few hours after administration of the prostaglandin may be incomplete.

It is compulsory for you to attend a follow-up visit 12 to 15 days after administration of Mifegyne to check that your pregnancy has indeed been terminated.

In the event of a failure, the termination of pregnancy or the evacuation of placental debris can only be obtained by surgical methods.

3. As with any termination of pregnancy uterine bleeding (metrorrhagia) will occur in almost all cases. This is sometimes very heavy and may in that case involve an emergency treatment. You should not move too far away from the prescribing centre until the follow-up visit and the doctor will indicate to you where you should telephone or go in the event of an emergency.

4. Abdominal pain requiring treatment, nausea, vomiting, diarrhoea or malaise can occur in some cases after administration of the prostaglandin, which therefore entails observation for a few hours in the prescribing centre.

5. THE FOLLOW-UP VISIT ALLOWS TO CHECK WHETHER THE PREGNANCY HAS BEEN TERMINATED. IN FACT IF THE PREGNANCY WERE TO CONTINUE AFTER ADMINISTRATION OF MIFEGYNE AND THE PROSTAGLANDIN THE FOETUS OR THE FUTURE CHILD ARE LIABLE TO BE MALFORMED.

6. The occurrence of a further pregnancy is possible immediately after the termination of the pregnancy: if you do not wish to have a further pregnancy, contraception must be instituted early on.

7. If you belong to a Rhesus negative blood group, Rhesus immunisation must be prevented.

8. Exceptional cases of cardiovascular accidents have been reported after the injection of a prostaglandin. Consequently the Mifegyne-prostaglandin analogue method is contra-indicated where there is an increased cardiovascular risk from the following factors: smoking, hyperlipidaemia, diabetes, hypertension, cardiovascular history age over 35 years.

9. You must refrain from SMOKING and drinking alcohol during the two days between administration of Mifegyne and administration of the prostaglandin, and on the day of administration of the prostaglandin.

In addition, the study may be discontinued:

a) from medical reasons of which the doctor will be the judge,

b) or at your own request, without any justification being required of you.

A uterine evacuation will then be undertaken at your request and under medical supervision.

In the event of an emergency, or for any other question relating to this study, you can contact by telephone:

. Dr. on number:

APPEARS THIS WAY
ON ORIGINAL

PRACTICAL PROCEDURE OF THE METHOD

DAY OF THE FIRST CONSULTATION

- You are requesting an abortion

- The first day of the last menstrual period is no more than 42 days previously.

- From this Day 0 you have one week for reflection (in accordance with the law on abortion).

ONE WEEK LATER - 2nd STAGE:

- You confirm your request for an abortion.

- You have no contra-indication to the use of Mifegyne and prostaglandin.

- You have read the information notice on Mifegyne, you have obtained any additional information which you require, and you have signed the form confirming that you have been informed.

- You swallow 3 tablets of Mifegyne in the presence of the doctor (Day 1)

- You will return home with a further appointment 48 hours later, knowing where to telephone or to go in the event of an emergency.

- The uterine bleeding usually begins one or two days later.

TWO DAYS LATER (DAY 3):

- You return to the prescribing centre.

- The prostaglandin is administered (2 tablets in a single dose)

- You remain resting for a few hours in the centre and then you return home with, if necessary, a prescription for an oral contraceptive.

- Expulsion of the ovum occurs while you are in the centre or within the following few days.

- The bleeding persists usually until the follow-up visit.

FOLLOW-UP VISIT: 5 to 13 days after administration of the prostaglandin.

- You return to the prescribing centre for the follow-up visit: the doctor will check that expulsion is complete. In the event of an on-going pregnancy or incomplete expulsion the prescriber will recommend a surgical technique (aspiration).

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WRITTEN INFORMED CONSENT

Protocol No.:

Study title:

APPEARS THIS WAY
ON ORIGINAL

I, the undersigned: _____

living at: _____

_____

_____

agree, in full awareness of the facts and of my own volition, to participate in the medical research

conducted by Doctor _____

The medical information obtained during the study is confidential. My identity will not be disclosed in the reports or publications to which this study may give rise.

I am aware that I may refuse to participate in this research or that I may withdraw my consent at any time without incurring any liability on my behalf.

I certify that the objective of the research, the conditions of its performance and its duration have been clearly indicated to me, together with the restrictions and foreseeable risks, including in the event of the research being terminated before its completion. A summary of this information has been given to me.

Treatment number
allocated

|_|_|_|

Place: _____

Date _____

Signature of the subject, preceded by
"Read and approved"

- *The original is to be kept by the investigator for a minimum of 10 years*

APPENDIX 2

- Serious adverse event record form.

APPEARS THIS WAY
ON ORIGINAL

CLINICAL TRIAL
SERIOUS ADVERSE EVENT FORM

➤ TO BE COMPLETED IN CASE OF :

- Life-threatening event
- Death
- Cancer/congenital anomaly
- Event leading to hospitalisation or prolongation of hospitalisation
- Event resulting in chronic condition/sequelae
- Overdose

WHATEVER RELATIONSHIP TO STUDY DRUG

➤ The first copy must be sent to the monitor, the second must be kept by the investigator, the third must be enclosed in the case record form.

➤ Please be as complete and as precise as possible when describing the course of the patient's condition.
If possible, please join a copy of the relevant investigations and forward a hospitalisation report when available.

CLINICAL TRIAL
SERIOUS ADVERSE EVENT FORM

STUDY/INVESTIGATOR

Protocol number : ⌊⌊⌊⌊⌊⌊⌊⌊⌊⌊

Center number : ⌊⌊⌊⌊

Indication : _____

_____

Investigator's name : _____

Address : _____ Country : _____

_____

_____

PATIENT

Number allocated in the study ⌊⌊⌊⌊⌊⌊

Local drug surveillance number ⌊⌊⌊⌊⌊⌊⌊⌊⌊⌊

Initials	Age		Sex	Weight		Height	
⌊⌊⌊	⌊⌊ y	⌊⌊ m	M ⌊ F ⌊	⌊⌊⌊ kg	⌊⌊⌊ g	⌊⌊ m	⌊⌊ cm

Occupation : _____ Ethnic origin : _____

Previous relevant history : _____

_____

_____

_____

Previous Intolerance to drugs : No ⌊ Yes ⌊ which drugs ? _____

_____

Unknown ⌊

ADVERSE EVENT

Date of onset ⌊⌊ D ⌊⌊ M ⌊⌊ Y

Description : _____

_____

Hospitalisation (or prolongation of hospitalisation) necessary ? ⌊ yes ⌊ no

Treatment : _____

_____

_____

OUTCOME : Complete recovery ⌊ Chronic condition or sequelae ⌊

Not yet resolved ⌊ Unknown ⌊

Death ⌊

➤ Date ⌊⌊ D ⌊⌊ M ⌊⌊ Y

➤ Autopsy ⌊ yes ⌊ no

➤ Cause of death _____

CASE SUMMARY

(Precise description of history with respect to the adverse event)

STUDY DRUG

Name _____

or Code ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋

Daily dose regimen : dose ⌊_⌋⌊_⌋ unit _____ frequency _____

Route _____

Therapy date : from ⌊_⌋⌊_⌋ D ⌊_⌋⌊_⌋ M ⌊_⌋⌊_⌋ Y to ⌊_⌋⌊_⌋ D ⌊_⌋⌊_⌋ M ⌊_⌋⌊_⌋ Y Ongoing ⌊_⌋

Action taken with study drug after the event :

Continued same dose ⌊_⌋ Stopped ⌊_⌋ Decreased ⌊_⌋ NA* ⌊_⌋

Immediate results :

Improvement ⌊_⌋ No change ⌊_⌋ Aggravation ⌊_⌋ Uninterpretable ⌊_⌋ NA* ⌊_⌋

Rechallenge

No ⌊_⌋ Yes ⌊_⌋ NA* ⌊_⌋

Date : ⌊_⌋⌊_⌋ D ⌊_⌋⌊_⌋ M ⌊_⌋⌊_⌋ Y

Recurrence of event :

No ⌊_⌋ Yes ⌊_⌋ NA* ⌊_⌋

Uninterpretable ⌊_⌋

* Not applicable

APPEARS THIS WAY ON ORIGINAL

CONCOMITANT DRUGS

Drug	Daily dose	From	To	Indication

CAUSAL RELATIONSHIP

Doctor's assessment :

Unrelated ⌊_⌋ Unlikely ⌊_⌋

possible ⌊_⌋ probable ⌊_⌋ highly probable ⌊_⌋

not assessable ⌊_⌋ explain why _____

This form was filled out ➤ on ⌊_⌋⌊_⌋ D ⌊_⌋⌊_⌋ M ⌊_⌋⌊_⌋ Y

➤ by (print)

➤ position

➤ signature : 110 d

➤ Monitor's name and signature : _____

APPENDIX 3

Declaration of Helsinki

APPEARS THIS WAY
ON ORIGINAL

WORLD MEDICAL ASSOCIATION
DECLARATION OF HELSINKI

Recommendations guiding physicians in biomedical research involving human subjects.

Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, and amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983, and the 41st World Medical Assembly, Hong Kong, September 1989.

Introduction

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration", and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

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 especially 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 implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may effect the environment, and the welfare of animals used for research 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 physician 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. Physicians 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 for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

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 interests 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. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians 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 physician is obliged to preserve the accuracy of the results. Reports on experimentation not in accordance with the principles laid down in the 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 bastain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician 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 physician who is not engaged in the investigation and who is completely independent of this official relationship.

120

11. In the 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. Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

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 physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, reestablishing 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 a patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician 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 physician 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. NONTHERAPEUTIC 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 physician to remain the protector of the life and health of that person on whom biomedical research is 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 to research if in his/her or their judgement 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.

APPENDIX 4

Insurance

APPEARS THIS WAY
ON ORIGINAL

Postal address: [ ]

TO WHOM IT MAY CONCERN

_____ certifies that the insurance policy No. _____ underwritten with the _____ a French Insurance Company, for the companies of the ROUSSEL UCLAF GROUP, and specifically for

ROUSSEL SANTE R. ET D.

covers:

- the legal liability incurred by the insured companies arising from experiments in respect of:

  . research pertaining to medicinal products,

  . relating to the product licence procedure,

- the legal liability incurred by the investigators working:

  . in research on behalf of the insured companies,

  . for the product licence procedure on behalf of the same companies.

In application of the regulation in force concerning biomedical research conducted in France, this insurance covers, up to the limit of the sums laid down in the policy, the liability of the sponsor for harmful consequences of the research to the person participating in it, without prejudice to the possible claims against parties other than the investigators and their colleagues where these are acting on behalf of the insured companies, and where this biomedical research is conducted in accordance with this regulation.

The present certificate does not commit the insurer beyond the limits of the policy to which it refers.

Paris, 15th January 1991

The Managing Director

APPEARS THIS WAY
ON ORIGINAL

APPENDIX 1

PROTOCOL
RANDOMISATION
STUDY BOOK

Final Version, November 27, 1985

Protocol F/85/.486/34

DOUBLE-BLIND DETERMINATION IN HEALTHY VOLUNTEERS OF THE OPTIMUM TIME INTERVAL BETWEEN A SINGLE 600-mg DOSE OF RU 38486 AND A 1-mg DOSE OF DEXAMETHASONE REQUIRED TO INHIBIT THE SUPPRESSIVE EFFECT OF DEXAMEHTASONE ON THE CIRCULATING WHITE BLOOD CELLS

Investigator:

[ ]

Study Leader:
Dr. Andre ULMANN

Clinical Research Assistant:
_____

DIRECTION MEDICALE
ROUSSEL-UCLAF
102-111 Route de Noisy
93230 ROMAINVILLE
Tel.: (1) 48 43 93 10

DOUBLE-BLIND DETERMINATION IN HEALTHY VOLUNTEERS, OF THE OPTIMUM TIME INTERVAL BETWEEN A SINGLE 600-mg DOSE OF RU 38486 AND A 1-mg DOSE OF DEXAMETHASONE REQUIRED TO INHIBIT THE SUPPRESSIVE EFFECT OF DEXAMETHASONE ON THE CIRCULATING WHITE BLOOD CELLS.

RU 38486 (RU 486, Mifepristone)is an original product synthesised by the Roussel-Uclaf research department and shown to bind hormone receptors . Pharmacological studies in the animal have shown that this product has an anti-progesterone, anti-glucocorticoid and slight anti-androgen activity.

A first single-dose clinical pharmacology study showed that the doses administered (50 to 400 mg) were extremely safe according to the clinical and laboratory findings and that from a dose of 200 mg, RU 38486 administered at 2 a.m. caused a significantly greater increase in cortisol and LPH than placebo between 7 and 11 a.m (1).

This increase in cortisol and LPH was interpreted as an effect of the antiglucocorticoid action of the product on the pituitary gland. This is the easiest effect to demonstrate after a single dose of the product.

A second study showed that RU 3486 inhibited the fall in circulating eosinophils induced by the administration of dexamethasone, with a dose-response effect (2).

As it is easy to demonstrate the antiglucocorticoid effects of RU 486, it is important to determine the duration of this effect. The measurement of the inhibitory effect of dexamethasone on the eosinophils at variable times after RU 486 administration makes it possible to answer this question .

To date, approximately 500 patients have received RU 486 and no notable clinical side effect or change in a laboratory parameter has been reported. A safety study (3), involving the administration of increasing single doses of RU 486 up to 2 g, failed to demonstrate any adverse effect.

1. Purpose

To study the effect of a single 600-mg dose of RU 486, 1h, 13h or 25 h before studying the action of 1 mg of dexamethasone on the circulating eosinophils.

2. Experimental Design

2.1 Latin square, double-blind trial on 10 healthy subjects receiving in succession, at one week intervals, one of the following five treatments (order determined by drawing lots):

Treatment number	First Day Time		Second Day Time	
	7.30a.m	7.30p.m	7.30a.m	8.30 a.m
1	P	P	P	P
2	P	P	P	DEX
3	P	P	RU 486	DEX
4	P	RU 486	P	DEX
5	RU 486	P	P	DEX

(P = RU 486 Placebo, p = dexamethasone placebo, DEX = Dexamethasone)
In each case, the eosinophils were counted at 7.30a.m and 1.30 p.m.

APPEARS THIS WAY
ON ORIGINAL

3. CHOICE OF SUBJECTS

3.1. 10 subjects will participate in the study after giving their informed consent in writing.

3.2. Inclusion Criteria

Subjects must meet the following criteria :

- Men aged from 18 to 40 years,
- Body weight not deviating by more than + or - 10% from the ideal weight for their age and height ,
- Normal findings in the clinical examination and normal laboratory values.

3.3. Exclusion Criteria

The following subjects will be excluded from the study:

- those with a history of allergy or hypersensitivity to drugs,
- those regularly using drugs or having been treated in the 3 months before the test with a drug with known toxicity (cf chloramphenicol) or with a very slow elimination from the organism ,
- those abusing tobacco or alcohol,
- those having suffered a serious acute illness during the month before the test,
- those with a history of gastro-intestinal, hepatic or renal disorders liable to interfere with the absorption, metabolism or excretion of the product .

4. CONDUCT OF THE TRIAL

4.1. Inclusion visit

-Clinical examination,

-Laboratory tests. All findings should be normal.

4.2. Diet

The subjects will follow their normal diet.

4.3. Alcohol and drugs

- The consumption of alcohol will not be permitted for 24 hours before each dose of the product and up to the end of each test.
- No drug should be absorbed during the 8 days before the study and throughout its duration.

4.4. Conduct of the Study

The protocol will comprise, per subject, 5 treatment periods, each separated by one week.

4.5. Conduct of each test

4.5.1 First Day :

- administration of RU 486 or its placebo at 7.30 a.m and 7.30p.m.

4.5.2 Second Day:

- 7.30 a.m : administration of RU 486 or its placebo and blood sample for measuring the eosinophils and hormone assays.
- 8.30a.m : administration of dexamethasone or its placebo .
- 1.30 p.m : new blood sample for determination of the eosinophils and hormone assays.

The subjects will continue their usual activity during each test.

5. PRODUCT

5.1. Dexamethasone (dectancyl):

This will be supplied in the form of tablets containing 0.5 mg of active principle . The corresponding placebo will be in the form of tablets with an identical appearance.

5.2. RU 486:

This will be supplied in the form of tablets containing 200 mg of active principle. The corresponding placebo will be in the form of tablets with an identical appearance.

5.3. Packaging :

The products tested, in their verum and placebo forms will be supplied by Roussel-Uclaf in the packaging described below.

According to the study design with two equivalent latin squares :

- 10 cartons will be prepared labelled as follows :
  subject number (from 1 to 10)
  name of subject :

- each carton will contain 5 packages labelled with the treatment intake number :
  subjet number :
  1st (or 2nd , or 3rd ...) intake

- each of these packages will contain four bottles labelled with the day and time :

  subject number :
  intake number :
  day 1 or 2:
  time 7.30 a.m or 7.30 p.m or 7.30 a.m or 8.30 p.m :

A third batch of five cartons, numbered from 11 to 15 will be prepared according to the same principles, following a latin square equivalent to the two first batches. These cartons will only be used if (an) additional subject(s) is/are included in the study to replace excluded subjects.

The investigator will then contact RU (Dr. Ulmann ——— who will tell him which number from 11 to 15 should be assigned to the new subject , so that he receives treatments in the same frame as that initially assigned to the replaced subjet (maintenance of the latin square).

5.4. Assignment of treatment :

Each patient will be assigned a number. He will be assigned one carton corresponding to this number and containg the five cartons corresponding to the five intakes .

6. CRITERIA OF EVALUATION

6.1. Efficacy

- measurement of the circulating eosinophils and neutrophils : this will be carried out by the ———————————————

  A differential count will be carried out on each sample using a regularly calibrated ——————— counter.

  The differential white cell count will be made by counting 200 WBC per smear , with 5 smears per sample. The smears will be made using a spinner and will be read after Wright staining by an automatic blood cell counter ———————
- Hormone assays : cortisol, ACTH , LPH, at 7.30 a.m and 1.30 p.m , assayed by the ———————. Endocrinology department , and plasma RU 486 determined by Roussel-Uclaf at Romainville.

6.2. Safety

The absence of change in any of the following parameters measured on inclusion and at the end of the final intake will be checked :

Hemogram
SGOT-SGPT
Blood glucose
creatinine

7. CONDUCT IN THE CASE OF ADVERSE EFFECTS

The subjects are free to drop out of the study at any moment.

In the case of any unusual sign or symptom, the investigator will take the necessary measures and immediately inform the Roussel-Uclaf study leader:

Dr. A; Ulmann
Direction Medicale a Romainville
Tel. 843. 93 10 3420

8. STATISTICAL ANALYSIS

This will be carried out by the Biometry department of the Roussel-Uclaf medical department.

9. DROPS-OUT

Any subject dropping out of the trial, for whatever reason, will be replaced by a subject receiving the same treatment in order to respect the latin square design, according to the procedure described in paragraph 5.3.

10. ETHICS COMMITEE

In accordance with the current rules of the Roussel-Uclaf medical department, this protocol will be submitted to the Roussel-Uclaf ethics commitee. The investigator may, if he so desires, submit the protocol to the ethics commitee of his choice.

APPENDIX I

1. Protocol
2. Randomisation
3. Patient record form

INVESTIGATION OF THE SINGLE ORAL DOSE OF DEXAMETHASONE SUPPRESSING THE ANTIGLUCOCORTICOID EFFECT OF A SINGLE ORAL DOSE OF 400 MG OF RU 486

1. INTRODUCTION

1.1. Description of the compound

RU 486 is a steroid possessing antiprogesterone, antiglucocorticoid and anti-androgen properties without direct action on the oestrogen and mineralocorticoid systems. It has already been administered in single oral doses of 800 mg to healthy volunteers without appreciable side effects. On this occasion it revealed its antiglucocorticoid effect, one sign of which was the rise in plasma ACTH, LPH and cortisol concentrations.

More detailed information is available in the "Investigators' Brochure" of RU 486 which summarises the main results obtained with the compound.

1.2. Aim of the study

- To discover the single oral dose of dexamethasone which suppresses the antiglucocorticoid action of a single dose of 400 mg of RU 486.

- To confirm the good clinical and biological tolerance of RU 486.

1.3. Context of the study

The reason behind the study is to know, when the compound is used for its antiprogesterone effect, what dose of glucocorticoid is capable of lifting the peripheral glucocorticoid blockade caused by RU 486 in case of necessity.

2. DESCRIPTION OF THE STUDY

2.1. Study design

- A single group of 10 subjects.
- Crossover for 5 sequences of treatment separated by an interval of 7 days using a Latin square design.
- Randomised double-blind.
- Independent observer for the main criteria.
- Double dummy technique.

2.2. Allocation of treatment

Allocation will be by two equivalent randomised Latin squares, each corresponding to a subgroup of five subjects.
The subjects shall be numbered from 1 to 10 in the order in which they are recruited.
The randomisation code shall be kept by the ROUSSEL UCLF Biometrics department. Individual sealed envelopes for each subject containing the details of the treatment received at each sequence (i.e. a total of 50 envelopes) shall be given to the investigator in case decoding is necessary in an emergency.

3. SELECTION OF SUBJECTS

3.1. Inclusion criteria

- Healthy male subjects
- Aged 18 to 35 years.

3.2. Exclusion criteria

- Abnormality at the full clinical examination done within the 15 days prior to the study.
- Abnormality in the laboratory examinations done a week before the beginning of the study and including:

  . Complete blood count
  . Platelet count
  . Prothrombin level
  . Partial activated thromboplastin time
  . SGOT and SGPT
  . Alkaline phosphatase
  . Blood bilirubin
  . Blood creatinine
  . Blood cholesterol
  . Blood triglycerides
  . Blood glucose
  . Electrolyte composition of the blood

  These examinations shall be done on an empty stomach in the morning.

  . glycosuria and albuminuria (reagent strips)

- Contra-indication to the administration of glucocorticoids or anti-glucocorticoids
- Current drug treatment of whatever nature
- Alcoholic intoxication
- Smoking more than 10 cigarettes per day or the equivalent.

3.3. Recruitment of subjects

Healthy subjects working on the premises of the study, who shall be contacted directly by the investigator.

3.4. Number of subjects

It is planned to obtain 10 complete observations.

4. TREATMENTS

4.1. Test compounds

4.1.1. RU 486

Form: scored tablet, either the active treatment containing 50 mg of active ingredient, or placebo, according to the treatment allocation schedule,

Dose: 8 tablets, correponding to 400 mg of RU 486 for the active treatment, in a single dose,

Route: Oral

Conditions of administration: On the evening of day 0 at 10 p.m., after 2 hours' fast following a light evening meal, with 250 ml of water, the 8 tablets being swallowed all at once.

4.1.2. Dexamethasone

Form: tablet, either the active treatment containing 0.50 mg of active ingredient, or placebo, according to the treatment allocation schedule,

Dose: 8 tablets, some of which will possibly be placebo, so as to be able to administer 0, 1, 2 or 4 mg of dexamethasone depending on the treatment period and according to the treatment allocation schedule.

Conditions of administration: In the evening of day 0 at 12 a.m. in a single dose with 250 ml of water, i.e. 2 hours after administration of RU 486, with the subjects not having swallowed anything since then and remaining fasted until the following morning.

4.1.3. Treatment schedule

On the basis of two equivalent randomised Latin squares with 5 columns and 5 lines, each corresponding to a group of 5 subjects, each subject shall receive in 5 treatment periods:

- RU 486 placebo and dexamethasone placebo
- RU 486 active substance and dexamethasone placebo
- RU 486 active substance and 1 mg of dexamethasone
- RU 486 active substance and 2 mg of dexamethasone
- RU 486 active substance and 4 mg of dexamethasone

4.2. Supply of compound

The test compounds, active and placebo, will be provided by ROUSSEL UCLAF in the packaging described below:

In accordance with the design of two equivalent randomised Latin squares:

- 10 boxes shall be prepared, bearing the following information:

. the subject's number: Subjet no. 1,2,3,4,5,6,7,8,9 or 10
. the subject's name, to be completed by the investigator on the subjects' admission : SUBJECT'S NAME _____

- each box shall contain 5 packages indicating the number of the treatment sequence: first, second, third, fourth or fifth treatment sequence.

- each of these packages shall contained 2 sealed sachets with the following information, depending on whether they contain RU 486 or dexamethasone:

Either: RU 486: take all the tablets contained in this sachet at once with 250 ml of water at 10 p.m. after 2 hours' without eating or drinking.

Or: DEXAMETHASONE: take all the tablets contained in this sachet at once with 250 ml of water at 12 midnight, again on an empty stomach including all fluids. Do not eat or drink anything until the blood sample the following morning.

- The sachets shall contain 8 tablets of RU 486 or dexamethasone in accordance with the labelling; these tablets shall include placebo tablets in varying proportions according to the treatment allocation list.

A third batch of 5 boxes, numbered 11 to 15, shall be prepared on the same principle, based on a Latin square equivalent to the first 2. These boxes shall only be used if one or more additional subjects are included in the study to replace excluded subjects (see] 9).

The investigator shall then contact ROUSSEL UCLAF [redacted], who shall indicate which number from 11 to 15 should be allocated to the new subject so that he receives the treatments in the same order as that originally allocated to the substituted subject (maintaining the Latin square).

4.3. Storage of compounds

These shall be stored by the investigator under the conditions stipulated for each of the compounds (ordinary temperature) and away from other compounds used for clinical purposes on the premises where the study is conducted.

4.4. Concurrent treatment

No concurrent treatment, whatever the duration, is permitted during the study; this applies also to commonly used comfort analgesics, such as aspirin. Any deviation shall be noted in the patient record form and shall result in the exclusion of the subject concerned who shall then be replaced.

5. CRITERIA OF ASSESSMENT

. The criterion used shall be the plasma assay of cortisol, ACTH and LPH, done at 9 a.m. and 12 noon on the 3 days (days 1, 2 and 3) after administration of the compounds.

These assays shall be done as a block from correctly identified frozen plasma by a technician who is unaware of the treatment schedule of each subject.

The results shall be transcribed by the investigator into the record forms.

. Plasma shall be taken and frozen for assay of plasma RU 486 and dexamethasone on the morning of day 1 at 9 a.m. (2 tubes containing 5 ml of blood each).

6. STUDY PROCEDURE

6.1. Recruitment and selection

The subjects shall have an initial clinical and laboratory examination in the 15 days preceding the beginning of the study (cf.] 3). The decision to admit the patient shall be taken once all the results of these examinations are known.

6.2. Identification of the patients

The patients shall be identified by their number in the study (cf] 2.2) and the first 3 letters of their surname, except in the event of a possible confusion in which case the first 4 letters and/or the Christian name will be used.

6.3. Procedure

- Each treatment period shall last 4 days; by convention day 0 shall signify the day of administration of the compounds and days 1, 2 and 3 the days of follow-up and hormonal assays.

6.3.1. For each treatment period

- Each subject at each of his 5 successive treatment periods (cf.] 2.2., 4.1) shall receive the treatment given to him by the investigator on the evening of day 0.
- On days 1, 2 and 3 the samples for the assay of cortisol, ACTH and LPH shall be taken at 9 a.m. and 12 noon. The subjects shall have fasted since the previous evening and shall receive a low-fat breakfast after the blood sample at 9 a.m.
- In addition, on the morning of day 1 a blood sample shall be taken at 9 a.m., decanted and frozen in 2 separate tubes to assay plasma RU 486 and dexamethasone.

6.3.2. For the whole study

Each of the 5 treatment periods shall be at an interval of 7 days from the previous one.

Laboratory examinations (cf.] 3.2) shall be done between the 3rd and 4th treatment periods (after day 3 of the 3rd period) and also after day 3 of the 5th period. Any abnormality in these examinations will result in them being repeated at fortnightly intervals until normalisation, independently of any treatment which might be required by this abnormality.
Any anormality in the interim examination, except in the case of an abnormality without pathological significance, would result in the study being suspended for the subject in question.

7. PROTOCOL MODIFICATIONS AND DEVIATIONS.

Any modifications made to the original protocol shall be detailed in the appendix to the present protocol, together with the date of the amendment. Such modifications may only be made with the express agreement of ROUSSEL UCLAF.
Any deviations from the protocol shall be recorded on the patient record form.

8. SIDE EFFECTS

These shall be recorded on non-specific forms supplied to the subjects in the study. On days 0, 1, 2 and 3 they shall note the following:

- description of the symptom,
- date and time of occurrence,
- any treatment,
- duration of the abnormality.

Any side effect or serious incident, whatever the cause, occurring in a subject participating in the study shall be reported immediately to ROUSSEL UCLAF.

9. SUBJECTS DISCONTINUING THE STUDY

Any subject discontinuing the study shall be replaced by a new subject who shall receive all the 5 study treatments in the order allocated to the substituted subject.

The reason for any withdrawals shall be detailed in the corresponding record form.

10. STATISTICAL ANALYSIS

10.1. The data shall be analysed by the Biometrics department of ROUSSEL UCLAF. The data relating to the assessment criteria (assays of cortisol, ACTH, LPH) shall be processed by analysis of variance allowing for the repetition of measurements in the same subject and the Latin square design. The data relating to the tolerance (laboratory tests and side effects) shall be analysed, depending on the case, by analysis of variance or non-parameteric tests. Statistical analysis will be done by SAS (Statistical Analysis System) software.

10.2. Subjects discontinuing the study shall not be included in the overall analysis. Part data relating to them shall be used as far as possible, particularly in evaluating the tolerance of treatment.

10.3. Data processing

10.3.1. Corrections

These may only be made by the investigator at his discretion and signed by him. The reason for any corrections shall then be specified.

10.3.2. Dispatch

The complete observations shall be forwarded to Roussel-Uclaf as a block at the end of the study.

10.3.3. Report

This shall be prepared in close co-operation between Roussel-Uclaf and the investigator.

11. ORGANISATION

11. Agreements and consent, ethics, undertakings

11.1.1. The subjects' consent shall be obtained before the beginning of the study after he has been as fully and truthfully informed as possible as to the treatments and course of study.

They shall be informed in particular that they may withdraw from the study at any time without having to provide a reason.

11.1.2. In conducting this study, the investigator and Roussel-Uclaf undertake to respect at the least the requirements relating to clinical research activities as stipulated in the Declaration of Helsinki.

11.1.3. The protocol shall be submitted to an independent Ethical Committee for their approval.

11.1.4. Confidentiality - Publication

The data relating to this study are the property of Roussel-Uclaf and shall be considered as confidential by the investigator. If their scientific value justifies publication, this shall only be done with the express agreement of Roussel-uclaf.

11.2. Financing of the study

The study shall be financed by Roussel-Uclaf who shall be responsible for the following:

- The cost of laboratory and hormonal examinations in the study,
- The cost of the work of clinicians involved in the study,
- The payment of a part-time technician, recruited on the site by the investigator and whose presence is necessary for the proper conduct of the study throughout its duration,
- The payment of the subjects
- Any additional expenses required for the proper conduct of the study not anticipated in this section.
- These items shall be the subject of a separate agreement between Roussel-Uclaf and the investigator.

11.3. Timetable

- Duration of the study: 4 months
- Scheduled commencement in December 1984
- Report: after statistical analysis of the data = Q 3 1985

11.4. Study follow-up

The study shall be monitored at regular intervals by the Roussel-Uclaf clinical research staff throughout its course by means of site visits and telephone communication in order to ensure the proper conduct of the experiment in compliance with the protocol.

All the patient record forms shall be completed and signed by the investigator. Missing information shall be indicated as such with the reasons for this omission.

11.5. End of the study

11.5.1. The material (drugs) not used at the end of the study shall be returned immediately to Roussel-Uclaf.

11.5.2. The volunteers shall if necessary be followed up by the investigator, aided by competent persons, for the length of time necessary for any pathological state occurring under treatment to resolve itself.

Investigator's signature

Professor F. GIRARD

Paris,

APPEARS THIS WAY
ON ORIGINAL

2 MATERIAL AND METHODS

2.1 PROTOCOL

The protocol is given in full in appendix IV.

2.1.1 Subjects

In accordance with the recommendations for clinical trials in man set out in the "Declaration of Helsinki", the study was conducted in healthy female volunteers who, after being fully informed of the action of the active ingredient and the nature and conditions of the study, had given their informed consent.

The 12 subjects who participated in this study were women aged from 20 to 40 years old, of child-bearing age, with a menstrual cycle of a constant length and free from any clinically detectable diseases, hypersensitivity to drugs or disorders of the gastro-intestinal tract, kidney or liver liable to interfere with the absorption, metabolism or excretion of the compound. The subjects received no medication in the week prior to administration or during the study. In addition, subjects had either to have an IUD which had been inserted at least 6 months previously, or have undergone tubal ligation, or not have any partner during the study. A mandatory pregnancy test was performed during the 24 hours before administration of RU 38 486, except in the case of subjects with a tubal ligation. In the event of a positive assay of B.H.C.G (human chorionic gonadotropin) the subject had to be excluded from the study.

2.1.2 Dosage form

The compound was presented by the [redacted] in tablets containing 50 mg of active ingredient, reference RG 129934106.

2.1.3 Method of administration

Each subject received the 3 doses of RU 38 486 (50 mg 1 tablet, 150 mg 3 tablets and 450 mg 9 tablets) successively in random order according to a Latin square design. Twenty-four hours before each dose a pregnancy test was performed and in the event of a negative test the active ingredient was administered between the third day before the expected date of menstruation and the second day after this date. The subjects admitted to the study had fasted for at least 10 hours and the tablet or tablets were administered with 150 ml of water. A solid fast was maintained for four hours after administration, after which time a light lunch was served. The subjects were able to resume their normal eating habits eight hours after administration of RU 38 486.

2.1.4 Blood samples

Blood samples of varying volume (5 or 10 ml) were taken by veni- puncture and collected in dry heparinised tubes at the following times:

10 ml at time 0 (five minutes before administration)
5 ml at times: 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 4.0, 8.0, 12 and 24 hours after administration
10 ml at times: 36, 48, 60, 72, 96 and 120 hours after administration.

The samples were centrifuged and the plasma separated, frozen and stored at -20°C until assay.

2.2 ASSAY OF RU 38 486 AND THREE OF ITS METABOLITES

After organic extraction of the plasma, RU 38 486 and three of its metabolites (RU 42 633, the N-monodemethylated derivative, RU 42 848, the N-didemethylated derivative, and RU 42 698, the hydroxylated derivative on the propargyl group) were assayed by two high performance liquid chromatography methods with ultraviolet detection. Two types of column were used, as the first supplier ——— could not guarantee that its supplies were of a constant quality.

2.2.1 Equipment

2.2.1.1 Chromatographic apparatus

- [illegible]
- [illegible] automatic injector, [illegible]
- [illegible]
  - Circulation cell: 12 µl
  - Optical pathlength: 10 mm
  - Wavelength: 304 nm
  - Sensitivity: 0.01 AUFS
- Stainless steel µ [illegible] column, [illegible]
  - Dimensions: 300 mm x 3.9 mm internal ∅
  - Support: micro-Porasil
  - Bonded layer: octadecylsilane groups
  - Bonding rate: 1 microMole.m^2
  - Particle size: distribution about 10 µm
  - Stability range: pH3 to pH8
- Or stainless steel [illegible] column, [illegible]
  - Dimensions: 150 mm x 4.6 mm internal ∅
  - Support: Zorbax-Sil
  - Bonded layer: octadecylsilane groups
  - Particle size: distribution about 5 µm
  - Stability range: pH3 to pH8
- Recording and calculation: single channel integrator-calculator comprising an alphanumeric keyboard and a [illegible] recorder-printer.

2.2.1.2 Extraction equipment

- 20 ml extraction tubes (ground glass stoppers)
- 5 ml round-bottomed tubes [illegible] disposable)
- [illegible] sampling vials (for [illegible] injector)
- [illegible] horizontal shaker, [illegible]
- [illegible] refrigerated centrifuge
- Brand manual piston pump
- Millipore filter unit
- [illegible] ultrasonic bath.

2.2.1.3 Reagents

- Ethanol, spectrographic grade, ———
- Acetonitrile, spectrographic grade, ———
- Redistilled ethyl acetate
- Demineralised water filtered on a Millipore 0.45 µm filter
- Pic B7 (heptane sulphonic acid) ———, No 85103
- Sodium dihydrogen phosphate with 2 molecules of water ($NaH_2PO_4, 2H_2O$) ———
- Lyophilised plasma (National Blood Transfusion Centre)

2.2.1.4 Reference products

- RU 38 486 batch 6 3E 0551
- RU 42 633 (N-monodemethylated derivative) batch 1
- RU 42 848 (N-didemethylated derivative) batch 1
- RU 42 698 (hydroxylated derivative on the propargyl group) batch 1
- R 2323 (Gestrinone) batch 68 3E 0222
- RU 39 974 batch 1

The chemical structure of the compounds listed is given in figure 1 overleaf.

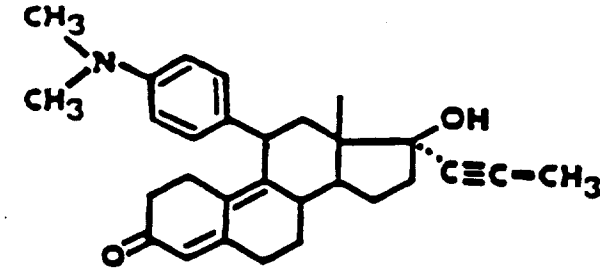
2.2.2 Techniques employed

2.2.2.1 Plasma processing

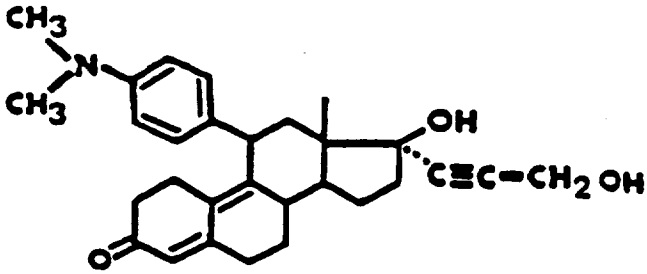
To 1 ml of plasma to be assayed* are added:

- 1 ml of filtered demineralised water
- 0.1 ml of methanolic solution of internal standard, 8 $\mu g.ml^{-1}$ of R 2323 or 3 $\mu g.ml^{-1}$ of RU 39 974.
- 10 ml of redistilled ethyl acetate.

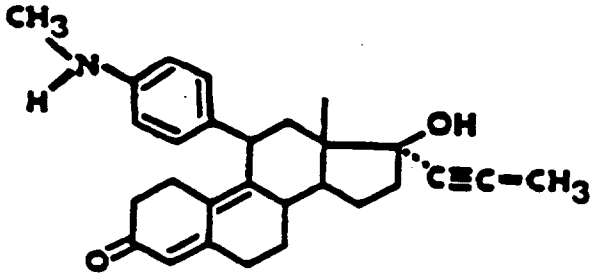
* If the plasma concentrations are greater than 250 $ng.ml^{-1}$, a lower volume of plasma made up to 1 ml with demineralised water was used. The results obtained were then multiplied by the dilution factor. The extraction protocol was the same for both chromatographic methods.



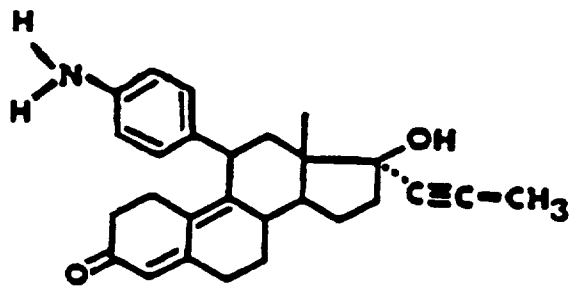
RU 38 486



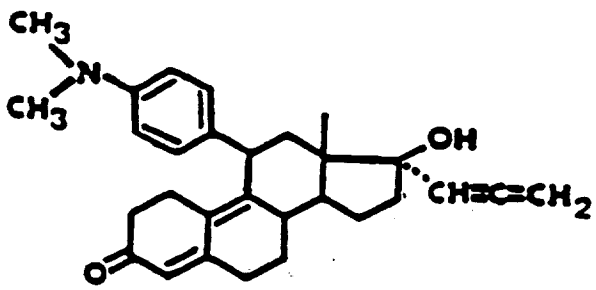
RU 42 698



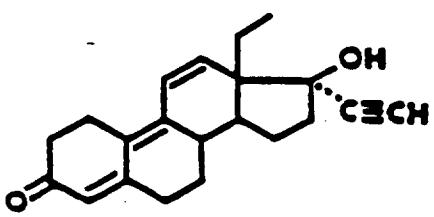
RU 42 633



RU 42 848



RU 39 974



R 2323

Chemical structure of the compounds mentioned in the report

FIGURE 1

After homogenisation, the tube was shaken mechanically for 10 minutes on the ——, at 300 revolutions per minute and then centrifuged at 4000 revolutions per minute for 10 minutes. The organic phase was removed and the extraction repeated once. The solvent phases were evaporated together to about 2 ml under a nitrogen current on a water bath at 37°C and then decanted into a 5 ml haemolysis tube and evaporated to dryness under a nitrogen jet. The dry extract was dissolved in 0.1 ml of methanol, the solution decanted into a small-volume cone for the —— automatic injector and two injections of 0.04 ml were made into the column.

2.2.2.2 Calibration curve

The calibration curve was established by spiking reconstituted lyophilised plasma (National Blood Transfusion Centre) with RU 38 486, RU 42 633, RU 42 698 and RU 42 848 so that it contained concentrations equal to 0, 10, 20, 40, 60, 80, 100, 200 and 250 ng of each compound per ml of plasma. With the exception of point 0 (plasma control), all the points on the plasma curve were spiked uniformly with 0.1 ml of internal standard solution, either 8000 $ng.ml^{-1}$ of R 2323 or 3000 $ng.ml^{-1}$ of RU 39 974. The plasma was processed as above and each point determined in duplicate.

The calibration ranges were obtained by plotting the curves of the plasma concentrations against the ratios of the heights of the peaks R_1, R_2, R_3 and R_4:

$$\frac{\text{Height of the peak of the compound to be assayed}}{\text{Height of the peak of the internal standard}}$$

Over the range of concentrations used, this plot is a straight line. In practice, the calibration curves were fitted by linear regression in the form of the least squares so as to obtain the equation of the regression line $Y = ax + b$ in which:

Y = plasma concentration of RU 38 486 or its metabolites (expressed in $ng.ml^{-1}$)

X = ratio R_1, R_2, R_3, R_4

a = slope of the regression line

b = intercept of the line with the Y axis

The plasma concentrations of the samples to be analysed were calculated directly from these equations.

2.2.2.3 Conditions of chromatographic analysis

- Method

The five compounds, RU 38 486, RU 42 633, RU 42 848, RU 42 698 and the internal standard were chromatographed on a reversed-phase column ——— group). Two stationary phases were used, μ ——— , labelled A, requiring the formation of pairs of ions, and ——— , labelled B, not necessitating the formation of a complex to obtain satisfactory separation. The internal standard used with the first stationary phase was R 2323 and with the second RU 39 974.

- Characteristics

With the two supports, the compounds were chromatographed in an isocratic system at a flow rate of 1 $ml.min^{-1}$ and a temperature of 20°C. In both cases, the extract to be analysed was injected automatically —— injector) in a volume of 40 μl and the compounds detected under ultraviolet light at 304 nm. The chromatogram tracing was recorded at a chart speed of 0.5 $cm.min^{-1}$. With column A —— the inlet pressure was 90 bars and with column B —— 125 bars.

- Composition of the mobile phases and observed retention times :

. Mobile phase with support A (μ ———

Acetonitrile	3.5 V
Filtered demineralised water	6.5 V

+ 1 vial of Pic B7 per litre of mobile phase (the final concentration of heptane sulphonic acid thus being 0.005 M).

Under these conditions the retention times were:

RU 42 968	7.3 minutes
RU 42 848	12.8 minutes
RU 42 633	15.2 minutes
RU 38 486	17.7 minutes
R 2323 (I.S.)	24.4 minutes

. Mobile phase with support B

0.1 M aqueous solution of sodium dihydrogenphosphate, $2H_2O$ 5 V

Acetonitrile 3 V

Ethanol 2 V

Under these conditions the retention times were:

RU 42 968	8.6 minutes
RU 42 848	6.1 minutes
RU 42 633	11.1 minutes
RU 38 486	21.2 minutes
RU 39 974 (I.S.)	26.6 minutes

- Evaluation of RU 38 486 and its metabolites

RU 38 486, the metabolites and the internal standard were identifed by their retention times. The heights of the chromatographic peaks were measured to determine the ratios R_1, R_2, R_3 and R_4, defined as follows:

$$\frac{H \text{ of RU 42 698}}{H \text{ internal standard}}$$

$$\frac{H \text{ of RU 42 848}}{H \text{ internal standard}}$$

$$\frac{H \text{ of RU 42 633}}{H \text{ internal standard}}$$

$$\frac{H \text{ of RU 38 486}}{H \text{ internal standard}}$$

The plasma concentrations of RU 38 486 and the metabolites were calculated directly from the equations of the calibration curves (calculations done on the ~~~ computer). The limit of detection corresponded to the first point on the calibration range, i.e. ~ ng.ml^{-1} for all the compounds assayed.

2.2.3 **Recovery and reproducibility**

Three series of 10 plasma samples were spiked so as to contain 50, 100 and 200 ng.ml^{-1} of RU 38 486 and of each metabolite.

The recovery was determined by measuring the radioactivity in the case of RU 38 486 and by comparison with the height of the chromatographic peak of the metabolites injected directly onto the HPLC column for the other compounds.

The following values ($\pm$ SEM) were observed:

98 $\pm$ 0.2% for RU 38 486
87 $\pm$ 0.8% for RU 42 698
70 $\pm$ 1.7% for RU 42 633
47 $\pm$ 0.6% for RU 42 848

In calculating the plasma concentrations, it was not necessary to take these yields into account as the calibration curves were established from spiked plasma, which thus accounted for the extraction yield.

2.3 PHARMACOKINETIC ANALYSIS

The parameters adopted for the pharmacokinetic analysis were the following, by compound assayed and by dose:

2.3.1 **Peak plasma concentration, C_{max}, in mg.l^{-1}**

2.3.2 **Peak plasma concentration divided by the corresponding dose administered, C_{max}/Dose, in mg.l^{-1}/mg.**

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

2.3.4 Area under the plasma concentration curve, AUC, in $mg.l^{-1}.h$

Calculated by the trapezoidal rule

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

2.3.5 Area under the plasma concentration curve divided by the corresponding dose administered, AUC/Dose, in $mg.l^{-1}.h/mg$.

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

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

2.3.7 Elimination half-life, t 1/2, in h

An exponential function was fitted by computer to the plasma concentrations observed after the absorption and distribution phases in the linear part on a semi-logarithmic plot.

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

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

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

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

The half-life was obtained from the equation

$$t\ 1/2 = \frac{\ln 2}{\lambda 1}$$

2.4 STATISTICAL ANALYSIS

The mean and standard error of the mean were calculated for the plasma concentrations by time for each dose and each compound assayed.

2.4.1 Comparison of the pharmacokinetic parameters by compound in terms of dose

The pharmacokinetic parameters by compound were subjected to a 3-way analysis of variance with the mean, variance and standard error of the mean of the parameters being calculated for each component.

The components were: dose effect, subject effect and period effect. The variance was thus broken down into:

Source	df
Dose	2
Subject	11
Period	2
Residual	20
Total	35

2.4.1.1 C_{max}-AUC

The variance of the dose effect was broken down to test the regression and the deviation from linearity between C_{max} or AUC and the dose.

If the regression was significant and the deviation from linearity non-significant, the slope and the intercept of the regression line were calculated and the intercept compared with 0.

2.4.1.2 Cmax/Dose - Tmax - AUC/Dose - MRT - t 1/2

If the analysis of variance revealed a significant dose effect, the means of the parameters in terms of dose were compared with one another by Tukey's test using the residual variance of the analysis of variance.

2.4.2 **Comparison of the pharmacokinetic parameters by dose in terms of compound**

The following pharmacokinetic parameters, C_{max}, T_{max}, AUC, MRT and t 1/2 per dose, were subjected to a 2-way analysis of variance, with the mean, variance and standard error of the mean of the parameters being calculated for each component.

The variance was broken down as follows:

Source	df
Compound	3
Subject	11
Residual	33
Total	47

If the analysis of variance showed a positive compound effect, the means of the parameters by compound were compared with one another by Tukey's test using the residual variance of the analysis of variance.

2.3.2. Oral administration

4 ml of solution were administered with 150 ml of water to subjects in the upright position who remained standing for 2 minutes.

2.3.3. Intravenous administration

The solute was diluted with a 0.9% solution of NaCl in water (1/24, V/V). 100 ml of this dilution were infused for 1 hour at a constant rate by means of an infusion pump to subjects in the supine position. The detailed conditions of administration are described in Appendix I.

2.4. Samples

Blood samples were collected on dry lithium heparinate 0.25 h before and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 4, 8, 12, 24, 48 and 72 hours after oral or intravenous administration. The plasma was separated, frozen at -20°C and stored at this temperature until analysis.

2.5. Assay of RU 38 486

After extraction, the compound was separated by HPLC and the concentration measured by densitometry at 304 nm. The analytical method is detailed in Appendix II and the results of the quality controls performed during these measurements are given there also.
The limit of detection was ___ $mg.l^{-1}$. Only RU 38 486 was assayed.