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25. Antihormonal

PHARMACOKINETICS AND METABOLISM OF RU 486

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Summary—The effects of dose on the initial pharmacokinetics and metabolism of an antiprogesterone steroid RU 486 (mifepristone) were studied in healthy female volunteers after administration of RU 486 as a single dose of 50–800 mg. The concentrations of RU 486 and its monodemethylated, dimethylated and hydroxylated non-demethylated metabolites were measured specifically after Chromosorb^R-column chromatography by HPLC. Their relative binding affinities to the human uterine progesterone receptor were also determined.

Micromolar concentrations of the parent compound in blood were reached within the first hour after oral administration. The pharmacokinetics of RU 486 followed two distinct patterns in a dose-dependent fashion. With a low dose of 50 mg the pharmacokinetics followed an open two-compartment model with a half-life of over 27 h. With the doses of 100–800 mg the initial redistribution phase of 6–10 h was followed by zero-order kinetics up to 24 h or more. Importantly, after ingestion of doses higher than 100 mg of RU 486 there were no significant differences in plasma concentrations of RU 486 within the first 48 h, with the exception of plasma RU 486 concentrations at 2 h. After single oral administration of 200 mg unchanged RU 486 was found 10 days later in two subjects. The elimination phase half-life with this dose, calculated between day 5 and 6, was 24 h.

Micromolar concentrations of monodemethylated, didemethylated and non-demethylated hydroxylated metabolites were measured within 1 h after oral administration of RU 486. In contrast to plasma RU 486 concentrations, circulating plasma concentrations of metabolites increased in a dose-dependent fashion. With higher doses the metabolite concentrations were close to, or even in excess to the parent compound. The relative binding affinities of RU 486, monodemethylated, didemethylated and hydroxylated metabolites (progesterone = 100%) to the human progesterone receptor were 232, 50, 21, and 36, respectively.

The existence of a high affinity–limited capacity serum binding protein would explain the long half-life and the observed diverging dose-dependent pharmacokinetics. The extravasation of RU 486 after the saturation of serum binding sites would explain the blunted serum peak concentrations of RU 486 with higher doses. The return of the drug back to circulation thereafter explains the zero-order kinetics. High concentrations of circulating metabolites capable of binding to the progesterone receptor suggest a significant contribution of these steroids in the overall antiprogestational action.

INTRODUCTION

RU 486, a recently synthesized 19-norsteroid derivative, presents a potent antiprogestin [1] which also exhibits antiglucocorticoid properties [2]. In addition to its use in basic research, this compound has a potential for clinical applications, particularly in the area of fertility regulation.

Clinically, RU 486 has been used with moderate success for termination of an early pregnancy of less than 7 week's duration [3, 4]. The overall success rate ranges between 70 and 80%. Higher success rates approaching 100% have been achieved neither by increasing the dose nor the length of the treatment. The reason why some women fail to respond to RU 486 is not known. Better outcome has been claimed when a prostaglandin is combined with the RU 486 therapy [5].

One possible explanation why some women do not respond to RU 486 therapy could be related to differences in the pharmacokinetics and/or metabolism of the drug. Animal studies have shown that the first steps of RU 486 metabolism (Fig. 1) are dealkylation of the dimethylaminophenyl ring at the C-11 position and hydroxylation of 17-propynyl-chain [6]. The demethylated metabolites can be further hydroxylated or acetylated [6]. We measured the plasma concentrations of RU 486 and its monodemethylated, didemethylated and hydroxylated metabolites after oral ingestion of various doses. A Chromosorb^R-column chromatography system was used prior to high performance-liquid chromatography (HPLC) to separate the parent RU 486 from its immunologically cross-reacting metabolites [7]. The latter system was thereafter used for the quantitation of RU 486 and its three most proximal metabolites [8]. To assess the possible role of demethylated and hydroxylated metabolites in the human, we also studied the binding of RU 486 and its metabolites to the human progesterone receptor.

EXPERIMENTAL

RU 486 (17β - hydroxy - 11β - (4 - dimethylaminophenyl)-17α - (1 - propynyl) - estra - 4,9 - dien

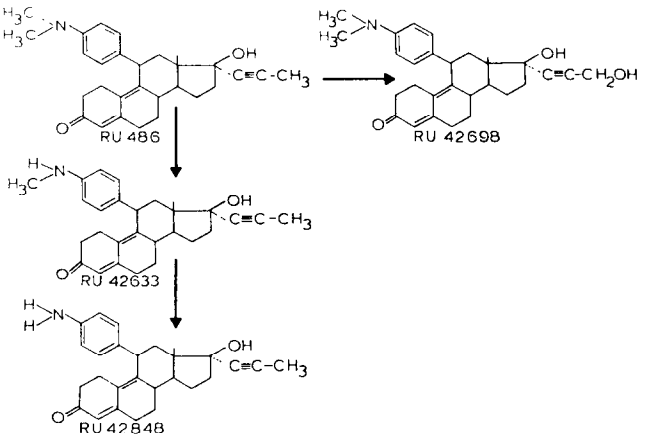

Fig. 1. Molecular structures of RU 486 and its monodemethylated (RU 42633), didemethylated (RU 42848) and alcoholic non-demethylated (RU 42698) metabolites.

- 3 - one), the monodemethylated metabolite RU 42633 (17β - hydroxy - 11β - (4 - monomethylaminophenyl) - 17α - (1 - propynyl) - estra - 4,9 - dien - 3 - one), the didemethylated metabolite RU 42848 (17β - hydroxy - 11β - (4 - aminophenyl) - 17α - (1 - propynyl) - estra - 4,9 - dien - 3 - one), and the hydroxylated metabolite RU 42698 (17β - hydroxy - 11β - (4 - dimethylaminophenyl) - 17α - (1 - propynol) - estra - 4,9 - dien - 3 - one), were kindly donated by Roussel-UCLAF Research Center (Romainville, France).

Healthy volunteers, whose ages ranged from 22 to 35 yr, participated in the study. They had regular menstrual cycles (28+/−3 days) and weighed between 45 and 70 kg. They were either surgically sterilized or used an IUD for contraception. The volunteers had not taken any steroid medication within the last 6 months.

There were 6 groups each comprising 3–5 subjects. Each subject received orally a single administration of either 50, 100, 200, 400, 600 or 800 mg of RU 486 (50 mg tablets supplied by Roussel-UCLAF, Romainville). The drug was administered 6–8 days after the LH-surge. Blood samples were obtained at $-\frac{1}{2}$, 0, $+\frac{1}{2}$, 1, 2, 4, 6, 10, 24 and 48 h, except those subjects who received 50 or 200 mg of RU 486. In these groups, blood samples were collected daily for 1 week and in the latter group also on days 10 and 14.

The concentrations of RU 486 and its metabolites were measured by HPLC [7] after Chromosorb^R-column chromatography [7, 8]. The relative binding affinities of RU 486 and its metabolites to the human uterine progesterone receptor were measured as described by Haukkamaa [9]. Statistical analysis was carried out using the two-tailed *t*-test of Welch.

RESULTS

Pharmacokinetics

Figure 2 gives the individual plasma concentrations of RU 486 in three volunteers after ingestion of 50 mg of RU 486. The elimination was remarkably slower in one study subject. However, these profiles fit into an open two-compartment model, and the half-life of disappearance varied between 16 and 36 h.

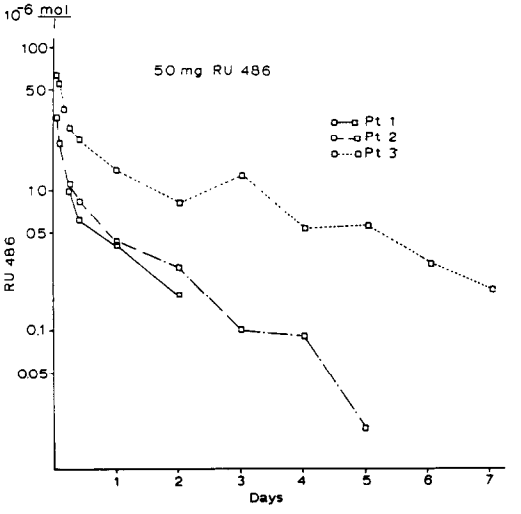

Fig. 2. Individual plasma concentrations of RU 486 after oral ingestion of 50 mg of RU 486 in 3 female volunteers.

Figure 3 shows the mean (+SEM) of plasma RU 486 concentrations in 5 women after ingestion of 200 mg of RU 486. The highest concentration was measured at 1 h. After the initial redistribution within 6 h, a plateau was reached until 24–48 h. Half-life between days 5 and 6 was about 24 h. The mean concentrations at 1 and 2 days were 1.8 and 1.4 μM, respectively.

Plasma RU 486 concentrations (mean + SEM) within the first 48 h after ingestion of 100, 400, 600 and 800 mg of RU 486 are depicted in Fig. 4, panels a, b, c and d, respectively. The peak RU 486 levels were measured at 1 h. Concentrations within 24 h did not significantly differ between various doses, with the exception of the 2-h value between the 100 and 800 mg dose ($P < 0.05$). Furthermore, after the

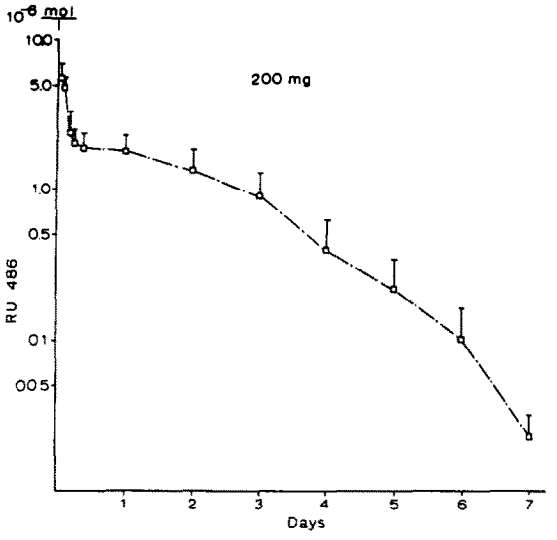

Fig. 3. Plasma concentrations of RU 486 (mean + SEM) in 5 female volunteers after oral ingestion of 200 mg of RU 486.

initial redistribution within the first 6 h, plasma RU 486 concentrations plateaued over the next 24 h or more. The duration of the plateau was proportional to the amount of the dose ingested.

Metabolism

Plasma concentrations (mean + SEM) of the monodemethylated, didemethylated and hydroxylated metabolites after oral intake of 100, 400, 600 and 800 mg of RU 486 are also depicted in Fig. 4, panels a, b, c and d, respectively. Micromolar concentrations of the metabolites were circulating already 1 h after ingestion of RU 486. When the dose was increased from 100 to 800 mg the concentrations of the metabolites increased in a dose-dependent fashion. The concentrations of the monodemethylated and alcoholic metabolites very much paralleled those of the parent compound. Their highest concentrations were measured 2–4 h after the intake. On the other hand, the amount of the didemethylated metabolite gradually increased within the first 10 h. Indeed, a single dose of 400 mg RU 486 or more resulted in plasma concentrations close to or even higher than those of the parent compound. The concentrations of the monodemethylated metabolite were always higher than those of the parent compound. From 2 h onward following the ingestion of 400 mg of RU 486, plasma levels of the didemethylated and hydroxylated metabolites were close to or even higher than the levels of the parent compound.

Progesterone receptor binding

The relative binding affinities of RU 486 and its metabolites to the human uterine progesterone receptor are compared to progesterone in Table 1. The metabolites display a lower but definite binding affinity as compared with the parent compound.

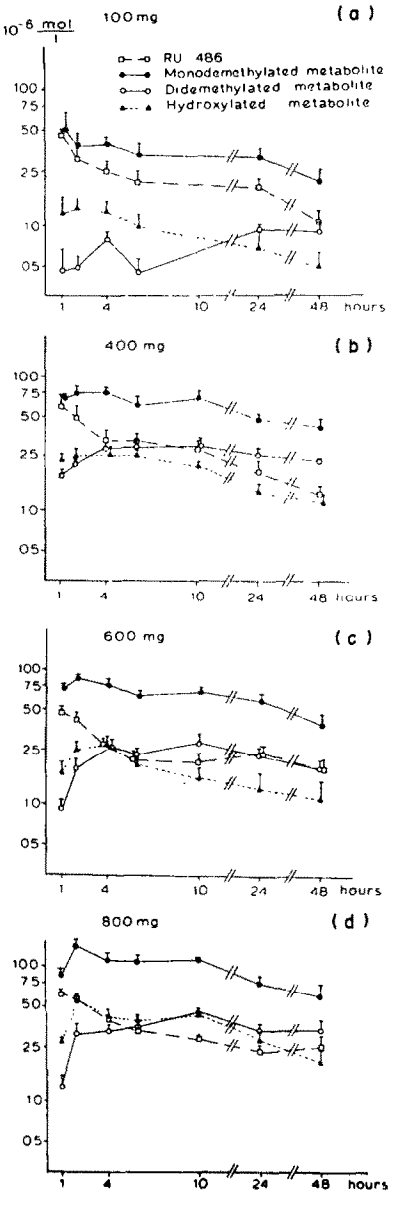

Fig. 4. Mean (+SEM) plasma concentrations of RU 486 (□) monodemethylated (●), didemethylated (○), and hydroxylated nondemethylated (▲) metabolites after oral ingestion of 100 mg (panel a), 400 mg (panel b), 600 mg (panel c) and 800 mg (panel d) of RU 486 in 5 female volunteers.

Table 1. The relative binding affinities of the steroids investigated for human myometrial and endometrial progesterone receptor

Compound	Relative affinity %*
ORG-2058	869
RU 486	232
Progesterone	100
RU 42633	50
RU 42698	36
RU 42848	21

*Relative to progesterone (= 100%).

DISCUSSION

In the radioimmunoassay for RU 486, developed by Salmon and Mouren [10], the mono- and didemethylated as well as hydroxylated metabolites of RU 486 (Fig. 1) cross-react 60, 84 and 0.8%, respectively [10]. Thus after oral administration the RIA measurement of plasma RU 486 gave blunted plasma peak concentrations over a period of 6 h [11]. It was not possible to judge to what extent this was due to slow absorption of the drug or to the contribution of the cross-reacting metabolites. We developed a Chromosorb^{R}-column chromatography to separate RU 486 from its immunologically cross-reacting metabolites. The eluates were subsequently used for the quantitative measurement of RU 486 by RIA or HPLC [7], or for the measurement of the metabolites by HPLC [8].

The pharmacokinetic results of Deraedt *et al.*[6] obtained with 100 mg or tritiated RU 486 given orally to 3 male volunteers indicated a rapid absorption, with peak concentrations within 1 h and a rapid metabolism thereafter by demethylation. Our results confirm these findings, and the peak concentrations of RU 486 were measured 1 h after ingestion of each dose. Interestingly, after ingestion of doses higher than 100 mg of RU 486 there were no significant differences in plasma concentrations of RU 486 within the first 48 h, with the exception of plasma RU 486 concentrations at 2 h. Furthermore, after the 6-h redistribution, plasma concentrations of RU 486 plateaued for 24–48 h, demonstrating zero-order pharmacokinetics during that time. The length of the zero-order kinetics was directly related to the dose. Thus the terminal plasma half-life of RU 486 could not be estimated from plasma concentrations measured within 48 h after these dosages. Studies by Philibert *et al.*[12] indicate that human orosomucoid is the specific high-affinity carrier protein of RU 486. This would explain the difference in the pharmacokinetics obtained between low and high doses of RU 486. The 50 mg dose of RU 486 does not saturate the binding sites of orosomucoid. Therefore a small apparent initial volume of distribution and first-order kinetics are obtained with small doses [6]. When the dose is increased, the orosomucoid binding sites become saturated and the extravasation of RU 486 into tissues starts. The higher the dose exceeding the orosomucoid binding capacity, the greater is the extravasation, and the longer is the zero-order pharmacokinetics substantiated by the return of RU 486 from the tissues into circulation.

Due to insensitivity of HPLC compared to RIA, measurable concentrations of RU 486 were only found up to 7 days, with an apparent terminal half-life of 24 h. By using RIA, RU 486 could still be found in plasma 10 days after administration of 200 mg RU 486 (data not shown).

The concentrations of the monodemethylated metabolite were always higher than those of the parent compound, in a dose-dependent fashion. This indicates that the first demethylation reaction has a high capacity. The second demethylation was slower, reaching peak concentrations only 10 h after the ingestion. Hydroxylation presented another important pathway of RU 486 metabolism. Peak levels were reached rapidly, the ratio of hydroxylation/monodemethylation remained relatively stable at 0.33 througout the 48 h measured in the single dose studies.

The relative binding affinity of RU 486 (relative to progesterone) to the human progesterone receptor was 232%, whereas those of the demethylated and hydroxylated metabolites were 21–50%. The biological role of these metabolites in the overall antiprogesterone action of RU 486 cannot be exactly evaluated by the *in vitro* data. Deraedt *et al.*[6] calculated the abortive effect of these metabolites in the rat. The effective dose (ED_{100}) was 3 times higher for the monodemethylated and hydroxylated metabolites than for RU 486, whereas the didemethylated metabolite was ineffective. When a single high dose of RU 486 is administered, the amount of the metabolite pool, in relation to the parent compound, increases, and therefore can display a significant role in the overall antiprogesterone action.

In conclusion, our results demonstrate micromolar concentrations of RU 486 and its metabolites with high affinity to the human progesterone receptor after a wide range of oral administration. Whereas the metabolites increased, there was no significant increase in plasma RU 486 concentrations in relation to the increased dose. These findings indicate significant extravasation of RU 486 after high dose administration and support, together with the long half-life of RU 486, the presence of a high affinity-limited capacity binding protein in serum.

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