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Pharmacological properties of mifepristone: toxicology and safety in animal and human studies

Regine Sitruk-Ware[a,*], Irving M. Spitz[a,b]

[a]*Center for Biomedical Research, Population Council Regine Sitruk-Ware Center for Biomedical Research, 1230 York Avenue, 6th Floor, New York, NY 10021, USA*
[b]*Institute of Hormone Research, Shaare Zedek Medical Center, Jerusalem, Israel*

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Abstract

Roussel Uclaf in partnership with the INSERM unit of Prof. E.E. Baulieu first discovered mifepristone (RU486) as part of a large research program on steroidal compounds with antihormone properties. Exhibiting a strong affinity to the progesterone and the glucocorticoid receptors, mifepristone exerted competitive antagonism to these hormones both in in vitro and in animal experiments. Due to its antiprogesterone activity, it was proposed that mifepristone be used for the termination of early human pregnancy. Mifepristone, at a dose of 600 mg initially used alone, was then used with a subsequent low dose of prostaglandin that led to a success rate of 95% as a medical method for early termination of pregnancy (TOP). Its use was extended to other indications, such as cervical dilatation prior to surgical TOP in the first trimester, therapeutic TOP for medical reasons beyond the first trimester, and for labor induction in case of fetal death in utero. The efficacy and safety of this treatment has been confirmed based on its use for over a decade, with close adherence to the approved recommendations. This paper describes the safety studies conducted in animals as well as the safety follow-up and side effects reported with use of the compound in various indications either approved or unapproved. The rationale for warnings and contraindications for use of the product are also explained. At lower doses, the molecule has proven promising for contraceptive purposes with few reported side effects. However, development of the product for this indication would require long-term studies. Although political and philosophical obstacles have delayed research, the use of mifepristone for other potential indications in gynecology or oncology should be investigated.

Keywords: Mifepristone; Pharmacology; Toxicology; Safety

1. Introduction

Mifepristone is an orally active synthetic steroid with antiprogesterone and antiglucocorticoid activities. To date, mifepristone is approved in several countries for use in four indications: early termination of pregnancy (TOP), cervical dilatation prior to surgical TOP, preparation for prostaglandin-induced TOP during the second-trimester, and expulsion of a dead fetus during the third trimester. Although the molecule has several possible indications due to its unique properties [1], its potential has not been fully realized; the controversy and philosophical debate involving mifepristone has resulted in opposition to further research of this compound. In spite of the controversy, there is continued interest in investigating the properties of antiprogestins, including its contraceptive properties.

2. Structure and physical properties

Mifepristone, 17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(prop-1-ynyl)-estra-4,9-dien-3-one, is derived from the estrane progestins (Fig. 1). Its molecular weight is 429.5. It is insoluble in water, but very rapidly dissolves in the gastric milieu when ingested orally. The product is available in the form of tablets containing 200 mg of active ingredient, and remains stable after 3 years at ambient temperature [2].

* Corresponding author. Fax: +1-212-327-7678.
E-mail address: regine@popcbr.rockefeller.edu (R. Sitruk-Ware).

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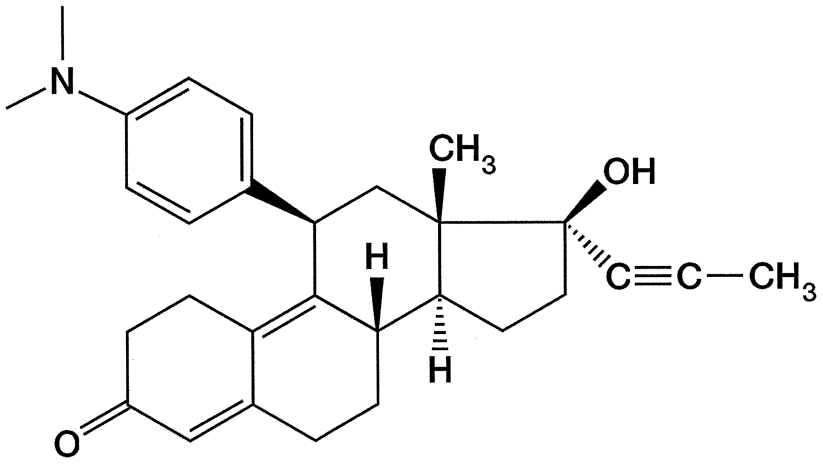

Fig. 1. Chemical structure of mifepristone.

3. Mechanism of action and interaction with steroid receptors

Mifepristone acts at the receptor level, binding strongly to the progesterone and glucocorticoid receptors, and to a lesser extent to the androgen receptor. Mifipristone is a potent antiprogesterone and antiglucocorticoid and a weak antiandrogen. Its relative binding affinity for progesterone, glucocorticoid and androgenic receptors is approximately five times greater than progesterone, three times greater than dexamethasone and four times less than testosterone, respectively [3]. In contrast, it is without affinity for the mineralocorticoid and estrogen receptors. Mifepristone's binding association constant for these receptors (Ka at steady state) is clearly greater than those of the natural steroids, progesterone and corticosterone, and of the glucocorticoid receptor agonist, dexamethasone. The metabolites of mifepristone also bind to the progesterone receptor, with binding of the monodemethylated, hydroxylated and didemethylated metabolites being 50, 36 and 21, respectively, relative to 100 for progesterone.

Mifepristone, like progesterone, enters target cells and reaches its receptors; however, it interacts differently from progesterone and may produce different conformational changes in the receptor. By occupying the progesterone receptor in the nucleus, progesterone modifies the receptor's shape, enabling it to bind to chromatin, and this binding leads to gene transcription and protein synthesis. Mifepristone antagonizes these effects by occupying the receptor without stimulating gene transcription [4,5].

The physiological effects of progesterone are mediated by its interaction with intracellular progesterone receptors (PRs) that are expressed as two protein isoforms, PR-A and PR-B, from a single gene. Both PRs have domains for DNA binding, hormone binding and activation [6]. PR-A and PR-B respond differently to progesterone as well as progesterone antagonists (PA) [7]. Activation of transcription always occurs with PR-B but often not with PR-A. Indeed, PR-A may function as a transdominant repressor not only of PR-B-mediated transcription, but also of estrogen receptor-mediated transcription. This may explain the antiestrogenic activity of certain PAs.

Antiprogestins may function as pure antagonists to the PR or as mixed agonist-antagonist molecules, also known as progesterone receptor modulators (PRMs). Mifepristone behaves as a pure antagonist on the McPhail test [1] that measures steroidal effect on the rabbit endometrium.

The complex of mifepristone with the PR inhibits transcription resulting in the down-regulation of progesterone-dependent genes [4]. As compared with other more recently synthesized antiprogestins, mifepristone is predominantly an antagonist with minimal agonist activity [8]. Several PAs including mifepristone, administered at low doses in the monkey, were shown to exert antiproliferative effects in the endometrium [9,10]. Whether this effect is due to a partial progesterone agonistic effect, or an overexpression of the androgen receptor is unclear [9].

4. Toxicology in animals

Roussel Uclaf conducted a comprehensive toxicology program in the mid-1980s demonstrating the safety of the molecule and allowing mifepristone to be used in humans. Most of the program focused on the development of indications using single-dose administration of the compound. Therefore, toxicology studies were conducted with durations of animal exposure not exceeding 6 months [11]. The compound was shown to have no mutagenic potential and no toxic effect up to 1000 mg/kg in acute administration performed in several species.

In subchronic toxicity studies conducted in rodents for 30 days and 26 weeks, daily doses up to 200 mg/kg or 125 mg/kg, respectively, displayed no toxicity but induced effects related to the antihormonal effects of the compound. The antiprogesterone effects resulted in: frequent estrus, a

decrease in uterine weight and in mammary development, and suppression of menstruation and a decrease in serum progesterone in monkeys. Antiglucocorticoid effects were observed with an increase in kidney and adrenal weights in rats and in monkeys and increases in serum adrenocorticotropic hormone (ACTH) and cortisol concentrations. An antiandrogenic effect was observed in male rats with a decrease in prostate and seminal vesicle weights. Monkeys were more sensitive to the antiglucocorticoid effect of the molecule. Although doses of 4 mg/kg did not have any effect, doses of 15 or 20 mg/kg induced increases in serum cortisol and in ACTH levels.

In summary, 1-month and 6-month treatments in rats and monkeys revealed no true toxicity that could not be attributed to the antiglucocorticoid, antiprogesterone and antiandrogenic activities of mifepristone. No long-term toxicity and carcinogenicity studies were performed because the treatment was developed for a single-dose use for the indications approved to date.

The molecule has been shown to induce fetal loss at 0.5 mg/kg in mice, 1 mg/kg in rats and 2 mg/kg in rabbits. Doses below these levels must be used to prevent the occurrence of fetal loss in the animals and to study the development of exposed embryos.

Embryotoxicity studies were conducted in rodents at these subabortive doses, and the surviving rat and mice fetuses showed no anomaly [11]. In rabbits, isolated anomalies were observed but were not dose-dependent and, therefore, could not be directly correlated to the drug. Rare malformations involving the encephalon were observed in another study [12] in which pregnant female rabbits were treated with a low dose of mifepristone (0.08–0.33 mg/kg/day). These abnormalities were attributed by the authors to a uterine retraction effect related to the antiprogesterone activity of mifepristone before or during the formation of the chondrocranium, rather than to a direct effect of the product on the embryo. Indeed, supplementary treatment with progesterone (100 mg/kg) totally suppressed the abortifacient effect of mifepristone, and under these circumstances no malformations were observed.

In a neonatal exposure study in rats, the administration of a subcutaneous dose of mifepristone up to 100 mg/kg on the first day after birth had no adverse effect on future reproductive function in males or females. The onset of puberty was observed to be slightly premature in female rats neonatally exposed to mifepristone [13].

5. Pharmacodynamic effects

5.1. Antiprogestational effects

In animal experiments conducted in vivo, at doses ranging from 3 to 10 mg/kg depending on the test, mifepristone totally inhibited all standard biological responses of exogenous progesterone, such as preparation of the endometrium for nidation in the rabbit, the process of decidualization which occurs at the time of implantation, and maintenance of pregnancy in the female rat. By antagonizing endogenous progesterone, it displayed antinidatory and abortifacient activity in the rat at doses of 10 mg/kg, whatever the treatment period, except for days 1, 2 and 15 when the compound only proved effective at about 10 times the dose. Mifepristone also exerted abortifacient activity in the mouse. In the monkey, it caused menstruation when administered during the luteal phase, regardless of the treatment period [3,4].

Thirty normal female volunteers received a single dose of mifepristone (5, 10, 25, 50, 75, 100, 150, 200, 250, 300 or 400 mg) between days 2 and 6 after the luteinizing hormone (LH) surge. An endometrial biopsy was performed 3 days after mifepristone intake. Mifepristone induced inhibition of glandular secretory activity, degenerative changes in glandular cells, vascular changes (reduction of stromal edema and stromal extravasation) and an increase in glandular mitotic activity. These endometrial responses were significantly related to the dose of mifepristone administered [14].

5.1.1. Effects of mifepristone on ovulation

Mifepristone, 3 mg/kg/day, was administered for 4 days to 6 normal women as soon as a dominant follicle had emerged. Mifepristone treatment provoked a fall in estradiol concentrations with a regression in the dominant follicle. LH and follicle-stimulating hormone levels had a tendency to diminish but subsequently increased with reinitiation of folliculogenesis and occurrence of an LH ovulatory surge 13 days later [15].

Three women received mifepristone, 25 mg/day, on days 1 to 14 of the cycle, and 5received 25 mg/day on days 1 to 21 [16] During mifepristone treatment, concentrations of estradiol remained low, indicating inhibition of folliculogenesis. When mifepristone treatment was discontinued, there was an increase in estradiol levels followed by a rise in progesterone levels, indicating the occurrence of ovulation. During the follicular phase, when administered immediately before the ovulatory peak of LH, mifepristone caused a major delay in this peak, resulting in increased duration of the follicular phase, without affecting the luteal phase [4] administration of mifepristone during the follicular phase thus interrupts normal follicular development resulting in delayed ovulation. This effect is probably related to the antigonadotropic activity of mifepristone.

Mifepristone at a dose of 1 mg/day interferes with endometrial development while allowing for the occurrence of biphasic ovarian cycles and regular bleeding [17]. However, it also prevents ovarian cyclicity in a high proportion of treatment months; this is associated with increased endometrial growth in some subjects, which may be of concern.

5.1.2. Effect of mifepristone on uterine contractility

During early pregnancy, the uterus is inactive, probably due to the inhibitory effect of progesterone. Doses of ≥1

mg/kg of mifepristone have been shown to antagonize the endometrial and myometrial effects of progesterone in women. During pregnancy, the compound sensitizes the myometrium to the contraction-inducing activity of prostaglandins [2,18].

The time interval between mifepristone and the appearance of uterine contractions is 24–36 h. Simultaneously with increased contractility, sensitivity to prostaglandin increases about fivefold. These data provide the rationale for combining mifepristone and a low dose of prostaglandin for termination of early pregnancy. In an earlier study [18], the increased sensitivity of the uterus to prostaglandin appeared 24 h after the start of mifepristone treatment and was maximal at 36 and 48 h. Pretreatment with mifepristone did not increase uterine sensitivity to oxytocin.

5.2. Antiglucocorticoid effects

Mifepristone has antiglucocorticoid properties and has antagonized the effects of dexamethasone in a number of models. Mifepristone totally inhibited effects of dexamethasone, such as inhibition of ACTH secretion, as well as its thymolytic action and diuretic effects [1].

In humans, mifepristone also exerts antiglucocorticoid activity. The antiglucocorticoid effect of mifepristone is exerted both on the central actions of cortisol (inhibition of feedback control of cortisol over its own production evidenced by an increase in ACTH and lipotropin hormone) and on the peripheral effects (suppression of cutaneous vasoconstriction, or decrease in circulating eosinophils induced by glucocorticoids) [19–24]. Mifepristone binds to the glucocorticoid receptor in human mononuclear leukocytes with an affinity about threefold higher than that of dexamethasone [1].

The antiglucocorticoid effects of mifepristone are dose-dependent and are apparent at single doses of mifepristone in the order of 4–6 mg/kg. The antiglucocorticoid effect lasts for at least 24 h after a single dose of mifepristone given at midnight [21]. Mifepristone at a dose of 400 mg (6 mg/kg) antagonized the suppressive effect of 1 mg of dexamethasone on the hypothalamo–pituitary–adrenal axis (HPA) [23]. Administration of dexamethasone at doses >1 mg counteracts the antiglucocorticoid effects of a 6 mg/kg dose of mifepristone [23].

In conclusion, mifepristone displays antiglucocorticoid activity, which is expressed at doses of 400 mg and above (single administration). This antiglucocorticoid activity occurs centrally and peripherally [19–24]. However, no clinical or laboratory signs of adrenal failure have been observed during chronic administration of mifepristone to patients with normal adrenal function. This is probably related to compensation arising from hypersecretion of ACTH and cortisol, resulting from the central action of cortisol and weak agonist activity of mifepristone. Also, it must be kept in mind that mifepristone does not bind to the mineralocorticoid receptors, and hence the mineralocorticoid axis is unaffected by the product.

5.3. Antiandrogenic properties

The product also has modest antiandrogenic action. When the different activities of mifepristone are compared in the same species (rat), the ED_{50} for the antiprogesterone and antiglucocorticoid activities is about 3 mg/kg, whereas it is about 30 mg/kg for antiandrogenic activity [1].

6. Pharmacokinetic studies

6.1. Studies in animals

At active oral doses, mifepristone is absorbed satisfactorily in rats and monkeys. Its bioavailability is reduced by a moderate presystemic effect in rats (bioavailability: 39% of the dose), which is very much more pronounced in monkeys (bioavailability: 15% of the dose). In humans, the absolute bioavailability of a 20-mg dose is 69% [2,25–29]. After ingestion of doses of 100–800 mg, there is an initial redistribution phase of 6–10 h followed by a plateau for 24 h or more [26]. The terminal $t_{1/2}$ of mifepristone is 4 h in rats, 15 h in monkeys and 30 h in humans [3,25,29].

The clearance of mifepristone is 2.7 L/h/kg of body weight in rats and 1.45 L/h/kg of body weight in monkeys. These values are very much higher than in women (0.04 L/h/kg of body weight) principally because of a very reduced volume of distribution in women as a result of saturable high-affinity binding to α1-acid glycoprotein, a property not shared by corresponding animal species. However, the influence of the human protein, studied in vitro in the rat, is restricted to this phenomenon and does not affect the elimination rate of mifepristone or its concentration in target tissues, the uterus and thymus, and consequently does not alter its activity.

6.2. Metabolism and excretion

The metabolism of mifepristone is initiated by rapid demethylation and hydroxylation in man, rat and monkey [3]. Metabolism of mifepristone occurs primarily via pathways involving N-demethylation and terminal hydroxylation of the 17-propynyl chain. In vitro studies conducted with human liver microsomes have shown that CYP450 3A4 is largely responsible for the oxidative metabolism of mifepristone [30]. The three major metabolites identified in humans are: (a) RU 42 633, which is the metabolite most widely found in plasma and is the N-monodemethylated metabolite; (b) RU 42 848, which results from the loss of two methyl groups from the 4-dimethylaminophenyl in position 11β and (c) RU 42 698, which results from terminal hydroxylation of the 17-propynyl chain [2,25] (Fig. 2).

Following oral administration of 100 mg or more to

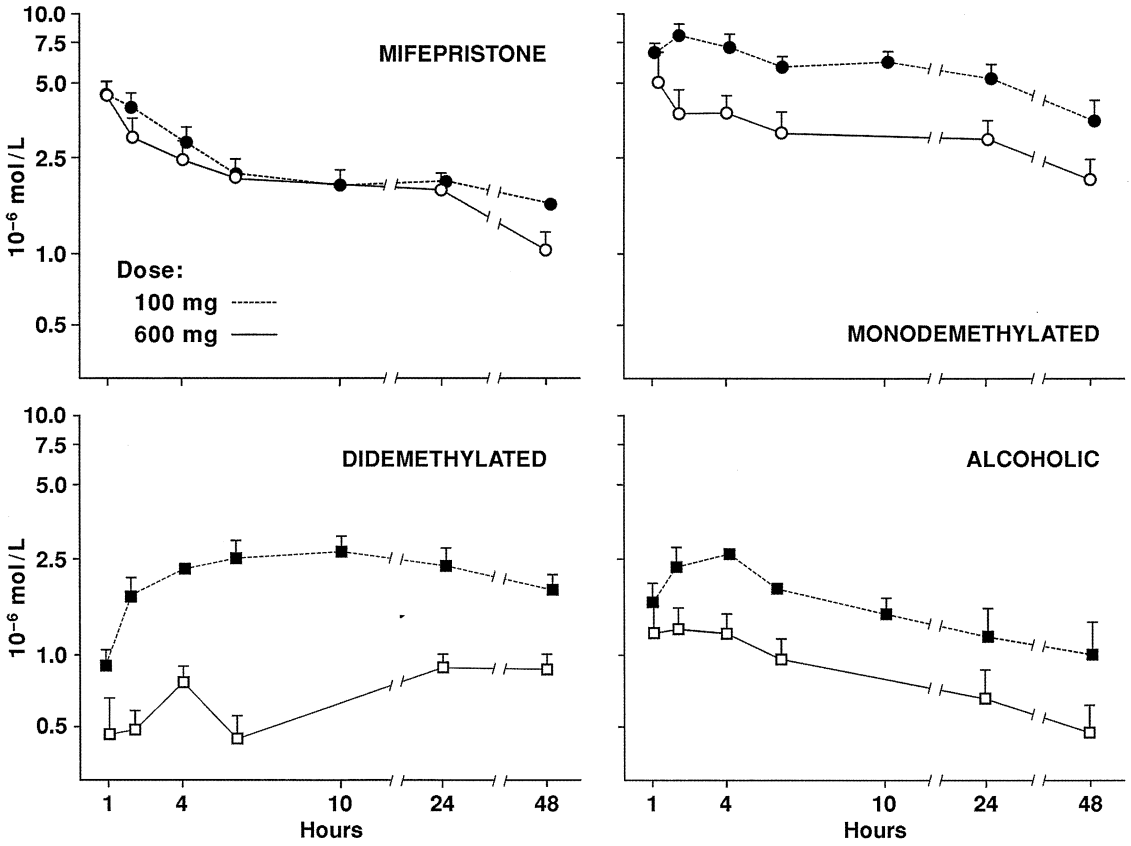

Fig. 2. The mean (±SEM) concentration of mifepristone and three of its metabolites after oral administration of 100 mg and 600 mg in five subjects. The upper panels illustrate mifepristone (left) and the monodemethylated (RU 42 633) metabolite (right). Circulating levels of the didemethylated (RU 42 848) and alcoholic nondemethylated (RU 42 698) derivatives are shown in the left and right lower panels, respectively. The concentrations of mifepristone were similar after these doses, but the concentrations of metabolites were higher after the 600 mg dose of mifepristone. Concentrations of the monodemethylated metabolites are higher than those of the parent compound at all time points. From Robbins A, Spitz I. Mifepristone: clinical pharmacology. Clin Obstet Gynecol 1996;39:436–50. Reprinted with permission from Lippincott-Raven Publishers.

humans, constant serum concentrations of mifepristone, but increasing concentrations of the monodemethylated, didemethylated and hydroxylated metabolites, are found [28]. Within the dose range of 100–600 mg, serum concentrations of the monodemethylated metabolite exceed those of the parent drug; in addition, following oral administration of doses beyond 400 mg, levels of didemethylated and hydroxylated metabolites exceed those of mifepristone. Although their affinity for the receptors and their potency are less than those of mifepristone, these metabolites may contribute to the overall effects of the drug in view of their high concentrations in serum [31].

Excretion is essentially fecal (about 92% of the total excreted). Mifepristone was excreted by rats and monkeys after undergoing almost complete biotransformation [25]. The presence in human plasma of the N-mono- and N-didemethylated metabolites and of the 22-hydroxylated metabolite shows that the two primary routes are also active in women. The demethylated and hydroxylated metabolites are further metabolized and excreted in bile, but in humans only a very small fraction of mifepristone can be detected in urine. The major difference between the pharmacokinetics of mifepristone in women and in animals, therefore, lies in the binding to human α1-acid glycoprotein, a characteristic that is absent in all the other species tested, including those currently used in pharmacology and toxicology. The only consequences are much higher plasma concentrations of mifepristone in women at active oral doses and the nonlinearity of the kinetics observed clinically.

6.3. Studies in women

Various assay methods have been employed in the measurement of serum mifepristone and its metabolites; these include radioimmunoassay (RIA), radioreceptor-assay (RRA), and assays based on high-performance liquid chromatography (HPLC) [26–29,31].

Following single-dose administration of mifepristone (600 mg), to healthy female volunteers, mean maximum plasma concentrations were about 2.0 mg/L at 1.35 h (t_{max}). After oral ingestion, mifepristone is rapidly absorbed, and the time to peak serum concentration (t_{max}) is approximately 1–2 h (Table 1). When analyzed by specific RIA or HPLC, t_{max} is similar within the dose range of 200–600 mg. Peak

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