

Acta Medica Okayama

Volume 45, Issue 2

1991

Article 3

APRIL 1991

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Abstract

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KEYWORDS: RU38486, metabolites of RU38486, high performance liquid chromatography, ultra-violet spectrophotometric detection

*PMID: 1867115 [PubMed - indexed for MEDLINE]

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Automated Direct Assay System for RU38486, an Antiprogestosterone-Antiglucocorticoid Agent, and its Metabolites Using High Performance Liquid Chromatography

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An automated direct assay system using high performance liquid chromatography was developed for the measurement of RU38486 and its three metabolites (RU42698, RU42848, RU42633) in human serum. Serum concentrations of these compounds were measured up to 144h following single oral administration of 200 (200mg group, n = 3) or 400mg (400mg group, n = 3) of RU38486 to healthy female volunteers. The serum half-lives (200mg group-400mg group) of RU38486, RU42698, RU42848 and RU42633 were 31.8-33.1h, 41.2-39.3h, 33.9-36.6h and 29.2-36.6h, respectively. Our system could quantify them easily and simultaneously, and was considered to be valuable in studies on the relationship between the pharmacokinetics and the clinical effects of RU38486.

Key words : RU38486, metabolites of RU38486, high performance liquid chromatography, ultra-violet spectrophotometric detection

RU38486, 17 β -hydroxy-11 β -(4-dimethylaminophenyl)-17 α -(1-propynyl)-estra-4, 9-dien-3-one, has potent antiprogestosterone and antiglucocorticoid effects mediated by high affinity binding to the receptors and is expected to be used clinically (10). But the method of administration and the optimal dose have not been established. There were several reports concerned with the pharmacokinetics of RU38486 using radioimmunoassay, but those of the metabolites have not yet been precisely studied (1, 3). In this study, we quantified the serum concentrations of RU38486 and its three metabolites (RU42698, RU42848 and RU42633) by a newly developed direct assay system, which was modified from our direct assay

system for sex steroid hormones using high performance liquid chromatography (HPLC) (5, 8, 9).

Materials and Methods

Reagents. The standard materials of RU38486, RU42698, RU42848, RU42633 and R2323 (used as internal standard, I.S.) were supplied by Roussel-Uclaf, Paris, France. Their chemical structures are shown in Fig. 1. Potassium dihydrogen phosphate and the organic solvents were of analytical grade and were obtained from Katayama Chemical Industries, Osaka, Japan.

Samples. Blood samples were provided from Professor T. Yanaihara, Department of Obstetrics and Gynecology, Showa University School of Medicine, Tokyo, Japan, which were taken from six healthy female

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volunteers, aged from 20 to 39 years, at the mid luteal phase following oral administration of 200 (200 mg group, $n = 3$) or 400 mg (400 mg group, $n = 3$) of RU38486. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 4, 8, 24, 48, 72, 96, 120, 144, 168 h after the administration. The samples were centrifuged at $1,300 \times g$ for 10 min and the resulting supernatants were stored below -20°C until

assay.

Apparatus. The measurement system was modified from that of Suzuki *et al.* (5). The system contains a column-switching system controlled by a sequencer consisted of the pretreatment phase and the analytical phase. The pretreatment phase consisted of an autosampler (MODEL WISP 712, Waters, Massa-

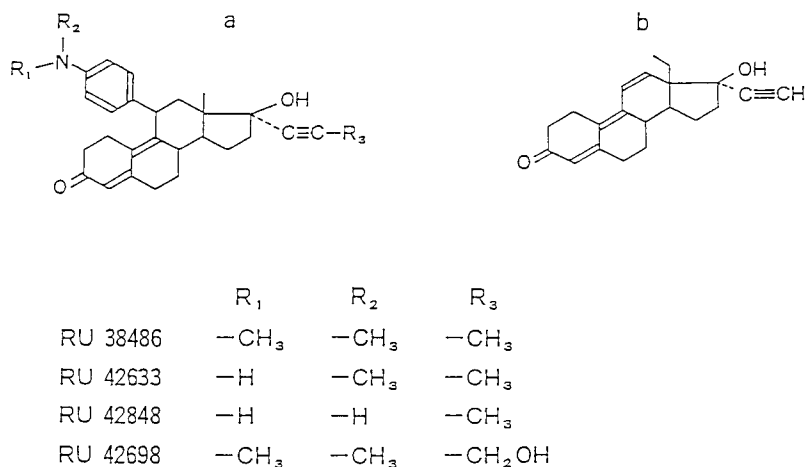


Fig. 1 Chemical structures of RU38486, its metabolites (RU42633, RU42848 and RU42698) (a) and R2323 (internal standard) (b).

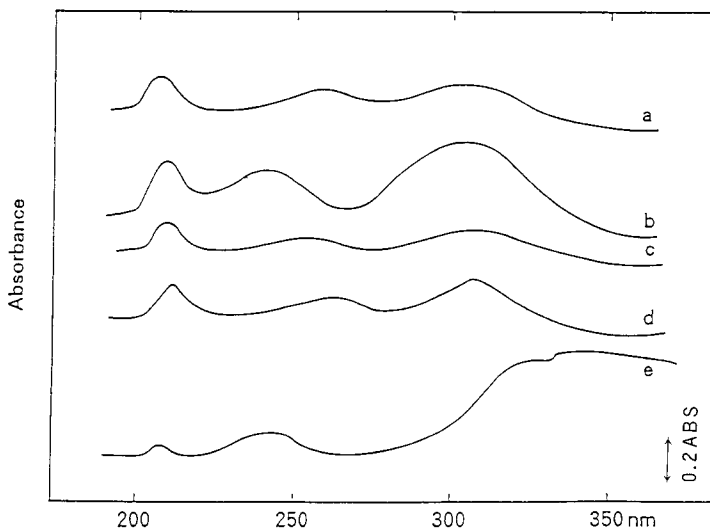


Fig. 2 The ultraviolet absorption spectra of RU42698 (a), RU42848 (b), RU42633 (c), RU38486 (d) and R2323 (e) in methanol. Concentrations: RU42698, $12.0 \mu\text{g/ml}$; RU42848, $30.6 \mu\text{g/ml}$; RU42633, $8.3 \mu\text{g/ml}$; RU38486, $6.6 \mu\text{g/ml}$; R2323, $20.8 \mu\text{g/ml}$.

chusetts, USA), and an autopretreatment system (Autoserumout, Sekisui Chemical Industries, Osaka, Japan) which consisted of a pump operated at a flow-rate of 0.6 ml/min, a pretreatment column (Serumout, 30 mm \times 4 mm I.D., Sekisui Chemical Industries) and a six-way electromagnetic valve to incorporate the pretreatment column alternatively into the pretreatment phase and the analytical phase. The analytical phase consisted of a pump operated at a flow-rate of 1.0 ml/min (Model 510, Waters), a degasser (Shodex DEGAS KT-21, Showa Denko, Tokyo, Japan), an analytical column (octadecyl silane-silica column, 300 mm \times 3.9 mm I.D. Microbondapak TM, Waters), an oven (CTE-22AV, Sekisui) regulated at $25.0 \pm 0.1^\circ\text{C}$, an ultraviolet spectrophotometric detector (UVD) (Model 490, Waters) used at wave lengths of 207 nm, 240 nm, 261 nm and 305 nm, electrochemical detector (ECD) (ECD-120, Sekisui) at an applied potential of +1.0 V vs. Ag/AgCl and a chromatogram data processor (type 7000B, System Instruments Corporation, Tokyo, Japan).

Chromatographic procedure. Fifty μl of R2323 solution in methanol (630 ng/50 μl , an internal standard, and 650 μl of water were added to 100 μl of each samples, and 400 μl of the mixture was injected into the pretreatment phase using the autosampler. RU38486 and its metabolites in a sample were adsorbed onto the pretreatment column and washed with water for 7 min.

Subsequently, the pretreatment column was incorporated into the analytical phase for 10 min. The eluent of the analytical phase was a mixture of 50 mM potassium phosphate buffer (pH 3.1) acetonitrile and methanol (20 : 9 : 6, v/v). RU38486 and its metabolites eluted from the pretreatment column were separated in the analytical column and detected by UVD and ECD. During this phase, the pretreatment column was incorporated again into the pretreatment phase, washed with 70 % methanol for 5 min, and then with water.

Results

Basic study. The ultraviolet absorption spectra of RU38486, RU42698, RU42848, RU42633 and R2323 are shown in Fig. 2. RU38486 exhibited UV-absorption at 206 nm, 261 nm and 305 nm (max.), RU42698 at 207 nm (max.), 261 nm and 304 nm, RU42848 at 209 nm, 242 nm and 303 nm (max.), RU42633 at 208 nm (max.), 251 nm, and 305 nm and R2323 at 203 nm, 239 nm and 328 nm (max.). RU42698, RU42848, RU42633 and R2323 shared a common high UV-absorption with RU38486 at 305 nm. The

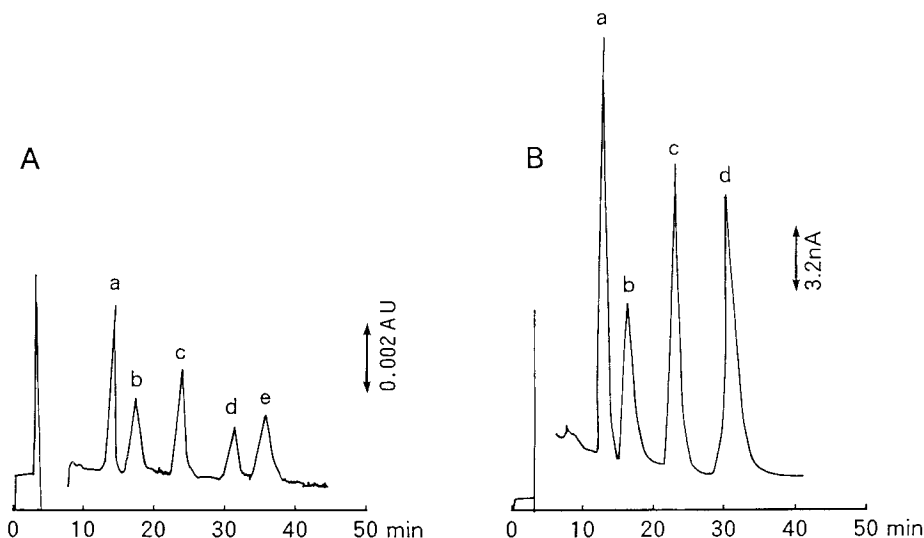


Fig. 3 Chromatograms of a mixture of the authentic samples of RU42698 (a), RU42848 (b), RU42633 (c), RU38486 (d) and R2323 (e) in 50 μl of serum (at the time of menstruation). Concentrations: RU42698, 463 ng/ml; RU42848, 880 ng/ml; RU42633, 364 ng/ml; RU38486, 265 ng/ml. A was detected with an ultraviolet detector at 305 nm and B with an electrochemical detector.

chromatogram obtained from a standard mixture of RU38486, its metabolites (RU42698, RU42848, RU42633) and R2323 are shown in Fig. 3. All these substances were well separated and detected by UVD. ECD could also detect them except for R2323. The detection limits (S/N ratio = 3) of RU42698, RU42848, RU42633 and RU38486 were 3.1, 3.4, 5.2 and 6.6 ng by UVD at the most sensitive wavelength, and 0.5, 0.5, 0.7 and 0.9 ng by ECD, respectively. ECD was able to assay the substances more sensitively than UVD was. In the routine work, however, we measured with UVD at the wave length of 305 nm, which was sensitive enough and most useful for the clinical application. The retention times of RU42698, RU42848, RU42633, RU38486 and R2323 were 12.9, 17.2, 23.0, 30.1 and 35.3 min, respectively. The relationships between the dose of each substance and the peak height detected on the chromatogram showed good linearity in the ranges from 7 to 110 ng for all these substances. From the study of the chromatogram ($n = 8$) of the standards directly injected into the analytical phase, and that of standards in serum ($n = 8$) (at the time of menstruation) injected into the pretreatment phase by the autosampler, the recovery rates of RU42698, RU42848, RU42633 and

RU38486, were 98.9, 99.5, 84.8 and 92.4 %, respectively. The intra-assay coefficients of variation (CV) of the peak height of RU42698, RU42848, RU42633 and RU38486 were 1.0, 1.6, 0.6 and 2.0 %, respectively.

Clinical results. A typical example of the chromatogram obtained from 50 μ l of a female volunteer's serum 1 h after the ingestion of 200 mg of RU38486 is shown in Fig. 4. The quantified concentrations of RU42698, RU42848, RU42633 and RU38486 were 169, 675, 1342 and 742 ng/ml, respectively using the internal standard method. Fig. 5 shows the pharmacokinetics of RU42698, RU42848, RU42633 and RU38486 in samples after the oral intake of 200 or 400 mg of RU38486. The fitted curve corresponded to an open two-compartment model for all compounds. The area under the serum level curve (AUC), maximum concentration (C_{max}), time of maximum concentration (T_{max}), and half life of elimination ($T_{1/2}$) are reported in Table 1. AUC of RU38486 and T_{max} of the RU42698 was significantly larger in the 400 mg group than in the 200 mg group ($p < 0.05$). AUC of RU42698 and $T_{1/2}$ of RU42633 tended to be larger in the 400 mg group than in the 200 mg group, but there were no significant differences.

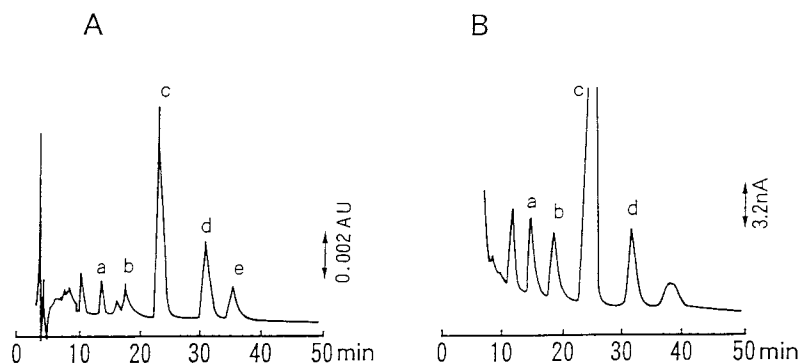


Fig. 4 Chromatograms obtained from 50 μ l of a volunteer's serum collected 1 h after the oral administration of 200 mg of RU38486. a: RU42698, b: RU42848, c: RU42633, d: RU38486, e: R2323. A, detected with an ultraviolet detector at 305 nm. B, detected with an electrochemical detector.

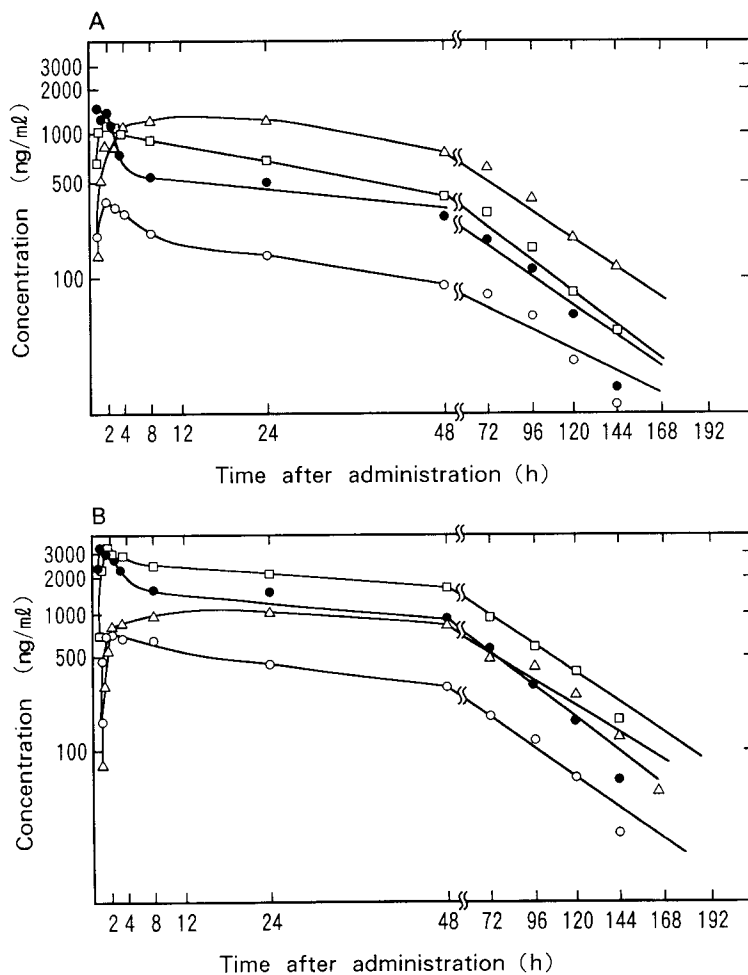


Fig. 5 Semilogarithmic plot of mean serum concentrations of RU38486 (●) and its metabolites (RU42698 (○), RU42848 (△) and RU42633 (□) in 6 female volunteers after oral administration of 200 mg (n = 3) (A) or 400 mg (n = 3) (B) of RU38486.

Table 1 Pharmacokinetic parameters of RU38486 and three metabolites^a

	Group	RU38486	RU42698	RU42848	RU42633
AUC (ng·h/ml)	200 mg	35155 ± 14846*	11962 ± 6297	91736 ± 16419	50155 ± 2476
	400 mg	116493 ± 37343*	36240 ± 11604	94602 ± 29576	183070 ± 73977
C _{max} (ng/ml)	200 mg	1586 ± 229	311 ± 112	1341 ± 236	1231 ± 188
	400 mg	3384 ± 1698	782 ± 441	1122 ± 34	3410 ± 1808
T _{max} (h)	200 mg	0.8 ± 0.5	1.0 ± 0.4*	13.3 ± 7.5	1.3 ± 0.2
	400 mg	1.3 ± 0.5	3.3 ± 0.9*	24.0 ± 0.0	1.7 ± 0.2
T _{1/2} (h)	200 mg	31.8 ± 2.7	41.2 ± 14.4	33.9 ± 2.7	29.2 ± 2.4
	400 mg	33.1 ± 5.1	39.3 ± 16.2	36.6 ± 7.4	36.6 ± 2.9

^a: Pharmacokinetic parameters of RU38486 and three metabolites (RU42698, RU42848 and RU42633) after oral administration of 200 mg (200 mg group, n = 3) or 400 mg (400 mg group, n = 3) of RU38486. Values are expressed as mean ± SD. *: Significantly different by student t-test.

Abbreviations: AUC = the area under the serum level curve; C_{max} = maximum concentration; T_{max} = time of maximum concentration; T_{1/2} = half life of elimination. * p < 0.05 mean ± SD

Discussion

RU38486 has both antiglucocorticoid and antiprogesterone activities of high potency, and is used clinically in France and China. RU38486 can therefore be applied in the symptomatic treatment of hypercortisolemia (11) and the regulation of fertility (12). Deraedt R. *et al.* reported that they succeeded in the synthesis of the four metabolites of RU38486 found in rats (1). We were supplied three of them, RU42698, RU42848 and RU42633 from Roussel Uclaf. In this study we assayed the serum concentration by an automated simultaneous direct assay system using HPLC.

There are several reports concerned with the measurement of RU38486 (1-4). However, because of the cross-reactivity of the putative metabolites of RU38486 in their RIA, the separation of RU38486 from metabolites was required for procedures such as thin layer chromatography after diethyl ether extraction (1-2) or column chromatography (2). We first reported the method to clearly separate RU38486 and its three metabolites by HPLC, and to quantify them simultaneously using UVD at the same wavelength of 305 nm in this report. The reliability of our separation and identification method was facilitated because we could measure them by UVD at four different wavelengths and by ECD simultaneously. The pharmacokinetic curve of RU38486 in serum corresponded to the open two compartment model in our study, that was same as reported by Deraedt *et al.* (1). Liu J.H. *et al.* reported that the pharmacokinetics of RU38486 corresponded to the non-compartment model, but they suggested that the cross-reactivity of the present RIA might affect the result (3). The half life of RU38486 was reported to be 23.7 ± 3.0 h by Deraedt *et al.* (1) or over 27 h by Heikinheimo *et al.* (2), but in our result it was 31.8 h (200 mg group) to 33.1 h (400 mg group). The half lives of other three metabolites were almost the same to that of RU38486. The rather long half lives of these compounds can be explained in part by its

binding to plasma protein, since approximately 95 % of circulating RU38486 was bound to both albumin (6) and alpha-1 acid glycoprotein (7). The effect of RU38486 was reported to be dose-dependent in the rat and rabbit (4). But the high concentrations and long half-lives of the metabolites also seem to be important because some metabolites, especially RU42633, were reported to have a potent antiprogesterone effect (1). Therefore our system seems to be very useful because it is important to study the concentration of RU38486 and its metabolites to determine the appropriate dose of RU38486.

Acknowledgments. The authors are grateful to Professor T. Yanaihara for providing the blood samples, and to Roussel Uclaf for providing the authentic powders of RU38486, RU42698, RU42848, RU42633 and R2323.

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Received October 18, 1990; accepted November 20, 1990.