Original Article

PHARMACEUTICAL STUDY OF SUPPOSITORY FORMULATIONS FOR IMPROVED *IN VIVO* KINETICS OF RIFAMPICIN

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Abstract [Purpose] To study rifampicin (RFP) suppository formulations that were prepared by adding an absorption promoter and a holding agent to conventional rifampicin suppositories in order to achieve higher blood drug levels. [Subjects and Methods] The subjects were 3 healthy volunteers who gave consent to participate in this study. Suppository formulations were prepared by adding sodium caprinate (the absorption promoter) to 600 mg, 750 mg, or 900 mg of RFP at about 3% of the suppository weight and sodium alginate (the holding agent) at 25% of the RFP content. Serum samples collected at 2, 6, and 10 hours after insertion of a suppository were subjected to RFP concentration analysis. [Results] In subject no. 1, the maximum concentration was 0.807 μg/ml, 1.093 μg/ml, and 1.291 μg/ml after administration of a suppository containing 600 mg, 750 mg, or 900 mg of RFP, respectively. [Discussion] Since an injectable RFP formulation has not been approved in Japan, formulation studies of RFP suppositories are important to achieve a better clinical response and to prevent the development of resistance. The present formulation that delivered a blood concentration of RFP considerably higher than 1 μg/ml was considered to have therapeutic potential. Its clinical utility will be examined in further formulation studies.

Key words: Pulmonary tuberculosis, Rifampicin, Suppository, Rectal absorption, Sodium caprinate, Sodium alginate

Introduction

Rifampicin (RFP) is a semisynthetic antibiotic of the ansamycin group that is a pivotal agent in the treatment of tuberculosis[1)~3)] When administered orally, RFP is readily absorbed from the small intestine and its maximum blood concentration occurs within 2 hours of ingestion[4)] This drug is distributed widely to various organs and to body fluids such as the lymph and cerebro-spinal fluid[5)]. At the National Hospital Organization Higashi Nagoya National Hospital (Higashi Nagoya Hospital), over 8,000 capsules of RFP are used every month to treat almost all tuberculosis patients, except those infected with RFP-resistant strains and those who suffer adverse reactions to this drug. There are also some patients who cannot receive oral or tube administration of RFP due to various conditions and they are given RFP suppositories that are prepared in-house. However, concern has been raised about the rectal absorption of RFP[6)], and development of a suppository formulation that improves the absorption of this drug is urgently needed in Japan where an injectable RFP formulation has not been approved. Against this background, we set out to prepare a suppository formulation that could achieve blood levels of RFP exceeding 1 μg/ml, which make the development of resistance very unlikely. We sought to improve the pharmacokinetics of the suppository formulation by increasing the dose of RFP and by adding an absorption promoter and a holding agent.

Subjects and Methods

(1) Subjects

The subjects of this study were 3 healthy volunteers who gave consent to participation. This study was approved by the institutional review board of Higashi Nagoya Hospital. In addition, regarding the ethical consideration, RFP suppository was used upon obtaining an agreement after having explained the purpose of this study, drug efficacy of this pharmaceutical,

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and ingredients in detail.

(2) Methods

(2-1) Materials

RFP granules from Rifadine capsule manufactured by Daiichi Pharmaceuticals were ground in a mortar to provide powder for use in this study. Vosco H-15 obtained from Maruishi Pharmaceutical Co., Ltd. (H-15) was used as the base for the suppository. The absorption promoter was sodium n-decanoate (sodium caprinate) from Kanto Chemical Co., Inc., and sodium alginate 80-120 obtained from Wako Pure Chemical Industries, Ltd. was used as the holding agent. Suppository containers with a volume of 2.25 m*l* were from Maruishi Pharmaceutical Co., Ltd.

(2-2) Preparation of RFP suppository formulations

RFP suppository formulations were prepared by the melting method. The formulations examined in the present study are presented in Table 1. To make up each formulation, H-15 was weighed into a beaker and melted in an ultrasonic bath at 50℃. Next, a weighed amount of sodium caprinate was ground to fine particles in an agate mortar. To 600 mg, 750 mg, or 900 mg of RFP powder, sodium caprinate powder prepared in this way and sodium alginate were added. Then the mixture was added to the melted H-15, and stirred at 50°C for 30 minutes in the ultrasonic bath. Finally, the mixture was used to fill the suppository containers so that it was molded to form suppositories, which were stored at 4 to 6°C with protection from light. The formulation containing 600 mg of RFP was designated as Formulation No.1, while those containing 700 mg and 900 mg of RFP were formulations No.2 and No.3, respectively. The conventional RFP suppository currently in use at our hospital was designated as No.4.

(2-3) Serum samples

Serum was harvested from blood collected at 2, 6, and 10 hours after insertion of an RFP suppository for determination of the RFP concentration. Serum samples were kept at 5 degrees Celsius until measurement. To remove proteins, 3 m*l* of acetonitrile was added to 1 m*l* of serum sample and the sample was mixed for 10 seconds. Then, after centrifuging the sample at 3,700 rpm for 15 minutes, 1 m*l* of supernatant was extracted for analysis.

(2-4) Reference standard and reagents

The RFP reference standard, acetonitrile for high-performance liquid chromatography (HPLC), distilled water for HPLC, methanol for HPLC, sodium dihydrogen phosphate $2H_2O$, disodium hydrogen phosphate $12H_2O$, and reference serum (control serum I WAKO/B) were all products of Wako Pure Chemical Industries, Ltd.

(2-5) Preparation of reference serum containing RFP

To 1 m*l* of the reference serum, sufficient RFP was added to make up concentrations of 2.5, 5, 7.5, 10.0, 12.5, and 15 μg/m*l*. Then the mixture was stirred for 10 minutes.

(2-6) Determination of the serumlevel of RFP

Serum concentrations of RFP were determined by HPLC.

The following HPLC instruments were used: a Prominence HPLC of Shimadzu Corporation (solvent delivery unit: LC-20AD, autosampler: SIL-20A, column oven: CTO-20A, UV-VIS detector: CTO-20A), a Shim-pack FC-ODS (4.6 mm I.D. × 150 mm: particle diameter: 3 μm) as the analytical column, and a guard column (4.6 mm I.D. × 10 mm). The analytical conditions were a column temperature of 40℃, a mobile phase of 40 mmol/L phosphate buffer (pH 6.7): acetonitrile: methanol (520 : 390 : 90 (v/v)), a flow rate of 1.1 m*l*/min, a detection wavelength of 340 nm, and an injection volume of 10 μl.

(2-7) Statistical analysis

All statistical analyses were performed by the paired t-test using JSTAT software.

Results

(1) Subjects

Three healthy male volunteers with no current illnesses or medications consented to participate in the present study. The average age was 35.0 years. The height of subject no. 1 was 160 cm; subject no. 2, 168 cm; and subject no. 3, 170 cm. The body weight of subject no. 1 was 60 kg; subject no. 2, 61 kg; and subject no. 3, 67 kg.

(2) HPLC

Measurement of serum RFP levels by HPLC gave chromatograms such as those shown in Fig. 1. The retention time of RFP was about 7.5 minutes and no interfering peaks were

Table 1 Rifampicin suppository formulations tested

Formulation	RFP content (mg)	VOSCO		Absorption enhancer	Holding agent	Designation
		H-15 (g)	E-75*(g)	Sodium n-decanoate	Sodium alginate	
1	600	1.22		0.067	0.15	RFP (600)-CA
2	300+450	1.75+1.53		0.067+0.067	0.077+0.113	RFP (750)-CA
	(750)	(3.28)		(0.134)	(0.19)	
3	300+600	1.75+1.22		0.067+0.067	0.077+0.15	RFP (900)-CA
	(900)	(2.97)		(0.134)	(0.227)	
4**	600	0.8	0.8			RFP (600)

*VOSCO E-75
**Current rifampicin suppository formulation

observed.

(3) Calibration curve and detection limit level

To the reference serum, the RFP reference standard was added at final concentrations of 2.5, 5, 7.5, 10, 12.5, and 15 $\mu g/ml$. Then the serum samples were assayed and the data were used to construct a calibration curve in which the area of RFP on the x-axis was plotted against the concentration of RFP on the y-axis (Fig. 2). The calibration curve passed through the origin and showed good linearity. The equation describing the curve was $f(x) = 0.00024 \times x$ ($r = 0.999$, $p = 0.003$), and there was a strong correlation between the RFP concentration in reference serum and the peak area. In addition, the detection limit level of RFP was 0.454 $\mu g/ml$.

(4) Profile of serum RFP levels with each formulation

Suppositories containing a mixture of RFP (600 mg, 700 mg, or 900 mg) with sodium caprinate and sodium alginate (RFP (600)-CA, RFP (700)-CA, and RFP (900)-CA, respectively) were inserted and serum RFP concentrations were measured after 2, 6, and 10 hours. The results are shown in Table 2. Note that the data at 6 hours after administration of RFP (900)-

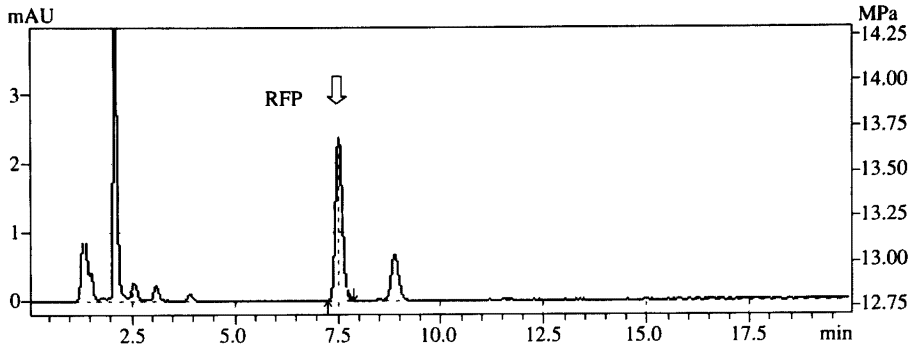

Fig. 1 HPLC chromatogram of RFP-spiked plasma

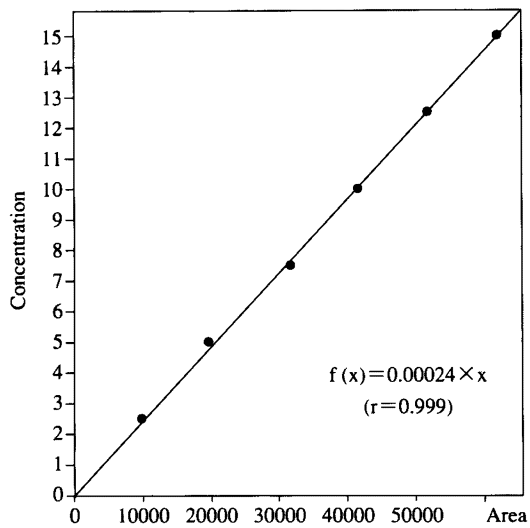

Fig. 2 Calibration curve for control RFP serum

Table 2 Serum RFP concentrations after administration of RFP suppositories

Subject	RFP (600)-CA			RFP (750)-CA			RFP (900)-CA		
	2 hr	6 hr	10 hr	2 hr	6 hr	10 hr	2 hr	6 hr	10 hr
1	0.089	0.807	0.736	0.317	1.093	0.739	0.221	1.291	1.078
2	0.104	0.471	0.363	0.178	0.544	0.361	0.108	0.311*	0.261*
3	0.062	0.126	0.09	0.075	0.175	0.159	0.114	0.235	0.193
AV±SD	0.085	0.468	0.396	0.190	0.604	0.419	0.148	0.612	0.511
	±	±	±	±	±	±	±	±	±
	0.017	0.278	0.265	0.099	0.377	0.240	0.052	0.481	0.402

*Data obtained following evacuation after value blood sampling at two hours

CA to subject no. 2 was obtained following defecation, which occurred after blood collection at 2 hours. The mean (mean ±standard deviation) serumlevel of RFP at 2, 6, and 10 hours after administration of RFP (600)-CA was 0.085± 0.017 μg/ml, 0.468±0.278 μg/ml, and 0.396±0.265 μg/ml, respectively. The mean serumlevel of RFP at 2, 6, and 10 hours after administration of RFP (750)-CA was 0.190± 0.099 μg/ml, 0.604±0.377 μg/ml, and 0.419±0.240 μg/ml, respectively. The mean RFP level at 2, 6, and 10 hours after administration of RFP (900)-CA was 0.148±0.052 μg/ml, 0.612±0.481 μg/ml, and 0.511±0.402 μg/ml, respectively. Although there was a decrease at 6 hours after administration of RFP (900)-CA in subject no. 2 due to defecation that reduced the mean RFP level, the serum concentration of RFP generally showed a dose-dependent increase. The changes of RFP over time for each suppository formulation are presented in Fig. 3 to 5. In all three subjects, the peak serum RFP concentration was seen at 6 hours after administration. Fig. 6 shows the serum concentration of RFP at 6 hours obtained with each formulation. Serum RFP levels exceeding 1 μg/ml were achieved in subject no. 1 after administration of both RFP (750)-CA and RFP (900)-CA (i.e., 1.093 μg/ml and 1.291 μg/ml, respectively).

Discussion

At present, the RFP suppository formulation used at Higashi Nagoya Hospital is prepared extemporaneously and contains a mixture of RFP from commercial capsules plus the suppository base H-15 or E-75. It is used for the treatment of tuberculosis in elderly patients who have difficulty in swallowing or patients in whom we cannot use feeding tube due to reflux or because they pull out the tube repeatedly. However, the peak

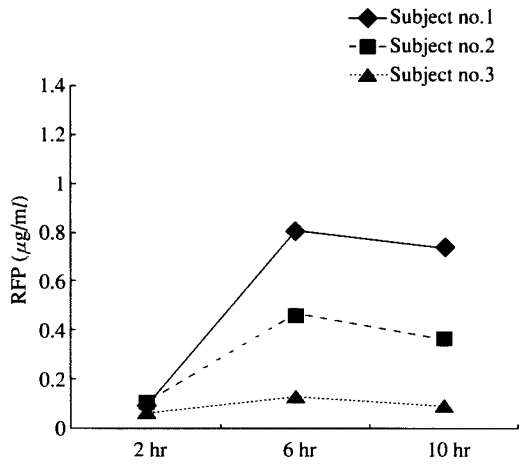

Fig. 3 Time course of serum RFP concentrations after administration of suppositories containing 600 mg of RFP (n=3)

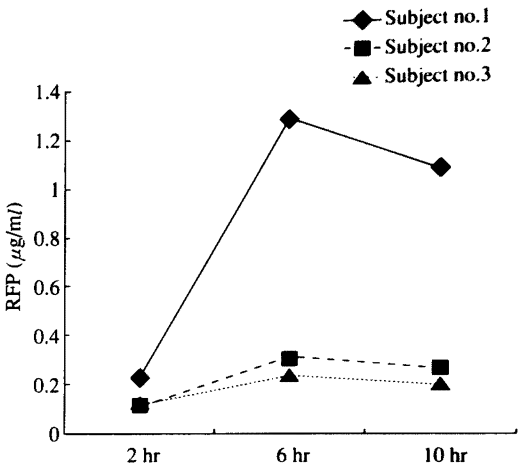

Fig. 5 Time course of serum RFP concentrations after administration of suppositories containing 900 mg of RFP (n=3)

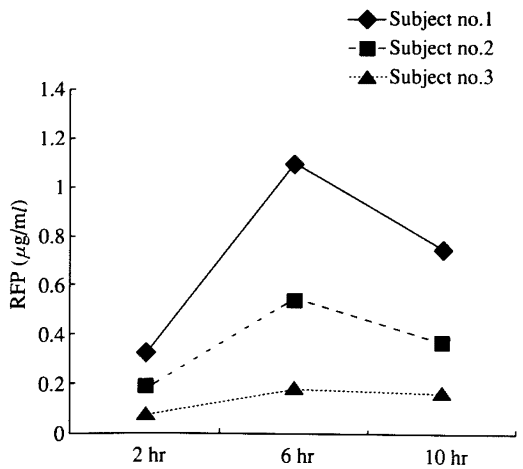

Fig. 4 Time course of serum RFP concentrations after administration of suppositories containing 750 mg of RFP (n=3)

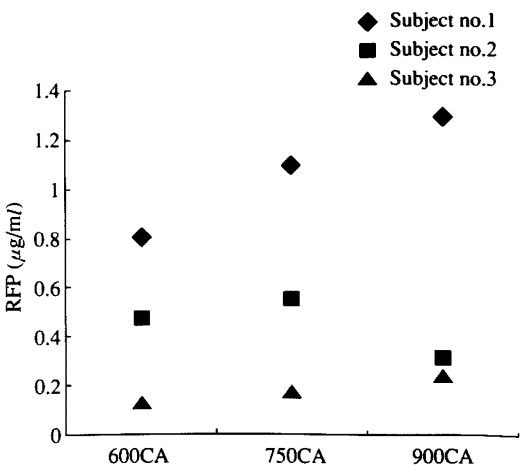

Fig. 6 Serum RFP concentrations at 6 hours after administration of suppositories containing 600 mg, 750 mg, and 900 mg of RFP

blood concentration of RFP achieved following administration as a suppository is relatively low compared with that after oral administration, and RFP was reported to be poorly absorbed from the rectum[6]. Accordingly, formulation studies on RFP suppositories have been performed[7]. We previously reported that the blood level of RFP increased dose-dependently after administration as a suppository[8]. In the present study, we sought to improve the pharmacokinetics of the suppository formulation by adding sodium caprinate as an absorption promoter and sodium alginate as a holding agent, aiming to achieve an increase of the blood level of RFP to above 1 μg/ml, at which resistance is not thought to develop[9]. The reason for adopting sodium caprinate as the absorption promoter was because this medium chain fatty acid salt (with 10 carbon atoms of various chain lengths) was reported to be the most effective absorption promoter for ampicillin suppositories by Nishimura et al.[10]. In addition, sodium caprinate has an effect on both the intracellular and intercellular routes of drug absorption according to Takahashi et al., and it was found to promote rectal absorption of drugs from suppositories[11]. Sodium caprinate is used in ampicillin suppositories (Herpen, Dainippon Sumitomo Pharma Co., Ltd.) and ceftizoxime suppositories (Epocelin, Choseido Pharmaceuticals), and its safety has been demonstrated in basic experiments during development, clinical studies, and post-marketing surveys. The reason for adopting sodium alginate as the holding agent is because it is abundant in seaweed such as konbu (kelp, *Lanminaria japonica*), wakame (brown seaweed, *Undaria pinnatifida*), hijiki (*Hizikia fusiformis*), and mozuku (*Nemacystus decipiens*), and has long been used clinically in extemporaneous formulations of morphine hydrochloride suppositories as a holding agent, with no adverse events being reported during use of formulations containing this substance[12]. In the present study, no adverse events caused by sodium caprinate or sodium alginate were reported either. The size of the suppository container allowed formulation of suppositories containing 600 mg of RFP at maximum. Therefore, RFP (300)-CA plus RFP (450)-CA were used for administration of 750 mg (RFP (750)-CA), while RFP (300)-CA plus RFP (600)-CA were used for administration of 900 mg (RFP (900)-CA).

The present study investigated methods for improving the rectal absorption of RFP from our in-house suppositories to achieve higher blood drug levels. Although the data from subject no. 2 at 6 hours after administration of RFP (900)-CA led to a reduction of the mean concentration (due to defecation), the serumlevel of RFP generally increased dose-dependently. At 6 hours after administration of RFP (600)-CA and RFP (750)-CA, the mean (mean$\pm$standard deviation) serumlevel of RFP was 0.468 ± 0.278 μg/ml and 0.604 ± 0.377 μg/ml, respectively, showing a 1.29-fold increase, while the serumlevels after RFP (600)-CA and after RFP (900)-CA were 0.468 ± 0.278 μg/ml and 0.612 ± 0.481 μg/ml, respectively, showing a 1.31-fold increase. At 10 hours after administration of RFP (600)-CA and RFP (750)-CA, the mean (mean$\pm$standard deviation) serumlevel of RFP 0.396 ± 0.265 μg/ml and 0.419 ± 0.240 μg/ml, respectively, showing a 1.06-fold increase, while the serumlevels after RFP (600)-CA and after RFP (900)-CA were 0.396 ± 0.265 μg/ml and 0.511 ± 0.402 μg/ml, respectively, showing a 1.29-fold increase. However, a significant difference was not observed in either group. This was considered to be attributable to the decrease of the serum RFP level in subject no. 2 due to defecation and the large differences of serum concentrations between subject no. 1 and subject no. 3. This marked difference between subjects 1 and 3 was considered to be due to individual variations in hepatic metabolism, but the reason needs to be examined in further studies. The same variation is also observed with blood levels of RFP after oral administration. In the present study, the times of blood collection were set at 2, 6, and 10 hours after administration. In previous studies, the time of 2 hours after administration was considered to be appropriate considering the absorption profile of RFP from the small intestine, the fact that the maximum blood level of RFP occurs within 2 hours after administration, and the good absorption of drugs from most suppositories. In the present study, however, it was assumed that the holding agent added to the suppository would maintain the blood level of RFP for longer and that the peak concentration would occur later. Addition of sodium alginate at 0.15 g to RFP (600)-CA, 0.19 g to RFP (750)-CA, and 0.225 g to RFP (900)-CA delayed the peak serum concentration of RFP until 6 hours after administration. While there have been no pharmacokinetic-pharmacodynamic analyses of RFP reported, it is assumed that maintenance of a serum concentration greater than 1 μg/ml means prolonged exposure of the tubercle bacillus to RFP. Availability of RFP against tuberculosis bacillus and the pharmaceutical devising of RFP suppository aimed at the evasion of acquired resistant tuberculosis bacillus by RFP are considered very important for tuberculosis treatment. Especially in our subject no. 1, the serumlevel of RFP at 6 hours after administration of RFP (750)-CA and RFP (900)-CA was 1.093 μg/ml and 1.291 μg/ml, respectively, with both being greater than 1 μg/ml. At 10 hours after administration of RFP (900)-CA, the serumlevel was still 1.078 μg/ml, showing that a high concentration was sustained. The above results suggested that rectal absorption of RFP from suppositories can be improved by addition of an absorption promoter and a holding agent to the suppository formulation. This could lead to improvement of the efficacy of therapy for patients with pulmonary tuberculosis who can not tolerate oral or tube administration. We plan to conduct further formulation studies, including a study on the optimum ratio of sodium caprinate, as well as assessing the clinical usefulness of RFP suppositories as an alternative to the oral formulation in Japan (where parenteral RFP is not approved).

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直腸吸収改善を目的として製剤改良したリファンピシン坐剤の検討

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要旨：〔目的〕Rifampicin (RFP) 坐剤に吸収促進剤および吸収保持剤を配合し血中RFP濃度上昇を目的として製剤化したRFP坐剤の検討を行った。〔対象および方法〕対象は研究に同意した3名のヘルスボランティアとした。RFP坐剤は600 mg，750 mg，900 mgのRFPに吸収促進剤であるカプリン酸ナトリウム坐剤重量の3%前後，吸収保持剤であるアルギン酸ナトリウムをRFP含量の25%添加した。各坐剤挿入後2時間，6時間，10時間後に採血した血清を検体として血中RFP濃度を測定した。〔結果〕Subject no. 1においてはRFP 600 mgを含有する坐剤で0.807 μg/ml，750 mgで1.093 μg/ml，900 mgで1.291 μg/mlとなるRFPの最高血中濃度が得られた。〔考察〕本邦においてRFP注射剤が使用できないため，RFP坐剤の製剤的検討が，治療効果を示すうえでも耐性菌の出現を回避するうえでも重要な課題であった。本研究においてRFP血中濃度が1 μg/mlを超えたことにより治療薬として使用可能であると考えられた。今後，さらに製剤的検討を加えて臨床的な有用性を検討していきたい。

キーワーズ：肺結核症，リファンピシン，坐剤，直腸吸収，カプリン酸ナトリウム，アルギン酸ナトリウム