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A new intramuscular formulation of rifamycin SV

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Summary

A new lyophilised formulation of Rifamycin SV, for intramuscular administration, has been developed. This differs from the existing commercial formulation (Rifocin) in that it has only 12 mg/ml polyvinylpyrrolidone K17 compared with 100 mg/ml in the Rifocin injection. It further promises to extend the shelf-life of the product from 2 years to a minimum of 4 years. The relative bioavailability of this new formulation was found to be equivalent (0.94), in sheep, to that of the Rifocin injection, although clear differences in the release pattern from the site of injection were observed. An ion-pair reversed-phase high-performance liquid chromatography assay procedure was also developed for the analysis of the drug in plasma samples.

Introduction

Rifamycin SV (RF SV) is an antibiotic used in the treatment of hepato-biliary infections, in tuberculous conditions, and in leprosy (Sensi, 1964). It is derived from rifamycin B (RF B), which is a natural fermentation product of *Streptomyces mediterranei*.

The intramuscular (i.m.) formulation of RF SV (Rifocin, Lepetit) contains polyvinylpyrrolidone (PVP) K17 at a concentration of 100 mg/ml. In recent years, foreign body granuloma have been reported in two patients treated over long periods of time with high doses of intramuscular drugs formulated with high molecular weight polyvinylpyrrolidone (Hölscher and Altmannsberger, 1982). As a result, the German and Austrian Health Authorities have proposed to limit the PVP content of i.m. formulations to 15 mg/ml with no more than 50 mg per injection and to prohibit the use of PVP grades in excess of K18.

Rifocin has been in clinical practice for about 22 years and despite continuous drug monitoring, foreign body granuloma have never been reported in patients treated with this i.m. injection. Nevertheless, alternative means of reformulating the i.m. injection with a reduced PVP content were examined.

This paper reports the development of a new lyophilised preparation in which the PVP concentration was reduced to 12 mg/ml. A cross-over study of the comparative bioavailability of this new preparation with that of the Rifocin injection

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was also performed, in sheep. In terms of analytical methodology, the standard method for the analysis of RF SV in biological fluids has been a microbiological assay procedure (Bergamini and Fowst, 1965). Although the level of sensitivity achieved is excellent, the procedure is non-specific and is therefore incapable of differentiating the activity of RF SV from that of its derivatives. Furthermore, the method is tedious, time-consuming, and the quantitative analysis involves the assessment of zones of inhibition which can be subject to inaccuracies. Hence, a reversed-phase ion-pair high-performance liquid chromatography assay procedure was developed for use as an analytical tool.

Materials and Methods

Materials

Rifamycin SV sodium salt, Rifamycin S (RF S), and Rifamycin B (RF B) were used as received from Gruppo Lepetit SpA (Italy). Rifocin ampoules containing 250 mg Rifamycin SV (RF SV) in 3 ml were also obtained from the above source. A benzophenone derivative, namely 2-hydroxy-5-chloro-4′-fluoro-benzophenone, was obtained from L.E.R.S., Paris, France and used as a suitable internal standard in HPLC measurements. Methanol, acetonitrile, and ethyl acetate, all of HPLC grade, were purchased from Romil Chemicals (U.K.). Dodecyltrimethylammonium bromide (C_{12}TAB) was obtained from Sigma (U.K.) and was used without further purification. Unless otherwise specified, all other reagents were of AnalaR quality.

The chromatography was performed on an automated Kontron (Switzerland) liquid chromatography system. This consisted of an MSI 660 sampler, a T-414 LC pump, a Uvikon 720 LC spectrophotometer, a Rheodyne 7010 sample injection valve fitted with a 100 μl sample loop, and a Spectra-Physics SP 4270 integrator. The chromatographic separation was performed on a 250 mm × 4.6 mm i.d. stainless steel column packed with Spherisorb S5 ODS1 (Phase Separations, U.K.). U.V./visible spectra of the compounds under consideration were determined on a Kontron Uvikon 810 spectrophotometer linked to a Uvikon 21 recorder.

For plasma extractions, two MT 16 minor vortex mixers (Chiltern Scientific Enterprises) and an MSE Centaur 2 centrifuge were used. A rotary evaporator (Rotavapor-R, Büchi) served to remove the organic solvent from the sample flasks.

Methods

(1) Composition of the new formulation

The existing i.m. commercial formulation of RF SV, Rifocin injection (Table 1) contains PVP K17 at 100 mg/ml. At this concentration, PVP serves to solubilise RF SV, to enhance its aqueous stability, and to prevent the precipitation (and possibly the formation) of the complex between drug and lidocaine. The physicochemical properties of RF SV were examined in detail with an

TABLE 1

Rifamycin SV formulations

Rifocin injection: [a]	
Rifamycin SV sodium salt q.s. to	250 mg activity RF SV
Ascorbic acid	25 mg
PVP K17	300 mg
Disodium EDTA	1 mg
Potassium metabisulphite	3 mg
Sodium hydroxide and sodium bicarbonate q.s. to	pH 6.6
Lidocaine hydrochloride monohydrate	10 mg
Water for injection q.s. to	3 ml
New formulation injection:	
Rifamycin SV sodium salt q.s. to	250 mg activity RF SV
Ascorbic acid	31.7 mg
PVP K17	47.6 mg
Disodium EDTA	1.3 mg
Potassium metabisulphite	3.8 mg
L-Arginine and hydrochloric acid q.s. to	pH 8.4
Lidocaine hydrochloride monohydrate	9.5 mg
Water for injection q.s. to	4 ml

[a] Unit pack information.

intent to reduce the PVP concentration to 12 mg/ml. The preformulation studies indicated that it was not possible to formulate the 250 mg dose of RF SV as an injectable solution without: (a) the addition of the amino acid, L-arginine, to enhance the solubility of the drug; (b) increasing its volume from 3 to 4 ml; and (c) changing its pH from 6.6 to 8.4. However, the decreased stability of RF SV in this liquid formulation coupled with the slow formation of the RF SV/lidocaine complex necessitated the use of a freeze-dried product to overcome these problems. The new proposed formulation, therefore, had the composition shown in Table 1. Following preparation, 4 ml aliquots of the new formulation were filled into 10 ml clear glass vials type 1 and were freeze-dried on an Edwards Minifast DO.5 freeze-dryer at Merrell Dow Research Institute, U.K. The head-space of the vials was back-filled with nitrogen before they were sealed and capped. For reconstitution purposes, 3.7 ml of water for injection was added to each vial to give a 4 ml injection of pH 8.4.

(2) Stability test programme

The aim was to determine the amount of RF SV remaining in the vials upon storage at different temperatures over a period of 5 years. At appropriate intervals, 5 vials were removed for analysis from the selected station. Following reconstitution, the solution was diluted (1 : 5000) in distilled water and the RF SV content determined by visible spectrophotometry at 445 nm (Scrutton, 1977) and also by ion-pair reversed-phase HPLC as described in section (4). Because the column behaviour can vary with time and because the stability test programme runs for 5 years, the HPLC system was recalibrated on every occasion of use.

(3) Intramuscular administration

Twelve Suffolk cross half-bred sheep were used in this study. The appropriate volumes of each formulation (equivalent to 250 mg of RF SV) were injected intramuscularly into the outer part of the mid-thigh. The injections were made at a depth of about 20 mm with 5 ml disposable syringes fitted with 21-gauge needles. On the first experimental day, 6 sheep received Rifocin and the other 6 received the new formulation. On the second experimental day, separated from the first by a wash-out period of 2 days, the cross-over part of the study was performed in which the formulations were administered into the previously uninjected hind-legs.

Blood samples of about 2 ml were collected from an in-dwelling Steriflex 14-gauge catheter (Vygon U.K.) in the jugular vein, into 5 ml heparinised tubes (Lithium Heparin, Laboratory Sales U.K.), immediately before the injection and at 15, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min post-injection. Blood samples were maintained at 4°C awaiting centrifugation. 1 ml samples of plasma were obtained, as soon as possible, by centrifuging the blood samples at 3000 rpm. Plasma samples were stored in 15 ml screw-neck glass extraction tubes fitted with PTFE-lined caps (Pyrex Disposable Screw Cap Culture Tubes, Corning) at -20°C awaiting analysis. Although the study involved 12 sheep, the data for one of them has been excluded from the calculations because of the difficulty exercised in obtaining blood from its in-dwelling catheter on one occasion. The weight ± S.D. of the remaining 11 sheep was 76.8 ± 13.5 kg.

(4) Chromatographic conditions

To separate the compounds under investigation, various solvent combinations were attempted and of these the system chosen for use had the composition: acetonitrile/ methanol/ McIlvaine buffer of pH 5.25 (Dawson et al., 1986) containing 5 mM C_{12}TAB (40 : 20 : 40 v/v/v). Prior to use, the mobile phase was filtered free from particulate matter on a glass ceramic sinter and degassed by helium. Further degassing was deemed unnecessary during the chromatography. The detector was set at 314 nm and the mobile phase was pumped at a flow-rate of 2 ml/min at a pressure of 180 bar. The volume of sample injected onto the column was held constant at 100 μl.

(5) Analysis of rifamycin SV in sheep plasma

RF SV and any related compounds were extracted from plasma samples by the following procedure: (1) the plasma, stored at -20°C, was allowed to thaw to room temperature; (2) 200 μl of a 10 mg/ml solution of ascorbic acid in distilled water was added to each tube to prevent the autoxidation of RF SV to RF S; (3) this was followed by the addition of 100 μl of 0.2 M hydrochloric acid to optimise the liquid–liquid extraction of RF SV; (4) 100 μl of a 49.5 μg/ml solution of the internal standard in methanol was added to each tube; (5) 3 ml of ethyl acetate was added to each tube and the mixture was vortexed for 1 min to achieve extraction; (6) the tubes were centrifuged at 2500 rpm for 5 min and the organic phase was transferred into clean 5 ml Quick-fit round-bottom flasks; and (7) the ethyl acetate was removed on the rotary evaporator and the solid residue was reconstituted in 200 μl of the mobile phase of which 100 μl was injected onto the column for analysis.

(6) Calibration of the plasma assay procedure

Standard stock solutions of Rifamycin SV were prepared in distilled water. These were used to spike 1 ml samples of blank sheep plasma, in triplicate, to produce a calibration curve in the concentration range 2–0.025 μg/ml. The samples were then carried through the above extraction procedure and the standard curve was obtained by plotting peak height ratios (drug/internal standard) against the corresponding concentration. The concentration of drug in unknown samples was determined by interpolation from the standard curve. The precision of the assay was determined by repeated analysis of plasma samples spiked with known amounts of Rifamycin SV.

(7) Pharmacokinetic analysis

The area under the plasma drug concentration–time curve, $AUC_{0-\infty}$, was calculated from the individual profiles using Eqn. 1:

$$AUC_{0-\infty} = AUC_{0-t'} + AUC_{t'-\infty} \qquad (1)$$

where t' represents the time when the last sample was taken. The first term, $AUC_{0-t'}$ was calculated using the trapezoidal rule (Gibaldi, 1984a) and the second term was calculated using Eqn. 2 (Chiou, 1978):

$$AUC_{t'-\infty} = C'/\beta \qquad (2)$$

where C' is the concentration of the last sample and β is the terminal first-order elimination rate constant. The mean residence time of the drug in the body, MRT, was calculated from Eqn. 3:

$$MRT = AUMC_{0-\infty}/AUC_{0-\infty} \qquad (3)$$

where the numerator is the area under the first moment plasma curve from time zero to infinity (Yamaoka et al., 1978). This term was calculated by the procedure given by Benet and Galeazzi (1979).

The terminal apparent first-order elimination rate constant was estimated from semilogarithmic plots of the concentration data from time 90 min to t' for each animal. The relative bioavailability, F_{rel}, was calculated by dividing the $AUC_{0-\infty}$ of the new formulation by that of Rifocin for each animal.

Results and Discussion

Optimizing performance of an HPLC system involves a good combination of separation and compactness of peaks together with a high speed of elution. Under aerobic conditions, RF SV is autoxidised to RF S (Fig. 1). In fact, RF SV and RF S are the reduced and oxidised forms, respectively, of a reversible oxidation–reduction system involving two electrons (Scrutton, 1977; Kono, 1982). To separate these two compounds and the internal standard, it was found necessary to use C_{12}TAB as an ion-pairing species in the mobile phase with resultant peak retention times of 3.9, 7.1 and 8.5 min for RF SV, RF S, and the internal standard, respectively (Fig. 2a). Further, RF B, present only as an impurity in the bulk substance of RF SV, also eluted at a retention time of 3.2 min.

The samples in which RF SV was in contact with the sheep plasma, both in vitro and in vivo, displayed a small unidentified peak adjacent to the drug's peak, as shown by the typical example in Fig. 2c. This was probably due to a metabolite of RF SV since the extracted blank plasma did not show it (Fig. 2b). Although the integrator employed often distinguished these two peaks, it

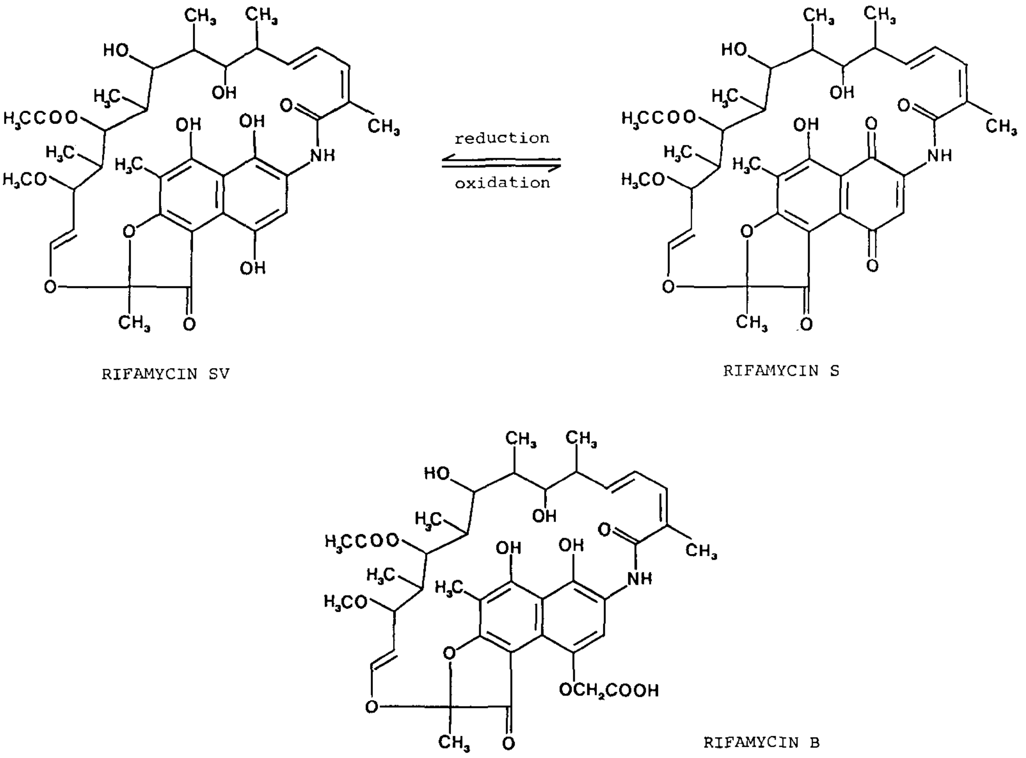

Fig. 1. Structures of Rifamycin SV, Rifamycin S and Rifamycin B.

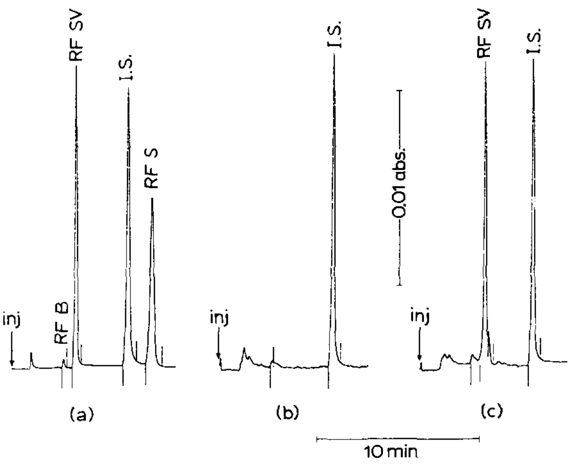

Fig. 2. Typical examples of the chromatograms obtained by the ion-pair reversed-phase HPLC procedure demonstrating: (a) separation of RF SV, RF S, RF B, and I.S.; (b) analysis of a sample of blank sheep plasma; and (c) analysis of a sample of sheep plasma collected 15 min after an i.m. injection of RF SV.

did not perform this universally and alteration of the integration parameters did not eliminate this problem.

Therefore, to avoid any interference with the data, it was decided to use the peak heights instead of the areas to construct the standard curve. The calibration curve obtained obeyed the linear equation: $y = 0.6952\ x - (5.678 \times 10^{-3})$ with a calculated correlation coefficient of 0.999. The precision of the assay procedure was examined at several sample concentrations and was found to be excellent (Table 2). Although RF SV and the internal standard were extracted almost totally from the plasma, the in-process losses resulted in an overall recovery of 60% and 74% for RF SV and the internal standard, respectively. The possible autoxidation of RF SV to RF S was prevented by the addition of ascorbic acid to the sample tubes and the chromatograms obtained, e.g. in Fig. 2c, confirmed this. The practical detection

TABLE 2

Reproducibility and accuracy of the assay procedure for rifamycin SV in plasma

Sample concentration (μg/ml)	Average of 6 assays (μg/ml)	Coefficient of variation (%)
0.025	0.023	6.5
0.10	0.103	3.1
0.50	0.495	2.1
1.00	1.01	2.9
2.00	1.99	1.8

limit of the chromatography was found to be 2 ng of RF SV per injection when the peak height was about 5 times the background noise.

Fig. 3 shows the plasma profiles obtained following the i.m. administration of the two formulations. It can be observed that the pattern of release of the drug from the i.m. site varies from one formulation to the other; the Rifocin injection produced a fast release of the drug into the general circulation with a mean $C_{\max}$ of 1.17 μg/ml and a mean $t_{\max}$ of 35.5 min (Table 3). However, these levels rapidly diminish and after 6 h there is little drug left in circulation (Fig. 3). By comparison, the new formulation gave a mean $C_{\max}$ of 0.62 μg/ml and a mean $t_{\max}$ of 81.8 min (Table 3). Here, the drug release from the i.m. site was maintained for a longer period which was reflected in the large standard deviation in the $t_{\max}$ value and in the higher postpeak plasma levels of the new formulation. This may also have reflected into the value calculated for the apparent first-order elimination rate constant, β; this was found to be smaller than that following the Rifocin injection although the magnitude of change was not statistically significant (Table 3). The mean values ($\pm$S.E.M.) of $AUC_{0-t'}$, $AUC_{0-\infty}$, and $AUMC_{0-\infty}$ for the two formulations are given in Table 3. Although the mean $AUC_{0-t'}$ for the new formulation was statistically ($P < 0.05$) different than that of Rifocin, the overall area under curve, $AUC_{0-\infty}$, was not. The mean relative bioavailability of the new formulation, F_{rel}, was calculated to be 0.94 with an S.E.M. of 0.18. Thus, in terms of extent, the two formulations can be described as equivalent. Another useful parameter which can be used to compare the two formulations is the mean residence time (MRT). The relative ranking of MRT for several formulations of the same drug mirrors their relative ranking with respect to drug release and absorption (Gibaldi, 1984b). The values calculated here show that the new formulation has a considerably longer MRT than the Rifocin injection (Table 3), which further confirms the different pattern of drug release from the i.m. site for the two formulations. The data obtained in sheep for Rifocin compare favourably with those obtained previously in humans upon the i.m. administration of a single 250 mg dose (Füresz and Scotti, 1961). The profiles are similar in terms of the rapid release of the drug from the i.m. site and its rapid elimination from the blood.

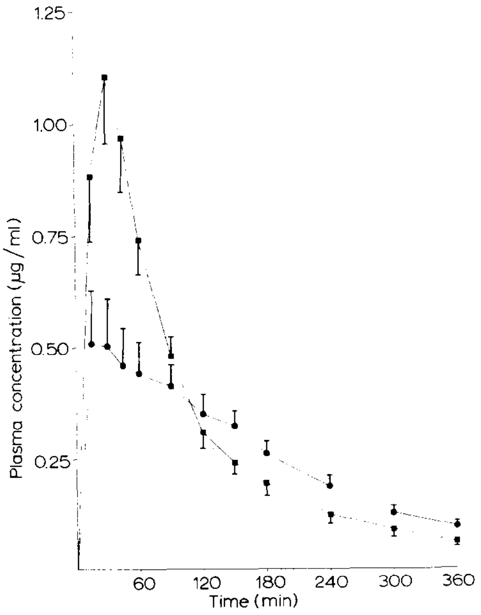

Fig. 3. Mean plasma levels obtained following the i.m. administration of two RF SV formulations to sheep (S.E. bars are shown, $n = 11$). ■, Rifocin injection; ●, new formulation injection.

The significant reduction in peak plasma levels associated with the new formulation should not reflect adversely on its clinical efficacy in the treatment of hepato-biliary infections because the drug is extensively excreted into the bile where it

TABLE 3

Mean pharmacokinetic parameters of rifamycin SV following intramuscular administration in sheep (n = 11)

	Rifocin injection		New formulation	
	mean	SEM	mean	SEM
C_{max} (μg/ml)	1.17	0.15	0.62 **	0.10
t_{max} (min)	35.5	4.6	81.8 *	19.7
$AUC_{0-t'}$ ($\mu g \cdot min \cdot ml^{-1}$)	115.1	9.6	97.9 *	9.4
$AUC_{0-\infty}$ ($\mu g \cdot min \cdot ml^{-1}$)	126.3	9.9	117.0	10.2
$AUMC_{0-\infty}$ ($\mu g \cdot min^2 \cdot ml^{-1}$)	17,554	2261	24,455	3506
$\beta \times 10^3$ (min^{-1})	8.109	1.44	5.747	0.46
MRT (min)	139.9	16.2	209.5 *	26.6
F_{rel}			0.94	0.18

* Significance of difference from Rifocin injection (as control) by Student's t-test: * $P < 0.05$, ** $P < 0.01$.

reaches concentrations several hundred fold those achieved in the general circulation (Füresz and Scotti, 1961; Acocella et al., 1968).

In terms of formulation, it proved difficult to remove the PVP entirely from the injectable solution without an associated marked loss in the stability and solubility of the drug and a decrease in local tolerance upon injection. Furthermore, it has been reported that in the absence of PVP, RF SV was poorly absorbed from the i.m. site with insignificant blood levels being achieved (Curci and Ninni, 1961). Thus, it appears that the addition of PVP to the formulation is necessary not only for pharmaceutical formulation but also for clinical efficacy. The proposed new formulation which contains 12 mg/ml PVP was found to be less stable as a liquid than the Rifocin injection and, therefore, lyophilisation was used to overcome the problem. In terms of product stability, the existing Rifocin injection has a 2 year shelf-life at room temperature. To date, i.e. after 12 months, the stability testing of the new formulation at all the temperature stations employed shows no loss in drug activity (Table 4). It is therefore anticipated that the new formulation can be used to increase the product shelf-life from 2 years to 4 years or more. This in turn would compensate for the increased cost in producing the new formulation as an alternative to the existing liquid injection. To conclude, a new formulation of acceptable physicochemical stability has been developed which gave comparable bioavailability, in terms of extent, to that of the Rifocin injection in the animal model used. The differences observed in the release pattern of the new formulation are not expected to affect the therapeutic efficacy of the drug in treating hepato-biliary infections. Future work will concentrate on the clinical assessment of the new formulation and on ensuring its therapeutic and biopharmaceutical equivalence to that of the Rifocin injection.

TABLE 4

Summary of new formulation stability

Lyovial content of Rifamycin SV, expressed as percentage of initial, by visible spectrometry (V) and by HPLC (H). The coefficients of variation are given in brackets, $n = 5$

Initial content	V: 100.0 (0.2%); H: 100.0 (1.4%)
12 months at 4°C	V: 101.2 (0.8%); H: 104.9 (2.2%)
12 months at 20°C	V: 100.4 (1.0%); H: 101.2 (3.8%)
12 months at 35°C	V: 100.4 (0.4%); H: 104.1 (2.4%)
12 months at 45°C	V: 100.4 (0.9%); H: 100.4 (4.0%)

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