Rifamycin SV systemic absorption in healthy volunteers administered as modified
release MMX® tablets

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Abstract

The new oral Rifamycin SV-MMX® 200 mg modified release tablets, designed to deliver rifamycin SV directly into the colonic lumen, offer considerable advantages over the existing immediate release anti-diarrhoeic formulations. In two pharmacokinetics studies in healthy volunteers the absorption, urinary excretion and faecal elimination of rifamycin SV after single and multiple dose regimen of the new formulation were investigated. Plasma concentrations >2 ng/mL were infrequently and randomly quantifiable after single and multiple oral dose. The systemic exposure to rifamycin SV after single and multiple oral doses of MMX® tablets under fasting and fed conditions or following a q.i.d. or a b.i.d. regimen could be considered as negligible. With both oral regimens the drug was confirmed to be systemically very poorly absorbable. The amount of systemically absorbed antibiotic excreted by the renal route is far lower than 0.01% of the administered dose both after single and multiple dose regimen. The absolute bioavailability calculated as mean percent ratio between total urinary excretion amounts (ΣXu) after single i.v. injection and after single oral dose under fasting conditions was 0.0410±0.0617.

The total elimination of the unchanged rifamycin SV with faeces was 87% of the administered oral dose. No significant effect of rifamycin SV on vital signs, ECGs or laboratory parameters was observed.

Keywords: non absorbable antibiotics, rifamycin SV, pharmacokinetics, healthy subjects, MMX®
1 INTRODUCTION

Rifamycin SV is a semi-synthetic antibiotic belonging to the class of ansamycins, obtained from rifamycin B, which is produced by fermentation of Streptomyces mediterranei n. sp. (4) (See Figure 1). The rifamycin producing strain was isolated in Lepetit Research Laboratories by Sensi et al. (20). Rifamycin SV, endowed with a broad-spectrum activity against Gram-positive and Gram-negative bacteria and mycobacteria, was introduced into clinical practice early in 1962 for parenteral use only and thus far has been employed in the treatment of infections due to staphylococci and other Gram-positive micro-organisms. Moreover due to the very high concentrations it reaches in the bile rifamycin SV has been also used in infections of the biliary tract sustained by less sensitive Gram-negative micro-organisms or mixed bacterial flora (4). Injectable and topical formulations of rifamycin SV sodium salt are commercially available in several countries with indication for cutaneous and soft tissue infections, osteomyelitis, bronchopulmonary, biliary tract infections and for staphylococcal septicaemias. Topical application is indicated for infections due to sensible pyogens: e.g. pyodermitis and dermatitis and infected wounds. Rifamycin SV activity is predominantly bactericidal by interference with bacterial protein synthesis. Rifamycins like other ansamycins inhibit the protein synthesis by binding the β-sub-unit of the bacterial DNA-dependent RNA-polymerase. Literature data reviewed by N. Bergamini and G. Fowst (4) reported that rifamycin SV administered as a single oral dose of 500 mg did not result in appreciable blood serum levels, thus behaving as a non absorbable antibiotic. Fürész et al. (11) found that after a single intramuscular injection approximately the 90% of the injected rifamycin SV was
eliminated with the bile. Rifamycin SV can hydrolyse in vivo and after de-esterification forms the 25-desacetyl-metabolite (1, 16).

Antimicrobial drugs represent an important treatment approach and remain the current standard of care for some types of enteric infection (9). The antimicrobial treatment has established value in the therapy for shigellosis, diarrhoea due to infection with enterotoxigenic *E. coli*, cholera, antibiotic-associated colitis due to *Clostridium difficile*, traveller’s diarrhoea, giardiasis and amoebiasis (9). In the early 80s it was indicated that the use of non-absorbable antibiotics represents an effective treatment of infectious acute diarrhoea with important safety and tolerability implications (9, 10). A non-absorbable antibiotic has the advantage to limit the antimicrobial effect to the intestinal lumen, avoiding systemic effects.

Rifaximin is a semi-synthetic derivative of rifamycin SV. The condensation of a pyridoimidazoyl moiety to the naphtoquinone nucleus of rifymcin renders rifaximin poor soluble and non-absorbable and enables it to achieve higher concentrations in the lumen (17, 15, 2). A great relevance has been given to the therapeutic use of rifaximin, which is present in the market with the name of Xifaxan® (USA) and Normix® (Europe). Xifaxan® tablets are indicated for the treatment of patients (≥12 years of age) with travellers’ diarrhoea caused by non-invasive strains of *E. coli* (24). Normix® indications extend to acute and chronic intestinal infections, diarrhoeic syndromes, cases of diarrhoea with altered balance of the intestinal microflora, prophylaxis of pre and post-operative infective complications of gastroenteric interventions and add-on therapy of hyperammonaenia (21). One of the main concerns about the use of non-absorbable antibiotics, is that these substances act throughout the entire intestine, affecting also the saprophytic beneficial flora that lives in the upper region of the gastrointestinal tract. An indiscriminate antibacterial and antimicrobial action may affect some biological activities mediated by the enteric saprophytic micro-organisms, e.g. metabolism of
cholesterol, bile acids and bilirubin, inactivation of tryptic activity, enterohepatic recycle, absorption of drugs and vitamins, “colonisation resistance” against opportunistic pathogens, like *Clostridium difficile*, which are often cause of nosocomial gastrointestinal illness (18, 8, 5, 19). The possibility to administer non-absorbable antibiotics in a new oral formulation designed to deliver the substances directly into the colonic lumen, offers consistent advantages over the existing formulations, minimizing the side-effects closely related to unwanted activity on the saprophytic flora living in the upper intestinal tract, and improving the drug efficacy due to direct topical delivery.

Rifamycin SV-MMX® (Cosmo Technologies Ltd., Dublin) is a new oral modified release formulation, manufactured as coated tablets, containing 200 mg of sodium rifamycin SV. The tablets are formulated using a multimatrix structure (MMX®), which allows the delivery of the active ingredient directly in the colon as already demonstrated by different pharmaco-scintigraphic studies (6, 7). By this technology the maximum local bioavailability of the active ingredient is achieved in the colonic region and the biological effect is optimised. Active pharmaceutical ingredient and excipients employed in the manufacturing of rifamycin SV tablets are well known in the pharmaceutical field for their selective functionality and are described in compendia texts. MMX tablets contain a double matrix system. Microparticles of active ingredient are dispersed in a lipophilic matrix, which in turn is dispersed throughout a hydrophilic matrix. This double matrix system creates a partially hydrophobic environment which hinders the penetration of aqueous fluids into the tablet core, thus slowing the rate drug dissolution. This double matrix core is coated with a pH dependent, gastroresistant polymer film. The MMX tablet film begins to disintegrate when pH is ≥7.0. With this pH sensitive coating, the MMX tablets arrive unaltered to the caecum where the release starts to take place. Once the coating disintegrates, the intestinal fluids interact with the hydrophilic matrix,
causing the tablet to swell and form an outer, viscous gel mass. The viscous gel mass slows the diffusion of the antibiotic from the tablet core into the colonic lumen. While the tablet progresses in the colon towards the rectum, debris of the gel mass disaggregate and release the antibiotic in proximity to the mucosa. MMX formulations were investigated in healthy volunteers with the aim of obtaining information on the gastrointestinal transit, disintegration and release of various drugs delivered in the colonic region. Colonic delivery was clearly demonstrated by pharmaco-scintigraphic investigations (16, 7).

Recently the efficacy and safety of rifamycin SV tablets administered q.i.d. for 3 days to patients with infectious diarrhoea were investigated. Rifamycin SV was not inferior to the reference treatment with Rifaximin® tablets administered according to the same dose regimen (A. F. D. Di Stefano, D. Binelli, W. Labuschagne, M. Mojapelo, E. van der Walt, M. Gani, S. Patel, L. Moro, submitted for publication). Results of this trial confirmed the effectiveness of the dose regimen of two 200 mg tablets administered b.i.d. (i.e. 800 mg/day ) for 3 consecutive days.

The present article reports the results of 2 Phase I studies aimed at investigating the absolute bioavailability, the systemic absorption and the elimination of rifamycin SV after single and multiple dose of Rifamycin SV-MMX® 200 mg tablets in healthy male and females volunteers. Rationale for these two Phase I trials was the need to add information about the PK and the bioavailability of rifamycin SV after single and multiple oral dose and after single i.v. dose in healthy subjects with the objective to confirm the lack of systemic absorption of orally administered rifamycin SV. Furthermore a new more sensitive LC-MS/MS analytical method was put in place thus allowing the detection of the analyte at concentrations lower than the previously adopted method.
2 METHODS

2.1 Study designs

Study A was a single dose, open-labelled, randomised, cross-over absolute bioavailability and food effect study. The study was aimed at investigating the absolute bioavailability of rifamycin SV after an oral dose of two tablets (400 mg) of Rifamycin SV-MMX® 200 mg administered under fasting conditions, compared with a single i.v. dose of 250 mg of rifamycin SV. The study consisted of 3 periods during which subjects were administered first rifamycin SV i.v., then orally under either fast conditions in the 2nd period and under fed conditions in the 3rd period or vice versa (cross-over design) to assess the possible interaction of food on the oral absorption of sodium rifamycin SV. The food effect investigation was carried out according to the FDA guideline (13).

Study B was an open-labelled, parallel group, multiple dose, bioavailability study aimed at investigating the pharmacokinetic profile of systemically available rifamycin SV in healthy male and female subjects receiving one Rifamycin SV-MMX® 200 mg tablet four times a day or two Rifamycin SV-MMX® 200 mg tablets twice a day. The latter represents the proposed therapeutic regimen in the infective pathologies of the colon. Blood and urine sampling schedule was decided according to the expected kinetic profile of the antibiotic (4). To determine the faecal elimination, stools were collected after the single oral dose under the fasting conditions for 3 consecutive days, while the administration of a laxative was planned with the aim of ensuring the complete collection of the antibiotic still present in the enteric lumen.
2.1.1  **Study population and criteria for inclusion**

Both studies were performed at the Phase I Unit of Cross Research S.A., Arzo, Switzerland. Healthy males and females were included into the 2 trials according to the following main inclusion criteria: (i) age of 18 to 55 y, (ii) a body mass index (BMI) between 18 and 29 kg/m$^2$, (iii) good health based on medical history, physical examination, a 12-lead electrocardiogram (ECG) and routine haematology and blood chemistry tests, (iv) use of highly effective contraceptive methods for at least 1 month prior to the study start for females (14), (v) willingness to provide written informed consent. Main exclusion criteria were (i) pregnancy, (ii) intake of any medication, (iii) a history of drug, caffeine (>5 cups coffee/tea/day) or tobacco (≥10 cigarettes/day) abuse, or alcohol consumption in excess of two drinks per day in males and one drink per day in females, as defined by the U.S.D.A. dietary guidelines (22).

Both studies were descriptive non comparative trials. Sample size of either study (N=24) was not obtained after formal calculation.

2.1.2  **Investigational treatments and dose regimen**

Besides the investigational product Rifamycin SV-MMX® 200 mg tablets, Rifocine® was used (rifamycin SV 500 mg injectable solution, Aventis Pharma S.A., Belgium) for the i.v. dosing. In study A during Period I all subjects received a single i.v. injection of 250 mg of rifamycin SV. After a wash-out period of 7-8 days, they received a single dose of two Rifamycin SV-MMX® 200 mg tablets administered alternatively under fed / fasting conditions in two
consecutive periods (Period II and Period III) separated by a wash-out of 14 days between administrations according to a randomised cross-over design. Each dose was administered with 240 mL of water.

In study B the subjects were randomised to receive Rifamycin SV-MMX® 200 mg tablets according to two different regimens:

- One 200 mg tablet administered q.i.d. (i.e. 800 mg/day) for 3 consecutive days starting from 12.00 ± 1 h of Day 1 (τ = 6 h), for a total of 12 doses;
- Two 200 mg tablets administered b.i.d. (i.e. 800 mg/day) for 3 consecutive days starting from 18.00 ± 1 h of Day 1 (τ = 12 h), for a total of 6 doses.

In both studies subjects were confined in the Phase I Unit during the whole study periods.

2.2 Ethical procedures

The documentation of the 2 studies was reviewed by the independent ethics committee of Canton Ticino and approved on 03MAR09. Ref. nr. 2143 and 2144. The Swiss Federal Health Authorities (Swissmedic) approved and authorised the protocol B on 21APR09 and protocol A on 04MAY09. Study A was assigned the reference number 2009DR1082 and study B was assigned the reference number 2009DR1075. Both studies were conducted in compliance with the Swiss ordinance on clinical trials of therapeutic agents and in accordance with the Declaration of Helsinki and the general principles of ICH Harmonised Tripartite Guidelines for GCP. Subjects of either study did not undergo any study procedure before signing the written informed consent form.
2.3 Pharmacokinetics variables and data analysis

The following PK parameters were measured and/or calculated for rifamycin SV, when feasible, using the validated software WinNonLin® 5.2 (Pharsight Corporation):

Single dose study:

Plasma:  $C_0$, $C_{\text{max}}$, $T_{\text{max}}$, $\text{AUC}_0-t$, $\text{AUC}_0-\infty$, $t_{1/2}$, $t_{\text{lag}}$, MRT; $\text{Cl}/F$, $V_d/F$, $F_{\text{abs}}$, defined as follows:

- $C_0$: Estimated drug concentration at dosing time (only for i.v injection)
- $C_{\text{max}}$: Maximum plasma concentration
- $T_{\text{max}}$: Time to attain peak concentration ($C_{\text{max}}$)
- $\text{AUC}_0-t$: Area under the plasma concentration curve from administration to the last observed concentration time $t$, calculated with the trapezoidal method
- $\text{AUC}_0-\infty$: Area under the concentration/time curve extrapolated to infinity (calculated as $\text{AUC}_0-t + C_t/\lambda_z$, where $C_t$ is the last measurable drug concentration)
- $t_{1/2}$: Half-life ($\ln 2/\lambda_z$, where $\lambda_z$ is the terminal elimination rate)
- $t_{\text{lag}}$: Lag time
- MRT: Mean residence time (calculated as $\text{AUMC}_0-\infty / \text{AUC}_0-\infty$, where $\text{AUMC}_0-\infty$ is the area under the moment curve extrapolated to infinity)
- $\text{Cl}/F$ and $\text{Cl}/F$: Total clearance (calculated as Dose / $\text{AUC}_0-\infty$)
- $V_d$ and $V_d/F$: Apparent oral volume of distribution (calculated as Dose / $\lambda_z \times \text{AUC}_0-\infty$)
F_{abs} calculated as ratio:

\[
\frac{AUC_{0-\infty} (\text{e.v.})}{\text{Dose (e.v.)}} \frac{AUC_{0-\infty} (\text{i.v.})}{\text{Dose (i.v.)}}
\]

211 Urine: \( X_u, \Sigma X_u, dX_u/dt, C_l \) defined as follows

- \( X_u \): Urinary amount of excretion in a collection interval
- \( \Sigma X_u \): Total urinary amount of excretion
- \( dX_u/dt \): Urinary excretion rate
- \( C_l \): Renal clearance (calculated as \( \Sigma X_u / AUC_{0-\infty} \))

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213 Faeces: \( X_f, \Sigma X_f, dX_f/dt \) defined as follows:

- \( X_f \): Faecal amount of elimination in a collection interval
- \( \Sigma X_f \): Total faecal amount of elimination
- \( dX_f/dt \): Faecal elimination rate

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215 Multiple dose study:

216 Plasma: \( C_{ss_{\text{max}}}, C_{ss_{\text{min}}}, T_{ss_{\text{max}}}, C_{ss_{\text{average}}}, AUC_{\tau}, t_{1/2}, \) PTF\% and swing\% for the curve

217 constructed after the last dose. Variables were defined as follows:

- \( C_{ss_{\text{max}}} \): Maximum plasma concentration
- \( C_{ss_{\text{min}}} \): Minimum plasma concentration
- \( T_{ss_{\text{max}}} \): Time to attain peak concentration (\( C_{\text{max}} \))
AUC$_\tau$ Area under the concentration/time curve during the selected dosing interval at steady state calculated with trapezoidal method ($\tau$=6 h for the q.i.d. dose regimen and $\tau$=12 h for the b.i.d. dose regimen)

$C_{ss_{average}}$ Average plasma concentration (calculated as $\frac{AUC_{\tau}}{\tau}$)

PTF% Peak-trough fluctuation, expressed as:

$$%\text{PTF} = \frac{C_{ss_{max}} - C_{ss_{min}}}{C_{ss_{average}}} \times 100$$

Swing% Swing, expressed as:

$$%\text{Swing} = \frac{C_{ss_{max}} - C_{ss_{min}}}{C_{ss_{min}}} \times 100$$

Urine: $X_u$, $\Sigma X_u$, $dX_u/dt$, $C_l$

Pharmacokinetic data were described. A non-compartmental model was applied to the pharmacokinetic analysis.

2.4 Sample collection, handling and analytics

Venous blood samples (8 mL) were collected from a forearm vein by a catheter, then transferred by a needle syringe in about 30 sec into K-EDTA tubes. Storage on ice did not last more than 20 min until centrifugation at 4°C for 10 min at 2,500 x g to obtain plasma. Each
plasma sample was immediately divided into 3 aliquots, transferred to pre-labelled polypropylene tubes and stored frozen at \( \leq -20^\circ C \) until analyses.

Urine was collected in containers refrigerated at approximately \( 4^\circ C \). At the end of each collection interval the urine volume was measured and after thorough mixing, aliquots were stored in polypropylene tubes at \( \leq -20^\circ C \) pending analysis.

Faeces were collected at each voluntary evacuation for 3 days and immediately frozen at \(-20^\circ C\). In the evening of Day 3, the subjects were administered 1 L of laxative (Moviprep\textsuperscript{®}, Norgine AG, Switzerland) and one 2\textsuperscript{nd} L of solution in the morning of Day 4. Afterwards the faeces were collected for 12 h.

Solid stool specimens were weighed, diluted in distilled water (1:3) and ascorbic acid 2 mg/mL was added. Ascorbic acid was added to prevent oxidation of the compound. Afterwards the samples were thawed in a bain-marie for about 60 min at \( 30^\circ C \), and finally homogenised using a Stomacher\textsuperscript{®} for 16 min to a medium speed.

Liquid stool was weighed, ascorbic acid (f 2 mg/mL) was added and the whole sample was frozen at \( \leq -20^\circ C \). Homogenisation of the liquid stool was performed as for the solid faeces. Homogenised samples were subdivided into aliquots stored at \( \leq -20^\circ C \) until assayed.

Rifamycin SV was determined in plasma, urine and faeces at ABL Analytisch Biochemisch Laboratorium, the Netherlands, using three different validated LC-MS/MS methods with a LLOQ of 2.00 ng/mL for plasma and urine and of 10.0 ng/g for faeces. A full validation of the methods was performed according to the current guidelines for bioanalytical method validation (12, 23). The long term stability of rifamycin SV in plasma, urine and faeces was tested during the analytical method validation. Samples were stored at \( \leq -18^\circ C \) at the laboratory facilities. Long term stability test results were that rifamycin SV stored frozen at \( \leq -
18° C is stable up to 152, 140 and 147 days in plasma, urine and faeces, respectively. The three LC-MS/MS methods for the determination of rifamycin SV in human EDTA plasma, human urine and human faeces samples produced accurate and precise results. With respect to the accuracy of the methods, the validation studies revealed absolute biases for the Quality Control (QC) samples at the levels LLOQ (LQC), Low (QC-Low), Medium (QC-Medium), High (QC-High) of 4.9, 1.8, 1.4, and 1.9% in plasma, 17.0, 8.2, 7.4, and 6.6% in urine and 18.5, 13.4, 9.4 and 8.9% in faeces, respectively. The precision results (expressed as total CV%) were as follows: 7.2, 4.7, 5.1, and 5.6% in plasma, 14.4, 4.9, 2.8, and 4.5% in urine, and 5.5, 3.0, 4.7, and 2.1% in faeces, for the LQC, QC-Low, QC-Medium, and QC-High samples, respectively.

Intra-run CVs (repeatability) were 6.1, 3.4, 3.8, and 3.9% in plasma, 13.8, 4.6, 2.8, and 4.7% in urine and 2.7, 1.7, 3.4, and 1.9% in faeces, for the LQC, QC-Low, QC-Medium, and QC-High samples, respectively. Rifamycin SV was determined in the study samples using the three validated methods. The accuracy and precision results of the QC samples at the levels Low, Medium and High were always within the acceptable value of 15%. The calibration range covered 2.00-2000 ng/mL in human plasma and urine and 10.0–10000 ng/g in human faeces, and included the determination of the parameters calibration, accuracy and precision, recovery, specificity, dilution and stability.

Plasma, urine and faeces samples were spiked with an internal standard (rifaximin). Subsequently, the samples were subjected to protein precipitation using acetonitrile (plasma and faeces samples only) and, afterwards, a solid phase extraction (SPE) was performed using Oasis® HLB SPE columns. The extracted samples were injected into the LC-MS/MS system for quantification. The samples were chromatographed on a Zorbax Extend® C18 LC column.
The mass-spectrometer was equipped with a Turbo Ion Spray interface and operated in the negative ion mode.

2.5 Safety variables

The primary safety measure was the recording of adverse events (AEs). Measurement of vital signs (BP and HR), ECG recording, full physical examination and routine blood chemistry and urinalysis laboratory tests were also performed.

3 RESULTS

3.1 Disposition of subjects

Study A: the first subject was enrolled on 12MAY09 and the last subject completed the trial on 11JUN09. 31 Caucasians were screened and 24 randomised. Twelve (12) subjects were males and 12 were females, aged 19 to 49 y, with a BMI=24.0±2.5 kg/m$^2$.

After dosing of Period II two males withdrew their consent for personal reasons and discontinued the study, while the other 22 completed the study as per protocol. However all the data of the 24 enrolled subjects were considered in both the PK and the safety analysis.

Study B: the first subject was enrolled on 27APR09 and the last subject completed the trial on 07MAY09. 30 Caucasians were screened and 24 randomised. Twelve (12) were males and 12 females, aged 19 to 55 y, with a BMI=23.8±3.0 kg/m$^2$. 
All the randomised subjects concluded the study as per protocol and were considered in the safety and PK analysis.

### 3.2 Rifamycin SV plasma concentration and urinary excretion after single i.v. dose

Scheduled blood sampling time points and urine and faeces collection intervals are reported in Table 1 for both study A and B.

After single i.v. dose of 250 mg of rifamycin SV the mean plasma rifamycin SV concentration vs. time profile is depicted in Figure 2. Main mean kinetic plasma parameters measured or calculated for the intravenous route of dosing are summarised in Table 2.

The elimination phase could be clearly defined and AUC could be extrapolated to infinity for all the subjects. Twelve h post-dose all 24 subjects had still rifamycin SV concentrations above the LLOQ of 2 ng/mL.

Mean urinary parameters are summarised in the Table 3. Urinary rifamycin SV was found at quantifiable concentrations in all samples of 24 subjects. The excreted fraction of the administered dose ranged between 1.0176% and 4.9129%.

### 3.3 Rifamycin SV plasma concentration and urinary excretion after single oral dose under fasting and fed conditions

After a single oral 400 mg dose given under fasting conditions the only quantifiable levels of rifamycin SV were measured 8 h post-dose in one subject (3.28 ng/mL) and 10, 12 and 14 h post-dose in another subject (3.68, 3.29 and 2.69 ng/mL respectively). In all other samples of
all the other subjects plasma rifamycin SV was BLQL. After a single oral dose given under fed conditions quantifiable levels of rifamycin SV were measured only 24 h post-dose in two subjects (2.26 and 2.60 ng/mL) and 18 and 20 h post-dose in another subject (2.32 and 2.60 ng/mL). No pharmacokinetic analysis of plasma concentrations was performed due to the scarcity of data. The overall evidence indicates a nil to negligible bioavailability of the orally administered antibiotic.

Urinary PK parameters (mean±SD) are summarised in Table 4. Urinary rifamycin SV was not quantifiable in any of the samples of 22 subjects during the collection interval of 0-6 h post-dose. During the following interval of 6-12 h post-dose 8 subjects had detectable concentrations of rifamycin SV in urine after administration under fasting conditions, whilst after administration under fed conditions detectable concentrations were found only in 2 subjects. During the 12-24 h post-dose collection interval 13 of 22 subjects had quantifiable concentrations of rifamycin SV after administration under fasting conditions, whilst after administration under fed conditions quantifiable concentrations were found for 8 subjects.

3.4 Rifamycin SV faecal elimination after single oral dose under fasting conditions

Main faecal PK parameters (mean±SD) are summarised in the Table 5. On average, the elimination during 0-24 h post-dose interval amounted to 74.56±115.99 mg corresponding to 18.64% of the administered dose, while the highest faecal elimination of the antibiotic occurred between 24-48 h post-dose (50.01% of dose) when all subjects showed quantifiable concentrations of rifamycin SV with the exception of two, who did not produce stools. During
the last 2 collection intervals between 48-72 h and 72-84 h post-dose all subjects could supply a stool sample and rifamycin SV was still detectable in all of them. Two out of 22 subjects showed a $\Sigma X_f < 200$ mg, i.e. <50% of the administered dose. These two females were unable to supply any stool sample during the interval 0-24 h post-dose, while in the following interval (24-48 h) the amount of the antibiotic eliminated was below 1 mg for both subjects. The reason for low recovery was likely a consequence of poor compliance by these subjects with the study procedures. After recalculation for 20 subjects $\Sigma X_f$ varied from a minimum of 67.0% of the dose (eliminated amount of 267.9 mg) to a maximum of 103.6% (eliminated amount of 414.6 mg) with a mean of 348.7 mg corresponding to 87.2% of the administered dose.

### 3.5 Rifamycin SV plasma concentration and urinary elimination after multiple oral dose regimens

After the 200 mg q.i.d. regimen the only quantifiable levels were found 6 h after the last dose in 3 subjects (3.54, 3.39 and 2.01 ng/mL). In all other samples of all the other subjects plasma rifamycin SV concentration was BLQL. Similarly, after the last dose of the 400 mg b.i.d. regimen quantifiable plasma levels of rifamycin SV were found randomly. On average the highest calculated concentration was $1.57\pm2.71$ ng/mL. This mean value refers to the 6-h post-dose sampling. At pre-dose the mean concentration was $1.41\pm2.49$ ng/mL with only 4 quantifiable and 8 BLQL values. Urinary PK parameters (mean±SD) are summarised in Table 4.
Urinary rifamycin SV was found generally at quantifiable concentrations in all samples. The excreted fraction of the administered dose did not vary on average with the dose. In the collection intervals of 0-6 h post-dose Xu % was between 0.0007 and 0.0009% of the administered dose. In the collection interval of 6-12 h post-dose the excreted fraction was between 0.0012 and 0.0018%. The excretion rate (dXu/Δt) did not vary from 0 to 12 h post-dose.

3.6 Safety

Six AEs occurred to four subjects during study A. Two episodes of headache, one episode of nausea, one episode of vomiting, one episode of toothache and one episode of erythema. No one of the 6 AEs was judged as related to the treatment with Rifamycin SV-MMX® 200 mg tablets. The occurrence of vomiting did not affect the oral dosing of the investigational product. No AE occurred after single i.v. injection of Rifocene®.

Four AEs occurred to four subjects during study B. Two episodes of headache, one episode of presyncope and one episode of phlebitis at the catheter site. No one of the recorded AEs was judged as relating to the treatment. No meaningful effect of Rifamycin SV-MMX® 200 mg tablets on vital signs, ECGs or laboratory parameters was observed in either study.
The overall evidence obtained is in agreement with the available literature (See Fürész et al. 11), which reports the high and fast liver uptake of the parenterally administered antibiotic and its almost complete (90%) elimination with faeces via the bile. In addition, the present work evidences the lack of systemic absorption of rifamycin SV when administered by oral route. This result was obtained for the first time in this study in healthy male and female subjects and for the first time with reliable more sensitive bioanalytical methods as compared with the old literature data (1), thus proving the character of non absorbable antibiotics for rifamycin SV. This characteristics of rifamycin SV, when compared to other rifamycins like rifampicin, rifabutin and rifapentin, may be explained either by its lower lipophilicity or by the absence of basic groups. On the contrary, rifamycin SV has an acid function (pKa=1.8) which confers to the molecule purely ionic protein binding properties (3).

Actually, the results of the present study show that the systemic exposure to rifamycin SV of subjects who received single oral doses of 400 mg of Rifamycin SV-MMX® under fasting and fed conditions and following multiple dose regimens is negligible. Quantifiable plasma levels (>2.00 ng/mL) of rifamycin SV were found in few subjects and were randomly distributed. Moreover the antibiotic concentrations were never higher than 10 ng/mL after either multiple dose regimen.

The urinary excretion of rifamycin SV accounts for a small percent of the i.v. administered dose (2.62±0.86.%) and represents less than 0.001% of the oral dose given either under single or multiple regimen. Urinary concentrations >2 ng/mL were found rarely. On average quantifiable concentrations of rifamycin SV were detected in more subjects after administration of the product under fasting conditions than under fed conditions. The amount
of systemically absorbed antibiotic, which is excreted by the renal route, is very low not exceeding the 0.0010% of the oral dose administered under fasting conditions, whereas the fraction decreased to 0.0004% after administration under fed conditions. Mean cumulative elimination within the administration intervals (τ = 6 and 12 h) was similar after the q.i.d. and the b.i.d. regimen. No significant difference was found between the cumulative elimination value 0.0036% after the q.i.d. regimen and 0.0027% after the b.i.d. regimen (p-value=0.1232).

The ratio between total urinary elimination (ΣXu) measured after single oral and intravenous dosing allowed calculating the absolute bioavailability in urine: \( F_{ab} = 0.0410 \pm 0.0617\% \).

The amount of rifamycin SV eliminated with faeces was on average 87% of the administered dose in the 84 h collection interval. The validation of the analytical method of rifamycin SV in faeces showed that the analyte is sufficiently stable in stool samples collected and handled according to the protocol and undergoing freezing and thawing sequentially. Actually, the recovery was highly variable probably due to the heterogeneity of the faecal matrix, the chemico-physical properties of rifamycin SV, that may lead to adhesion of the antibiotic to the intestinal wall and/or sequestration by the faecal debris. Very likely the recovery would balance, if the urinary and faecal elimination of the 25-desacetyl metabolite were considered.

In conclusion rifamycin SV, administered orally as modified release MMX® tablets, is poorly absorbed both after single and multiple dose regimen. Oral bioavailability of the antibiotic is negligible (<0.1%). Urinary excretion of rifamycin SV is remarkably lower than the 0.01% of the administered dose both after single and multiple dose regimen. Nearly the 90% of the oral dose is recovered from faeces. These overall features permit to classify rifamycin SV as a non-absorbable oral antibiotic per se. A non-absorbable antibiotic has the advantage to limit the antimicrobial effect to the intestinal lumen, avoiding systemic effects. The administration of
the non-absorbable rifamycin SV in the new oral MMX® formulation designed to deliver the
substances directly into the colonic lumen, offers consistent advantages over the existing
formulations, minimising the side-effects closely related to unwanted activity on the
saprophytic flora living in the upper intestinal tract, and improving the drug efficacy due to
direct topical delivery.

The results on PK of orally administered rifamycin SV formulated with the MMX®
technology add information on the new formulation proposed for the treatment of infectious
diarrhoea. The efficacy and safety of rifamycin SV tablets administered to patients with
infectious diarrhoea were proven in a recent trial in comparison with Rifaximin® (A. F. D. Di
Stefano, D. Binelli, W. Labuschagne, M. Mojapelo, E. van der Walt, M. Gani, S. Patel, L.
Moro, submitted for publication).
ACKNOWLEDGEMENTS

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The Sponsor reviewed and approved the study design, was informed about the collection of data and reviewed and approved the analysis and the interpretation of data. Cosmo Technologies Ltd. reviewed and approved the manuscript for publication.

AR reviewed and approved the design of the study, was responsible for the clinical activities and collected the data, LL participated in the design of the study and performed the pharmacokinetic analysis, MD was responsible for the bioanalysis, AA proposed the study design and reviewed the draft manuscript, AFDD managed the study co-ordination, wrote the clinical trial report and drafted the manuscript.

All authors read and approved the manuscript.
References


14. ICH harmonised tripartite guideline. Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals M3(R1), recommended for adoption at step 4 of the ICH process on 16 July 1997 and amended on 9 November 2000 by the ICH steering committee


24. Xifaxan® product leaflet, Copyright © Salix Pharmaceuticals, Inc., NOV07.
Table 1  Sampling time points and intervals for the determination of rifamycin SV in plasma, urine and faeces in study A and B

<table>
<thead>
<tr>
<th>Study A</th>
<th>Matrix</th>
<th>Sampling time points or intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>single i.v. injection (250 mg) of Rifocine®</td>
<td>plasma</td>
<td>pre-dose (0), 5, 10, 15, 30 and then 1, 1.5, 2, 4, 6, 8 and 12 h post-dose</td>
</tr>
<tr>
<td></td>
<td>urine</td>
<td>0-4, 4-8 and 8-12 h post-dose</td>
</tr>
<tr>
<td>single oral doses (400 mg) of Rifamycin SV-MMX® under fasting and fed conditions</td>
<td>plasma</td>
<td>pre-dose (0), 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 24 h post-dose</td>
</tr>
<tr>
<td></td>
<td>urine</td>
<td>0-6, 6-12 and 12-24 h post-dose</td>
</tr>
<tr>
<td>Faeces¹</td>
<td>pre-dose (0), 0-24, 24-48, 48-72 and 72-84 h post-dose</td>
<td></td>
</tr>
</tbody>
</table>

Study B

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sampling time points or intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>multiple oral doses (200 mg q.i.d. or 400 mg b.i.d. for 3 days) of Rifamycin SV-MMX®</td>
<td>plasma</td>
</tr>
<tr>
<td></td>
<td>urine</td>
</tr>
</tbody>
</table>

¹: only in fasting conditions

Table 2  Mean ± SD main rifamycin SV PK parameters measured and calculated after single i.v. dose of 250 mg of rifamycin SV (as Rifocine®) (N=24)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀=Cₘ₅₅₅ (ng/mL)</td>
<td>36 025.00 (±8569.32)</td>
</tr>
<tr>
<td>Tₘ₅₅₅ (min)</td>
<td>5 (±0)</td>
</tr>
<tr>
<td>AUC₀₅₅₅₅ (ng/mL x h)</td>
<td>11 840.50 (±4002.83)</td>
</tr>
<tr>
<td>AUC₀₅₅₅₅ (ng/mL x h)</td>
<td>11 865.21 (±4007.18)</td>
</tr>
<tr>
<td>t₀/2 (h)</td>
<td>3.04 (±0.73)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.49 (±0.06)</td>
</tr>
<tr>
<td>Clt (L/h)</td>
<td>23.29 (±7.25)</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>101.79 (±40.34)</td>
</tr>
</tbody>
</table>
Table 3  Mean ± SD of main urinary rifamycin SV PK parameters namely: the amount of excretion (Xu) expressed as both µg and %, rate of excretion (dXu/dt) and total amount of excretion (ΣXu) expressed as both µg and %, after single i.v. dose of 250 mg of rifamycin SV (as Rifocine®) (N=24)

<table>
<thead>
<tr>
<th>Interval</th>
<th>Xu (µg)</th>
<th>Xu (% of dose)</th>
<th>dXu/dt (µg/h)</th>
<th>ΣXu (µg)</th>
<th>ΣXu (% of dose)</th>
<th>Clr (L/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 h</td>
<td>6398.1±2132.5</td>
<td>2.56±0.85</td>
<td>1599.5±533.1</td>
<td>6539.1±2153.7</td>
<td>2.62±0.86</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>4-8 h</td>
<td>111.9±58.0</td>
<td>0.04±0.02</td>
<td>28.0±14.5</td>
<td>6601.0±2253.7</td>
<td>2.5±0.8</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>8-12 h</td>
<td>29.1±26.9</td>
<td>0.01±0.01</td>
<td>7.3±6.7</td>
<td>6539.1±2153.7</td>
<td>2.62±0.86</td>
<td>0.6±0.1</td>
</tr>
</tbody>
</table>

Table 4  Mean ± SD of main urinary rifamycin SV PK parameters namely: the amount of excretion (Xu) expressed as both ng and %, rate of excretion (dXu/dt) and total amount of excretion (ΣXu) expressed as both ng and %, after single (N=22) and multiple oral dose of Rifamycin SV-MMX® 200 mg tablets (N=12)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Interval</th>
<th>Xu (ng)</th>
<th>dXu/dt (ng/h)</th>
<th>ΣXu (ng)</th>
<th>ΣXu (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>single oral 400 mg fasting</td>
<td>0-6 h</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>3966.1±6166.3</td>
<td>0.0010±0.0015</td>
</tr>
<tr>
<td>6-12 h</td>
<td>1612.8±3338.4</td>
<td>268.8±556.4</td>
<td>6539.1±2153.7</td>
<td>2.62±0.86</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>12-24 h</td>
<td>2353.4±3221.4</td>
<td>196.1±268.5</td>
<td>6539.1±2153.7</td>
<td>2.62±0.86</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>single oral 400 mg fed</td>
<td>0-6 h</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>1500.5±3049.1</td>
<td>0.0004±0.0008</td>
</tr>
<tr>
<td>6-12 h</td>
<td>105.2±335.7</td>
<td>17.2±55.9</td>
<td>1500.5±3049.1</td>
<td>0.0004±0.0008</td>
<td></td>
</tr>
<tr>
<td>12-24 h</td>
<td>1397.3±2990.5</td>
<td>116.4±249.2</td>
<td>1500.5±3049.1</td>
<td>0.0004±0.0008</td>
<td></td>
</tr>
<tr>
<td>oral 800 mg (200 mg q.i.d.)</td>
<td>0-3 h</td>
<td>1873.9±1405.8</td>
<td>624.6±468.6</td>
<td>7172.7±4180.0</td>
<td>0.0036±0.0021</td>
</tr>
<tr>
<td>3-6 h</td>
<td>1611.1±931.2</td>
<td>537.0±310.4</td>
<td>7172.7±4180.0</td>
<td>0.0036±0.0021</td>
<td></td>
</tr>
<tr>
<td>6-12 h</td>
<td>3687.7±2377.6</td>
<td>614.6±396.3</td>
<td>7172.7±4180.0</td>
<td>0.0036±0.0021</td>
<td></td>
</tr>
<tr>
<td>oral 800 mg (400 mg b.i.d.)</td>
<td>0-3 h</td>
<td>3216.9±2437.2</td>
<td>1072.3±812.4</td>
<td>10 914.5±5309.3</td>
<td>0.0027±0.0013</td>
</tr>
<tr>
<td>3-6 h</td>
<td>2713.3±1249.5</td>
<td>904.4±416.5</td>
<td>10 914.5±5309.3</td>
<td>0.0027±0.0013</td>
<td></td>
</tr>
<tr>
<td>6-12 h</td>
<td>4984.3±3029.6</td>
<td>830.7±504.9</td>
<td>10 914.5±5309.3</td>
<td>0.0027±0.0013</td>
<td></td>
</tr>
</tbody>
</table>
Table 5  Mean ± SD of main faecal rifamycin SV PK parameters namely the faecal elimination (Xf) expressed as both µg and %, rate of elimination (dXf/dt) and the total elimination (ΣXf) expressed as both mg and %, after single oral dose of 400 mg of sodium rifamycin SV-MMX® under fasting conditions (N=22)

<table>
<thead>
<tr>
<th>Interval</th>
<th>Sample size</th>
<th>Xf (µg)</th>
<th>Xf (% of dose)</th>
<th>dXf/dt (µg/h)</th>
<th>ΣXf (mg)</th>
<th>ΣXf (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dose</td>
<td>--</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>N=22</td>
<td>N=22</td>
</tr>
<tr>
<td>0-24 h</td>
<td>N=19</td>
<td>74.559±115 987.1</td>
<td>18.6±999±28.9968</td>
<td>3106.7±4832.8</td>
<td>331.6±69.0</td>
<td>82.9±17.3</td>
</tr>
<tr>
<td>24-48 h</td>
<td>N=20</td>
<td>200.044.5±134 718.6</td>
<td>50.0111±33.6796</td>
<td>8335.2±5613.3</td>
<td>331.6±69.0</td>
<td>82.9±17.3</td>
</tr>
<tr>
<td>48-72 h</td>
<td>N=22</td>
<td>124.148.3±98 101.0</td>
<td>21.0371±24.5253</td>
<td>3506.2±4087.5</td>
<td>3506.2±4087.5</td>
<td>3506.2±4087.5</td>
</tr>
<tr>
<td>72-84 h</td>
<td>N=22</td>
<td>1250.0±2856.5</td>
<td>0.3125±0.7141</td>
<td>104.2±238.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NC: not calculated
Figure 1  Rifamycin SV chemical structure

Figure 2  Mean ± SD plasma rifamycin SV concentrations (ng/mL) vs. time profiles after single i.v. dose of 250 mg of rifamycin SV (as Rifocine®).

Logarithmic/linear scale