CENTER FOR DRUG EVALUATION AND RESEARCH

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

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CLINICAL PHARMACOLOGY and BIOPHARMACEUTICS REVIEW

SPONSOR: Rhone-Poulenc Rorer Pharmaceuticals Inc.
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Collegeville, PA 19426-0107

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I. INTRODUCTION

Synercid® is an association of two semisynthetic pristinamycin derivatives, RP 57669 or Quinupristin (derived from pristinamycin I) and RP 54476 or dalfopristin (derived from pristinamycin II) in the ratio 30:70 (w/w). Synercid® is an injectable Streptogramin developed for the treatment of severe Gram positive infections. It has been evaluated in Phase III comparative studies in [redacted] complicated skin and soft structures infections, and for the treatment of Vancomycin-resistant Enterococcus faecium (VREF) infections.

Synercid®'s pharmacokinetic and metabolic profiles have been determined in the animal species utilized in toxicology and pharmacology, employing methods adapted to the assays of the two components quinupristin and dalfopristin. Radioactivity measurements and specific assays [redacted] have been utilized in both studies in whole animals and in vitro. The results have enabled the characterization of the distribution, metabolic and elimination processes involved in Synercid®'s disposition, providing useful information for the evaluation of the safety and efficacy of the drug.

The aim of the present summary is to describe Synercid®'s pharmacokinetic profiles in man. This was first studied in a reference population of young healthy volunteers, then in at-risk populations represented by elderly volunteers, the obese subjects, and patients with liver and kidney failure. Potential for drug-drug interactions have been evaluated mainly by *in vitro* studies. Finally, the effects of Synercid® on cyclosporine pharmacokinetics were evaluated in vivo.

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GLOSSARY OF ABBREVIATIONS

(presented alphabetically)

AE	Adverse Event
AUC	Area under the drug plasma concentration-time curve calculated by trapezoidal rule extrapolation to infinity, by using the elimination half-life
AUC(0-t)	area under the drug plasma concentration-time curve from zero to the last detectable sampling (linear trapezoidal rule).
CL	Plasma clearance
Cmax	Maximum Drug Plasma Concentration
CYP450	Cytochrome P450 family
CV (%)	Coefficient of variation
D_5W	5% Dextrose in Water
DMPK	Drug Metabolism and Pharmacokinetics
FSR	Final Study Report
G6PDH	Glucose-6-Phosphate Dehydrogenase
GCP	Good Clinical Practice
glo. bio.	Global bioassay
sel. bio.	Selective bioassay
HPLC	High Performance Liquid Chromatography
HR	Heart Rate
IV	Intravenous
$\lambda 1$	Rate constant of distribution calculated by log-linear regression of the distribution concentra time data.
λz	Rate constant of elimination calculated by log-linear regression of the terminal concentration-data Lactate Dehydrogenase
LOQ	Limit of Quantitation
PI	Pristinamycin I or RP 57669
PII	Pristinamycin II or RP 54476
r1	Cmax on day 4 or 5 / Cmax on day 1
r2	AUC(0-t) on day 4 or 5 / AUC on day 1.
RBC	Red Blood Cell
RIA	Radioimmunoassay
RPR	Rhône-Poulenc Rorer
SD	Standard deviation
SEM	Standard Error of the Mean
T	Duration of perfusion
Tmax	Time to Maximum Plasma Concentration
$t½\lambda_1$	Apparent distribution half-life, calculated by $Ln2/\lambda_1$
$t½\lambda z$	Plasma Elimination Half-life
ULN	Upper Limit of Normal
Vss	Volume of distribution at steady-state (Vss = CL x (MRT - T/2))
Vz	Volume of distribution calculated by CL / λz
WBC	White Blood Cell
PAE	Post antibiotic effect

II. OVERALL SUMMARY

The Clinical Pharmacology file includes 30 studies (including two studies on the separate components of Synercid), performed in Europe, North America and Japan. All the studies are presented in Table 1 with their objectives, design, number of subjects and doses evaluated.

A summary of analytic methods and pharmacokinetic results for those studies with data is provided in Appendix I and II.

Both RP57669 (quinupristin) and RP54476 (dalfopristin) at physiologic pH are spontaneously converted into microbiologically active metabolites via non-enzymatic chemical reactions. Glutathione-conjugated (RP69012) and cysteine-conjugated (RPR100391) derivatives of quinupristin and the natural pristinamycin RP12536 formed by hydrolysis of dalfopristin are the major active metabolites formed *in vivo* as well as *ex vivo*. RP12536 itself is unstable in biological media. Therefore, specific conditions (acidic pH adjustment) are required for suitable blood sample collection in order to ensure the stability of the streptogramins.

The evaluation of the pharmacokinetics of Synercid was performed primarily using specific one for quinupristin and one for dalfopristin, which measure the *in vitro* activity of drug-derived ~~products~~

In the range of doses tested (5 - 15 mg/kg) in man, the pharmacokinetics of quinupristin are linear but those of dalfopristin are not. The clearance of dalfopristin is constant, but its volume of distribution may increase with dose. Nevertheless, the pharmacokinetics of dalfopristin in combination with its active metabolite RP12536 are linear. Quinupristin and dalfopristin are highly cleared drugs (plasma clearance: 0.7 - 1 and 0.7 - 1.2 l/h·kg respectively) with 'intermediate' volumes of distribution (V_β = 0.7 - 1 l/kg).

After a q12h or q8h administration at the 7.5 mg/kg dose tested in phase III trials, a comparable steady-state concentration is achieved by day two of treatment. Regardless of the dosage regimen, there is a similar moderate increase in C_{max} and AUC (around 20%) of parent drugs as compared to single-dose administration. This phenomenon is also observed for the drug metabolites, and is more pronounced for the cysteine-derivative of quinupristin. The elimination half-lives of the metabolites (1.8 hours for RP69012 and RPR100391, 1.3 hours for RP12536) are longer than that of the parent drugs (0.9 and 0.7 - 0.8 hours for quinupristin and dalfopristin respectively), but not enough to result in significant accumulation of drug (no significant trough levels detected). The lower plasma clearance of quinupristin and dalfopristin on multiple dosing could not be explained by any inhibitor effect of the drug on its own metabolism or accumulation based on half-life.

The plasma peak of each of the major circulating active metabolites is about 15 - 20% that of the parent drug, whereas the AUC is about 25 - 30, 30 - 40 and 30% of the parent drug AUC, for RP69012, RPR100391 and RP12536, respectively. The plasma level data obtained by selective bioassays for quinupristin and dalfopristin for quinupristin, RP69012, RPR100391, dalfopristin and RP12536, respectively.

Plasma protein binding is 55 to 78% for quinupristin and 11 to 26% for dalfopristin. After a 7.5 mg/kg dose, the extent of penetration of the drug in extracellular non-inflammatory fluid is moderate (around 40% in a suction blister fluid model), but blister levels of RPR100391 and RP12536 have been found to be more persistent than their plasma levels. RPR69012 is not present in the blister. Logistic issues related to drug instability in biological material or analytical issues related to drug assay specificity have limited the characterization of the tissue distribution of Synercid in man. Drug penetration in lung has been assessed in a mouse pneumonia model but not in man. Penetration in the cerebrospinal fluid has been assessed in a rabbit model of *S. Pneumoniae* meningitis, but not in man.

The major *in vitro* active metabolites of the drug have been identified. RP69012 and RPR100391 are the major components which have been identified in human plasma, urine and fecal extracts. Dalfopristin is extensively metabolized, and the major microbiologically active metabolite identified in plasma and urine is the natural pristinamycin RP12536. Major routes of metabolism are not dependent on CYP-450 or glutathione transferase enzyme activities. The elimination of these metabolites is not rate-limited by the elimination of the parent drug.

Fecal excretion constitutes the major elimination route for both compounds and their metabolites (75 - 77% of dose).

The pharmacokinetics of Synercid are not modified with age or gender. A small increase in drug levels is observed in obese patients.

The elimination of quinupristin active metabolites, but not of unchanged drug, and the elimination of unchanged dalfopristin are moderately impaired in severe renal failure patients. Synercid and its components are not dialyzable from blood across the peritoneal membrane.

The elimination of quinupristin and dalfopristin active metabolites is markedly impaired in patients with liver cirrhosis, whereas that of the parent drug is slightly (quinupristin) or not (dalfopristin) modified.

Dosage adjustment is not necessary based on age, gender or the presence of renal dysfunction; however, dosage reduction may be necessary in patients with severe hepatic insufficiency.

Synercid can inhibit the biotransformation of drugs metabolized by CYP3A4 isozymes, as indicated by *in vitro* data and confirmed in healthy subjects in a cyclosporine-Synercid interaction study. Coadministration of Synercid and drugs primarily metabolized through CYP3A4-mediated reactions (for example, cyclosporine, midazolam, nifedipine, alfentanyl, and cisapride) will change the pharmacokinetic profile of these drugs. In the case of cyclosporine, it is recommended that therapeutic drug level monitoring be performed. For other drugs for which such monitoring is not possible, the clinician must be alert to the possibility of a pharmacokinetic and possible pharmacodynamic interaction, decide whether their administration is necessary, and if so, carefully monitor the patient's clinical status for adverse pharmacodynamic effects.

III. RECOMMENDATIONS

(1) The protein binding study results are incomplete. All other studies, in terms of experimental design, performance, analysis and information provided, meet biopharmaceutics and clinical pharmacology requirements and are acceptable.

(2) Background information of Synercid indicates that the drug has a dual mode of action for the two components, Quinupristin (Q) and Dalfopristin (D). The combination of Quinupristin and Dalfopristin is synergistic and is 16-fold more potent than that of Quinupristin or Dalfopristin alone. Anti-infective activity was found to exist at 30:70 - 70:30 w/w ratio of Q:D. Metabolites of Quinupristin and Dalfopristin also contribute to synergy. Most MIC_{90} values are at 1 ug/ml. PAE of the drug varies between 0.8 to 9 hours and is drug concentration, bacteria and phonotype dependent. As indicated, Synercid clinical usage is mainly for seriously ill patients.

Therefore, combined Quinupristin and Dalfopristin (and active metabolites) are needed at the site of infection. Pharmacokinetic profiles of Quinupristin, Dalfopristin and active metabolites in seriously ill patients appear to be important. Due to the fixed ratio of Q:D in the dosage form (Q:D = 3:7 w/w), dose adjustment recommendations may be complicated.

(3) The following pharmacokinetic and pharmacodynamic characteristics of Quinupristin and Dalfopristin, that may have impact on its clinical application, were discussed at the Advisory Committee meeting on Feb. 19, 1998: (i) The differences in the elimination, distribution and accumulation kinetics of Quinupristin and Dalfopristin, (ii) The drug-drug interaction issue as demonstrated in the in vitro drug metabolism and in vivo Synercid-cyclosporine interaction studies, and (iii) the dose adjustment recommendation for hepatic impaired patients.

(4) A commitment to additional phase 4 studies is requested as outlined in the **SPECIFIC COMMENTS** Section.

(5) Please convey all **SPECIFIC COMMENTS** (see page 56) to the sponsor.

IV. BACKGROUND INFORMATION

A. Materials, methods, and list of clinical pharmacokinetic studies

All the clinical studies with pharmacokinetics conducted in man are listed in Table 1. The codes RP59500 and RP 57669/RP 54476, given in the references below, are the codes which have been used internally at Rhône-Poulenc Rorer to denote Synercid.

All the in vivo studies were performed after administration of quinupristin/dalfopristin (30/70), except the studies PRI 001 (administration of RP 57669) and PRI 002 (administration of RP 54476).

TABLE 1 : LISTING OF STUDY OBJECTIVES, DESIGN, NUMBER OF SUBJECTS AND DOSES EVALUATED

Study Number	Objective	Design	Doses tested	N. of subjects
Studies with separate components				
PRI001	Safety, PK	Double-blind, escalating-dose, placebo-controlled, randomized	single-dose, 0.25 to 12 mg/kg	42
PRI002	Safety, PK	Double-blind, escalating-dose, placebo-controlled, randomized	single-dose, 0.6 to 28.8 mg/kg	43
Studies with Synercid				
Pharmacokinetic and safety studies				
SYN003	Safety, PK	Double-blind, escalating-dose, placebo-controlled, randomized	single-dose, 1.4 to 29.4 mg/kg	26
SYN004	Safety, PK	Double-blind, placebo-controlled, randomized	single-dose, 5 to 15 mg/kg	81
SYN005	Safety, PK	Double-blind, placebo-controlled, randomized, 7 days	multiple-dose, 5 to 8 mg/kg q12h	16
JRV015	Safety, PK	Double-blind, placebo-controlled, randomized, 10 days	multiple-dose, 5 to 15 mg/kg q12h	34
SYN023	Safety	Double-blind, placebo-controlled, randomized, 5 days	multiple-dose, 5 mg/kg q12h	7
SYN024	Safety	Double-blind, placebo-controlled, randomized,1 to 5 days	multiple-dose, 15 mg/kg q12h	49
JRV006	PK, linearity	Open, cross-over, randomized	single-dose, 5, 10, 15 mg/kg	20
JRV124	Safety	Open, 4 or 5 days	multiple-dose, 7.5 mg/kg q12h or q8h	22
V126	PK, Safety, Gender Effect	Open	single-dose, 7.5 mg/kg	32
JRV128	PK, Safety	Open, 4 or 5 days	multiple-dose, 7.5 mg/kg q12h or q8h	20
V129	PK, Safety	Single-blind, placebo-controlled, randomized	single-dose, 5 to 12.5 mg/kg	30
V130	PK, Safety	Single-blind, placebo-controlled, randomized, 3 or 5 days	multiple-dose, 7.5 mg/kg q12h or q8h	15
Pharmacodynamic studies				
V136	Effect on fecal flora	Open, placebo-controlled, 5 days	multiple-dose, 7.5 mg/kg q12h	13
V137	Venous tolerance	Double-blind, controlled, randomized,2 days	multiple-dose, 7.5 mg/kg q8h	39
V142	Venous tolerance	Double-blind, controlled, randomized,2 days	multiple-dose, 7.5 mg/kg q8h	11
Excretion balance and distribution studies				
SYN111	PK, excretion balance	Open	single-dose, 500 mg labeled	6
SYN112	PK, excretion balance	Open	single-dose, 500 mg labeled	6

JRB104	Blister diffusion	Open	single-dose, 12 mg/kg	7
JRV123	Pulmonary diffusion	Open	single-dose, 7.5 mg/kg	15
V131	Blister diffusion	Open	single-dose, 7.5 mg/kg	12
V132	PMN diffusion	Open	multiple-dose, 7.5 mg/kg	14
V140	Pulmonary diffusion	Open	single-dose, 7.5 mg/kg	4
Pharmacokinetics in high risk subjects				
JRV007	PK, renal failure	Open, comparative	single-dose, 7.5 mg/kg	26
JRV008	PK, hepatic failure	Open, comparative	single-dose, 7.5 mg/kg	33
V125	PK, obese subjects	Open, comparative	single-dose, 7.5 mg/kg	27
V127	PK, elderly subjects	Open, comparative	single-dose, 7.5 mg/kg	24
V141	PK, CAPD	Open, comparative	single-dose, 7.5 mg/kg	16
Interaction study				
V138	PK, cyclosporine	Open, cross-over, 4 days	multiple-dose, 7.5 mg/kg q8h	25

PK = pharmacokinetics; PMN = polymorphonuclear neutrophils; CAPD = continuous ambulatory peritoneal dialysis

B. Bioanalyses were performed in the following laboratories

- Rhône-Poulenc Santé, Pharmacokinetic Section - Department of Biodynamics - Avenue Raymond Aron, 92165 Antony France

- Rhône-Poulenc Rorer R&D, Clinical Pharmacokinetics Section, Drug Metabolism and Pharmacokinetics Department, Antony (France),

[redacted]

- Rhône-Poulenc Rorer/RD/CRVA/SM Alfortville, Metabolism Section, Drug Metabolism and Pharmacokinetics Department / France.

[redacted]

C. Drug formulations and administered doses

The dosage levels evaluated in the clinical pharmacokinetic studies included those administered to evaluate Synercid®'s safety and activity, as well as those characteristic of clinical dosage regimens.

1. **Administration of 14C-RP 57669 or 14C-RP 54476**

2. **Administration of unlabelled RP 59500**

D. Pharmacokinetics and Statistical Methodology

Pharmacokinetic analysis of the data was carried out utilizing conventional methods, such as those described by M. Gibaldi and D. Perrier in Pharmacokinetics, 2nd edition Marcel Dekker, New-York/Basel, 1982. The experimental data were processed

The areas under the plasma concentration-time curves (AUC(0-t)) were computed using the trapezoidal rule. The elimination half-life of each compound was calculated from the absolute value of the slope of the terminal log-linear portion of the plasma concentration-time curve. Extrapolation of AUC to infinity was estimated by using the elimination half-life. Distribution half-life was estimated using either non-compartmental analysis if the fit between experimental data and calculated data was satisfactory or compartmental analysis using Powell algorithm and weight = $1/y^2$. Mean values are given with standard deviations.

Statistical analysis was conducted utilizing the SAS program.

Parts of the PK data were re-evaluated by the reviewer.

V. SINGLE DOSE PHARMACOKINETIC STUDIES

A. Study on the linearity of Synercid® (Study JRV 006)

The pharmacokinetics of the two components RP 57669 and RP 54476 of Synercid® were determined in a cross-over study performed in 18 healthy young male volunteers. Six groups of three subjects received 3 single doses of RP 59500 (5, 10 and 15 mg/kg) as an one-hour IV infusion, in a sequential fashion. Plasma levels of RP 57669, RP 54476 and the metabolite RP 12536 [redacted] Mean pharmacokinetic parameters of RP 57669, RP 54476 and RP 12536 are summarized in Table 2. Mean plasma concentration-time curves are illustrated in Figures 1 to 3 :

TABLE 2 : MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND RP 12536

[redacted]

Compound	Dose (mg/kg)	Cmax µg/ml	Tmax * h	AUC(0-t) µg.h/ml	AUC µg.h/ml	t½λ1 h	t½λz h	CL l/h.kg	Vz l/kg	Vss l/kg
RP 57669	5	1.20 ± 0.36	1	1.35 ± 0.28	1.41 ± 0.29	0.17 ± 0.04	0.93 ± 0.20	1.13 ± 0.38	1.37 ± 0.41	0.83 ± 0.42
	10	2.32 ± 0.48	1	2.91 ± 0.53	3.01 ± 0.51	0.19 ± 0.05	0.97 ± 0.20	1.03 ± 0.20	1.44 ± 0.42	0.79 ± 0.40
	15	3.58 ± 0.76	1	4.60 ± 0.91	4.69 ± 0.91	0.23 ± 0.11	0.96 ± 0.30	0.99 ± 0.19	1.37 ± 0.45	0.81 ± 0.34
RP 54476	5	4.55 ± 2.34	1	4.54 ± 1.96	4.57 ± 2.02	0.11 ± 0.03	0.39 ± 0.15	0.99 ± 0.69	0.54 ± 0.33	0.33 ± 0.36
	10	6.38 ± 2.76	1	7.21 ± 3.07	7.27 ± 3.08	0.15 ± 0.06	0.52 ± 0.22	1.19 ± 0.65	0.88 ± 0.53	0.43 ± 0.29
	15	8.46 ± 2.61	0.50	9.72 ± 3.43	9.71 ± 3.57	0.23 ± 0.05	1.09 ± 0.37	1.23 ± 0.49	1.80 ± 0.63	0.70 ± 0.34
RP 12536	5	0.94 ± 0.58	1	1.19 ± 0.63	1.24 ± 0.63	0.22 ± 0.10	0.91 ± 0.26	2.76 ± 1.51	-	-
	10	2.54 ± 0.84	1	3.55 ± 0.86	3.70 ± 0.89	0.39 ± 0.26	1.63 ± 0.66	1.53 ± 0.39	-	-
	15	3.75 ± 1.62	1	4.94 ± 1.89	5.14 ± 1.93	0.39 ± 0.10	2.17 ± 0.35	1.83 ± 0.83	-	-

FIGURE 1 : MEAN RP 57669 PLASMA CONCENTRATIONS VERSUS TIME

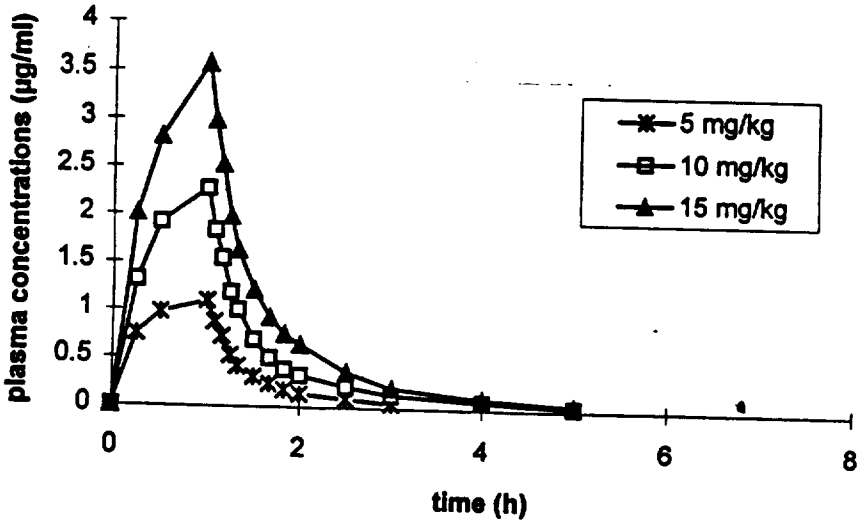


FIGURE 2 : MEAN RP 54476 PLASMA CONCENTRATIONS VERSUS TIME

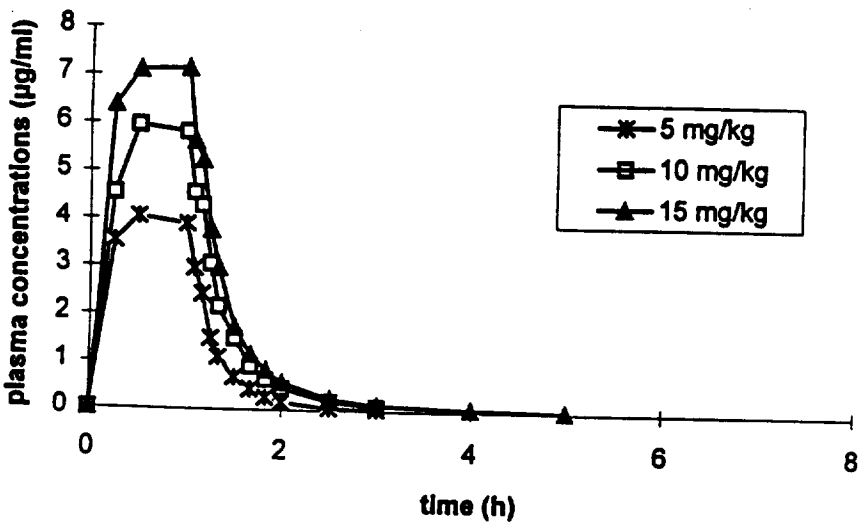
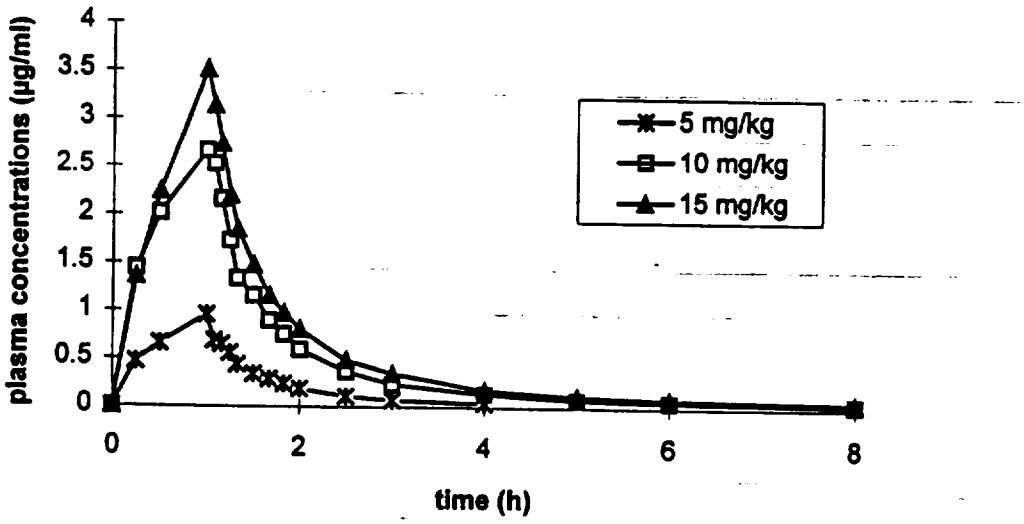


FIGURE 3 : MEAN RP 12536 PLASMA CONCENTRATIONS VERSUS TIME



The pharmacokinetic profiles of RP 57669 and RP 54476 were fitted to a two-compartmental open model. The pharmacokinetics of RP 57669 were dose-independent. Cmax and AUC increased proportionally to the dose. The pharmacokinetics of RP 54476 were dose-dependent, with smaller than expected increases in Cmax and AUC with increases in dose. However, the sum of AUCs for RP 54476 and RP 12536 (expressed as RP 54476 equivalents) increased proportionally with dose ; this was not observed with Cmax.

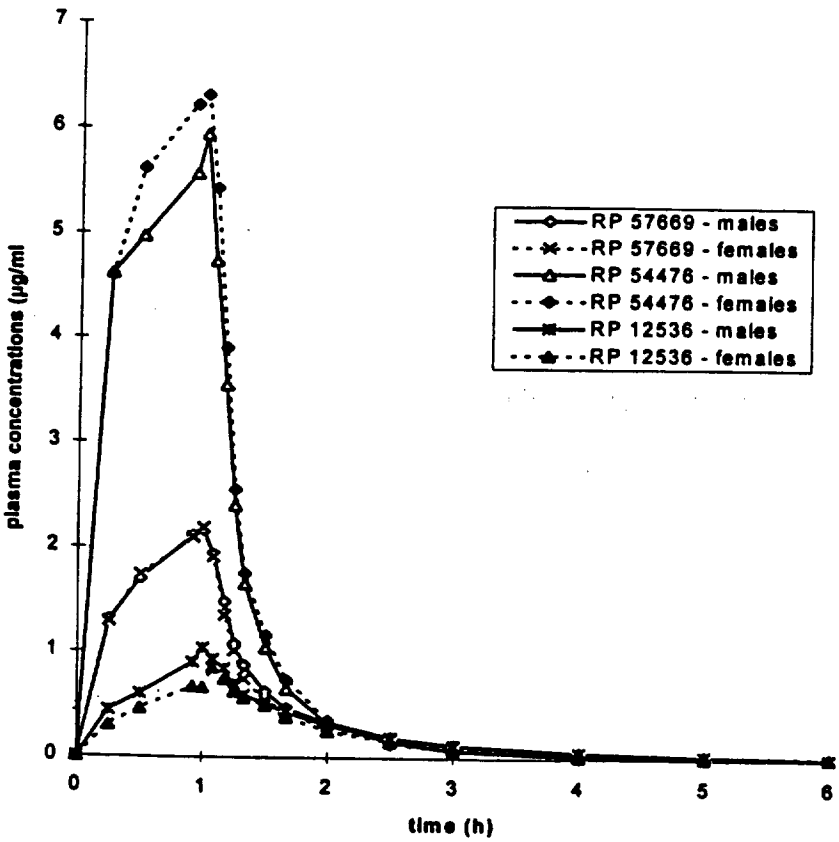
B. Gender effect (Study JRV 126)

The pharmacokinetics of the two components RP 57669 and RP 54476 of Synercid® were determined in an open single dose study to compare the pharmacokinetic profile in healthy young male and female volunteers. 24 subjects (12 males and 12 females) received a single dose of RP 59500 (7.5 mg/kg) as an one-hour IV infusion. Plasma levels of RP 57669, RP 54476 and the metabolite RP 12536 [redacted] Mean pharmacokinetic parameters of RP 57669, RP 54476 and RP 12536 are summarized in Table 3. Mean plasma concentration-time curves are illustrated in Figure 4:

TABLE 3 : MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND RP 12536 IN MALE AND FEMALE VOLUNTEERS

Compound	Sex	Cmax µg/ml	Tmax h*	AUC(0-t) µg.h/ml	AUC µg.h/ml	t½λ_1 h	t½λz h	CL l/h.kg	Vz l/kg	Vss l/kg
RP 57669	male	2.28 ± 0.32	1	2.72 ± 0.43	2.78 ± 0.45	0.19 ± 0.05	1.11 ± 0.37	0.83 ± 0.14	1.29 ± 0.31	0.63 ± 0.10
	female	2.26 ± 0.34	1	2.62 ± 0.48	2.67 ± 0.49	0.19 ± 0.09	1.26 ± 0.74	0.87 ± 0.17	1.54 ± 0.75	0.67 ± 0.26
RP 54476	male	6.10 ± 1.49	1	6.43 ± 1.41	6.45 ± 1.50	0.15 ± 0.04	0.84 ± 0.84	0.85 ± 0.18	0.91 ± 0.72	0.32 ± 0.06
	female	6.59 ± 1.40	0.92	6.92 ± 1.72	6.79 ± 1.66	0.17 ± 0.04	1.15 ± 0.94	0.82 ± 0.21	1.45 ± 1.45	0.35 ± 0.17
RP 12536	male	1.08 ± 0.51	1.08	1.51 ± 0.51	1.57 ± 0.52	-	1.21 ± 0.35	-	-	-
	female	0.87 ± 0.25	1.08	1.21 ± 0.27	1.33 ± 0.32	-	1.29 ± 0.40	-	-	-

FIGURE 4 : MEAN PLASMA CONCENTRATIONS OF RP 57669, RP 54476 AND RP 12536 VERSUS TIME IN MALE AND FEMALE VOLUNTEERS



RP 57669 and RP 54476 declined biexponentially with mean elimination half-lives of 1.11 and 0.84 hours in males and 1.26 and 1.15 hours in females, respectively. Plasma clearances were very high for both compounds, ranging between 0.82 and 0.87 l/h.kg. No statistically significant gender effect was observed on all the pharmacokinetic parameters estimated in this study.

VI. REPEATED DOSE PHARMACOKINETICS

A. Study JRV 128

Kinetic profiles of RP 57669 and RP 54476 were determined following multiple one-hour infusions of RP 59500 at the dose of 7.5 mg/kg to 20 healthy young male volunteers. These subjects were divided into two groups of 10 subjects: first group received b.i.d. administration, every 12 hours for 4 days, followed by a single administration on day 5 morning. The second group received t.i.d. administration, every 8 hours for three days, followed by a single administration on day 4 morning. Three analytical methods were used. Quinupristin (RP 57669) and its metabolites RP 69012, RPR 100391, and dalfopristin (RP 54476) and its metabolite RP 12536[redacted]Quinupristin and its derivatives and dalfopristin and its derivatives were also measured by two selective microbiological assays.

The results are summarised in Tables 4 to 6.

TABLE 4 : MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND THEIR MAIN METABOLITES FOLLOWING 1-HOUR IV INFUSION OF RP 59500 AT 7.5 MG/KG DOSE Q12H OR Q8H

Compound	Day	Cmax µg/ml	Tmax * h	AUC(0-t) µg.h/m	AUC µg.h/ml	t½ λ_1 h	t½λz h	CL l/h.kg	Vz l/kg	Vss l/kg
q8h group (n=10)										
RP 57669	D1	2.39 ± 0.70	0.92	2.57 ± 0.59	2.60 ± 0.59	0.13 ± 0.05	0.75 ± 0.20	0.90 ± 0.16	0.98 ± 0.33	0.48 ± 0.12
	D4	2.79 ± 0.55	0.92	3.17 ± 0.51	3.22 ± 0.54	0.15 ± 0.08	0.85 ± 0.12	0.71 ± 0.11	0.87 ± 0.17	0.45 ± 0.08
RP 69012	D1	0.31 ± 0.06	0.92	0.50 ± 0.12	0.53 ± 0.13	0.34 ± 0.08	1.83 ± 0.76	-	-	-
	D4	0.42 ± 0.08	1.17	0.83 ± 0.15	0.88 ± 0.16	0.48 ± 0.14	2.11 ± 0.26	-	-	-
RPR 100391	D1	0.25 ± 0.09	1.17	0.52 ± 0.18	0.56 ± 0.19	0.45 ± 0.15	1.81 ± 0.31	-	-	-
	D4	0.59 ± 0.16	1.17	1.25 ± 0.30	1.26 ± 0.26	0.57 ± 0.19	2.65 ± 0.58	-	-	-
RP 54476	D1	6.20 ± 1.90	0.50	6.41 ± 2.08	6.44 ± 2.08	0.12 ± 0.03	0.45 ± 0.10	0.87 ± 0.21	0.56 ± 0.15	0.25 ± 0.06
	D4	7.22 ± 1.85	0.92	7.77 ± 2.36	7.81 ± 2.36	0.16 ± 0.06	0.70 ± 0.39	0.73 ± 0.22	0.71 ± 0.37	0.24 ± 0.07
RP 12536	D1	0.82 ± 0.22	0.92	1.32 ± 0.38	1.39 ± 0.38	0.44 ± 0.16	1.26 ± 0.32	-	-	-
	D4	1.10 ± 0.18	0.92	1.90 ± 0.26	2.01 ± 0.27	0.36 ± 0.13	1.97 ± 0.75	-	-	-
q12h group (n=10)										
RP 57669	D1	2.31 ± 0.52	0.92	2.44 ± 0.50	2.53 ± 0.50	0.14 ± 0.04	0.82 ± 0.20	0.93 ± 0.21	1.10 ± 0.41	0.54 ± 0.17
	D5	2.53 ± 0.41	0.92	2.96 ± 0.24	3.01 ± 0.24	0.14 ± 0.03	0.87 ± 0.15	0.75 ± 0.06	0.95 ± 0.20	0.47 ± 0.09
RP 69012	D1	0.31 ± 0.09	0.92	0.52 ± 0.15	0.55 ± 0.16	0.39 ± 0.08	1.70 ± 0.41	-	-	-

Compound	Day	Cmax µg/ml	Tmax * h	AUC(0-t) µg.h/m	AUC µg.h/ml	$t_{1/2}\lambda_1$ h	$t_{1/2}\lambda z$ h	CL l/h.kg	Vz l/kg	Vss l/kg
	D5	0.38 ± 0.08	0.92	0.73 ± 0.18	0.79 ± 0.17	0.48 ± 0.16	1.85 ± 0.37	-	-	-
RPR 100391	D1	0.25 ± 0.07	1.25	0.54 ± 0.17	0.58 ± 0.18	0.44 ± 0.08	1.96 ± 0.39	-	-	-
	D5	0.49 ± 0.14	1.17	1.05 ± 0.23	1.11 ± 0.23	0.64 ± 0.15	2.06 ± 0.44	-	-	-
RP 54476	D1	5.92 ± 1.51	0.92	6.20 ± 1.54	6.24 ± 1.54	0.13 ± 0.03	0.61 ± 0.30	0.89 ± 0.24	0.78 ± 0.38	0.29 ± 0.06
	D5	6.81 ± 1.75	0.92	7.73 ± 2.22	7.78 ± 2.22	0.15 ± 0.05	0.79 ± 0.48	0.73 ± 0.22	0.93 ± 0.86	0.30 ± 0.11
RP 12536	D1	0.84 ± 0.17	0.92	1.32 ± 0.32	1.39 ± 0.34	0.40 ± 0.16	1.28 ± 0.30	-	-	-
	D5	1.11 ± 0.27	0.92	2.01 ± 0.65	2.09 ± 0.67	0.41 ± 0.16	1.57 ± 0.60	-	-	-

* : MEDIAN

TABLE 5 : MEAN PHARMACOKINETIC PARAMETERS OF RP 57669 AND RP 54476 IN COMBINATION WITH THEIR DERIVATIVES FOLLOWING 1-HOUR IV INFUSION OF RP 59500 AT 7.5 MG/KG DOSE Q12H OR Q8H

Compound	Day	Cmax µg/ml	Tmax * h	AUC(0-t) µg.h/ml	AUC µg.h/ml	$t_{1/2}\lambda_1$ h	$t_{1/2}\lambda z$ h
q8h group (n=10)							
RP 57669	D1	2.45 ± 0.82	0.92	3.55 ± 0.92	3.86 ± 0.93	0.23 ± 0.09	1.39 ± 0.44
	D4	3.20 ± 0.67	0.92	6.36 ± 1.06	7.20 ± 1.24	0.54 ± 0.14	3.07 ± 0.51
RP 54476	D1	6.93 ± 1.61	0.92	7.94 ± 2.33	8.16 ± 2.36	0.16 ± 0.07	0.77 ± 0.16
	D4	7.96 ± 1.30	0.92	10.29±2.21	10.57±2.24	0.15 ± 0.05	1.04 ± 0.20
q12h group (n=10)							
RP 57669	D1	2.26 ± 0.51	0.92	3.30 ± 0.72	3.65 ± 0.76	0.19 ± 0.07	1.31 ± 0.27
	D5	3.03 ± 0.71	0.92	5.49 ± 1.02	5.98 ± 1.05	0.25 ± 0.15	1.99 ± 0.74
RP 54476	D1	6.66 ± 1.29	0.92	7.83 ± 1.84	8.05 ± 1.87	0.13 ± 0.04	0.72 ± 0.14
	D5	8.51 ± 1.73	0.92	10.65±2.50	10.92±2.50	0.13 ± 0.03	0.89 ± 0.17

* : MEDIAN

TABLE 6 : MEAN RATIOS (± SD) OF CMAX (DAY 4/5) / CMAX (DAY 1) (R1) AND OF AUC(0-T) (DAY 4/5) / AUC (DAY 1) (R2)

Compound	Group	Selective bioassays		HPLC	
RP 57669	q8h				
	q12h				
RP 69012 (glutathione-conjugate)	q8h				
	q12h				
RPR 100391 (cysteine-conjugate)	q8h				
	q12h				
RP 54476	q8h				
	q12h				
RP 12536	q8h				
	q12h				

Mean plasma concentration-time curves are illustrated in Figures 5 and 6.

FIGURE 5 : MEAN PLASMA LEVELS OF RP 57669 AND ITS METABOLITES VERSUS TIME - T.I.D. REGIMEN

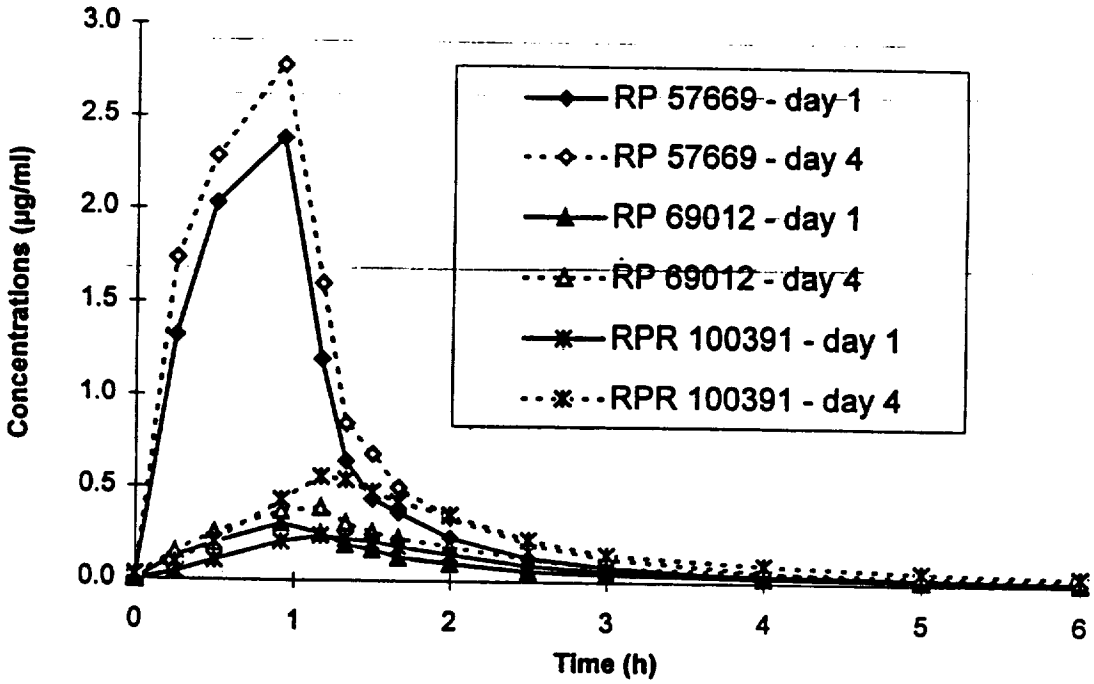
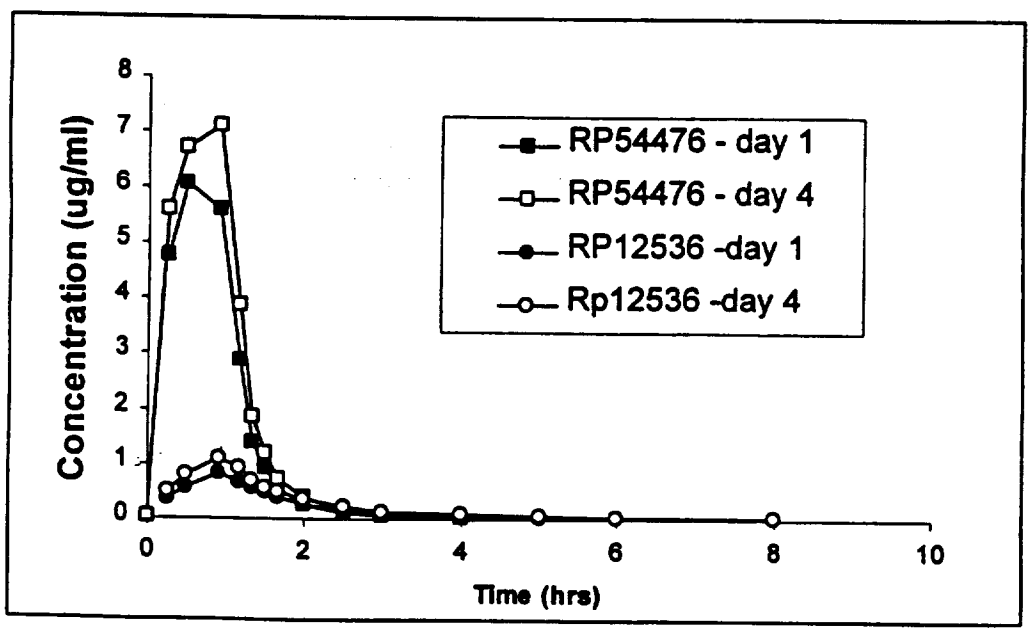
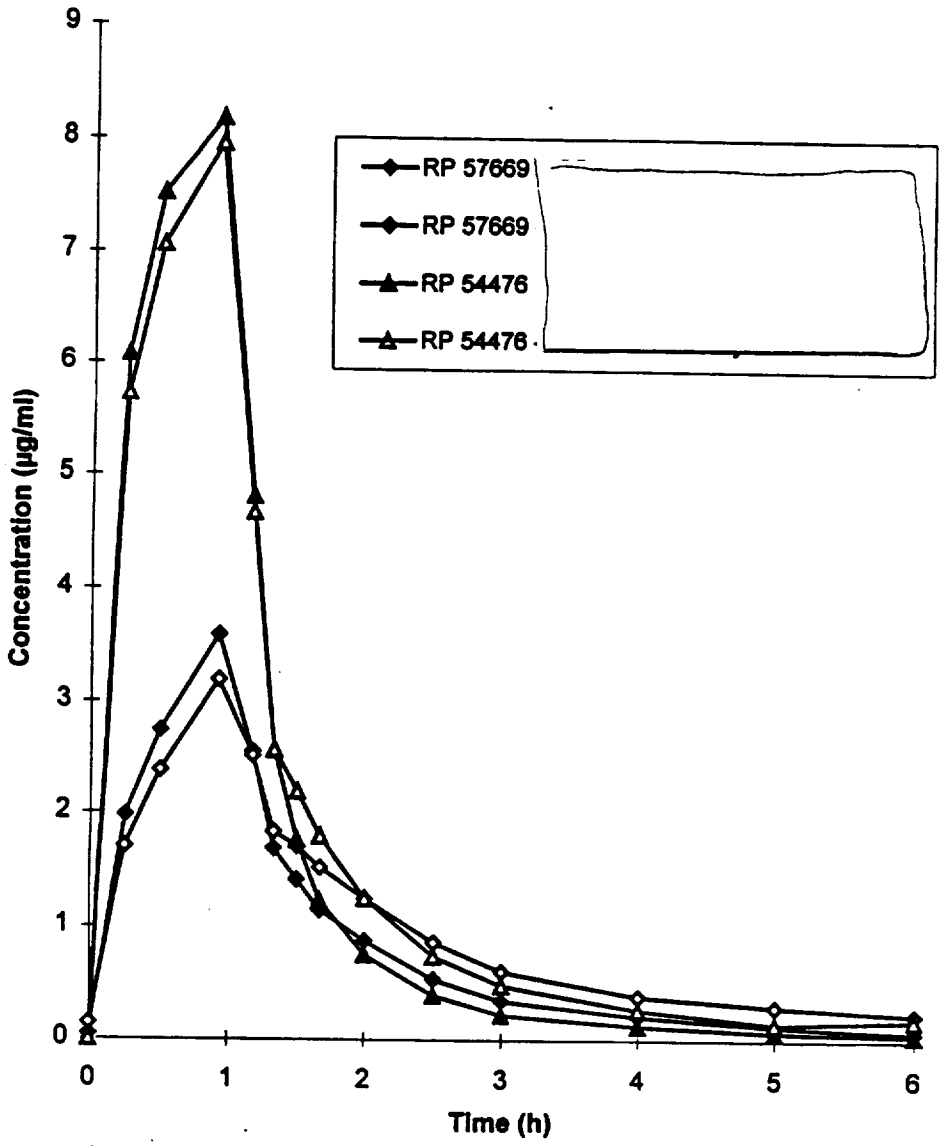


FIGURE 6 : MEAN PLASMA LEVELS OF RP 54476 AND ITS METABOLITE VERSUS TIME - T.I.D. REGIMEN



Bioassay values were comparable to the sum of plasma levels of parent compound and metabolite(s) both for RP 57669 and RP 54476, at the first sampling time until 1.33 h post-start of infusion, as shown by the Figure 7.

FIGURE 7 : MEAN PLASMA CONCENTRATIONS VERSUS TIME T.I.D. REGIMEN - ADMINISTRATION ON DAY 4



The kinetic profile corresponding to the sum of RP 57669, RP 69012 and RPR 100391 plasma concentrations was comparable to that obtained with the selective bioassay of RP 57669 in the first sampling times. That corresponding to the sum of RP 54476 and RP 12536 was also comparable to the kinetic profile of RP 54476 measured by selective bioassay in the first sampling times. Thereafter bioassay values were higher than those at 2 and 3 hours post-start of infusion. However, disparity between bioassay at the last sampling times concerned low levels

compared to the respective Cmax values: 3-hour plasma levels of RP 57669 and RP 54476 measured by selective bioassays corresponded to less than 15 % and 6 % of respective Cmax values. This explains why the AUC(0-t) values estimated from the sums of AUC(0-t) data (RP 57669 + RP 69012 + RPR 100391 for quinupristin and RP 54476 + RP 12536 for dalfopristin) [redacted] were comparable to global AUC(0-t) estimated by bioassay, as shown by the Table 7 :

TABLE 7 - MEAN RATIOS (± SD) OF SUMS OF AUC(0-T) VALUES OF UNCHANGED COMPOUNDS AND METABOLITES [redacted] AUC(0-T) DETERMINED BY SELECTIVE BIOASSAYS

Subject	RP 57669		RP 54476	
	day 1	day 4/5	day 1	day 4/5
q8h group	1.02 ± 0.09	0.84 ± 0.06	0.98 ± 0.11	0.96 ± 0.11
q12h group	1.08 ± 0.12	0.88 ± 0.11	0.96 ± 0.10	0.91 ± 0.12

A downward trend of mean ratios [redacted] bioassay $AUC_{(0-t)}$ values was observed for RP 57669 between the first and last day for both treatments. This result could be due to the presence of derivatives taken into account by the selective bioassay, different from RP 69012 and RPR 100391, in higher amounts on the last day of treatment than on the first day. This phenomenon did not seem present for RP 54476.

Plasma concentrations of RP 57669 and RP 54476 declined biexponentially with mean distribution half-lives, estimated after the first administration, of 0.13 and 0.12 hour in the q8h group and 0.14 and 0.13 hour in the q12h group, and mean elimination half-lives of 0.75 and 0.45 hour in the q8h group and 0.82 and 0.61 hour in the q12h group, respectively. Total plasma clearances for both compounds were very high (about 0.90 l/h.kg). Peak plasma levels of metabolites were reached practically at the same time as parent compounds (Tmax range : 0.92 h to 1.25 h). Areas under the plasma concentration-time curves of the metabolites of RP 57669, RP 69012 and RPR 100391 were comparable and approximately five times lower than that of parent compound. AUC of the natural pristinamycin PIIA (RP 12536) corresponded also to about 20 % of that of RP 54476. Apparent elimination half-lives of metabolites were about 1.8 hours for RP 69012 and RPR 100391 and 1.3 hours for RP 12536.

No regimen difference was observed between pharmacokinetic parameter values estimated either on day 1 or on days 4 or 5, [redacted] bioassay. This result indicates that q8h and q12h administrations lead to the same disposition profile after repeated administration. Comparable steady-state levels were reached from day 2 of both treatments, q8h and q12h.

Moderate increases of Cmax and AUC(0-t) values of unchanged RP 57669 and RP 54476 were observed between day 1 and day 4 or 5, which resulted from a moderate lower drug clearance (around 20%). This phenomenon is more pronounced for the metabolite cysteine conjugate RPR 100391 and to a lesser extent for the metabolites RP 69012 and RP 12536 than for the unchanged compounds. As a consequence, increases of Cmax and AUC(0-t) values of RP 57669 and RP 54476 in combination with their derivatives, estimated by selective bioassays, between day 1 and day 4 or 5, were more important than those estimated for unchanged compounds. These increases were observed rapidly after the beginning of the treatments, but could not be predicted by the high elimination rates (short half-lives)

of all drug-related components. Moreover, following both dosage regimens, pre-drug levels (for parent drugs and metabolites) were below or close to the limits of quantitation [redacted]

VII. DISTRIBUTION

A. PROTEIN BINDING

1. In vitro studies

The in vitro protein binding of the two components of RP 59500, Quinupristin (RP 57669) and Dalfopristin (RP 54476) was studied in animal plasma (rat and monkey) and in human serum, using radiolabelled compounds [redacted] at concentrations ranging from 1 to 15 µg/ml for Quinupristin and from 0.2 to 15 µg/ml for Dalfopristin, which represents 1 to 15 µM Quinupristin and 0.3 to 22 µM Dalfopristin. This concentration range covered the plasma level range observed in these animal species and in man. The in vitro protein binding of Quinupristin (RP 57669) was also studied in human isolated proteins such as human serum albumin (HSA), alpha$_1$-acid glycoprotein and lipoproteins.

Despite optimisation of assay conditions, parent compounds remained unstable during the assays. In the first experiment, Quinupristin, which had a purity of 90%, was converted into essentially RP 50309 and from the total radioactivity measured at the end of the assay, Quinupristin represented 58% in the rat, 51% in the monkey and 59% in man. Dalfopristin, which had a purity of 92%, was converted into essentially RP 12536 and from the total radioactivity measured at the end of the 15 µg/ml assay, Dalfopristin represented 58% in the rat and monkey and 55% in man.

a) RP 57669

In vitro plasma protein binding of the total radioactivity from Quinupristin (RP 57669) was high, 95 to 96% in the rat, 90 to 93% in the monkey and 88 to 94 % in man. Binding appeared not to be saturable in the range of concentrations tested. In a second study, the plasma protein binding of RP 57669 ranged between 55 and 78 %. The binding of RP 57669-related radioactivity to human serum albumin increased when the time of incubation increased, simultaneously to the degradation of RP 57669, suggesting that the degradation products may have greater affinity for HSA than the parent compound. In these conditions, the range of 55 to 78 % seems to be more predictive of the protein binding of the parent drug than that of 88 to 94 %. Addition of glutathione to the incubation medium did not change this binding. The extent of binding of ^{14}C-RP 57669 (and derivatives) to human serum albumin and human alpha$_1$-acid glycoprotein ranged from 30 to 33 % and from 7.2 to 41 %, respectively. The lipoproteins were not or weakly involved in the plasma protein binding of RP 57669.

b) RP 54476

In vitro plasma protein binding of the total radioactivity from Dalfopristin (RP 54476) was low and ranged from 10 to 28% in the rat, 11 to 36% in the monkey and 11 to 26% in man.

No interference of any of both compounds on the binding of the other one is to be expected.

The in vitro human serum protein binding values for warfarin at concentrations of 2.5 and 5 µg/ml were 99.3 and 99.1 %, respectively. When RP 59500 was added at therapeutic concentrations (2, 10

and 20 µg/ml) to warfarin at 2.5 or 5 µg/ml, the human serum protein binding of warfarin ranged from 98.8 to 99.3 %. Thus, RP 59500 did not alter the binding of warfarin in vitro to serum human protein.

2. **In vivo studies**

a) RP 57669 (study Syn 111)

The human plasma protein binding of RP 57669 was studied following single IV infusion of ^{14}C-labelled RP 59500 (^{14}C-RP 57669 : RP 54476, 30 : 70) at the dose of 500 mg to 6 healthy young male volunteers. Plasma protein binding of total radioactivity ranged from 23 % to 32 %. This large difference observed between in vitro and in vivo results was observed for human plasma but not for rat and monkey plasma. It could be due to the process to obtain protein-free ultrafiltrate. Samples were [redacted] counting by liquid scintillation.

b) RP 54476 (study Syn 112)

The human plasma protein binding of RP 54476 was studied following single IV infusion of ^{14}C-labelled RP 59500 (RP 57669 : ^{14}C-RP 54476, 30 : 70) at the dose of 500 mg to 6 healthy young male volunteers. The plasma protein binding of total radioactivity was relatively constant over [redacted] hour period after the drug administration from 50.3 % to 59.3 %. [redacted] hours after the drug administration and later, the protein binding of total radioactivity increased from 64.9 % to 85 % at time [redacted] This increase is probably related to the protein binding of metabolites with higher affinity for these proteins than drug derived species present during and within a short time after drug infusion.

B. **Distribution of total radioactivity in plasma and whole blood**

1. **RP 57669 (study SYN 111)**

Following 50-minute infusion of 500 mg of Synercid® (^{14}C-RP 57669 : RP 54476, 30 : 70) to six healthy young male volunteers, mean Cmax values of total radioactivity expressed as RP 57669 equivalents were 2.56 ± 0.13 µg/ml in plasma and 2.28 ± 0.14 µg/ml in blood. Both plasma and whole blood Cmax were achieved at the end of infusion [redacted] Plasma Cmax was slightly but significantly higher than whole blood Cmax ; the plasma Cmax / whole blood Cmax ratio was 1.13 ± 0.11. The plasma AUC / whole blood AUC ratio was 1.15, when AUC was calculated in the time interval 0 to [redacted] when AUC was calculated between time 0 and the last time of quantitation. This difference is due in part to a difference in sensitivity limits [redacted] This result indicates that drug-derived products do not concentrate in red blood cells.

2. **RP 54476 (study SYN 112)**

Following 50-minute infusion of 500 mg of Synercid® (RP 57669 : ^{14}C-RP 54476, 30 : 70) to six healthy young male volunteers, mean Cmax values of total radioactivity expressed as RP 54476 equivalents were 6.91 ± 1.14 µg/ml in plasma and 6.57 ± 0.96 µg/ml in blood. The mean plasma Cmax

/ whole blood Cmax ratio was 1.06 ± 0.12 and the mean plasma AUC / whole blood AUC ratio was 0.85 ± 0.09. This result indicates that drug-derived products do not concentrate in red blood cells.

C. Diffusion in blister fluid (study JRB 104)

RP 59500 pharmacokinetics and penetration into suction blister fluid were evaluated in six healthy young male volunteers following a single infusion of 12 mg/kg over one hour. Plasma and blister fluid concentrations were determined by global microbiological assay. The results are summarized in Table 8 :

TABLE 8 : MEAN PLASMA PHARMACOKINETIC PARAMETERS OF RP 59500 IN COMBINATION WITH THEIR DERIVATIVES AS ANALYSED BY GLOBAL MICROBIOLOGICAL ASSAY

Compound	Cmax µg/ml	Tmax * h	AUC(0-6h) µg.h/ml	AUC µg.h/ml	t½λz h
Blister fluid	2.41 ± 0.55	2	9.19 ± 2.03	-	-

* : MEDIAN

Bioavailability of RP 59500 into non-inflammatory interstitial fluid for the interval 0-6 hours corresponded to 82.5 ± 11.4 % of that estimated in plasma, suggesting a large diffusion in this medium. RP 59500 was still detectable at 6 hours post start of infusion in blister fluid in all 6 subjects and in plasma in 3 subjects.

D. Diffusion in blister fluid (study JRV 131)

RP 59500 pharmacokinetics and penetration into suction blister fluid were evaluated in twelve healthy young male volunteers following a single infusion of 7.5 mg/kg over one hour. Plasma and blister fluid concentrations [redacted] The results are summarized in Tables 9 to 11:

TABLE 9 : MEAN PLASMA PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND THEIR MAIN METABOLITES [redacted] (N=12)

Compound	Cmax µg/ml	Tmax * h	AUC(0-t) µg.h/ml	AUC µg.h/ml	Vz l/kg	Vss l/kg	t½λz h	CL l/h.kg
RP 57669	2.32 ± 0.58	1	2.79 ± 0.47	2.83 ± 0.47	0.96 ± 0.21	0.56 ± 0.14	0.81 ± 0.11	0.82 ± 0.13
RP 69012	0.272 ± 0.088	1	0.417 ± 0.091	0.442 ± 0.088	- -	- -	1.30 ± 0.26	- -
RPR 10039	0.198 ± 0.037	1.17	0.429 ± 0.080	0.457 ± 0.080	- -	- -	1.51 ± 0.41	- -
RP 54476	5.97 ± 2.20	0.5	5.95 ± 1.86	5.97 ± 1.86	0.69 ± 0.22	0.33 ± 0.10	0.51 ± 0.13	0.93 ± 0.20
RP 12536	0.702 ± 0.131	1	1.18 ± 0.25	1.24 ± 0.28	- -	- -	1.43 ± 0.73	- -

* : MEDIAN

TABLE 10 : MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND THEIR MAIN METABOLITES IN BLISTER FLUID (N=10)

Pharmacokinetics in blister fluid			
Compound	Cmax (µg/ml)	Tmax* (h)	AUC(0-t) (µg.h/ml)
RP 57669	0.443 ± 0.076	1	0.571 ± 0.121
RP 69012	BLQ (< 0.004 µg / 100 µl)	-	-
RPR 100391	0.194 ± 0.028	2	0.948 ± 0.280
RP 54476	0.595 ± 0.197	1	0.620 ± 0.189
RP 12536	0.481 ± 0.133	2	2.19 ± 0.49

* : median

TABLE 11 : PENETRATION RATIOS (%)

Compound	r1 (%) = Cmax in blister fluid / Cmax in plasma	r2 (%) = AUC(0-t) in blister fluid / AUC(0-t) in plasma
RP 57669		
RP 69012		
RPR 100391		
sum (RP 57669 + RPR 100391)		
RP 54476		
RP 12536		
sum (RP 54476 + RP 12536)		

Following the termination of the one-hour infusion of RP 59500 at the dose of 7.5 mg/kg to healthy volunteers, the mean maximum plasma concentrations of unchanged RP 57669 and RP 54476 were 2.32 and 5.97 µg/ml, respectively. Peak plasma levels of metabolites were reached practically at the same time as parent compounds (Tmax range : [redacted]). Areas under the plasma concentration-time curves of the metabolites of RP 57669, RP 69012 and RPR 100391 were comparable and approximately six times lower than that of parent compound. AUC(0-t) of the natural pristinamycin PIIA (RP 12536) corresponded to about 20 % of that of RP 54476. Apparent elimination half-lives of metabolites were longer than those of parent compounds, about [redacted] hours for RP 69012, [redacted] hours for RPR 100391 and [redacted] hours for RP 12536.

The mean maximum concentrations of unchanged RP 57669 and RP 54476 in blister fluid were 0.443 and 0.595 µg/ml, respectively. Median peak levels were reached 1 hour after the beginning of the infusion. Concentrations were detectable up to 2 hours after the beginning of the infusion. AUC(0-t) values of RP 57669 and RP 54476 in blister fluid corresponded to about 19 % and 11 % of those estimated in plasma, respectively. RP 69012 was not present in blister fluid, suggesting that it did not penetrate into blister fluid and/or that the metabolism of RP 57669 was different in blood and in blister fluid. Finally, the comparison of the kinetic profiles of RPR 100391 and RP 12536 in blister fluid to those estimated in plasma indicated that these two metabolites were more stable and (or) less rapidly eliminated in blister fluid than in blood, their AUC(0-t)s being about two times higher in blister fluid (Figures 8 and 9).

FIGURE 8 : MEAN CONCENTRATIONS OF RPR 100391 IN BLISTER FLUID AND IN PLASMA VERSUS TIME

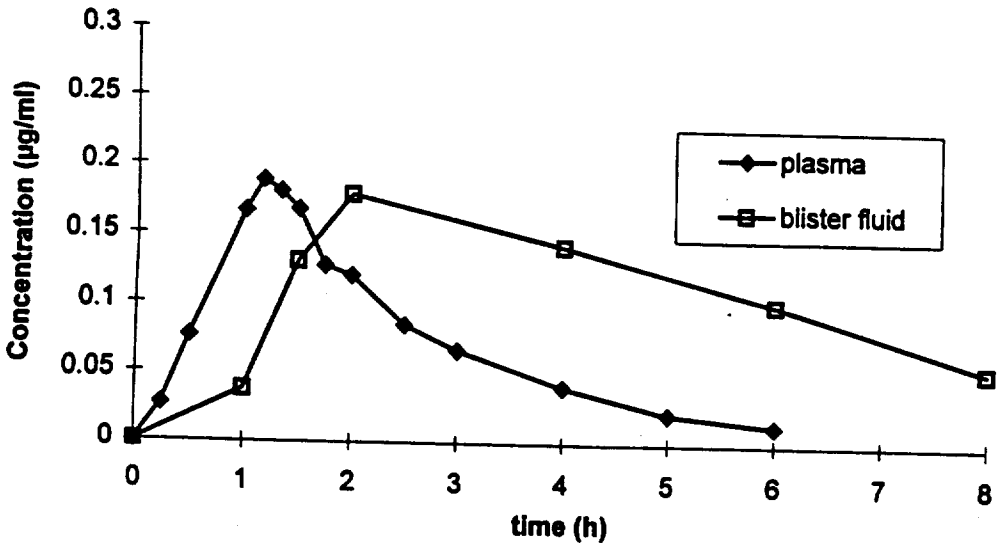
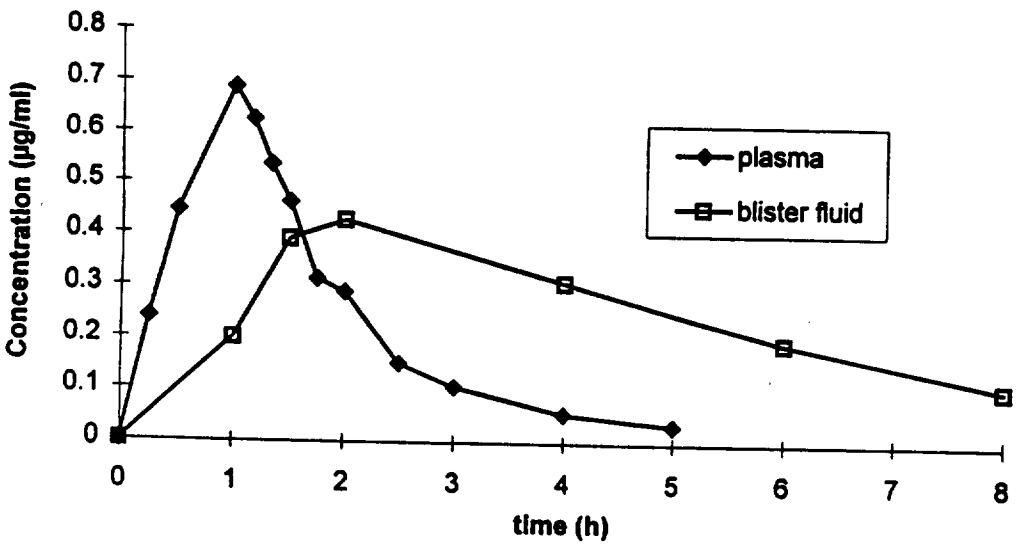


FIGURE 9 : MEAN CONCENTRATIONS OF RP 12536 IN BLISTER FLUID AND IN PLASMA VERSUS TIME -



Globally, the relative bioavailabilities of RP 57669 and RP 54476 and their metabolites in blister fluid, compared to that in plasma, were about 40 %.

E. Pulmonary diffusion (study JRV 123)

The pharmacokinetics of the two components RP 57669 and RP 54476 of Synercid were determined in an open, non-randomized, single dose study performed in 15 healthy young male non-smoking volunteers. Three groups of 6 subjects were enrolled, but only 15 subjects received a single dose of RP 59500 (7.5 mg/kg) as an one-hour IV infusion, since negative respiratory tree pharmacokinetic results were found. Bronchoscopies were performed at 1.5, 3 and 6 hours post-start of infusion in groups 1, 2 and 3, respectively. No detectable levels of compound were found in bronchoalveolar lavage fluid or cell pellets. This result could be due in part to the unstability of the compounds in this medium.

F. Penetration into non-infected respiratory tree (study JRV 140)

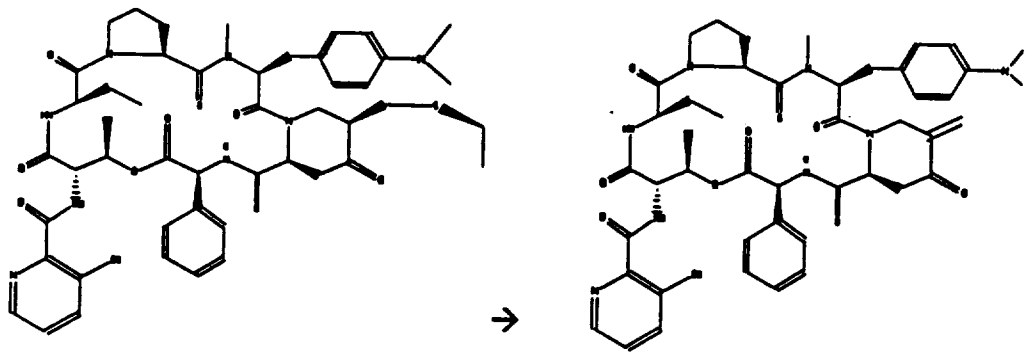
The concentrations of RP 57669, RP 69012, RPR 100391, RP 54476 and RP 12536 were determined in bronchoalveolar lavage fluid (BAL) and in plasma in an open, non-randomized, single dose study performed in 4 healthy young male smoking volunteers. The subjects received a single dose of RP 59500 (7.5 mg/kg) as an one-hour IV infusion. Bronchoscopies were performed at 0.25 hour (subject 1) or 1 hour (subject 2) or 3 hours (subject 3) or 6 hours (subject 4) after the end of infusion. BAL fluid levels were generally below the limit of quantitation, except for one data : RP 12536 concentration was 0.065 µg/ml (LOQ = 0.0625 µg/ml for RP 12536) after the second lavage performed 15 minutes after the end of infusion in subject 1. Because no detectable levels of compound were found in bronchoalveolar lavage fluid or cell pellets, the results obtained are limited to plasma data. Plasma levels [redacted] at the end of infusion for all subjects (n=4). Mean 1-hour plasma levels (µg/ml, ± SD) of RP 57669, RP 69012, RPR 100391, RP 54476 and RP 12536 are 3.00 ± 0.89, 0.40 ± 0.10, 0.24 ± 0.08, 7.97 ± 3.45 and 1.06 ± 0.34 µg/ml, respectively.

VIII. METABOLISM

A. Proposed metabolic pathways

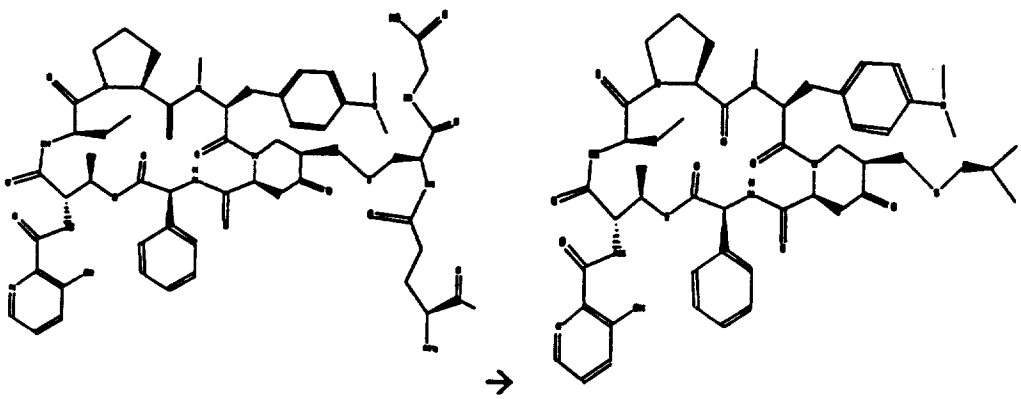
The major routes of biotransformation of RP 57669 and RP 54476 are presented thereafter.

a) Proposed metabolic pathways of RP57669:



RP 57669

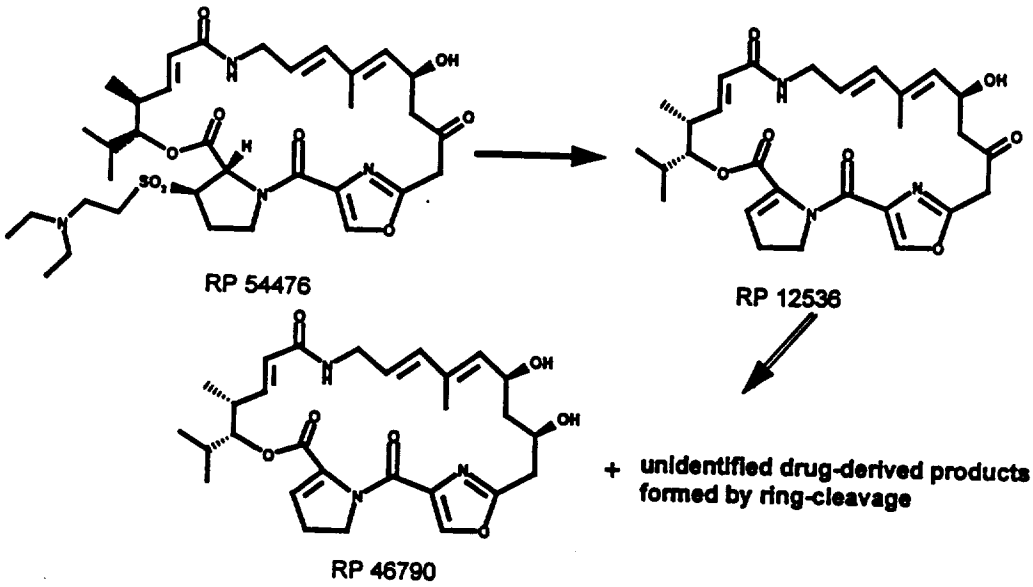
RP 50309



RP 69012

RPR 100391

b) Proposed metabolic pathways of RP 54476



B. In vivo metabolism of RP 57669 (study SYN 111)

The metabolism profile of RP 57669 was studied in plasma, urine and feces following single IV infusion of ^{14}C-labelled RP 59500 (^{14}C-RP 57669 : RP 54476, 30 : 70) at the dose of 500 mg to 6 healthy male volunteers.

1. Metabolism in plasma

The comparison of unchanged RP 57669 whole blood concentrations and total radioactivity equivalent concentrations showed only small differences until the end of infusion, as presented in Table 12 :

TABLE 12 : PHARMACOKINETIC PARAMETERS OF UNCHANGED RP 57669 IN WHOLE BLOOD AND OF TOTAL RADIOACTIVITY IN PLASMA AND WHOLE BLOOD

Then a more marked difference was noted, with a more rapid elimination of unchanged RP 57669 from blood. Two Phase II metabolites, RP 69012 (glutathione conjugate) and RPR 100391 (cysteine conjugate) were the main compounds found in plasma with unchanged RP 57669.

2. Metabolites in urine

12 % of the administered dose were eliminated in the 0-3 hour urine samples. RPR 100391, representing 4.68 % of the administered dose, was the main metabolite. Unchanged RP 57669

represented 4.22 % of the administered dose, whereas RP 69012, RP 60844 and RP 50309 represented 0.57, 0.41 and 0.27 % of the administered dose, respectively.

3. Metabolites in feces

59 % of the administered dose were eliminated in feces by 96 hours post dosing. 37 to 47 % could be extracted from feces and only 5.8 to 14.9 % [redacted] The main metabolite found in feces was the cysteine conjugate RPR 100391. It represented about 1.0 to 12 % of the extractable dose. Unchanged compound represented about 10 to 15 % and unknown metabolites 1.4 to 8.6 % of the extractable dose.

C. In vivo metabolism of RP 54476 (study Syn 112)

The metabolism profile of RP 54476 was studied in plasma, urine and feces following single IV infusion of ^{14}C-labelled RP 59500 (RP 57669 : ^{14}C-RP 54476, 30 : 70) at the dose of 500 mg to 6 healthy male volunteers.

1. Metabolism in plasma

Unchanged RP 54476 was rapidly cleared from blood, as presented in Table 13:

TABLE 13 : PHARMACOKINETIC PARAMETERS OF UNCHANGED RP 54476 IN WHOLE BLOOD AND OF TOTAL RADIOACTIVITY IN PLASMA AND WHOLE BLOOD

[redacted]

Unchanged RP 54476 was rapidly and extensively converted to RP 12536. This rapid conversion could have resulted from non acidic conditions used for the collection of plasma samples in the metabolism study. Both compounds were present in plasma as well as RP 46790 (reduced pristinamycin IIA) and a cysteine-conjugated metabolite of RP 12536.

2. Metabolism in urine

About 15 % of the administered dose were eliminated in the 0-3 hour urine samples. Only traces of RP 54476 and RP 46790 were observed. RP 12536 was the main metabolite, with 8.6 % of the administered dose. Unknown metabolites and suspected glutathione or cysteine conjugated metabolites represented 4.6 % of the administered dose.

3. Metabolism in feces

66 % of the administered dose were eliminated by 96 hours post dosing. The 7-day faecal excretion represented approximately 77 % of the administered dose. No metabolite could be identified in feces extracts, RP 54476 metabolites being not extractable in feces.

D. IN VITRO METABOLISM

The metabolism of ^{14}C-RP 57669 and ^{14}C-RP 54476 was studied in liver subcellular fractions of mouse, rat, monkey and man in the presence and absence of co-factors of the microsomal monooxygenases and of the cystolic glutathione S-transferases.

1. 14C-RP 57669

The main metabolic pathway for RP 57669 appeared to be a non-enzymatic glutathione-conjugation leading to RP 69012. This metabolite formed by loss of the thio-quinuclidinyl group might have been formed from a spontaneous reaction with glutathione and the electrophilic intermediate RP 50309. This reaction is non enzymatic and hence would proceed at a similar rate in all species. An irreversible protein binding of RP 57669-related residues was observed in the absence of gluthatione.

Microsomal monooxygenases are not responsible for the generation of protein-binding metabolites and no formation of any other phase I metabolites was observed for the four species in this study.

Other metabolites of RP 57669 may exist, possibly derived from the gluthathione-conjugate RP 69012.

The main in vitro biotransformation of RP 57669 is not enzyme-catalyzed and no important interspecies differences in the fate of these compounds were detected. The main primary metabolites would be the glutathione-conjugated metabolites RP 69012 (Table 14).

TABLE 14. IN VITRO METABOLISM OF 14C-RP 57669 IN MOUSE, RAT, MONKEY AND HUMAN LIVER SUBCELLULAR FRACTIONS

RP 57669 WAS INCUBATED IN THE PRESENCE OF MICROSOMES AND NADPH OR IN THE PRESENCE OF MICROSOMES ONLY : CONTROL. RESULTS ARE EXPRESSED IN % OF THE INCUBATED DOSE

	Type	none*		rat		mouse			monkey			man
		time (minute)		time (minute)		time (minute)			time (minute)			
		0.5	60	0.5	60	0.5	30	60	0.5	30	60	
RP 57669	NADPH		60	74	40	73	56	51	76	38	28	
	Control	70			51			40			48	
RP 50309	NADPH		12	ND	ND	9	9	16	ND	3	ND	
	Control	7			ND			ND			ND	
RP 69012	NADPH		ND	ND	ND	3	2	3	2	2	3	
	Control	ND			ND			3			3	
RP 68044	NADPH		10	10	14	4	6	7	6	17	17	
	Control	8			7			11			5	
Metab 3	NADPH			ND	ND	ND	ND	ND	ND	ND	ND	
	Control				ND			ND			7	
Protein bound	NADPH			2	28	9	21	27	8	17	24	
	Control				33			46			32	
Total	NADPH			86	82	98	94	104	92	77	72	
	Control				91			100			95	

*incubation in buffer

ND : not detected

2. ^{14}C-RP 54476

For RP 54476 , the major in vitro transformation pathway in all species is the non-enzymatic chemical reaction to RP 12536 or Pristinamycin IIA, and subsequently non-enzymatic glutathione-conjugation to metabolite C (Table 15).

An irreversible protein binding of RP 54476-related residues was observed in the absence of glutathione. Microsomal monooxygenases do not increase the formation of protein-binding residues, but they may catalyze the biotransformation of RP 54476 in the rat, the monkey and in man.

TABLE 15. IN VITRO METABOLISM OF ^{14}C-RP 54476 IN MOUSE, RAT, MONKEY AND HUMAN LIVER CYTOSOL

RP 54476 was incubated in the presence of cytosol and GSH or in the presence of cytosol and diethylmaleate (control). Results are expressed in % of the incubated dose

	Type	none*		rat			mouse			monkey			man
		time (minute)		time (minute)			time (minute)			time (minute)			time (minute)
		0.5	60	0.5	30	60	0.5	30	60	0.5	30	60	
RP 54476	GSH	22	2	20	4	ND	44	7	ND	21	4	ND	
	Control			15		ND	27		5	14		2	
RP 12536	GSH	5	5	6	5	3	7	6	5	6	5	4	
	Control			50		77	38		85	41		64	
Metab C	GSH	60	65	56	74	70	34	72	73	48	77	71	
	Control			ND		ND	ND		ND	ND		ND	
Metab Z	GSH	8	10	10	9	12	7	10	10	8	10	10	
	Control			ND		2	1		2	ND		ND	
Metab V	GSH	3	ND	3	ND	ND	7	ND	ND	3	ND	ND	
	Control			3		ND	5		ND	2		ND	
Metab Y1	GSH	ND	ND	3	ND	ND	ND	ND	ND	ND	ND	ND	
	Control			17		ND	10		ND	16		ND	
Protein bound	GSH	ND	ND	1	3	3	1	3	3	1	3	4	
	Control			7		12	7		15	4		8	
Total (% dose)	GSH	98	82	99	95	86	100	98	91	87	99	89	
	Control			94		91	88		107	77		74	

* INCUBATION IN THE PRESENCE OF GSH IN BUFFER ND : NOT DETECTED

E. In vitro activity of drug metabolites against Staphylococcus aureus, Streptococcus pneumoniae and Enterococcus faecium

1. Metabolites of RP 57669

RP 69012 and RPR 100391, main metabolites of RP 57669, show an in vitro activity similar to that of the parent drug (changes in MICs : x 1-4). The synergistic activity of RP 69012 and RPR 100391, each of them associated with RP 54476 is close that of Synercid (changes in MICs : x 1-4).

2. Metabolites of RP 54476

RP 12536, main metabolite of RP 54476 has an in vitro activity similar to the parent drug (changes in MICs : x 0.5-1). The synergistic activity of RP 12536 when associated with RP 57669, is similar to that of Synercid (changes in MICs : x 0.5-2).

F. EFFECT OF SYNERCID ON DRUG-METABOLIZING ENZYMES

1. In Rat

A 6 and 18 mg/kg/day doses of RP 59500, 6 mg/kg/day dose of RP 57669 and 12 mg/kg/day dose of RP 54476 given for 7 days to male rats (n = 6/dose) did not significantly modify the hepatic parameters: liver weight, microsomal or cytosolic proteins, total cytochrome P450 content, aniline and aminopyrine N-demethylase activities, except for liver weight ($p<0.05$) and for cytosolic protein ($p<0.01$) after administration of RP 57669 at 6 mg/kg/day. RP 59500 or each compound RP 57669 and RP 54476 are devoided of effect on hepatic drug-metabolism enzymes in the rat (Tables 16 and 17).

TABLE 16. EFFECT OF RP 59500 ON LIVER WEIGHT, PROTEIN AND CYTOCHROME P-450 CONTENT OF MALE RAT LIVER MICROSOMES AFTER INTRAVENOUS TREATMENT WITH RP 59500 DURING 7 DAYS

				Dose (mg/kg/day)			
				0 (control)	6	0 (control)	18
Liver weight*			%body weight	3.2 ± 0.2	3.0 ± 0.2	3.1 ± 0.2	2.9 ± 0.2
Protein*		(microsomal)	mg/g liver	21.0 ± 2.0	25.3 ± 4.5	20.9 ± 3.7	21.4 ± 3.5
		(cytosolic)	mg/g liver	79.5 ± 1.6	82.2 ± 5.0	77.8 ± 8.2	74.0 ± 6.2
P-450*			nmol/mg prot	0.45 ± 0.11	0.51 ± 0.0	0.62 ± 0.23	0.68 ± 0.21
complexed P-450*			nmol/mg prot	0	0	0	0
AH*		without KF	nmol/min.mg prot	0.63 ± 0.07	0.65 ± 0.0	0.61 ± 0.10	0.63 ± 0.09
		with KF		0.59 ± 0.05	0.59 ± 0.0	0.59 ± 0.07	0.59 ± 0.08
APND*		without KF	nmol/min.mg prot	2.33 ± 0.16	2.56 ± 0.2	2.07 ± 0.34	2.33 ± 0.45
		with KF		1.88 ± 0.24	2.14 ± 0.4	1.79 ± 0.37	1.84 ± 0.47
ECOD*	high affinity	without KF	nmol/min.mg prot	0.42 ± 0.03	0.44 ± 0.0	0.50 ± 0.10	0.54 ± 0.10
	low affinity			1.24 ± 0.12	1.39 ± 0.2	1.30 ± 0.16	1.45 ± 0.37
	high affinity	with KF	nmol/min.mg prot	0.25 ± 0.08	0.29 ± 0.0	0.40 ± 0.18	0.43 ± 0.15
	low affinity			1.14 ± 0.34	1.20 ± 0.2	1.05 ± 0.20	1.12 ± 0.36
Cytosolic glutathione S-transferase*			nmoles / min/mg	847 ± 115	770 ± 103	872 ± 150	972 ± 197

AH : Aniline hydroxylase; APND : Aminopyrine N-demethylase; ECOD : microsomal 7 ethoxycoumarin o-deethylase; KF : K ferricyanide; * - not significant difference between control and results at 6 and 18 mg/kg dose of RP 59500

TABLE 17. EFFECT OF RP 57669 AND RP 54476 ON LIVER WEIGHT, PROTEIN AND CYTOCHROME P-450 CONTENT OF MALE RAT LIVER MICROSOMES AFTER INTRAVENOUS TREATMENT WITH RP 57669 (6 MG/KG/DAY) AND RP 54476 (12 MG/KG/DAY DURING 7 DAYS)

				Dose (mg/kg/day)			
				0 (control)	6 (RP 57669)	0 (control)	12 (RP 54476)
Liver weight			%body weight	3.2 ± 0.2	3.2 ± 0.4*	3.0 ± 0.1	3.2 ± 0.2
Protein		(microsomal)	mg/g liver	21.0 ± 2.0	20.8 ± 2.1	22.6 ±4.0	21.2 ± 3.7
		(cytosolic)	mg/g liver	79.5 ± 1.6	70.6 ± 5.3**	76.1 ± 11.	66.2 ± 3.2
P-450			nmol/mg prot	0.45 ± 0.11	0.48± 0.16	0.79 ± 0.1	0.72 ± 0.13
complexed P-450			nmol/mg prot	0	0	0	0
AH		without KF	nmol/min.mg prot	0.63 ± 0.07	0.66 ± 0.10	0.58 ± 0.1	0.57 ± 0.07
		with KF		0.59 ± 0.05	0.62 ± 0.12	0.59 ± 0.1	0.58 ± 0.13
APND		without KF	nmol/min.mg prot	2.33 ± 0.16	2.69 ± 0.64	1.82 ± 0.2	2.33 ± 0.45
		with KF		1.88 ± 0.24	2.36 ± 0.99	1.69 ± 0.4	1.65 ± 0.47
ECOD	high affinity	without KF	nmol/min.mg prot	0.42 ± 0.03	0.43 ± 0.01	0.58 ± 0.0	0.55 ± 0.08
	low affinity			1.24 ± 0.12	1.24 ± 0.22	1.36 ± 0.1	1.47 ± 0.19
	high affinity	with KF	nmol/min.mg prot	0.25 ± 0.08	0.27 ± 0.22	0.56 ± 0.0	0.53 ± 0.08
	low affinity			0.99 ± 0.13	0.96 ± 0.10	1.12 ± 0.2	1.10 ± 0.33
7			nmoles/min/ mg	847 ± 115	770 ± 103	894 ± 183	1012 ± 167

AH : Aniline hydroxylase
APND : Aminopyrine N-demethylase
ECOD : microsomal 7 ethoxycoumarin o-deethylase
KF : K ferricyanide
* significant difference between control and results : $p < 0.05$
** significant difference between control and results $p<0.01$

G. Effect on Human cytochrome P450 (CYP450) isoenzymes

1. Effect of Synercid (RP 59500) on human liver cytochrome P450 isoenzymes in vitro.

The effect of Synercid (RP 59500) on the biotransformation rate of specific CYP450 substrates was studied in human liver microsomes in vitro. The cytochromes P450's CYP1A2, 2A6, 2C9, 2C19, 2D6, 2E1 and 3A45 were studied by the following enzymatic reactions: ethoxyresorufin o-deethylase, coumarin 7-hydroxylase, tolbutamide 4'-hydroxylase, (S)-mephenytoin 4'-hydroxylase, dextromethorphan o-demethylase, aniline p-hydroxylase and cyclosporin A biotransformation, respectively (Table 24). For each CYP450, two tests were performed : 1- specific substrate has been added simultaneously to Synercid (coincubation) and 2- specific substrate has been added after a 10 min incubation of Synercid with NADPH (preincubation). A concentration of 100 µg/ml Synercid was used in a first screening.

This concentration is approximately equivalent to ten fold the plasma Cmax in human and corresponds to 29 µM RP 57669 and 101 µM RP 54476. A concentration-dependent study was run when inhibition was detected. Substrates concentrations were chosen near the Km value of their respective CYP450 dependent metabolization. This study demonstrated that Synercid did not interact with ethoxyresorufin o-deethylase (CYP1A2), coumarin 7-hydroxylase (CYP2A6) nor aniline p-hydroxylase (CYP2E1). At Cmax, a 19.4 % inhibition was observed on tolbutamide 4'-hydroxylase (CYP2C9), a 17.4 % inhibition was obtained on (S)-mephenytoin 4'-hydroxylase (CYP2C19) and a 4.8 % inhibition was detected on dextromethorphan o-demethylase (CYP2D6). These effects should not be clinically relevant. Therefore, the concomitant administration of Synercid with compounds metabolized by CYP1A2, 2A6, 2C9, 2C19, 2D6 and 2E1 would not be expected to alter their hepatic metabolic clearance.

TABLE 18 : EFFECT OF SYNERCID ON P450S ACTIVITIES (COMPARED TO METHANESULFONATE ACTIVITIES)

Name of Enzyme activity (Substrate Concentration)	P450	COINCUBATION Synercid concentration 1 µg/ml (≈ 0.1 Cmax)	10 µg/ml (≈ Cmax)	100 µg/ml (≈ 10 Cmax)	PREINCUBATION OF SYNERCID Synercid concentration 1 µg/ml (≈ 0.1 Cmax)	10 µg/ml (≈ Cmax)	100 µg/ml (≈ 10 Cmax)
Ethoxyresorufin o-deethylase (1.25 µM)	1A2			+13.9 %			0 %
Coumarin 7-hydroxylase (3 µM)	2A6			+9.5 %			-5.8 %
Tolbutamide 4'-hydroxylase (200 µM)	2C9	-3.6 %	-5.7 %	-19.6 %	-7.6	-19.4 %	-51.4 %
(S)-Mephenytoin 4'-hydroxylase (100µM)	2C19	0.1 %	-5.5 %	-19.4 %	-24.3 %	-17.4 %	-15.2 %
Dextromethorphan o-demethylase (5 µM)	2D6	+6.2 %	+7.0 %	-26.0 %	+11.5 %	-4.8 %	-28.8 %
Aniline p-hydroxylase (60 µM)	2E1			-13.3 %			-5.4 % **
Cyclosporine biotransformation* (1 µM)	3A4/5	-24.5 %	-69.6 %	-97.5 %	***	***	***

SYNERCID CONCENTRATION : 10 µG/ML CORRESPONDS TO 2.93 µM RP 57669, 10.13 µM RP 54476 AND 13.06 µM METHANESULFONATE.

Empty squares : not studied. 1 : 10 min with NADPH. ≈ : approximatly. ** : % of control without methanesulfonate.

*** : Same inhibition level as coincubation.

IX. IN VITRO DRUG-DRUG INTERACTIONS

A. With Cyclosporin

The effect of RP 57669/ RP 54476 and of both components separately on human cyclosporin A (CsA) biotransformation was studied in vitro in liver microsomes from three male subjects.

To assay CsA biotransformation, 3H-labelled drug at 1µM was incubated with human liver microsomes in the presence of NADPH.

RP 57669/RP 54476 was a potent inhibitor of CsA metabolism on liver microsomes from all subjects with an IC50 of 2.1 ± 1.5/7.1 ± 4.9 µM (mean ±SD) (Tables 19 and 20). Both components of RP 57669/RP 54476 were inhibitors of CsA metabolism , with an IC50 of 9.8 ± 7.5 µM for RP 57669 and 5.7 ± 1.5 µM for RP 54476. The values for the inhibition of the formation of two major metabolites were similar : 2.6 ± 2.4/8.7 ± 8.1 µM for RP 57669/RP 54476, 19.6 ± 5.1 µM for RP 57669 and 7.7 ± 2.8 µM for RP 54476. For one subject the inhibition constants were also determined. The Ki (±S.E.) was 1.1 ± 0.3/3.4 ± 0.9 µM for the mixture RP 57669/ RP 54476, 6.1 ± 1.3 µM for RP 57669 alone and 4.0 ± 0.7 µM for RP 54476 alone.

Non-linear iterative regression analysis showed little difference in the residual error between a competitive and a non-competitive inhibition model. However, Dixon plots were more in agreement with non-competitive inhibition.

Glutathione (5mM) reversed only 16 to 29% of the inhibition by the different compounds. Preincubation time in the presence of inhibitors did not have a notable effect on inhibition. This suggests that formation of reactive metabolites and nonspecific inactivation is not the principal mechanism of inhibition.

TABLE 19 : PARENT DRUG BIOTRANSFORMATION

Inhibitor	IC_{50} (± S.E.) in µM			
	883-H1-17	992A-H1-5	931-H1-1	Mean (± SD)
RP 57669	1.3 ± 0.7	15.4 ± 0.1	12.8 ± 2.7	9.8 ± 7.5
RP 54476	4.8 ± 6.7	7.5 ± 1.0	4.9 ± 0.9	5.7 ± 1.5
RP 57669/RP 54476	3.5 ± 0.7/11.8± 2.3	0.6 ± 0.7/2.1± 2.3	2.2 ± 0.3/7.3± 1.1	2.1 ± 1.5/7.1± 4.9

TABLE 20 : METABOLITE D + E FORMATION

Inhibitor	IC_{50} (± S.E.) in µM			
	883-H1-17	992A-H1-5	931-H1-1	Mean (± SD)
RP 57669	16.1 ± 2.2	25.4 ± 10.0	17.2 ± 3.8	19.6 ± 5.1
RP 54476	6.3 ± 7.3	5.9 ± 2.4	10.9 ± 4.8	7.7 ± 2.8
RP 57669/RP 54476	0.8 ± 1.1/2.58± 3.5	5.3 ± 0.7/17.8± 2.3	1.7 ± 1.0/5.7± 3.3	2.6 ± 2.4/8.7± 8.1

X. IN VIVO DRUG-DRUG INTERACTIONS

A. With Cyclosporin (study JRV 138)

The impact of repeated administration of Synercid® on cyclosporine pharmacokinetics was studied in 24 healthy volunteers. 300 mg (three capsules of 100 mg) of cyclosporine were administered either alone with an infusion of 250 ml D5W solution 1.5 hours later or on day 3 of the study, following administration of 7.5 mg/kg Synercid® q8h on days 1 and 2 and administration of the same dose of Synercid® 1.5 hours after cyclosporine intake on day 3 of treatment. Cyclosporine concentrations were determined using a specific radioimmunoassay.

The mean pharmacokinetic parameters are given in Table 21.

TABLE 21 : MEAN PHARMACOKINETIC PARAMETERS OF CYCLOSPORINE WITH OR WITHOUT SYNERCID®

		Cmax ($\mu g.ml^{-1}$)	Tmax (h)	AUC ($\mu g.h.ml^{-1}$)	t1/2λz (h)	CL/f ($l.h^{-1}.kg^{-1}$)	Vz/f ($l.kg^{-1}$)
Cyclosporine alone	mean	0.978	2	5.276	13.2	0.772	13.0
	SD	0.271	-	1.774	10.9	0.207	7.5
Cyclosporine with Synercid®	mean	1.302	2	8.664	21.7	0.470	13.2
	SD	0.333	-	2.382	13.0	0.146	4.5

The primary influence of RP 59500 on the pharmacokinetics of cyclosporine was to increase the AUC by a median of 63 % ; the range was from a 5 % decrease to a 222 % increase. This was accompanied by a median 34 % decrease in CL/f and a median 77 % increase in t½λz. Treatment with RP 59500 was highly significant.

a) Effect of Synercid® on Nifedipine, Midazolam [redacted] biotransformation in human liver microsomes in vitro

The effect of Synercid® on the metabolism of several CYP3A4 substrates (nifdipine, midazolam [redacted]) was studied in human liver microsomes. For all three assays, each of three substrate concentrations was incubated with a series of Synercid® concentrations. Dixon plots showed that RP 57669 / RP 54476 inhibited the oxidation of nifedipine and midazolam with a non-competitive mechanism, and inhibited the oxidation [redacted] with a competitive mechanism. The Ki values of RP 57669 / RP 54476 estimated from the Dixon plots were 8.5/28.3, 5/17 and 5/17 µM for the oxidation of nifedipine, midazolam [redacted] respectively. These Ki values are comparable to one another and are lower than the mean steady-state levels of Synercid® in humans given at a therapeutic dose of 7.5 mg/kg. The co-administration of Synercid® with other CYP3A4 substrate drugs would be expected to produce elevated drug levels due to decreased metabolism.

XI. EXCRETION

A. Excretion balance of RP 57669 (study Syn 111)

The excretion balance of RP 57669 was studied following single IV infusion of ^{14}C-labelled RP 59500 (^{14}C-RP 57669 : RP 54476, 30 : 70) at the dose of 500 mg to 6 healthy male volunteers. Elimination occurred essentially via fecal excretion, with 69 % of the administered dose eliminated by 96 hours post-dosing. The 7-day fecal excretion represented approximately 75 % of the administered dose. Approximately 15 % of the administered dose was eliminated by the urinary tract at the end of the 7-day period. The elimination was rapid, as 80 % of the urinary radioactivity was eliminated within 3 hours post-dosing. Elimination half-life of total radioactivity calculated from cumulated urinary excretion data was 33.3 ± 2.1 hours.

B. Excretion balance of RP 54476 (study Syn 112)

The excretion balance of RP 54476 was studied following single IV infusion of ^{14}C-labelled RP 59500 (RP 57669 : ^{14}C-RP 54476, 30 : 70) at the dose of 500 mg to 6 healthy male volunteers. Elimination occurred essentially via fecal excretion, with 66 % of the administered dose eliminated by 96 hours post-dosing. The 7-day fecal excretion represented approximately 77 % of the administered dose. Approximately 18.7 % of the administered dose was eliminated by the urinary tract at the end of the 7-day period. The elimination was rapid, as 79 % of the urinary radioactivity was eliminated within 3 hours post-dosing. Elimination half-life of total radioactivity calculated from cumulated urinary excretion data was 31.6 ± 5.6 hours.

XII. AT RISK POPULATIONS

Possible pharmacokinetic changes associated with the varying degrees of physiological and/or pathological modifications characterizing populations termed « at risk » were studied in elderly volunteers, in obese subjects as well as in patients with kidney and liver failure (cirrhosis). The dose of RP 59500 was 7.5 mg/kg as a single 1-hour infusion in a 250 ml infusion volume. It corresponded to that used in Phase III studies.

A. Pharmacokinetics of RP 59500 in elderly people (JRV 127)

This study was conducted as an open parallel group, single-center in 12 healthy elderly volunteers (6 males and 6 females, 69 to 74 years old) and 12 healthy young volunteers, sex and weight matched to elderly volunteers. The age of elderly volunteers ranged between 69 and 74 years of age, and the age of young volunteers ranged between 19 and 32 years of age.

TABLE 22 : MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND RP 12536 IN YOUNG AND ELDERLY VOLUNTEEERS

		Cmax ($\mu g.ml^{-1}$)	Tmax (h)	AUC(0-t) ($\mu g.h.ml^{-1}$)	AUC ($\mu g.h.ml^{-1}$)	$t_{1/2\lambda_1}$ (h)	$t_{1/2\lambda_z}$ (h)	CL ($l.h^{-1}.kg^{-1}$)	Vz ($l.kg^{-1}$)	Vss ($l.kg^{-1}$)
RP57669 young	mean	2.82	0.92*	3.22	3.27	0.12	1.14	0.72	1.20	0.45
	sd	0.52	-	0.72	0.72	0.03	0.51	0.19	0.64	0.15
RP 57669 elderly	mean	2.96	0.92*	3.39	3.44	0.13	1.05	0.66	1.01	0.40
	sd	0.42	-	0.41	0.41	0.05	0.59	0.09	0.59	0.12
test(p)		0.4789		0.4811	0.4887	0.6186	0.4700	0.5824	0.3706	0.3548
RP54476 young	mean	7.12	0.92*	7.30	7.33	0.12	0.61	0.75	0.65	0.22
	sd		-	1.46	1.47	0.04	0.15	0.17	0.19	0.05
RP54476 elderly	mean	8.16	0.92*	8.19	8.33	0.11	0.54	0.72	0.55	0.22
	sd	4.59	-	4.14	4.33	0.03	0.24	0.22	0.32	0.06
test(p)		0.7290		0.8174	0.9020	0.5431	0.4037	0.8024	0.1561	0.9802
RP12536 young	mean	1.08	0.92*	1.54	1.60	-	1.38	-	-	-
	sd	0.26	-	0.40	0.41	-	0.44	-	-	-
RP12536 elderly	mean	1.24	0.92*	1.89	2.04	-	2.87	-	-	-
	sd	0.24	-	0.42	0.44	-	1.09	-	-	-
test(p)		0.0888		0.0432	0.0190		0.0005			

* MEDIAN

RP57669 and RP54476 plasma levels declined biexponentially with mean elimination half-lives of 1.05h and 0.54h in elderly volunteers and 1.14h and 0.61h in young volunteers respectively. Plasma clearance was very high for both compounds, ranging between 0.66 to 0.75 l.h-1.kg-1. No statistically significant age effect was shown for the pharmacokinetic profiles of RP 57669 and RP 54476. There was a slight increase of AUC of RP 12536 in the elderly volunteers (by a factor of 1.2). This increase is of minor influence on the kinetics of RP 59500 as RP 12536 AUC represents about 25% of AUC of RP 54476 in the elderly volunteers.

Overall, the age effect has no influence on the pharmacokinetics of parent drugs.

B. Pharmacokinetics of RP 59500 in obese (study JRV 125)

The pharmacokinetics of a single 1-hour infusion of Synercid® 7.5 mg/kg of total body weight were studied in 12 healthy obese and 15 non-obese healthy males matched for age and height. The age of obese volunteers ranged between 25 and 65 years, and the age of normal healthy volunteers ranged between 19 and 50 years.

TABLE 23: MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND RP 12536

		Cmax ($\mu g.ml^{-1}$)	Tmax (h)	AUC(0-t ($\mu g.h.ml^{-1}$)	AUC ($\mu g.h.ml^{-1}$)	t1/2λ1 (h)	t1/2λz (h)	CL ($l.h^{-1}.kg^{-1}$)	Vz ($l.kg^{-1}$)	Vss ($l.kg^{-1}$)
RP57669	mea	2.92	0.92*	3.63	3.69					
obese	SD	0.40	-	0.51	0.49	0.18	1.07	0.62	0.97	0.49
RP57669	mea	2.32	0.92*	2.87	2.92	0.06	0.21	0.08	0.29	0.11
non-obese	SD	0.60	-	0.64	0.64	0.16	0.86	0.80	0.99	0.58
test	p	0.0266		0.0052	0.0045	0.05	0.13	0.16	0.26	0.12
RP54476	mea	9.30	0.92*	9.91	9.96	0.4216	0.0121	0.0038	0.8464	0.064
obese	SD	1.25	-	1.10	1.10	0.18	0.88	0.53	0.69	0.21
RP54476	mea	8.19	1.00*	7.59	7.34	0.05	0.38	0.07	0.35	0.03
non-obese	SD	2.03	-	2.11	1.96	0.15	0.70	0.76	0.74	0.27
test	p	0.1340		0.0039	0.0014	0.04	0.26	0.20	0.24	0.07
RP12536	mea	1.29	1.08*	1.95	2.04	0.1148	0.1674	0.0034	0.3926	0.008
obese	SD	0.35	-	0.38	0.39	-	1.52	-	-	-
RP12536	mea	0.98	1.00*	1.47	1.56	-	0.48	-	-	-
non-obese	SD	0.22	-	0.41	0.46	-	1.66	-	-	-
test	p	0.0741		0.0082	0.0149	-	0.77	-	-	-
							0.6148			

Cmax, AUC(0-t) and AUC corrected by a factor of LBW/TBW

			Cmax(N) ($\mu g.ml^{-1}$)	AUC(0-t)(N) ($\mu g.h.ml^{-1}$)	AUC(N) ($\mu g.h.ml^{-1}$)
RP57669	obese	mean±SD	2.02±0.29	2.50±0.37	2.55±0.37
	non- obese	mean± SD	1.87±0.47	2.31±0.50	2.35±0.50
	test	p*	0.3816	0.3048	0.3076
RP54476	obese	mean±SD	6.41±0.80	6.84±0.77	6.87±0.76
	non- obese	mean± SD	6.59±1.52	6.10±1.61	5.89±1.41
	test	p*	0.7258	0.1736	0.0589

N : NORMALISED (CORRECTION BY A FACTOR LBM/TBW) ;

* : PARAMETRIC ANALYSIS (T-TEST PROCEDURE)

Cmax and AUC of RP 57669 and AUC of RP 54476 were statistically significantly higher in obese subjects than in controls, which resulted from significantly lower clearances (decrease by factors of 1.29 and 1.43, respectively). When these parameters were corrected to the lean body mass, no statistically significant difference was observed between obese subjects and controls. Distribution volumes during the terminal phase of quinupristin and dalfopristin, expressed as l/kg of TBW, were comparable in the two groups, suggesting that quinupristin and dalfopristin distribute less in the fat part of the body than in the non fat body mass.

C. Pharmacokinetics of RP 59500 in patients with severe renal insufficiency (study JRV 007)

Kinetic profiles of quinupristin and dalfopristin were studied in 13 healthy male and female volunteers and 13 male and female patients with severe renal insufficiency. The age of patients ranged between 32 and 67 years, and the age of healthy volunteers ranged between 29 and 65 years. The choice of patients with severe chronic renal failure to participate in this study was justified by the low renal excretion of the two compounds observed previously in the balance of excretion studies. Mean creatinine clearances measured at the baseline were 16.69 ± 5.65 ml/min (range : 6 to 28 ml/min) in enrolled patients and 92.7 ± 30.3 ml/min (range : 53.4 to 156 ml/min) in healthy volunteers. Thirteen patients and thirteen subjects (five females and eight males in each group) were enrolled in this study and were evaluable for safety and pharmacokinetics. Healthy volunteers were age-, weight-, and sex-matched to each individual patient volunteer in order to determine the influence of renal impairment only on the pharmacokinetics of Synercid®.

The mean pharmacokinetic parameters [redacted] selective bioassay methods, are given in Table 24.

D. Pharmacokinetics of RP 59500 in patients who require continuous ambulatory peritoneal dialysis (CAPD) (study JRV 141)

Kinetic profiles of quinupristin, dalfopristin and their metabolites were studied in 8 male and female patients undergoing CAPD and in 8 healthy male and female volunteers (three females and five males in each group). Healthy volunteers were age-, weight-, and sex-matched to each individual patient volunteer. The age of patients ranged between 19 and 67 years, and the age of healthy volunteers ranged between 23 and 62 years. Infusion of Synercid in patients started as soon as the dialysate has been infused into the peritoneum. The dialysate remained in the peritoneum for 6 hours. Four 50 ml samples of dialysate were collected at 1.25, 2, 3 and 6 hours post-start of infusion. Blood samples were collected up to 10 hours following administration. Parent compounds (RP 57669 and RP 54476), in vitro active glutathione- and cysteine-conjugated metabolites of RP 57669 and the pristinamycin IIa RP 12536

The ranges of concentrations of RP 57669, RP 69012, RPR 100391, RP 54476 and RP 12536 are given in Table 25. The mean pharmacokinetic parameters, are given in Table 26.

TABLE 25 : RANGE OF CONCENTRATIONS (µG/ML) OF RP 57669, RP 69012, RPR 100391, RP 54476 AND RP 12536 IN DIALYSATE SAMPLES

Compound		Time (h) 1.25	2	3	6	Peritoneal Clearance (l/h)
RP 57669	min					
	max					
RP 69012	min					
	max					
RPR 100391	mean					
	SD					
	min					
	max					
RP 54476	min					
	max					
RP 12536	mean					
	SD					
	min					
	max					

* Concentrations under limit of quantification are reported as 0

Limits of quantification are 0.200 µg/ml for RP 57669, RP 54476 and RP 12536 and 0.100 µg/ml for RP 69012 and RPR 100391

Following IV administration, dialysis clearance was negligible for RP 57669, RP 54476 and their respective metabolites. For Synercid®, peritoneal clearance is a relatively insignificant part of total body clearance.

TABLE 26 : MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND RP 69012, RPR 100391RP 12536 FOLLOWING 1-HOUR IV INFUSION OF SYNERCID® AT 7.5 MG/KG DOSE IN PATIENTS UNDERGOING CAPD (N=8) AND IN HEALTHY VOLUNTEERS (N=8)

Compound		Cmax (µg/ml)	Tmax (h)*	AUC(0-t) µg.h/ml	AUC µg.h/ml	t½λz (h)	CL (l/h.kg)	Vz (l/kg)
RP 57669	patients	2.89 ± 0.85	1	3.34 ± 0.96	3.39 ± 0.96	0.83 ± 0.13	0.71 ± 0.20	0.85 ± 0.29
	healthy volunteers	2.60 ± 0.43	1	2.84 ± 0.37	2.90 ± 0.41	0.93 ± 0.15	0.79 ± 0.12	1.07 ± 0.27
	test (p)	0.4249	-	0.2805	0.2810	0.2258	0.3855	0.1857
RP 69012	patients	0.320 ± 0.064	1.17	0.585 ± 0.145	0.653 ± 0.203	2.39 ± 1.34	-	-
	healthy volunteers	0.405 ± 0.128	1	0.633 ± 0.233	0.722 ± 0.250	1.75 ± 0.61	-	-
	test (p)	0.1229	-	0.6309	0.6616	0.3233		
RPR 100391	patients	0.232 ± 0.079	1.25	0.563 ± 0.185	0.690 ± 0.215	2.38 ± 1.06	-	-
	healthy volunteers	0.289 ± 0.093	1.17	0.580 ± 0.229	0.656 ± 0.252	1.44 ± 0.39	-	-
	test (p)	0.2091	-	0.8763	08327	0.1732		
RP 54476	patients	8.52 ± 3.52	1	9.66 ± 4.52	9.72 ± 4.53	0.76 ± 0.29	0.67 ± 0.36	0.68 ± 0.30
	healthy volunteers	7.09 ± 2.70	1	7.51 ± 2.84	7.60 ± 2.81	0.71 ± 0.18	0.77 ± 0.30	0.77 ± 0.34
	test (p)	0.3758	-	0.2721	0.2797	0.7316	0.5750	0.5658
RP 12536	patients	0.974 ± 0.318	1	1.55 ± 0.46	1.62 ± 0.48	0.84 ± 0.39	-	-
	healthy volunteers	1.05 ± 0.29	1	1.47 ± 0.28	1.54 ± 0.28	1.15 ± 0.23	-	-
	test (p)	0.6286	-	0.6816	0.6976	0.0880		

Plasma pharmacokinetic profiles of RP 57669, RP 54476 and their metabolites in patients were similar or close to those achieved in healthy volunteers. In the light of these results, Synercid® dosage reduction seems to be unnecessary in these patients.

E. Pharmacokinetics of RP 59500 in patients with liver cirrhosis (study JRV 008)

The pharmacokinetics of a single 1-hour infusion of Synercid® 7.5 mg/kg were studied in 16 cirrhotic patients classified according to Child-Pugh score (8 with A score , cirrhosis without ascites ; 8 with B score, cirrhosis with ascites) and 17 healthy volunteers matched to patient volunteers for sex, age and weight. The age of patient volunteers ranged between 37 and 65 years, and the age of healthy volunteers ranged between 34 and 64 years.

The mean pharmacokinetic parameters [redacted] selective bioassay methods, are given in Table 27.

TABLE 27 : MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND RP 12536 FOLLOWING 1-HOUR IV INFUSION OF SYNERCID® AT 7.5 MG/KG DOSE IN HEALTHY VOLUNTEERS AND PATIENTS WITH HEPATIC INSUFFICIENCY

RP57669 (mean ± SD)									
	Cmax (µg/ml)	Tmax (h)*	AUC(0-t) µg.h/ml	AUC µg.h/ml	t1/2l1 (h)	t½λz (h)	CL (l/h.kg)	Vz (l/kg)	Vss (l/kg)
patients	3.16 ± 0.85	0.92 -	3.44 ± 0.69	3.50 ± 0.69	0.12 ± 0.04	0.91 ± 0.37	0.67 ± 0.16	0.85 ± 0.27	0.40 ± 0.12
healthy volunteers	2.72 ± 0.67	0.92 -	2.84 ± 0.71	2.90 ± 0.72	0.13 ± 0.05	0.91 ± 0.37	0.82 ± 0.20	1.04 ± 0.36	0.44 ± 0.14
test (p)	0.1120	-	0.0305	0.0290	0.4094	0.8958	0.0194	0.1170	0.4533
RP54476 (mean ± SD)									
	Cmax (µg/ml)	Tmax (h)*	AUC(0-t) µg.h/ml	AUC µg.h/ml	t1/2l1 (h)	t½λz (h)	CL (l/h.kg)	Vz (l/kg)	Vss (l/kg)
patients	7.34 ± 2.65	0.92 -	7.37 ± 2.98	7.36 ± 3.21	0.10 ± 0.04	0.69 ± 0.63	0.86 ± 0.40	0.87 ± 0.92	0.29 ± 0.24
healthy volunteers	7.24 ± 2.28	0.50 -	7.30 ± 2.04	7.36 ± 2.04	0.11 ± 0.04	0.45 ± 0.40	0.77 ± 0.21	0.45 ± 0.35	0.19 ± 0.06
test (p)	0.9153	-	0.9459	0.9992	0.4290	0.1956	0.4742	0.2828	0.4633

RP12536 (mean ± SD)					
	Cmax (µg.ml-1)	Tmax (h)*	AUC(0-t) (µg.h.ml-1)	AUC (µg.h.ml-1)	t½λz (h)
patients	1.35 ± 0.41	0.92 -	2.14 ± 0.60	2.23 ± 0.63	2.00 ± 1.27
healthy volunteers	1.27 ± 0.69	0.92 -	1.54 ± 0.48	1.62 ± 0.51	1.69 ± 0.91
test (p)	0.6810	-	0.0086	0.0113	0.7648

RP57669 (1) (mean ± SD)						
Selective bioa	Cmax (µg.ml-1)	Tmax (h)*	AUC(0-t) (µg.h.ml-1	AUC (µg.h.ml-1	t½λ1 (h)	t½λz (h)
patients	4.33 ± 0.90	0.92 -	10.48 ± 3.27	11.39 ± 3.53	0.23 ± 0.08	3.03 ± 1.01
healthy volunteers	2.97 ± 0.66	0.92 -	3.67 ± 0.85	4.00 ± 0.96	0.14 ± 0.07	1.08 ± 0.30
test (p)	0.0004	-	0.0002	0.0002	0.0078	0.0003
RP 54476 (2) (mean ± SD)						
Selective bioa	Cmax (µg.ml-1)	Tmax (h)*	AUC(0-t) (µg.h.ml-1	AUC (µg.h.ml-1	t½λ1 (h)	t½λz (h)
patients	9.46 ± 1.90	0.92 -	13.16 ± 3.41	13.67 ± 3.47	0.17 ± 0.02	1.91 ± 0.57
healthy volunteers	7.86 ± 1.72	0.92 -	9.05 ± 2.01	9.33 ± 2.05	0.13 ± 0.04	1.00 ± 0.26
test (p)	0.0490	-	0.0070	0.0056	0.0138	0.0001

SD = Standard Deviation ; (1) : RP 57669 and its derivatives ; (2) : RP 54476 and its derivatives.
* : median

The disposition profiles of unchanged RP 57669 and RP 54476 were comparable in patients with hepatic insufficiency and in healthy volunteers. However, the clearances of RP 57669 and RP 54476 derivatives in patients were markedly impaired, as suggested by the bioassay data : the mean AUCs of RP 57669 and RP 54476 in combination with their respective derivatives (determined by selective bioassays) were about 2.8 and 1.5 times higher than those estimated in healthy subjects, respectively. When comparing mean AUC values selective bioassays, the active derivatives of RP 57669 and RP 54476, taken into account by the bioassays, represented about 38 %

and 27 % of unchanged drugs in healthy volunteers and 225 % and 86 % of unchanged drugs in patients with hepatic insufficiency.

These results are in agreement with the fact that unchanged RP 57669 and RP 54476 are cleared by non-enzymatic processes. It is likely that hepatic/biliary clearances of the active derivatives taken into account by selective bioassays, are significantly reduced.

In the light of these results, and from a pharmacokinetic point of view, a modification of the dosage regimen in patients with hepatic insufficiency could be all the more recommended as, in healthy volunteers, following a q12h regimen, mean AUC(0-t)s of derivatives at steady-state were significantly higher than AUCs on day 1.

XIII. JAPAN STUDIES

A. Single dose study (Japanese study JRV 129)

The pharmacokinetics of the two components RP 57669 and RP 54476 of Synercid were determined in a single dose, placebo-controlled ascending dose study performed in 32 healthy volunteers. Four groups of 8 subjects received a single dose of RP 59500 (5, 7.5, 10 or 12.5 mg/kg) as an one-hour IV infusion. Plasma levels of RP 57669, RP 54476 and the metabolite RP 12536 [redacted] compared to those obtained by global microbiological assay (taking into account both compounds RP 57669 and RP 54476) and selective microbiological assays allowing the determination of unchanged drugs in combination with their derivatives. The urinary and fecal excretions of RP 59500, RP 57669 and RP 54476 were measured by global and selective bioassays (Tables 31 and 32). Mean pharmacokinetic parameters of RP 57669, RP 54476 and RP 12536 are summarized in Tables 28 to 30.

BLE 28 : MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND RP 12536 [redacted]

Compound	dose mg/kg	Tmax h	Cmax µg/ml	AUC(0-t) µg.h/ml	AUC µg.h/ml	t½λ1 h	t½λz h	CL l/h.kg	Vz l/kg	Vss l/kg
RP 57669	5	1.00*	1.87 ± 0.20	2.12 ± 0.24	2.17 ± 0.26	0.13 ± 0.05	0.83 ± 0.23	0.70 ± 0.08	0.82 ± 0.17	0.42 ± 0.08
	7,5	1.00*	3.08 ± 0.60	3.44 ± 0.64	3.49 ± 0.64	0.15 ± 0.07	0.93 ± 0.24	0.67 ± 0.16	0.92 ± 0.44	0.49 ± 0.30
	10	1.00*	4.46 ± 0.85	5.10 ± 0.87	5.15 ± 0.88	0.12 ± 0.02	0.90 ± 0.11	0.60 ± 0.10	0.76 ± 0.09	0.38 ± 0.08
	12,5	1.00*	5.47 ± 1.25	6.56 ± 1.00	6.62 ± 0.99	0.14 ± 0.02	0.89 ± 0.05	0.58 ± 0.09	0.74 ± 0.09	0.39 ± 0.10
RP 54476	5	0.75*	4.96 ± 0.33	5.07 ± 0.53	5.10 ± 0.52	0.11 ± 0.04	0.60 ± 0.24	0.69 ± 0.06	0.61 ± 0.25	0.21 ± 0.03
	7,5	1.00*	7.17 ± 3.80	7.13 ± 3.34	7.16 ± 3.34	0.13 ± 0.03	0.56 ± 0.13	0.83 ± 0.26	0.65 ± 0.22	0.25 ± 0.09
	10	0.50*	9.13 ± 2.08	9.98 ± 2.07	10.04 ± 2.06	0.17 ± 0.04	0.97 ± 0.24	0.72 ± 0.15	1.06 ± 0.47	0.26 ± 0.09
	12,5	0.75*	11.66 ± 2.96	13.26 ± 3.25	13.32 ± 3.26	0.20 ± 0.05	0.99 ± 0.17	0.69 ± 0.14	0.97 ± 0.23	0.27 ± 0.06
RP 12536	5	1.00*	0.78 ± 0.13	1.08 ± 0.13	1.12 ± 0.13	-	1.00 ± 0.24	-	-	-
	7,5	1.00*	1.18 ± 0.62	1.52 ± 0.52	1.58 ± 0.52	-	1.18 ± 0.35	-	-	-
	10	1.00*	1.65 ± 0.27	2.68 ± 0.52	2.78 ± 0.51	-	1.32 ± 0.17	-	-	-
	12,5	1.04*	2.17 ± 0.50	3.78 ± 0.69	3.93 ± 0.72	-	1.27 ± 0.12	-	-	-

TABLE 29: MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND RP 59500 - SELECTIVE AND GLOBAL BIOASSAY

Compound	dose mg/k	Tmax h	Cmax µg/ml	AUC(0-t) µg.h/ml	AUC µg.h/ml	t½λz h
RP 59500 (global Bioassay)	5	1.00*	5.16 ± 0.75	6.30 ± 0.72	6.58 ± 0.70	0.93 ± 0.21
	7,5	1.00*	7.10 ± 0.72	8.77 ± 1.38	9.08 ± 1.39	1.11 ± 0.09
	10	1.00*	10.77 ± 1.70	14.81 ± 1.53	15.18 ± 1.54	1.24 ± 0.07
	12,5	1.00*	15.00 ± 3.05	19.96 ± 2.47	20.43 ± 2.52	1.21 ± 0.06
RP 57669 (selective Bioassay)	5	1.00*	1.67 ± 0.11	1.88 ± 0.13	- -	1.13 ± 0.31
	7,5	1.00*	2.33 ± 0.21	2.98 ± 0.29	3.19 ± 0.31	1.31 ± 0.32
	10	1.00*	3.93 ± 0.33	5.43 ± 1.11	6.19 ± 1.21	1.37 ± 0.14
	12,5	1.00*	4.63 ± 1.52	6.85 ± 1.16	7.18 ± 1.13	1.36 ± 0.12
RP 54476 (selective Bioassay)	5	1.00*	3.01 ± 0.49	3.35 ± 0.69	3.47 ± 0.71	0.61 ± 0.06
	7,5	1.00*	4.14 ± 0.56	4.86 ± 0.91	5.02 ± 0.92	0.90 ± 0.13
	10	1.00*	6.30 ± 0.92	7.88 ± 1.01	8.08 ± 1.03	1.10 ± 0.12
	12,5	1.00*	9.54 ± 3.17	11.77 ± 2.35	11.97 ± 2.39	1.09 ± 0.09

TABLE 30 : MEAN ± SD CUMULATIVE URINARY EXCRETION (0-24H)

Dose	Global bioassay	Selective bioassays	
	RP 59500	RP 57669	RP 54476
5 mg/kg	49.4 ± 6.5 mg	21.0 ± 4.8% 19.8 ± 4.2 mg	10.8 ± 1.1% 23.9 ± 2.3 mg
7.5 mg/kg	88.7 ± 21.6 mg	28.9 ± 2.9% 40.0 ± 6.9 mg	15.3 ± 4.0% 49.7 ± 15.6 mg
10 mg/kg	136 ± 21.7 mg	26.8 ± 4.1% 59.3 ± 9.7 mg	14.7 ± 1.5% 76.3 ± 12.2 mg
12.5 mg/kg	194 ± 46.7 mg	35.1 ± 8.7% 83.5 ± 23.3 mg	17.2 ± 3.1% 95.7 ± 21.8 mg

Results are expressed in % of total administered dose and in mg

TABLE 31 : MEAN (±SD) FECAL CUMULATIVE EXCRETION (0-48H)

Dose	Global bioassa	Selective bioassays	
	RP 59500	RP 57669	RP 54476
7.5 mg/kg	8.2 ± 4.3 mg	8.6 ± 4.5% 12.0 ± 6.6 mg	0. 7± 0.3% 2.3 ± 1.24 mg
10 mg/kg	10.9 ± 6.7 mg	5.5 ± 4.3% 12.9 ± 8.0 mg	1.1 ± 1.2% 3.5 ± 2.1 mg
12.5 mg/k	4.4 ± 3.8 mg	2.6 ± 2.2 % 6.3 ± 5.3 mg	0.2 ± 0.2% 1.4 ± 1.2 mg

Results are expressed in % of total administered dose and in mg

Following IV administration of Synercid® at the doses of 5, 7.5, 10 and 12.5 mg/kg, pharmacokinetic profiles of RP 57669, RP 54476 declined biexponentially. Total plasma clearances of both compounds were higher than 0.58 l/h.kg.

[redacted] C_{max} and AUC values of RP 57669, RP 54476 and RP 12536 increased practically proportionally to the dose. Clearances were not modified with the dose. The distribution volume of RP 57669 was not modified with the dose, but that of RP 54476 observed following administration of 10 and 12.5 mg/kg doses was about 1.7 times higher than that obtained after administration of 5 and 7.5 mg/kg doses, resulting from higher elimination half-lives calculated from plasma concentrations estimated at different time ranges (3 to 5 hours following administration of 5 and 7.5 mg/kg and 4 to 6 hours following administration of 10 and 12.5 mg/kg dose).

When measured by global and selective bioassays, C_{max}, AUC(0-t) and AUC of RP 59500, RP 57669 and RP 54476 increased with the dose. This increase was linear for AUC of RP 59500 and RP 54476. The disposition profile of RP 59500, RP 57669 and RP 54476 did not seem to be influenced by the dose.

The mean cumulative urinary excretions of RP 57669 and RP 54476, as measured by the global and selective bioassay were in the range [redacted] respectively.

The mean cumulative fecal excretions of RP 57669 and RP 54476 expressed in % of the administered dose were in the range [redacted] RP 57669 [redacted] RP 54476.

B. Repeated dose study (Japanese study JRV 130)

This study was conducted as an single-blind randomized placebo-controlled single-center trial in 16 healthy male volunteers between 20 and 38 years of age. Two separate parallel groups of healthy volunteers were planned. Group 1 received 9 infusions of 7.5 mg/kg of RP 59500 (6 subjects) or placebo (2 subjects) q12h for 5 days as 1-hr I.V. infusion in a volume of 250 ml, Group 2 received 7 infusions of 7.5 mg/kg of RP 59500 (6 subjects) or placebo (2 subjects) q8h for 3 days as 1-hr I.V. infusion in a volume of 300 ml. Plasma levels of RP 57669, RP 54476 and the metabolite RP 12536 [redacted] compared to those obtained by global microbiological assay (taking into account both compounds RP 57669 and RP 54476) and selective microbiological assays allowing the determination of unchanged drugs in combination with their derivatives. The urinary and fecal excretions of RP 59500, RP 57669 and RP 54476 were measured by global and selective bioassays. Mean pharmacokinetic parameters of RP 57669, RP 54476 and RP 12536 are summarized in Tables 32 and 33. Mean cumulative urinary recovery amounts (expressed as mg equivalents of the analyte) and fecal excretion are summarized in Tables 34 and 35, respectively.

TABLE 32 : MEAN PHARMACOKINETIC PARAMETERS OF RP 57669, RP 54476 AND RP 12536 FOLLOWING 1-HOUR IV INFUSION OF RP 59500 AT 7.5 MG/KG DOSE Q12H OR Q8H

q8h group (n=6 on day 1 n= 5 on day 3, except for RP 12536 n=4 on day 3)

compound	Day	Cmax µg/ml	Tmax h	AUC(0-t) µg.h/ml	AUC µg.h/ml	$t_{1/2l_1}$ h	$t_{1/2\lambda z}$ h	CL l/h.kg	Vz l/kg	Vss l/kg
RP 57669	D1	2.018 ± 0.282	1	2.39 ± 0.36	2.45 ± 0.36	0.12 ± 0.03	0.90 ± 0.08	0.93 ± 0.14	1.21 ± 0.08	0.68 ± 0.10
	D3	2.256 ± 0.247	1	2.90 ± 0.34	3.00 ± 0.37	0.11 ± 0.05	1.10 ± 0.37	0.76 ± 0.09	1.21 ± 0.49	0.65 ± 0.15
RP 54476	D1	6.559 ± 2.257	1	6.71 ± 2.39	6.75 ± 2.38	0.12 ± 0.05	0.61 ± 0.30	0.87 ± 0.32	0.82 ± 0.63	0.30 ± 0.14
	D3	6.829 ± 1.702	0.5	7.24 ± 1.19	7.27 ± 1.20	0.09 ± 0.04	0.72 ± 0.26	0.74 ± 0.12	0.74 ± 0.19	0.26 ± 0.05
RP 12536	D1	0.952 ± 0.194	1	1.46 ± 0.39	1.56 ± 0.42	-	1.65 ± 0.68	-	-	-
	D3	1.050 ± 0.238	1	1.89 ± 0.42	2.07 ± 0.49	-	1.87 ± 0.54	-	-	-

* : median

q12h group (n=6 on day 1 and n=5 on day 5)

	Day	Cmax µg/ml	Tmax h	AUC(0-t) µg.h/ml	AUC µg.h/ml	$t_{1/2l_1}$ h	$t_{1/2\lambda z}$ h	CL l/h.kg	Vz l/kg	Vss l/kg
RP 57669	D1	2.910 ± 1.078	1	3.38 ± 1.16	3.44 ± 1.16	0.13 ± 0.06	1.10 ± 0.18	0.71 ± 0.22	1.11 ± 0.33	0.49 ± 0.12
	D5	3.102 ± 0.819	1	3.88 ± 1.13	3.95 ± 1.12	0.18 ± 0.04	1.07 ± 0.17	0.61 ± 0.16	0.95 ± 0.36	0.47 ± 0.17
RP 54476	D1	8.056 ± 2.005	0.5	7.94 ± 2.05	7.97 ± 2.05	0.10 ± 0.05	0.54 ± 0.11	0.69 ± 0.17	0.54 ± 0.14	0.18 ± 0.05
	D5	9.281 ± 2.169	1	9.12 ± 2.49	9.17 ± 2.49	0.14 ± 0.06	1.00 ± 0.50	0.62 ± 0.21	0.83 ± 0.40	0.23 ± 0.06
RP 12536	D1	1.398 ± 0.390	1	2.01 ± 0.58	2.13 ± 0.62	-	2.04 ± 0.58	-	-	-
	D5	2.010 ± 0.479	1	2.60 ± 0.43	2.79 ± 0.43	-	1.85 ± 0.60	-	-	-

* : MEDIAN

TABLE 33 : MEAN PHARMACOKINETIC PARAMETERS OF RP 59500 (GLOBAL BIOASSAY) AND RP 57669 AND RP 54476 IN COMBINATION WITH THEIR DERIVATIVES (SELECTIVE BIOASSAYS) FOLLOWING 1-HOUR IV INFUSION OF RP 59500 AT 7.5 MG/KG DOSE Q12H OR Q8H

with calibration standards added to acidified plasma

q8h group (n=6 on day 1 and n=5 on day 3)

compound	Day	Cmax µg/ml	Tmax * h	AUC(0-t) µg.h/ml	AUC µg.h/ml	t½λz h
RP 59500	D1	8.72 ± 0.32	1	11.0 ± 0.8	11.4 ± 0.8	1.55 ± 0.39
	D3	10.1 ± 1.0	1	14.6 ± 1.6	15.3 ± 1.8	1.70 ± 0.08
RP 57669	D1	2.38 ± 0.27	1	3.06 ± 0.34	3.38 ± 0.40	1.08 ± 0.21
	D3	2.59 ± 0.11	1	4.57 ± 0.66	4.92 ± 0.69	1.52 ± 0.24
RP 54476	D1	5.51 ± 0.86	1	6.23 ± 0.91	6.39 ± 0.90	0.96 ± 0.12
	D3	5.85 ± 0.76	1	7.82 ± 1.03	8.12 ± 1.03	1.74 ± 0.11

* : median

q12h group (n=6 on day 1 and n=5 on day 5)

compound	Day	Cmax µg/ml	Tmax * h	AUC(0-t) µg.h/ml	AUC µg.h/ml	t½λz h
RP 59500	D1	10.9 ± 2.9	1	14.0 ± 3.9	14.3 ± 4.0	1.28 ± 0.23
	D5	13.2 ± 2.6	1	18.2 ± 3.4	18.9 ± 3.4	1.69 ± 0.15
RP 57669	D1	3.02 ± 0.91	1	3.77 ± 1.40	4.81 ± 1.71	1.17 ± 0.15
	D5	4.64 ± 0.63	1	6.83 ± 0.90	7.21 ± 0.97	1.43 ± 0.25
RP 54476	D1	6.72 ± 2.10	1	8.03 ± 2.40	8.24 ± 2.45	0.94 ± 0.17
	D5	7.43 ± 1.82	1	9.54 ± 2.60	9.94 ± 2.72	2.06 ± 0.42

* : MEDIAN

with calibration standards added to whole blood

q8h group (n=6 on day 1 and n=5 on day 3)

compound	Day	Cmax µg/ml	Tmax * h	AUC(0-t) µg.h/ml	AUC µg.h/ml	t½λz h
RP 59500	D1	6.95 ± 0.36	1	8.69 ± 0.65	9.06 ± 0.68	1.29 ± 0.22
	D3	7.87 ± 0.72	1	11.6 ± 1.3	12.1 ± 1.4	1.80 ± 0.13
RP 57669	D1	2.12 ± 0.22	1	2.66 ± 0.31	2.96 ± 0.47	1.13 ± 0.22
	D3	2.38 ± 0.17	1	4.00 ± 0.58	4.32 ± 0.61	1.38 ± 0.26
RP 54476	D1	3.86 ± 0.48	1	4.26 ± 0.5	4.41 ± 0.57	0.75 ± 0.10
	D3	4.14 ± 0.61	1	5.33 ± 0.84	5.54 ± 0.87	1.25 ± 0.37

* : median

q12h group (n=6 on day 1 and n=5 on day 5)

compound	Day	Cmax µg/ml	Tmax * h	AUC(0-t) µg.h/ml	AUC µg.h/ml	t½λz h
RP 59500	D1	8.48 ± 1.90	1	11.0 ± 3.0	11.3 ± 3.0	1.08 ± 0.16
	D5	9.96 ± 2.05	1	13.5 ± 2.5	13.9 ± 2.6	1.38 ± 0.17
RP 57669	D1	2.64 ± 0.84	1	3.37 ± 1.33	4.08 ± 1.40	1.14 ± 0.08
	D5	3.48 ± 0.46	1	4.98 ± 0.80	5.27 ± 0.82	1.38 ± 0.24
RP 54476	D1	4.64 ± 1.43	1	5.49 ± 1.68	5.65 ± 1.69	0.84 ± 0.18
	D5	4.59 ± 1.62	1	5.78 ± 1.92	5.94 ± 1.93	0.96 ± 0.15

* : median

Mean ratios (± SD) of Cmax (day 3/5) / Cmax (day 1) (r1) and of AUC(0-t) (day 3/5) / AUC (day 1) (r2) selective bioassays (with calibration standards added to acidified plasma)

analytical meth	compound	group	r1 = Cmax D3/5 / Cmax D1	r2=AUC(0-t) D3/5 / AUC D1
[redacted]	RP 57669	q8h	1.14 ± 0.15	1.22 ± 0.09
		q12h	1.03 ± 0.31	1.06 ± 0.21
	RP 54476	q8h	1.00 ± 0.22	1.06 ± 0.29
		q12h	1.14 ± 0.20	1.12 ± 0.22
	RP 12536	q8h	1.18 ± 0.26	1.21 ± 0.17
	RP 57669	q12h	1.38 ± 0.52	1.12 ± 0.16
		q8h	1.13 ± 0.17	1.34 ± 0.15
[redacted]		q12h	1.51 ± 0.27	1.57 ± 0.32
	RP 54476	q8h	1.06 ± 0.21	1.21 ± 0.19
		q12h	1.04 ± 0.10	1.07 ± 0.10

TABLE 34 : MEAN CUMULATIVE URINARY RECOVERY AMOUNTS (EXPRESSED AS MG EQUIVALENTS OF THE ANALYTE)

q8h group (n=6 on day 1 and n=5 on days 2 and 3)					
compound (dose, mg/kg)	day 1 (0 - 8 h)		day 2 (0 - 24 h)		day 3 (0 - 24 h)
RP 57669 (2.25 mg/kg)	25.8 ± 5.8		110 ± 14		37.4 ± 7.3
RP 54476 (5.25 mg/kg)	47.3 ± 8.2		187 ± 19		58.1 ± 7.2
q12h group (n=6 on days 1, 2 and 3 ; n=5 on days 4 and 5)					
compound	day 1 (0 - 8 h)	day 2 (0 - 24 h)	day 3 (0 - 24 h	day 4 (0 - 24 h	day 5 (0 - 24 h
RP 57669	52.4 ± 14.9	120 ± 39	113 ± 29	137 ± 22	78.4 ± 16.1
RP 54476	36.4 ± 10.4	61.7 ± 18.2	98.4 ± 25.2	122 ± 25	50.2 ± 9.7

TABLE 35 : MEAN CUMULATIVE FECAL EXCRETION (EXPRESSED AS MG QUIVALENTS)

q8h group (n=6)				
compound	day 2 (0 - 24 h)	day 4 (0 - 24 h)	. 1 week after (0 - 24 h)	3 weeks after (0-24 h)
RP 57669	11.9 ± 9.7	19.6 ± 11.3	0.043 ± 0.097	0.000
RP 54476	4.65 ± 4.31	7.95 ± 6.00	0.000	0.000
q12h group (n=5)				
compound	day 3	day 6	1 week after	3 weeks after
RP 57669	3.18 ± 2.44	15.3 ± 14.5	1.32 ± 1.80	0.088 ± 0.148
RP 54476	0.613 ± 0.576	3.59 ± 4.00	0.019 ± 0.047	0.000

Plasma concentrations of RP 57669 and RP 54476 declined biexponentially with mean distribution half-lives, estimated after the first administration, of about 0.12 hour for both compounds and mean elimination half-lives of about 1.0 and 0.6 hour, respectively. Total plasma clearances for both compounds were very high (higher than 0.69 l/h.kg). Peak plasma level of the metabolite of RP 54476 (RP 12536) was reached practically at the same time as parent compound (Tmax range : 1.00 h to 1.08 h).

Mean area under the plasma concentration-time curve of RP 12536 corresponds to about 20 % of that of RP 54476. Apparent elimination half-life of RP 12536 was about 1.8 hours.

Regimen difference, due to a subject effect, was observed between pharmacokinetic parameter values [redacted] bioassay. In the same way, for the same reasons, on the last day of treatment, the difference between the two regimens remained stable suggesting that q8h and q12h administrations lead to the same disposition profile after repeated administration.

Slight increases of Cmax and AUC(0-t) values of unchanged RP 57669 and RP 54476 (increase range : 8 to 21 % for AUC(0-t)s), [redacted] were observed between day 1 and day 3 or 5, which resulted from slight decreases of clearance. Slightly more pronounced increases of Cmax and AUC(0-t) values of RP 57669 and RP 54476 in combination with their respective derivatives, estimated by selective bioassays, were observed between day 1 and day 3 or 5. These increases were observed rapidly after the beginning of the treatments, but could not be predicted by the high elimination rates (short half-lives) of all drug-related components. Moreover, following both dosage regimens, pre-drug levels (for parent drugs and metabolites) were below or close to the limits of quantitation [redacted]

The kinetic profiles of RP 57669, RP 54476 and RP 12536 in Japanese healthy volunteers, are similar to those estimated in Caucasian healthy volunteers.

XIV. CONCLUSIONS

A. Conclusions on the studies in healthy volunteers

Pharmacokinetic profiles of RP 57669 and RP 54476 were determined in young healthy volunteers after administration of 1-hour intravenous infusion of single and repeated administrations. Main pharmacokinetic characteristics are as follows:

- Plasma concentrations of RP 57669 and RP 54476 declined biexponentially with mean distribution half-lives of 0.10 to 0.15 hour for both compounds and mean elimination half-lives of 0.75 to 1.26 hours for RP 57669 and 0.45 to 1.15 hours for RP 54476.

- RP 57669 and RP 54476 are characterised by moderate distribution volumes (ranges of mean values were 0.92 to 1.54 l/kg for RP 57669 and 0.56 to 1.45 l/kg for RP 54476) and high total plasma clearances (ranges of mean values were 0.71 to 0.93 l/kg for RP 57669 and 0.74 to 0.93 l/kg for RP 54476). These characteristics account for the short elimination half-lives, around 1 h.

Binding to plasma proteins in vivo ranges from 23 to 32 % for RP 57669 derived products and 50 to 59 % for RP 54476 derived products. In vitro human plasma protein binding ranges from 55 to 78 % for RP 57669 and from 11 to 26 % for RP 54476. These results were obtained simultaneously with a partial degradation of the unchanged compounds. The binding of RP 57669-related radioactivity to human serum albumin increased when the time of incubation increased, simultaneously to the degradation of RP 57669, suggesting that the degradation products may have greater affinity for HSA than the parent compound.

- The pharmacokinetics of RP 57669 was dose-independent. Cmax and AUC increased proportionally to the dose. The pharmacokinetics of RP 54476 were dose-dependent, with smaller than expected increases in Cmax and AUC with increases in dose.

However, the sum of AUCs for RP 54476 and RP 12536 (expressed as RP 54476 equivalents) increased proportionally with dose; this was not observed with Cmax.

- AUC(0-t) values of unchanged RP 57669 and RP 54476 in blister fluid corresponded to about 19 % and 11 % of those estimated in plasma, respectively. However, the relative bioavailabilities of RP 57669 and RP 54476 and their metabolites in blister fluid, compared to that in plasma, were about 40 %.

- The main in vitro biotransformation of RP 57669 is not enzyme-catalyzed and no important interspecies differences in the fate of these compounds were detected. The main metabolic pathway for RP 57669 appeared to be a non-enzymatic glutathione-conjugation leading to RP 69012. Another metabolite of RP 57669, possibly derived from the gluthathione-conjugate RP 69012 is RPR 100391 (cysteine conjugate of RP 57669). In plasma, the sum of AUC values of these two metabolites represent about 40 % of AUC of RP 57669 on day 1 and 65 % on last day of treatment.

- For RP 54476, the major in vitro transformation pathway in all species is the non-enzymatic chemical reaction to RP 12536 or Pristinamycin IIA, and subsequently non-enzymatic glutathione-conjugation. In plasma, AUC values of RP 12536 represents about 22 % of AUC of RP 54476 on day 1 and 26 % on last day of treatment.

RP 57669/RP 54476 was a potent inhibitor of cyclosporine A metabolism in liver microsomes with an IC50 of 2.1 ± 1.5/7.1 ± 4.9 µM. Both components of RP 57669/RP54476 were inhibitors of CsA metabolism , with an IC50 of 9.8 ± 7.5 µM for RP 57669 and 5.7 ± 1.5 µM for RP 54476.

- Excretion of total radioactivity following administration of ^{14}C-RP 57669, RP 54476 (30 : 70) or RP 57669, ^{14}C-RP 54476 (30 : 70) are mainly fecal, whereas urinary excretions represent around 15 and 19 %, respectively.

- after repeated administration of 7.5 mg/kg b.i.d. or t.i.d., moderate increases of Cmax and AUC(0-t) values of unchanged RP 57669 and RP 54476 were observed between day 1 and day 4 or 5, which resulted from a reduced drug clearance. This phenomenon is more pronounced for the metabolite cysteine conjugate RPR 100391 and to a lesser extent for the metabolites RP 69012 and RP 12536 than for the unchanged compounds. As a consequence, increases of Cmax and AUC(0-t) values of RP 57669 and RP 54476 in combination with their derivatives, estimated by selective bioassays, between day 1 and day 4 or 5, were more important than those estimated for unchanged compounds. These increases were observed rapidly after the beginning of the treatments, but could not be predicted by the high elimination rates (short half-lives) of all drug-related components. Moreover, following both dosage regimens, pre-dose levels (for parent drugs and metabolites) were below or close to the limits of quantitation

B. Conclusions on the studies in populations at risks

The results of the pharmacokinetic studies in elderly volunteers, in patients with liver cirrhosis and in patients with renal insufficiency have shown no modification of the kinetic profiles of unchanged RP 57669 and RP 54476 following single dose administration. This finding is in agreement with the fact that unchanged RP 57669 and RP 54476 are cleared by non-enzymatic processes. However, the clearances of RP 57669 and RP 54476 derivatives in patients were impaired, as suggested by the bioassay data : the mean AUCs of RP 57669 and RP 54476 in combination with their respective derivatives (determined by selective bioassays) were about 2.8 and 1.5 times higher in patients with liver cirrhosis than those estimated in healthy subjects, respectively, and about 1.38 and 1.3 times higher in patients with renal insufficiency than those estimated in healthy subjects, respectively. In elderly volunteers, AUC of RP 57669 and RP 54476 were similar to those estimated in young subjects and AUC of the metabolite RP 12536 was about 1.3 times higher in elderly volunteers than in young subjects.

Following single intravenous 1-hour infusion in patients undergoing CAPD, dialysis clearance was negligible for RP 57669, RP 54476 and their respective metabolites. Peritoneal clearance is a relatively insignificant part of total body clearance.

No statistically significant gender effect was observed on all the pharmacokinetic parameters estimated in this study.

C. Conclusions on the interaction study with cyclosporine

The primary influence of RP 59500 on the pharmacokinetics of cyclosporine was to increase the AUC by a median of 63%; the range was from a 5% decrease to a 222% increase. This was accompanied by a median 34% decrease in CL/f and a median 77% increase in $t_{½\lambda z}$. Multiple doses of RP 59500

significantly increase the AUC of cyclosporine given a single 300mg dose to healthy volunteers. Considering the toxicity associated with cyclosporine, a reduction in cyclosporine is indicated when RP 59500 and cyclosporine are combined, however considering the variability of this interaction, serum concentration monitoring of cyclosporine will be necessary.

XV. OVERALL CONCLUSIONS ON THE PHARMACOKINETIC STUDIES IN MAN

The results provided in the present report enable describing RP 57669 and RP 54476 pharmacokinetic profiles in man in both normal and pathological situations.

RP 57669 and RP 54476 were found to be unstable in citrated human blood at room temperature, requiring specific conditions for blood sample collection. On the basis of all the results obtained, it may be concluded that pharmacokinetics of RP 57669 and RP 54476 associated to its active metabolite RP 12536 are linear for the dose range studied. In young healthy volunteers, moderate increases in plasma levels have been observed in the course of repeated administration of 7.5 mg/kg b.i.d. or t.i.d.. They are more pronounced for the metabolite cysteine conjugate RPR 100391 and to a lesser extent for the metabolites RP 69012 and RP 12536 than for the unchanged compounds. These increases were observed rapidly after the beginning of the treatments, but could not be predicted by the high elimination rates (short half-lives) of all drug-related components. Moreover, following both dosage regimens, pre-drug levels (for parent drugs and metabolites) were below or close to the limits of quantitation. Under these administration conditions, steady-state was achieved by day 2 of treatment for both dosage regimens, with plasma peaks of around 2.7, 7.0 and 1.1 µg/ml for RP 57669, RP 54476 and RP 12536, obtained at the end of the 1-hour infusion. Plasma peaks of RP 57669 and RP 54476 in combination with their respective derivatives, determined by selective bioassays, were 3.1 and 8.2 µg/ml, respectively.

Bioavailability of unchanged RP 57669 and RP 54476 in blister fluid corresponded to about 19 % and 11 % of those estimated in plasma, respectively. That of RP 57669 and RP 54476 with their metabolites in blister fluid, compared to that in plasma, was about 40 %. In vitro plasma protein binding ranged from 55 to 78 % for RP 57669 and from 11 to 26 % for RP 54476, but these results were obtained simultaneously with a partial degradation of the unchanged compounds. The binding of RP 57669-related radioactivity to human serum albumin increased in parallel with the amount of degradation products present in the incubation medium.

The biotransformations of RP 57669 and RP 54476 resulted essentially from non-enzymatic chemical reactions. RP 57669 was transformed into RP 69012 and RPR 100391, which were found as active as parent compound in vitro. RP 54476 was transformed into active RP 12536 or natural Pristinamycin IIA, and subsequently extensively metabolized in unidentified drug-derived products. RP 57669 and RP 54476 were cleared rapidly from the plasma, with a plasma clearance of about 0.7 l/h.kg. The elimination plasma half-lives were around 1 hour for both compounds. Excretion was essentially fecal.

The pharmacokinetic consequences of the different degrees of physiological and/or pathological modifications characterizing populations considered at risk were studied in elderly volunteers, obese subjects and in patients with kidney and liver failure (cirrhosis). The degree of change in the pharmacokinetic profile characteristic of each of these populations was analysed by comparing their profile to that of a reference group of healthy volunteers sex-, age- and weight-matched to patient

volunteers. The result of these pharmacokinetic studies show that the disposition profiles of unchanged RP 57669 and RP 54476 were comparable in patients and in healthy volunteers, but the clearances of RP 57669 and RP 54476 derivatives in patients were moderately impaired in patients with renal insufficiency and markedly impaired in patients with liver failure. Dialysis clearance was negligible for RP 57669, RP 54476 and their respective metabolites in patients undergoing CAPD.

Synercid® does not alter the binding of warfarin in vitro to serum human protein. Synercid® affects cyclosporine kinetics, inhibiting strongly the CYP3A dependent cyclosporine metabolism in human liver microsomes. Synercid® inhibits the oxidation of nifedipine, midazolam [redacted] in human liver microsomes, suggesting that the co-administration of Synercid® with other CYP3A4 substrate drugs would be expected to produce elevated drug levels due to decreased metabolism.

XVI. SPECIFIC COMMENTS:

(1) The protein binding study is incomplete. The drug is not stable in room temperature. The 2 hours in vitro centrifugation at 4500g for determining protein binding is too long and makes the results not reliable.

An additional protein binding study with modified methodologies is suggested.

(2) The coexistence of both quinupristin and dalfopristin at their effective ratio is significant in producing bactericidal activity. When the concentration of quinupristin is lower than MICs, it is an effective resistance inducer and leads to bacterial growth in the absence of dalfopristin. However, the following observations indicate that the distribution characteristics of the two components of Synercid, quinupristin and dalfopristin, is different and suggest that in some tissues quinupristin may exist without the dalfopristin:

(i) the elimination half-life of dalfopristin (0.5 hour) is less than quinupristin (1 hour). This may result in a more prolonged plasma concentration profile of quinupristin than dalfopristin.

(ii) the volume of distribution of quinupristin is twice as much as that of dalfopristin as seen in all the studies with PK measurements. This suggests that the distribution of the two components in the body differs and may indicate that quinupristin distributes throughout the body more widely and homogeneously than dalfopristin.

(iii) the degree of accumulation of the two components are different too. As compared to single dose study results, the $T_{1/2}$ and AUC of quinupristin is double that after single dose while the $T_{1/2}$ and AUC of dalfopristin is increased with smaller fraction.

(iv) forty percent of the drug resides in tissue fluids as suggested by the results from the blister fluid concentration study. The peak concentration ratio and the AUC ratio of quinupristin/dalfopristin in blister fluid is 50/50 while in plasma it s 30/70. This again suggests that the distribution and tissue penetration of quinupristin is better than that of dalfopristin.

(v) Also, as reported, the distribution of dalfopristin is heterogeneous (**Fantin, B., R Leclercq, et. al.** 1994. In vivo activity and penetration of the two components of the streptogramin RP

59500 in cardic vegetations of experimental endocardities. *Antimicrob. Agents Chemother.* 38:432-437).

Therefore, bacteria in some tissues, or in disease conditions, may be exposed at least transiently only to quinupristin without dalfopristin.

(3) As indicated, this drug will be mainly used in seriously ill patients. In severe hepatic impaired patients, the pharmacokinetic profile is different than that of normal healthy volunteers, and the pharmacokinetic differences between quinupristin and dalfopristin in hepatic impaired patients are enlarged compared to healthy volunteers. Hepatic impairment increases the AUC of quinupristin by 180% while the increase in the AUC of dalfopristin is only by 40%. In these patients, dose adjustment is needed. However, consider the facts that quinupristin is more effected by hepatic impairment, more tissue accumulation, longer t1/2 and wider distribution than dalfopristin, dose adjustment by using the current formulation will be a concern.

(4) For hepatically impaired patients, the sponsor recommended a simple reduced dose of 5 mg/kg when patients are not tolerable to the usual 7.5mg/kg dose. However, it is difficult to determine what it is meant to be "not tolerable" and the 5mg/kg dose recommendation is not supported by any pharmacokinetic or clinical data. Therefore,

the sponsor should conduct a kinetic study to demonstrate hepatic function–dose–pharmacokinetic profile relationship. The study results then may be used as a base for dose adjustment recommendation for hepatic impaired patients.

(5) In human, in vitro, Synercid significantly affects CYP3A4 enzyme activity. Both quinupristin and dalfopristin are potent inhibitors of CsA metabolism on liver CYP3A4 substrates. Dose adjustment is needed for patients taking other medications that are CYP3A4 substrates. This concern should be incorporated in the labelling. Therefore,

The sponsor should be requested to commit to conduct in vivo clinical study(s) to systemically evaluate appropriate drug-drug interactions during phase 4.

(6) The drug is intend to be used in pediatric patients as needed.

Pharmacokinetic studies should be conducted in pediatric patients to establish appropriate dose and dose regimens in this population.

(7) The population pharmacokinetic study results submitted via fax on 2/10/98 needs to be reviewed.

The sponsor should formally submit the study report for review. The reviewer is also looking forward to receiving the results of on-going studies regarding lung tissue penetration and the pharmacokinetics of Synercid in renal impaired patients.

LABELLING:

Labelling comments have been forwarded to the medical review division and discussed in team meetings and FDA-sponsor meetings. A revised document with suggested changes is attached.

/S/ 3/5/98

He Sun, Ph.D.
Division of Pharmaceutical Evaluation III

RD/FT Initialled by Frank Pelsor, Pharm. D. /S/ 3/5/98

cc:
NDA 50,748 and 50,747
HFD-520 (Clinical, CSO)
HFD-340 (Viswanathan)
HFD-880 (Pelsor, Sun)
HFD-880 Div. File NDA 50,748 (Synercid®)
CDR (Attn.: Barbara Murphy)

15 *pages have been removed here because they contain confidential information that will not be included in the redacted portion of the document for the public to obtain.*