RU 29 246, THE ACTIVE COMPOUND OF THE CEPHALOSPORIN PRODRUG-ESTER HR 916

III. PHARMACOKINETIC PROPERTIES AND ANTIBACTERIAL ACTIVITY *IN VIVO*

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The pharmacokinetics of the broad spectrum cephem RU 29 246 and its prodrug-ester HR 916 B were investigated in mice, rats and dogs and compared to those of cefpodoxime proxetil, cefuroxime axetil and cefixime. HR 916 B is well absorbed following oral administration and efficiently converted to the antibacterially active form. In mice, mean peak blood levels of 31.1 μg/ml of the parent compound were recorded within 20 minutes after oral administration of a single dose equivalent to 40 mg/kg RU 29 246. The bioavailability calculated on the basis of the areas under the concentration-time curves (AUC) and the urinary recoveries was about 90%. In rats, peak blood levels of 14.5 μg/ml were obtained 1 hour after an oral 20 mg/kg dose. The bioavailability was calculated as 70%. In dogs, 40% of an oral 10 mg/kg dose was recovered in the urine within 24 hours. C_{max} was 15.9 μg/ml at 4.6 hours. Mean elimination half-lives of RU 29 246 were 0.35, 0.5 and 2.1 hours in mice, rats and dogs, respectively. After an oral HR 916 B dose equivalent to 50 mg/kg of RU 29 246, tissue concentrations at 0.5 hour ranged between 0.8 μg/g in brain and 95.7 μg/g in murine kidneys. These values of HR 916 B are similar to, or distinctly higher than, those of the reference compounds. Of the oral cephalosporins tested, HR 916 B had the most balanced antibacterial spectrum. With ED_{50}s of between 0.9 and 11.5 mg/kg against staphylococci, its activity was similar to that of the additional reference compound cefaclor and higher than that of cefuroxime. Cefixime and cefpodoxime proxetil displayed low antistaphylococcal activity or were inactive.

In septicemias with *Enterobacteriaceae*, cefixime and cefpodoxime proxetil were more potent than HR 916 B and cefaclor. Cefuroxime axetil was inactive against most of these infections. HR 916 B was also highly effective against murine lung infections caused by *Klebsiella pneumoniae* DT-S or *Streptococcus pneumoniae* 1147.

The aminothiazolyl moiety combined with an oxyimino moiety in the acyl side chain of cephem antibiotics leads to a significant enhancement of their intrinsic activity, a broader antibacterial spectrum and resistance to enzymatic hydrolysis by β-lactamases[1]. Recently, there has also been great progress in the development of enterally absorbable cephalosporins. Most of these compounds, however, have only moderate activity against staphylococci or show low bioavailability after oral dosing[2~12].

HR 916 B is the pivaloyloxyethyl-ester of the oxyimino-aminothiazolyl cephalosporin RU 29 246 which makes oral administration of this broad spectrum antibiotic possible.

This study compares the pharmacokinetic properties of RU 29 246, HR 916 B and representative oral cephalosporins in rodents and dogs. In addition, the chemotherapeutic efficacy of five oral cephalosporins was determined in experimentally induced systemic and localized infections in mice.

Materials and Methods

Antibiotics

HR 916 B (tosylate) and its parent compound RU 29 246 as well as cefpodoxime (the parent compound of cefpodoxime proxetil) were synthesized at Hoechst AG laboratories, Frankfurt, FRG. Cefpodoxime proxetil (CS 807), was obtained from the manufacturer (Sankyo, Japan)

The other reference compounds cefaclor, cefuroxime axetil and its parent compound cefuroxime, and cefixime were obtained commercially.

Pharmacokinetic Studies

Mice: NMRI albino mice weighing 20 ± 2 g received the test compounds orally or subcutaneously dissolved or suspended in sterile water at a concentration equivalent to 40 mg (potency)/kg body weight (0.1 mg/10 g b.w.). The pH of the solutions ranged from pH 5.2 to 6.5, this is the pH range where the compounds tested showed highest activity. The animals were fasted for 16 hours prior to dosing but were allowed free access to water. At intervals between 10 and 240 minutes after dosing, blood samples were taken from a cut at the tip of the tails using 10 μl capilary tubes (Wiretrol, Drummond). Blood clotting was prevented by rinsing the tubes with 3.8% sodium citrate solution. As shown in pilot studies, these traces of sodium citrate had no influence on the bioassay. The blood samples were stored at 4°C until assayed for antibiotic levels. The blood concentrations were determined immediately after the end of the study, *i.e.* at 4 hours after dosing. Within 24 hours no loss in activity of the antibiotics tested could be detected. Urine was collected over an 18-hour period from another group of mice kept in metabolism cages[13].

Rats: Wistar rats weighing 100 ± 10 g received the test compounds dissolved in sterile water orally or subcutaneously at a concentration of 20 mg (potency)/kg. Blood and urine samples were collected as for mice.

Dogs: Male beagle dogs (Hoe: BEAK) weighing 20 ± 4 kg received an intravenous injection of RU 29 246 (10 mg/kg) dissolved in 0.2 M sodium bicarbonate solution. HR 916 B was administered orally in snap-fit gelatine capsules. The dogs were fasted for 16 hours prior to dosing but were allowed free access to water.

Blood samples were withdrawn from the *vena cephalica antebrachii* prior to administration and intervals after dosing.

Blood was allowed to clot for 30 minutes at room temperature, samples were centrifuged for 15 minutes at 3,000 rpm, and the separated serum was kept frozen at -20°C until assayed for antibiotic levels. The bladders of the dogs were emptied by catheterization before test compounds were administered. Urine was collected in fractions over a 24-hour period after administration of the compound. The volumes of the pooled samples were measured and the amount of active substance determined microbiologically.

Tissue Samples from Mice

0.5 and 1.0 hour after administration of an oral 50 mg/kg groups of eight mice were killed by exsanguination. Heart, lungs, liver, kidneys, spleen, brain and femoral muscles were removed.

The tissue samples from each of the mice were homogenised in phosphate buffer, pH 6.0, (ratio of 1 : 5) and large components removed by centrifuging at 3,000 rpm. Supernatants were pipetted off and stored frozen at -20°C. The haemoglobin content was determined in aliquot portions of the samples[13].

As shown in pilot studies, the method showed good reproducibility for all compounds tested. The recovery rates in supernatants of tissue homogenates ranged from 97.3% (kidney) to 107.3% (spleen) for RU 29 246, from 92.4% (liver) to 108.6% (heart) for cefpodoxime, from 104.9% (heart) to 112% (thigh muscle) for cefuroxime and from 91.4% (spleen) to 106.7% (kidney) for cefixime.

Bioassay and Pharmacokinetic Analysis

Concentrations of the parent compounds RU 29 246, cefpodoxime and cefuroxime in the blood, serum, urine and the supernatants of tissue homogenates were determined microbiologically by the agar diffusion test using Mueller-Hinton agar supplemented with 10% sheep blood and seeded with

Streptococcus pyogenes A 77 as the indicating organism. *Escherichia coli* V 6 311/65 served as test organism for cefixime. The standard solutions were prepared with blood, serum, urine or supernatants of tissue homogenates, respectively. The concentration range of the standard solution was selected to cover the expected measurable values.

10 μl (mice, rats) or 100 μl (dogs) of the samples and the standard solutions were pipetted into prepared wells (4 and 11 mm diameter, respectively) in the agar plates. After a diffusion period of 1 hour (blood, serum) or 8 hours (urine, supernatants of the tissue homogenates) at 4°C, the agar plates were incubated for 18 hours at 37°C.

The diameters of the zones of inhibition — in the case of *S. pyogenes* A 77 characterized by the lack of haemolysis — were measured. The detection limit was about 0.2 μg/ml for RU 29 246 and cefpodoxime and 0.05 μg/ml for cefuroxime and cefixime. Serum, blood, urine and tissue concentrations were calculated by regression analysis using the standard curves in which the logarithms of the concentrations were proportional to the areas of the inhibition zones. Serum concentration-time data after intravenous administration were approximated to the sum of two e-functions describing an open two-compartment model. For subcutaneous and oral application a Bateman-function was used. Curve fitting was carried out by non-linear regression analysis using a computer program[13].

Protection Tests in Mice

Animals: Male and female mice (strain Hoe: NMRKf, SPF71), weighing 18~22 g, served as experimental animals. The mice were kept in groups of eight and received grain feed and tap water *ad libitum*.

Microorganisms: Ten Gram-positive and ten Gram-negative pathogens were used as test organisms (Table 4). The strains were suspended in 15% skimmed milk and kept in liquid nitrogen.

Table 1. Pharmacokinetic parameters (mean ± SD) in rodents (n = 6).

Species and dose	Compound	Route	$t_{1/2}$ (hours)	C_{max} (μg/ml)	t_{max} (hours)	$AUC_{0\sim4h}$ (μg·hour/ml)	$UR_{0\sim18h}$ (%)
Mice	RU 29 246	s.c.	0.34±0.07	45.7± 9.9	0.46±0.13	41.8± 5.5	94
(40 mg (potency)/kg)	HR 916 B	p.o.	0.74±0.32	31.1± 8.4	0.35±0.10	35.2± 6.7	78
	Cefpodoxime	s.c.	0.38±0.06	47.9±12.8	0.39±0.14	38.2± 8.9	57
	Cefpodoxime proxetil	p.o.	0.59±0.12	26.4± 7.1	0.35±0.14	28.0± 4.4	43
	Cefuroxime	s.c.	0.35±0.11	28.2± 1.72	0.45±0.08	27.2± 7.9	75
	Cefuroxime axetil	p.o.	0.67±0.23	11.9± 4.1	0.26±0.10	13.4± 3.2	53
	Cefixime	s.c.	1.00±0.18	54.3±10.4	0.18±0.1	81.1± 9.8	100
	Cefixime	p.o.	0.80±0.08	10.4± 2.0	1.16±0.13	28.7± 3.0	19
Rats	RU 29 246	s.c.	0.47±0.09	31.9± 2.7	0.64±0.08	46.6± 9.0	71
(20 mg (potency)/kg)	HR 916 B	p.o	0.68±0.22	14.5± 5.3	1.19±0.30	31.3± 7.8	83
	Cefpodoxime	s.c.	0.61±0.15	32.7± 4.4	0.51±0.08	46.2± 9.2	85
	Cefpodoxime proxetil	p.o.	0.75±0.34	9.4± 1.9	1.13±0.08	21.0± 4.4	39
	Cefuroxime	s.c.	0.55±0.10	15.1± 2.5	0.50±0.06	19.1± 2.5	66
	Cefuroxime axetil	p.o.	0.49±0.13	6.7± 1.7	0.68±0.08	10.3± 2.1	33
	Cefixime	s.c.	1.20±0.54	37.0± 7.1	0.77±0.18	95.5±17.7	92
	Cefixime	p.o.	1.13±0.25	7.1± 1.7	1.90±0.31	32.1±12.9	29

Table 2. Pharmacokinetic parameters of the active metabolite RU 29 246 (mean ± SD) in dogs (n = 4) (dose: 10 mg (potency)/kg body weight).

Compound	Route	$t_{1/2}$ (hours)	C_0; C_{max} (μg/ml)	t_{max} (hours)	$AUC_{0\sim7h}$ (μg·hour/ml)	$UR_{0\sim24h}$ (%)
RU 29 246	i.v.	2.1±0.2	226.0±37.0		177.0±31.7	86.0±9.7
HR 916 B	p.o.	2.8±1.2	15.9± 6.9	4.6±2.2	68.7±29.1	39.2±8.2

Susceptibility testing: The susceptibility of the infecting organisms used was determined by the agar-dilution method with Mueller-Hinton agar (Difco) as test medium. In the case of the streptococcal strains, the agar was supplemented with 10% horse blood. Agar plates containing serial dilutions of the antibiotics or their parent compounds were inoculated with a Denley Multipoint inoculator which delivered 5×10^5 colony forming units (cfu) of a stationary culture of the strain concerned. The minimum inhibitory concentrations (MICs) were taken as the lowest concentrations of the antibiotics that suppressed visible growth after 24 hours incubation at 37°C.

Protection tests: In the experimentally induced septicemia, NMRI mice of both sexes weighing 18~20 g were infected intraperitoneally with 0.3 ml bacterial suspension in hog gastric mucin[14]. The streptococcal strains used were suspended in meat extract bouillon without mucin.

Depending on the infecting organism, the challenge inoculum contained 4 to 500 times the LD_{100} of the pathogens. A group of eight untreated animals served as control. Control animals died between 6 and 48 hours after infection, depending on the type of bacteria injected. Eight mice were used for each of the serial two-fold dose concentrations of the cephalosporins tested.

Treatment was oral, immediately and four hours after infection. The median effective dose (ED_{50}, mg/kg/total dose) was calculated by probit analysis from the number of surviving mice on day 10.

Experimental Pneumonia with *Klebsiella pneumoniae* DT-S and Time-kill Curve Studies in Pneumonic Mice

Murine pneumonia was produced as previously described[14]. In brief, up to 100 NMRI mice were placed in an exposure chamber and challenged for 40 minutes with an aerosol containing the infecting organism *Klebsiella pneumoniae* DT-S. The bacterial suspension (4 ml) was nebulized by means of a nebulizer (Vaponefrin Pocket Nebulizer, USV pharmaceutical Co., U.S.A.). Pneumonia was allowed to become well-established before being treated with the different antibiotics. The compounds were administered orally, in serial two-fold dose concentrations at 18, 19 and 20 hours after aerosol exposure. Eight control mice were untreated. The number of dead mice was recorded daily. The ED_{50} was calculated by probit analysis.

In studies on the bactericidal activity *in vivo*, mice with experimentally induced pneumonia were administered orally with 50 mg/kg of HR 916 B, cefpodoxime proxetil or cefixime 20 hours after challenge[14].

Groups of eight mice were killed prior to and at intervals between 1 and 48 hours after antibiotic administration. The lungs were excised, diluted in nine times their weight of PBS, homogenized and plated. After incubation of the plates for 20 hours at 37°C, the cfu were counted.

Lung Infections with *Streptococcus pneumoniae*

The technique of intratracheal inoculation of *Streptococcus pneumoniae* 1147 was detailed previously by ESPOSITO and PENNINGTON[15].

Mice were anesthetized by ip injection of Nembutal (Pentobarbital Sodium, Abbott), and their trachea was cannulated with a blunt-tipped metal spinal needle (no. 22 gauge). After intratracheal inoculation of 0.05 ml of a pneumococcal suspension containing 8 $\log_{10}$ cfu/ml of *S. pneumoniae* 1147, the cannula was removed. Treatment with the oral cephalosporins was initiated 6 hours after infection.

Results

Pharmacokinetic Studies in Laboratory Animals

Mice: As shown in Table 1 and Fig. 1 the pharmacokinetic profiles in murine blood of the parent compounds RU 29 246 and cefpodoxime following a subcutaneous dose of 40 mg/kg are very similar. However, after oral administration of their prodrug-esters HR 916 B and cefpodoxime proxetil the pharmacokinetic values of the drugs were different.

The bioavailability calculated on the basis of AUCs after subcutaneous and oral dosing was 84% for RU 29 246 and 73% for cefpodoxime. Mean urinary recoveries from 0 to 18 hours after oral dosing were

Fig. 1. Mean blood levels of RU 29 246 in mice (n=6) after a single subcutaneous or oral dose of 40 mg (potency)/kg body weight.

■ RU 29 246 sc, ● HR 916 B po.

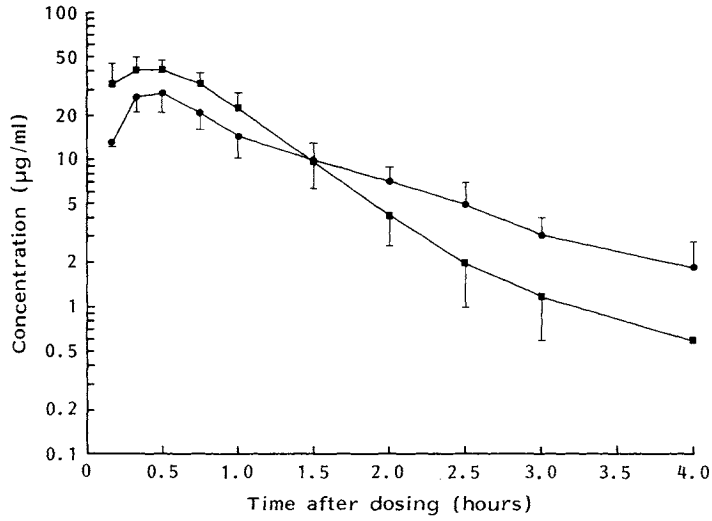
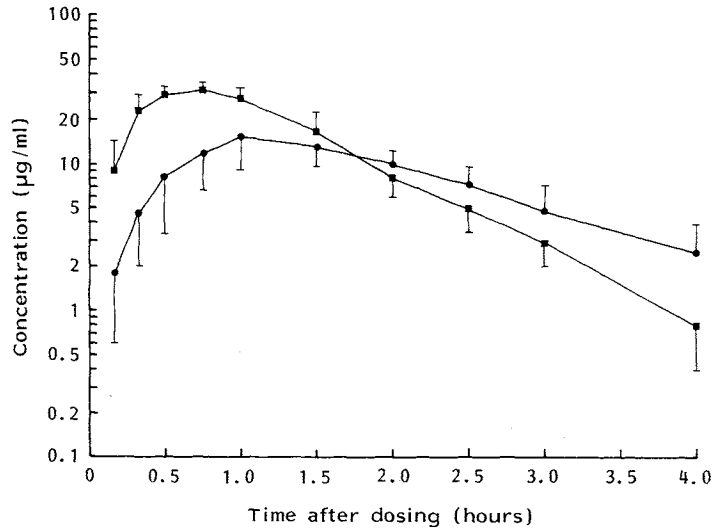


Fig. 2. Mean blood levels of RU 29 246 in rats (n=6) after a single subcutaneous or oral dose of 20 mg (potency)/kg body weight.

■ RU 29 246 sc, ● HR 916 B po.



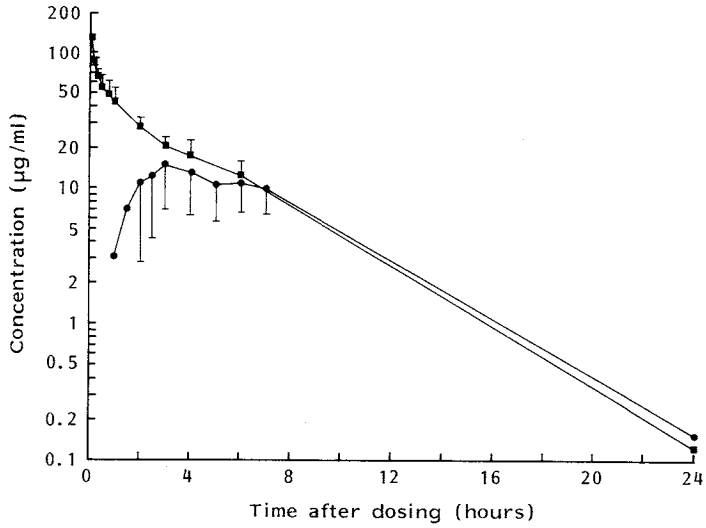
78% for RU 29 246 but only 43% for cefpodoxime.

Cefuroxime axetil and cefixime showed low absorption after oral administration with only 53% and 19% of the given dose being recovered in the urine.

Rats: Profiles of the mean blood concentrations in rats given a single sc dose of RU 29 246 or an oral dose of HR 916 B are illustrated in Fig. 2. The pharmacokinetic parameters are given in Table 1. As demonstrated in mice, the blood levels of RU 29 246 (when given orally as its ester compound) were

Fig. 3. Mean serum levels of RU 29 246 in dogs (n=4) after a single intravenous or oral dose of 10 mg (potency)/kg body weight.

■ RU 29 246 iv, ● HR 916 B po.



higher than those of the reference compounds cefpodoxime and cefuroxime. As shown in mice, cefixime displayed very high blood levels in rats after subcutaneous administration. Urinary recoveries after oral dosing were 83% for RU 29 246 but only 29 to 39% for cefpodoxime, cefuroxime and cefixime.

Table 3. Blood and tissue concentrations of RU 29 246 after a single oral dose of 50 mg (potency)/kg body weight of HR 916 B.

Tissue	Concentrations in μg/ml or μg/g			
	0.5 hour	%	1.0 hour	%
Blood	24.1± 6.0	(100)	20.0± 2.6	(100)
Heart	5.1± 0.4	(21)	1.5± 0.4	(8)
Lungs	13.5± 3.4	(56)	5.8± 1.8	(29)
Liver	20.8± 5.4	(86)	4.6± 1.7	(23)
Kidney	95.7±36.0	(397)	23.2± 6.2	(116)
Thigh muscle	5.1± 1.0	(21)	1.5± 0.3	(8)
Spleen	1.9± 0.3	(8)	0.8± 0.2	(4)
Brain	0.8± 0.3	(3)	N.D.	—

N.D.: Not detectable.

Dogs: Intravenous administration of RU 29 246 to dogs at a dosage of 10 mg/kg resulted in high initial blood levels (Fig. 3 and Table 2). Disappearance from the systemic circulation was biphasic showing a rapid distribution phase followed by a slower elimination phase with a half-life of 2.1 hours. Twenty-four hours after dosing serum concentrations of 0.1 μg/ml were still detectable. Peak levels after oral administration of HR 916 B (C_{max}=15.9 μg/ml) were reached relatively late (t_{max}=4.6 hours). The bioavailability and the urinary recovery from 0 to 24 hours after administration were 38.8 and 39.2%, respectively.

Tissue Distribution in Mice

High concentrations of RU 29 246 in the kidneys of up to 95.7 μg/g at 0.5 hour and 23.2 μg/g at 1.0 hour after oral administration of a 50 mg/kg dose of HR 916 B in the kidneys, indicate that the kidneys are the main route of elimination for this compound. Cefpodoxime and cefuroxime had high concentrations in both kidneys and liver, whereas cefixime was concentrated mainly in the liver

Concentrations of RU 29 246 between 1.9 and 13.5 μg/g were detected in spleen, thigh muscle, heart and lungs of the test animals at 0.5 hour after dosing, which represents 7.8 to 56% of the

Table 4. Comparative *in vivo* activity of the oral cephalosporin antibiotics HR 916 B (tosylate), cefaclor, cefuroxime axetil, cefpodoxime proxetil and cefixime against experimentally induced septicemia in mice.

Infective organism	Challenge dose (cfu/mouse)	Median effective dose (ED_{50}, in mg/kg)				
		HR 916 B	Cefaclor	Cefuroxime axetil	Cefpodoxime proxetil	Cefixime
Staphylococcus aureus Giorgio	1.0×10^6	2.40	1.43	13.15	24.41	>50.00
S. aureus SG 511	1.0×10^8	6.40	2.38	35.30	>50.00	>50.00
S. aureus 1806	3.0×10^8	11.47	2.37	19.20	38.74	>50.00
S. aureus KN4	1.5×10^8	4.29	58.70	15.73	28.59	>50.00
S. aureus A 9537	1.0×10^7	0.94	0.84	4.57	12.18	>50.00
S. aureus 20240	8.0×10^7	6.87	>50.00	18.87	>50.00	>50.00
Streptococcus pyogenes A77	3.9×10^3	0.15	0.50	0.29	0.18	2.29
S. aronson B	1.3×10^3	0.13	1.10	0.14	0.10	4.42
S. agalactiae B	4.0×10^4	0.41	4.81	0.80	0.40	11.28
S. pneumoniae 1147	1.5×10^4	0.17	3.83	0.29	0.14	3.41
Escherichia coli 078	1.1×10^4	1.73	1.29	1.20	0.43	0.32
E. coli TEM[a]	2.0×10^8	83.64	>100.00	>100.00	39.46	83.12
Salmonella typhimurium MZ II	2.5×10^3	3.13	2.03	5.74	0.49	0.55
Klebsiella pneumoniae M 477	1.4×10^7	3.48	6.10	6.55	0.57	0.58
K. pneumoniae DT-S	3.1×10^3	5.64	2.73	25.60	2.13	0.39
K. pneumoniae ATCC 10031	1.0×10^8	9.72	4.76	>50.00	6.92	0.55
K. pneumoniae 1976 E[b]	1.5×10^6	8.54	42.99	>50.00	4.72	<0.39
Enterobacter cloacae M 417	5.5×10^6	12.20	>50.00	>50.00	3.05	0.51
Proteus mirabilis ATCC 14273	7.0×10^7	8.51	4.86	17.22	0.78	0.36
Pasteurella multocida 6525	2.3×10^5	3.20	2.04	6.48	0.74	0.34

[a] TEM I-β-lactamase producing strain.
[b] SHV I-β-lactamase producing strain.

corresponding level in the blood. Levels of 0.8 and 5.8 μg/g could still be found at one hour, which is 4 to 29% of the blood concentration.

The relative values for cefpodoxime were 2.5 to 15.1 μg/g at 0.5 hour and 1.3 to 9.1 μg/g at one hour, for cefuroxime 2.1 to 7.5 μg/g at 0.5 hour and 0.6 to 2.8 μg/g at one hour. Cefixime showed delayed tissue penetration, so that one hour after dosing tissue levels were higher than those at 0.5 hour (not shown).

Chemotherapeutic Activity against Experimental Infections in Rodents.

Protection Tests in Mice

With MICs of <0.002 to 0.50 μg/ml against Gram-positive infecting organisms (streptococci of the serogroup A and B, *S. pneumoniae* 1147 and methicillin-sensitive *S. aureus* strains (MSSA)) RU 29 246 was clearly more active than cefaclor, cefuroxime, cefpodoxime and cefixime. MICs of RU 29 246 against various Enterobacteriaceae and *Pasteurella multocida* were <0.25 μg/ml, the MIC against *Enterobacter cloacae* M 417 was 1.25 μg/ml.

The results of the protection tests in mice are summarized in Table 4. When given orally as its prodrug-ester HR 916 B, the high *in vitro* activity of RU 29 246 against MSSA and streptococci was reflected by good chemotherapeutic efficacy against murine staphylococcal and streptococcal septicemia.

With ED_{50} values of between 0.84 to 2.38 mg/kg, the reference compound cefaclor displayed comparable activity in four out of six *S. aureus* infections, but was clearly inferior to HR 916 B in the case of septicemia due to *S. aureus* KN4, 20240 and the four streptococcal strains tested.

Cefuroxime axetil, cefpodoxime proxetil and cefixime were either significantly less potent in protecting

mice against MSSA infections or were inactive, the ED_{50}s ranged from 4.57 to >50.00 mg/kg.

Whereas streptococci of the serogroups A and B and *S. pneumoniae* were influenced by low concentrations of cefuroxime axetil and cefpodoxime proxetil, comparatively high cefixime doses of between 2.29 to 11.28 mg/kg were required to cure infections caused by these bacteria.

The most effective agent against murine septicemia due to Enterobacteriaceae and *Pasteurella multocida* 6525 was cefixime followed by cefpodoxime proxetil with ED_{50}s of 0.32 to 0.58 mg/kg and 0.43 to 6.92 mg/kg, respectively. These compounds were, therefore, 2 to 24 times more potent than HR 916 B and cefaclor. *E. cloacae* M 417 was susceptible to HR 916 B, cefpodoxime proxetil and cefixime, but resistant to cefaclor.

Although the MICs of the compounds tested were low against *E. coli* TEM (0.125 to 0.25 μg/ml), septicemia caused by this strain was difficult to treat. The therapeutic response of cefpodoxime proxetil was higher than that of HR 916 B and cefixime against this infection and cefaclor was inactive. Cefuroxime given orally as its prodrug-ester cefuroxime axetil showed some efficacy against five out of ten of the Gram-negative pathogens but was less active against *K. pneumoniae* DT-S. It was inactive against *K. pneumoniae* ATCC 10031 and *E. cloacae* M 417 and the β-lactamase producers *E. coli* TEM and *K. pneumoniae* 1976 E.

Experimental Pneumonia in Mice

Table 5 compares the therapeutic efficacy of the five oral cephalosporins against murine *Klebsiella* and pneumococcal lung infections.

With an ED_{50} value of 59.7 mg/kg, HR 916 B was three times less active than cefixime against *K. peumoniae* DT-S, but two times more potent than cefpodoxime proxetil. Cefuroxime axetil and cefaclor, however, showed only marginal efficacy. Even after administration of high doses of up to 600 mg/kg (total dose) only a few animals survived the infection. The ED_{50}s were 379.5 mg/kg for cefuroxime axetil and 710.7 mg/kg for cefaclor.

Following a single oral dose of 50 mg/kg HR 916 B, cefpodoxime proxetil or cefixime given 20 hours after challenge, the three compounds all displayed, over the first 6 to 8 hours, similar intrapulmonary killing of *K. pneumoniae* DT-S and caused a mean reduction of viable bacteria in the lungs of pneumonic mice to 1/1,000 of the pretreatment level (not shown).

With cefixime no further reduction of the challenge organism occurred. With HR 916 B and cefpodoxime proxetil, however, a regrowth of bacteria was observed so that mean bacterial counts in lungs at 24 hours were only 1.0 $\log_{10}$ cfu/ml lower than in the control group.

Against murine lung infections caused by intratracheal inoculation of the animals with *Streptococcus pneumoniae* 1147, HR 916 B and cefpodoxime proxetil had similar activity, whilst the other reference compounds tested, cefuroxime axetil, cefaclor and cefixime showed only low antipneumococcal efficacy in this localized

Table 5. Therapeutic activity of HR 916 B (tosylate), cefpodoxime-proxetil, cefuroxime-axetil, cefaclor and cefixime on experimentally induced lung infections in mice.

Compound	Median effective dose (ED_{50}, mg/kg/total dose)	
	K. pneumoniae DT-S[a]	*S. pneumoniae* 1147[b]
HR 916 B (tosylate)	59.7	3.3
Cefpodoxime proxetil	131.3	3.7
Cefuroxime axetil	379.5	15.7
Cefaclor	710.7	>100.0
Cefixime	19.9	31.2

[a] Mice infected with *K. pneumoniae* DT-S were treated at 18, 19 and 20 hours after challenge.
[b] Mice infected with *S. pneumoniae* 1147 were treated once, 6 hours after challenge.

infection (Table 5).

Discussion

Recently, several new oral cephalosporins, such as cefixime, or cephalosporin prodrug-esters, such as cefpodoxime proxetil, have been developed. Although these antibiotics or their parent compounds, respectively, display high activity against various streptococci and *Enterobacteriaceae*, especially in comparison to older agents such as cephalexin or cefaclor, their potency against staphylococci remains relatively weak[3~10]. In contrast, the broad spectrum of the aminothiazolyl-cephalosporin RU 29 246 also includes methicillin-susceptible staphylococci and is clearly more active against this species than cefixime, cefpodoxime, cefuroxime and cefaclor[16].

In addition, as shown in this study, when administered orally as its prodrug-ester HR 916 B (tosylate), the resulting pharmacokinetic profile of RU 29 246 clearly differentiates itself from cefpodoxime proxetil, cefuroxime axetil and cefixime. HR 916 B is rapidly absorbed from the gastro-intestinal tract and subsequently converted into the antibacterially active compound RU 29 246, which is itself very poorly absorbed. With a urinary recovery of 84% of the oral bioavailability of HR 916 B in rodents is superior to that of the reference compounds. Peak levels and areas under the curve were distinctly higher than those of the three other oral cephalosporins tested. Good penetration of RU 29 246 from the blood stream into various tissues was observed, and tissue concentrations up to 86% of the corresponding blood values were obtained. These absorption and penetration characteristics are sufficient to achieve blood and tissue concentrations well above the MICs for the majority of Gram-positive and Gram-negative infective organisms. The comparatively good *in vitro*-activity and pharmacokinetic properties of RU 29 246 combined to produce high efficacy rates in animals models of experimentally induced pneumonia and septicemia.

In conclusion, both RU 29 246 and its enterally absorbable cephalosporin prodrug-ester HR 916 B, possess a more balanced antibacterial *in vitro* and *in vivo* spectrum with greater Gram-positive activity than currently available oral cephalosporins. This extended spectrum, combined with their pharmacokinetic properties warrant further development of these compounds.

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