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CLINICAL THERAPEUTICS

Ceftriaxone Absorption Enhancement for Noninvasive Administration as an Alternative to Injectable Solutions

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ABSTRACT Neonatal sepsis is a major cause of infant mortality in developing countries because of delayed injectable treatment, making it urgent to develop noninjectable formulations that can reduce treatment delays in resource-limited settings. Ceftriaxone, available only for injection, needs absorption enhancers to achieve adequate bioavailability via nonparenteral administration. This article presents all available data on the nonparenteral absorption of ceftriaxone in humans and animals, including unpublished work carried out by F. Hoffmann-La Roche (Roche) in the 1980s and new data from preclinical studies with rabbits, and discusses the importance of these data for the development of noninjectable formulations for noninvasive treatment. The combined results indicate that the rectal absorption of ceftriaxone is feasible and likely to lead to a bioavailable formulation that can reduce treatment delays in neonatal sepsis. A bile salt, chenodeoxycholate sodium salt (Na-CDC), used as an absorption enhancer at a 125-mg dose, together with a 500-mg dose of ceftriaxone provided 24% rectal absorption of ceftriaxone and a maximal plasma concentration of 21 μg/ml with good tolerance in human subjects. The rabbit model developed can also be used to screen for the bioavailability of other formulations before assessment in humans.

KEYWORDS rectal route, third-generation cephalosporins, pediatric, antibiotic, pediatric drug therapy

Sepsis is a common and lethal syndrome in neonates; it is the third leading cause of neonatal death, after prematurity and intrapartum-related complications (1, 2). Prompt injectable administration of effective antibiotics is lifesaving but seldom possible in the rural tropics (3). In resource-limited settings, where most deaths from neonatal sepsis occur, facilities that provide appropriate injectable treatments are often hours or days away. Treatment delays can be fatal. Estimates suggest that ~7.3% of neonatal deaths may be associated with drug-resistant Gram-negative bacterial strains (4). As neonatal sepsis can result from septicemia, pneumonia, or meningitis, a safe, affordable, broad-spectrum antibiotic with excellent central nervous system (CNS) penetration that can be given as early as possible in the community is needed. Ceftriaxone (CRO) meets these criteria, but it is only available for injectable use.

CRO is a widely used broad-spectrum cephalosporin that exhibits potent activity against Gram-positive and Gram-negative bacteria (5–7). It has a good safety record in neonates (8) and good CNS penetration (9), and use of a single daily dose is possible because CRO has much slower elimination than other third-generation cephalosporins, penicillins, or carbapenems. The European Committee on Antimicrobial Susceptibility

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Testing currently classifies the breakpoint for ceftriaxone sensitivity to be equal to or below 1 mg/liter (10), but at the time of the studies by F. Hoffmann-La Roche (Roche) in the 1980s, the breakpoint applied was 4 μg/ml in CRO-sensitive strains, as cited in Roche's Rocephin product information leaflet (11), and the higher breakpoint is also used in this paper to facilitate comparison. The pharmacokinetics of CRO are linear, but CRO binding to human serum proteins, mainly to albumin, is concentration dependent (12–16). However, despite the saturable protein binding of CRO, Stoeckel et al. (17) showed that the systemic and renal clearances of the free fraction are constant and do not change with dose.

The protein binding of CRO is also age dependent and is about 70% in neonates, which increases through childhood and into adulthood (90% to 95%) (18). A single dose provides effective antimicrobial plasma concentrations for more than 24 h in adults and more than 48 h in neonates. The elimination half-life ($t_{1/2}$) of CRO recorded in neonates is double that in adults (18.6 h for neonates less than 8 days of age versus 8 h for adults) (8, 18, 19). CRO is excreted primarily in urine and bile (14). The quantity of CRO excreted unchanged in urine depends on the route of administration and the number of administered doses; it can vary from 36% to 65% (mean, 46%) and is higher, approximately 70%, in newborns (18, 20). The mechanism of urinary excretion is mainly glomerular filtration (80%) and, to a lesser extent, tubular excretion (20%), with an additional 10% to 20% being excreted in bile (18, 21).

With regard to extravascular administration, CRO is classified as a Biopharmaceutics Classification System (BCS) class 3 drug; i.e., it presents high aqueous solubility but has a low level of permeation across biological membranes. For this reason, it exists only as an injectable pharmaceutical formulation. Consequently, it requires an absorption enhancer to achieve significant plasma concentrations when an alternative noninjectable route of administration is envisaged.

When administered orally, CRO degrades in gastric fluid and has poor absorption through the intestinal tract, making the use of absorption enhancers to improve oral absorption essential (22–24). Furthermore, oral administration of antibiotics is not recommended in premature neonates (25).

Rectal administration is a simple, safe, and acceptable method for treating sick children who cannot take oral medication. Rectal CRO could be lifesaving if a formulation with adequate bioavailability and stability were developed for prereferral use in low- and middle-income countries for the treatment of sick neonates with critical illness, i.e., convulsions, unconsciousness, shock, or an inability to feed. These infants have the highest risk of death and no treatment option before arriving at a health care facility. A therapeutic rectal formulation could prevent neonatal mortality by reducing lethal delays in a potentially fatal rapidly progressing illness, as well as increase access, acceptance, and adherence to treatment.

To assist in this endeavor, the originator of CRO, F. Hoffmann-La Roche, made available its unpublished reports to the World Health Organization (WHO) under confidentiality agreements. It was therefore possible to study CRO rectal absorption from prior (unpublished) research carried out by Roche to design and execute new work and compare the earlier results with those of new bioavailability experiments. The data presented here are largely based on a series of preclinical studies conducted in rabbits and baboons and clinical studies conducted in humans, and inferences made from these data provide support for a decision to develop a rectal formulation of ceftriaxone. The long-term aim of the research project was the development of rectal bioavailable formulations for the treatment of serious neonatal sepsis.

RESULTS

Effect of an absorption enhancer in improving bioavailability. (i) Part 1: preliminary screening of enhancers for CRO rectal bioavailability in rabbits. For ethical reasons, 30 absorption enhancers (described in the section "Formulations" in Materials and Methods) were screened by Roche on a small number of young adult male and female New Zealand White rabbits. In water, without any absorption en-

TABLE 1 Bioavailability and plasma concentrations of ceftriaxone from various enhancer systems in rabbits[a]

Type of formulation	Enhancer system	Mean % bioavailability (*n*)	Mean C_{max} (μg/ml)	Mean C_8 (μg/ml)
Control	Water	4.5 (3)	8.7	4.6
Suspension	Capmul MCM-90 + 2.5% Na-glycocholate	50.8 (4)	107.3	18.9
Suspension	Caprilic acid	50.7 (2)	100.0	21.8
Suspension	2.5% Na-glycocholate + water	39.3 (4)	100.9	16.3
Suspension	Caprilic acid + 2.5% Na-glycocholate	39.1 (2)	63.7	16.7
Capsule	Imwitor 742 + 2.5% Na-glycocholate	34.9 (2)	52.9	18.2
Suspension	2.5% Na-glycocholate + soya oil	33.8 (2)	64.0	17.2
Suspension	Capmul MCM-90	33.5 (3)	72.2	14.0
Suspension	5% Na-glycocholate + water	32.8 (2)	95.0	14.2
Suspension	Capmul MCM-90 + Alcolec-S	32.7 (2)	70.5	9.3
Suspension	2.5 % Na-glycocholate + Captex 300	26.7 (2)	57.5	9.8
Suspension	Capmul MCM-90 + 2.5% Na-glycocholate + Alcolec-S	25.0 (2)	38.1	17.5
Capsule	Capmul MCM-90	22.9 (2)	43.3	10.6
Capsule	Capmul MCM-90 + 2.5% Na-glycocholate + Alcolec-S	22.0 (2)	31.2	19.4
Suspension	5-Methoxy salicylic acid + 1.5% Na-glycocholate + water	21.5 (2)	47.2	8.7
Suspension	5-Methoxy salicylic acid + water	18.2 (2)	35.2	6.2
Suspension	Capmul MCM-90 + Alcolec-S	15.9 (2)	23.1	9.4
Suspension	Imwitor 460 + 2.5% Na-glycocholate	14.7 (3)	24.9	7.8
Suspension	Monocaprylate + PEG 400	14.4 (2)	23.0	5.3
Capsule	Na-glycocholate	11.9 (2)	33.9	6.5
Suspension	Captex 300	7.7 (2)	17.4	2.3
Suspension	Linolenic acid	6.6 (2)	11.9	3.9
Suspension	Azone	5.1 (2)	5.7	3.0
Suspension	Jojoba oil	4.6 (2)	15.8	3.1
Suspension	Na-salicylate + water	4.2 (2)	6.7	3.3
Suspension	Soybean oil	3.9 (2)	4.5	3.0
Capsule	Imwitor 742	3.8 (2)	16.8	7.1
Suspension	2.5% Na-glycocholate + Alcolec-S	3.8 (2)	5.4	2.7
Suspension	PEG 400	3.8 (2)	4.3	3.8
Suspension	Propylene glycol	3.6 (2)	4.8	2.6
Suspension	Emulphor	3.2 (2)	8.9	3.1
Capsule	Imwitor 460	2.1 (2)	8.4	6.1
Suspension	Alcolec-S	1.9 (2)	3.3	2.7

[a]*n*, number of animals; C_{max}, maximal plasma concentration; C_8, plasma concentration after 8 h; PEG, polyethylene glycol.

hancer, the rectal bioavailability of CRO was 4.5% in rabbits (Table 1). Among 32 suspensions or hard-shell capsules (Table 1), 10 preparations exhibited absolute rectal bioavailability greater than 30% and 23 preparations provided plasma levels of over 4 μg/ml during the 8-h experimental period. For most formulations, the peak plasma levels occurred within 30 min. Caprilic acid and Na-glycocholate with Capmul MCM-90 gave the highest bioavailability results (bioavailability, >50%) and the highest maximal plasma concentrations (C_{max}; ≥100 μg/ml). Na-glycocholate alone in water gave 39% bioavailability and a comparable C_{max}. The effect of glycocholate did not increase with its quantity; indeed, in this preliminary study, a 2.5% content was a more efficient absorption enhancer than a 5% content, with 39 and 33% rectal bioavailability in water, respectively. Generally, complex multicomponent absorption enhancer systems were not found to be superior because of the presence of surfactants (like Alcolec-S or Imwitor 742) or glyceride derivatives (like Imwitor 460, Captex 300), and fatty acids did not enhance rectal bioavailability compared to that achieved with glycocholate and Capmul MCM-90. Glycocholate and Capmul MCM-90 were considered for further studies.

(ii) Part 2: optimization of bile salts as absorption enhancers of CRO in baboons. Six bile salts, namely, sodium salts of glycocholate, taurocholate, deoxycholate, taurodeoxycholate, ursodeoxycholate, and chenodeoxycholate sodium salt (Na-CDC), were assessed by Roche as absorption enhancers in baboons in suppository formulations of CRO with different drug-to-enhancer ratios against a control enhancer-free formulation (Table 2). The control formulation showed bioavailability below 4%. Bioavailability in the presence of bile salts was improved except when using ursodeoxycholate. For a drug/enhancer ratio of 4:1, bioavailability enhancement was obtained in

TABLE 2 Use of bile salts in enhancing the rectal absorption of ceftriaxone in baboons[a]

Bile salt	Drug/enhancer ratio	C_{max} (μg/ml)	T_{max} (min)	C_8 (μg/ml)	Bioavailability (%)
Control		4.4	68	1.6	3.8
Na-glycocholate	4:1	43.9	60	9.6	33.0
Na-glycocholate	3:1	38.4	98	8.8	20.3
Na-glycocholate	1:1	39.6	83	12.9	59.9
Na-glycocholate	1:2	48.2	105	15.0	68.0
Na-taurocholate	4:1	38.4	38	8.3	37.0
Na-deoxycholate	4:1	50.0	90	18.0	43.0
Na-taurodeoxycholate	4:1	54.9	53	19.7	46.0
Na-taurodeoxycholate	2:1	48.8	38	10.3	55.6
Na-taurodeoxycholate	1:1	60.9	38	8.8	41.3
Na-ursodeoxycholate	4:1	4.6	109	1.9	4.5
Na-chenodeoxycholate	8:1	38.8	135	11.1	38.6
Na-chenodeoxycholate	4:1	75.1	90	25.3	69.6

[a]The data for the pharmacokinetic parameters represent the mean values ($n = 4$). C_{max}, maximal plasma concentration; T_{max}, time to C_{max}; C_8, plasma concentration after 8 h.

the following order: Na-CDC > taurodeocycholate > deoxycholate > taurocholate > glycocholate > ursodeoxycholate. Bioavailability was not related to the dose of biliary salt in a linear manner. The highest bioavailability with the lowest enhancer quantity appeared to be Na-CDC with 69.9% rectal bioavailability in a 4:1 drug-to-Na-CDC ratio, with significant CRO blood levels being achieved within 5 min and C_{max} being achieved within 1 to 2 h of dosing.

Na-CDC was further kept for human studies, but the optimal ratio would need to be evaluated in humans starting from 4:1 CRO/Na-CDC, which was found to be optimal in baboons.

(iii) Part 3: human studies of rectal absorption of ceftriaxone from suppositories. The mean values of the human pharmacokinetic parameters observed in the various Roche clinical studies using rectal, intravenous, and oral treatments and the absorption enhancer Na-CDC are summarized in Tables 3 and 4. CRO was rapidly absorbed after both rectal and oral administrations; however, only the rectal formulation provided therapeutically relevant plasma concentrations and a trough concentra-

TABLE 3 Pharmacokinetic parameters for 500 mg ceftriaxone by rectal, enteral, and intravenous treatment[d]

Characteristic or parameter	Value	Value(s) by the following route:		
		Rectal	Enteral	Intravenous
Protocol characteristics				
Amt of Na-CDC (mg)/formulation		125	250	0
No. of volunteers		6	4	6
Pharmacokinetic parameters				
C_{max} (μg/ml)	Mean	20.7	8.4	91.9[a]
	RSD (%)	26	17	21
T_{max} (h)	Mean	1.0	0.25	0.6[b]
	RSD (%)	52	0	33
AUC (μg·h/ml)	Mean	169	56	711
	RSD (%)	25	27	14
β (h^{-1})	Mean	0.097	0.089	0.08
	RSD (%)	24	29	14
$t_{1/2}$ (h)[c]	Mean	7.4	8.3	8.8
	RSD (%)	20	28	12
Bioavailability (%)	Mean	23.8	8.0	
	RSD (%)	30	36	

[a]C_{max} is the extrapolated initial concentration.
[b]Time of infusion.
[c]Recalculated to the arithmetic mean.
[d]The protocol (protocol N2998A, 1985) involved humans receiving 500 mg CRO. RSD, relative standard deviation.

TABLE 4 Comparison of the mean biopharmaceutic parameters of ceftriaxone following rectal administration of one 500-mg ceftriaxone suppository containing 15 or 30 mg of sodium chenodeoxycholate and two 500-mg ceftriaxone suppositories containing 0, 62.5, or 125 mg of sodium chenodeoxycholate with or without Capmul MCM-90 in humans[d]

Characteristic or parameter	Value	Value obtained by the following protocol:						
		Protocol N3119C		Protocol N3025B				
Protocol characteristics								
Formulation		Rectal	Rectal	i.v.	Rectal	Rectal	Rectal	Rectal
CRO dose (mg)		500	500	500	1,000	1,000	1,000	1.000
Amt of Na-CDC (mg)/500 mg CRO		15.0	30.0	0	0	62.5	62.5	125.0
Amt of Capmul MCM-90 (mg)/suppository							375	
No. of volunteers		5	6	6	6	5	5	6
Pharmacokinetic parameters								
C_{max} (μg/ml)	Mean[a]	7.3	9.4	80.2	8.1	22.0	17.2	23.8
	RSD (%)	38	50	5	41	33	22	22
T_{max} (h)	Mean	0.9	0.8	0.5[c]	1.4	1.4	1.1	1.0
	RSD (%)	15	24	14	27	17	20	24
AUC (μg·h/ml)	Mean	69	94	699	90	220	193	256
	RSD (%)	37	42	22	41	27	22	30
$t_{1/2}$	Mean[a]	6.9	7.0	9.2	9.2	8.3	9.1	8.4
	RSD (%)	50	42	39	30	35	25	18
β (h^{-1})	Mean[a]			0.075	0.075	0.083	0.076	0.073
	RSD (%)			29	24	34	32	16
Bioavailability (%)	Mean[b]	9.9	13.4		6.5	16.0	14.0	18.1
	RSD (%)	NA	NA		36	28	16	19

[a]Harmonic mean.
[b]Mean bioavailability was obtained as (mean AUC by rectal administration/mean AUC by i.v. administration) × 100.
[c]Time of infusion.
[d]The total ceftriaxone dose was 1,000 mg (protocol N3025B) or 500 mg (protocol N3119C) CRO for rectal administration and 500 mg for i.v. administration. Bioavailability was recalculated so that all measurements are comparable. However, because CRO protein binding is not linear, the bioavailabilities between different dosages could not be compared directly. RSD, relative standard deviation; NA, not applicable.

tion that was still above the breakpoint used at the time of the study (4 μg/ml) at 12 h. This substantially greater rectal absolute bioavailability compared favorably with the oral bioavailability (24% versus 8%; Table 3), and the relative rectal-versus-oral bioavailability was 297%. The rectal route was therefore chosen by Roche as optimal for CRO bioavailability. The intravenous (i.v.) route was used as a bioavailability control.

All further work with rectal formulations used a constant 500-mg dose of CRO per suppository but varied the doses of Na-CDC and assessed whether the addition of Capmul MCM-90 improved the bioavailability; the results are summarized in Table 4. One or two suppositories were administered depending on the formulation (cf. Table 4), with the total CRO dose being 500 mg or 1,000 mg, respectively. A low dose of Na-CDC did not improve the bioavailability above 13.4%, nor did use of the same formulation, but various Na-CDC particle sizes did (raw data not shown). Consequently, dose-ranging studies to optimize the ratios of Na-CDC were implemented. Bioavailability studies showed that when a 500-mg intravenous dose was infused over a 30-min period, the maximal plasma concentration ranged from 62.7 to 122.8 μg/ml (mean, 80.2 μg/ml) and the area under the concentration-time curve (AUC) was 699 μg·h/ml (cf. Table 4). For the rectal formulations, CRO concentrations were not detected until 45 min postdosing without an absorption enhancer but were generally measurable at 10 min postdosing when CRO was combined with Na-CDC (0 to 125 mg Na-CDC per suppository). The time to C_{max} (T_{max}) generally occurred at about 1 h postdosing whatever absorption enhancer dose was used. AUC and bioavailability values increased with the dose of Na-CDC in a nonlinear dose-related manner, with the highest absorption of 18.1% being achieved with the highest dose of Na-CDC tested, which corresponded to a 4:1 CRO/Na-CDC ratio. In view of the results obtained in baboons, higher ratios of Na-CDC were not tested. The addition of Capmul MCM-90 to Na-CDC did not significantly improve the pharmacokinetic parameters.

TABLE 5 Pharmacokinetic parameters obtained with suppository formulation in rabbits[b]

Characteristic or parameter	Value	Value for the following route of administration:	
		Rectal	Intravenous
Characteristics of the protocol			
Amt of Na-CDC (mg)/suppository		125	0
No. of animals		5	6
Pharmacokinetic parameters			
C_{max} (μg/ml)	Mean	28	203[a]
	RSD (%)	82	34
T_{max} (h)	Mean	0.10	
	RSD (%)	67	
AUC (μg·h/ml)	Mean	56	281
	RSD (%)	43	33
β (h^{-1})	Mean	0.26	0.35
	RSD (%)	23	11
$t_{1/2}$ (h)[a]	Mean	2.7	2.0
	RSD (%)	26	10
Bioavailability (%)	Mean	19.9	
	RSD (%)	40	

[a]Recalculated to the arithmetic mean.
[b]The protocol involved rabbits receiving CRO at 20 mg/kg of body weight and was performed at the University of Bordeaux. RSD, relative standard deviation.

New rabbit bioavailability study of the same formulation used for human testing. Roche also evaluated the bioavailability of a fatty suppository formulation in humans. The recent replication of this reference formulation in our rabbit model made it valuable to compare any number of new formulations against the reference, with the knowledge that this reference gave significant bioavailability in humans. A rabbit study was undertaken at the University of Bordeaux with this objective.

Table 5 provides the absolute bioavailability results recently obtained in rabbits using the rectal formulation (4:1 CRO/Na-CDC) in a glyceride suppository base (Suppocire AML, equivalent to Witepsol H15) for comparison with prior human bioavailability data obtained with the exact same formulation.

The analysis of rabbit i.v. profiles showed a mean initial concentration (concentration at time zero [t_0]) of 203 $\pm$ 70 μg/ml (Table 5). The elimination rate constant (β) was 0.35 $\pm$ 0.04 h^{-1} and led to a mean elimination half-life of 2.0 $\pm$ 0.2 h. The mean AUC from time zero to infinity (AUC_0^∞)was 281 $\pm$ 92 μg·h/ml. Following rectal administration, a rabbit mean maximal plasma concentration (C_{max}) of 28 $\pm$ 23 μg/ml was obtained within 6 $\pm$ 4 min. The elimination rate constant determined from data obtained after administration by the rectal route was low compared to that determined from data obtained after administration by the i.v. route (0.26 $\pm$ 0.06 h^{-1} versus 0.35 $\pm$ 0.04 h^{-1}) and led to a mean elimination half-life of 2.7 $\pm$ 0.7 h. An AUC_0^∞ of 56 $\pm$ 24 μg·h/ml by the rectal route provides a bioavailability of 19.9% $\pm$ 8.4% of that obtained by the i.v. route.

DISCUSSION

Most third- and fourth-generation cephalosporins are administered by injection twice or thrice daily (26). CRO possesses a broad spectrum of antibacterial activity, is very effective against common bacteria causing sepsis and other clinically important infections, and is unique among third-generation cephalosporins in possessing an unusually prolonged elimination half-life which allows for once daily dosing (14, 27). These special characteristics of CRO make the drug more useful than other cephalosporins for community-based single daily treatment of serious invasive infections. Clinical efficacy is dependent on the free drug concentrations in serum, and in critically ill patients, where it is necessary to maximize killing throughout the dosing regimen, a longer time with concentrations above the breakpoint concentration of the organism is vital (28–31). Therefore, in our development of efficacious rectal CRO formulations,

both rapid absorption and higher bioavailability are critical parameters. The rabbit model was recently developed at the University of Bordeaux to screen new rectal formulations for comparison with the suppository reference formulation previously tested by Roche in humans. The purpose was to select the most bioavailable formulation for further human pharmacokinetic assessment based on the rabbit bioavailability results.

In all Roche studies (preclinical and clinical), CRO showed low permeation and therefore required an absorption enhancer. The absorption of rectal CRO in the absence of an absorption enhancer achieved only 4.5% bioavailability in rabbits, 3.8% in baboons, and 6.5% in humans. The use of the absorption enhancers Na-glycocholate and Capmul MCM-90 increased bioavailability to over 50% in rabbits, thus justifying further work in primates and humans. When biliary salts (Na-CDC) showed 69% bioavailability in baboons (4:1 CRO/Na-CDC ratio) with good tolerability, human bioavailability was tested with and without biliary salts as rectal and enteral treatments (Table 3). The Roche choice of a biliary salt derivative as the absorption enhancer for CRO is in line with the findings of recent studies, where an oral drug carrier derived from deoxycholic acid enhanced CRO bioavailability in monkeys (24). A lack of compatibility of Capmul MCM-90 with CRO was evidenced (32), confirming the choice of Na-CDC as an absorption enhancer.

The target of the Roche clinical program was to develop a nonparenteral (e.g., rectal, oral) dosage form of CRO. The Roche human studies had clear results favoring rectal over oral administration (the relative rectal bioavailability compared to oral bioavailability was 287%). Investigations of the absorption enhancer dose showed that rectal absorption improved in a nonlinear dose-related manner without modifying $t_{1/2}$ or T_{max} but achieved plasma concentrations above the breakpoint concentration (of 4 μg/ml used at the time of the study) for several hours. Given the current classification of levels below 1 μg/ml being sensitive (10), the kinetic profiles (data not shown) indicate that plasma concentrations remain above the 1-μg/ml current breakpoint throughout the 24-h administration interval and, therefore, until the next treatment dose. Neither granulometric modifications of Na-CDC, the melting point of suppository base excipients (data not shown in detail), nor the addition of another absorption enhancer at a lower dose of Na-CDC significantly influenced rectal CRO bioavailability. Thus, among the bioavailability improvement strategies tested by Roche, Na-CDC alone emerged as the most promising absorption enhancer, with the highest dose tested (125 mg) achieving 18.1% bioavailability at a 1-g CRO dose and 23.8% bioavailability at a 0.5-g CRO dose, with the difference between these results being attributed to the nonlinear protein binding of CRO. The absorption enhancer effect could be explained by the fact that bile salts are amphiphilic and the polar and nonpolar domains are separated along the longitudinal axis of the bile acid molecules (24). CRO is hydrophilic and has a low octanol/water partition coefficient (log K; which is -2.10 ± 0.19) (6). Bile salts are ionically complexed with CRO to enhance its absorption (24).

The considerable work done by Roche in the mid to late 1980s in the development of extravascular therapeutic options for CRO was the starting point of our work in 2015. Roche data from studies in humans and our data from studies in rabbits provide a calibrator for estimating human bioavailability because the same formulation was tested in rabbits and adults. The bioavailability of 18.1% in adults obtained by Roche was achieved with a dose of 0.5 g and provided a C_{max} of 21 μg/ml at 1 h postdose when ceftriaxone was combined with 125 mg Na-CDC absorption enhancer. Plasma levels of over 2 μg/ml were observed for at least 12 h postdose. Both of these concentrations are manyfold greater than or equal to the breakpoint for many pathogens.

The formulation tested in Roche preclinical and clinical studies was a fatty suppository which was unstable under zone IVa hot humid/tropical zone conditions required for testing stability (33), and this weakness underpinned our development of alternative rectal formulations that would be stable and therefore more practical in the tropics. Our recent bioavailability results with these alternative formulations, all with an absorption

enhancer, confirmed that rabbit plasma concentrations were within the same range as those obtained earlier with the Roche 125-mg Na-CDC formulation, suggesting that we would likely be able to confirm the Roche human bioavailability results in future work.

The usual parenteral adult dose used as the reference because it reaches the systemic circulation immediately is 2 g, and the pediatric intravenous dose is 25 or 37.5 mg/kg of body weight for serious miscellaneous infections or 50 mg/kg for meningitis with or without a loading dose. The age-dependent kinetics of ceftriaxone have been studied for most infections and consistently show that 1- to 8-day-old sick infants have a substantially slower clearance (18.6 $\pm$ 6.9 h) than children 1 to 12 months of age (7.2 $\pm$ 3.2 h) or adults (7.3 $\pm$ 1.6 h), because of their poorly developed renal and hepatic clearance pathways at birth (19). Along with high peak concentrations in 1- to 8-day-old newborns, there is a higher free fraction of ceftriaxone at bactericidal levels for most common pathogens that lasts 24 h in serum and tissue and for 12 h in cerebrospinal fluid. If comparable levels and an extended duration of antimicrobial activity are documented with rectal administration in humans, this would favor single daily rectal administration and might even be curative in neonatal sepsis.

Conclusion. These data provide the first published information on the rectal bioavailability of CRO in several animal species and in humans. They indicate that CRO achieves as much as 24% relative rectal bioavailability in adult humans only when formulated in association with an absorption enhancer.

The pharmacokinetics of intravenous CRO have been studied extensively in human subjects of different ages, and its bioavailability varies with age. The age-associated changes result from the improvements in renal and hepatic function that occur in the first 2 weeks of life (18). The time above the breakpoint is the most relevant pharmacodynamic outcome predictor (29–31), and although this depends on acute-phase pharmacokinetics and the pathogen, our objective has been the development of a rectal formulation which can achieve reliable absorption and a long time above the breakpoint concentration for likely invading pathogens in neonates.

The good rectal bioavailability shown in recent preclinical and earlier human studies favors the probability that one of our rectal formulations may achieve therapeutic concentrations in the target population of critically ill neonates. These results are a proof of concept for the rectal administration of CRO with absorption enhancers. For further pharmaceutical development, a target product profile, defined with the World Health Organization (WHO), will consist of a low-cost, stable, CRO rectal formulation that could achieve drug concentrations above the breakpoint for an extensive portion of the dosing interval among neonates with sepsis.

MATERIALS AND METHODS

Materials. Ceftriaxone disodium 3.5 hydrate pharmaceutical raw material was supplied by Roche Chemical Research, Basel, Switzerland. All excipients were of pharmaceutical grade. All solvents and high-performance liquid chromatography (HPLC) materials were of analytical grade. *In vivo* studies used medical equipment provided by medical suppliers.

Ethics. Ethical guidelines in force at the time of the studies were used for all *in vivo* investigations. The details are given in each paragraph according to the species used for *in vivo* studies.

Formulations. In the mid-1980s, over 30 research formulations were tested by F. Hoffmann-La Roche in rabbits to screen various enhancers (bile salts as sodium glycocholate, glycerides as Capmul MCM-90, fatty acids as caprilic acid) alone or in combination. The preparations for absorption enhancer screening were made as rectal suspensions, hard gelatin capsules for rectal administration, and suspensions for oral administration (Table 1). The bile salts (Na-CDC, Na-deoxycholate, Na-taurocholate, Na-glycocholate, Na-taurodeoxycholate, and Na-ursodeoxycholate) were further tested as absorption enhancers for CRO in baboons, with rectal suppositories being prepared by conventional methods of fusion, i.e., inclusion of powders into a premelted suppository base under stirring and then pouring of the melted blend into molds. Metal molds of different sizes were used, and Witepsol H15 was used as the suppository base (Table 2).

The influence of the Na-CDC quantity and particle size on the rectal absorption of CRO was examined in human subjects. Suppositories of 2 g with 500 mg CRO prepared with 0.0, 15.0, 30.0, 62.5, or 125.0 mg of Na-CDC and Witepsol H15 at 1.400 g, 1.385 g, 1.370 g, 1.3375 g, and 1.275 g, respectively, were prepared as described above. The effect of Na-CDC on CRO absorption was also evaluated in humans using Witepsol H15 suppositories and oral suspensions. The formulations (Table 3) consisted of 500 mg CRO using intravenous injections, 500 mg CRO, 125 mg Na-CDC, and 1.275 g Witepsol H15 for rectal administration, and 500 mg CRO, 250 mg Na-CDC, and 1.150 g Witepsol H15 for oral administration in

humans. Smaller Na-CDC quantities associated or not with Capmul MCM-90 were also tested (Table 4) and compared to the i.v. administered control.

Formulations for rectal administration in rabbits prepared at the University of Bordeaux used 20% CRO, 5% Na-CDC, and 75% Suppocire AML. Mixtures were prepared with conventional fusion methods. Samples were molded in a 1-ml syringe. After complete cooling at room temperature followed by refrigeration (4°C), the mass of the suppository (and, hence, the dose of CRO administered) was adjusted to each individual animal's body weight, sealed within aluminum-aluminum blisters, and refrigerated at 4°C until rectal administration. Suppositories were taken out of the refrigerator 30 min prior to administration for insertion at room temperature. The intravenous reference formulation was the commercially available Rocephin i.v. solution (Table 5).

***In vivo* studies. (i) Rabbit experiments at the University of Bordeaux.** In 2013, animal experiments were performed in the animal laboratory of the University of Bordeaux, DETERCA (French national authorization no. A33-0.63-235), after obtaining national experiment authorization, including ethical committee agreement. Male New Zealand White adult rabbits (Eurolap, Gosné, France) were used. The rabbits were acclimatized for at least 1 week in a quarantine room, and 24 h before the beginning of the experiment (day −1), the rabbits were fasted and weighed but had water *ad libitum*. On the day of the experiment, rabbits were placed in restraining cages in the experiment room. One 24-gauge catheter was placed on the ear vein of each animal for blood sampling. The rectum was emptied of feces using a urinary catheter. Groups of 6 animals were administered a dose of 20 mg CRO/kg, individually adjusted to the weight of each rabbit, via either the rectal or the i.v. route. The drug was administered rectally using a homemade device (a non-sharp-edged cut syringe). For intravenous administration, the CRO solution was extemporaneously reconstituted on day 0 of treatment by adding 10 ml of water for injection to 1 g of CRO powder (Rocephin; Roche). The final CRO concentration was 100 mg/ml. Then, the injection volumes were taken out with a 1-ml syringe, and the CRO solution was intravenously administered as a slow injection (1 min) via a catheter placed in the ear vein of the ear opposite the blood sample collection site. The time of midinjection was considered t_0. Blood samples of approximately 1 ml (at t_0), 1.5 ml (from 5 to 60 min), or 2 ml (from 120 to 1,440 min) were taken by opening the catheter and collecting freely flowing blood in 4-ml lithium heparinized tubes at t_0 and 5, 15, 30, 45, 60, 120, 240, 480, and 1,440 min. Samples were immediately centrifuged at 2,500 rpm for 10 min. Plasma was placed in two 1.5-ml Eppendorf tubes, one of which was used for HPLC analysis and the other of which was used as a backup, and immediately frozen at −20°C. To avoid plugging by blood coagulation, 0.5 ml sterile heparinized (125 IU/ml) saccharose (1.5-mg/ml) solution was placed into the catheter. After the blood sampling at 4 h, the rabbits were removed from their restriction boxes and were let free in housekeeping cages, but their ears were taped together with Scotch tape in order to protect the catheter. Then, at 8 h, they were placed back in their restriction boxes to take blood samples. Thereafter, the catheters were removed and the rabbits were placed back in the housekeeping cages overnight. For the last sample (24 h), a new catheter was placed in the ear vein. At the end of blood sampling, the rabbits were sacrificed by lethal injection via the catheter, using 3 ml of 200-mg/ml sodium pentobarbital after a 0.3-ml injection of acepromazine (Calmivet; Vetoquinol).

(ii) Rabbit experiments by Roche. The rectal absorption of CRO was determined in male and female adult New Zealand White rabbits (weight, 3 kg) supplied by Hare (Hewitt, NJ) or Dutchland (Denver, PA, USA) in accordance with internal ethical standards for animal experimentation in force at the time of the study. The animals were fasted for 48 h prior to the administration of the drug at a dose of 20 mg/kg. One capsule was placed into the rabbit's rectum and inserted with the plunger of a 1-ml syringe. For nonencapsulated forms, the appropriate volume of each preparation was administered into the rabbit's rectum using a 1-ml syringe attached to a 6-cm conical tip. The syringe was removed from the tip, filled with air, and reattached to the tip, and the air was pushed through to ensure delivery of the drug into the rectum. One-milliliter syringes fitted with 25-gauge needles attached were rinsed with heparin and were used to withdraw approximately 0.5 to 1 ml of blood from the main artery of the ear. The blood samples were obtained at 0 min (prior to rectal administration) and 5, 30, 60, 120, 240, and 360 min following rectal administration. Samples were collected in 3-ml heparinized Vacutainer tubes and centrifuged at 3,200 rpm for approximately 6 min. The plasma was withdrawn and frozen at −20°C until analysis.

(iii) Baboon experiments by Roche. Baboon studies were performed by Roche using mature adult male and female baboons (*Papio annubis*; weight, 12 to 30 kg) obtained from and housed at the Charles River Research Primate Corporation, Port Washington, NY, USA. All handling of the animals, dosing, and bleeding were done by personnel at the Charles River Research Primate Corporation in accordance with internal ethical standards for animal experimentation in force at the time of the study.

Prior to the rectal administration of the drug, adult baboons were fasted for 24 h. The baboons (n = 4) were sedated with ketamine hydrochloride by intramuscular injection (5 to 10 mg ketamine per kg of body weight) prior to i.v. drug administration. Five milliliters of an aqueous solution of CRO at 20 mg/kg was injected into the superior saphenous vein on the back of the leg at t_0 using a 1-ml disposable tuberculin syringe and a 26-gauge needle.

A suppository was administered postsedation with the use of a glass rod. The rectum was taped shut for 20 min to prevent expulsion or leakage of the suppository mass. Blood samples were obtained from the femoral region at 0 min (prior to drug administration) and at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480, and 1,440 min following intravenous administration. The sampling schedule was 0 min (predose) and 5, 30, 60, 120, 240, 360, 480, 600, 720, and 1,440 min following rectal administration. One-milliliter blood samples were withdrawn with a preheparinized 3-ml syringe fitted with a 22-gauge

needle and were placed into heparinized 1.5-ml Eppendorf centrifuge tubes. The samples were centrifuged at 12,000 rpm for 1 min, and the plasma was withdrawn and frozen until analysis via bioassay.

(iv) Human experiments by Roche. Human volunteer studies were performed by Roche between 1985 and 1988 to determine the human absorption of those formulations which had proven best in improving absorption in primates. In all human studies, the subjects were required to be in good general health, as determined by the findings of their baseline history, physical examination, and laboratory examination. All clinical protocols (protocols N2998A, N3119C, and N3025B) were approved by the local institutional review board, and written, informed consent was obtained from each subject prior to participation in the study (Tables 3 and 4).

In the first study (Johns Hopkins Hospital, Baltimore, MD, 1985, protocol N2998A), only Na-CDC and Witepsol H15 were chosen as absorption enhancers after results were available from studies with baboons. Pharmacokinetics were examined in a three-way, open-label, crossover study using six healthy adult male subjects and a single 500-mg dose of CRO given intravenously, rectally, or enterally with a washout period of at least 2 days between doses. Alcohol and other medications were not permitted. A single rectal CRO dose of 500 mg was prepared with 125 mg Na-CDC and 1.275 g Witepsol H15, whereas the oral formulation used 250 mg Na-CDC and 1.15 g Witepsol H15 (Table 3). The intravenous dose was infused over 30 min, and oral administration was achieved by inserting the suppository into the duodenum using a pediatric endoscope. The subjects reported to the inpatient Clinical Research Unit by 4 p.m. on the day before the initiation of the study. They fasted from midnight until noon of the day of drug administration, when lunch was served. The subjects refrained from smoking on the morning of drug administration. They were evaluated to be eligible on the basis of clinical criteria and the findings of hematology, blood chemistry analysis, and urinalysis. Prior to drug administration, a heparin lock was inserted for blood sampling. Plasma was obtained at t_0 (predose, control) and 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 360, and 480 min postdosing and analyzed by HPLC.

A second human study (Johns Hopkins Hospital, Department of Clinical Pharmacology, Baltimore, MD, 1985, protocol N3119C) was an open-label, 3-way, randomized study in which 6 healthy male volunteers were allocated to 3 regimens (all rectal) with a washout period of at least 2 days between each dose. The influence of the Na-CDC quantity on the rectal absorption of CRO was examined (Table 4) using suppositories of 500 mg CRO and CRO/Na-CDC ratios of 4:1, 8:1, 16:1, and 32:1 (i.e., 125, 62.5, 30, and 15 mg of Na-CDC, respectively). Suppositories containing 500 mg CRO were prepared with 62.5 mg coarse Na-CDC and 1.34 g Witepsol H15, 15 mg coarse Na-CDC plus 1.38 g Witepsol H15, or 15 mg fine Na-CDC plus 1.38 g Witepsol H15. All subjects reported to the inpatient Clinical Research Unit 12 h before dosing and fasted, except for water, from midnight until 4 h after each treatment. Volunteers were evaluated to be eligible on the basis of clinical criteria and the findings of hematology, blood chemistry analysis, and urinalysis. Samples were taken at t_0 (predose, control) and 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480, 600, 720, 1,440, and 2,880 min after the initiation of drug administration. Samples were immediately centrifuged, and plasma was transferred to labeled vials and stored at −17°C until analysis.

In a third human study (Johns Hopkins Hospital, Department of Clinical Pharmacology, Baltimore, MD, 1986, protocol N3025B), 5 different formulations of 500 mg of CRO were given rectally with Na-CDC and Witepsol H15. The bioavailability obtained by rectal administration was compared with that obtained by intravenous infusion in a 6-way, open-label, crossover study using six healthy adult male volunteers with a washout period of at least 2 days between doses. The subjects reported to the inpatient Clinical Research Unit 12 h before dosing, fasted, except for water, from midnight until 4 h after each treatment, and remained on-site for the duration of the study. As before, they were evaluated to be eligible on the basis of clinical criteria and the findings of hematology, blood chemistry analysis, and urinalysis. Subjects were sampled at t_0 (predose, control) and 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480, 600, 720, 1,440, and 2,880 min after the initiation of drug administration. Plasma was separated in a cooled centrifuge and frozen and stored in a freezer at −17°C until analysis.

HPLC method for plasma analysis for levels of ceftriaxone. (i) Analysis of rabbit plasma at the University of Bordeaux. The HPLC system (SpectraSystem) consisted of a solvent delivery pump (P1000), an autosampler (AS3000), a diode array detector (SN4000), and a vacuum degasser (Biotech AB). The software used to control the HPLC chain was Chromeleon, update 7.1.2. The method was based on ion-pairing reverse-phase liquid chromatography with a stationary phase of YMC C_{18} on a 150- by 4.6-mm column with 4-μm particles and a YMC C_{18} 10- by 4.6-mm guard column with 4-μm particles and an associated thermostat set at 20°C. The mobile-phase flow rate was 1.0 ml/min, and the composition was 40% acetonitrile and 60% aqueous phase. The final concentrations in the mobile phase of the buffer were 15 and 15 mM for hexadecyltrimethylammonium bromide and phosphate buffer (KH_2PO_4, K_2HPO_4, pH 7.5), respectively. Analyses were performed at 20°C, and the UV detector was set at 240 nm.

CRO was extracted from 200 μl-plasma samples by addition of 400 μl of acetonitrile for protein precipitation, followed by addition of 400 μl dichloromethane to remove the acetonitrile and lipidic components of the matrix. After centrifuging, the organic phase was discarded and 5 μl of the remaining aqueous phase was injected into the chromatograph. The lower limit of HPLC sensitivity was equal to 1.5 μg/ml. The interassay and intra-assay precisions for a concentration range of 1.5 to 150 μg/ml were ±15.9% ($n = 15$) and ±3.9% ($n = 15$), respectively. Extraction recovery was 100.1% for a concentration range of 2 to 200 μg/ml. Total plasma concentrations were obtained.

(ii) Analysis of human, baboon, and rabbit plasma by Roche. The concentrations of total CRO were determined by a modified chromatographic method described by Cuisinaud et al. (34). The HPLC system consisted of a model 6000 A pumping system, an automatic sample injector (model 710A), and a model 440 dual-channel UV detector at 270 nm (Waters Associates, Milford, MA). The method was

based on ion-pairing reverse-phase liquid chromatography with a stationary-phase 30-cm by 4.6-mm (inside diameter [i.d.]) stainless steel column prepacked with 10 μm Chromegabond C_{18} (E.S. Industries, Marlton, NJ). A hand-packed stainless steel guard column (2 cm by 3.9 mm [i.d.]) containing 15 to 40 μm Chromegabond C_{18} (ES Industries) was placed directly before the analytical column. The mobile-phase flow rate was at 2.0 ml/min, and the composition was 60% acetonitrile and 40% aqueous phase. The final concentrations in the mobile phase of the buffer were 10 and 50 mM for hexadecyltrimethylammonium bromide and phosphate buffer (KH_2PO_4, K_2HPO_4, pH 7.0), respectively. The analyses were performed at room temperature. The UV detector was set at 270 nm.

CRO was extracted from 250-μl plasma samples by addition of 750 μl of water and 2,000 μl of acetonitrile for protein precipitation. After shaking for 10 min and centrifuging, the organic phase was discarded and 50 μl of the retained aqueous phase was injected into the chromatograph. The lower limit of HPLC sensitivity was 1.0 μg/ml. The interassay and intra-assay limits for a concentration range of 2 to 200 μg/ml were $\pm$2.6% ($n = 15$) and $\pm$4.7% ($n = 15$), respectively. Extraction recovery was 101.6% for a concentration range of 2 to 200 μg/ml.

(iii) CRO stability in frozen plasma at the University of Bordeaux. Our preliminary stability results for CRO at 150 μg/ml in plasma at -20°C showed that CRO recovery was 101.9% at 1 week, 96.8% at 1 month, and 93.4% at 2 months. The results were in line with those in the literature, where CRO was found to be stable in serum for 85 and 89 days at -20 and -40°C, respectively (35). Therefore, for this study, all plasma samples were analyzed before 1 month.

Pharmacokinetic data analysis (Roche and the University of Bordeaux). The maximal concentration (C_{max}) and the time to reach the maximal concentration (T_{max}) were taken directly from the concentration-time profiles, whereas the area under the concentration-time curves (AUC) were calculated using the trapezoidal rule. The percent bioavailability was calculated by using the following formula: [(AUC rectal or enteral/AUC i.v.) $\times$ (dose i.v./dose rectal or enteral)] $\times$ 100, where AUC rectal or enteral is the area under the concentration-time curve for the rectal or oral data, respectively; AUC i.v. is the area under the concentration-time curve for the intravenous data; dose i.v. is the dose used for the i.v. route; and dose rectal or enteral is the dose used for the rectal or enteral route, respectively. The half-life ($t_{1/2}$) was defined as the time necessary for the blood concentration to decrease to 50% of the initial value and was determined on the elimination part of the plasma profile. The elimination rate constant (β) was obtained as follows: $\beta = \ln(2)/t_{1/2}$.

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The authors alone are responsible for the views expressed in this publication, and they do not necessarily represent the decisions, policy, or views of the WHO.

REFERENCES

1. Bhutta ZA, Darmstadt GL, Hasan BS, Haws RA. 2005. Community-based interventions for improving perinatal and neonatal health outcomes in developing countries: a review of the evidence. Pediatrics 115:519–617. https://doi.org/10.1542/peds.2004-1441.
2. Obiero CW, Seale A, Berkley J. 2015. Empiric treatment of neonatal sepsis in developing countries. Pediatr Infect Dis J 34:659–661. https://doi.org/10.1097/INF.0000000000000692.
3. Seale AC, Obiero CW, Berkley J. 2015. Rational development of guidelines for management of neonatal sepsis in developing countries. Curr Opin Infect Dis 28:225–230. https://doi.org/10.1097/QCO.0000000000000163.
4. Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen JA, Klugman K, Davies S. 2016. Access to effective antimicrobials: a worldwide challenge. Lancet 387:168–175. https://doi.org/10.1016/S0140-6736(15)00474-2.
5. Beam TR. 1985. Ceftriaxone: a beta-lactamase-stable, broad-spectrum cephalosporin with an extended half-life. Pharmacotherapy 5:237–253. https://doi.org/10.1002/j.1875-9114.1985.tb03423.x.
6. Lee S, Kim SK, Lee DY, Chae SY, Byun Y. 2006. Pharmacokinetics of a new, orally available ceftriaxone formulation in physical complexation with a cationic analogue of bile acid in rats. Antimicrob Agents Chemother 50:1869–1871. https://doi.org/10.1128/AAC.50.5.1869-1871.2006.
7. Beskid G, Christenson J, Cleeland R, DeLorenzo W, Trown P. 1981. In vivo activity of ceftriaxone (Ro 13-9904), a new broad-spectrum semisynthetic cephalosporin. Antimicrob Agents Chemother 20:159–167. https://doi.org/10.1128/AAC.20.2.159.
8. Mulhall A, De Louvois J, James J. 1985. Pharmacokinetics and safety of ceftriaxone in the neonate. Eur J Pediatr 144:379–382. https://doi.org/10.1007/BF00441782.
9. Steele RW, Eyre LB, Bradsher RW, Weinfeld RE, Patel IH, Spicehandler J. 1983. Pharmacokinetics of ceftriaxone in pediatric patients with meningitis. Antimicrob Agents Chemother 23:191–194. https://doi.org/10.1128/AAC.23.2.191.
10. European Committee on Antimicrobial Susceptibility Testing. 2018. Testing breakpoint tables for interpretation of MICs and zone diameters. European Committee on Antimicrobial Susceptibility Testing.
11. Theriaque. 2018. Theriaque, French public medical database. www.theriaque.org. Accessed 23 July 2018.

12. Owens HM, Dash AK. 2003. Ceftriaxone sodium: comprehensive profile. Profiles Drug Subst Excip Relat Methodol 30:21–57. https://doi.org/10.1016/S0099-5428(03)30002-4.
13. Abu TMM, Ghithan J, Abu-Taha MI, Darwish SM, Abu-Hadid MM. 2014. Spectroscopic approach of the interaction study of ceftriaxone and human serum albumin. J Biophys Struct Biol 6:1–12. https://doi.org/10.5897/JBSB2013.0045.
14. Richards DM, Heel RC, Brogden RN, Speight TM, Avery GS. 1984. Ceftriaxone. A review of its antibacterial activity, pharmacological properties and therapeutic use. Drugs 27:469–527. https://doi.org/10.2165/00003495-198427060-00001.
15. McNamara PJ, Gibaldi M, Stoeckel K. 1983. Volume of distribution terms for a drug (ceftriaxone) exhibiting concentration-dependent protein binding. Eur J Clin Pharmacol 25:399–405. https://doi.org/10.1007/BF01037955.
16. Patel IH, Chen S, Parsonnet M, Hackman MR, Brooks MA, Konikoff J, Kaplan SA. 1981. Pharmacokinetics of ceftriaxone in humans. Antimicrob Agents Chemother 20:634–641. https://doi.org/10.1128/AAC.20.5.634.
17. Stoeckel K, Mcnamara PJ, Brandt R, Plozza-Nottebrock H, Ziegler WH. 1981. Effects of concentration-dependent plasma protein binding on ceftriaxone kinetics. Clin Pharmacol Ther 29:650–657. https://doi.org/10.1038/clpt.1981.90.
18. Hayton WL, Stoeckel K. 1986. Age-associated changes in ceftriaxone pharmacokinetics. Clin Pharmacokinet 11:76–86. https://doi.org/10.2165/00003088-198611010-00005.
19. Schaad UB, Stoeckel K. 1982. Single-dose pharmacokinetics of ceftriaxone in infants and young children. Antimicrob Agents Chemother 21:248–253. https://doi.org/10.1128/AAC.21.2.248.
20. Schaad UB, Hayton WL, Stoeckel K. 1985. Single-dose ceftriaxone kinetics in the newborns. Clin Pharmacol Ther 37:522–528. https://doi.org/10.1038/clpt.1985.82.
21. Karachalios G, Charalabopoulos K. 2002. Biliary excretion of antimicrobial drugs. Chemotherapy 48:280–297. https://doi.org/10.1159/000069712.
22. Beskid G, Unowsky J, Behl CR, Siebelist J, Tossounian JL, McGarry CM, Shah NH, Cleeland R. 1988. Enteral, oral, and rectal absorption of ceftriaxone using glyceride enhancers. Chemotherapy 34:77–84. https://doi.org/10.1159/000238551.
23. Cho SW, Lee JS, Choi SH. 2004. Enhanced oral bioavailability of poorly absorbed drugs. I. Screening of absorption carrier for the ceftriaxone complex. J Pharm Sci 93:612–620. https://doi.org/10.1002/jps.10563.
24. Jeon O-C, Hwang SR, Al-Hilal TA, Park JW, Moon HT, Lee S, Park JH, Byun Y. 2013. Oral delivery of ionic complex of ceftriaxone with bile acid derivative in non-human primates. Pharm Res 30:959–967. https://doi.org/10.1007/s11095-012-0932-0.
25. Mulhall A. 1985. Antibiotic treatment of neonates—does route of administration matter? Dev Pharmacol Ther 8:1–8. https://doi.org/10.1159/000457016.
26. Bijie H, Kulpradist S, Manalaysay M, Soebandrio A. 2005. In vitro activity, pharmacokinetics, clinical efficacy, safety and pharmacoeconomics of ceftriaxone compared with third and fourth generation cephalosporins: review. J Chemother 17:3–24.
27. Pacifici GM. 2011. Pharmacokinetics of cephalosporins in the neonate: a review. Clinics (Sao Paulo) 66:1267–1274. https://doi.org/10.1590/S1807-59322011000700024.
28. Craig WA. 2003. Basic pharmacodynamics of antibacterials with clinical applications to the use of β-lactams, glycopeptides, and linezolid. Infect Dis Clin North Am 17:479–501. https://doi.org/10.1016/S0891-5520(03)00065-5.
29. Frimodt-Møller N. 2002. How predictive is PK/PD for antibacterial agents? Int J Antimicrob Agents 19:333–339. https://doi.org/10.1016/S0924-8579(02)00029-8.
30. Barger A, Fuhst C, Wiedemann B. 2003. Pharmacological indices in antibiotic therapy. J Antimicrob Chemother 52:893–898. https://doi.org/10.1093/jac/dkg482.
31. Mouton JW, Dudley MN, Cars O, Derendorf H, Drusano GL. 2005. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. J Antimicrob Chemother 55:601–607. https://doi.org/10.1093/jac/dki079.
32. Kauss T, Marchivie M, Phoeung T, Gaubert A, Désiré A, Tonelli G, Boyer C, Langlois M-H, Cartwright A, Gomes M, White N, Gaudin K. 2017. Preformulation studies of ceftriaxone for pediatric non-parenteral administration as an alternative to existing injectable formulations. Eur J Pharm Sci 104:382–392. https://doi.org/10.1016/j.ejps.2017.04.010.
33. ICH. ICH quality guidelines; Q1A-Q1F stability. ICH, Geneva, Switzerland. http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html. Accessed 21 September 2018.
34. Cuisinaud G, Ferry N, Seccia M, Bernard N, Sassard J. 1980. Determination of nalidixic acid and its two major metabolites in human plasma and urine by reverse-phase high performance liquid chromatography. J Chromatogr 181:399–406. https://doi.org/10.1016/S0378-4347(00)81142-1.
35. De Diego Glaría M, Moscciati GG, Ramos RG, Riquelme MM. 2003. Stability of ceftriaxone in water and cerebrospinal fluid determined by high-performance liquid chromatography. J Sep Sci 26:939–942. https://doi.org/10.1002/jssc.200301306.