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Pharmacokinetic Interaction between Itraconazole and Ceftriaxone in Yucatan Miniature Pigs

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Since ceftriaxone and itraconazole are highly protein bound, are excreted via a biliary pathway, and are in vitro modulators of the efflux pump P glycoprotein, a pharmacokinetic interaction between these antimicrobial agents can be hypothesized. Therefore, we evaluated the pharmacokinetics of itraconazole and ceftriaxone alone and in combination in a chronic model of catheterized miniature pigs. Itraconazole does not influence ceftriaxone kinetic behavior. The mean areas under the concentration-time curve (AUC) were 152.2 μg · h/ml (standard deviation [SD], 22.5) and 129.2 μg · h/ml (SD, 41.2) and the terminal half-lives were 1.1 h (SD, 0.3) and 0.9 h (SD, 0.2) when ceftriaxone was given alone and combined with itraconazole, respectively. Regarding itraconazole kinetics, ceftriaxone was shown to alter the disposition of the triazole. Contrary to what was expected, the AUC (from 0 to 8 h) decreased from 139.3 ng · h/ml with itraconazole alone to 122.7 ng · h/ml with itraconazole and ceftriaxone combined in pig 1, from 398.5 to 315.7 ng · h/ml in pig 2, and from 979.6 to 716.6 ng · h/ml in pig 3 (P of <0.01 by analysis of variance).

Itraconazole is an orally active antifungal triazole demonstrating a broad spectrum of activity; its pharmacokinetic profile in humans is characterized by variable and moderate bioavailability (<60%) after oral administration of the commercial form, high serum protein binding (99.8%), mainly albumin, and a long terminal half-life (15 to 20 h) (7, 10). Itraconazole is extensively metabolized by various pathways, mainly oxidative, into a very large number of products (10). The major metabolite in humans is hydroxyitraconazole. Fecal excretion represents 54% of an administered dose at one week after dosing (9).

Ceftriaxone belongs to the broad-spectrum cephalosporin family and exhibits a long half-life (6 to 9 h) (23) and high serum protein binding (95.3% at 25 μg/ml). Ceftriaxone is partially eliminated by the kidneys (60 to 70% of the total dose) (22). The other elimination pathway is biliary excretion of the intact drug, which is then degraded by the intestinal microflora and eliminated in the feces (1).

One phenotype of multidrug resistance (MDR) to certain anticancer agents is associated with hyperexpression of P glycoprotein (P-gp), encoded in humans by the gene *MDR*1 (20). P-gp is thought to act as a transmembrane energy-dependent efflux pump that expels the drug from the cell and decreases intracellular drug concentration and hence cytotoxic activity (12, 20). Besides in tumors, P-gp has been found to be expressed in healthy tissues and particularly in organs involved in the behavior of drugs (3, 14, 24). Hence, P-gp is located in a polarized manner on the luminal surfaces of hepatocytes and duct cells, kidney proximal cells, and enterocytes (24). The physiologic functions of P-gp remain unclear, but P-gp's specific location and properties indicate that it could be a factor that limits intestinal absorption, as well as a feature that participates in the biliary and renal secretion, of drugs (14). P-gp's expulsing function can be modulated in vitro by a large variety of unrelated drugs, such as verapamil, cyclosporin, and quinidine (4), as well as ceftriaxone (5) and itraconazole (6). Independently of their original pharmacological properties, these agents enhance the accumulation of the cytotoxic drug and thereby partially restore antitumoral activity. The modulator is believed to bind to P-gp, preventing the expulsion of the drug.

Ceftriaxone and itraconazole are likely to be combined in the treatment of patients coinfected with bacteria and fungi. Since ceftriaxone and itraconazole are highly protein bound, are partially eliminated via a biliary pathway, and are modulators of the P-gp pump, a pharmacokinetic interaction can be hypothesized.

The aim of the present study was to explore a pharmacokinetic interaction between itraconazole given orally and ceftriaxone administered intravenously in Yucatan miniature pigs. This pig has become a popular model in biomedical research due to its small size, slow growth rate, and physiological and metabolic similarities to humans (2, 18). This species displays cardiovascular, gastrointestinal, and renal systems that approximate those of humans (18). Curiously, the miniature pig remains seldom used in experimental pharmacokinetics, partially due to its maintenance requirements and the cost of the animal. In our laboratory, we have developed a chronic model of catheterized miniature pigs allowing long-term venous access and serial blood sampling (13, 15). The interaction between itraconazole and ceftriaxone was evaluated at steady state for the triazole at doses similar to those administered in clinical practice. Steady-state itraconazole concentrations are reached after 2 weeks of oral administration in humans.

Animals. The investigations were done with three healthy female adult Yucatan miniature pigs, aged about 1 year, obtained from Charles River (Saint-Aubin les Elbeuf, France). They were housed individually in loose boxes in a single room lit with daylight, fed once daily with miniature pig food (UAR, Epinay-sur-Orge, France), and given water ad libitum. Their weight averaged 33.5 kg (standard deviation [SD], 3.6 kg) and remained stable throughout the study. After an overnight fast,

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TABLE 1. Pharmacokinetic parameters of ceftriaxone alone and combined with itraconazole[a]

Pig	Ceftriaxone				Ceftriaxone and itraconazole			
	C_{max}[b] (μg/ml)	AUC (μg · h/ml)	$t_{1/2}$ (h)	CL (ml/min)	C_{max} (μg/ml)	AUC (μg · h/ml)	$t_{1/2}$ (h)	CL (ml/min)
1	233.3	127.4	1.1	130.8	257.1	106.3	1.1	156.8
2	512.5	157.9	0.8	105.5	415.2	176.8	0.8	94.2
3	309.0	171.4	1.4	96.2	279.1	104.4	0.7	159.5
Mean (SD)	351.6 (144.4)	152.2 (22.5)	1.1 (0.3)	110.8 (17.9)	317.1 (85.6)	129.2 (41.2)	0.9 (0.2)	136.8 (36.9)

[a] Ceftriaxone was administered intravenously at 1 g/12 h, and itraconazole was given orally for 3 weeks at 200 mg/day.
[b] C_{max}, maximum concentration of the drug in serum.

the swines were anesthetized with halothane and then propofol. Vascular access was established by surgical placement of a polyurethane catheter (60 cm by 2 mm) in the external jugular vein. The device exits the midline dorsal neck. The catheter was flushed every 2 days with 5 ml of a sterile normal saline and heparin solution (10 U of heparin per ml of normal saline). During catheter maintenance or pharmacokinetic studies, the conscious pig was comfortably placed in a special sling (19).

Study design. Pharmacokinetic investigations were initiated at least 3 weeks after surgery. After an overnight fast, two 100-mg itraconazole capsules (Sporanox; Janssen Laboratories, Val de Reuil, France) placed in a homemade cream bun were administered orally to the animals before a full meal for 3 weeks. During the next 5 days (day 22 to day 26), ceftriaxone (Rocéphine; Roche Laboratories, Neuilly, France) was coadministered every 12 hours by short intravenous injection through the jugular catheter at a dose of 1 g. Following a 10-day washout period, ceftriaxone (1 g) was administered as a single bolus injection (day 36). On the day of ceftriaxone pharmacokinetic studies, the injection was performed through a small catheter set previously in an ear vein. The pharmacokinetic profile of itraconazole was evaluated at the end of the third week (day 21) and 5 days later (day 26). Ten-milliliter blood samples were obtained via the jugular catheter at the following times: 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 24, and 48 h after triazole ingestion.

Ceftriaxone pharmacokinetic properties were determined on day 26 and 36. Five-milliliter blood samples were also obtained via the catheter at 0.0167, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, and 8 h after drug injection. All blood specimens were collected in glass tubes and centrifuged immediately. Serum samples were then removed and stored at −70°C until analysis.

Drug assays. (i) Chemicals. Pure specimens of itraconazole, hydroxyitraconazole, and internal standard (R 51012) were kindly supplied by Janssen Laboratories. Ceftriaxone was obtained commercially (Rocéphine). All of the solvents used were of high-performance liquid chromatography (HPLC) grade.

(ii) Ceftriaxone analysis. Ceftriaxone concentrations in serum were determined by an HPLC method, as previously described (11). Briefly, serum (250 μl) was deproteinized with 500 μl of acetonitrile. After 10 min of shaking at 28 rpm and 10 min of centrifugation at 2,000 × *g*, 3.4 ml of methylene chloride was added to the supernatant. The mixture was shaken by rotation for 10 min (28 rpm) and then centrifuged for 10 min at 2,000 × *g*. An aliquot (5 μl) of the upper layer was injected into the chromatograph. Separation was performed with a C_{18} high speed reversed-phase column (inner diameter, 70 by 4.6 mm; particle size, 3μm; Beckman).

The mobile phase mixture consisted of 24 mM hexadecyl trimethylammonium bromide–phosphate buffer (pH 7.0)–acetonitrile (45:5:50, vol/vol/vol). The spontaneous pH was 8. At a flow rate of 2 ml/min, ceftriaxone had a retention time of 2.8 min and a limit of detection of 0.1 μg/ml of serum. The precision expressed as a coefficient of variation (CV) was 2.4% (at 5 μg/ml) and 2.1% (at 150 μg/ml) for the intraday assay and 3.3% (at 5 and 150 μg/ml) for the interday assay.

(iii) Itraconazole and hydroxyitraconazole analysis. Itraconazole and hydroxyitraconazole were analyzed in serum by an HPLC method originally developed by Janssen Laboratories (25) that was modified as described previously (13). Serum samples (1 ml) were spiked with 100 μl of internal standard (0.6 μg of methanol per ml), 0.5 ml of borate buffer (pH 10), and 4 ml of a heptane-isoamylic acid mixture (90:10).

After 12 min of shaking at 28 rpm and 10 min of centrifugation at 2,000 × *g*, the upper layer was collected in tubes (first rinsed with methanol) and then dried under a stream of nitrogen at 60°C. The extraction residues were redissolved in 70 μl of HPLC elution solvent, and 50 μl was injected into the chromatograph. The chromatograph consisted of a model 126 programmable solvent delivery module (Beckman), a model 210 sample injection valve with a 50-μl loop (Beckman), and a model 166 programmable wavelength detector (Beckman). Chromatograms were processed with a Gold chromatographic data system (Beckman). The assay was carried out with a 150- by 4.6-mm (inner diameter) C_{18} column filled with 5-μm-diameter particles (Ultrasphere ODS; Beckman).

The mobile phase mixture was acetonitrile–methanol–25 mM ammonium acetate (55:25:20, vol/vol/vol). The spontaneous pH was 7.4. The flow rate was 1.2 ml/min. Retention times for itraconazole and R 51012 were 4.7 and 6.3 min, respectively. The limit of detection of itraconazole in serum was 2.5 ng/ml. The intraday CVs were 4.2% (at 20 ng/ml) and 3% (at 1,500 ng/ml). The interday CVs were 10% (at 25 ng/ml) and 1% (at 1,500 ng/ml).

Pharmacokinetic analysis. Kinetic interaction was determined by comparison of the following parameters: the area under the concentration-time curve (AUC), the terminal half-life ($t_{1/2}$), and the systemic clearance (CL).

The AUC from 0 to 8 h (AUC_{0-8}) was calculated for both drugs by using the trapezoidal rule. For itraconazole, the AUC represents the triazole exposure between two injections of ceftriaxone. The 12-h point was excluded corresponding to the peak of the following injection of the cephalosporin. The $t_{1/2}$ was estimated by linear regression analysis with computerized software (Siphar, Simed, France). The CL was calculated as the intravenous dose divided by the AUC.

Statistical analysis. The pharmacokinetic interaction between the two drugs was analyzed by analysis of variance.

(i) Ceftriaxone kinetics. The pharmacokinetic parameters of ceftriaxone given alone and in combination with itraconazole in the three pigs are presented in Table 1. Itraconazole did not influence ceftriaxone kinetic behavior, as the mean AUCs were 152.2 μg · h/ml (SD, 22.5) and 129.2 μg · h/ml (SD, 41.2), the $t_{1/2}$s were 1.1 h (SD, 0.3) and 0.9 h (SD, 0.2), and the CLs were

TABLE 2. Steady-state AUC_{0-8} of itraconazole alone and combined with ceftriaxone[a]

Pig	AUC (ng · h/ml)		Decrease (%)
	Itraconazole	Itraconazole and ceftriaxone	
1	139.3	122.7	12
2	398.5	315.7	21
3	979.6	716.6	27
Mean (SD)	505.8 (430.3)	385.0 (302.9)	24

[a] Itraconazole was given for 3 weeks as a 200-mg/day oral dose; ceftriaxone was administered intravenously at 1 g/12 h.

110.8 ml/min (SD, 17.9) and 136.8 ml/min (SD, 36.9) when ceftriaxone was given alone and with itraconazole, respectively.

(ii) Itraconazole kinetics. Table 2 displays the pharmacokinetic data for itraconazole administered alone and combined with intravenous ceftriaxone. Wide interanimal variability was observed. Regarding each concentration data point, a significant decrease ($P = 0.0001$) was evident when ceftriaxone was coadministered. The AUC_{0-8} decreased from 139.3 to 122.7 ng · h/ml in pig 1, from 398.5 to 315.7 ng · h/ml in pig 2, and from 979.6 to 716.6 ng · h/ml in pig 3. Hydroxyitraconazole was undetectable in all serum samples.

Given the kinetic properties of itraconazole and ceftriaxone and the modulating activity of both drugs on P-gp expelling function, we hypothesized the likelihood of a pharmacokinetic interaction between the triazole and the cephalosporin. More precisely, an increased retention of itraconazole could be expected (i.e., increased absorption and delayed elimination) based on the modulation of the P-gp pump by ceftriaxone. The reverse could also be expected, with itraconazole able to delay biliary excretion of ceftriaxone. P-gp has been detected at the luminal sites of enterocytes, hepatocytes, and proximal renal cells and in capillary endothelial cells of the testes and brain (3, 24). It has been suggested that P-gp could be a factor that limits intestinal absorption of vinblastine (14), pristinamycin I_A (21), and digoxin (16) and that it participates in the biliary excretion of colchicine (14) and daunorubicin (14). In addition, inhibition of the P-gp pump may be related to kinetic interactions that have been observed between doxorubicin and cyclosporin (14) or digoxin and quinidine (14).

In our study, hydroxyitraconazole was undetectable in all serum samples, confirming our previous finding (13). Hydroxyitraconazole is a minor metabolite in the miniature pig (13). High interindividual variability of the itraconazole AUC was observed. Hence, we used each animal as its own reference to compare the kinetic profile of itraconazole administered alone and in combination with ceftriaxone. The main expression of the influence of ceftriaxone on itraconazole disposition was a significant decrease of the triazole concentrations, a result contrary to what was expected. Ceftriaxone has been found to modulate the MDR1 phenotype in human sarcoma cells (5). The reversal of resistance was dose dependent and was obtained with a concentration range of 0.25 to 1 mM (i.e., 150 to 600 mg/liter). The peak concentration values obtained in the three pigs (mean, 371 mg/liter) were consistent with a modulating activity. Nevertheless, the concentration of the drug in serum rapidly decreased and the duration of modulation was perhaps not sufficient for altering itraconazole disposition. We cannot support that P-gp, in vivo, is a feature that participates in the kinetic behavior of itraconazole. Contrary to what was expected, ceftriaxone was shown to decrease itraconazole systemic exposure. The increase of bile flow after administration of ceftriaxone (for about 5 h after each dose) suggests the cephalosporin may be choleretic (8). This effect is particularly obvious after repeated administration (8). Another study has shown that ceftriaxone may increase the hepatic blood flow compared to controls (17). Itraconazole is eliminated by the bile both as a metabolite and in an unchanged form (10). Hence, the elimination of the triazole may be increased by the choleretic effect of ceftriaxone. This may partly explain the decrease of itraconazole AUC when the cephalosporin is coadministered. The clinical significance of this interaction remains to be determined.

Regarding the influence of itraconazole on ceftriaxone kinetics, the triazole was found not to alter the disposition of the cephalosporin. It has been stressed that the concentration of itraconazole obtained in the miniature pig was far lower than that required for the modulation of P-gp in vitro. Hence the potential alteration of ceftriaxone kinetics by itraconazole via inhibiting P-gp could not be established.

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