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Concentrations in Plasma and Tissue Penetration of Ceftriaxone and Ornidazole during Liver Transplantation

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Plasma and epiploic fat drug concentrations and fat penetration of ceftriaxone and ornidazole given for antimicrobial prophylaxis were studied in 11 patients scheduled for liver transplantation. Ceftriaxone (1 g) and ornidazole (500 mg) were infused during 30 min after the induction of anesthesia. Arterial blood and epiploic fat samples were collected at 30, 60, and 120 min and then every 90 min following the end of the infusion until closure of the peritoneum. Blood samples were immediately centrifuged, and plasma and fat were stored at −35°C until analysis. Ceftriaxone and ornidazole concentrations were determined by high-performance liquid chromatography. Surgery lasted 440 ± 84 min and required a mean of 9.5 units of packed erythrocytes and 13 units of fresh frozen plasma. Plasma ceftriaxone concentrations decreased from 89 ± 34 to 41 ± 16.5 μg/ml from the beginning of the operation until the time of closure of the peritoneum. Corresponding levels in epiploic fat decreased from 8.7 ± 3.3 to 4.5 ± 3.5 μg/g. Ornidazole concentrations ranged, respectively, between 8.7 ± 2.5 and 4.9 ± 1.7 μg/ml in plasma samples and 4.6 ± 1.2 and 2.5 ± 1.1. μg/g in fat samples. Rates of penetration into the omentum remained at about 9% for ceftriaxone and between 50 and 70% for ornidazole. Tissue ceftriaxone concentrations were, in all cases, greater than typical MICs for 90% for *Escherichia coli* and *Klebsiella* isolates tested (MIC_{90}s). They were insufficient in 40% of patients after 60 min with regard to the MIC_{90}s for *Staphylococcus aureus*. Tissue ornidazole concentrations were not superior to MIC_{90}s for anaerobes after 30 min in 50% of patients. These results show that a single dose of 1 g of ceftriaxone provides adequate coverage against gram-negative bacteria and that 1 g instead of 500 mg of ornidazole may provide a protective effect against anaerobes during liver transplantation. Prophylaxis against *S. aureus* and *Streptococcus faecalis* requires more specific antibiotics. Prophylaxis for patients with significant blood loss or initial severe renal or hepatic failure needs further evaluation.

Liver transplantation is a long surgical procedure lasting between 6 and 10 h. The surgical procedure requires an opening of the biliary tract to perform primary bile duct anastomosis or choledochojejunostomy. Therefore, this kind of surgery may be considered to be of class II risk with regard to postoperative infection, as defined by Polk and Lopez-Mayor (14). Abdominal surgery with a class II risk involves "clean contaminated" procedures, with which the rate of infection without prophylactic antibiotherapy ranges between 10 and 20%. Other factors, including the need for massive transfusion, the perioperative use of an extracorporeal venovenous bypass, and immunosuppressive therapy, contribute to the enhancement of this risk. For these reasons, antibiotic prophylaxis appears to be justified and is commonly used during liver transplantation. Different drug regimens, acting mainly against gram-negative aerobes, anaerobes, and sometimes *Staphylococcus aureus*, are given for 48 to 72 h postsurgery in most centers. However, their efficacy is questionable since there is a high frequency of early postoperative infections, reaching 40 to 80% in some studies (4, 7, 11, 13). The effectiveness of prophylaxis depends upon the achievement of adequate concentrations of antimicrobial agents in the tissues at risk. This activity should be maintained during the whole surgical procedure and requires an easy route of drug administration. Only a few studies evaluating the pharmacokinetics of drugs during liver transplantation are available in the literature. The aim of the present study was to evaluate plasma and epiploic fat drug concentrations obtained after a single injection of 1 g of ceftriaxone (intravenously [i.v.]) and 500 mg of ornidazole (i.v.) in patients scheduled for liver transplantation. Ceftriaxone is a cephalosporin antibiotic with good activity against gram-negative aerobes including the members of the family *Enterobacteriaceae* (3). Ornidazole is a nitroimidazole derivative commonly used for antimicrobial prophylaxis in patients undergoing gut surgery (12); it acts against anaerobes and especially *Bacteroides fragilis*. Both agents have well-known long pharmacokinetic elimination half-lives, which is valuable for long surgical procedures.

MATERIALS AND METHODS

Eleven consecutive patients with normal preoperative creatinine levels were studied. Approval from the Institutional Ethical Committee was obtained, and all patients gave informed consent. Those treated with antibiotics before the operation or who described a history of previous intolerance to the antibiotics chosen for study were excluded from the study. Single doses of 1 g of ceftriaxone (i.v.) and 500 mg of ornidazole (i.v.) were infused at a constant rate flow during 30 min after the induction of anesthesia and after the hemodynamic monitoring of the patient, including the insertion of a 7F pulmonary artery catheter and a radial artery

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catheter, had begun. Ten milliliters of arterial blood was collected in heparinized tubes at 30, 60, and 120 min and then every 90 min following the end of infusion until the time of peritoneum closure. Blood samples were immediately centrifuged (6,700 × *g*) for 10 min (E 80; Jouan, Saint-Nazaire, France). Plasma was removed and was stored at −35°C until analysis. Epiploic fat samples (1 cm^3) were taken by the surgeons at the same time periods as plasma samples were obtained and were frozen. Determinations of plasma and tissue antibiotic concentrations were performed later by high-performance liquid chromatography (HPLC).

Ceftriaxone was extracted from plasma after the addition of cefoperazone as an internal standard agent. A total of 400 μl of acetonitrile was added to 200 μl of plasma, and the mixture was vortexed for 30 s and centrifuged (15 min, 5,300 × *g*). The supernatant was collected and evaporated under nitrogen. The residue was diluted with 100 μl of water (pH 7.5 adjusted by the addition of 1 M NaOH). A total of 20 μl of the aqueous phase was injected into the column. Epiploic fat samples were processed as follows. Each sample was ground at ice-cold temperature in 4 ml of methanol and 400 μl of dichloromethane by using an automatic grinder (Ultra Turrax; Ika-Werk, Stauffen, Germany). Then, the sample was centrifuged (15 min, 5,300 × *g*). Methanol was collected and evaporated under nitrogen. The residue was washed in 500 μl of water (pH 7.5), and 1 ml of dichloromethane was added in order to eliminate the lipids. After a new centrifugation (10 min, 5,300 × *g*), 20 μl of the aqueous supernatant was collected and was injected into the column. Recoveries were 80 and 84% for plasma and fat samples, respectively.

Ornidazole was extracted from plasma on an Extrelut column. One milliliter of plasma was added to 5 μg of an internal reference agent (Ro 7-0913) and 250 μl of ammoniac (25/100). Elution of the lipophilic components was achieved with dichloromethane (5 ml). After evaporation under nitrogen, the eluate was diluted with 100 μl of a methanol-water (50/50; vol/vol) solution before injection onto the chromatography column. Epiploic fat samples were processed as follows. Tissue samples (1 g) were ground at ice-cold temperature after the addition of dichloromethane (5 ml) and 5 μg of an internal reference agent (Ro 7-0913). The mixture was frozen before collection of the organic phase. The latter was added to 5 ml of acidic water (pH 1 by the addition of 1 M HCl), and the mixture was vortexed for 10 min and centrifuged (10 min, 4,000 × *g*). The organic phase was eliminated, and the aqueous phase was diluted in 5 ml of alkalinized dichloromethane (pH 10 adjusted with 1 M NaOH). After evaporation under nitrogen, the residue was diluted with 100 μl of a methanol-water solution (50/50; vol/vol). Twenty microliters was injected onto the column. Recoveries were 95 and 62% from plasma and fat samples, respectively.

The antibiotic concentrations in plasma and omentum were assayed by HPLC with a Beckman model 112 pump (Gold system; Beckman, San Ramon, Calif.), a 100RP-18 end-capped Lichrosphere column (5 μm, 125 by 4 mm) coupled to a 100RP-18 Lichrosphere column (5 μm, 25 by 4 mm; Merck) and a UV detector (313 nm for ornidazole and 280 nm for ceftriaxone). The mobile phase used for ceftriaxone determination consisted of acetonitrile and a buffered aqueous phase titrated at pH 7.5 (23/77). For plasma samples, the within-day (10, 50, and 75 μg/ml) and between-day (10, 75, and 100 μg/ml) repeatability assays were 1.73, 1.84, and 1.78% and 10.5, 5.3, and 3.2%, respectively. For epiploic fat samples, the within-day (7.5 and 10 μg/g) and between-day (5, 7.5, and 10 μg/g) repeatability assays were 8.02 and 7.97% and 15.2, 10.5, and 7.4%, respectively. For plasma samples, the linearity between 10 and 100 μg/ml was $y = 0.033x - 0.039$ ($r = 0.99$). For tissue samples, the linearity between 5 and 20 μg/g was $y = 0.065x + 0.035$ ($r = 0.99$). The lower limit of quantification was 0.05 μg/g.

The phase for ornidazole assessment consisted of acetonitrile at a concentration of 12% (by volume) in an aqueous phase buffered at pH 3. For plasma samples (0.5, 5, and 8 μg/ml), the within- and between-day (6 days) repeatability assays were 6, 1, and 1.1% and 3.4, 1.2, and 0.6%, respectively. For tissue samples (0.5, 2.5, and 5 μg/g), the within- and between-day repeatability assays were 7.6, 4.6, and 1.6% and 15.5, 9.5, and 3.5%, respectively. For plasma samples, the linearity between 0.1 and 20 μg/ml was $y = 0.148x + 0.0037$ ($r = 0.99$). For tissue samples, the linearity between 0.1 and 1 μg/g was $y = 0.531x + 0.083$ ($r = 0.99$). The lower limit of quantification was 0.05 μg/ml.

Plasma and fat antibiotic concentrations were plotted against time. Evaluation of tissue penetration was performed by calculation of the epiploic fat to plasma concentration ratio at each time period. Results are expressed as means and ranges for demographic data and means ± standard deviations for antibiotic concentrations.

TABLE 1. Demographic data and surgical time periods

Parameter	Mean (range)
Age (yr)	44 (21–62)
Wt (kg)	67 (46–84)
Duration of operation (min)	440 (330–600)
Onset of venous bypass (min)	119 (60–180)
Revascularization time (min)	231 (150–330)
Peritoneum closure time (min)	328 (240–450)
Packed erythrocytes (units)	9.5 (3–28)
Fresh frozen plasma (units)	13 (6–32)
Crystalloids (liters)	3 (2–5)

RESULTS

The present study included 10 men and 1 woman. An extracorporeal venovenous bypass was used in all cases during the anhepatic phase, including the removal of the sick liver and the connection of the vena cava and portal vein. The mean age of the patients was 44 years, and the mean duration of the operation was 440 min. Mean blood product requirements were 9.5 units of packed erythrocytes and 13 units of fresh frozen plasma added to 3,000 ml of crystalloids. Only four patients were evaluated at 390 min (Table 1). Plasma ceftriaxone concentrations decreased by 50% from 30 to 390 min (Table 2). They remained greater than 20 μl/ml during the whole procedure. Omentum drug concentrations dropped rapidly between 30 and 60 min following the end of infusion and more slowly after 1 h. They reached values of less than 2 μg/g in only one patient. The lowest value reached in that patient was 0.8 μg/g, at the end of surgery. The penetration rate into tissue remained stable at about 9% during the procedure. The different surgical steps, including the onset of venous bypass and the revascularization of the new liver, had no effect on the decrease in plasma and fat drug concentrations.

Plasma ornidazole concentrations decreased from 9 to 5 μg/ml from the beginning to the end of the procedure (Table 2). Epiploic fat antibiotic levels remained at about 4 μg/g. However, epiploic fat drug concentrations were less than 4 μg/g in two and five patients at 30 and 60 min, respectively,

TABLE 2. Plasma and epiploic fat drug concentrations determined by HPLC at 30, 60, and 120 min and then every 90 min after the end of infusion of ceftriaxone and ornidazole during liver transplantation in 11 patients[a]

Sample time (min)	Ceftriaxone concn in:		Ornidazole concn in:	
	Plasma (μg/ml)	Epiploic fat (μg/g)	Plasma (μg/ml)	Epiploic fat (μg/g)
30	92.7 ± 35.7	8.7 ± 3.3	9.11 ± 2.57	4.64 ± 1.20
60	80.5 ± 37	5.8 ± 2.9	7.96 ± 1.43	3.98 ± 1.14
120	68.2 ± 30.7	5.3 ± 1.4	6.93 ± 1.20	4.28 ± 1.51
210	58.7 ± 25.7	4.2 ± 2.7	5.80 ± 1.73	3.70 ± 1.34
300	45 ± 19.6	3.6 ± 3.5	4.97 ± 1.67	3.25 ± 1.43
390	43.5 ± 17.3[b]	7.3[c]	5.10 ± 1.42[d]	2.48 ± 1.10[e]

[a] Ceftriaxone was administered i.v. at 1 g; ornidazole was administered i.v. at 500 mg. Values are means ± standard deviations.
[b] $n = 4$.
[c] $n = 2$.
[d] $n = 4$.
[e] $n = 3$.

after the end of the infusion. Penetration rates were stable, ranging from 50 to 70%. As for ceftriaxone, clamping and unclamping did not modify the aspect of the curves. No bacterial infections occurred in these patients within the first few postoperative days.

DISCUSSION

Prophylactic administration of antibiotics in the 2 h before surgery reduces the risk of wound infection (5, 10). Previous publications soon reported the reduction in postoperative infectious complications from 20 to 5% in surgery with a class II risk (14). However, the rate of infection established in immunocompromised liver transplant patients is very high during the first few postoperative days (4, 6, 7, 11, 13). Infection occurs despite the administration of various conventional antibiotics such as piperacillin (4), pefloxacin (4), cefotaxime (11, 13, 17), ampicillin (11), and tobramycin (13, 17), with (17) or without concomitant digestive tract decontamination. Gram-negative bacteria (*Escherichia coli*) and gram-positive bacteria (*S. aureus*) seem to be equally involved. Among the anaerobes, *B. fragilis* represents the most frequent contaminating pathogen (11, 13). These infectious complications illustrate the difficulties encountered by providing classical schemes of prophylactic antibiotherapy in new surgical fields such as liver transplantation without pharmacokinetic support. The real goal of prophylaxis is to reach and maintain blood and tissue drug concentrations at levels that allow the inhibition of growth of contaminating agents throughout the surgical procedure (2).

The present study aimed at evaluating the efficacy of our own regimen, associating ceftriaxone and ornidazole for antibiotic prophylaxis during liver transplantation. These drugs were chosen because of their known pharmacokinetic properties and their spectra of activity (3, 12, 16). Plasma and epiploic fat drug concentrations were determined until the end of the surgical procedure. No long-term study was attempted because of the specificity of the surgery, including the use of an extracorporeal venovenous bypass and the total absence of liver function after the removal of the sick liver and during the period of connection of the graft, making pharmacokinetic calculations hazardous to the patients. The administration of 1 g of ceftriaxone resulted in adequate blood and tissue drug concentrations throughout surgery, despite the well-known 95% protein binding of ceftriaxone. Plasma and epiploic fat drug levels were effective at all time periods and in all patients to cover against *E. coli* and *Klebsiella* species (MIC for 90% of isolates tested, 0.1 μg/ml), which are frequently involved in postoperative infections (9). This could be explained in part by an increase in the amount of free drug activity as albumin concentrations decreased in these cirrhotic patients. However, the lack of adequate coverage against *S. aureus* (MIC for 90% of isolates tested, 4 μg/ml) may require the use of additional, more specific antibiotics such as vancomycin, which consequently was added to the protocol in other patients. Epiploic fat ornidazole concentrations were too low in the majority of patients to cover anaerobes (1), especially *B. fragilis* (MIC for 90% of isolates tested, 4 μg/ml). These results led us to increase the initial infused dose to 1 g instead of 500 mg.

Notwithstanding the previous remarks, ceftriaxone and ornidazole, with their well-known long half-lives and their constant penetration rates, appear to be of interest for use as prophylaxis during liver transplantation. The penetration rates found in the present study are similar to those described in other studies during classical gut surgery (12). However, our results might also take into account the fact that perioperative conditions during liver transplantation may modify blood and/or tissue drug concentrations. As demonstrated previously, plasma volume and tissue vascularization are modified by vascular clampings, abnormalities in tissue oxygenation (15), and fluid replacement. Dupon et al. (8) reported inefficient concentrations of piperacillin in plasma when piperacillin was administered at an initial dose of 4 g and then repeated injections of 2 g every 4 h in patients scheduled for liver transplantation without extracorporeal venovenous bypass. Dosing regimens for patients with massive blood losses, a long duration of surgery, and initial renal or hepatic failure require further evaluation. Moreover, even proven perioperative efficacy may not guarantee an adequate antimicrobial effect during the postoperative period.

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