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Behavior of Antibiotics during Human Necrotizing Pancreatitis

C. BASSI,[1]* P. PEDERZOLI,[1] S. VESENTINI,[1] M. FALCONI,[1] A. BONORA,[1] H. ABBAS,[1] A. BENINI,[2] AND E. M. BERTAZZONI[2]

Surgical[1] and Pharmacological[2] Departments, University Hospital, University of Verona, Borgo Roma, 37134 Verona, Italy

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The aim of the study was to verify whether antibiotics excreted by the normal pancreas are also excreted in human necrotizing pancreatitis, reaching the tissue sites of the infection. Twelve patients suffering from acute necrotizing pancreatitis were treated with imipenem-cilastatin (0.5 g), mezlocillin (2 g), gentamicin (0.08 g), amikacin (0.5 g), pefloxacin (0.4 g), and metronidazole (0.5 g). Serum and necrotic samples were collected simultaneously at different time intervals after parenteral drug administration by computed tomography-guided needle aspiration, intraoperatively, and from surgical drainages placed during surgery. Drug concentrations were determined by microbiological and high-performance liquid chromatography assays. All antibiotics reached the necrotic tissues, but with varying degrees of penetration, this being low for aminoglycosides (13%) and high in the case of pefloxacin (89%) and metronidazole (99%). The concentrations of pefloxacin (13.0 to 23 μg/g) and metronidazole (8.4 μg/g) in the necrotic samples were distinctly higher than the MICs for the organisms most commonly isolated in this disease; the concentrations in tissue of imipenem (3.35 μg/g) and mezlocillin (8.0 and 15.0 μg/g) did not always exceed the MICs for 90% of strains tested, whereas the aminoglycoside concentrations in necrotic tissue (0.5 μg/g) were inadequate. Repeated administration of drugs (for 3, 7, 17, and 20 days) seems to enhance penetration of pefloxacin, imipenem, and metronidazole into necrotic pancreatic tissue. The choice of antibiotics in preventing infected necrosis during necrotizing pancreatitis should be based on their antimicrobial activity, penetration rate, persistence, and therapeutic concentrations in the necrotic pancreatic area. These requisites are provided by pefloxacin and metronidazole and to a variable extent by imipenem and mezlocillin.

Superinfection of necrotic tissue in the course of acute necrotizing pancreatitis is a decisive prognostic factor with regard to morbidity and mortality (2, 3, 5, 7, 20, 25, 33, 34, 40).

This has prompted attempts to identify the patients at septic risk (3, 5, 20), the microorganisms responsible for the superinfection (1), the times of infection in the natural history of the disease (3, 20), the therapeutic measures to be adopted (1–3, 5, 20, 34), and possible effective prophylaxis (7, 9, 10, 19, 23, 25, 33).

The ideal antibiotic for therapy and/or prophylaxis should be targeted at the microorganisms responsible for the septic complications and should reach the infection site in therapeutic concentrations.

To date, several studies have been conducted to evaluate the penetration of various different antibiotics into healthy pancreatic tissue and juice (4, 7–11, 28, 35–38). None of these studies has been conducted with humans in the course of disease, and to the best of our knowledge, the effective ability of antibiotics to penetrate the infection risk areas has never been assessed.

Such areas of the retroperitoneum are characterized by the presence of necrotic fragments of pancreas and perinecrotic tissues with solid or semisolid consistency, exudate, transudate, pus, enzymes, and pancreatic juice. We defined the combination of these substances as necrotic pancreatic sample (NP), which represents an ideal environment for bacterial colonization.

The aim of the present study was to evaluate the degree of penetration of a number of antibiotics into the NP obtained from patients with acute necrotizing pancreatitis, by preintraoperative and postoperative procedures.

* Corresponding author. Mailing address: Department of General Surgery, University of Verona, Borgo Roma Hospital, 37134 Verona, Italy. Phone: 39-45-8074671. Fax: 39-45-583852.

MATERIALS AND METHODS

Patients. The patient sample comprised 12 patients (9 males, 3 females; mean age: 42 years, range: 34 to 69; mean body weight: 71.2 kg, range 61.1 to 76.3 kg) suffering from acute necrotizing pancreatitis (mean Ranson score, 4.2), diagnosed on the basis of clinical routine, biochemical, and computed tomography parameters. Samples were collected over a period ranging from day 13 to day 43 after onset of the acute attack. All patients gave their informed consent. Approval of the University Human Research Review Committee was obtained for the study.

Drug administration. In view of the fact that all the patients admitted to the study were seriously ill, the choice of antibiotics was dictated by susceptibility tests rather than predetermined protocol. Gram-negative bacilli (*Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Acinetobacter* spp. in seven patients), gram-positive cocci (*Enterococcus faecalis* in two patients, *Staphylococcus aureus* in three patients), and anaerobes (*Bacteroides* spp. in two patients) were identified in the necrotic samples; in some cases, there were mixed infections, or in other cases, the pathogen microorganisms changed during the course of disease.

Mezlocillin (2 g; Baypen R; Bayer-Leverkusen, Milan, Italy), imipenem-cilastatin (0.5 g–0.5 g; Tienam; Merck, Sharp & Dohme, Rome, Italy), pefloxacin (0.4 g; Peflacin; Rhone-Poulenc Rorer, Milan, Italy), and amikacin (0.5 g; Bbk8; Bristol Italy Sud, Rome, Italy) were administered to patients in single intravenous (i.v.) doses by 10 to 15 min of infusion. Gentamicin (0.08 g; Gentalyn; Schering-Plough, Milan, Italy) was administered in a 5-min bolus three times a day (t.i.d.).

TABLE 1. Concentrations of antimicrobial drugs in serum and NPs of patients with necrotic acute pancreatitis

Drug	Dose (g [i.v.])	Patient	Time (h)	Serum (μg/ml)	Necrotic samples[a]		Tissue/serum concn ratio
					Drainage	IO	
Imipenem	0.5	A	2.0	2.23	1.4		0.63
		B	0.3	50.0	ND[b]	ND	
			1.0	ND		0.39[c]	
			1.5	ND		1.38	
			2.0	ND		3.28	
		C	2.5	14.5	3.35[d]	ND	0.23
		D	5.3	0.33	0.19	0.12[e]	0.57
							0.36
Mezlocillin	2	B	2.0	34.5	0.65	ND	0.019
		H	2.0	37.0	15.0	8.08	0.40-0.22
		I[f]	2.0	26.6	25.3		0.95
Amikacin	0.5	E	1.5	7.6	0.93		0.12
		H	2.0	14.9	0.56		0.037
Gentamicin	0.08 × 3 (× 7 days)	F	2.0	0.6	0.15		0.21
		G[g]	2.0	2.8	0.5		0.18
Pefloxacin	0.4	H	2.0	2.28	2.05		0.89
					3.75[e]		1.64
	0.4 × 2 (× 7 days)	F	Before[h]	6.3	9.3		1.48
			2.0	9.0	13.0		1.44
	0.4 × 2 (× 3 days)	N	4.5	3.2		13.3	4.15
	0.4 × 2 (× 17 days)	L	6.0	ND		21.5	
	0.4 × 2 (× 20 days)	M	6.0	6.0		14.1–23.0[i]	2.35–3.83
Metronidazole	0.5 × 3 (×7 days)	F	Before	1.22	4.07		3.34
			2.0	2.53	4.45		1.75
		G[g]	Before	6.36	6.30		0.99
			2.0	13.53	8.48		0.63

[a] Values (micrograms per gram) are expressed as the mean of six determinations. IO, intraoperative pancreatic tissue sample.
[b] ND, not determined.
[c] Simultaneous levels in perinecrotic pancreatic tissue = 1.11 μg/g.
[d] Computed tomography-guided fine-needle aspiration.
[e] Hemorrhagic fragments.
[f] Pseudocyst.
[g] Sole kidney.
[h] Before, determination done before the first drug administration on the seventh day.
[i] Hemorrhagic tissue sample.

Metronidazole (Deflamon; SPA, Milan, Italy) was given at a dose of 0.5 g t.i.d. by 30 min of infusion. Only gentamicin and metronidazole were administered in combination (gentamicin followed by metronidazole t.i.d. for 7 days before sample collection). In no case did we observe interference with the quantitation of either metronidazole or its metabolites. Metronidazole cannot interfere with the microbiological determination of gentamicin because of its activity against anaerobic microorganisms.

Patients F, N, L, and M were treated with pefloxacin (0.4 g) every 12 h for 1 week, and 3, 17, and 20 days, respectively, before serum and pancreatic levels were determined.

After i.v. administration of the single dose of imipenem, patient B was maintained on treatment with repeated intramuscular doses of 0.5 g every 8 h for 5 days. Samples were collected before and 2 h after the first drug administration on day 5 (imipenem) and day 7 (gentamicin, metronidazole, and pefloxacin) of multiple-dose treatments. For patients N, L, and M, the collection of samples was possible only during surgery, at 4.5 and 6 h.

A 48-h pharmacological washout period was scheduled prior to new drug treatment in the same patient (H and F).

Serum and necrotic samples. Serum was obtained by puncture of an antecubital vein before and 2 h after drug administration; NPs were collected at the same times. NPs were obtained intraoperatively and/or from material drained postoperatively (34) and/or by computed tomography-guided needle aspiration before surgery. In the cases treated with surgical irrigation, the lavages were stopped 24 h before drug administration and NP was obtained by direct aspiration from the drainages. In these cases, the samples consisted of necrotic pancreatic fragments suspended in fluid.

Serum samples and NPs were maintained in an ice bath and processed as soon as possible (generally 20 to 30 min after collection). The various sampling times are given in Table 1. Serum samples were obtained after centrifugation (1,000 rpm). NP was homogenized and centrifuged (3,000 rpm, 4°C); the supernatant was separated and assayed; the residual material was resuspended in sterile saline, manually homogenized, and then assayed. Samples obtained from patients treated with imipenem were immediately stabilized by addition of a 1:1 mixture of MES (morpholineethanesulfonate buffer, pH 6) plus ethylene glycol in order to reduce possible degradation of the drug (32). Residual samples were stored at −80°C.

Drug assay. We used the microbiological method instead of chemical determination of drugs in order to evaluate the total antimicrobial drug activity present in NP. The microbiological determination of metronidazole was unsuitable owing to low sensitivity and poor reproducibility.

(i) Microbiological method. Antimicrobial drugs were assayed by the agar-well-diffusion microbiological method. *Bacillus subtilis* ATCC 6633 (spore suspension; Difco Laboratories, Detroit, Mich.; final concentration of inoculum, 0.02%) as test organism was seeded in brain heart infusion agar (Difco) for determining imipenem and mezlocillin concentrations and in Isosensitest Agar (Oxoid, UNIPATH LTD, Basingstoke,

Hampshire, England) for determining amikacin and gentamicin concentrations. Pefloxacin was assayed with *E. coli* Kp 05124 (final concentration of overnight culture, 0.15%) in Isosensitest Agar (Oxoid). Agar (110 ml) was poured into sterile, level, square plastic Nunc bioassay plates (245 by 245 mm; Intermed, Kamstrup, Denmark). After solidification, 80-mm-diameter wells were punched out and filled with 50 μl of samples. Amikacin was also assayed by fluorescence polarization immunoassay (TDx; Abbott, Chicago, Ill.).

After overnight incubation at 37°C, the diameters of the inhibition zones were measured. Antibiotic concentrations were determined in relation to the diameters of the inhibition zones yielded by a standard series of known concentrations of antibiotics in pooled human serum and pancreatic tissue. The standard tissue concentrations were prepared with rat (Charles River) pancreas for ethical and technical reasons. Our patients were treated with antibiotics before surgery, and other pancreatic patients (i.e., pancreatic head cancer) underwent perioperative prophylaxis according to a protocol approved by our Ethical Committee. We consider this method acceptable in view of the reliability of our results.

Standard concentrations of imipenem were obtained in MES-ethylene glycol (1:1 mixture). Standard curves were prepared daily in appropriate specimens by spiking aliquots with the described antibiotics in serum, pancreatic juice, and rat pancreatic tissue. The final concentrations of different antibiotics ranged from 0.03 μg/ml (imipenem) to 50 μg/ml (amikacin and metronidazole). Standard and samples were processed in the same way as described above and assayed in triplicate. Metronidazole samples were stored at −80°C for 3 weeks and analyzed on the same day. Previous experiments showed that metronidazole at −80°C is stable over a period of 6 months. The sensitivity limits in serum and tissue were the following: 0.03 μg/ml for imipenem, 0.1 μg/ml for mezlocillin, 0.5 μg/ml for amikacin, 0.1 μg/ml for gentamicin, and 0.2 μg/ml for pefloxacin.

The imipenem assay was linear over a range of 0.07 to 40.0 μg/ml (r^2 = 0.92, correlation coefficient calculated by linear least-square regression); the within-day coefficient of variation was 1.8 and 0.8% for the lowest and highest serum concentrations, respectively, and 0.1% for tissue; the between-day coefficient of variation was 10.5% for the lowest and 4.9% for the highest serum values and 7.9 and 9.8% for the lowest and highest tissue concentrations, respectively. The mezlocillin assay was linear over a range of 0.6 to 20.0 and 35 to 500 μg/ml (r^2 = 0.92); the within-day coefficient of variation was 4.5 and 1.3% for lowest and highest serum concentrations, respectively; the between-day coefficient of variation was 2.8% for the lowest and 8.6% for the highest serum values. The amikacin assay was linear over a range of 2.5 to 20.0 μg/ml (r^2 = 0.91); the within-day coefficient of variation was 2.1 and 4.0% for the lowest and highest serum concentrations, respectively; the between-day coefficient of variation was 9.1% for the lowest and 4.6% for the highest serum values. The gentamicin assay was linear over a range of 2.5 to 20.0 μg/ml (r^2 = 0.92); the within-day coefficient of variation was 1.9 and 3.3% for the lowest and highest serum concentrations, respectively; the between-day coefficient of variation was 2.9% for the lowest and 0.3% for the highest serum values. The aminoglycoside assay by TDx shows a limit sensitivity of 0.8 μg/ml for amikacin and 0.2 μg/ml for gentamicin; the inter- and intraday coefficients of variation were 1.37 and 1.74% for low and high values, respectively.

The pefloxacin assay was linear over a range of 1.0 to 20.0 μg/ml (r^2 = 0.91); the within-day coefficient of variation was 0.5% for different concentrations in serum and tissue. The between-day coefficient of variation was 8.0% for the lowest and 2.5% for the highest serum values and 6.7% and 2.8% for the lowest and highest tissue levels, respectively. Samples and standard concentrations were assayed in triplicate. Results are expressed in micrograms per milliliter or gram of tissue.

(ii) Metronidazole assay. Concentrations of metronidazole and its hydroxymetabolite (OH-MZ) were determined by a specific high-performance liquid chromatographic assay, as described by Nilsson-Ehle et al. (31).

Apparatus consisted of a Beckman pump (model 112-solvent delivery module) and a Spherisorb ultrasphere S5-ODS2 column (5-μm particle size, 25 cm by 4.6 mm, reverse phase). Light absorbance was measured with a model 450 variable-wavelength detector (Gilson) (absorbance detection = 320 nm).

For samples, one part of serum or necrotic pancreatic homogenate was mixed with one part of 5% perchloric acid by rapid stirring. After centrifugation, the clear supernatant was filtered through a 0.6-μm filter and 40 μl was injected into chromatographic apparatus. The eluting solvent was methanol–acetonitrile–0.005 M KH_2PO_4 (pH 4) (4:3:93 [vol/vol]); flow rate was 1.5 ml/min. Retention times were 10.88 min for metronidazole and 5.76 min for OH-MZ. Standard solutions were prepared in plasma and in pancreatic tissue and assayed along with samples. The standard curves were linear over a range of 0.1 to 50 μg/ml (r^2 = 0.99). The sensitivity limit was 0.1 μg/ml; the intra-assay coefficient of variation was 2.5 and 3.1% for lowest and highest values, respectively. The efficiency of the extraction method was tested by comparing serum and tissue samples with aqueous standards. The recovery from biological samples was (99 ± 2%) for both high and low concentrations. Concentrations in samples were determined by integrated area-under-the-concentration-time-curve analysis with a Hewlett-Packard integrator (model 3390 A) in the external standardization mode.

All samples were assayed in triplicate. Results are expressed in micrograms per milliliter or gram of tissue, as arithmetic mean.

RESULTS

The concentrations of various antimicrobial substances determined in serum and in NP are shown in Table 1. No antimicrobial activity was detected in samples collected before single-dose administration of the drugs.

Imipenem. After a single i.v. administration of imipenem, the concentrations in serum and NP after 2 h were 2.23 μg/ml and 1.4 μg/g, respectively, in patient A and 14.5 μg/ml and 3.35 μg/g, respectively, in patient C (after 2.5 h).

The imipenem concentrations in the necrotic pancreatic tissue from patient B were 0.39 μg/g at 1 h after the end of infusion, 1.38 μg/g after 1.5 h, and 3.28 μg/g after 2 h, thus showing a gradual penetration of the drug, reaching good levels in tissue after 2 h.

In the same patient, the determination performed 1 h after the end of infusion in a tissue sample from a perinecrotic area apparently unaffected by inflammatory processes yielded a higher concentration (1.11 μg/g). Imipenem penetrates into necrotic-hemorrhagic tissue: after 5.30 h, the concentration of the drug was inadequate against the organisms responsible for pancreatic infection (patient D), though the pancreatic tissue-to-serum ratio was quite similar to the ratio evaluated at 2 h (patient C). The imipenem concentrations in necrotic tissue samples were similar to those determined in the perinecrotic fluid collected intraoperatively.

Mezlocillin. Two hours after administration, mezlocillin was

present in the sera of the three patients studied in concentrations which were still quite high with a little intersubject variability (26.6 to 37.0 μg/ml).

The concentrations in NP were different: 0.65 μg/g in patient B and 15 μg/g in patient H in the material obtained postoperatively from the surgical drainage. In patient H, the concentration in necrotic pancreatic tissue collected intraoperatively was approximately 8 μg/g. The tissue-to-serum ratio of mezlocillin is fairly low (0.40 to 0.22), and the concentrations do not always exceed the MICs for potential pathogenic microorganisms. There was clearly a great difference between these two patients. By contrast, it can be seen that in the patient (I) with a pseudocyst communicating with the main duct the penetration was excellent (95.1%); the concentrations attained in the pancreatic juice of the pseudocyst and in serum were similar.

Aminoglycosides. Two hours after administration, the concentrations of gentamicin and amikacin in NP were very low and below the MICs for 50% of strains tested for the organisms most commonly present in such infection sites. The degree of tissue-to-serum ratio ranged from 0.037 for amikacin to 0.18 to 0.21 for gentamicin after 2 h of drug administration.

Amikacin, 1.5 h after administration, appeared to be capable of penetrating rapidly into NP, though the concentrations remained low (0.93 μg/g).

Similarly, gentamicin administration t.i.d. for a week showed a low degree of penetration into NP (0.18 to 0.21 tissue-to-serum ratio).

In patient G, who presented higher levels in serum than patient F owing to lack of a kidney, the penetration did not increase and a trend similar to that of the other patients was observed. The determination of aminoglycoside concentrations by microbiological and immunoenzymatic methods yielded comparable results.

Pefloxacin. After administration of a single dose of 400 mg of pefloxacin, we observed a good penetration into NP (0.89 tissue-to-serum ratio). The NP from patient H presented a substantial number of fragments, which were analyzed after centrifugation, homogenization, and recentrifugation; the drug concentration was 3.75 μg/g, which is higher than that determined in the corresponding supernatant. In this case, the NP-to-serum ratio was 1.64.

After repeated administrations of 0.4 g every 12 h for 1 week (patient F), the concentrations of pefloxacin 12 h after the last dose were high both in serum (6.3 μg/ml) and in NP (9.3 μg/g); 2 h after the administration of the first dose of 400 mg i.v. on the seventh day there were a further rise in levels in serum to 9 μg/ml and a parallel rise in concentrations in tissue (13.0 μg/g). Following multiple doses, pefloxacin penetration into NP was very high (ratio = 1.44 to 1.48). Necrotic pancreatic tissues collected intraoperatively showed a similar range of values (from 13.3 to 23.0 μg/g) after 3, 17, and 20 days of treatment. Levels in tissue and tissue-to-serum ratio were two to four times higher than those obtained after a single dose. The concentrations in tissue in patient M were 14.1 and 23.0 μg/g 6 h after the first dose on the 20th day: the highest values correspond to a second sample collected from a different site of the necrosis.

Metronidazole. Following multiple administrations, metronidazole achieved and maintained elevated concentrations in serum and NP. On the seventh day, NP concentrations averaged 4.07 μg/g in patient F and 6.3 μg/g in patient G before the first daily dose, which induced double serum levels and high pancreatic concentrations. Higher levels in serum (13.53 μg/ml, patient G, sole kidney) led to a nonproportional increase in metronidazole concentrations in NP (8.48 μg/g).

The hydroxymetabolite (OH-MZ) was detected in serum (1.3 μg/ml, patient F, and 2.4 μg/ml, patient G) and in NP at relatively low levels (1.1 to 1.4 μg/g). The degree of penetration into NP was 76% 2 h after metronidazole administration (patient F), while at 8 h, prior to drug administration, the penetration was 87.8% in patient F and 62.6% in patient G. These concentrations and the degree of penetration of OH-MZ into NP were not significantly different from those observed in healthy pancreatic juice (4).

Multiple-dose administration. Repeated i.v. administration thus seems to enhance penetration of pefloxacin and metronidazole into NP. The penetration of imipenem also appears to be favorably affected: after a single intramuscular administration (patient B), the tissue-to-serum ratio in NP corresponded to 0.04 to 0.05 after 2 and 4 h, but increased to 0.09 after repeated intramuscular administration of 0.5 g–0.5 g t.i.d. for 6 days. The concentrations of the antibiotic in drainage material ranged from 0.29 to 0.89 μg/g depending upon the dose administered. Thus, in this case, the absolute antibiotic concentration values proved lower than the MICs for numerous pathogens involved in infected pancreatic necrosis.

DISCUSSION

Deaths from acute necrotizing pancreatitis are due in as much as 80% of the cases to late septic complications (40). Sepsis originates from superinfection of pancreatic and peripancreatic collections which in toto represent an ideal environment for bacterial infection, particularly enteric gram-negative microorganisms (1–3, 34). Antibiotic prophylaxis of sepsis due to acute pancreatitis, though appealing (7), has thus far proven uneffective in clinical trials (19, 23).

On the other hand, the availability of several new broad-spectrum molecules, the best knowledge of the bacterial flora involved in pancreatic sepsis, and the evidence that only a few antibiotics can penetrate pancreatic juice and tissue at therapeutic concentrations mandate a critical reappraisal of this issue (4, 7–9, 11, 25, 28, 35–38). Animal experiments have led to conflicting results (14, 41–45); however, few data have been reported on the behavior of antibiotics during necrotizing pancreatitis in humans. Recently, Büchler et al. (10) reported the drug penetration in perinecrotic vital tissue of eight patients with necrotizing pancreatitis. Our data, obtained from clinical investigation, show that a number of antibiotics penetrate at adequate and therapeutic concentrations into the infection risk areas, that is, into necrotic tissues and fluid collections.

Necrosis and inflammation affect drug penetration to a considerable extent. In these conditions, while the serum drug levels are generally within normal ranges (4, 8, 25, 28, 32), the physiological interindividual variability is further enhanced by the disease. This is supported by the concentration of antibiotics in necrotic pancreatic tissue compared with nonnecrotic samples. Imipenem concentrations are nearly three times greater in perinecrotic pancreatic tissue than in necrotic samples. Similarly, the mezlocillin levels were higher in pseudocysts than in necrotic fluid.

Other factors of variability should be the number and the consistency of necrotic fragments present in the samples as well as the presence of hemorrhagic component, in which variable contamination with blood is always present. Such contamination may be considered of limited importance considering that drugs with low diffusibility such as aminoglycosides are not present at adequate concentrations in hemorrhagic samples, despite parallel high levels in serum. Hypovolemia, edema, fluid sequestration, and increase in

enzymes are additional variability factors in the course of acute pancreatitis.

The degree of antibiotic penetration in both healthy and necrotic human pancreatic tissue depends mostly on (i) physicochemical properties of the drug (molecular size, structure, polarity, pK_a, ionization, lipid solubility); (ii) individual pharmacokinetic characteristics; (iii) presence of necrosis and inflammation; and (iv) administration route and dosage schedules (7, 9, 11). Since on the basis of the infection trend and consequently of the antimicrobial susceptibility tests, it proved necessary in the same patient to change the therapeutic regimen, we were able to study the degree of penetration of drugs with different physicochemical and pharmacokinetic characteristics in the same NP. In patient H, amikacin is the drug which shows the poorest penetration capacity (0.037 tissue-to-serum ratio), while mezlocillin and pefloxacin show a mild (ratio = 0.4 and 0.2) and good (ratio = 0.89) penetration, respectively. The multiple administration (patient F) determined a similar behavior: gentamicin showed the lowest degree of penetration (ratio = 0.21), and pefloxacin maintained high levels in tissue and enhanced tissue-to-serum ratio (1.44), while metronidazole presented the highest capacity of penetration (ratio = 1.75).

The limited diffusibility of aminoglycosides (12) is also observed in the course of pancreatic necrosis (7, 9), and this probably depends on their polycationic structure, ionization, and poor intrinsic liposolubility. Low levels in tissue are further diminished by interaction with cell membranes, and macromolecules as well as inflammatory and necrotic components (leukocytes, pus, etc.) (26, 46). Mezlocillin and imipenem belong to the same class of moderately liposoluble acids and therefore present the same characteristics of penetration into pancreatic juice (8, 15, 38), as rapid distribution and elimination (27); for this reason, the trough concentrations (at 6 h) may be lower than MICs for 90% of susceptible bacteria commonly found in pancreatic infections. How long the concentrations of antibiotic in the infection site remain above the MIC is a decisive factor for the therapeutic efficacy of beta-lactams (16, 18). Pefloxacin is characterized by good antimicrobial activity against most gram-negative bacilli and a favorable pharmacokinetic profile, with a long half-life and extensive extravascular distribution (21); it therefore shows easy diffusion, readily penetrates the cell membranes, and reaches therapeutic concentrations in the necrotic site, i.e., the infection risk area, as well as in pancreatic tissue obtained intraoperatively. The quinolones, which are weak organic lipophilic acids, partition extensively intracellularly (17, 42) and are partly ionized at alkaline pancreatic pH (range, 7.5 to 8.5), causing an ion-trapping effect. The size of pefloxacin, the pK_a (6.3, 7.6), and its amphoteric configuration at neutral pH are compatible with the possibility of a gradual release from cells to extracellular fluid or pancreatic juice. Moreover, the mechanisms of pancreatic secretion may influence pefloxacin concentrations in tissue. In addition, at high pH values, drugs such as pefloxacin with piperazine at C-7 are negatively charged and more active in an alkaline environment (43). The small molecular size of metronidazole, a weak base, and its liposolubility and extensive tissue distribution are very favorable characteristics for maintaining long-lasting concentrations in NP above the MICs for 50% of strains tested for most anaerobic bacteria.

These results may also be modified by the dosage schedules and administration route of antibiotics. According to our experience, the degree of penetration of imipenem and pefloxacin can be improved by using multiple doses and more effective ways of administration. The pefloxacin penetration into pancreatic tissue increased following multiple-dose administration by two to three times the single dose. The i.v. route appeared to be more effective than the intramuscular route and allowed better penetration with high concentrations in NP at least for imipenem. In the case of aminoglycosides, repeated administrations by i.v. route do not improve their penetration capacity.

Although we have established that a number of antibiotics effectively penetrated into the infected necrosis of the pancreas, the experimental conditions did not allow us to define how they reached the necrotic foci. The latter are known to be isolated at an early stage by vascular microthrombosis phenomena (34). In our opinion, the pancreatic juice itself might be a useful carrier for the antibiotic in such situations: pancreatic juice leaking via the ductal lesions may presumably reach the parenchymal necrotic focus and thus the infection risk areas. This hypothesis is borne out by the findings that antibiotics with good excretion in pancreatic juice (4, 8, 11, 28, 35–38) were the same ones which presented adequate penetration into NP (7, 9, 10, 25). The presence of antibiotics in pancreatic juice is the result of a further membrane passage and depends on blood and/or pancreatic levels, drug characteristics, pH environment, and pancreatic secretion mechanisms. Bicarbonate, electrolytes, enzymes, and proteins as well as some weak organic acids (sulfomerazine) are actively secreted by pancreas (13). The acinar unit (consisting of almost 200 secretory cells), the anastomoses directly connecting the cytoplasm of adjacent cells, and the paracellular channels make the pancreas a special organ. Fluorescent organic molecules with molecular weight ranging from 300 to 700, ions, metabolites, and nucleotides can pass from cell to cell (39). Therefore, the antibiotic present in healthy or nonnecrotic pancreatic cells could also gain access to the vicinity of necrotic tissue by intercellular anastomoses, which may represent an alternative route for drainage of secretions during pancreatic disease (24), or by paracellular pathway (13) or via damaged ducts. Moreover, the lymphatic (6, 14, 30) and/or intestinal (27) and/or peritoneal (22, 29) pathways may constitute alternative routes for the diffusion of antibiotics in the retroperitoneal area.

In order to recommend clinical use, any final assessment of the antibiotics tested must be based on the overall picture of their antimicrobial activity against pancreatic pathogens, degree of diffusibility, attained concentrations, penetration, and persistence in the infection site. These requisites seem adequately provided in the necrotizing pancreatitis by pefloxacin and metronidazole and, to a variable extent, by imipenem and mezlocillin. This evaluation agrees with the conclusions achieved by Büchler and colleagues (10). These results obtained in relatively few patients are supported by the positive clinical results observed in a larger patient sample. Recently, we found a reduction in the incidence of pancreatic sepsis in acute pancreatitis in a 2-year randomized multicenter clinical trial in 74 patients (33).

In conclusion, the antibiotics which proved capable of penetrating into the pancreas in physiological conditions maintained this ability in the course of acute disease with a necrotic component. Their use in the prophylaxis of necrotizing pancreatitis appears useful (33) but requires further confirmations in controlled clinical trials.

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REFERENCES

1. **Bassi, C., M. Falconi, R. Girelli, F. Nifosì, A. Elio, N. Martini, and P. Pederzoli.** 1989. Microbiological findings in severe pancreatitis. Surg. Res. Commun. **5**:1–4.
2. **Bassi, C., S. Vesentini, F. Nifosì, R. Girelli, M. Falconi, A. Elio, and P. Pederzoli.** 1990. Pancreatic abscess and other pus harboring collections related to pancreatitis: a review of 108 cases. World J. Surg. **14**:505–512.
3. **Berger, H. G., R. Bittner, S. Block, and M. Büchler.** 1986. Bacterial contamination of pancreatic necrosis. A prospective clinical study. Gastroenterology **91**:433–438.
4. **Bertazzoni, M. E., P. Pederzoli, M. Falconi, C. Bassi, and S. Vesentini.** 1987. Pharmacokinetic of metronidazole in patients with pancreatic fistula, p. 162–164. *In* B. Berkarda and H. P. Kummerle (ed.), Progress in antimicrobial and anticancer chemotherapy, vol. I. Ecomed, Munich.
5. **Block, S., M. Büchler, R. Bittner, and H. G. Berger.** 1987. Sepsis indicators in acute pancreatitis. Pancreas **2**:499–505.
6. **Bonner-Weir, S.** 1993. The microvasculature of the pancreas, with emphasis on that of the islets of Langherans, p. 759–768. *In* V. L. W. Go, E. Di Magno, J. D. Gardner et al. (ed.), The pancreas: biology, pathobiology and disease, 2nd ed. Raven Press, New York.
7. **Bradley, E. L.** 1989. Antibiotics in acute pancreatitis. Current status and future directions. Am. J. Surg. **158**:472–478.
8. **Brattström, C., A. S. Malmborg, and G. Tydén.** 1989. Penetration of imipenem into human pancreatic juice following single intravenous dose administration. Chemotherapy. **35**:83–87.
9. **Büchler, M., P. Malfertheiner, H. Friess, and H. G. Berger.** 1989. The penetration of antibiotics into human pancreas. Infection **1**:20–25.
10. **Büchler, M., P. Malfertheiner, H. Friess, R. Isenmann, E. Vaneck, H. Grimm, P. Schlegel, T. Friess, and H. G. Berger.** 1992. Human pancreatic tissue concentration of bactericidal antibiotics. Gastroenterology **103**:1902–1908.
11. **Burns, G. P., T. A. Stein, and L. S. Kabnik.** 1986. Blood/pancreatic juice barrier to antibiotic excretion. Am. J. Surg. **151**:205–208.
12. **Carson, H. B., A. S. Heller, T. B. Koch, P. Walczak, and J. Schentag.** 1983. Antibiotic penetration in abdominal infection: a case of tobramycin failure responsive to moxalactam. Drug Intell. Clin. Pharm. **17**:277–279.
13. **Case, R. M., and B. E. Argent.** 1993. Pancreatic duct cell secretion. Control and mechanisms of transport, p. 301–350. *In* V. L. W. Go, E. Di Magno, J. D. Gardner et al. (ed.), The pancreas: biology, pathobiology and disease. 2nd ed. Raven Press, New York.
14. **De Broe, M. E.** 1973. Antibiotics in renal lymph. Arch. Int. Pharmacodyn. **201**:193–194.
15. **Demol, P., M. V. Singer, D. D. Bernemann, G. Linzenmeier, and H. Goebell.** 1983. Excretion of azlocillin and mezlocillin by the normal pancreas and in acute pancreatitits in dogs and rats. Digestion **27**:93–99.
16. **Drusano, G. L., P. A. Ryan, H. C. Standiford, M. R. Moody, and S. C. Schimpff.** 1984. Integration of selected pharmacologic and microbiologic properties of three new β-lactam antibiotics: a hypothesis for rational comparison. Rev. Infect. Dis. **6**:357–363.
17. **Easmon, C. S. F., J. P. Crane, and A. Blowers.** 1986. Effect of ciprofloxacin on intracellular organisms: in vitro effects and in vivo studies. J. Antimicrob. Chemother. **18**(Suppl. D):43–48.
18. **Fantin, B., J. Leggett, S. Ebert, and A. Craig.** 1991. Correlation between in vitro and in vivo activity of antimicrobial agents against gram-negative bacilli in murine infection model. Antimicrob. Agents Chemother. **35**:1413–1422.
19. **Finch, W. T., J. L. Sawjers, and S. Schenker.** 1976. A prospective study to determine the efficacy of antibiotics in acute pancreatitis. Ann. Surg. **183**:667–671.
20. **Gerzof, F. G., P. A. Banks, A. H. Robins, W. Johnson, S. J. Specler, S. M. Wetzner, J. M. Snider, R. E. Langevin, and M. E. Jay.** 1987. Early diagnosis of pancreatic infection by computed tomography guided aspiration. Gastroenterology **93**:1915–1920.
21. **Gonzales, J. P., and J. M. Henwood.** 1989. Pefloxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs **37**:628–668.
22. **Gutschow, K., E. R. Weissenbacher, I. Wachter, F. D. Deininger, P. Muth, and F. Sorgel.** 1991. Pharmacokinetics of ceftriaxone in lymphatic fluid and serum following radical hysterectomy. Program Abstr. 17th Int. Congr. Chemother. Berlin, 23 to 28 June, abstr. 961.
23. **Howes, R., G. D. Ziderna, and J. D. Cameron.** 1975. Evaluation of prophylactic antibiotics in acute pancreatitis. J. Surg. Res. **18**:197–200.
24. **Kern, H.** 1993. Fine structure of the human esocrine pancreas, p. 9–19. *In* V. L. W. Go, E. Di Magno, J. D. Gardner et al. (ed.), The pancreas: biology, pathobiology and disease, 2nd ed. Raven Press, New York.
25. **Koch, K., B. Drewelow, R. Reding, and A. K. Riethling.** 1991. Die pancreasgangigkeit von antibiotica. Chirurg **62**:317–322.
26. **Lüllmann, H., and B. Vollmer.** 1982. An interaction of aminoglycoside antibiotics with Ca binding to lipid monolayers and to biomembranes. Biochem. Pharmacol. **31**:3769–3773.
27. **Mac Gregor, R. R., G. A. Gibson, and J. A. Bland.** 1986. Imipenem pharmacokinetics and body fluid concentrations in patients receiving high-dose treatment for serious infections. Antimicrob. Agents Chemother. **29**:188–192.
28. **Malmborg, A. S., C. Brattström, and G. Tydén.** 1990. Penetration of pefloxacin into human allograft pancreatic juice. J. Antimicrob. Chemother. **29**:393–397.
29. **Mikamo, H., K. Izumi, K. Ito, Y. Yamada, and T. Tamaya.** 1992. Drug concentrations in pelvic retroperitoneal space exudate after the combined administration of cephems and amikacin. Drug Invest. **4**:459–465.
30. **Naber, K. G.** 1978. Renal lymph concentration of antibiotics. Scand. J. Infect. Dis. **14**:164–165.
31. **Nilsson-Ehle, I., B. Ursing, and P. Nilsson-Ehle.** 1981. Liquid chromatography assay for metronidazole and tinidazole: pharmacokinetic and metabolic studies in human subjects. Antimicrob. Agents Chemother. **19**:754–760.
32. **Norrby, S. R., K. Alestig, F. Ferber, J. L. Huber, K. H. Jones, F. M. Kahan, M. A. P. Meisinger, and J. D. Rogers.** 1983. Pharmacokinetics and tolerance of *N*-formimidoyl thienamycin (MK0787) in humans. Antimicrob. Agents Chemother. **23**:293–299.
33. **Pederzoli, P., C. Bassi, S. Vesentini, and A. Campedelli.** 1993. Antibiotic prophylaxis of septic complications in acute necrotizing pancreatitis. A randomized multicenter clinical trial with imipenem. Surg. Gynecol. Obstet. **176**:480–483.
34. **Pederzoli, P., C. Bassi, S. Vesentini, R. Girelli, G. Cavallini, M. Falconi, F. Nifosì, A. Riela, and A. Dagradi.** 1990. Retroperitoneal and peritoneal drainage and lavage in the treatment of severe necrotizing pancreatitis. Surg. Gynecol. Obstet. **170**:197–203.
35. **Pederzoli, P., M. Falconi, C. Bassi, R. Girelli, S. Vesentini, N. Martini, and A. Messori.** 1989. Ofloxacin penetration in bile and pancreatic juice. J. Antimicrob. Chemother. **23**:805–807.
36. **Pederzoli, P., M. Falconi, C. Bassi, S. Vesentini, F. Orcalli, F. Scaglione, A. Messori, and N. Martini.** 1987. Ciprofloxacin penetration in pancreatic juice. Chemotherapy **33**:397–401.
37. **Pederzoli, P., M. Falconi, N. Martini, G. Cavallini, C. Bassi, and F. Orcalli.** 1985. Rifampicin and ceftazidime concentration in pure pancreatic juice. Digestion **32**:210–212.
38. **Pederzoli, P., F. Orcalli, M. Falconi, L. Bozzini, and N. Martini.** 1986. Penetration of mezlocillin into pancreatic juice. J. Antimicrob. Chemother. **17**:397–398.
39. **Petersen, O. H.** 1993. Electrophysiology of acinar cells, p. 191–218. *In* V. L. W. Go, E. Di Magno, J. D. Gardner et al. (ed.), The pancreas: biology, pathobiology and disease, 2nd ed. Raven Press, New York.
40. **Renner, I. G., W. T. Savage, J. W. Pantja, and V. J. Renner.** 1985. Death due to acute pancreatitis: a retrospective analysis of 405 autopsy cases. Dig. Dis. Sci. **30**:1005–1018.
41. **Rubistein, E., J. Haspel, E. Klein, G. Ben-Ari, R. Swartzkopf, and A. Tadmor.** 1980. Effect of pancreatitis on ampicillin excretion in pancreatic fluid of dogs. Antimicrob. Agents Chemother. **17**:905–907.
42. **Schentag, J. J.** 1989. Clinical significance of antibiotic tissue penetration. Clin. Pharmacokinet. **16**(Suppl. 1):25–31.

43. **Smith, J. T., and C. S. Lewin.** 1988. Chemistry and mechanisms of action of the quinolone antibacterials. p. 23–82. *In* V. T. Andriole (ed.), The quinolones. Academic Press, New York.
44. **Studley, J. G. N., J. J. Schentag, and W. G. Schenk.** 1981. Effect of bile induced pancreatitis on tobramicin excretion in pancreatic fluid. Ann. Surg. **193:**649–659.
45. **Studley, J. G. N., J. J. Schentag, and W. G. Schenk.** 1982. Excretion of cefalothin and cefamandole by the normal pancreas and in acute pancreatitis in dogs. Antimicrob. Agents Chemother. **22:**262–265.
46. **Vaudaux, P., and F. A. Waldvogel.** 1980. Gentamicin inactivation in purulent exudates: role of cell lysis. J. Infect. Dis. **142:**586–593.