Pefloxacin penetration into human necrotic pancreatic tissue

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Antibiotic prophylaxis may be useful in acute necrotising pancreatitis, a disease associated with a considerable incidence of infectious complications. The aim of this study was to assess pefloxacin penetration into necrotic pancreatic tissue during human necrotic pancreatitis. Ten patients (mean age 53.2 ± 17.4 years) with severe acute pancreatitis (mean Ranson score 4.3) were studied. Pefloxacin was administered at a dose of 400 mg bd every 12 h by iv infusion (bolus, 15 min). Intraoperative samples of necrotic pancreatic tissue and blood were collected simultaneously 1, 2, 4.5, 6, 8.5 or 10 h after the last pefloxacin administration in patients treated for 1, 3, 4, 7, 8, 17 or 20 days. Drug concentrations were determined by the microbiological agar-well diffusion method (Escherichia coli Kp 05124 as test micro-organism in Isosensitest Agar). Levels in serum ranged from 20 to 90 mg/L (at 2 and 6 h, respectively), in necrotic pancreatic tissue from 20 to 290 ng/g depending on different sampling time. Maximum tissue peak concentrations appeared between 4 and 6 h. The necrotic pancreatic tissue/serum concentration ratio ranged from 0.9 to 5.1, values depending on tissue sample collection. Therapeutic concentrations (20-60 µg/g) above the MIC of potentially pathogenic enteric microorganisms were still present in necrotic pancreatic tissue 10 h after the last drug administration. Pefloxacin appeared to concentrate in necrotic pancreatic tissue, without appreciable accumulation after multiple-dose administration. The pefloxacin concentrations in necrotic pancreatic tissue showed high variability, depending on the degree of necrosis, inflammation and sample vascularization. Our results provided evidence of good, prompt penetration of pefloxacin into necrotic pancreatic tissue. Pefloxacin seems to exhibit favourable pharmacokinetic and pharmacodynamic properties for pancreatic infections.

Introduction

Acute pancreatitis is a disease associated with a considerable incidence (40%-60%) of infectious complications bearing high rates of morbidity and mortality. Most infections are caused by Gram-negative enteric micro-organisms; 20% by Gram-positive organisms and 15% by anaerobes (Widdison, 1994). The use of antibiotic prophylaxis in severe pancreatitis complicated by pancreatic necrosis can be useful (Pederzoli et al., 1993; Sainio et al., 1995). The choice of antimicrobial drugs, timing and dosage schedules, i.e. the optimisation of drug administration are open questions requiring further investigation.
Our previous studies (Bassi et al., 1994) have demonstrated that some antibiotics, including the fluoroquinolones, have appropriate characteristics to penetrate necrotic pancreatic tissue, but drug concentrations are very variable. These concentrations depend on the different degrees of illness of the patients and on the dose and sampling collection time. The majority of factors affecting tissue concentration of antibiotics are present in this disease: tissue characteristics, alkaline pH, inflammation, ischaemia, fibrin clots, vascular microthrombosis, and infection. Moreover, pancreatic exocrine tissue is characterized by secretory functions, acinar units, with anastomoses directly connecting the cytoplasm of adjacent cells, and paracellular channels making it a special organ (Kern, 1993). Molecules can pass from cell to cell (Petersen, 1993). Therefore, the concentrations of antibiotics in pancreatic tissue (and more in necrotic tissue) are very difficult to predict.

Pefloxacin is a fluoroquinolone antimicrobial agent with a wide spectrum of activity, good tissue diffusibility, long serum half-life (10–12 h), and parenteral and oral administration (Gonzalez & Henwood, 1989). These characteristics provide a rationale for using pefloxacin to prevent acute pancreatitis infections. In a preliminary study pefloxacin administered to a few patients with acute necrotising pancreatitis showed good penetration into necrotic pancreatic tissue (NPT). The aim of the study was to assess the degree of pefloxacin penetration into necrotic pancreatic tissue in sufficient patients with acute necrotising pancreatitis, to define the main source of variability: collection time, total dose administered, and/or duration of treatment.

Patients and methods

Subjects

Ten patients with severe acute necrotising pancreatitis diagnosed on the basis of clinical examination, biochemical tests and computed tomography scans were enrolled in the study, i.e. eight males and two females with a mean age of 53.2 ± 17.4 years (mean ± S.D.) and a mean body weight of 65.8 kg (range 52–93). The severity of pancreatitis as determined by Ranson score (Ranson et al., 1974) was 4.3 (mean) at 24 h after admission. One patient with a necrotic pseudocyst was included. All patients gave their informed consent. Approval of the University Human Research Review Committee was obtained for the study.

Pefloxacin (Peflacin, Rhône-Poulenc Rorer, Milan, Italy) was administered prophylactically 400 mg bd every 12 h by iv infusion (bolus, 15 min). Venous blood and tissue samples were collected simultaneously from each patient at 1, 2, 4.5, 6, 8.5 or 10 h after the last pefloxacin administration in each patient treated for 1, 3, 4, 7, 8, 17 or 20 days (one or two patients each time). Samples of NPT were obtained intraoperatively in seven patients and by fine needle aspiration in another three patients (A, B and L). NPT samples were maintained in an ice bath and processed as soon as possible (generally 20–30 min after collection). Serum samples were obtained after centrifuging at (1020 g). NPT was homogenized and centrifuged (3279 g, 4°C); the supernatant was separated; the residual material was resuspended in sterile saline, manually homogenized and centrifuged, and the supernatant separated. All samples were frozen at −80°C until assay.
Drug assay

We used a microbiological assay to measure the levels of active drug. Pefloxacin was assayed by the standard large-plate agar-well-diffusion method using *E. coli* Kp 05124 (final concentration of overnight culture 0·15%) as test micro-organism in Isosensitest Agar (Oxoid Unipath Ltd, Basingstoke, England), as previously described in detail (Bassi et al., 1994).

Standard concentrations of pefloxacin were prepared in rat pancreatic tissue and serum and processed along with samples. The sensitivity limits in serum and tissue were 0·2 mg/L and the pefloxacin assay was linear over a range of 1·0—20 mg/L \( (r = 0·95) \); the within-day coefficient of variation was 1·3% and 1·7% for the highest and lowest serum concentrations, respectively, and 0·5% for different concentrations in tissue. The between-day coefficient of variation was 5·9% and 7·3% for the highest and lowest serum concentrations, respectively, and 8·6% and 8·8% for the highest and lowest tissue concentrations, respectively. Samples and standard concentrations were assayed in triplicate.

Results are mg/L or µg/g of tissue.

Results

Pefloxacin reached necrotic pancreatic tissue at adequate, microbiologically active concentrations (Figure).

Serum concentrations after a single intravenous dose were 2·28 mg/L at 2 h. Furthermore, during the course of repeated administrations pefloxacin mean serum concentrations increased, ranging from 4·5 (after 10 h, patient H) to 9·12 mg/L at 6 h (patients D and G). Concentrations in intraoperative necrotic pancreatic tissue ranged from 7·25 µg/g (after 1 h, patient I) to 29·0 µg/g (patient H) following multiple-dose administration. High variability was observed in tissue concentrations among patients as well as in different necrosis sites. In three patients (F, D, and G) samples collected from perinecrotic sites showed very high pefloxacin concentrations (49·0 µg/g, patient...
G, \(54.0 \mu g/g\), patient D and \(82.9 \mu g/g\), patient F) depending on lower degree of necrosis, inflammation and better vascularization of pancreatic samples. There was no apparent relation between serum levels and tissue concentrations.

Pefloxacin given as a single dose (patient B) showed good penetration in sample fluid obtained by fine-needle aspiration \(2.05 \text{ mg/L}\) and a better rate into necrotic fragments \(3.75 \mu g/g\) 2 h after administration. Six days after multiple administration (patient A) tissue concentrations were high, registering \(9.3 \mu g/g\) 12 h after the second administration, i.e. just before the first administration on day 7 which produced a tissue concentration of \(13.0 \mu g/g\) 2 h later. The penetration in fluid appeared prompt, without any appreciable accumulation following seven days of treatment.

The time to reach peak concentrations in necrotic tissue was 4–6 h after the last dose, after which drug concentrations in NPT tended to decrease. Steady-state concentrations were achieved within 72 h. The effect of prolonged treatments produced no pefloxacin increase in serum, as shown in the Table: concentrations corresponded to \(9.2 \text{ mg/L}\) 6 h after 1 and 20 days of treatment, \(7.5 \text{ mg/L}\) and \(5.6 \text{ mg/L}\) at \(4.5 \text{ h}\) after 3 and 8 days, respectively. NPT concentrations of pefloxacin increased with the length of treatment, corresponding to \(10.1 \text{ mg/L}\) and \(28.7 \mu g/g\) at \(4.5 \text{ h}\) after 3 and 8 days of treatment, respectively. At \(6 \text{ h}\) NPT levels were \(10.5\) and \(26.9 \mu g/g\) after 1 and 20 days of drug administration, respectively.

The patient with a pseudocyst (L) treated with a single dose administration (400 mg) showed serum levels of \(3.6\) and \(2.0 \text{ mg/L}\) after 1 and 2 h, respectively, and no drug in pseudocyst fluid. Therapeutic concentrations, above the MICs of potentially pathogenic micro-organisms, were present in NPT and serum 8–10 h after pefloxacin administration. Microorganisms were not isolated during NPT assay. New organisms isolated during drug administration were recovered from surgical drains, namely: *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Candida glabrata* in three of seven patients who underwent surgery.

The penetration capacity of pefloxacin (expressed as NPT to serum ratio) was good in both drainage samples and tissue. The ratio was 0.89 and 1.4 in drainage fluid and NPT two hours after pefloxacin administration, increased to 5.1–5.9 after 4.5 and 6 h, and was maintained at 4.3–4.6 values after 8.5–10 h. The multiple-dose administration produced a slight increase in both serum and tissue levels without any appreciable influence on the degree of penetration into the pancreas. Pefloxacin concentrations in pancreatic tissue were 1–5 times greater than those in serum. Tissue to serum ratio changed in relation to time of sample collection.

If we arrange the data according to days of treatment we observe that the NPT to serum ratio following multiple-doses was maintained at the same levels after prolonged
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treatment (8 and 20 days). Pefloxacin penetration capacity was similar for treatments administered for 1 or 3 weeks. Therefore, pefloxacin appeared to concentrate in necrotic pancreatic tissue without appreciable accumulation (Table). At least 3 days of treatment are advisable to obtain a constant degree of drug penetration into necrotic pancreatic tissue.

Discussion

Our results confirm good, prompt penetration of pefloxacin into necrotic pancreatic tissue. Pefloxacin quickly reaches extracellular fluid as shown by fine-needle aspirate, and penetrates into necrotic pancreatic tissue reaching maximum concentrations 4–6 h after administration. Repeated multiple-dose administration produced serum concentrations similar to those observed in healthy subjects, and concentrations in necrotic pancreatic tissue 1–5-fold higher than in serum. Time to peak in necrotic tissue and serum concentrations of pefloxacin in patients with acute pancreatitis corresponded to the pharmacokinetic profile of pefloxacin in normal subjects (Gonzalez & Henwood, 1989). The physiological fluctuations observed among different subjects were emphasised by the pathology. The substantial variability in necrotic pancreatic tissue concentrations reported in our previous study (Bassi et al., 1994) for different antibacterial drugs has also been confirmed in this study in a larger number of patients (treated with the same drug). In our conditions, while the drug serum levels are generally within normal ranges, the physiological interindividual variability in tissue concentrations is further enhanced by the disease. Pefloxacin seems to concentrate in pancreatic tissue without a direct relation to serum levels, thus the serum levels are not fully predictive of tissue concentrations. The number of doses administered and the duration of therapy assured high pancreatic levels, although the amount remains difficult to predict quantitatively from serum concentrations. Tissue to serum ratios changed in relation to timing of collection of samples at steady state after multiple-dose, according to pefloxacin pharmacokinetics. No significant tissue accumulation was noted from the first dose to the steady state on chronic dosing also in healthy volunteers (Nix & Schentag, 1988; Gonzalez & Henwood, 1989). Therefore, the high variability found in pefloxacin tissue concentrations depended mainly on the degree of necrosis and the patient's conditions.

The fluoroquinolones are relatively small, lipophilic molecules, weak acids with an amphoteric configuration. These properties result in a rapid, extensive distribution in tissue and fluids throughout the intracellular and extracellular compartments (Fitton, 1992). The intracellular concentration depends on the degree of drug ionisation at pancreatic pH, and the characteristics of the membranes. Studies carried out on the bacterial cytoplasmic membrane have shown that the penetration of pefloxacin across the membrane and intracellular accumulation are related to the electrochemical configuration of the drug and cellular pH. Both the zwitterionic form and undissociated drug penetrate into cells (Furet et al., 1992). This behaviour of quinolones can be applied to different biological membranes, including the pancreatic acinar cell. In presence of necrotizing pancreatitis (Bertazzoni Minelli, 1994), additional factors such as the anatomy (acinar unit) and functions of the pancreas and, even more so, membrane integrity and intracellular pH can contribute to define the amount of active drug present in the cell and its permanence at the site of infection. However, on the basis of our data the intracellular disposition of fluoroquinolones also seems to be
confirmed in the course of necrotising pancreatitis as well as the inability of pefloxacin to penetrate across the membrane of specialised tissues such as pseudocysts. In necrotic pancreatic tissue pefloxacin is partly ionised: in this form pefloxacin undergoes the intracellular ion-trapping to a limited extent. Probably, intra-extracellular exchanges occur which are so rapid as to prevent pefloxacin from accumulating, or the amount of drug capable of penetrating is reduced in the presence of necrosis.

Very high concentrations of quinolones are obtained in tissue far exceeding the minimum inhibitory concentrations for most aerobic and anaerobic bacteria (Gonzalez & Henwood, 1989), whenever the concentrations of fluoroquinolones assayed in tissue may not reflect the free concentration available at the site of infection. The binding of the quinolones to cells and the degree of antibiotic activity at intracellular sites has not been defined (Tulkens, 1991).

After surgery three patients developed further infections during treatment. Micro-organisms not susceptible or resistant to pefloxacin were isolated from drained pancreatic necrosis, namely: E. faecalis, P. aeruginosa and C. glabrata. Since pefloxacin, by virtue of its pharmacokinetic characteristics, is capable of exerting an antibacterial effect on intestinal aerobic Gram-negative bacteria (Korten & Murray, 1993), the risk of infections due to translocation of intestinal bacteria should be reduced. For instance, the prophylactic use of new quinolones has been proposed for selective gut decontamination in the prevention of nosocomial infections in intensive care units (Potgieter & Hammond, 1995) as well as for prophylaxis of infections related to acute pancreatitis (Luiten et al., 1995). The treatments seem to be effective, though, it is interesting to note the increasing colonisation by staphylococci and enterococci in intensive care unit patients and the occurrence of E. faecalis in post-operative infections (Dellamonica & Bernard, 1993).

Though pefloxacin concentrations and degree of penetration are difficult to predict, we can conclude that as a result of the pharmacodynamic properties, its degree of diffusibility, the concentrations obtained, and its penetration and persistence in the infection site, pefloxacin appears to be ideally suited for prophylaxis during human acute pancreatitis. Clinical trials are warranted for the thorough evaluation of this indication.

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References


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