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Activity of Moxifloxacin, Imipenem, and Ertapenem against *Escherichia coli*, *Enterobacter cloacae*, *Enterococcus faecalis*, and *Bacteroides fragilis* in Monocultures and Mixed Cultures in an *In Vitro* Pharmacokinetic/Pharmacodynamic Model Simulating Concentrations in the Human Pancreas

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The activities of moxifloxacin, imipenem, and ertapenem against pathogens causing severe necrotizing pancreatitis were studied in an *in vitro* pharmacokinetics/pharmacodynamics (PK/PD) model. *Escherichia coli*, *Enterobacter cloacae*, *Enterococcus faecalis*, and *Bacteroides fragilis* were exposed in monocultures and mixed cultures to concentrations of the three agents comparable to those in the human pancreas. Moxifloxacin was more active than the two carbapenems in monocultures and mixed cultures, reducing the numbers of CFU more drastically and more rapidly.

Infectious complications are the major determinant of morbidity and mortality in patients with severe acute pancreatitis (SAP), in particular in patients with necrotizing pancreatitis. Although polymicrobial infections caused by anaerobes and aerobes, including *Escherichia coli*, *Enterobacter cloacae*, *Enterococcus faecalis*, and *Bacteroides fragilis* are frequent in patients with SAP (2, 4, 13, 19), antibiotic prophylaxis is not recommended; however, in case of infectious complications, treatment with, e.g., a fluoroquinolone or a carbapenem is indicated (1, 4, 12, 16, 21, 23, 25).

Thus, we assessed the pharmacokinetics/pharmacodynamics (PK/PD) of moxifloxacin, imipenem, and ertapenem against SAP pathogens by simulating the regimens that either are recommended for treatment of SAP (carbapenems) or are commercially available (moxifloxacin).

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Drug concentrations were deduced by log-linear regression from published data following infusions of 400 mg moxifloxacin (22) and 1,000 mg each of imipenem (3, 18) and ertapenem (24). The following maximal total drug concentrations (C_{max}), time to C_{max} (T_{max}), and elimination half-life ($t_{1/2}$) in human pancreatic tissue were deduced from these published data: for moxifloxacin, C_{max} = 4.6 mg/liter, T_{max} = 5 h, and $t_{1/2}$ = 8; for imipenem, C_{max} = 16 mg/liter, T_{max} = 0.25 h, and $t_{1/2}$ = 1.3 h; for ertapenem, C_{max} = 1.3 mg/liter, T_{max} = 0.5 h, and $t_{1/2}$ = 4 h. For calculation of free drug concentrations, the following was considered. First, binding of antibiotics to serum proteins was assessed (moxifloxacin, 40% [20]; imipenem, 20% [11]; ertapenem, 95% [17]). Second, all agents bind predominantly to albumin. Third, albumin concentrations in serum amount to 4.5%, compared to 1.3% in pancreatic juice (7, 8). This difference in albumin content was considered for the calculation of free drug concentrations in serum and pancreatic tissue, respectively.

E. coli ATCC 11775, *E. cloacae* ATCC 13047, *E. faecalis* ATCC 19433, and *B. fragilis* ATCC 25285 served as indicator strains. The *Enterobacteriaceae* were cultivated aerobically in cation-adjusted Mueller-Hinton broth, and *B. fragilis* in monoculture as well as *B. fragilis* and *E. coli* in mixed culture were grown anaerobically in brucella broth (Oxoid GmbH, Wesel, Germany). The pH was adjusted to 7.2, which corresponds to the physiologically relevant pH value in pancreatic juice (7, 8). Pre- and postexposure MICs (Table 1) were determined according to CLSI standards (9, 10) using the relevant American Type Culture Collection (ATCC) control strains. Moxifloxacin (batch BXO1X6E), imipenem (batch 0885170), and ertapenem (batch 0932340) were provided by the manufacturers. The open one-compartment model according to Grasso et al. (14) was used to simulate the free pancreas concentrations. Samples for drug monitoring and viable count determinations were withdrawn at 0, 0.5, 1, 2, 4, 6, 8, and 10 h and at the end of the study at 24 h. Drug concentrations in the system were monitored in the absence and presence of bacteria; actual drug concentrations were almost identical under both conditions and were on average within 1.2 to 2.4% of the desired profiles. The

TABLE 1 Drug susceptibilities of the four indicator strains

Drug[a]	Pre-exposure/postexposure MIC (mg/liter)			
	E. coli	*E. cloacae*	*E. faecalis*	*B. fragilis*
MXF	0.06/0.06	0.06/0.06	0.25/0.25	0.25/0.25
IMI	0.06/0.06	0.25/0.25	0.5/0.5	0.125/0.125
ERTA	0.015/0.03	0.25/0.25	8.0/8.0	0.25/0.25

[a] MXF, moxifloxacin; IMI, imipenem; ERTA, ertapenem.

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TABLE 2 Antibacterial activities of moxifloxacin (MXF), imipenem (IMI), and ertapenem (ERTA) against mixed bacterial cultures in an *in vitro* model simulating free drug concentrations in pancreatic tissue

Drug	*E. coli* + *E. cloacae*		*E. coli* + *E. faecalis*		*E. coli* + *B. fragilis*	
	$\log_{10}$ ΔCFU (h)[a]	Regrowth (fold) after 24 h	$\log_{10}$ ΔCFU (h)[a]	Regrowth (fold) after 24 h	$\log_{10}$ ΔCFU (h)[a]	Regrowth (fold) after 24 h
MXF	−>6 (4)/−>6 (4)	+2/+6	−>6 (2)/−4 (8)	+2/+2.3	−>6 (2)/−3 (6)	+2/0
IMI	−4 (6)/−3 (6)	+2/+4	−5.5 (10)/−2 (8)	+3/+3	−5 (6)/−2.5 (8)	+2/0
ERTA	−5.3 (4)/−5.3 (4)	+3.1/+6.6	−4.5 (4)/+1.6 (4)	+3.4/+2.5	−5.4 (4)/−2.1 (6)	+2/0

[a] Maximal reduction (negative values) or growth (positive values) of viable counts in the indicated time.

indicator strains (inoculum, 6.0 to 6.7 $\log_{10}$ CFU/ml) grew equally well in monocultures, with growth rates (k) ranging from 0.19 (*E. cloacae*) to 0.25 h^{-1} (*B. fragilis*), and in mixed cultures of *E. coli* plus *E. cloacae* ($k = 0.2 + 0.2\ h^{-1}$), *E. coli* plus *E. faecalis* ($k = 0.21 + 0.22\ h^{-1}$), and *E. coli* plus *B. fragilis* ($k = 0.23 + 0.29\ h^{-1}$), respectively. The lower limit of detection of viable counts was 2 $\log_{10}$ CFU/ml.

Viable counts of *E. coli* and *E. cloacae* growing in monocultures were eliminated by moxifloxacin within 4 and 10 h, respectively; moxifloxacin reduced the inocula of *E. faecalis* and *B. fragilis* by 1.9 and 2.7 $\log_{10}$ CFU/ml within 6 h; regrowth was not observed. Imipenem reduced viable counts of *E. coli* and *E. cloacae* growing in monocultures by 4 $\log_{10}$ units within 2 h and those of *E. faecalis* and *B. fragilis* by 3 $\log_{10}$ units each within 8 h. The latter two species regrew by 5 $\log_{10}$ units. Ertapenem reduced CFU of *E. coli*, *E. cloacae*, and *B. fragilis* in monocultures by 5.8 $\log_{10}$ each in 8 h, whereas viable counts of *E. faecalis* were not affected. Regrowth was noted for *E. coli* by 4.5, for *E. cloacae* by 5.3, and for *B. fragilis* by 3.8 $\log_{10}$ units.

The activities of the three study drugs against mixed cultures are summarized in Table 2. Moxifloxacin was more active against *E. cloacae*, *E. faecalis*, and *B. fragilis* growing in mixed cultures than against monocultures, reducing the CFU more extensively and more rapidly. However, all species except *B. fragilis* regrew in mixed cultures, whereas they did not regrow in monocultures. The two carbapenems were less active in mixed cultures than in monocultures; first, viable counts were reduced more slowly, and second, regrowth was recorded in almost every case, except of *B. fragilis*. MICs of pre- and postexposure isolates were identical despite regrowth of bacteria having been exposed to the three study drugs (Table 1). Moxifloxacin tended to reduce viable counts of all species growing in mixed cultures more effectively and more rapidly than imipenem or ertapenem. The different activities of the agents tested in either monocultures or mixed cultures cannot be due to different growth conditions or competition for nutrient supply, as the growth rates of the indicator strains were almost identical under both conditions.

Data generated in the experiments described above simulating free concentrations in the human pancreas demonstrate that moxifloxacin and the two carbapenems were active against the major bacterial species causing infectious complications in patients with SAP. There is a 40 to 60% risk of pancreatic infections in patients with necrotizing pancreatitis, so antibacterial treatment is justified. Actually, carbapenems or ciprofloxacin are recommended (1, 5, 12, 21, 25). However, in contrast to ciprofloxacin, moxifloxacin is active against anaerobes; furthermore, its pharmacokinetic profile is more favorable than that of ciprofloxacin, as the concentrations of moxifloxacin in pancreatic tissue exceed the corresponding serum concentrations 3-fold (22), compared to a 1:1 ratio for ciprofloxacin (6, 15). In comparison to ertapenem, which exerts no activity against enterococci, moxifloxacin is variably active against *E. faecalis*. Furthermore, moxifloxacin was found to affect viable counts of the pathogens in mixed cultures more effectively and more rapidly than the two carbapenems. Thus, moxifloxacin may be superior to imipenem and ertapenem and could be a therapeutic alternative for an antibacterial treatment in patients with severe necrotizing pancreatitis.

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REFERENCES

1. **Bai Y, Gao J, Zou DW, Li ZS.** 2008. Prophylactic antibiotic cannot reduce infected pancreatic necrosis and mortality in acute necrotizing pancreatitis: evidence from a meta-analysis of randomized controlled trials. Am. J. Gastroenterol. **103**:104–110.
2. **Baron TH, Morgan DE.** 1999. Acute necrotising pancreatitis. N. Engl. J. Med. **340**:1412–1417.
3. **Bassi C, et al.** 1994. Behavior of antibiotics during human necrotizing pancreatitis. Antimicrob. Agents Chemother. **38**:830–836.
4. **Beger HG, Bittner R, Block S, Büchler MW.** 1986. Bacterial contamination of pancreatic necrosis: a prospective clinical study. Gastroenterology **91**:433–438.
5. **Beger HG, Gansauge F, Poch B, Schwarz M.** 2009. The use of antibiotics for acute pancreatitis: is there a role? Curr. Infect. Dis. Rep. **11**:101–107.
6. **Büchler M, et al.** 1992. Human pancreatic tissue concentration of bactericidal antibiotics. Gastroenterology **103**:1902–1908.
7. **Ciba-Geigy AG.** 1977. Teilband Körperflüssigkeiten, p 131–135. *In* **Wissenschaftliche Tabellen Geigy, 8th ed. Ciba-Geigy AG, Basel, Switzerland.**
8. **Ciba-Geigy AG.** 1977. Teilband Hämatologie und Humangenetik, p 121–144. *In* **Wissenschaftliche Tabellen Geigy, 8th ed. Ciba-Geigy AG, Basel, Switzerland.**
9. **Clinical and Laboratory Standards Institute (CLSI).** 2007. M11-A7: methods for antimicrobial susceptibility testing of anaerobic bacteria: approved standard. Seventh edition. Clinical and Laboratory Standards Institute, Wayne, PA.
10. **Clinical and Laboratory Standards Institute (CLSI).** 2009. M07-A8: methods for dilution antimicrobial susceptibility testing for bacteria that grow aerobically: approved standard. Eighth edition. Clinical and Laboratory Standards Institute, Wayne, PA.
11. **Clisshold SP, Tood PA, Campoli-Richards DM.** 1987. Imipenem/cilastatin: a review of its antibacterial activity, pharmacokinetic properties, and therapeutic efficacy. Drugs **33**:183–241.
12. **Dellinger EP, et al.** 2007. Early antibiotic treatment for severe acute necrotizing pancreatitis. A randomized double-blind, placebo controlled study. Ann. Surg. **245**:674–683.
13. **Garg PK, Khanna S, Bohidar NP, Kapil A, Tandon RK.** 2001. Incidence, spectrum and antibiotic sensitivity pattern of bacterial infections among patients with acute pancreatitis. J. Gastroenterol. Hepatol. **16**:1055–1059.
14. **Grasso B, Meinardi G, de Carneri I, Tamasala V.** 1978. New in vitro

model to study the effect of antibiotic concentration and rate of elimination of antibacterial activity. Antimicrob. Agents Chemother. **13**:570–576.
15. **Isenmann R, Friess H, Schlesinger P, Fleisscher K, Büchler MW.** 1994. Penetration of ciprofloxacin into the human pancreas. Infection **22**:343–346.
16. **Isenmann R, et al.** 2004. Prophylactic antibiotic treatment in patients with predicted severe acute pancreatitis: a placebo controlled, double blind trial. Gastroenterology **126**:997–1004.
17. **Livermore DM, Sefton AM, Scott GM.** 2003. Properties and potential of ertapenem. J. Antimicrob. Chemother. **52**:331–344.
18. **MacGregor RR, Gibson GA, Bland JA.** 1986. Imipenem pharmacokinetics and body fluid concentrations in patients receiving high-dose treatment for serious infections. Antimicrob. Agents Chemother. **29**:188–192.
19. **Schmid SW, Uhl W, Friess H, Malfertheiner P, Büchler MW.** 1999. The role of infection in acute pancreatitis. Gut **45**:311–316.
20. **Stass H, Kubitza D.** 1999. Pharmacokinetics and elimination of moxifloxacin after oral and intravenous administration in man. J. Antimicrob. Chemother. **43**(Suppl B):83–90.
21. **Talukdar R, Vege SS.** 2009. Recent developments in acute pancreatitis. Clin. Gastroenterol. Hepatol. **7**:53–59.
22. **Wacke R, et al.** 2006. Penetration of moxifloxacin into the human pancreas following a single intravenous or oral dose. J. Antimicrob. Chemother. **58**:994–999.
23. **Werner J, Hartwig W, Büchler MW.** 2007. Antibiotic prophylaxis: an ongoing controversy in the treatment of severe acute pancreatitis. Scand. J. Gastroenterol. **42**:667–672.
24. **Wittau M, et al.** 2006. Intraabdominal tissue concentration of ertapenem. J. Antimicrob. Chemother. **57**:312–316.
25. **Yao L, Huang X, Shi Y, Zhang G.** 2010. Prophylactic antibiotics reduce necrosis in acute necrotizing pancreatitis: a meta-analysis of randomized trials. Dig. Surg. **27**:442–448.