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INACTIVATION OF ANTIMICROBIAL AGENTS INSIDE THE DIGESTIVE TRACT

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SUMMARY

Inactivation of antimicrobial agents in the human intestinal tract is a frequent event and may significantly influence the impact of the antimicrobial agent on the normal microflora. Inactivation by bacterial enzymes, or specific or unspecific binding to bacteria and faecal material are common phenomena which lowers the active *in vivo* concentration in the intestines. The most common and thoroughly studied mechanism is the irreversible enzymatic inactivation of beta-lactam agents by beta-lactamases. Treatment with beta-lactam agents is often associated with an increase in the amount of beta-lactamase producing strains in the normal intestinal microflora. An inverse correlation between beta-lactamase activities in faeces, compared with the concentration of drug in the intestines and the level of ecological disturbance in the normal intestinal microflora, have been shown during administration of oral cephalosporins. Highly erythromycin resistant enterobacteria occurs frequently during treatment with erythromycin. The mechanism of this resistance is enzymatic inactivation of erythromycin, but it is uncertain whether the activities of these enzymes are sufficient to significantly reduce the high intestinal levels of erythromycin obtained after peroral administration. Oral administration of quinolones causes minor selective alterations in the intestinal microflora despite very high drug levels in faeces. Quinolones like norfloxacin have been shown to be reversibly inactivated in the intestinal tract by binding to bacteria and faecal material. Metronidazole have been reported to be significantly inactivated, probably by binding, by several susceptible and resistant organisms commonly found in the normal microflora. Sonicated cell extracts of *Enterococcus faecalis* have also been found to inactivate metronidazole *in vitro*. Intestinal inactivation of antimicrobial agents is probably of great importance with respect to reducing the ecological disturbances and maintaining the colonisation resistance of the normal intestinal microflora.

INTRODUCTION

It is well known that besides invaluable clinical achievements, treatment with antimicrobial agents against bacterial infections also may cause unwanted effects on the normal gastrointestinal microflora (*Edlund* and *Nord*, 1993). The ecological disturbances in the normal intestinal microflora after antimi-

crobial administration can be described from different aspects. Quantitative changes in the microflora are common, such as repression of susceptible bacterial groups, which may lead to a decreased colonisation resistance. Overgrowth of potentially pathogenic microorganisms belonging to the normal microflora like enterococci or yeasts may occur, as well as superinfections with exogenic potentially pathogenic bacteria like *Clostridium difficile*, which may cause life-threatening infections. Establishment of resistant strains, either by selection of intrinsically resistant microorganisms, or by selection of bacteria with acquired resistance, is also a common finding after antimicrobial therapy. Resistant factors may further be spread among other bacteria and bacterial groups by transposons, plasmids or other genetic material (*Hawkey*, 1986; *Quintiliani* and *Courvalin*, 1995). Several antimicrobial agents have also the ability to act as inducers and promote a temporary increase in levels of bacterial produced drug-inactivating enzymes.

The human microflora is a huge potential reservoir of potentially pathogenic microorganisms which under certain circumstances may translocate and cause infections in other sites of the body. Bacteria from the intestinal microflora dominate as causative agents in post-operative infections, and resistance among them may seriously complicate the prophylaxis and treatment of intra-abdominal infections (*Nord* et al., 1986; *Hawkey*, 1986).

The potential of an antimicrobial agent to disturb the normal intestinal microflora is related to the antibacterial spectrum of the agent, the route of administration, dose and pharmacokinetic properties (*Bergan*, 1986). The microflora can be influenced by high intestinal drug concentrations due to incomplete absorption of orally administered agents and by secretion of the antimicrobial agent in the bile or from the intestinal mucosa. Inactivation of the agent in the intestinal tract by enzymes or by binding of the drug to intestinal content may also significantly influence the impact of the antimicrobial agent on the normal microflora. In the present paper, different mechanisms of inactivation of antimicrobial agents in the digestive tract will be discussed.

ENZYMATIC INACTIVATION OF ANTIMICROBIAL AGENTS

Enzymes that inactivate or modify antimicrobial agents were detected almost in parallel with the discovery of antimicrobial agents. Primarily, these enzymes protect the enzyme-producing bacteria themselves, or co-infecting bacteria in mixed infections. Members of the normal microflora that produce large amounts of these enzymes, may also inactivate antimicrobial agents at the site of infection, and thereby protecting the infecting bacteria against the killing activity of the antibiotic (*Neu*, 1994; *Quintiliani* and *Courvalin*, 1995).

Beta-lactam agents

The most common and thoroughly studied mechanism is enzymatic inactivation of beta-lactam agents by beta-lactamases. Beta-lactamases are enzymes which irreversibly hydrolyse the beta-lactam ring of penicillins, cephalosporins, monobactams and carbapenems (*Livermore*, 1995; *Quintiliani* and *Courvalin*, 1995). The ability to produce beta-lactamases is widely spread among microorganisms. The beta-lactamases of Gram-positive bacteria are mainly extracellularly excreted, while

Gram-negative bacteria accumulate their beta-lactamases in the periplastic space, although extracellular release does occur. The enzyme production may be either chromosomally or plasmid mediated and may be constitutive or inducible. Nearly 200 beta-lactamases of various bacteria differing in substrate profiles, potential for inhibition, and physical characteristics have been described. Several classification schemes have been proposed, the most recent and comprehensive of which was developed by *Bush* (1995). Enzymes from aerobic microorganisms are well recognised, but beta-lactamases from various anaerobic bacteria such as *Bacteroides, Fusobacterium, Porphyromonas, Prevotella* and *Clostridium* strains are now commonly reported (*Aldridge,* 1995; *Finegold,* 1995). Bacteria belonging to the *Bacteroides fragilis* group are dominating in the intestinal microflora. Recent studies have shown that at least 90% of all strains in the *B. fragilis* group produce beta-lactamases. Twenty-five percent of these strains produce high levels of enzymes (*Cornick* et al., 1990). Most beta-lactamases from the *B. fragilis* group are constitutive chromosomal cephalosporinases, although penicillinases also have been described, as well as enzymes capable of hydrolysing the most stable beta-lactams such as cefoxitin and imipenem (*Cuchural* et al., 1986; *Hedberg* et al., 1992). Chromosomal beta-lactamases are widely present in enterobacteria and are inducible, with the exception of enzymes produced by *Escherichia coli*. Very low levels of the inducible enzyme are present in the absence of antibiotics, but temporary high levels can be observed in the presence of an inducing compound. Beta-lactamase derepressed mutants can be selected from inducible populations during therapy with weak inducers. Such compounds kill the inducible cells but allow survival and overgrowth of derepressed mutants, which arise at frequencies as high as 10^{-5} in inducible bacterial populations (*Livermore*, 1995). Cefoxitin, imipenem and meropenem are examples of beta-lactamase-stable strong inducers, while ampicillin, amoxicillin and narrow-spectrum cephalosporins are beta-lactamase-labile strong inducers. Plasmid-mediated beta-lactamase production genes are widely spread in bacteria belonging to the normal intestinal microflora, and are responsible for most of the ampicillin resistance now seen in 50% of clinical *E. coli* isolates (*Livermore*, 1995). Plasmid-mediated beta-lactamases are also widespread among staphylococci, and high-level ampicillin resistance in enterococci has recently occurred, and is now reported world-wide, as a result of genes encoding for beta-lactamases acquired from staphylococci (*Shlaes*, 1993). Extended spectrum beta-lactamases which attack many of the newer "beta-lactamase-stable" cephalosporins and penicillins, are now increasingly being reported. To overcome the clinical problem of beta-lactamase resistance, a combination of a labile beta-lactam agent with a beta-lactamase inhibitor like clavulanic acid, sulbactam or tazobactam can be used. The success of this strategy depends on how efficient the inhibitor is, how much enzyme the bacteria produce, the stability of the protected drug, the permeability and intrinsic susceptibility of the organisms, and surrounding conditions, especially the pH (*Livermore*, 1995).

Treatment with beta-lactam agents is often associated with an increase in the number of beta-lactamase producing strains in the normal intestinal microflora. In a study where amoxicillin was given perorally to 10 healthy volunteers, the colonisation of beta-lactamase producing amoxicillin resistant enterobacteria were strongly induced

Table 1: Relationship between the concentration of cephalosporins in faeces, beta-lactamase activities and ecological disturbances in the intestinal microflora, in 34 subjects receiving peroral cephalosporins

Number of patients	Drug concentration in faeces	β-lactamase activity	Overgrowth of: Enterococci	Yeasts	*C. difficile*
7	- [a]	++	-	-	-
10	-	+	+	(+)	-
4	+ [b]	+	+	+	-
5	-	-	+	(+)	+
2	+	-	+	-	+
6	++[c]	-	++	+	++

[a] -: negative; [b] +: positive; [c] ++: strongly positive

during administration (*Brismar* et al., 1993). In another study where 14 patients with *Helicobacter pylori* infection were treated with omeprazole plus amoxycillin for two weeks, a significant selection of resistant enterobacteria and enterococci were observed, and 10 patients increased their intestinal beta-lactamase production during treatment (*Stark* et al., 1996). The faecal levels of amoxicillin were under the detection limit in all samples from these studies, probably partly due to enzyme inactivation. The ecological effects in the intestinal microflora by 7 to 10 days administration of three oral cephalosporins - cefuroxime axetil (n=10), cefpodoxime proxetil (n=10) and ceftibuten (n=14) - to healthy volunteers lead to significant increases in beta-lactamase production during administration (Table 1). There was an inverse correlation between enzyme activity in faeces during administration compared with the concentration of drug in the intestines and the level of ecological disturbances in the normal intestinal microflora (*Edlund* et al., 1994). The results of these studies imply that from an ecological point of view, the increase in beta-lactamase activity during beta-lactam administration is probably an advantage because it helps to preserve the colonisation resistance and thus, protects against invading pathogens.

In another study, the induction ability of cefoxitin on beta-lactamase production was studied in aerobic and anaerobic microorganisms isolated from the intestinal microflora (*Stark* et al., 1995). It was shown that the enzyme production was highly enhanced in the presence of sub-inhibitory concentrations of the inducer, in pure bacterial broth cultures. However, when the induction assay was performed with the inducible bacterial strains *(Citrobacter freundii, Bacteroides ovatus* and *Clostridium butyricum)* incubated in faecal suspension, the induction ability was strongly reduced. Thus, it seems that faeces inhibits the beta-lactamase induction of aerobic and anaerobic bacteria.

Macrolides

Macrolide antimicrobial agents are generally bacteriostatic agents that inhibit bacterial RNA-dependent protein synthesis (*Yao* and *Moellering,* 1995). Erythromycin is the prototype of this group; newer semisynthetic agents are clarithromycin, azithromycin, dirithromycin and roxithromycin. Macrolides are mainly active against Gram-positive bacteria. By contrast, enterobacteria are

naturally resistant to low levels of these drugs, due to cell impermeability (MIC of erythromycin against most enterobacteria ranges between 2 and 256 mg/l) (*Quintiliani* and *Courvalin*, 1995). High drug concentrations are obtained in the intestinal tract after peroral administration of clinical recommended doses, and oral administration of erythromycin, clarithromycin and roxithromycin has been reported to markedly alter the aerobic and anaerobic intestinal microflora (*Brismar* et al., 1992; *Edlund*, 1993). However, highly resistant enterobacteria (MIC ≥500 mg/l) occur frequently during treatment with erythromycin. The mechanism for this high-level resistance to erythromycin in enterobacteria has been shown to be enzymatic inactivation of erythromycin, either by production of erythromycin esterases which destroy the lactone rings of 14-membered macrolides, or by a phosphorylation catalysed by a 2'-phosphotransferase (*Quintiliani* and *Courvalin*, 1995). These highly resistant enterobacteria are often isolated from the intestinal microflora after peroral erythromycin administration (*Andremont*, 1986), but it is uncertain whether the activities of these enzymes are sufficient to significantly reduce the high intestinal levels of erythromycin obtained after peroral administration.

BINDING OF ANTIMICROBIAL AGENTS TO FAECAL MATERIAL

Quinolones

Quinolone antimicrobial agents (ciprofloxacin, enoxacin, fleroxacin, norfloxacin, ofloxacin, pefloxacin and sparfloxacin) exhibit a bactericidal antibacterial activity resulting from inhibition of the essential enzyme DNA gyrase. The function of this enzyme is required for DNA replication, transcription, recombination, DNA repair and transposition (*Yao* and *Moellering*, 1995). The *in vitro* activity of quinolones is primarily directed against aerobic and anaerobic facultative Gram-negative bacteria. Peroral administration of quinolones results in elimination or strong suppression of intestinal enterobacteria (*Edlund* et al., 1987; *Edlund* and *Nord*, 1993). Ciprofloxacin and ofloxacin also affect intestinal enterococci and anaerobic microorganisms to a minor degree (*Bergan* et al., 1986; *Edlund* et al., 1988). Resistance to quinolones can be mediated in two different ways; alterations in the A subunit of the DNA gyrase or alterations in the bacterial outer membrane protein, thus affecting uptake of the drug into the bacterial cell. The latter case seems to be associated with reduced susceptibility to other groups of antimicrobial agents such as chloramphenicol, cefoxitin and tetracycline. Resistance against quinolones is still rather uncommon, although findings of resistant *Pseudomonas*, staphylococci and enterobacteria are increasingly reported (*Quintiliani* and *Courvalin*, 1995). Several clinical studies on the newer quinolones have shown that peroral administration results in very high concentrations in the intestinal tract. Peak faecal concentrations of norfloxacin of 120 to 2,700 mg/kg after therapeutic doses have been reported, which is, with few exceptions, far exceeding the MIC's for all intestinal microorganisms (*Edlund* et al., 1987). Thus, despite high drug levels in faeces, the main part of the aerobic Gram-positive and the anaerobic flora remains unaffected after norfloxacin administration. The same pattern is true for other quinolone agents (*Edlund* and *Nord*, 1993).

It has earlier been shown by using ^{14}C labelled norfloxacin, that nor-

floxacin is reversibly inactivated in the intestinal tract by binding to faecal material (*Edlund* et al., 1988). The specific binding was reversible, saturated after 90 min of incubation at 37°C, and increased linearly with faecal concentration. The maximal binding capacity (B_{max}) of the specific binding was 0.12 μmol/g and of the unspecific binding 11.8 μmol/g faeces. The binding capacity of unlabelled ciprofloxacin, norfloxacin, enoxacin, ofloxacin and pefloxacin to faeces was studied by competitive assays, and was shown to be in the same range as that of ^{14}C norfloxacin. The results of norfloxacin binding to pure bacterial suspensions, suggest that the main part of the binding is to the bacterial fraction of faeces.

Nitro-imidazoles

Nitro-imidazoles (metronidazole, tinidazole and ornidazole) have their bactericidal activity inside the cell. The nitro group is reduced by a nitroreductase enzyme in the cytoplasm, generating highly cytotoxic intermediate compounds of free radicals which disrupt the DNA of the cell (*Yao* and *Moellering*, 1995). Nitro-imidazoles exhibits an excellent activity against all anaerobic bacteria but are inactive against all aerobic and facultative anaerobic bacteria. Orally administered nitro-imidazoles are well absorbed and have been shown to cause only minor alterations in the oral and intestinal microflora (*Heimdahl* et al., 1980; *Edlund* and *Nord*, 1993). The main mechanisms involved in resistance against metronidazole is slower penetration of the drug into the cell. It has been shown that metronidazole can be inactivated by aerobic bacteria, not susceptible to metronidazole (*Edwards* et al., 1979; *Quintiliani* and *Courvalin*, 1995). The phenomenon that metronidazole treatment of anaerobic infections occasionally results in clinical failure, although the pathogenic strain isolated is sensitive to the drug *in vitro*, has been studied by several groups. *Edwards* et al. reported that several organisms commonly found in the normal microflora, such as *E. coli* and *Klebsiella aerogenes*, were capable of absorbing and inactivating significant amounts of metronidazole *in vivo*, and suggested that metronidazole binds to particular fractions of the cells in a chemically unmodified form (*Edwards* et al., 1979). Time-kill curves and radioisotope experiments with ^{14}C-metronidazole have also revealed that the drug is taken up by both susceptible and resistant bacteria (*Ralph* and *Clarke*, 1978; *Tally* et al., 1981). *Nagy* and coworkers have studied the *in vitro* inactivation of metronidazole by 20 different clinical isolates of *Enterococcus faecalis* (*Nagy* and *Földes*, 1991). In this study, susceptible *B. fragilis* strains and metronidazole were cultured in broth medium, with or without *E. faecalis* strains. All of the tested *E. faecalis* strains were able to protect the *B. fragilis* group strains against the killing effect of metronidazole at a concentration 4-8 times higher than normal MIC. When *E. faecalis* strains were cultured anaerobically for 24 h in the presence of 4 mg metronidazole/l, no metronidazole could be detected subsequently in the culture supernatants by HPLC. Sonicated cell extracts of *E. faecalis* were found to inactivate metronidazole to the same extent, whereas culture supernatants had no such effect. Due to the findings that cell free extracts of the *E. faecalis* strain were able to inactivate metronidazole, the authors suggest that this mode of metronidazole inactivation differs to the binding mode of inactivation described earlier, and may be enzymatic. Further studies are needed to investigate whether these phenomena of metronidazole inactivation by microorganisms commonly found in the normal intestinal microflora contribute to the

fact that very low or undetectable intestinal levels of metronidazole are found after peroral administration.

CONCLUSION

Antimicrobial agents may be inactivated in the human intestinal tract by several mechanisms. Inactivation by bacterial enzymes, or specific or unspecific binding to bacteria and faecal material are common phenomena which lowers the active *in vivo* concentration in the intestines. Intestinal inactivation of antimicrobial agents is probably of great importance with respect to reducing the ecological disturbances and maintaining the colonisation resistance of the normal intestinal microflora.

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