The impact of anaesthesia and perioperative antibiotic treatment on postoperative infections

PhD Thesis

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1. Abbreviations

Amp	ampicillin
ASA	American Society for Anaesthetists
carb	carbenicillin
CFLP	Carnworth Farm Lane Petter
cfu	colony forming unit
Cm	chloramphenicol
cys	cysteine
DMEM	Dulbecco`s modified Eagle`s medium
DPBS	Dulbecco`s phosphate buffered saline
GTN	glyceryl trinitrate
HA	haemagglutination
HBSS	Hanks' balanced salt solution
Hly	haemolysin
HPLC	high pressure liquid chromatography
i.p.	intraperitoneally
i.v.	intravenous
Km	kanamycin
met	methyl transferase
MRHA	mannose resistant haemagglutination
MSHA	mannose sensitive haemagglutination
Nal	Nalidixic acid
PBS	phosphate buffered saline
PMN	polymorphonuclear leukocyte
p.o.	per os
pro	proline
^{r}	resistant
SNP	sodium nitroprusside
Tc	tetracycline
TIVA	total intravenous anaesthesia
Tn	transposon

2. Introduction

2.1 General introduction

The Greek-derived noun 'anaesthesia', implying insensitivity to touch and therefore to surgical trauma, was suggested by Oliver Wendell Holmes, professor of anatomy in Boston and famous author, in a letter to William Thomas Green Morton. Morton was the first to demonstrate the administration of ether for surgical operations in 1846 (Van Aken et al., 1998; Kitz and Vandam, 1990). That was the date of birth of modern anaesthesia.

Whereas in the early days of surgery the major concern of the anaesthetist was merely survival of the patient, whilst providing adequate anaesthesia and analgesia, nowadays the anaesthetist's task has broadened to encompass the protection of the patient against adverse effects of the surgical trauma in general and to help reducing perioperative morbidity.

Infection is still one of the leading causes of perioperative morbidity and mortality. The postoperative infection rate of patients undergoing clean elective surgery can be as low as 0.7% (Schmalzried et al., 1991), but after major trauma it can be as high as 80% (Daschner, 1985).

Factors determining the occurrence of postoperative infections include the type and length of surgery, the presence of underlying diseases (Murray et al., 1991), perioperative blood transfusion (Waymack et al., 1989), and perioperative hypothermia (Kurz et al., 1996).

Anaesthesia has been long suspected to contribute to infection in the postoperative period (Rubin, 1904; Brown and Petroff, 1919; Bruce 1967; Hilburger et al., 1997).

Bacterial virulence factors and mechanisms to help invading humans and how our immune system tries to overcome infections have been extensively studied (Gunzenhaeuser et al., 1991).

But how much do we know about the effects of anaesthesia on postoperative infections and about the effects of anaesthetics on bacteria?

Taking into consideration the number of operations under general anaesthesia (more than 6000/year only in the main teaching hospital of Pécs University) and the high cost of prolonged hospital stay due to infectious complications explain the importance of this topic.

The anaesthetist, in taking care of the patient before, during and after the operation, should have a sufficient knowledge of this subject. Preventing these infections requires an understanding of their pathogenesis.

The occurrence of infections most often involves a three-step process: first, colonisation of a patient's mucosa or skin with a potential pathogen; second, access of the pathogen to a site where it may invade tissues, often in association with a foreign body such as an intravascular catheter or an endotracheal tube; and third, an imbalance among the pathogen's virulence factors and the host's defence factors, which eventually results in the infection.

The process leading to infection is complex and many microbial, host and environmental factors are implicated. The topics that are involved in the Thesis will be discussed in detail while those that are not connected with it will only be mentioned.

2.2 Source of bacteria in postoperative infections

Bacteria colonising patients in the perioperative period are generally acquired in one of two ways: endogenously from the patient's own flora or exogenously from the immediate hospital environment.

2.2.1 Endogenous source of bacteria in postoperative infections

Most of the infections in the postoperative period and/or associated with long term ventilation originate from the patient's own flora, – what is referred to as primary or secondary endogenous infection (Stoutenbeek and van Saene, 1990; Murray et al., 1991; Barana et al., 1992; Sato et al., 1996; Inglis et al., 1998). The number of bacteria carried by man and animal hosts greatly outnumber the host cells (Linton and Hinton, 1990). Fortunately, this enormous bacterial population cause no detectable illness, indicating that there must be a well-sustained balance between the host and the commensal microflora. This balance may be maintained by the production of bacterial and/or host cytokine-like molecules. These agents can either inhibit the release or activity of proinflammatory cytokines or induce anti-inflammatory cytokines (Henderson and Wilson, 1996a).

There are data supporting this view. Bacteria produce various cytokine-inducing proteins, carbohydrates and lipids (Henderson and Wilson, 1996b; Henderson et al., 1996). Mice with inactivated genes for interleukin-2 (IL-2) or IL-10 (anti-inflammatory cytokines) developed severe colitis (Sadlack et al., 1993) or enterocolitis (Kühn et al., 1993). There is a growing number of studies investigating the effects of anaesthesia and surgery on cytokines. Detailed discussion of this topic falls beyond the Thesis and is extensively covered in a recent review (Sheeran and Hall, 1997).

There is no data concerning the direct impact of anaesthetics excreted into the gastrointestinal tract at low concentration on the bacterial composition of the bowels and on their activity.

The most important factor that can change the commensal flora is antibiotic treatment (Burman, 1980).

In the healthy individual different mechanisms are in operation to keep our commensal flora under control (Murray et al., 1991).

2.2.2. Host defence mechanisms against endogenous bacteria and the effect of anaesthesia

Integrity of anatomy and physiology

In connection with anaesthesia this first barrier against skin flora is disrupted by peripheral, central venous, and arterial cannulae (Walton et al., 1975; Opie, 1980; McLaws et al., 1998), epidural (Barreto, 1962), intradural and intrapleural catheters. Tracheal intubation is a relatively traumatic procedure causing mucosal damage. The incidence of bacteremia due to orotracheal intubation has been reported to be 0.0% to 5.3% (Berry et al., 1973; Hansen et al., 1989; Goldstein et al., 1997).

The presence of a nasogastric tube impairs the function of the gastrooesophageal sphincter, leading to migration of gastric flora into the oesophagus and oropharynx (Wynne and Modell, 1977).

Physiological pH

The low pH of the gastric fluid is bactericidal for most bacteria. Gastric acidity contributes to the carriage defence of the alimentary canal (Arnold, 1933). For fear of acid aspiration we frequently elevate the gastric pH. Increasing its pH above 4 allows the multiplication of enteral bacteria (Daschner et al., 1988).

Mechanical clearance

Motility is an important factor contributing to the mechanical removal of micro-organisms (Dack and Petran, 1934). The propulsive movements of the gut, generated by the migrating myoelectric complex (Vantrappen et al., 1977) are responsible for the efficacy of clearance (Aitkenhead, 1988). Different anaesthetics have different effect on bowel motility. Thiopentone (Healy et al., 1981) and neostigmine (Wilkins, 1970) increase, diazepam (Birnbaum et al., 1970), and volatile anaesthetic agents decrease it (Condon et al., 1987). Morphine increases intraluminal pressure, but decreases the frequency of propulsive movements (Schang et al., 1986) and prolongs intestinal transit time (Yukioka et al., 1987). Translocation of enteric micro-organisms from the intestinal tract to extraintestinal sites has been proposed as an early step in the development of gram-negative sepsis. In rat experiments it was clearly demonstrated that parenteral morphine sulphate induced gut stasis, enhancing bacterial overgrowth and bacterial translocation from the gut to ileocoecal mesenteric lymph nodes (Runkel et al., 1993; Kueppers et al., 1993). Tumour necrosis factor and morphine have synergistic effects on impairing gut barrier function (Leslie et al., 1994).

Mucosal cell renewal

The alimentary canal is metabolically the most active cell mass in the body, repopulating its entire mucosal surface every 2-3 days under normal circumstances. Micro-organisms adhering to the mucosal surfaces are removed by this high cell turnover (Dreizen et al., 1956). Halothane prolongs the time required for DNA synthesis in rat epithelial cells of the small-bowel; thus it may affect intestinal cell renewal (Bruce and Traurig, 1969).

2.2.3 Bacteria of exogenous origin in postoperative infections

In exogenous infections the organism is introduced into the patient from the environment.

As soon as we open an ampoule, there is a hazard of contamination. Glass particle contamination of the contents of single-dose glass ampoules can occur upon opening (Sabon et al., 1989). As high as 66% of the ampoules can be contaminated with particles from the external ampoule surface liberated upon opening (Kempen and Treiber, 1990). It was confirmed later that bacterial contamination could occur when a glass ampoule was opened (Zacher et al., 1991). The use of multiple dose vials and intravenous anaesthetic techniques further increase the risk of infection. Not only ampoules but syringes in anaesthetic use can also be contaminated (Blogg et al., 1974; Lessard et al., 1988).

There are reports of nosocomial infections and epidemics where contaminated anaesthetics were blamed (Siboni et al., 1979; Veber et al., 1994).

These observations urged anaesthetists and microbiologists to test drugs used in anaesthetic practice to determine if they support or inhibit bacterial growth. Most of them proved to be bacteriostatic or bactericidal while propofol, diazemuls, and calcium gluconate supported bacterial growth. Although the number of studies is large, interestingly enough many medications given in anaesthetic practice as infusions have never been investigated in respect of their effects on bacterial growth (Bátai et al., 1999a).

Contaminated anaesthetic machines and gases may be a source of bacteria and cross infection too, which has to be considered (Old et al., 1972; Albrecht and Dryden, 1974; Nielsen et al., 1978).

2.3. Anaesthesia and the first stage of the development of infection

Transport of pathogenic bacteria of either endogenous or exogenous origin to a surface followed by initial adhesion and attachment later to epithelial cells is thought to be the initial process leading to colonisation of the mucosal surfaces of the body that may cause infection (Johanson et al., 1979). Bacterial adherence is cell-specific. Different epithelial cells within anatomic sites as confined as the human oropharynx have striking differences in suitability for adherence by individual bacterial species (Gibbons and van Houte, 1975). This cell specificity of bacteria appears to be mediated by the attraction of species-specific microbial adhesins to complementary host cell-specific receptors (Beachey, 1981). Adhesins are microbial surface antigens that frequently exist in the form of filamentous projections designated as pili or fimbriae (Duguid and Old, 1980). Adhesins bind to specific receptors on epithelial-cell membranes (Neidhardt et al., 1990). At least three factors are important determinants of whether colonisation will result from a given exposure: the availability of suitable mucosal binding sites, the presence of attachment sites on the invading bacteria, and the presence of a favourable milieu.

The first experiment concerning changes on bacterial cell surface under the influence of an anaesthetic was published as early as 1911. These investigations showed that ether 2% in NaCl 0.85% had no effect on bacterial agglutination *in vitro* (Graham, 1911). Among the drugs used in anaesthesia, local anaesthetics may influence bacterial adherence, however, no experiments have addressed this topic so far. Studies have already verified that local anaesthetics alter the function of membranes in both eukaryotic and prokaryotic cells. In *Escherichia coli* the block of both processing and translocation of periplasmic proteins (Lazdunski and Pages, 1979), the inhibition of synthesis and processing of outer membrane proteins (Gayda et al., 1979; Pugsley et al., 1980), and the inhibition of carbohydrate transport (Granett and Villarejo, 1981) were found. Promethazine is the only drug used in anaesthesia known to inhibit

the adhesion of *E. coli* to tissue culture cells (Molnár et al., 1983). Benzodiazepines may also alter bacterial cell surface characteristics as micro-organisms can bind them via cell surface receptors (Lummis et al., 1991; Baker and Fanestil, 1991).

Disposable materials are essential for treating and monitoring anaesthetised and critically ill patients. Many of these items are made of polyvinylchloride to which bacteria readily adhere. The colonisation of these endotracheal tubes may be a significant factor in the pathogenesis of nosocomial pulmonary infection (Sottile et al., 1986). Intravenous catheters have been studied extensively by electron microscopy and have been shown to colonise rapidly with multiple glycocalyx-producing organisms, including *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas. aeruginosa*, and *Acinetobacter* (Peters et al., 1982; Sheth et al., 1983a; Sheth et al., 1983b).

2.3.1. Host defence mechanisms against bacterial adherence and the effect of anaesthesia

Host defence mechanisms aimed at blocking the adherence of microorganisms to epithelial surfaces have evolved to the same degree as microbial mechanisms favouring adherence. A primary function of the antibody-forming system of mucosal surfaces is to discourage adherence of unwelcome microorganisms through the production of specific secretory IgA (Williams and Gibbons, 1972). Productions of lysozyme and epithelial-receptor analogues increase the host's resistance against the adherence of certain microorganisms (Williams and Gibbons, 1975).

The mechanism of secretory immunoglobulin A provided defence is thought to be the coating of micro-organisms to prevent bacterial adherence (van Saene and van der Waaij, 1979). Bacterial adherence to tracheal cells correlates inversely with sputum IgA levels (Niederman et al., 1986).

Serum immunoglobulin concentrations decrease during surgery and in the early postoperative period (Rem et al., 1980; Grob et al., 1988; Khan et al., 1988; Lockwood et al., 1993). A temporary decrease in Ig synthetic capacity could not explain the decreased Ig concentrations in the immediate postoperative period on account of the long biological half-lives of IgG (20 - 28 days), IgM (5 days) and IgA (6 days). Haemodilution, loss of protein into extravascular tissues and immunoglobulin consumption may be responsible for this decrease.

Following propofol or isoflurane anaesthesia, the secretion rate of total protein, amylase, lysozyme, total peroxidase, thiocyanate and IgG in saliva is decreased owing to a marked decrease in the salivary flow. However, no alterations were found in the secretion rate of myeloperoxidase, IgA and IgM. These findings show that nonimmunological oral mucous host defences are altered after major surgery under general anaesthesia, but immunoglobulin responses are better maintained (Lähteenmäki et al., 1998).

Nutrient deprivation, although generally not a major obstacle to bacterial growth on most epithelial surfaces (Gibbons and van Houte, 1975), is important insofar as it relates to iron (Bullen et al., 1974). The availability of this essential nutrient to microbes is limited as a result of the production of iron-binding proteins by the host.

The effect of anaesthetics on this protein has never been investigated. However, the possibility of anaesthetic-protein interaction was suggested as early as 1875 by Claude Bernard (Bernard, 1875). In 1915 Harvey reported the first interaction of this kind. The odd thing about it that it is a bacterial-anaesthetic interaction as well. He reported that inhalational anaesthetics reversibly depressed the luminescence of certain marine bacteria (Harvey, 1915). The enzyme involved is bacterial luciferase. The anaesthetic is competing with a high molecular weight aldehyde for a receptor site on the enzyme molecule (Adey et al., 1973). Franks and Lieb had further observations on this protein-anaesthetic interaction (Franks and Lieb, 1984). Later their work became the basis of protein theories of general

anaesthetic action. There is little doubt today that anaesthetics can directly interact with a variety of proteins (Eckenhoff and Johansson, 1998).

Other antibacterial substances produced by the host may further impair microbial growth on some surfaces. Saliva, gastric and pancreatic juice, bile and mucus contain antibacterial products including lysozyme and enzymes to control bacterial adherence (MacFarlane and Mason, 1972). The antibacterial effect of unconjugated bile is probably at least partially responsible for the low number of microorganisms present in the small intestine (Binder et al., 1974).

Anaesthesia may interfere with host defence mechanisms at this level as well. Salivary flow markedly decreases after isoflurane or propofol anaesthesia (Lähteenmäki et al., 1998).

One of the most important host defence mechanisms against pathogens is the presence of the indigenous flora (Mackowiak, 1982).

The commensal flora covering the mucosal surfaces and skin contributes to host defence mechanisms via bacterial interference (Sanders et al., 1977).

There are various exogenous factors affecting the balance between the host and the normal flora.

The most important exogenous factor is antimicrobial treatment. Antimicrobial agents are capable of causing the most rapid and most radical changes in the normal flora (Sanders et al., 1976; Archer, 1991). They may kill members of the normal flora and they may impair the adherence of micro-organisms to epithelial cells, even when present in subinhibitory concentrations (Vosbeck et al., 1979). Furthermore, as certain bacterial strains develop resistance to antimicrobial agents, they undergo concomitant changes in membrane proteins, associated with an altered ability to colonise mammalian epithelial cells (Onderdonk et al., 1981). By depressing the normal flora they may facilitate plasmid transfer as well (Jones and Curtiss, 1970). Finally, antimicrobial agents may actually promote colonisation by certain resistant micro-organisms due to inhibition of the growth of sensitive microbial competitors.

At present we have little knowledge of the interaction between antibiotics and medications used in anaesthesia. *P. aeruginosa* exposed to subinhibitory doses of tetracaine *in vitro* became susceptible to otherwise ineffective concentration of erythromycin (Leung and Rawal., 1977). Subinhibitory concentrations of lignocaine hydrochloride enhanced the sensitivity of *E. coli, S. typhimurium*, and *P. aeruginosa* to novobiocin and nalidixic acid (Ohsuka et al., 1994). The *in vitro* synergetic effects of chlorpromazine with aminoglycosides, β-lactams, tetracycline, vancomycin and quinolones have also been reported (Amaral et al., 1992). There is *in vivo* evidence for the effectiveness of promethazine. Children with frequently recurring pyelonephritis were given a combination of gentamicin and promethazine or gentamicin alone. The treatment was more effective with the combination (Molnár et al., 1990).

Most of the drugs used in anaesthesia are excreted into the gastrointestinal tract at low concentrations where they meet with large numbers of bacteria and these interactions may have some importance.

Underlying disease also changes normal flora. Chronically ill patients such as those with diabetes, alcoholism or chronic bronchitis while carry one or more of the community micro-organisms, may also carry abnormal flora (Table 1) (Jordan et al., 1976; Mackowiak et al., 1978; Mackowiak et al., 1979; Murray et al., 1991).

Data on the impact of anaesthesia on the oropharyngeal and gastrointestinal bacterial adherence is sparse and none of the studies could exclude the effects of underlying diseases, surgery, or the antibiotics given in the perioperative period (Glover and Jolly, 1971; Niederman et al., 1983).

Table 1. Classification of micro-organisms based on their intrinsic pathogenicity.

	Intrinsic pathogenicity	Flora
1. Indigenous flora Oropharynx: peptostreptococci, *Veillonella* spp viridans streptococci Gut: *Bacteroides, Clostridium* spp., enterococci, *Escherichia coli*? Vagina: peptostreptococci, *Bacteroides* spp., lactobacilli Skin: *Propionibacterium acnes, Staphylococcus epidermidis*	LOW PATHOGENIC	NORMAL
2. Community micro-organisms Oropharynx: *Streptococcus pneumoniae, Haemophilus influenzae, Branhamella catarrhalis* Gut: *Escherichia coli* Oropharynx and gut: *Staphylococcus aureus* *Candida* spp.	POTENTIALLY PATHOGENIC	NORMAL
3. Hospital micro-organisms *Klebsiella, Proteus, Morganella, Enterobacter, Citrobacter, Serratia* spp., *Pseudomonas, Acinetobacter* spp.	POTENTIALLY PATHOGENIC	ABNORMAL
4. Epidemic micro-organisms *Neisseria meningitidis* *Salmonella* spp.	HIGHLY PATHOGENIC	ABNORMAL

2.4. The effect of anaesthesia on the second stage of the development of infection

The second stage towards infection is the access of the pathogen to a site where it may invade tissues, often in association with a foreign body such as an intravascular catheter or an endotracheal tube.

Bacteria can enter host cells by phagocytosis and some bacteria have evolved mechanisms for entering host cells that are not naturally phagocytic. Bacteria attaching to the host cell surface may cause changes in the host cell

cytoskeleton that result in their engulfment by the host cell (Goldberg and Sansonetti, 1993).
The only agent commonly used in anaesthesia that has known effects – probably indirect – on this stage of the infection process is morphine sulphate. It has already been mentioned that it induces gut stasis, enhances bacterial overgrowth and bacterial translocation from the gut to ileocecal mesenteric lymph nodes (Runkel et al., 1993; Kueppers et al., 1993).
The increasing demand for the growing use of intravascular, intrapleural, intradural and extradural catheters in anaesthetic practice have a great contribution to the high incidence of nosocomial infections (Craven et al., 1988).

2.5. The third stage of the development of infection and the involvement of anaesthesia

The last stage is an imbalance among the pathogen's virulence factors and the host's defence factors, which eventually results in the infection.
The pathogens have various virulence factors, including α-haemolysin (Emődy et al., 1982a, Emődy et al., 1984a), fimbrial and non fimbrial adhesins, capsules, M protein, immunoglobulin A proteases, exotoxins and other extracellular aggressins (Cockayne, 1997).
Promethazine is the only drug used in anaesthesia that is known to reduce the expression of a virulence factor of *E. coli* (Molnár et al., 1983).
The possible effects of anaesthesia on the immune system have been discussed from as early as 1904 (Rubin, 1904), and well covered in extensive reviews (Salo, 1992; McBride, 1996). There are both *in vitro* and *in vivo* evidences that most of the anaesthetics depress various components of the immune system at therapeutic concentrations. Some of the effects last for several days (Salo, 1992; McBride, 1996).

The detailed discussion of the effects of anaesthesia on the immune system falls beyond the topic of the Thesis.

2.6. The treatment of perioperative infections

The detailed discussion of the treatment of perioperative infection falls beyond the topics of the Thesis. The importance of the commensal flora and how antibiotics affect it has already been mentioned in Chapter 2.3.1. (pages 15-16). However, when our measures fail to prevent perioperative infections antibiotics give the basis of therapy. However, there is an important issue that is quite often neglected. It is not enough to administer the right antibiotic; it must reach the site of infection at an appropriate concentration to ensure successful treatment.

2.6.1. The role of antibiotics accumulated in polymorphonuclear leukocytes

Polymorphonuclear leukocytes (PMNs) play a complex role in the fight against bacteria. PMNs incorporate bacteria during their defence activity against infections. Bacteria can be protected from antibodies and from certain antibiotics inside the PMNs and survive inside these cells. This phenomenon is proved in the case of staphylococci (Rogers and Tompsett, 1952), brucellae (Schaffer et al., 1953), salmonellae (Rhodes and Hsu, 1974), and organisms responsible for atypical pneumonia (*Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumophila* and rickettsiae) (Schlick, 1993). Bacteria growing inside human cells may be the source of chronic diseases such as chronic granulomatous disease (Dinauer and Orkin, 1992). Antibiotics penetrating into PMNs play an important role in killing intracellular microorganisms. Granulocytes accumulate e.g. chloramphenicol, erythromycin, azitromycin, doxycycline and clindamycin while beta-lactams and aminoglycosides hardly enter these cells

(Forsgren and Bellahsene, 1985; Bonnet and Van der Auwera, 1992). Most of the antibiotics are released from the cells in antibiotic free environment but 40% of doxycycline remains intracellularly after 24 hours (Forsgren and Bellahsene, 1985). PMNs also deliver intracellularly accumulated antibiotics to the site of infection (Deysine et al., 1979).

The effect of anaesthetics on the intracellular antibiotic content of PMNs has not been investigated yet.

3. Aims of the Thesis

It is well known that anaesthesia has some influence on postoperative infections. So far, however, the main concern was the impact of anaesthesia on the immune system and how the practice of anaesthesia influenced anatomical barriers. In the Thesis I investigated other aspects how anaesthesia and perioperative antibiotic administration may influence the occurrence of infections in the postoperative period.

I. The effect of drugs used in anaesthesia and perioperative antibiotic treatment on the endogenous and exogenous source of postoperative infections.

I/1. What are the effects of drugs used in anaesthesia on bacterial growth?

The first goal of the Thesis was to extend our knowledge in respect, which drug is safe or not as far as iatrogenic infections are concerned in anaesthesia and intensive care.

The impact of glyceryl trinitrate (GTN), sodium nitroprusside (SNP), atracurium, and cisatracurium on bacterial growth was examined.

I/2. What is the effect of antibiotic treatment – simulating natural circumstances – on the *in vivo* selection of resistant haemolytic *E. coli* clones?

Numerous authors have proved the possibility of *in vivo* plasmid transfer (Smith, 1969; Jones and Curtiss, 1970; Anderson et al., 1973; Petrocheilou et al., 1976; Smith, 1977; Gyles et al., 1978). The experiments were performed on gnotobiotic animals or on animals freed of members of the *Enterobacteriaceae*

by pre-treatment with antibiotics. Could antibiotics help selecting recombinants when natural circumstances are simulated (antibiotic treatment starts after the infection)?

I/3. Could antibiotic treatment enhance the emergence of intergeneric transfer of plasmids?

Plasmids frequently encode virulence factors. Alpha-haemolysin of *E. coli* is a virulence factor in extraintestinal manifestations. The possibility of *E. coli* haemolysin plasmid transfer into *Proteus morganii* was examined.

II. Does anaesthesia affect bacterial adherence?

Bacterial adherence is thought to be the first step in infection. However, there is no data on the impact of anaesthetics on this process. In order to elucidate the effects of surgery and the antibiotics given perioperatively *in vitro* experiments were performed first.

II/1. Does halothane affect bacterial haemagglutination?

II/2. Does halothane affect fimbria mediated bacterial adherence to human epithelial cells *in vitro*?

II/3. Is there any change in human oral bacterial flora following general anaesthesia, surgery, and perioperative antibiotic treatment?

III. Could anaesthesia influence perioperative antibiotic therapy by altering intracellular antibiotic concentrations?

4. Materials and methods

4.1. Bacterial strains, plasmids, culture media, and culture conditions

Strains for testing sensitivity to drugs used in anaesthesia

Sensitivity of clinical isolates of *S. aureus* from wound infection, *E. coli* from blood culture, and *P. aeruginosa* from tracheal aspirate were tested.

Strains and culture media for R plasmid transfer

The bacterial strains used in this work were *E. coli* J53pTE1 pro^-, met^-, Nal^r (Km^r, Cm^r) and the prototrophic *E. coli* P673pTE5 O139:K82 (Hly^+, Amp^r, $Carb^r$). Organisms were cultivated in nutrient broth (Difco) on blood agar containing 5% (v/v) sheep blood, or on Endo agar. Antimicrobials were used in the media at the following concentrations: carbenicillin, 250 μg mL^{-1}; kanamycin, 30 μg mL^{-1}; ampicillin, 30 μg mL^{-1}; and chloramphenicol, 30 μg mL^{-1}.

Strains and culture media for the intergeneric transfer of the haemolysin plasmid

The bacterial strains used in this work were *E. coli* J53p673Tn1-pro^-, met^-, Nal^r (Hly^+, Tc^r, $Carb^r$, Amp^r) in which the Hly plasmid is labelled with the ampicillin transposon Tn1 derived from pMR5 and *P. morganii* 2380-cys^-, Tc^r, $Colimycin^r$ a natural isolate from human faeces giving mannose-resistant haemagglutination (MRHA) with sheep erythrocytes. Nutrient broth (Difco), blood agar plates containing 5% sheep blood and Endo agar plates were used for cultivation. The concentrations of antibiotics applied were as follows: carbenicillin 250 μg mL^{-1} and colimycin 1000 IU mL^{-1}.

Strains for the haemagglutination studies

E. coli J53p673Tn1 was used for mannose sensitive haemagglutination (MSHA) and *E. coli* J96 (O'Hanley et al., 1983) was used for mannose resistant haemagglutination (MRHA) with human erythrocytes.

Strains and culture conditions for the *in vitro* adherence assay

E. coli strain with fimbrial adhesin K88 (serotype 0141: K85, K88a,c) (Ørskov et al., 1964) was grown unshaken in nutrient broth (Oxoid) for 16 h at 37°C to maximise the expression of the adhesins (Payne et al., 1993a). *E. coli* strain NG7C (serotype O105: H8) is known to bind fibronectin (Ljungh et al., 1990), and was cultured on CFA agar at 37°C (Ljungh et al., 1991). Bacteria then were washed in Dulbecco's phosphate buffered saline (DPBS) three times, and resuspended in DPBS at the appropriate density (5×10^8 colony forming unit / mL [cfu mL^{-1}]).

Strains for the bioassay to measure doxycycline concentrations

Bacillus subtilis ATCC 6633 was used as the test bacterium.

4.2. Bacterial sensitivity to drugs used in anaesthesia

Bacterial suspensions were prepared by modifying the method described previously (Berry et al., 1993; Sosis and Braverman, 1993). Mueller-Hinton broth (Oxoid) was inoculated with each organism and incubated overnight at 37°C. The cultures were diluted to a density of 0.5 McFarland units (1.5×10^8 mL^{-1}) with sterile nonbacteriostatic saline 0.9%. Each bacterium solution was further diluted 1:1000 with sterile saline 0.9%. A 0.2 mL aliquot of each diluted bacterial suspension was added to sterile sealed culture vials containing 20 mL of the tested drug solutions or the controls. Five vials of each tested drugs and controls were inoculated. It gave an approximate initial concentration of 10^3 colony

forming units (cfu) mL^{-1}. All diluted suspensions were vortexed for 1 min between each aliquot removal. After inoculation, the culture vials were kept in incubators at both 20°C and 37°C. Each vial was vortexed for 5 sec and a 10µL sample was then removed and plated on Mueller-Hinton agar (Oxoid) at the following times after inoculation: 0, 3, 6 and 24 h. The plates were then incubated at 37°C for 24 h. The numbers of cfu were counted. Five parallels were performed.

The tested pharmaceutical preparations were 100 µg mL^{-1} glyceryl trinitrate (GTN) in alcohol 10% (Nitrolingual®; Pohl-Boskamp, Hohenlockstedt, Germany); 100 µg mL^{-1} sodium nitroprusside dissolved in 5% glucose (SNP) (Nipride®; Roche, Basle, Switzerland),10 mg mL^{-1} atracurium besylicum (Tracrium®, GlaxoWellcome) and 2 mg mL^{-1} cisatracurium besylicum (Nimbex®, GlaxoWellcome).
The control solutions were selected as the solvents of the tested drugs: glucose 5% and alcohol 10%. Mueller-Hinton broth (Oxoid) controls were also applied to assess bacterial viability.
The chemical structure of the tested drugs are shown in Figures 1 - 3.

Figure 1. The chemical structure of glyceryl trinitrate

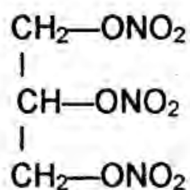


Figure 2. The chemical formula of sodium nitroprusside

$$Na_2[Fe(CN)_5NO]$$

Figure 3. The chemical structure of atracurium and cisatracurium

* The asterisks denote the centers of stereoisomerism.

4.3. Halothane exposure

Halothane (Fluothane®, Zeneca Pharma, Wilmslow, UK) (Figure 5) in 40 % oxygen in air containing 5 % carbon dioxide was administered with a fresh gas flow of 600 mL/min using a Fluotec Mark III vaporiser and the apparatus described previously (Wardley-Smith and Nunn, 1971) (Figure 2). A gas monitor (Capnomac®; Datex, Helsinki, Finland) was used to determine the concentration of the anaesthetic exiting the chamber (Figure 4).

Figure 4. Apparatus for halothane exposure.

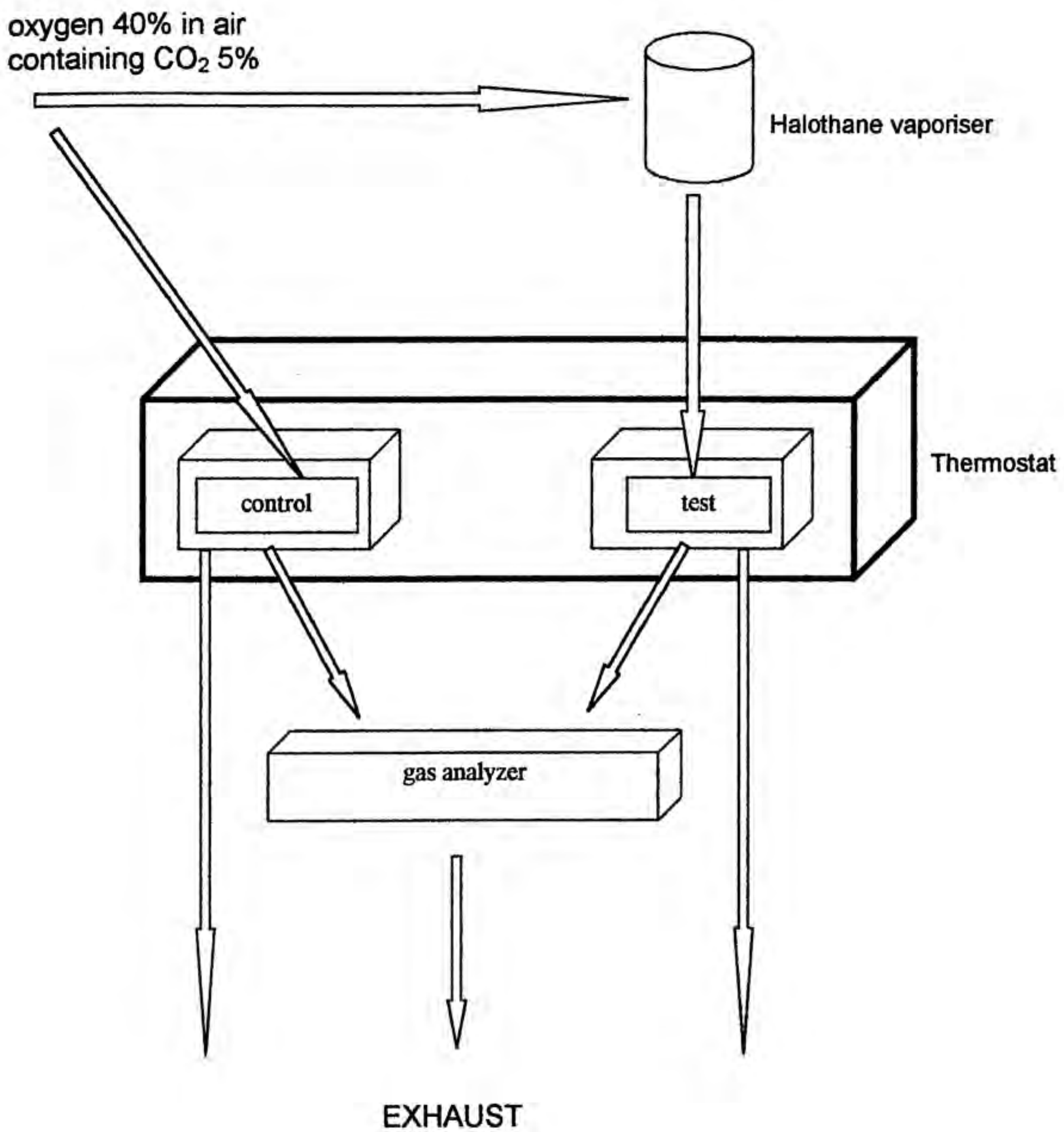
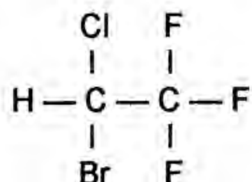


Figure 5. The chemical structure of halothane



Halothane exposure for the haemagglutination and adherence assays

The apparatus described above was used (Figure 4). Two percent halothane in 40% oxygen in air containing 5% carbon dioxide was administered for 2 hours with a fresh gas flow of 600 mL/min. The vapour was flown over the Petri dishes.

Halothane exposure for the intracellular doxycycline content assay

The apparatus described above was used (Figure 4). 2.5% halothane in 40% oxygen in air containing 5% carbon dioxide was administered for 1 hours at 37°C with a fresh gas flow of 600 mL/min. The vapour was flown over the samples.

4.4. R plasmid transfer

***In vitro* transfer of R plasmid**

The frequency of transfer was tested in nutrient broth as described earlier (Emődy et al., 1980). Controls were performed in the presence of carbenicillin and kanamycin to determine the influence, if any, of these selective agents on the transfer process. Five parallels were performed.

***In vivo* transfer of R plasmid**

Twenty twelve-week-old CFLP outbred mice (Gödöllõ, Hungary) weighing 25 g and free of both carbenicillin- and kanamycin-resistant *E. coli* were kept in separate cages throughout the experiment. The cages were replaced daily and the animals were given pelleted food and water ad lib. Twenty animals were each infected through an oesophageal cannula with 0.5 mL nutrient broth culture containing approx. 3 X 10^9 cells of the donor (J53pTE1) and 1 hour later with the same number of cells of the recipient (P673pTE5). The possibility of bi-directional transfer was excluded by the fact that pTE5 was a derivative of a non-conjugative haemolysin plasmid (Waalwijk et al., 1982). Starting the next day 2.5 mg carbenicillin and 2.5 mg kanamycin were administered daily to each of 10 mice intraperitoneally for a period of 7 days. The other 10 mice were injected with saline. Faecal samples were taken and plated onto carbenicillin-kanamycin blood agar both directly and after 48-h enrichment in nutrient broth containing these selective agents. Faecal samples were collected on days 2, 3, 4, 5, 7, 8, 11 and 16 (80 samples were collected from both the antibiotic treated and the control animals, one from each animal on the sampling days). The resistant recombinants which appeared were further identified by haemolytic activity, agglutinability in O139:K82 immunoserum, prototrophic property, and resistance to chloramphenicol. The resistance to ampicillin - being parallel with carbenicillin resistance - was controlled too. Representative colonies were examined for the presence of plasmids by agarose gel electrophoresis (Eckhardt, 1978). To rule out the possibility of mating between donor and recipient cells during the enrichment procedure faecal samples obtained from mice which had received only donor or recipient cells - and treated, respectively, with either kanamycin or carbenicillin only - were mixed and assayed as described above. The total *E. coli* count, and the numbers of donor, recipient and recombinant cells per faecal pellet were estimated as follows: fresh faecal pellets were macerated by vortexing for 3 min in 1.0 mL PBS. The samples were then serially diluted in saline, and 0.5-mL portions of appropriate dilutions were plated onto pre-dried

Endo agar plates supplemented with the appropriate selective agent(s) when necessary. Plates were incubated at 37°C until colonies were suitable for counting. Counts are expressed as the number of viable cells in the 1.0 mL faecal homogenate.

4.5. Intergeneric transfer of the haemolysin plasmid

In vitro transfer of Hly plasmid

The frequency of transfer was tested in nutrient broth as described earlier (Emődy et al., 1980). Parallels completed with carbenicillin + colimycin at the time of mating were tested to check whether transfer occurs in the presence of these selective agents. Five parallels were performed.

In vivo transfer of Hly plasmid

12-week-old CFLP outbred mice (Gödöllő, Hungary) weighing 25 g were kept in sterile jars throughout the experiment. The jars were replaced daily and the animals were given sterile food. 50 mg of streptomycin was administered through a cannula introduced into the oesophagus on two consecutive days to deprive the animals of faecal *Enterobacteriaceae* flora. On the third day faecal samples were plated onto Endo agar plates. If no growth occurred mice were given a 0.5 mL shaken nutrient broth culture of the donor and 1 h later that of the recipient through the oesophagus cannula (5-8 X 10^9 bacteria each). Next day faecal pellets were taken. Then mice were killed and the small and large intestines were removed aseptically and all samples put into carbenicillin-colimycin nutrient broth separately, homogenised with sterile glass sticks and vortexed. After cultivation at 37°C for 48 h the samples were plated onto carbenicillin-colimycin blood agar and Hly^+ transconjugants were searched for. Direct plating of faecal samples was not performed. Hly^+ colonies were tested for urease and phenylalaninedeaminase activities (Cowan and Steel, 1965) and

MRHA with sheep erythrocytes (Kuch et al., 1980). The number of donors and recipients in faecal pellets was counted using carbenicillin Endo agar and colimycin Endo agar, respectively.

4.6. Lung toxicity assay

4-week-old CFLP outbred mice (Gödöllő, Hungary) weighing 10-12 g were infected intranasally with 0.05 mL overnight nutrient broth culture (approx. 3 X 10^9 bacteria mL^{-1}) under superficial ether anaesthesia. The death rate and pathomorphological changes were recorded for 24 h after infection. In positive cases dyspnoe and convulsions developed, with signs of acute asphyxia. Death mostly occurred 1-4 h after infection. Post-mortem examination revealed haemorrhagic lung oedema. Ten animals were applied for each strain.

4.7. Chicken embryo virulence assay

Ten-day-old Tetra B hybrid stock chicken embryos (Baksa, Hungary) were infected allantoically with 0.1 mL volumes of PBS dilutions of overnight nutrient broth cultures. Inocula containing approx. 10^3, 10^2, and 10^1 bacteria were each given to groups of 5 embryos. Death was recorded over a period of 48 h after challenge. Because the results were not suitable for LD_{50} calculation, the number of survivors per total number of infected embryos is indicated.

4.8. Haemagglutination

Three percent (v/v) erythrocyte suspension in Dulbecco's PBS (DPBS) was used for the haemagglutination (HA) assays (Old, 1985). Bacteria were grown on CFA

agar for 24 h at 37°C. The bacteria were suspended in PBS to approximately 5 X 10^{10} cfu mL^{-1}. Then halothane 2% was administered for two hours (see details in Chapter 4.3.). The slide agglutination test was performed by mixing 10 µl of erythrocytes with an equal volume of bacterial suspension on a slide. Mannose resistant HA (MRHA) and mannose sensitive HA (MSHA) were examined by mixing the erythrocyte suspension with bacteria suspended in PBS containing 2% (w/v) α-methyl-D-mannopyranoside. The haemagglutination reaction was read with the naked eye.

Four groups were created. Group I was the control, nor the erythrocytes neither the bacteria were halothane treated. In group II only the erythrocytes were exposed to halothane. In group III only the bacteria were exposed to halothane and mixed with untreated erythrocytes. In group IV both bacteria and erythrocytes were treated with the anaesthetic.

4.9. Preparation of the HEp-2 monolayers

HEp-2 cells (American Type Culture Collection [ATCC] CCL 23) (Moore et al., 1955) were grown in Dulbecco`s modified Eagle`s medium (DMEM) containing heat-inactivated fetal calf serum 10% and antibiotics (penicillin 100 IU mL^{-1} and streptomycin 100 µg mL^{-1}) on the surface of $25cm^3$ tissue culture flasks (Greiner) in a humidified atmosphere containing CO_2 5%. After removal of the supernate the monolayer was rinsed with trypsin-EDTA (trypsin 0.5 g L^{-1}, EDTA 0.2 g L^{-1}, NaCl 0.85 g L^{-1} in calcium and magnesium-free Hanks's buffered salt solution) and then treated with trypsin-EDTA (5mL) until the cells became detached from the flask (10-15 min at 37°C). The cells were centrifuged and resuspended for counting in a Buerker counting chamber. The number of cells was adjusted to 10^5 mL^{-1} and 1 mL of this suspension was placed in each Petri dish for tissue cultures (size 35/10 mm, Greiner) and incubated overnight which resulted in a monolayer covering over 90% of the dish base.

HEp-2 cells were stained with trypan blue, and viability was assessed by light microscopy.

4.10. Bacterial adherence assay

Following two washes of the HEp-2 monolayers with 2 mL of DPBS, the bacterial suspension (10^9 colony forming units in 2 mL DPBS) was added. HEp-2 monolayers with bacteria were incubated for 3 hours at 37°C. Non-adherent bacteria were removed by aspiration and washing of the monolayer three times with DPBS (2mL) at 37°C. Monolayers were fixed with methanol and Giemsa stained. The percentage of epithelial cells with more than 40 adhering bacteria was evaluated by light microscopy (Vaahtontemi et al., 1992). 25 individual experiments were performed.

The experimental protocol consisted of pretreating either the epithelial cells or the bacteria or both with halothane 2% for 2 hours prior to coincubation for 3 hours. Four groups were established. Group I served as control receiving only vehicle gas consisting of oxygen 40% in air containing carbon dioxide 5%. Group II consisted of halothane exposed HEp-2 cells and control bacteria. Group III consisted of control HEp-2 cells and halothane exposed bacteria, whereas in group IV both the HEp-2 cells and bacteria were halothane treated.

4.11. Total intravenous anaesthesia (TIVA)

Twenty ASA grade 1 or 2 (see Appendix) female patients aged 16-24 (average 17.6) years scheduled for elective surgery were studied. Patients on antibiotics, smokers, diabetics, alcoholics, patients with dentures or those with overweight were excluded. Cotrel - Dubousset scoliosis operation, posterior approach with

wake up test was performed. Duration of surgery was 3 - 5 hours (average: 3.6 h).

Details of TIVA:

Premedication: diazepam 10 mg p.o. the night before surgery,
diazepam 10 mg p.o. two hours before surgery.

At induction of anaesthesia: alfentanil $25 \mu g\ kg^{-1}$,
propofol $1\ mg\ kg^{-1}$,
atracurium $0.5\ mg\ kg^{-1}$.
cefuroxime $20\ mg\ kg^{-1}$.

Maintenance of anaesthesia: alfentanil $10\text{-}50\ \mu g\ kg^{-1}\ h^{-1}$,
propofol $10 - 8 - 6\ mg\ kg^{-1}\ h^{-1}$,
atracurium $0.3\ mg\ kg^{-1}\ h^{-1}$,
oxygen 40% in air, $1\ L\ min^{-1}$.

Following wake up test midazolam 5 mg was given i.v.

At the end of anaesthesia: neostigmine $0.05\ mg\ kg^{-1}$;
atropine $0.015\ mg\ kg^{-1}$.

4.12. Preparation of buccal epithelial cells and the evaluation of attached bacteria

Human epithelial cells were collected by scraping buccal mucosal surfaces with a moisturised wooden applicator stick. The applicators were twirled in sterile saline to dislodge the cells. Unattached bacteria were removed by washing the cell suspension four times in saline. Direct smears were prepared from each epithelial cell suspension and stained for 30 minutes with Giemsa.

The number of bacteria attached to the epithelial cells was determined by light microscopy at X 900 magnification.

Consequently, 200 cells were counted for each sample. The percentage of cells covered with more than 40 attached bacteria were compared (Vaahtontemi et al, 1992) at the beginning and at the end of surgery.

4.13. Isolation of human polymorphonuclear granulocytes (PMNs) and exposure to halothane *in vitro*

Thirty mL anticoagulated (with EDTA) venous blood was collected from 30 male patients (ASA grade 1 or 2; age 18 - 62, average 48). Patients suffering from malignancy, haematological disorders or chronic inflammatory disease, patients on antibiotics, smokers, diabetics, or alcoholics were excluded.

Granulocytes were isolated by combining centrifugation and sedimentation in ficoll gradient (Böyum, 1968; Klempner and Styrt, 1981). The granulocytes were resuspended in Hanks' balanced salt solution (HBSS). Aliquots were made and the cell count was checked in each part to be comparable. Control cells were exposed to oxygen 40% in air containing carbon dioxide 5%. Cells in the treatment group were exposed to halothane 2.5% in oxygen 40% in air containing carbon dioxide 5%. The halothane exposure was performed at 37°C for 1 hour as described above. Then doxycycline was added to achieve a concentration of 2 $\mu g\ mL^{-1}$. After 1 hour long incubation at 37°C antibiotics remaining extracellularly were removed by washing the cells in HBSS four times. Before disrupting the cells the viability of PMNs was checked by the tripan blue exclusion test and more than 90% of all cells proved to be alive in both the control and treatment group. Intracellular doxycycline was released from the PMNs by freezing in liquid nitrogen and thawing four times.

4.14. Measurement of doxycycline by HPLC

HPLC data: packing C18; column 25 cm; eluent 24% acetonitrile, 38% methanol, 15% tetramethyl-ammonium chloride 0.1 M/H_2O, 23% H_3PO_4 0.1 M/Na_2HPO_4 0.005 M/H_2O 3:1; flow rate 1.5 cm^3 min^{-1}; detection λ=355nm.

4.15. Measurement of doxycycline by bioassay

The bioassay was performed by the agar diffusion method (Collins et al., 1989). *B. subtilis* ATCC 6633 was used as the test bacterium. Test bacteria were inoculated into agar plates melted and cooled to 45°C. Then wells (approx. 0.4 cm^3) were deepened into the media. The wells then were filled with the samples containing the antibiotic. As the antibiotic diffused into the agar there were zones where the test bacteria that had been sensitive for the given antibiotic did not grow. The diameter of this zone was proportional to the amount of the antibiotic in the sample. Controls with known amounts of doxycycline were used to get standards.

4.16. Statistical analysis

Using paired or unpaired Student's t test or analysis of variance where appropriate were used for statistical analysis. Individual comparisons between group means were made using Scheffe's procedure. $P < 0.05$ was regarded as significant.

5. Results and Discussion

5.1. Effects of drugs administered in the perioperative period on the exogenous and endogenous bacterial sources of perioperative infection

5.1.1. Effects of glyceryl trinitrate and sodium nitroprusside on bacterial growth

All strains grew in Mueller-Hinton broth at both 20°C and 37°C. *P. aeruginosa* and *E. coli* multiplied in glucose 5% while the count of *S. aureus* did not change significantly.

Glyceryl trinitrate reduced the number of all bacteria at both temperatures.

Sodium nitroprusside had less bactericidal activity. It reduced the bacterial counts of *S. aureus* at both temperatures. The number of *E. coli* was growing first and reduced after 6 hours, but always exceeded the starting value at 20°C. It was bacteriostatic against *P. aeruginosa* at 37°C and supported its growth at 20°C (Table 2-4) (Bátai et al., 1999b).

The alcohol or glucose content of the drugs may contribute to the observed changes in bacterial growth but can not explain it alone. Bacterial counts regarding all strains were higher at any time in the control samples than in the corresponding GTN or SNP solution at both 20°C and 37°C.

Table 2. Growth of *Staphylococcus aureus* in glyceryl trinitrate and in sodium nitroprusside.

	glyceryl trinitrate		sodium nitroprusside	
T / t (h)	20°C	37°C	20°C	37°C
0	12.2 ± 3.03	15.2 ± 3.8	16.4 ± 5.94	14.8 ± 9.31
3	3.4 ± 2.07*	0.4 ± 0.54*	16.4 ± 5.72	9.2 ± 7.04
6	0.4 ± 0.54*	0.2 ± 0.44*	10.6 ± 4.63*	5.8 ± 2.48*
24	0*	0*	7.4 ± 2.70*	0.6 ± 0.54*

Results are means ± SD of colony forming units in 10 μL sample, n = 5.
*, result is significantly different from 0 time, at $p < 0.05$;
T, temperature of incubation;
t, time of incubation.

Table 3. Growth of *Pseudomonas aeruginosa* in glyceryl trinitrate and in sodium nitroprusside.

	glyceryl trinitrate		sodium nitroprusside	
T / t (h)	20°C	37°C	20°C	37°C
0	23.8 ± 5.71	23.6 ± 6.65	26 ± 4.69	25 ± 7.74
3	14.2 ± 4.32*	0*	67.4 ± 5.12*	29 ± 10.07
6	6.6 ± 1.51*	0*	69.6 ± 2.96*	26 ± 6.08
24	0*	0*	>500*	24.2 ± 7.72

Results are means ± SD of colony forming units in 10 μL sample, n = 5.
*, result is significantly different from 0 time, at $p < 0.05$;
T, temperature of incubation;
t, time of incubation.

Table 4. Growth of *Escherichia coli* in glyceryl trinitrate and in sodium nitroprusside.

	glyceryl trinitrate		sodium nitroprusside	
T / t (h)	20°C	37°C	20°C	37°C
0	17 ± 2.91	20 ± 3.74	23.60 ± 4.66*	20.8 ± 4.65
3	14.2 ± 2.58	0*	> 250*	116.8 ± 22.35*
6	12.8 ± 3.19	0*	207 ± 21.12*	197.2 ± 33.11*
24	4.8 ± 1.48*	0*	158 ± 11.51*	1.6 ± 1.81*

Results are means ± SD of colony forming units in 10 μL sample, n = 5.
*, result is significantly different from 0 time, at $p < 0.05$;
T, temperature of incubation;
t, time of incubation.

Our data suggest that while GTN inhibits, SNP can either inhibit or support bacterial growth. Glyceryl trinitrate at 37°C had the most pronounced antibacterial effects. On the other hand, *P. aeruginosa* grew in SNP at 20°C.
The Gram negative strains were more resistant to the tested drugs than the Gram positive bacterium. In both GTN and SNP solutions the inhibition of bacterial growth was more evident at 37°C than at 20°C. This effect of temperature is in accordance with previous studies examining the effects of local anaesthetics on bacteria (James et al., 1976; Taki et al., 1988).
Both GTN and SNP can serve as nitric oxide (NO) donors (Wendt et al., 1978; Arnold et al., 1984; Moncada et al., 1991). Nitric oxide is an important component of the host response to infection (Nathan and His, 1991). Nitric oxide may directly kill bacteria (Nathan and His, 1991) or it may play a role in the limitation of the available intracellular iron (Lancaster and Hibbs, 1990.). In this study SNP showed less antimicrobial activity than GTN. Sodium nitroprusside is photochemically degraded to NO (Arnold et al., 1984) and it was light protected as used in clinical practice. In this way the spontaneous NO production was reduced which could explain its reduced antimicrobial activity.

5.1.2. Effects of atracurium and cisatracurium on bacterial growth

Both strains grew in Mueller-Hinton broth at both 20°C and 37°C.
Both drugs killed *P. aeruginosa* after 3 hours at 37°C and after 6 hours at 20°C. They were less effective against *S. aureus*. They had more pronounced antibacterial effects at 37°C against both strains (Table 5, 6) (Kerényi et al., 1996).

Table 5. Growth of *Staphylococcus aureus* in atracurium and in cisatracurium.

	atracurium 10 mg mL^{-1}		cisatracurium 2 mg mL^{-1}	
T / t (h)	20°C	37°C	20°C	37°C
0	13.2 ± 4.23	14.2 ± 3.65	14.4 ± 4.94	15.1 ± 9.01
3	5.2 ± 2.07*	8.4 ± 2.54*	8.4 ± 4.72*	8.9 ± 4.52
6	5.6 ± 3.1*	6.2 ± 3.44*	9.6 ± 4.63*	3.8 ± 1.39*
24	2.4 ± 2.1*	0*	4.4 ± 2.70*	0*

Results are means ± SD of colony forming units in 10 μL sample, n = 5.
*, result is significantly different from 0 time, at $p < 0.05$;
T, temperature of incubation;
t, time of incubation.

Table 6. Growth of *Pseudomonas aeruginosa* in atracurium and in cisatracurium.

	atracurium 10 mg mL^{-1}		cisatracurium 2 mg mL^{-1}	
T / t (h)	20°C	37°C	20°C	37°C
0	20.8 ± 6.73	19.6 ± 6.49	24.1 ± 5.12	21.8 ± 6.82
3	3.2 ± 1.32*	0*	4.4 ± 2.12*	0*
6	0*	0*	0*	0*
24	0*	0*	0*	0*

Results are mean ± SD of colony forming units in 10 μL sample, n = 5.
*, result is significantly different from 0 time, at $p < 0.05$;
T, temperature of incubation;
t, time of incubation.

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Atracurium and cisatracurium are non-depolarising muscle relaxants frequently used at intensive care units and for various surgical procedures. Cisatracurium is one of the 10 stereoisomers present in the commercially available preparation of atracurium. Cisatracurium is three to five times more potent than atracurium. They gained widespread popularity, as their administration is safe for most patients including those in renal and/or hepatic failure. The pharmacokinetic model of atracurium and cisatracurium is unusual, as Hofmann degradation (cleavage of the link between the quaternary nitrogen ion and the central chain

at normal body pH and temperature) occurs in both the central and peripheral compartments (Calvey and Williams, 1997).

Atracurium and cisatracurium are quaternary amines. Protamine sulphate (Teichman et al., 1993; Teichman et al. 1994) is another quaternary amine that possess antibacterial properties. Quaternary amines can also be found among disinfectants (Simpson, 1997). The quaternary amine structure of atracurium and cisatracurium may contribute to their antibacterial effects.

We evaluated bacterial growth at both 20°C and 37°C. As far as infection control is concerned, results obtained at 20°C should be considered. Our results suggest that while *S. aureus* or *P. aeruginosa* cannot survive in GTN if contaminated the infusion, *E. coli* can survive in it for at least 24 hours. Sodium nitroprusside can even support the growth of bacteria. Atracurium and cisatracurium killed *P. aeruginosa* within 6 hours while *S. aureus* could survive in them at a reduced number even after 24 hours at 20°C.

The fact that the examined pharmaceutical preparations does not support the growth of *S. aureus* is particularly important as staphylococci were isolated most frequently from contaminated syringes used in anaesthesia (Magee et al., 1995).

The use of intravenous catheters is associated with a 2 - 19 % frequency of catheter-related septicemia (Surenderilit, 1982; Miller et al., 1984; Prager and Silva, 1984; Sitzmann et al., 1985). These infections have been reported to result from migration of bacteria from the skin around the catheter (Bjornson et al., 1982; Snydman et al., 1982; Maki and Ringer, 1987; Cooper et al., 1988; Hampton and Sheretz, 1988), contamination at the time of catheter-insertion because of poor sterile technique (Mermel et al., 1991), contamination of the catheter hub during their routine use (Linares et al., 1985; Sitges-Serra et al., 1985; Mermel et al., 1991), hematogenous seeding of the catheter and

contamination of the infusate (Linares et al., 1985; Hampton and Sheretz, 1988; Maki et al., 1988).

Various efforts have been made to reduce catheter associated infections. Reduced bacterial colonisation has been reported with the use of sulfadiazine and chlorhexidine impregnated venous catheters (Modak and Sampath, 1992; Greenfeld et al., 1995).

Infusions like atracurium, cisatracurium, GTN or SNP that possess antibacterial properties may help preventing venous catheters from bacterial colonisation.

Results obtained at 37°C can be interesting for different reasons. The continuous emergence of multi-resistant strains makes effective antibiotic therapy difficult. Non-antibiotic drugs may contribute to the treatment of resistant infection (Kristiansen and Amaral, 1997). It is unlikely that the examined drugs alone could help treating infections as their concentrations *in vivo* are far below compared to those in these *in vitro* tests. At present we have scarce knowledge on the interaction between antibiotics and medications used in anaesthesia and intensive care but the few results available are encouraging. There is evidence of synergistic effects of chlorpromazine with aminoglycosides, β-lactams, tetracycline, vancomycin and quinolones (Amaral et al., 1992), promethazine with gentamicin *in vivo* (Molnár et al., 1990), and in the case of lignocaine with some antibiotics *in vitro* (Ohsuka et al., 1994).

Whether GTN, SNP, atracurium or cisatracurium have any additive or synergistic effects with antibiotics in clinical settings needs further investigations.

5.1.3. Effect of synergistic combination of antibiotics on the emergence of multiresistant bacteria

The appearance of resistant haemolytic *E. coli* clones was studied in mice treated with a synergistic combination of antibiotics. The R plasmid donor and the haemolytic recipient were given orally. The animals then were treated intraperitonealy with 2.5 mg carbenicillin and 2.5 mg kanamycin pro die for 7 days.

In nutrient broth the frequency of transfer was 4.9×10^{-4} for the donor and 3.6×10^{-4} for the recipient, respectively. No transfer could be detected when 3.0×10^{7} donor and 3.0×10^{7} recipient cells were mated in nutrient broth containing carbenicillin and kanamycin. Table 6 shows the results of the *in vivo* mating experiments. In faecal samples of animals treated with antibiotics, kanamycin-resistant haemolytic recombinants were found in 13 cases (16.1% of all samples) by direct plating and in 27 cases (33.7% of all samples) after enrichment. Only haemolytic colonies appeared on carbenicillin-kanamycin blood agar, and all of them reacted with O139:K82 immuneserum. Both the Hly plasmid and plasmid pTE1 could be detected in representative clones tested by agarose gel electrophoresis. In some animals the excretion of recombinants seemed to be continuous while in others it was intermittent. In control animals (infected only but not treated with antibiotics) a single positive culture occurred, only after enrichment.

Five mice each were monocontaminated with either the donor or the recipient and given accordingly either kanamycin or carbenicillin only. No recombinants could be isolated when faecal samples taken daily from pairs of the above mice were enriched together.

Quantitative assays showed that one day after the ingestion of bacteria the number of donor cells per faecal pellet (6.6×10^{5}) and that of recipient cells (6.8×10^{5}) could have been sufficient for the transfer of pTE1. The next day - one day after starting with combined antibiotic treatment - the numbers of recipient

cells excreted per faecal pellet were greater than the *in vitro* transfer frequency although the number of donor cells was 2.2 X 10^3 or less in the faecal pellets tested. In mice Nos. 7 and 8, where excretion of transconjugants was the most prominent, the number of kanamycin-resistant transconjugants in faecal pellets increased from $<10^3$ to 10^7 during the antibiotic treatment. Even at day 11 – 3 days after stopping antibiotic treatment – the number of carbenicillin-kanamycin resistant colonies was equal to the number of total *E. coli* excreted. Five days later no recombinant could be detected in the faeces of the animals (Table 7) (Emődy et al., 1984b).

Table 7. The effect of antibiotic treatment on the *in vivo* selection of resistant haemolytic *Escherichia coli* clones in mice

Days after ingestion of donor [a] and recipient [b]	Excretion of resistant recombinants			
	Direct plating [c]		Enrichment [d]	
	1-10 [e]	11-20 [e]	1-10 [e]	11-20 [e]
2	7	–	1,3,7,8,9	12
3	7	–	3,7,8,9	–
4	2,7	–	1,3,7,8,9	–
5	7,8	–	2,3,7,8	–
7	7,8	–	2,7,8,10	–
8	7,8	–	7,8	–
11	2,7,8	–	2,7,8	–
16	–	–	–	–
Total positive	13	0	27	1

[a] *E. coli* J53pTE1 pro^-, met^-, Nal^r/Km^r, Cm^r/.
[b] *E. coli* P673pTE5 O139:K82 /Hly^+, Amp^r, $Carb^r$/.
[c] Direct plating of faecal samples onto Km-Carb blood agar plates.
[d] Enrichment of faecal samples in Km-Carb nutrient broth before plating onto Km-Carb blood agar plates.
[e] Designation of mice: 1-10, treated i.p. with 2.5 mg kanamycin and 2.5 mg carbenicillin for 7 days starting 1 day after infection; 11-20, controls treated i.p. with saline.

Plasmids are extrachromosomal genetic elements found in a variety of bacterial species that generally confer some evolutionary advantage to the bacterial cell (i.e., resistance to antibiotics, production of haemolysin). Plasmids are double-stranded, closed DNA molecules ranging in size from 1 to 200 kilobases (King and Stansfield, 1997).

Numerous authors have proved the possibility of *in vivo* plasmid transfer both in animal models (Jones and Curtiss, 1970; Smith, 1977; Gyles et al., 1978) and in man (Anderson et al., 1973; Petrocheilou et al., 1976). Most of the animal experiments were performed either on gnotobiotic animals or on animals freed of members of the *Enterobacteriaceae* by pre-treatment with antibiotics (Reed et al., 1969; Jones and Curtiss, 1970; Milch and Nguyen, 1981). To simulate natural circumstances no pre-treatment was applied in our model. Combined antibiotic treatment was started one day after the infection, so that numbers of donor and recipient cells were suitable for *in vivo* plasmid transfer in the first 24 h of the experiment. Later the counterselecting agents repressed especially the K-12 derivative donor cells very effectively. In view of this fact and the results of control experiments it is very unlikely that transfer occurred later *in vivo* or in the selective enrichment medium. That is, clones of recombinants could exist and multiply without the antagonistic effect of the resident *E. coli* flora in mice treated with antibiotics. In control animals transconjugants could have been formed in comparable numbers but they were presumably not able to compete with the multiplication of the resident bacterial flora. Our results also suggest that these selective effects of the antibiotics given in the perioperative period could last for some days after the administration of antibiotics had been stopped. The persistence and spreading of such recombinants seem to be easier in hospital wards (Burman, 1980) where great quantities of antibiotics are administered regularly. The synergistic broad-spectrum β-lactam-aminoglycoside combination

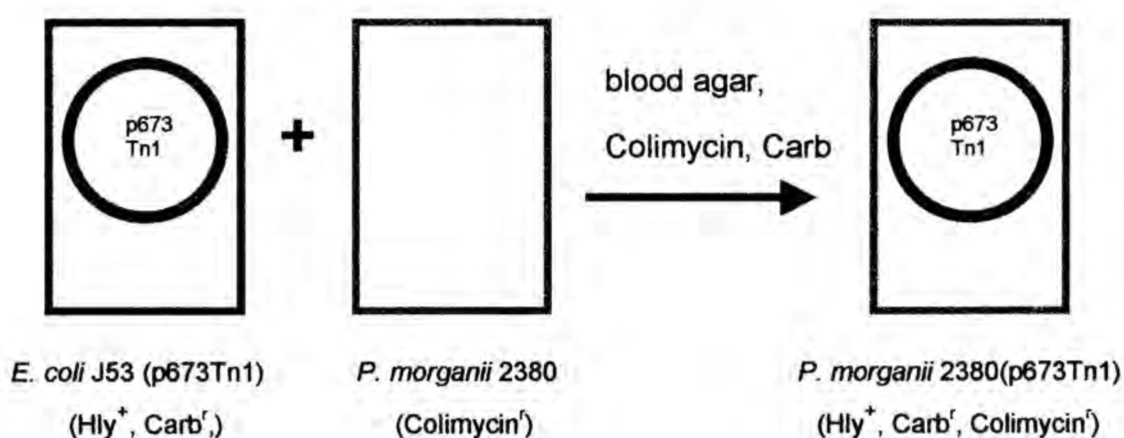
can act more effectively than a single antibiotic when curing an actual infection or to reduce the intestinal flora. It can, however, unfavourably select multiresistant clones of potential extra-intestinal pathogens incidentally present in the gut, and resistant to the antibiotics administered.

5.1.4. Intergeneric transfer of *E. coli* haemolysin plasmid into *Proteus morganii* in the mouse intestine after antibiotic treatment

Before commencing experiments on mice we checked whether transfer of Hly plasmid could be detected between *E. coli* and *P. morganii* mated in nutrient broth if selection is facilitated by the presence of Tn1 on the Hly plasmid. Hly^+ transconjugants could be isolated only by as low a frequency as approximately 2×10^{-9} for both donor and recipient cells. As transconjugants occurred only on selective plates poured with undiluted mating mixture it was unlikely to get recombinants by direct plating of faecal samples in the *in vivo* experiments. Therefore a selective enrichment medium containing carbenicillin and colimycin was used. Before performing transfer experiments in mice it was necessary to know whether transfer of Hly plasmid might occur in this medium interfering with the evaluation of the *in vivo* transfer. Ten independent matings were performed in carbenicillin-colimycin nutrient broth but no growth occurred at all when mating mixtures were plated onto ampicillin-colimycin blood agar.

In vivo transfer experiments were performed on 20 streptomycin treated mice. Hly^+ derivatives of *P. morganii* 2380 were isolated from 9 samples: 3 from the faeces, 2 from the small intestines and 4 from the large intestines. All transconjugants were isolated after enrichment and no transfer could be detected by direct plating. Figure 6 shows the intergeneric transfer of the Hly plasmid.

Figure 6. Intergeneric transfer of the Hly plasmid.



In the next experiments the virulence of a representative of Hly^+ transconjugants (*P. morganii* 2380p673Tn1) was compared with that of the donor and recipient. As shown in Table 7, the Hly^+ derivative exhibited a high degree of virulence both in mouse lung toxicity assay and chicken embryo virulence test (Table 8) (Emődy et al., 1983).

Table 8. Virulence conferred by Hly plasmid in *Proteus morganii* 2380

	Mouse lung toxicity		Chicken embryo virulence	
Strains	Number died/ Number injected	Mortality (%)	Number died/ Number injected	Mortality (%)
J53p673Tn1 Hly^+ donor	0/12	0	5/15	33
Pm 2380 Hly^- recipient	0/30	0	2/15	13
Pm 2380p673Tn1 Hly^+ transconjugant	27/30	90	14/15	93

Numerous studies support the idea that alpha-haemolysin of *E. coli* may be a virulence factor in extraintestinal manifestations (Green and Thomas, 1981; Van den Bosch et al., 1981; Waalwijk et al., 1982). Haemolytic strains exhibit a high degree of virulence in different animal models whereas nonhaemolytic derivatives obtained either by mutagenesis or plasmid elimination were no longer virulent in the same test systems (Kétyi et al., 1978; Waalwijk et al., 1982; Van den Bosch et al., 1982). Plasmids coding for haemolysin production confer virulence when transferred into suitable nonhaemolytic recipients (Kétyi et al., 1978; Emődy et al., 1980; Waalwijk et al., 1982; Van den Bosch et al., 1982). Alpha-haemolysin of *P. morganii* might play a role in extraintestinal infections similar to that of *E. coli* alpha-haemolysin (Emődy et al., 1982b).

Data presented in this study show that genetic information of alpha-haemolysin is exchangeable between *E. coli* and *P. morganii in vivo* in a favourable milieu when antibiotics depress the normal flora.

Expression of transferred genes was shown not only by the appearance of haemolysin production but also by demonstration of an enhanced virulence in animal models. It is not surprising that expression of virulence is better in the transconjugant than in the donor being an *E. coli* K-12 derivative with an R-like surface character. The Hly^+ transconjugant of *P. morganii* 2380 exhibited the same degree of virulence as Hly^+ wild-type *P. morganii* strains (Emődy et al., 1982b).

The method described in this study may be useful to detect *in vivo* transconjugants of a poorly selectable plasmid (Hly) transferred at a very low frequency.

5.2. Effects of anaesthesia on bacterial adherence

5.2.1. Effect of halothane on bacterial haemagglutination

It has already been demonstrated that bacterial growth rate is not affected at the applied anaesthetic concentration under these experimental conditions (Wardley-Smith and Nunn, 1971).
Halothane exposure (halothane 2% in oxygen 40% containing carbon dioxide 5% for two hours) did not affect mannose resistant or mannose sensitive haemagglutination.

5.2.2. Effect of halothane on bacterial adherence *in vitro*

Following halothane exposure bacterial counts and HEp-2 cell viability did not differ from the control group.

In the control group (Group I) 145 ± 15.2 cells (72.5%) were covered with more than 40 bacteria (*E. coli* K88). The number of cells covered with more than 40 *E. coli* K88 in group II (Hep-2 cells exposed to halothane) was reduced to 80 ± 15.1 cells (55% of control) ($p < 0.05$). A 37% reduction as compared to control ($p < 0.05$) was observed in group IV (both HEp-2 cells and bacteria were exposed to halothane).

No significant difference was found between group III (where only the bacteria were exposed to halothane) and the control, and between group II and IV.
Similar reductions in the number of adhered bacteria were obtained with *E. coli* NG7C (Table 9) (Bátai and Kerényi, 1999c).

Table 9. Effect of halothane on the adherence of *E. coli* to HEp-2 cells

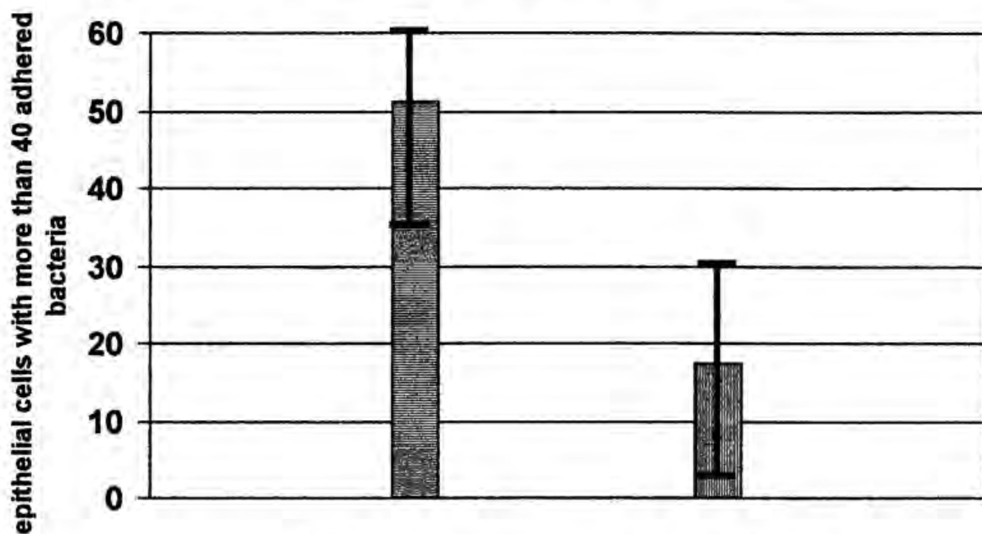
strains	***E. coli* K88** epithelial cells covered with more than 40 bacteria (200 cells were counted)	***E. coli* NG7C** epithelial cells covered with more than 40 bacteria (200 cells were counted)
Group I	145 ± 15.2	165.8 ± 32.6
Group II	80 ± 15.1 * ‡	92.6 ± 19.3 * ‡
Group III	156.8 ± 25.6	138.3 ± 21.1
Group IV	91.4 ± 17.6 * ‡	86.1 ± 21.5 * ‡

Values are means ± SD, n = 25.
Group I, control;
Group II, halothane exposed HEp-2 cells and untreated bacteria;
Group III, untreated HEp-2 cells and halothane exposed bacteria;
Group IV, both HEp-2 cells and bacteria exposed to halothane.
* Significantly different ($P < 0.05$) from Group I.
‡ Significantly different ($P < 0.05$) from Group III.

5.2.3. Changes in the oral commensal flora following prophylactic antibiotic administration and surgery under general anaesthesia

Before induction of anaesthesia 51.2 ± 15.8 epithelial cells were covered with more than 40 bacteria (25.6% of all cells). By the end of the operation the number of epithelial cells covered with more than 40 bacteria was reduced to 17.4 ± 13.5 (8.7% of all cells) ($p < 0.05$) (Figure 7) (Bátai et al, 1996).

Figure 7. Changes in the oral commensal flora following prophylactic antibiotic administration and surgery under general anaesthesia.



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Haemagglutination provides a means for detecting and classifying specific adhesins (Duguid and Old, 1980). The impact of halothane on the expression of bacterial adhesiveness was examined by mannose resistant and mannose sensitive haemagglutination of *E. coli*. *E. coli* strains have different fimbrial adherence factors such as Type 1, Dr/075X, S, Pap, and Curli thin aggregative fimbriae (Patti et al., 1994). In the presence of mannose, Type 1 fimbriae are inhibited (Parry and Rooke, 1985).

This experiment showed that halothane 2% does not affect the expression of structures needed for the haemagglutination of *E. coli*.

Our results suggest that halothane reduces bacterial adherence to human epithelial cells *in vitro*. It seems likely that at the applied concentration the

adhesion at the level of the epithelial cells must be affected as haemagglutination was not influenced by halothane and no significant change was found in bacterial adherence after only the bacteria were exposed to halothane.

Medical interest in bacterial adherence dates back to 1955 (Duguid and Smith, 1955). Since then, adherence has been considered to represent the initial step in the pathogenesis of most bacterial infections (Niederman, 1990).

In previous studies seeking connection between anaesthesia and the attachment of bacteria to human epithelial cells many factors (surgical trauma, anaesthetic technique, postoperative ventilation, and antibiotics) may have contributed to the results (Redman and Lockey, 1967; Johanson et al., 1980).

In order to assess the isolated effect of an anaesthetic agent on bacterial adherence we choose an *in vitro* model that proved to give reproducible results in microbiological research (Tavendale and Old, 1985). Among the different assay systems used for measuring bacterial adhesion, tissue-culture methods are considered reliable in respect of their technical simplicity, ease of performance and high reproducibility (Vosbeck and Mett, 1983). We tested two strains of *E. coli* that bind to host cells or to the extracellular matrix by different adhesins (Ørskov et al., 1964; Ljungh et al., 1990; Smith, 1992).

The first experiment concerning changes on bacterial cell surface under the influence of an anaesthetic was published as early as 1911. These investigations showed that ether 2% in NaCl 0.85% had no effect on bacterial agglutination *in vitro* (Graham, 1911). The work of Duguid et al. showed that "fimbriae" of *E. coli* were responsible for haemagglutination (Duguid et al., 1955).

It has also been demonstrated that halothane reduces the expression of sugar binding receptors on human granulocytes (Bárdosi et al., 1990; Bárdosi et al., 1992). The receptor for *E. coli* K88 adhesin is a glycolipid and a recent study proved that the minimum structure of the K88 receptor was β1-linked galactose (Payne et al., 1993b). These observations may explain our results that suggest that a reduced number or function of the epithelial cell surface receptors may be

responsible for the decrease in bacterial adherence to human epithelial cells after halothane exposure.

The *in vivo* results showed that surgery of long duration under general anaesthesia with a single dose of a cephalosporin antibiotic administered at the beginning of the procedure reduces the number of bacteria attached to buccal epithelial cells. It is worth mentioning that conventional bacteriological culture methods do not distinguish between attached and non-attached bacteria. Thus the presence of an organism in a patient's throat and its subsequent culture from a swab does not necessarily establish that it associated with the mucosal surface, and possibly deprives pathogens from cell surface attachment sites.

The effects of an anaesthetic on bacterial adherence may be of interest from several aspects.
These *in vitro* and *in vivo* data showing changes in bacterial adherence following the exposure of bacteria and/or epithelial cells to general anaesthetics may promote better understanding of the pathomechanism of postoperative infections. Our normal flora protects us from colonisation by potential pathogens (Sanders et al., 1977), and it is an essential element of maintaining host defence mechanisms. Changes in the normal human bacterial flora eventually will result in increased susceptibility to infections.
Questions whether anaesthesia alone causes any change in the normal protective oral flora or in the adherence of pathogenic bacteria in clinical settings need further investigations.

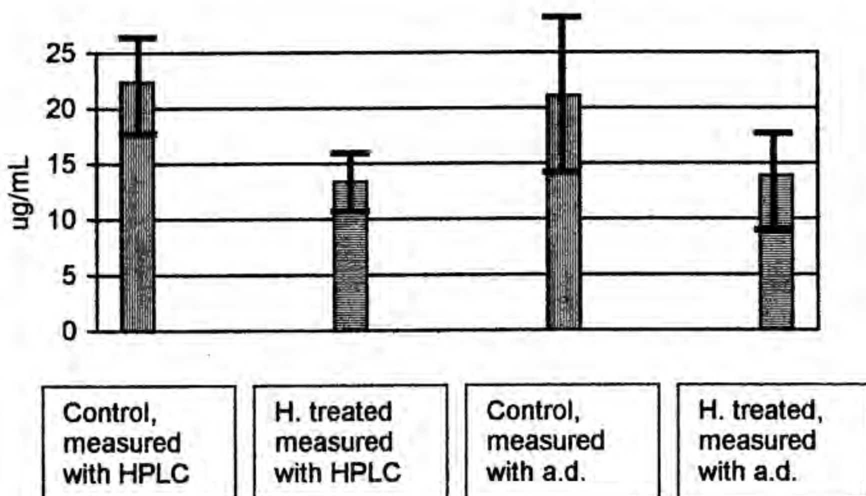
On the other hand, observation suggesting changes on receptor expression on human cells after anaesthetic exposure might contribute to the better understanding of the mechanisms of anaesthetic action.

5.3. The effect of anaesthesia on the perioperative antibiotic therapy

5.3.1. The effect of halothane on the doxycycline content of human polymorphonuclear leukocytes (PMNs) *in vitro*

Halothane, HBSS or oxygen 40% in air containing carbon dioxide 5% did not affect the biological activity of doxycycline assessed by the bioassay. Freezing doxycycline in liquid nitrogen had no influence on its activity.
The concentration of doxycycline accumulated in untreated PMN was 21.1 ± 6.3 $\mu g\ mL^{-1}$ measured with the biological assay and 22.4 ± 5.6 $\mu g\ mL^{-1}$ measured with HPLC.
The biological assay revealed that the antibiotic content in the halothane treated PMN reduced to 13.9 ± 3.1 $\mu g\ mL^{-1}$ ($p<0.05$) (66% of the control). Similar alterations were found with HPLC; the doxycycline content was 13.4 ± 2.4 $\mu g\ mL^{-1}$ (60% of the control) ($p<0.05$) in the halothane treated group (Figure 8) (Bátai et al., 1987).

Figure 8. Doxycycline content of polymorphonuclear leukocytes following halothane treatment.



HPLC, high pressure liquid chromatography;
H. halothane;
a.d. agar diffusion method
Results are mean ± S.D.
n = 30.

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Our study suggests that halothane significantly decrease the intracellular doxycycline content of human PMNs detected by two independent methods. Doxycycline was chosen for its favourable pharmacokinetic properties. It is accumulated inside PMNs and not released rapidly in antibiotic free environment (Forsgren and Bellahsene, 1985).

The mechanism of action is not known and it may have many possible explanations. Passive diffusion probably plays a more important role than active transport in the uptake of lipophilic tetracyclines (Kivman et al., 1983). Halothane binds to the lipid membranes at various locations (Washington et al., 1984) and

may alter its characteristics. Halothane fluidizes lipid bilayers (Mastrangelo et al., 1978) and it has been suggested that even small changes in lipid fluidity may profoundly change membrane function (Koblin, 1990). Halothane also influences soluble (Franks and Lieb, 1984) and membrane proteins (Tas et al., 1987).

There are data suggesting that uptake of tetracyclines is associated with their intracellular binding (Katiyar and Edlind, 1991), mainly to mitochondria (Kivman et al., 1985), while mitochondrial functions are also affected by halothane (Cohen, 1973).

Antibiotic treatment can only be effective if it reaches sufficiently high levels at the site of infection. Polymorphonuclear leukocytes play an important role in delivering antibiotics to the site of infection (Deysine et al., 1979). As halothane exposure reduces their intracellular antibiotic content *in vitro*, it may influence perioperative antibiotic therapy.

6. New observations

1. Glyceryl trinitrate infusion is safe as far as infection control is concerned. It reduces the number of *S. aureus*, *E. coli* and *P. aeruginosa* at both 20°C and 37°C.

2. Sodium nitroprusside has less antibacterial activity than GTN and in special circumstances may facilitate the development of postoperative infections. It reduced the bacterial counts of *S. aureus* at both 20°C and 37°C and that of *E. coli* only at 37°C. It is bacteriostatic against *P. aeruginosa* at 37°C and supports its growth at 20°C.

3. Both atracurium and cisatracurium are unlikely to pose a risk for postoperative infection. They killed *P. aeruginosa* after 3 hours at 37°C and after 6 hours at 20°C. They were less effective against *S. aureus*.

4. The antibacterial effect of GTN, SNP, atracurium and cisatracurium is more pronounced at 37°C than at 20°C.

5. Synergistic combination of antibiotics given parenterally following infection can select antibiotic resistant haemolytic transconjugant bacteria *in vivo*.

6. Genetic information of alpha-haemolysin is exchangeable between *E. coli* and *P. morganii in vivo* and antibiotic treatment may select for the transconjugants.

7. The inhalational anaesthetic halothane does not influence the mannose sensitive or the mannose resistant haemagglutination of *E. coli*.

8. The inhalational anaesthetic halothane decreases the adherence of *E. coli* to human epithelial cells *in vitro*.

9. The number of bacteria attached to human oral epithelial cells decreases following surgery under general anaesthesia (TIVA) with antibiotic prophylaxis.

10. Halothane decreases the intracellular doxycycline content of human polymorphonuclear leukocytes *in vitro*.

11. Halothane does not influence the antimicrobial effect of doxycycline *in vitro*.

12. Freezing and thawing does not influence the antimicrobial effect of doxycycline.

7. Conclusions

Postoperative infection is a serious complication of surgical procedures. The cause of postoperative infections is multifactorial. The patient's preoperative condition, the site of surgery, the surgical trauma, blood transfusion, perioperative hypothermia and anaesthesia all determine its occurrence. Anaesthesia has long been suspected to contribute to infection in the postoperative period. So far the main concern has been the depression of the immune system which allows an invading organism to become established. The majority of investigators have studied granulocyte and lymphocyte functions and revealed depressed immune functions.

In the Thesis other possible causes of postoperative infection were discussed. Intravenous medications may be contaminated in the operating theater being the source of postoperative infections. This issue has long been examined, however, many drugs we use as a bolus injection or/and as infusion have never been tested as far as infection control is concerned. In the Thesis GTN, SNP, atracurium and cisatracurium has been added to the list of drugs tested concerning bacterial growth. Atracurium cisatracurium and GTN proved to be safe while SNP supports the growth of some bacteria at room temperature.

Adherence of pathogen bacteria to mucous membranes is thought to be the initial step in infection. There are many defence mechanisms working against the adherence of potentially pathogenic micro-organisms and anaesthesia affects most of them. An important host defence mechanism is the presence of the resident micro-flora. Drugs or procedures altering bacterial adherence influence host defence mechanisms. However, the effects of anaesthesia on this prerequisite of infection have not been investigated yet.
Our results suggest that general anaesthesia reduces bacterial adherence to epithelial cells. Data showing changes in the adhesive properties of epithelial

cells after anaesthetic exposure may promote better understanding of the pathomechanism of postoperative infections. Commensal flora protects us from colonisation by potential pathogens and it is an essential element of maintaining host defence mechanisms. Any changes in the normal human bacterial flora may result in increased susceptibility to infections.
Questions whether anaesthesia alone can be responsible for changes in the normal protective oral flora or in the adherence of pathogenic bacteria in clinical settings need further investigations.

Should our measures fail to prevent infection in the postoperative period, despite their side effects, antibiotics will provide the basis of treatment. We must always remember that the use of antibiotics, especially their synergistic combinations may help the appearance of multiresistant strains and/or more virulent clones. On the other hand, antibiotics can only help if they get to the site of infection at a sufficient concentration. Anaesthetics may interact with the effect of antibiotics at this level.

The incidence of postoperative infections is still high. The more we know about the impact of anaesthesia on the process leading to infection, the more opportunity we have to reduce it.

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List of publications

1. Emődy L, **Bátai I**, Kerényi M, Székely J, Polyák L. Anti-*Escherichia coli* alpha-haemolysin in control and patient sera. *Lancet* 1982; **ii**: 986.
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10. **Bátai I**, Kerényi M. Impact of halothane on bacterial adherence in vitro. *Acta Anaesthesiol Scand* 1999; **43**: 760-763. **IF: 1.036**

Appendix

ASA Physical Status

Anaesthesiologists have worked for many years to devise a means to classify preoperative conditions. Total operative risk also depends on the proposed surgery and skill of the surgeon, but the classification index was strictly limited to a definition of preoperative physical status. The original ASA-sponsored scheme included six categories and was revised in 1961 (Dripps et al., 1961) to its present form of five categories (Table).

Table ASA Physical Status

Category	Description
I	Healthy patient
II	Mild systemic disease - no functional limitations
III	Severe systemic disease[a] - definite functional limitation
IV	Severe systemic disease[a] that is a constant threat to life
V	Moribund patient not expected to survive 24 hours with or without operation

[a] Whether or not the systemic disease is the disease for which the patient is undergoing surgery.

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