Factors affecting antimicrobial agents in an anaerobic abscess

The optimal treatment of anaerobic infections includes the selection of an appropriate antibiotic and the surgical drainage of purulent collections (Finegold et al., 1975). The surgical dictum that 'pus must be drained' has been known for centuries but drainage alone is not adequate because in the pre-antibiotic era one-third of the patients died of the infection despite surgical therapy. The need for this dual therapeutic approach is dictated by the pathological characteristic of these infections which is tissue destruction, usually progressing to discrete abscesses. Recent studies have shown that the environment in an anaerobic abscess is hostile to action of white cells and the activity of many antibiotics. It is important to have some knowledge of white cell function as well as antimicrobial activity.

A decrease in polymorphonuclear leukocyte (PMN) function under anaerobic conditions has been documented (Mandell, 1974). This most likely results from the lack of an oxygen dependent metabolic burst which is important in phagocytosis and bacterial killing by PMNs (Babior, 1978). Because anaerobic abscesses are devoid of oxygen, the PMN is at a disadvantage. Additionally, inhibition of PMN phagocytosis of aerobic bacteria *in vitro* by certain anaerobic organisms has been described (Ingham, Sisson, Tharagonnet, Selkon & Codd, 1977). The greatest effect was seen with *Bacteroides fragilis* and *Bacteroides melaninogenicus* and it was postulated that the anaerobes are protecting the aerobes in mixed infections. Further work is necessary to define the role of the immunological response to anaerobic infection in the development and resolution of abscesses. A prerequisite to this and a better understanding of antibiotic activity in anaerobic abscess is to further define the characteristics of these infections which is tissue destruction, usually progressing to discrete abscesses. Recent studies have shown that the environment in an anaerobic abscess is hostile to action of white cells and the activity of many antibiotics. It is important to have some knowledge of white cell function as well as antimicrobial activity.

Microbiological observations of anaerobic abscesses have revealed several features which may adversely affect the action of certain antimicrobial agents and be favorable to the action of others. First, the quantity of organisms is large, usually numbering 10^8 to 10^9 colony forming units (CFUs) per ml (Bartlett, Sullivan-Sigler, Louie & Gorbach, 1976). The high inoculum affects the activity of the β-lactam antibiotics including the penicillins and the cephalosporins. Second, the infections are polymicrobial with an average of 5 organisms including 3 anaerobes and 2 aerobes which may act synergistically in forming the
abscess and in maintaining it (Finesgold et al., 1975). Third, there is a low oxidation-reduction potential, measured as eH, indicating a very low oxygen tension (Hays & Mandell, 1974). Anaerobiosis has a deleterious effect on selected agents such as the aminoglycosides, while it favors the activity of other drugs like metronidazole. Little is known about the effect that pH and divalent cation concentration have on the activity of antimicrobial agents in this environment.

The high inoculum found in anaerobic abscesses raises important questions concerning the validity of the current common methods of in vitro susceptibility testing of anaerobes which, as currently applied, usually employ low inocula. This fact may explain the dichotomy often observed between the in vitro and in vivo activity, especially with the beta-lactam antibiotics. We have shown that there is a decrease in the inhibitory activity of penicillin G and the cephalosporins against B. fragilis as the inoculum is increased (O'Keefe, Tally, Barza & Gorbach, 1978; Tally, O'Keefe, Sullivan & Gorbach, 1977). One notable exception is the lack of inoculum effect with cefoxitin. These studies suggest that the resistance encountered at high inocula is due to beta-lactamase present in B. fragilis rather than to the lack of cell division at high bacterial counts (Tally et al., 1977; Darland & Birnbaum, 1977; Olsson, Nord & Wadstrom, 1976).

The importance of beta-lactamases on the in vivo activity of the penicillins was explored in a rabbit model utilizing intraperitoneal capsules in which a pure B. fragilis infection could be established (O'Keefe et al., 1978). The mean per cent penetration (concentration in capsule divided by serum level x 100) of bioactive penicillin into uninfected capsules was 19.9% whereas the mean per cent penetration was only 1.5% into capsules infected with high numbers of organisms. Using labeled penicillin, however, there was substantial penetration into the infected site (12%) but the drug had lost its bioactivity. The inactivation of the penicillin was found to be inoculum-dependent with little decrease in drug concentration when bacterial counts were below 10^4 CFU/ml. Degradation of penicillin within the site of infection may explain the clinical observation that even high doses of penicillins often fail to control anaerobic infections involving B. fragilis when the organism displays intermediate susceptibility to the antibiotic. Similar results have been obtained with cefalothin (O'Keefe, Tally, Sullivan & Gorbach, 1977) while cefoxitin is resistant to the rapid inactivation in vivo. The results of these studies demonstrate that for a beta-lactam drug to be effective in anaerobic infections with B. fragilis it must penetrate into the abscess and be resistant to breakdown by the beta-lactamases. At this time cefoxitin is the only such agent available.

The polymicrobial nature of anaerobic infections increases the likelihood that there will be organisms in the abscesses which are capable of inactivating antibiotics. In addition to B. fragilis, facultative organisms may also be involved. The inactivation is not limited to the beta-lactams, but includes agents such as chloramphenicol. Studies in England and Japan indicated that anaerobic bacteria, particularly Clostridia and Bacteroides, are capable of breaking down chloramphenicol (O'Brien & Morris, 1971; Kanazawa, Kuramata & Miyamura, 1969). These observations have been further studied in vitro by Louie, Bartlett, Onderdonk & Gorbach, (1977). They demonstrated that the Clostridia and B. fragilis inactivate chloramphenicol primarily by reduction of the p-nitro group on the six carbon ring (Louie et al., 1977). This inactivation may explain the decreased activity of this agent in the rat intra-abdominal abscess model and the report of the failure of chloramphenicol in the treatment of serious anaerobic sepsis (Thadepalli, Gorbach & Bartlett, 1977).

The physiochemical characteristics of anaerobic abscesses, including the low oxidation-reduction potential, pH, the presence of divalent cations and short chain organic acids, may have a significant effect on antimicrobial activity. However, only the effect that anaerobiosis has on antibacterial activity has been adequately studied. The lack of oxygen in anaerobic abscesses is known by direct measurement of low oxidation reduction potentials and from the fact that obligate anaerobic organisms are unable to initiate growth unless eH is from -100 to -400 millivolts. These reduced conditions are known to adversely affect the activity of most aminoglycoside antibiotics. In 1946 Bondi et al. showed that the activity of streptomycin was significantly decreased by incubation in an anaerobic jar and recent data demonstrate that gentamicin and tobramycin are 4 to 20 times less active under anaerobiosis (Bondi, Dietz & Spalding, 1946; Verklin & Mandell, 1977). Bryan and Van Den Elzen, in studying
the mechanisms of aminoglycoside uptake in \textit{E. coli}, have provided an explanation for these observations (Bryan & Elzen, 1976). They found that there is an energy-requiring oxygen-dependent second stage in the transport of aminoglycosides into \textit{E. coli}. Because the oxygen concentration is very low in an anaerobic environment, the second phase of aminoglycoside uptake is adversely affected at sites of anaerobic infection. These observations explain the universal resistance of obligate anaerobes to the aminoglycosides since they lack the oxidative transport pathways necessary to assimilate sufficient intracellular concentrations of these drugs.

Although anaerobiosis is deleterious to the activity of aminoglycosides, a low oxidation reduction potential is a prerequisite to metronidazole's antimicrobial activity (Lindmark & Muller, 1976). This compound is the most bactericidal agent known against anaerobes. Its activity is linked to bacterial reduction of the nitro group on the imidazole ring. The enzymatic reaction requires a very reduced environment which is present in anaerobic infections. Metronidazole's antibacterial action is not affected by either inoculum size nor growth rate and it penetrates tissues well (Tally et al., 1978). The novel characteristics of this agent open new avenues for investigation for the development of antimicrobial agents: i.e. drugs which are converted to an active form by the bacterium in a unique environment. Few data are available on the role that the other physicochemical characteristics of anaerobic abscess including pH, and divalent cation concentrations have upon the activity of antibiotics. Further study of these characteristics are essential to an understanding of the mechanisms of antimicrobial bacterial inactivation in anaerobic abscesses.

Based on the above microbiological observations, new antimicrobial agents should possess several characteristics to be effective in anaerobic infection. These include the ability to penetrate abscesses, resistance to the inactivating enzymes present in the infected site, activity against high inocula of organisms and activity under anaerobic conditions. Three drugs currently fulfill these criteria, clindamycin, cefoxitin and metronidazole. Alternative drugs are needed because of the problem of emerging resistance (especially with clindamycin in Europe), the resistance of some strains of \textit{B. fragilis} to cefoxitin and metronidazole's lack of activity against the aerobic component of mixed infections. Understanding the mechanism of action of these antibiotics coupled with further knowledge of the microbiological and physicochemical characteristics of anaerobic infections will help chemists and pharmacologists to develop new agents which would be safe and effective in serious anaerobic infections. If such drugs can be developed then the need for destructive surgical drainage may be reduced and in some instances eliminated.

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References


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Prevention of Infection In leukaemia

It is well recognized that infection is a major cause of morbidity and mortality in patients with acute leukaemia. Fatal infection occurs in up to 25% of patients before therapy has had a sufficient trial (Smith et al., 1977). In a recent trial of prophylactic oral nonabsorbable antibiotics in acute leukaemia, 52% of the control group died of infection (Schimpff et al., 1975). Patients with acute leukaemia are more susceptible to many types of pathogens; nevertheless, bacteria account for the great majority of serious infections (The EORTC International Antimicrobial Therapy Project Group, 1978; Gurwith, Brunton, Lank, Ronald & Harding, 1978). Although increased susceptibility to infection in acute leukaemia has many causes including decreased antibody formation, impaired cellular immunity, mucosal ulcerations, and inevitably granulocytopenia, there is little doubt that granulocytopenia is the most important defect. It is clear that the rate of infection is directly related to duration and degree of granulocytopenia (Bodey, Buckley, Sathe & Freireich, 1966; Gurwith et al., 1978).

The most frequently investigated strategy of infection prevention in acute leukaemia has been oral nonabsorbable antibiotics with or without the additional use of a protected environment, where exposure to any microorganism is limited through the use of life islands, laminar air flow isolation systems, sterilized food, etc. While the usefulness of protected environments has been investigated in at least 12 trials (Levine, 1976), only a limited number of these have been prospectively controlled (Yates & Holland, 1973; Schimpff et al., 1975). Infection was reduced but a significantly improved remission rate was found in only one (Schimpff et al., 1975).

Prophylactic oral nonabsorbable antibiotics used alone have also been widely investigated but of four recent large prospectively controlled trials (Levine et al., 1973; Yates & Holland, 1973; Schimpff, Greene, Young, Fortner, Jepsen, Cusack, Block & Wiernik, 1975; Storrning, McElwain, Jameson, Wiltshaw, Spiers & Gaya, 1977), only the most recent two demonstrated a significant decrease in infection (Schimpff et al., 1975; Storrning, McElwain, Jameson & Wiltshaw, 1977). In none of these studies has an improvement in long-term (> 1 year) survival resulted with either method of infection prevention. There are some obvious disadvantages with both. Although not always emphasized, prophylactic oral nonabsorbable antibiotics produce a variety of unpleasant gastrointestinal side effects, and frequently patients reject their use (Bodey & Rosenbaum, 1974; Schimpff et al., 1975). Those oral nonabsorbable antibiotic regimens which include gentamicin are extremely expensive and may promote the development of gentamicin resistant organisms. Although protected environments have not been as psychologically disturbing as was expected, some patients have rejected them for this reason and/or because of the concomitant nonabsorbable antibiotics. They are also extremely expensive, and unlikely to be available to more than a small minority of patients with acute leukaemia.

Simple but prudent techniques for limiting infection include limited (single room) isolation, minimizing the use of indwelling...