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The influence of antibiotics on gut colonization

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Animal and human studies have suggested the concept of "colonization resistance" of the gastro-intestinal tract, which can be decreased by administration of antibiotics that inhibit the anaerobic portion of the normal flora of the gut. This effect can be prevented by the production, by resistant members of the flora, of bacterial enzymes that inactivate or destroy the antibiotic in question. Possible changes in the prevailing gut flora and the implications for the incidence of different infecting agents and for antibiotic therapy are discussed.

Introduction

It has been observed frequently that colonization and infection by nosocomial Gram-negative bacilli are likely to follow administration of broad spectrum antibiotics. For example, Myerowitz, Medeiros & O'Brien (1971) found that 75% of patients with hospital-associated bacteraemia caused by Gram-negative bacilli had received antibiotics previously, as compared with 9% of non-hospital associated cases. In such observations, it is difficult to rule out other factors, such as the severity of the underlying illness in the antibiotic-treated patients. We report here the findings in animal experiments, in ward observations and in human volunteer studies that have led us to the concept of colonization resistance (CR), the ability of the normal gut flora to combat colonization by extrinsic bacteria, a property which can be upset by antibiotic administration.

Effect of antibiotics on the infective dose in mice

In these experiments (van der Waaij, Berghuis-de Vries & Lekkerkerk-Van der Wees, 1971; van der Waaij & Berghuis, 1974) groups of 20–30 conventionally raised mice were given oral doses of strains of Enterobacteriaceae. The number of bacteria necessary to establish gut colonization (which was observed two weeks later) in 50% of mice was compared with that necessary when the mice received an antibiotic to which the strain was resistant. In untreated mice the 50% infective dose was high, at least 10^7 bacteria, while after antibiotic treatment 10^2 or fewer bacteria would set up colonization.

Subsequent experiments in mice and in humans (van der Waaij *et al.,* 1977) indicated that suppression of the strictly anaerobic gut flora by the antibiotic is necessary for a significant suppression of colonization resistance. Antibiotics that

produce high concentrations of unchanged drug in the gut contents, either because they are not completely absorbed or because they are secreted into the gut in bile or other fluids, created the greatest effect. For example it was shown that parenteral, as well as oral, ampicillin decreased the colonization resistance in mice (van der Waaij, Berghuis & Lekkerkerk, 1972).

Effect of antibiotics on spread of resistant bacteria in mice

The spread of resistant Gram-negative bacilli between cage mates was investigated in pairs of groups consisting of 15 mice. One group was treated with an antibiotic while the other remained untreated as a control; one mouse in each group was given an oral colonizing dose of a Gram-negative bacillus (of the Enterobacteriaceae) resistant to the antibiotic in question. Spread from mouse to mouse was greatly increased by the antibiotic administration; for example, all 15 mice treated with ampicillin were excreting the resistant Gram-negative bacillus after ten days, while no spread had occurred in the control group (van der Waaij, 1979).

However, when ampicillin was given for more than four weeks, the effect on the colonization resistance was reduced; there was no spread when the bacterium was introduced into one member of the group after ampicillin had been given for six weeks, even if high daily doses were given (Hofstra, W., personal communication). This effect was associated with the appearance of a strongly β-lactamase-secreting *Clostridium* strain in the gut flora. Bacterial enzymes may rapidly degrade various antibiotics, so that they have no effect on the colonization resistance (Veringa & van der Waaij, 1984).

Spread of Gram-negative bacilli in patients

Fortuitously, in 1971, a similar "experiment" to that carried out in mice occurred in the prophylactic use of antibiotics in a unit occupied by ten chronically ill paediatric patients. One child developed diarrhoea caused by *Salmonella typhimurium*, which was sensitive to ampicillin, and it was decided, rightly or wrongly, to treat all patients with ampicillin prophylactically, with the aim of preventing spread. Each patient received 2 g of ampicillin daily, orally, for three weeks. We were able to examine twice a week, the faeces of all the patients, identify and biotype the Enterobacteriaceae isolated from them, and determine their antibiotic sensitivity pattern, and, if the specimens were large enough, estimate the ampicillin concentration.

Thirteen of the 14 biotypes of Enterobacteriaceae (including the *Salmonella* strain) that were isolated from the faeces of the patient at the onset of ampicillin treatment disappeared from the faecal cultures during the first week of treatment. All 14 biotypes were sensitive to ampicillin. From day 2 onwards, however, ampicillin-, and often multiply-, resistant Gram-negative biotypes were isolated. The number of ampicillin-resistant Gram-negative biotypes in the faeces of the patients increased almost linearly with time. By the end of the third and last week of treatment, 17 different ampicillin-resistant biotypes had been isolated. Five of these biotypes were found in the stools of most (eight) patients. They had presumably spread among the group in the ward as had happened in the mouse experiments.

In one patient, the original Gram-negative flora did not change during ampicillin treatment. This patient continued to excrete her original ampicillin-sensitive

Escherichia coli biotype and did not acquire new biotypes of Gram-negative bacteria during the three weeks of treatment. In the faeces of nine of the ten patients, ampicillin concentrations of 10–45 mg/kg faeces were found; concentrations differed among patients and among samples from the same patient. In the patient who retained the ampicillin-sensitive strain of *E. coli* during treatment, no ampicillin could be detected in any sample. Unfortunately, at the time of this investigation only one possible explanation for this finding was considered: that the patient might not have taken her medication; this was, however denied by the attending nurses. We did not consider the possibility that the patient's microflora might have degraded the ampicillin after ingestion. No assay was performed to determine the presence of β-lactamase in the faeces of the patients who participated in the study.

Antibiotic-degrading enzymes

Recently we have demonstrated antibiotic-degrading enzymes in faecal suspensions from 12 of 13 healthy volunteers, who had not received antibiotics recently. In particular, ampicillin was rapidly degraded by five of the specimens. Similar results have been described by Hazenberg *et al.* (1983).

Discussion

The investigations in animals and the observations in humans suggested to us that broad spectrum antibiotics, active against the anaerobic flora of the intestinal tract, decrease colonization resistance. Restraint in the administration of these agents is needed. Other influences, such as induction therapy for malignancy, or virus infections, which affect the mucous membrane or the secretions into the gut (saliva, mucus, etc.) may also reduce the colonization resistance.

The effect of antibiotics can be prevented by the presence of resistant bacteria that produce enzymes able to inactivate or destroy the antibiotics. Unfortunately we did not investigate the occurrence of antibiotic-degrading enzymes at the time of our observations on the spread of resistant Gram-negative bacilli from patient to patient. It is possible that, with the use of these antibiotics in the interim, bacteria with the ability to produce enzymes that inactivate the antibiotic are now occurring more frequently in the population. One might speculate that this could be a factor accounting for the relative decline in infections by Gram-negative bacilli in the immunocompromised patient (Hennemann, 1985).

Recently attention has been directed to the oropharynx, where this antibiotic-degrading effect is less likely to occur. A decrease in the colonization resistance of the oropharynx has been described in patients undergoing chemotherapy for leukaemia (Fainstein *et al.*, 1981) or in intensive care units. In the future, it will be important to study the effects of antibiotics on the oral flora and to determine any influence of bacterial enzymes in this very different ecological situation.

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