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Oral dose and faecal concentration of antibiotics during antibiotic decontamination in mice and in a patient

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SUMMARY

In both mice and one patient, a similar correlation was found between the oral dose of a 'nonabsorbable' aminoglycoside antibiotic selected for antibiotic decontamination of the digestive tract and the resultant faecal concentration. In mice, absorption of the orally-given antibiotics could only be demonstrated in animals treated with extremely high doses of 1440 mg. per kg. body weight per day. Evidence of absorption of gentamycin was found to occur in a patient after doses as low as 70 mg. per kg. per day.

INTRODUCTION

In man as well as in animals bacterial infections during and as a consequence of a severe decrease in defence capacity are more often prevented by previous antibiotic decontamination of the digestive tract. The latter is decontaminated because it appears to be the main source of bacterial invasion in such individuals. The antibiotics used for decontamination are administered orally and are not or only minimally absorbed from the digestive tract. The antibiotics employed for decontamination in man are sometimes selected on the basis of a sensitivity test (Gunn & Gould, 1965) but are often selected without prior information concerning the sensitivity pattern of the patient's microflora and are supplied in empirical dosages (Schwartz & Perry, 1966; Bodey, Loftis & Bowen, 1968; Preisler, Goldstein & Henderson, 1970). In our laboratory, decontamination of animals is always preceded by a sensitivity test on their faecal microflora (van de Waaij, de Vries & Lekkerkerk, 1970). In this way, an optimum mixture of antibiotics is selected in a first phase of the sensitivity test. In a second phase, the minimum bactericidal concentration of the selected antibiotics for the intestinal flora to be eliminated is determined. It is still a matter of trial and error to determine at which dose the antibiotics must be supplied in order to achieve at least the minimum bactericidal concentration in the intestines. Therefore, the relation between various oral dosages of neomycin and the resulting faecal concentration was investigated in mice while similar observations were made in a patient treated with gentamycin. We only once had a patient with an extremely resistant microflora so that oral treatment had to be started with quite high doses. Dose reduction could for the same reason

only be performed stepwise so that the concentrations resulting from three doses could be studied.

The relation between the faecal concentration and the concentration of the antibiotics in the serum was also determined both in the patient and in mice.

MATERIAL AND METHODS

Mice

The animals were conventional female ND2 mice of 12 weeks of age with an average body weight of 35 grams. They were housed in separate cages with individual drinking water bottles for the estimation of the daily water (antibiotic) intake. Autoclaved pelleted food (Hope Farms) was supplied *ad libitum*. The animals were maintained under strict isolation conditions in autoclaved cages inside a 2% peracetic acid-sterilized laminar cross-flow bench (van der Waaij & Andreas, 1971).

Bacteriological culturing

Fresh faeces were taken daily for culturing in Brain Heart Infusion broth and in Brewer's semi-solid thioglycolate medium at 37° C. The cultures were incubated for 1 week before they were determined negative when no evidence of growth was seen. If growth was observed, the cultures were subcultured for pure culturing and subsequent identification.

Patient

A 12-year-old boy, weight 30 kg., was decontaminated and maintained in a down-flow isolator (van der Waaij, Vossen & Korthals Altes, 1973) under strict bacteriological isolation conditions. The child was suffering from severe Pemphigus vulgaris and frequent periods of bacteraemia due to *Staphylococcus aureus*. This strain was resistant to all antibiotics except gentamycin after $1\frac{1}{2}$ years in hospital, which justified our unconventional approach to treatment. To minimize bacterial infection of the skin lesions, antibiotic decontamination was done as well as skin disinfection by twice daily bathing in 0·5% solution of chlorhexidine in water. Faeces and blood were sampled daily for culturing and the estimation of the antibiotic concentration. Blood was also investigated biochemically to monitor liver and kidney function. The biochemical tests involved were: serum creatinin concentration, and the S.G.O.T., S.G.P.T. and alkaline phosphatase tests.

Antibiotic treatment and sampling

On the basis of a sensitivity test (van der Waaij *et al.* 1970), a combination of neomycin and bacitracin was found to be most suitable for decontamination of the mice. According to the results of a sensitivity test of the same type, the patient was treated with a combination of gentamycin and bacitracin. The mice were treated in groups of 28 animals; each group received 1·25, 2·5, 5·0, or 10·0 mg. of neomycin per ml. of water in combination with 2·5 mg. bacitracin and 100 μg. pimaricin per ml. According to the daily water intake this equalled, respectively, 180, 360, 720, and 1440 mg. neomycin/kg. body weight per day.

Immediately after daily sampling in eight mice per dose, the faeces were suspended 1/10 in tryptose phosphate (T.P.) broth (DIFCO) for the determination of the neomycin concentration. The animals were killed in groups of four on days 2, 3, 4, 5, 7 and 12 of treatment. For the determination of the antibiotic concentrations in the serum, blood was sampled immediately after killing.

The patient was given 2 g. of gentamycin every 6 hr. (about 280 mg./kg./day) for 4 successive days. Thereafter, the dose was reduced to 1 g. four times a day (about 140 mg./kg./day) again for 4 days. Treatment of the patient was then continued with 0·5 g. gentamycin 4 times a day (about 70 mg./kg./day) for 15 days. Bacitracin was given in combination with gentamycin from the beginning of treatment in a dose of 1 g. and nystatin in a dose of 500,000 i.u. during the first 8 days; during the second treatment period of 15 days, these doses were, respectively, 0·5 g. and 500,000 i.u. administered four times a day.

Faecal samples from the patient were also suspended 1/10 in T.P.-broth for the determination of the gentamycin concentration.

Antibiotic concentration assay

The concentration of neomycin and gentamycin in the faeces and the serum was determined by the microcup dilution method described by Goss & Cimyotti (1968). This test gave good reproducible results after it had been standardized and reference curves were determined (W. D. H. Hendriks *et al.*, to be published). The test was performed with a strain of *Escherichia coli* which was sensitive to 1 μg./ml. gentamycin and to 3 μg./ml. neomycin. A concentration of 10^4 *E. coli* cells per ml. of T.P.-broth, with tetrazolium tetrachloride (T.T.C.) as indicator, was used for the test. This assay was not applicable to bacitracin, since we failed to isolate a streptococcus strain with an adequate sensitivity for bacitracin which reduced the medium sufficiently for a change in the T.T.C. Other methods for determining the bacitracin concentration in the faeces also failed to give sufficient accuracy.

RESULTS

Mice

The relation between the oral dose of neomycin, and the faecal concentration is presented in Figs. 1 and 2. It took 7–10 days before the final concentration was reached in the colon as evidenced by the concentration in the faeces (Fig. 1).

The oral intake of the antibiotic mixtures was in the normal range of 4–5 ml. per day after it had been lower in the first 3–4 days of treatment in the mice treated with neomycin doses up to 720 mg./kg./day. The dose of 1440 mg./kg./day gave more difficulties in this respect. Some animals refused to take it in sufficient amounts for several days, so that they had to be replaced by others.

Apart from the mice treated with 170 mg. neomycin the faecal cultures remained sterile from the second day. However, the majority of the mice had sterile faeces from the third day. The rest of the animals gave sterile cultures on day four. It was also interesting that the higher doses of 720 and 1440 mg. per kg. body weight per day resulted in progressively higher faecal concentrations than did the lower doses (Fig. 1). In the group treated with 180 mg./kg./day, two animals that were

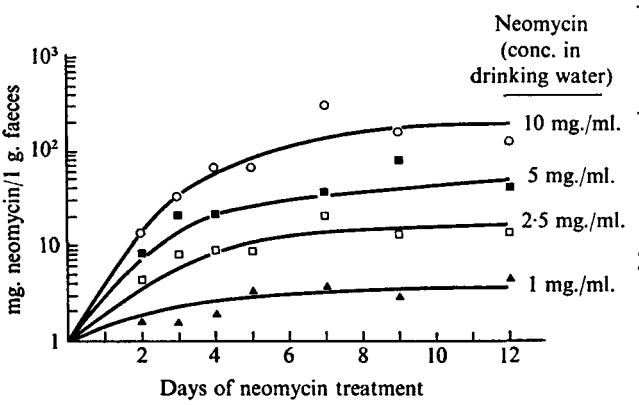

Fig. 1

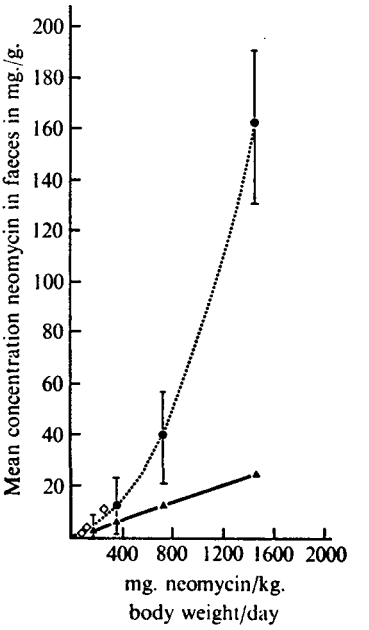

Fig. 2

Fig. 1. Mean concentration of neomycin in the faeces determined for 12 days after the onset of treatment in groups of eight mice treated with different oral doses.

Fig. 2. Mean concentration and S.D. of neomycin in the faeces of mice treated for 12 days as well as the average gentamycin concentration in the faeces of the patient in relation to the oral doses given. ●- - - -●, Mice; ◇- - - -◇, patient (gentamycin concentration in mg./g. of faeces); ▲——▲, calculated concentration of neomycin: $\frac{\text{daily dose per mouse}}{\text{mean weight intestinal contents}}$.

killed on day 5 had persistent positive cultures for *Klebsiella aerogenes*. The group receiving this dose for 7 days had sterile cultures from day 4 of treatment on, while in the animals killed at day 12, one animal remained positive for klebsiella for 1 more day.

The serum concentration of the mice treated with the high oral doses of 1440 mg./kg./day varied between 5 and 9 μg./ml. when they were killed on days 7 and 12. The serum concentration of neomycin in all animals treated with the lower doses was lower than 3 μg./ml. No neomycin could be detected in serum of these mice with the *E. coli* strain used for the serum concentration assay.

The food intake was noticeably reduced in the mice treated with the highest dose of neomycin, while it appeared normal in the others. However, this was not specifically investigated.

Patient

Regardless of the quite high dosing of gentamycin in the first 4 days of treatment, low serum concentrations were found during that period (Fig. 3). Only on day 7 was a peak concentration of 8 μg./ml. seen. Thereafter, the serum gentamycin concentration declined during the period that the patient was treated with 4 g. a

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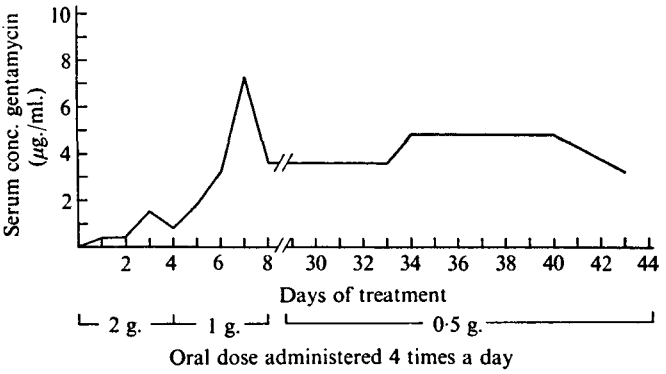

Fig. 3. Serum concentration of gentamycin in the patient during treatment with three different doses.

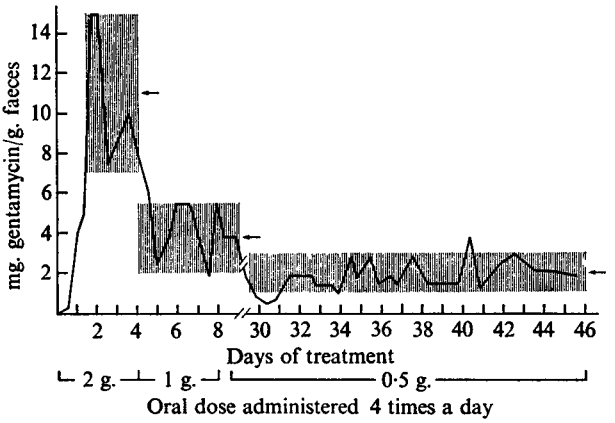

Fig. 4. Gentamycin concentration in the faeces of the patient during treatment with three different doses (the arrows indicate the mean concentrations plotted in Fig. 2).

day to a rather constant value of 4–5 μg./ml. Kidney and liver function tests, performed twice weekly, did not reveal abnormalities during decontamination. The relation between the daily dose of gentamycin per kg. body weight and the average faecal concentration in the patient seems similar to that found in the mice (Fig. 2).

The effect of decontamination of the digestive tract in combination with twice daily bathing in a 0·5 % solution of chlorhexidine (I.C.I.) in water was dramatic. The skin lesions existing at the beginning of the treatment healed in the course of 2 weeks, while there was no formation of new blisters on the skin. The stool cultures were sterile after 48 hr. of treatment and remained so during treatment.

DISCUSSION

The present study indicates that the oral administration of non-absorbable antibiotics for decontamination of the digestive tract of the mouse can safely be started with doses of neomycin as high as 720 mg. per kg. body weight per day (5 mg./ml. of drinking water) if indicated by the sensitivity test. However, the use

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of much higher doses seems to be contra-indicated and it is obvious that if treatment is started with the higher doses, the dose should be reduced as soon as possible. As a rule, we decrease the neomycin concentration in the drinking water usually to 2·5 mg./ml. or lower after one week of treatment without recurrence of bacterial growth.

The quite long interval between the beginning of treatment and the establishment of a steady concentration in the faeces in the mouse (Fig. 1) is probably the result of the reduced water intake during the first 3 days of treatment. The enlargement of the caecum, however, which was three-fold in the first week, could also contribute to this effect. In other words, a relatively small dose is suspended in a gradually increasing volume.

The gentamycin concentration in the faeces of the patient reached a plateau on the second day of treatment. A similar relatively rapid build up of another orally administered aminoglycoside antibiotic (kanamycin) in the faeces of man was reported by Cohn (1958). In 12 surgical patients selected on the basis of being in a good general condition and having no lesions in the large bowel, kanamycin reached a steady maximum concentration after one day of treatment. This rapid establishment of the final antibiotic concentration in the faeces may be due to the fact that Cohn's patients were given enemas before treatment, while our patient was not. They were treated with 1 gram every 6 hr. The average faecal concentration was 5 mg./gram. Assuming that Cohn treated adults, this is in agreement with our observations in the patient and the mice.

Woodward, Herrmann & Shadomy (1964) treated conventional rats with a dose of neomycin varying between 160 and 200 mg. per kg. body weight in the diet. It took only 3 days in the rats before the faecal neomycin concentration had reached its final concentration range of 4–4·5 mg. per gram. The difference in build up of the concentration of the antibiotic in the faeces between mice and rats can probably be ascribed to the fact that the rats apparently took the dose of antibiotics from the first day, while the mice had a reduced intake in the first three days. The bitter taste of bacitracin may have contributed to the initial reduced intake.

Another important observation in our study was that the antibiotic concentration in the large bowel following the higher dosages was much higher than was to be expected on the basis of thorough mixing of the daily dose with the intestinal contents (Fig. 2, calculated curve). A daily dose of 720 mg. of neomycin/kg. body weight per day, for example, would then result in an intestinal concentration of approximately 12·5 mg/g. (which is about 25 % of what we found after 12 days of treatment) when the intestinal (colon) contents with an average weight of 2 gram are replaced daily. The exponential rise of the neomycin concentration in the faeces is difficult to explain. Since it has also been observed in monkeys by Hendriks *et al.* (1974), we assume that the antibiotics are inactivated by one or more substances in the intestinal (faecal) contents. It is, for example, known that the antibacterial activity of oligosaccharide antibiotics is decreased by phosphate, citrate, chloride, aluminium and magnesium as well as by other chemicals like cysteine, glutathione, glucose, ascorbic acid, hydroxylamine, semicarbazide, peptone,

and complexes like nucleic acid, thymonucleoproteins etc. (Heilmeyer, 1965). The cephalosporin antibiotics on the other hand are inactivated by protein substances. The consequence of our findings for antibiotic decontamination is that a considerable part of low oral doses will be inactivated. This means that doubling the dose will result in more than double the concentration in the intestinal contents. Also the chemical composition of the diet may be of influence; the more inhibiting substances the greater the loss of antibacterial activity.

The pharmacodynamics of orally supplied nonabsorbable antibiotics are presently under investigation by W. D. H. Hendriks *et al.* in our laboratory. This study is performed in monkeys in order to obtain more detailed information. The monkey was selected since it may be a better model for man than the mouse.

An investigation of this kind can only be performed systemically in experimental animals owing to the fact that administration of high doses of toxic (after absorption) antibiotics which was done in the present study, is not permitted in man without a strict indication. In addition to the pharmacodynamics of oral dosing of 'nonabsorbable' antibiotics, several parameters such as possible toxic effects on remote organs including the liver, kidneys, and bone marrow will be determined.

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