Oral Neomycin Dosage Schedules for Suppression of Ammonia Production by Bowel Flora

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To better define a minimal but optimal dose of oral neomycin to suppress ammonia production by bowel flora, several dosage regimens were examined in normal healthy volunteers. Fecal urease activity was quantitatively determined and was used as an indirect measure of intrinsic ammonia production by bowel flora. Large doses of neomycin were found to exert inhibition of fecal urease for many days. There was considerable variation in enzymatic activity among subjects even after adjustments were made for protein content of the stool. Depending on the dose, there was a 1- to 3-day lag in neomycin effect on stool urease activity and several days of continued effect. The most effective regimen of those studied was a loading dose of 6 g of neomycin given in three divided doses on day 1, followed by 1 g twice daily.

Since the discovery of neomycin in 1949 by Waksman and Lechevalier (16), its use has been limited to either topical or oral preparations due to its toxicity. Although gastrointestinal absorption of orally administered neomycin is reported to be as low as 0.58% on average (2), complications following the oral use of neomycin have been reported. These side effects include severe hearing loss (1, 7, 9), renal toxicity (1), and malabsorption syndrome (6, 8). Deafness, associated with topical neomycin irrigation (10) and intrapleural irrigation (15), also has been demonstrated. This antibiotic, therefore, must be given with care especially when there is preexisting renal failure.

Oral use of neomycin for treatment of hepatic coma was first described by Dawson and associates in 1956 (5), and it has been the most popular means of treatment for the condition ever since. Daily doses recommended for hepatic precama or coma have ranged from 2 to 10 g (1, 4, 5, 7, 9). The blood levels of neomycin in patients with normal renal function are usually low or not detectable, but may rise to toxic levels in the presence of renal failure (11, 13).

Oral neomycin is not always effective for hepatic encephalopathy since some patients may be colonized with neomycin-resistant, urea-splitting microorganisms, such as Klebsiella or Proteus (12). Recently, Conn and associates compared lactulose and neomycin in a randomized double-blind study of chronic portal systemic encephalopathy. Lactulose and neomycin demonstrated almost equal efficacy (4). Lactulose therefore can be a useful drug to replace neomycin. Nevertheless, an adequate study showing a quantitative determination of a minimal but effective oral neomycin dose to suppress the ammonia production by bowel flora is lacking.

MATERIALS AND METHODS

Chemicals. Urea, potassium phosphates (monobasic and dibasic), and sodium hydroxide were purchased from Fisher Scientific Co. Ammonium chloride standard was obtained from Orion Chemical Co. Neomycin sulfate, 0.5-g tablets (The Upjohn Co.), were obtained from the hospital pharmacy stocks.

Subject selection. Normal healthy adult volunteers of either sex who had not received any antibiotics at least for 2 weeks before the initiation of study were enrolled after obtaining a signed informed consent. There were no dietary restrictions. In most studies, stool specimens were collected starting 1 week before the initiation of neomycin and continued throughout the course of 1-week therapy and approximately 4 weeks after the treatment.

Urease assay. A 1-g fecal specimen, freshly collected, was suspended in 0.04 M phosphate buffer, pH 7.4, to make a 1% suspension, and homogenized in a Sorvall mixer. A volume of the homogenate (0.25 ml) was added to an assay mixture containing 0.25 M urea and 0.04 M phosphate buffer to give a final volume of 10 ml. The mixture was incubated at 37°C for 30 min. Preliminary studies revealed that ammonia was produced linearly during this period. The reaction was stopped by adding 0.1 ml of 10 M NaOH. The ammonia produced was measured by an Orion analyzer ammonia electrode (model 95–10) as described by Byrne and Power (3). Ammonia standard solutions were made...
from a stock solution of 0.1 M ammonium chloride (Orion Chemical) in a range of $10^{-3}$ to $10^{-6}$ M and similarly treated as the test mixture. Specific activity was expressed as moles of ammonia formed per gram of protein per hour. Protein was determined by the method of Lowry et al. (14).

RESULTS

In vitro effect of neomycin on fecal urease activity. Fresh stool specimens were obtained from four healthy subjects and incubated in the presence of urea (as described above) for 4 h in the presence or absence of neomycin. As shown in Table 1, the antibiotic did not inhibit enzymatic activity.

In vivo effect of 1-day treatment with neomycin. Nine healthy volunteers were given 6 g of neomycin orally in four divided doses in 24 h. A significant fall in stool urease activity was noted only on day 3 after receiving the drug (Fig. 1). Neomycin was effective with a significant decrease from baseline up to day 8 after the dose. Two subjects continued to have low levels of urease activity in their stools for 30 and 50 days, respectively. In view of these findings, further studies were conducted to determine the optimal dose of neomycin.

Experimental dose schedules. Groups of four to five subjects were given the following dosage schedule: 1 g twice daily for 7 days (Fig. 2); 1 g three times daily for 7 days (Fig. 3); 2 g three times on day 1 and 1 g twice daily for 6 more days (Fig. 4). At least 1 month of rest was allowed for subjects if they reentered the study.

Considerable variation was noted among the subjects in each experiment. In general, however, there tended to be a delay of 2 to 3 days before urease activity fell in all subjects. Also, depending on the dose, urease activity remained diminished for several days after stopping treatment. The most consistent effect on urease activity was observed in subjects given the large loading dose. In all but one of the five subjects in this group, urease activity was not detectable or was at very low levels from day 3 to day 7 of treatment.

DISCUSSION

Direct measurement of stool urease activity provides a laboratory correlate of the in vivo efficacy of various agents used to treat or prevent hepatic coma by blocking this enzyme. As might be predicted, neomycin does not exert an im-
mediate effect on enzyme. Rather, by preventing the multiplication of sensitive bacteria, the antibiotic interferes with synthesis of new enzyme. Thus, the efficacy of the drug must depend on evacuation of organisms containing preformed enzyme. This is the most likely explanation for the delayed effect on urease activity in the stools of subjects given the drug and the tendency for the effect to persist for several days after it is stopped. Neomycin given orally or by enema should not be expected to exert an immediate effect in patients in hepatic coma.

The considerable variability of response by subjects to administration of neomycin is not unexpected since bowel transit times and mixing of the drug with bowel contents are also highly variable. For this reason, it is difficult to establish an optimal dose. In the current studies, a loading dose of 6 g given over 24 h followed by a small dose of 1 g twice daily appeared to exert the most marked effect on fecal urease activity. The low maintenance dose may be important in preventing toxic side effects and obtaining patient compliance.

The most effective immediate measures for treatment of patients with impending hepatic coma would seem to be those which thoroughly evacuate the fecal mass. Local instillation of urease inhibitors into the evacuated colon would theoretically be of additional help by blocking residual enzyme in the colonic lining. Wolpert et al. (17) demonstrated that cleansing by colonic perfusion rapidly reduced ammonia concentration in the colon. This effect was augmented by oral neomycin given for 3 days (8 g per day) preceding colonic perfusion. Addition of acetohydroxamic acid, a potent urease inhibitor to the perfusate, had no effect on colonic ammonia concentration and outputs. However, administration of acetohydroxamic acid by mouth was followed by a significant decrease in ammonia output. Thus, the efficacy of local application of urease inhibitors into the colon remains to be proven.

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LITERATURE CITED

Fig. 3. Ammonia production by stools of subjects given 1 g of neomycin orally three times daily.

Fig. 4. Ammonia production by stools of subjects given 2 g of neomycin orally three times daily on day 1, followed by 1 twice daily.
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