Effect of Orally and Parenterally Administered Neomycin on Plasma Lipids of Human Subjects

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THE apparent relationship between circulating cholesterol and atherosclerosis has stimulated interest in the study of factors influencing blood cholesterol levels. In this regard a number of dietary factors have been studied, and the results of these studies have been the subject of recent reviews.[1–3]

It has been reported that neomycin therapy depresses serum cholesterol levels[4] and increases the excretion of bile acids[5] in human subjects. A recent report from this laboratory[6] has corroborated and extended these observations by showing that treatment with a mixture of polymyxin B and bacitracin also reduced serum cholesterol values and caused increased excretion of bile acids and other fecal sterols. However, no conclusive evidence has been presented as to the mechanism of action of these antibiotics. It has been proposed[6] that the depression of serum cholesterol levels resulting from orally administered neomycin may be due to one or more of the following: (1) a systemic effect; (2) a malabsorption state; (3) a change in the intestinal bacterial flora; or (4) a combination of these factors.

Steiner et al.[7] recently presented data demonstrating that neomycin administered intramuscularly failed to influence the serum cholesterol levels of patients with atherosclerosis. These investigators thought that orally administered neomycin depressed serum cholesterol levels by "a local effect in the gastrointestinal tract or its content." This conclusion is supported by the data to be presented in this report which suggest that neomycin therapy depresses plasma cholesterol levels by directly influencing the absorption of lipids and sterols.

EXPERIMENTAL

The subjects employed for this study were six healthy, young adult male volunteers ranging in age from twenty to twenty-four years (mean age twenty-two and a half years). The average initial weight of the subjects was 76.1 kg. (67.4 to 89.2 kg.). The subjects were housed in a metabolic ward and were allowed out of the ward for supervised activity.

The diet employed consisted of four menus of equivalent composition which were rotated on a daily basis throughout the study. Each subject received a weighed serving which was consumed completely. By analysis the diets supplied 161 gm. of protein, 123 gm. of fat, 23 gm. of ash and 3,452 calories per day. Therefore, the fat in this diet, calculated to be derived in equal amounts from animal and plant sources, supplied 32 per cent of the calories. Approximately 35 per cent of the fatty acids were saturated; the remainder were mono- and polyunsaturated. An adequate quantity of foods was purchased initially to last for the entire study to lessen the possibility of dietary variations during the course of the study. In addition to the diet, the subjects were allowed water, coffee and a noncaloric carbonated beverage *ad libitum*.

The only variable throughout the study was the administration of neomycin. The study lasted for

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The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

TABLE I

Influence of Experimental Treatment on Plasma Lipids, Sterol and Bile Acid Excretion, Stool Coliform Counts and Body Weights of Human Subjects

Neomycin Administration		Plasma Liquids (mg./100 ml.)			Daily Fecal Excretion		Coliform Organisms in Stool (organisms/gm.)	Final Body Weight (kg.)	Apparent Lipid Digestibility* (%)
Daily Dose	Method	Cholesterol	Lipid Phosphorus ×25	Glycerides	Digitonin Precipitable Sterols (gm.)	Bile Acids (mg.)			
				Period I					
None	···	227 ± 15	232 ± 15	85 ± 35	1.55 ± 0.14	764 ± 163	75 × 10^5 (1)	76.6 ± 7.4	97.84 ± 0.44
				Period II					
200 mg.	Parenteral	206 ± 16	205 ± 20	90 ± 37	1.31 ± 0.30	1,939 ± 351	381 × 10^7 (0)	77.0 ± 7.3	98.30 ± 0.28
				Period III					
None	···	200 ± 12	232 ± 24	73 ± 18	1.30 ± 0.44	765 ± 292	79 × 10^4 (1)	77.6 ± 6.9	98.23 ± 0.41
				Period IV					
200 mg.	Oral	191 ± 15	205 ± 12	76 ± 27	1.55 ± 0.24	1,130 ± 311	300 × 10^5 (4)	77.7 ± 6.8	97.93 ± 0.24
				Period V					
2 gm.	Oral	150 ± 16	165 ± 21	72 ± 32	1.99 ± 0.41	1,680 ± 467	245 × 10^5 (4)	77.7 ± 6.6	95.68 ± 0.24
				Period VI					
None	···	177 ± 19	204 ± 21	85 ± 22	1.41 ± 0.30	1,936 ± 643	91 × 10^6 (0)	77.7 ± 6.9	97.69 ± 0.31

NOTE: Values presented are mean values for six subjects ± standard deviation. Values in parentheses represent the number of individuals having negative coliform counts.

* Apparent digestibility = [(lipid intake − fecal lipid excretion)/lipid intake] × 100.

eighty-four days and consisted of six experimental periods. The first was a twenty day adjustment period; during the second (twelve days) each subject was given 200 mg. per day of neomycin intramuscularly (50 mg. every six hours). The third (twenty days) served as a control period; during the fourth 200 mg. per day of neomycin was administered orally for twelve days (50 mg. every six hours). The dose of neomycin was increased to 2 gm. per day (500 mg. every six hours) for twelve days in period V, and period VI was an eight day recovery period.

Every fourth day throughout the study, fasting blood samples were drawn (5 ml. of heparinized blood) from each subject by venipuncture. Each of these samples was analyzed for cholesterol and lipid phosphorus content, and alternate samples were analyzed for total glycerides. A chloroform:methanol extract of plasma was analyzed for amount of cholesterol and phospholipids. The method of Searcy and Bergquist[8] was employed for determination of total cholesterol and the procedure of Fiske and Subbarow[9] for lipid phosphorus. Total glycerides were determined by the method of Van Handel and Zilversmit[10] as modified by Leveille et al.[11]

Continuous four day pooled stool collections were carried out with the aid of charcoal markers. Each pooled sample was homogenized with water and made up to a known volume. Aliquots of the stool samples were extracted with chloroform:methanol (2:1) and the extract analyzed for digitonin precipitable sterols.[12] A second aliquot of the homogenized stool sample was dried at 60°c. under vacuum; the dried stool was extracted in a continuous extractor for four hours with 6 vol. of ethanol; the ethanol extract was then analyzed for content of tri- and dihydroxy bile acids using the

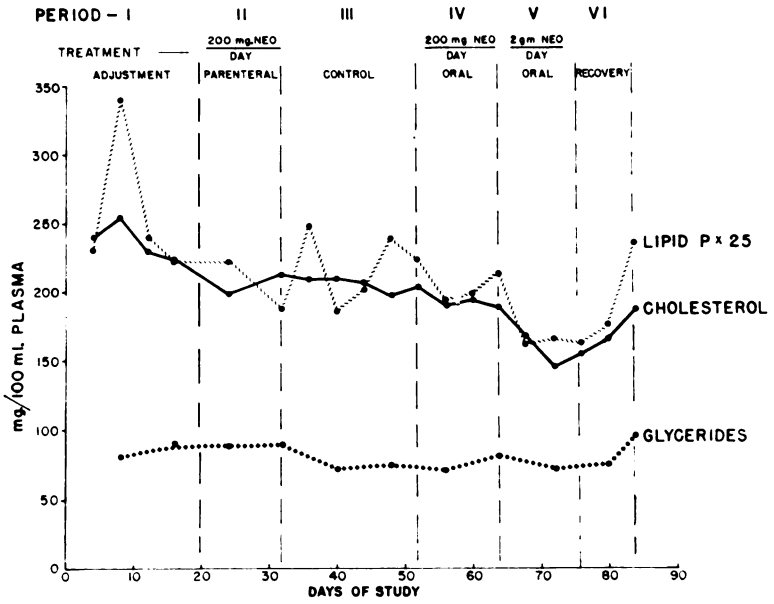

FIG. 1. Effect of dose and method of administration of neomycin on plasma lipid levels of human subjects. Each point represents the mean value for six subjects.

procedure outlined by Haust and Beveridge.[13] A third aliquot of the stool samples was analyzed for total lipids by an acid hydrolysis method.[14]

Once during each four day stool collection period, a 1 gm. aliquot of fresh homogenized stool was used for microbiologic study. The samples were diluted serially with sterile saline solution; aliquots were introduced into tubes of a differential media for coliform organisms (violet red bile agar) and pour plates made after mixing. Coliform colonies were counted after incubation at 36°c. for twenty-four and forty-eight hours.

The data on blood lipids were evaluated statistically by comparing the mean of the last two values obtained during each period using the paired t test. The values for stool lipids were also statistically evaluated by the paired t test method using a mean of the last three values for each period.

RESULTS

The data presented in Table I demonstrate the influence of neomycin therapy on plasma lipid levels, stool sterol content and fecal coliform counts. Also presented in Table I are the changes in body weight noted between periods. Figure 1 shows the changes in plasma lipids observed throughout the study.

Plasma cholesterol values decreased significantly during the adjustment period (period I). Following the adjustment period, the subjects were given 200 mg. per day of neomycin parenterally (50 mg. every six hours); this treatment, however, did not influence the plasma cholesterol level. The difference between the value obtained for plasma cholesterol in period II and the control period (period III) was not statistically significant. In period IV (200 mg. per day of neomycin given orally), the plasma cholesterol level was not significantly different from that observed during period III. It is of importance to note that four of the six subjects given 200 mg. per day of neomycin orally had stools which were negative for coliform organisms. In period V the subjects received 2.0 gm. per day of neomycin orally, and again only four of the six subjects demonstrated negative stools for coliform organisms. It is also of significance to note that the stools of the same four subjects were again negative in periods IV and V. As can be seen from Table I, the plasma cholesterol level did fall significantly during period V when compared to the values in periods III or IV

($P < 0.001$). During period VI, the blood cholesterol level was still significantly lower than the control value obtained during period III ($P < 0.025$) but was significantly higher than that of period V ($P < 0.001$). The changes in plasma cholesterol were observed in all subjects; the values for period V (2 gm. neomycin per day given orally) were 25 per cent (−12 to −33 per cent) lower than that for period III (control period) and 21 per cent (17 to 25 per cent) lower than those for period IV (200 mg. neomycin per day orally). The cholesterol level of all six subjects rose by 18 per cent (12 to 28 per cent) during the recovery period (period VI) as compared to the level in period V (2 gm. neomycin per day given orally).

The plasma lipid phosphorus level generally paralleled the cholesterol level except that there was much more variability among subjects as evidenced by the greater standard deviation for this measurement. The parameters of sterol excretion analyzed for in the stool, bile acids and digitonin precipitable sterols tend to generally follow the same trend. There was usually an increased excretion accompanying the decrease in plasma cholesterol levels during period V when compared to period III. Lipid digestibility, as can be seen from the values presented in Table I, decreased significantly during period V (2.0 gm. neomycin per day given orally) as compared to period III, the control period ($P < 0.001$). During the recovery period (period VI) lipid digestibility was not significantly different than during the control period (period III) but was significantly higher than during period V (2.0 gm. neomycin per day given orally) ($P < 0.005$).

It is also noteworthy to examine the elevation in coliform count when neomycin was administered parenterally. Plasma glycerides remained essentially unchanged throughout the study (Table I and Fig. 1). However, as with phospholipid measurements, there was a considerable variation among subjects as evidenced by the magnitude of the standard deviation of the glyceride values (Table I).

COMMENTS

As stated previously, the study described herein was designed in an attempt to elucidate the mechanism(s) responsible for the hypocholesteremic effect of neomycin therapy on human subjects.

The fact that parenterally administered neomycin was ineffective in altering plasma cholesterol levels suggests that the small amount of orally administered neomycin which is normally absorbed[15] does not depress plasma cholesterol values through a systemic effect. These findings are in accord with those of Steiner et al.[7] The lack of effect on plasma cholesterol levels of 200 mg. per day of orally administered neomycin (an adequate amount of the antibiotic to suppress coliform counts in four of the six subjects) does not support the concept that the reduction in the cholesterol level is due to a disturbance of the intestinal flora. That the hypocholesteremic effect of neomycin is not mediated through its influence on the intestinal flora is also supported by the decreased plasma cholesterol levels noted in all six subjects, including two demonstrating positive coliform counts, given 2.0 gm. neomycin per day. The possibility remains that bacteria other than coliform organisms might be responsible for the plasma lipid changes observed and that these organisms are not influenced by neomycin in the same manner as are coliform organisms.

The values obtained for bile acid excretion show considerable variability, not only between periods but between subjects as evidenced by the magnitude of the standard deviation for the values presented (Table I). Thus, difficulties inherent to the procedure itself and the fact that the method employed measures only di- and trihydroxy cholanic acids, make this measurement less significant in interpreting the results of the present study.

The influence of orally administered neomycin on lipid digestibility is of considerable importance in the interpretation of the results obtained. As shown in Table I, the oral administration of 2 gm. of neomycin per day depressed lipid digestibility significantly, an observation which is in excellent agreement with the results of a previous study from this laboratory.[6] Recognizing that digitonin precipitable sterols are not necessarily an accurate reflection of total sterol excretion, the fact that this parameter of sterol excretion did signifi-

cantly increase with the oral administration of 2 gm. per day of neomycin is strongly indicative of an effect of neomycin on sterol absorption.

That oral neomycin administration induces a malabsorption syndrome has been conclusively shown;[16,17] however, the mechanism through which absorption is impaired is not clear. Jacobson et al.[16] have postulated that the antibiotic might exert an inflammatory effect on the intestinal mucosa, thereby impairing mucosal permeability to foodstuffs. In a second report, these authors present evidence strongly supporting this concept; in patients treated with neomycin changes in the intestinal mucosa qualitatively similar to those seen in idiopathic steatorrhea developed.[18] The data presented in this report would fit this hypothesis; however, further study is needed to demonstrate clearly how treatment with neomycin influences intestinal absorption and plasma cholesterol levels.

Interpretation of these data leads to the conclusion that neomycin probably depresses plasma cholesterol levels in human subjects by interfering with the intestinal absorption of lipids and sterols and possibly the recirculation of bile acids. Further study will be required to demonstrate this conclusively and to establish whether this effect of neomycin is merely part of a malabsorption syndrome brought about by the antibiotic. It is plausible that the observed hypocholesteremic effect of neomycin is mediated through the malabsorption syndrome resulting from oral neomycin administration.[16] The data presented show that neomycin does not exert its hypocholesteremic influence systemically.

SUMMARY

Neomycin, when administered intramuscularly to human subjects at the rate of 200 mg. per day, did not influence plasma cholesterol levels. This same dose administered orally also was without influence, although in four of the six subjects fecal coliform counts were suppressed. When the amount of neomycin administered orally was increased to 2.0 gm. per day, the same four subjects had stools which were negative for coliform organisms; however, all six subjects demonstrated a highly significant decrease in plasma cholesterol levels. The 2.0 gm. per day level of neomycin also significantly depressed lipid digestibility.

The data are interpreted to indicate that neomycin therapy lowers plasma cholesterol levels by interfering with normal intestinal absorption of lipids and sterols and possibly the recirculation of bile acids and that the hypocholesteremic effect of neomycin is not the result of a systemic effect.

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