The Effect of Para-Aminobenzoic Acid on the Serum Cholesterol Level in Man

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CURRENT medical thinking implicates cholesterol in the pathogenesis of atherosclerosis. Epidemiologic data suggests that a positive correlation exists between serum cholesterol and athersclerosis. By inference, reduction of the serum cholesterol level may inhibit the development of atherosclerosis, and at the present time several pharmacologic agents are in clinical use for the purpose of reducing the serum cholesterol level in man. The present paper reports on the use of para-aminobenzoic acid for this purpose, and inquires into the means whereby this drug as well as nicotinic acid reduce the serum cholesterol level in man. Para-aminobenzoic acid has been listed in the U. S. Pharmacopeia for a number of years, having been used principally in the treatment of rickettsial infections and a variety of "collagen diseases." Insofar as we could determine, there has been no previous report on the effect of this drug upon serum cholesterol.

Our interest in para-aminobenzoic acid for lowering serum cholesterol levels was stimulated by studies of bile acid pharmacology indicating that nicotinic acid tended to promote conjugation of bile acids with taurine rather than with glycine.[1] The bile acids have been shown to be important oxidation and excretion products of cholesterol. Since atherosclerosis seems to develop less readily in animals conjugating bile acids predominantly with taurine than in those conjugating with glycine,[2] we thought that any agent which tended to make glycine less available for conjugation with bile acids might in some manner promote excretion of cholesterol. Para-aminobenzoic acid was selected because previous reports on its use in human therapeutics had shown that it had no unpleasant side effects or serious toxic action.

METHOD

Serum cholesterol levels were determined by the ferric chloride method employing the modification described by Herrmann.[3] Bile acid determinations were made upon bile obtained from the duodenum by aspiration. Bile was separated upon celite columns into its taurine and glycine conjugated fractions. The stationary phase used here was 70 per cent glacial acetic acid in water. For removal of the glycoconjugates the moving phase was ethylene dichloride:Skellysolve B 4:1, and for removal of the tauroconjugates methanol was used as the moving phase. Total bile acids were determined for each of the conjugation fractions either by titration with 0.01 normal NaOH or alternately by spectrophotometry in 65 per cent sulfuric acid.

During the treatment period, all subjects took para-aminobenzoic acid as the sodium salt at a dosage level of 2.0 gm. four times a day. The drug was administered in capsules, each capsule containing 0.5 gm.

The subjects were hospitalized for a variety of medical conditions at the Indianapolis Veterans' Hospital. Although their hospitalizations were primarily for diagnostic purposes, we do not believe that their day to day condition changed sufficiently to distort the results of this study. During the control period, blood specimens were obtained daily from each subject for four consecutive days for a total of forty individual determinations on the ten subjects studied. During the treatment period,

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TABLE I
Effect of Para-Aminobenzoic Acid on Serum Cholesterol Level

Subject	Cholesterol Level (mg./100 ml.)	
	Control (four days)	Treatment (five days)
B	275.8	249.0
C	220.0	168.2
D	327.3	313.6
G	210.3	211.6
In	164.0	127.6
McI	245.0	213.8
M	401.3	355.4
Ri	179.0	160.0
So	210.0	192.4
W	266.8	268.4
Average	250.0 ± 67.5	226.0 ± 63.9
	p = <0.005 and >0.001	

TABLE II
Effect of Para-Aminobenzoic Acid on Serum Cholesterol Level

Subject	Cholesterol (mg./100 ml.)			
	Control	Treatment	Control	Treatment
	Four Days	*Five Days*	*Three Days*	*Ten Days*
D	327.3 (4)	313.6 (4)	304 (1)	320.0 (8)
M	401.3 (4)	355.4 (4)	454 (1)	317.1 (8)
W	266.8 (4)	268.4 (4)	276 (1)	271.0 (8)
	Four Days	*Ten Days*		
A	264.3 (4)	220.0 (4)	...	...
T	124.8 (4)	133.0 (4)	...	...
	Four Days	*Ten Months*	*One Month*	
Im	232.7 (3)	169.0 (4)	221.5 (2)	...

NOTE: Figures in parentheses represent number of separate determinations during stated period.

which followed immediately, blood specimens were obtained daily for five consecutive days for a total of fifty determinations on the ten subjects.

RESULTS

Results of the therapeutic use of para-aminobenzoic acid, with reference to serum cholesterol levels, are shown in Tables I and II. Data shown in Table II were obtained from subjects studied on a less formally planned basis than those for whom data are presented in Table I. Three subjects (D, M and W) listed in Table II are also limited in Table I. Three subjects (A, T and Im) were ambulatory during the period of study, and blood was drawn on a somewhat irregular basis. In Table II the figures in parentheses represent the number of individual cholesterol determinations made during each of the separate periods of observation. By combining the data in Tables I and II and treating each subject as a separate unit the average control values were 240.3 mg./100 ml. and the treatment values 212.6 mg./100 ml. On this basis, the average composite drop was 11.5 per cent.

During the course of this study, no serious toxicity to para-aminobenzoic acid was noted. During the first few days the patients experienced a mild epigastric discomfort immediately after ingestion of the drug, but this never interfered with medication. Although previous reports have emphasized the gastrointestinal symptoms which follow the administration of para-aminobenzoic acid, we noted no vomiting, diarrhea or other toxic symptoms which would interfere with ingestion of the drug. Daily weights were not recorded. We were not impressed by anorexia as an effect of the drug. We noted no flushing or itching, such as frequently occurs following the administration of nicotinic acid. Para-aminobenzoic acid has been reported to be a hypoglycemic agent, and syncopal attacks have been reported following its administration.[4] We noted no dizziness or syncope in any of our subjects. We did not determine blood glucose levels during our study; and there were no diabetic patients in our series.

The results shown in Table III were obtained from a smaller number of subjects who received benzoic acid as the sodium salt at a dosage level of 8 gm. a day during two consecutive weeks. No decrease in serum cholesterol levels was noted.

COMMENTS

At present the method whereby para-aminobenzoic acid acts to lower serum cholesterol appears to be unclear. The rather wide

TABLE III
Effect of Benzoic Acid on Serum Cholesterol

Subject	Cholesterol (mg./100 ml.)	
	Control (five days)	Treatment (five days)
H	309.0	326.4
St	288.0	274.8
Sh	229.6	225.3
Re	175.0	189.6
Su	152.0	145.0
Average	230.7 ± 61.2	232.2 ± 63.5
	p = n.s.	

TABLE IV
Change in Bile Acid Conjugation in Response to Treatment

Treatment	Control (%)		Treatment (%)	
	Glycine	Taurine	Glycine	Taurine
Nicotinic acid (2 gm.) (5/7 patients with reduced glycine conjugation)	79.8	20.2	63.3	36.7
Para-aminobenzoic acid (8 gm.) (11/11 patients with reduced glycine conjugation)	71.0	29.0	47.2	52.8
Benzoic acid (8 gm.) (6/7 patients with increased glycine conjugation)	65.6	34.4	74.8	25.2

variability in individual responses to administration of the drug and the tendency to greater responses in persons with higher control cholesterol values are to be noted.

As previously mentioned, we undertook the study of para-aminobenzoic acid because we believed that its mode of action might be similar to that of nicotinic acid. Both of these drugs are removed from the body to a great extent by conjugation with glycine, nicotinic acid conjugating to form nicotinuric acid and para-aminobenzoic acid to form para-aminohippuric acid. In contrast, nicotinamide is methylated prior to excretion and does not draw upon the glycine pool. The extensive studies of Haslewood[2] have shown, in general, that in animals which conjugate their bile acids with taurine rather than with glycine, the tendency to develop atherosclerosis is much less than in animals which predominantly conjugate their bile acids with glycine. Young children principally conjugate taurine, whereas adults characteristically do not.[5] Patients with myxedema in particular conjugate bile acids predominantly with glycine.[6] By analogy it might be surmised that an agent which would promote taurine conjugation would affect bile acid metabolism in such a way that cholesterol excretion, or conversion of cholesterol to bile acids, might be aided and atherosclerosis less likely to develop.

On the assumption that the effect exerted by nicotinic acid and para-aminobenzoic acid upon serum cholesterol is mediated by their effect upon bile acid conjugation, benzoic acid, which conjugates with glycine to form hippuric acid, was similarly studied.

The lack of a comparable effect is documented in Table III. As a further means of studying the effects of these drugs, the relative percentages of taurine and glycine conjugated bile acids in duodenal bile were determined in subjects who had received the various pharmacologic agents. These results are summarized in Table IV. In these studies, nicotinic acid was given as a single oral dose of 2 gm. as the acid, while para-aminobenzoic and benzoic acids were given as a single oral dose of 8 gm. each as the sodium salt. Here the tendency for nicotinic and para-aminobenzoic acids to be associated with the increased taurine conjugation is apparent, whereas with benzoic acid no such effect was seen. The rather striking differences between benzoic acid on the one hand, and para-aminobenzoic and nicotinic acid on the other, would at the present time appear to cast some doubt upon the hypothesis of glycine depletion as a significant mediating influence in the lowering of serum cholesterol levels.

With regard to other mechanisms whereby para-aminobenzoic acid and nicotinic acid lower serum cholesterol levels, inhibition of cholesterol synthesis should be considered. The extensive work of Goldstein has been quoted by Kritchevsky.[7] In studying a variety of aromatic acids, Goldstein found that salicylic acid was most effective in inhibiting

hepatic cholesterol synthesis. Benzoic and para-aminobenzoic acids were equally effective; nicotinic acid was not studied.

Friedman and Byers[8] have reported the apparent inhibition of appetite in animals fed therapeutic doses of nicotinic acid, and they have suggested this as the means for its effect upon human serum cholesterol levels. In our own study, as already stated, we were not impressed by anorexia in association with administration of para-aminobenzoic or of benzoic acids, and others have not reported on it following administration of nicotinic acid in man. As previously noted, we did not obtain daily weights of our subjects during the course of our study.

A pure vitamin effect may be considered as explaining the mode of action of nicotinic and para-aminobenzoic acids. In this connection, however, the amounts required for an effect upon serum cholesterol are quite in excess of those required if vitamins are considered to be catalysts which are required only in minute amounts. Similarly, the lack of effect of nicotinamide renders this line of thought difficult to accept. In addition, the rapid response of cholesterol levels to changes in drug administration (most notable in subject M) do not seem to reflect a vitamin effect.

Considering nicotinic and para-aminobenzoic acids as antimetabolites or as antibiotics, a somewhat better case can be made. The conjugated bile acids reaching the lower part of the intestine are hydrolyzed by bacterial action.[9] Their further modification by bacterial action characteristically results in the production of considerable amounts of deoxycholic acid, which in man is not a primary bile acid produced by hepatic oxidation of cholesterol.[10] Deoxycholic acid produced in the intestine recirculates through the enterohepatic circulation, and its presence in the liver may inhibit formation or excretion of other bile acids.[11] In this connection, it may be noted that the administration of neomycin to man has been reported to be associated with lowering of the serum cholesterol levels concomitant with disappearance of deoxycholic acid from the intestine.[12]

An alternate possibility explaining the mode of action of nicotinic and para-aminobenzoic acids might be that these agents are not active at the level of bile acid conjugation but are involved in the metabolism of the sulfur-containing amino acids as they relate to cholesterol metabolism. Mann, Andrus and Stare[13] reported originally on the induction of atherosclerosis in the monkey by sulfur deprivation. The addition of sulfur-containing amino acids to the diet has produced conflicting results depending apparently on the identity of the supplement and the partcular animal species studies. Most recently, Seidel, Nath and Harper[14] have reviewed this subject and have reported on the serum cholesterol lowering effect of various sulfur-containing amino acids in the rat. Methionine was most notably effective in this respect, taurine being much less so. If a similar effect obtains in man, and if nicotinic acid and para-aminobenzoic acid, but not benzoic acid, are active at the methionine level, it would follow that the increased taurine conjugation of bile acids following the administration of these agents is not a primary effect of their administration. Preliminary studies by ourselves, involving the feeding of taurine as a dietary supplement in man, suggest that it is without effect on the serum cholesterol level.

SUMMARY

Serum cholesterol levels in thirteen male subjects were determined during periods of medication with para-aminobenzoic acid at a dosage of 8 gm. daily. The average control value of 240.3 mg./100 ml. fell to 212.6 mg./100 ml. during the period of medication, a fall of 11.5 per cent. This change, while of limited significance, was not seen following administration of benzoic acid in a comparable manner.

In an attempt to determine possible mechanisms responsible for this difference the conjugation of bile acids in bile was studied. Following the administration of para-aminobenzoic acid and also nicotinic acid, there was an increase in the proportion of taurine to glycine conjugated bile acids. An opposite effect followed the administration of benzoic acid.

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