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Research Article

The hypocholesterolemic effect of the hydrophobic surfactant, poloxalene 2930, was studied in the rabbit to determine whether this agent prevents experimentally produced atherosclerosis. Male rabbits were divided into four groups and fed a control diet (group A) or an atherogenic diet (groups B, C, and D) for 10 wk. Diets of groups C and D were supplemented with 0.5 and 1% poloxalene 2930, respectively. Animals in group B developed significantly greater levels of cholesterol in the serum and aorta compared with group A. Addition of poloxalene 2930 to the diets of groups C and D prevented significant elevations in cholesterol concentrations of both serum and aorta compared with group B with values for group D being essentially similar to those observed in group A. Groups C and D also had significant increases of fecal excretion of both neutral fat and neutral steroids as compared with either groups A or B. There were no atherosclerotic lesions of the aortas from group D. Aortas from rabbits in group B had numerous atheromatous plaques while one rabbit each from groups A and C had several very small atheromatous lesions. These results demonstrate that poloxalene 2930 reduces the rise of serum cholesterol in rabbits in response to an atherogenic diet and prevents the development of atherosclerosis. This hypocholesterolemic effect is likely mediated by the […]

Hydrophobic Surfactant Treatment Prevents Atherosclerosis in the Rabbit

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ABSTRACT The hypocholesterolemic effect of the hydrophobic surfactant, poloxalene 2930, was studied in the rabbit to determine whether this agent prevents experimentally produced atherosclerosis. Male rabbits were divided into four groups and fed a control diet (group A) or an atherogenic diet (groups B, C, and D) for 10 wk. Diets of groups C and D were supplemented with 0.5 and 1% poloxalene 2930, respectively. Animals in group B developed significantly greater levels of cholesterol in the serum and aorta compared with group A. Addition of poloxalene 2930 to the diets of groups C and D prevented significant elevations in cholesterol concentrations of both serum and aorta compared with group B with values for group D being essentially similar to those observed in group A. Groups C and D also had significant increases of fecal excretion of both neutral fat and neutral steroids as compared with either groups A or B. There were no atherosclerotic lesions of the aortas from group D. Aortas from rabbits in group B had numerous atheromatous plaques while one rabbit each from groups A and C had several very small atheromatous lesions. These results demonstrate that poloxalene 2930 reduces the rise of serum cholesterol in rabbits in response to an atherogenic diet and prevents the development of atherosclerosis. This hypocholesterolemic effect is likely mediated by the effect of this surfactant on the small intestine.

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INTRODUCTION

Hydrophobic poloxalenes composed of 90% polypropylene oxide, as the hydrophobic component, and 10% polyethylene oxide, as the hydrophilic component, have been shown to inhibit lipid absorption in the rat (1) and the swine (2). The effect of this agent was observed to be on the intracellular process of chylomicron formation or secretion causing absorbed lipid to accumulate in the enterocytes (2, 3). In both the rat (1) and the swine (2) this action on the small intestine was associated with a reduction of serum cholesterol levels. Such an agent might have potential use for treatment of various hyperlipidemic conditions if it were shown to be free of serious toxic effects.

A somewhat similar poloxalene to that previously studied has been in use in veterinary medicine for the past decade. This surfactant, poloxalene 2930 (Bloatguard, Smith, Kline, and French Laboratories, Philadelphia, PA) has been marketed as a feed additive to prevent gastric bloating in cattle. Prior toxicology studies plus the experience with this surfactant in veterinary medicine indicate poloxalene 2930 is well tolerated in animals. This agent has a 30% hydrophilic (polyethylene oxide) content. As the toxicity of poloxalenes is believed to be related to their hydrophobic content, it might be expected that poloxalene 2930 would be better tolerated than the more hydrophobic poloxalenes previously studied (1–3).

We have recently tested poloxalene 2930 in the rat and have observed it to have an effect on the small bowel similar to that demonstrated with the more hydrophobic poloxalenes. As poloxalene 2930 is known to be well tolerated, we were interested in studying

its potential for treatment of hypercholesterolemia associated with accelerated atherosclerosis. The presently described experiments present our initial attempt with poloxalene 2930 to prevent atherosclerosis using rabbits fed a high fat-high cholesterol diet.

METHODS

Male New Zealand White rabbits weighing 1.6–2.0 kg were divided into four groups. Group A (n = 5) was fed ground regular chow that contains essentially no cholesterol and is low in fat content (2% by weight). Group B (n = 8) was fed an atherogenic diet prepared from regular ground chow by the addition of cholesterol (0.5% wt/wt) and peanut oil (3% wt/wt). Group C (n = 7) and group D (n = 8) received the atherogenic diet supplemented with poloxalene 2930, 0.5 and 1% (wt/wt), respectively. Poloxalene 2930 was provided by Menley and James Laboratories, Philadelphia, PA. During 10 wk of dietary treatment animals were individually housed and received 150 g of chow daily. Food intake was continuously monitored and changes in body weight were recorded at weekly intervals. Feces were collected over a 3-d period for analyses during the final week of the study.

At termination of the experiment animals were fasted 18 h and were exsanguinated by a puncture of the abdominal aorta while under ketamine (25 mg/kg)-xylazine (2 mg/kg) anesthesia. The aorta was removed, opened longitudinally, and weighed. Macroscopic inspection of the intima was done to record and measure atheromatous plaques. The aorta was then longitudinally cut into two equal parts. One part was taken for morphologic studies using standard techniques for light microscopy and the other portion used for chemical determination of cholesterol content. As part of the light microscopic studies, atherosclerotic lesions of the aorta were quantified as intimal proliferation in the aortic arch, the thoracic, and the abdominal aorta. The thickness of these lesions was measured and expressed as a ratio of the thickness of the entire wall. The portion of the aorta used for chemical analysis was extracted for lipid (4) and the cholesterol in the extract was determined (5).

Fecal samples were dried and weighed. An aliquot was extracted for lipid (6) and the fatty acid content of the lipid phase determined (7). Content of cholesterol and the bacterial metabolites of cholesterol (neutral steroids) (8) and protein nitrogen (9) were determined on separate fecal aliquots from each rabbit.

Blood samples obtained at the time of killing were centrifuged to separate the serum component. Lipoprotein fractions from each serum sample were separated by a single centrifugation in a discontinuous gradient formed with KBr-NaCl according to the method of Chapman et al. (10). This allowed for separation of very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) fractions. The VLDL fraction of group B, however, was a broad band representing contamination with intermediate density lipoproteins (IDL). No attempt was made in this study to separate IDL from VLDL and thus the value for VLDL cholesterol in group B represents a combination of VLDL and IDL cholesterol. Cholesterol content of each lipoprotein fraction was measured by an enzymatic assay using kits (Sigma Chemical Co., St. Louis, MO).

For statistical analysis of the results multiple comparisons were performed by Fisher's protected least significance difference as modified by Satterthwaite's rule to allow for unequal population variances of the groups (11). This method of statistical analysis allows the mean for each group to be compared to the mean for every other group to determine whether significant differences exist.

RESULTS

Food intake in all groups was comparable and averaged 138 g/d. Weight gain was the greatest for group B animals that were on the high fat diet but the difference of weight gain between this group and group A was not statistically significant (1.22±0.33 kg vs. 0.91±0.35 kg, $P < 0.05$). Weight gains for group C (0.83±0.34 kg) and for group D (0.82±0.21 kg) were fairly comparable to that observed for group A but were significantly less than that for group B ($P < 0.05$ for each comparison).

The data on fecal neutral fat and neutral steroid excretion are presented in Table I. Expressed as a percentage of dietary fat intake, the amount of neutral fat excreted by animals in group B was comparable to that from group A, while the animals in groups C and D excreted two times and four times as much neutral fat, respectively. Similarly, the animals in groups C and D had significantly increased excretion of neutral steroids compared with either group A or B. The animals in group D excreted essentially 100% of the dietary cholesterol intake (Table I). The fecal protein content was comparable in all groups and ranged from 1.04–1.35 g/d.

The results of the cholesterol analysis of serum and aorta are presented in Table II. Cholesterol concentrations of each of the lipoprotein fractions from group B were significantly increased compared with group A. The levels of VLDL and LDL cholesterol in group C were significantly less than those of group B, but still greater than in group A. Cholesterol levels from all three of the lipoprotein fractions from group D were basically indistinguishable from those of group A.

Aorta total cholesterol concentrations expressed as milligrams per gram tissue were similar in groups A, C, and D, but significantly increased in group B (Table II).

All rabbits in group B demonstrated macroscopic atherosclerosis (Fig. 1), with the greatest changes observed to be in the aortic arch and the least changes in the thoracic aorta. The thickness of the atherosclerotic plaques in the arch as demonstrated by light microscopy contributed up to 50% of the aortic wall thickness. On the contrary, only one rabbit in the A and C groups had minimal atherosclerosis in the arch area. No atherosclerotic lesions were observed in group D (Fig. 1).

DISCUSSION

Prior studies on hydrophobic surfactants indicate that they have a selective action on the enterocytes inhib-

TABLE I
Fecal Excretion of Neutral Fat, Neutral Steroids, and Protein

Neutral fat (*g/d*)	A 0.34±0.15	<	B 0.78±0.35	<	C 1.35±0.33	<	D 3.26±0.36
Neutral fat (*% of intake*)	A 9.6±4.4	<	B 10.9±4.2	<	C 23.1±8.7	<	D 48.0±7.6
Neutral steroids (*mg/d*)	A 31.7±12.3	<	B 217.5±85.6	<	C 571.8±79.9	<	D 706±94.1
Neutral steroids (*% of intake*)			B 31.2±10.6	<	C 86.0±15.1	<	D 101.4±15.0
Protein (*g/d*)	A 1.16±0.53	<	C 1.18±0.17	<	D 1.30±0.20	<	B 1.36±0.33

Results for each analysis are presented in increasing values and are presented as mean±SD. Results of statistical comparisons between groups are represented by a horizontal bar(s) above the groups. A continuous bar above more than one group heading indicates no statistically significant difference between the means for these groups ($P > 0.05$). A group(s) under a separate bar has a mean significantly different compared with the mean(s) of the group(s) not under this bar ($P < 0.05$).

iting secretion of chylomicrons into intestinal lymph while secretion of very low density sized lipoproteins into lymph continues to be observed (12). The onset of action of these agents (12) and the reversal of their effect on secretion of chylomicrons (13) is impressively rapid. The hydrophobic surfactants probably have no effect on absorption of various materials that are transported into the body via the portal vein. The fact that protein absorption in the present studies was normal, as indicated by results of fecal protein nitrogen excretion, supports this impression as do former studies showing these surfactants do not increase fecal bile acid secretion (2, 14). The specific and unique effect of hydrophobic surfactants results in increased fecal losses of neutral fat and cholesterol as shown by the present experiments and prior studies (3).

Treatment of rabbits exposed to an atherogenic diet with poloxalene 2930 was associated with lower cholesterol levels in circulating VLDL and LDL compared with values observed for rabbits on the atherogenic diet without surfactant. Indeed VLDL and LDL cholesterol levels were grossly elevated in group B rabbits

TABLE II
Cholesterol Concentrations in Serum Lipoproteins and the Aorta

Total serum cholesterol (*mg/dl*)	D 59.5±16.1	<	A 65.8±22.2	<	C 187.9±93.6	<	B 912.2±221.4
VLDL cholesterol (*mg/dl*)	A 8.1±4.6	<	D 12.8±6.3	<	C 27.2±14.7	<	B 477.1±290.9
LDL cholesterol (*mg/dl*)	D 22.3±8.2	<	A 36.9±21.5	<	C 122.2±80.1	<	B 392.0±128.1
HDL cholesterol (*mg/dl*)	A 20.7±7.2	<	D 24.2±10.4	<	C 36.8±5.5	<	B 43.1±14.4
Cholesterol in aorta (*mg/g*)	A 0.67±0.21		D 0.83±0.18		C 0.86±0.25		B 1.81±0.58

Results expressed as mean±SD and are presented in increasing values. Results of statistical comparisons between groups are represented by a horizontal bar(s) above the groups. A continuous bar above more than one group heading indicates no statistically significant difference between the means for these groups ($P > 0.05$). A group(s) under a separate bar has a mean significantly different compared with the mean(s) of the group(s) not under this bar ($P < 0.05$).

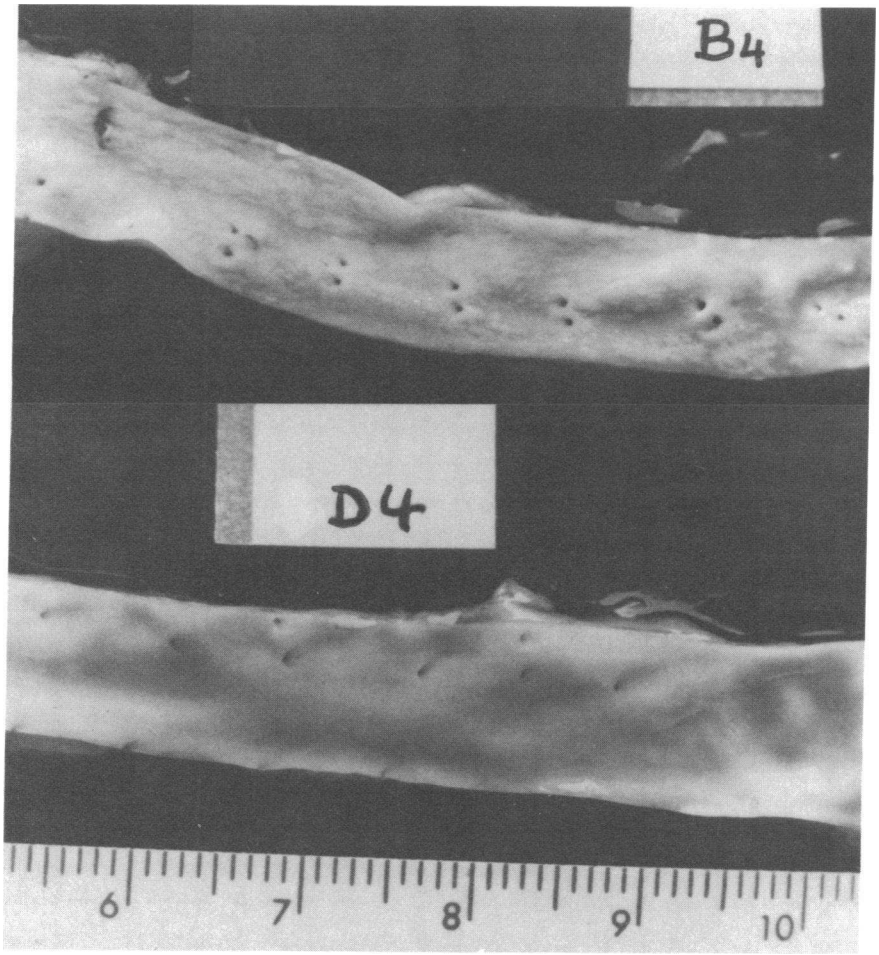

FIGURE 1 Photograph of the endothelial surface of the aorta from a rabbit from group B and one from a group D rabbit.

given the atherogenic diet without poloxalene 2930. HDL cholesterol was also elevated in group B as compared with values observed in rabbits in group D given the higher dose of poloxalene 2930 and to those in group A on regular rabbit chow. HDL cholesterol was only moderately elevated, however, in group B rabbits as compared with the more impressive increases of VLDL and LDL cholesterol concentrations observed in this group of rabbits. Values of cholesterol concentrations of circulating lipoproteins from rabbits of group D receiving the higher dose of poloxalene 2930 were no different from values from rabbits on regular chow (group A). In association with these reductions of cholesterol concentrations in circulating VLDL and LDL produced by poloxalene 2930, essentially no atherosclerosis was observed in rabbits treated with this agent over a 10-wk period (groups C and D), while rabbits treated with the atherogenic diet alone (group B) had obvious atherosclerosis of the aorta by the end of this dietary treatment period.

Based on the data for fecal fat excretion it would appear that the effect of poloxalene 2930 on serum cholesterol levels and the prevention of atherosclerosis was mediated by the effects of this surfactant on the function of the small intestine. Thus, rabbits in group D absorb daily about the same amount of neutral fat as group A (52% of 7.5 g vs. 90% of 3 g). The net amounts of absorbed cholesterol in these two groups as determined from the food intake and fecal neutral steroid output data was also similar.

Whether the effects of poloxalene 2930 on fecal lipid excretion is the only explanation for the observed pro-

tection against the development of atherosclerosis observed in rabbits of groups C and D await subsequent investigations. Results of group C suggest, however, that there may be other effects of poloxalene 2930. Total serum cholesterol levels were three times greater in group C than those observed for group D and yet the concentrations of cholesterol in the wall of the aortas were nearly identical in these two groups. It is possible, therefore that poloxalene 2930 may also affect the distribution of cholesterol between the serum compartment and the peripheral tissues. Also, if poloxalene 2930 itself is absorbed into the body, it may have a more systemic pharmacologic effect on cholesterol transport. Investigations using ^{14}C-labeled poloxalene 2930 are now in progress in our laboratory to determine whether any of this agent is indeed absorbed.

Despite the fact that the mechanism of action of poloxalene 2930 on cholesterol transport and metabolism is not completely known at this time, it is obvious from these studies that this surfactant has a profound effect on lipid absorption and can completely prevent experimentally induced atherosclerosis. Also no obvious toxic effects of this agent was observed during this study except that rabbits on the surfactant gained less weight than those on the atherogenic diet without surfactant (group B). This was at least partially the result of excessive fecal losses of dietary fat produced by poloxalene 2930. As this study was done with a single species and a relatively limited number of rabbits, it is possible that subtle toxic side effects of poloxalene 2930 were overlooked that might have been more obvious if larger numbers of animals or different species were used.

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REFERENCES

1. Bochenek, W. J., and J. B. Rodgers. 1977. Effect of polyol detergents on cholesterol and triglyceride absorption. Hypolipemic action of chronic administration of hydrophobic detergent. *Biochim. Biophys. Acta.* **489:** 503–506.
2. Brunelle, C. W., W. J. Bochenek, R. Abraham, D. N. Kim, and J. B. Rodgers. 1979. Effect of hydrophobic detergent on lipid absorption in the rat and on lipid and sterol balance in swine. *Dig. Dis. Sci.* **24:** 718–725.
3. Tso, P., J. A. Balint, and J. B. Rodgers. 1980. Effect of hydrophobic surfactant (Pluronic L-81) on lymphatic lipid transport in the rat. *Am. J. Physiol.* **239:** G348–G353.
4. Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226:** 497–509.
5. Zak, B. 1957. Simple rapid microtechnic for serum total cholesterol. *Am. J. Clin. Pathol.* **27:** 583–588.
6. Van de Kamer, J. H., H. T. B. Hiunink, and H. A. Weyers. 1949. Rapid method for determination of fat in feces. *J. Biol. Chem.* **177:** 347–355.
7. Kyriakides, E. C., B. Paul, and J. A. Balint. 1972. Lipid accumulation and acid lipase deficiency in fibroblasts from a family with Wolman's disease, and their apparent correction in vitro. *J. Lab. Clin. Med.* **80:** 810–816.
8. Miettinen, T. A., E. H. Ahrens, and S. M. Grundy. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral sterols. *J. Lipid Res.* **6:** 411–424.
9. Natelson, S. 1957. Microtechniques of Clinical Chemistry. Charles C. Thomas, Publisher, Springfield, IL. 277–282.
10. Chapman, M. J., S. Goldstein, D. Lagrange, and P. M. Laplaud. 1981. A density gradient ultracentrifugal procedure for the isolation of major lipoprotein classes from human serum. *J. Lipid Res.* **22:** 339–358.
11. Cochran, W. G., and G. W. Snedecor. 1980. Statistical Methods. The Iowa State University Press, Ames, Iowa. 7th edition. 228–234.
12. Tso, P., J. A. Balint, M. B. Bishop, and J. B. Rodgers. 1981. Acute inhibition of intestinal lipid transport by Pluronic L-81 in the rat. *Am. J. Physiol.* **241:** G487–G497.
13. Tso, P., K. L. Buch, J. A. Balint, and J. B. Rodgers. 1982. Maximal lymphatic triglyceride transport rate from the rat small intestine. *Am. J. Physiol.* **242:** G408–G415.
14. Bochenek, W. J., and J. B. Rodgers. 1980. Comparison of hydrophobic surfactant and cholestyramine on lipid and sterol balance in the rat. *Exp. Mol. Pathol.* **33:** 223–230.