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STUDIES ON THE PHARMACOLOGICAL CONTROL OF HYPERLIPEMIA IN EXPERIMENTAL NEPHROTIC SYNDROME*

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Abstract—The hyperlipemia of puromycin aminonucleoside induced nephrotic syndrome (nephrosis) was found to provide a model for testing antilipemic drugs in the rat. Increased serum concentrations of cholesterol, triglycerides, phospholipids (mainly lecithin) and alpha and beta-lipoprotein lipids were effectively controlled in acute studies by two drugs—ethyl chlorophenoxyisobutyrate (Atromid-S) and sodium betabenzalbutyrate (Kata-Lipid)—after 7 days' treatment. Moderate hepatomegaly occurred with nephrosis, and was further increased by chlorophenoxyisobutyrate but not by betabenzalbutyrate, adding indirect support to the hypothesis that these drugs may have different modes of action. Cholestyramine, D-thyroxine and 13,437-Su had no effect on nephrotic hyperlipemia in this acute study, although hepatomegaly was caused by 13,437-Su.

Nephrotic hyperlipemia in man usually remains untreated although accelerated atherosclerosis with increased morbidity and mortality is a feature of the disease. Methods successful in controlling nephrotic hyperlipemia in the rat should be put to clinical trial in man.

ACUTE puromycin aminonucleoside (PA) induced nephrotic syndrome (nephrosis) in the rat has been used successfully since 1955[1] as a model of human nephrosis, with histological changes resembling minimal or nil change glomerulonephritis on light and electron microscopy.[2–5] Acute PA-nephrosis in the rat is accompanied by acute hyperlipemia and hypercholesteremia[6–10] and the plasma lipid pattern appears potentially atherogenic.[8,9,11–13] A number of investigations have been made into the mechanism of hyperlipemia[7,8,14–16] which is primarily due to a remarkable increase in the rate of synthesis of lipoproteins by the liver[8,17,18] together with a decreased rate of liberation of lipoprotein lipase and decreased removal of circulating lipids by adipose tissue.[14, 15, 19–21] No investigations appear to have been made of the pharmacological control of nephrotic hyperlipemia in rat or man. The fact that human nephrosis has a similar pattern of hyperlipemia, associated with an increased mortality due to the increased and early incidence of atherosclerosis[7, 9, 22–25] has prompted the present study, in PA-nephrotic rats, of the effects of drugs known to interfere with lipid synthesis or transport.

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TABLE 1. EFFECT OF DRUGS ON THE LIVER WEIGHT AND ON HYPERLIPEMIA OF PA-NEPHROTIC RATS AT 22 days

Treatment:	Liver wt. / Body wt. (% of PA value)	Serum concentrations (mg% : mean ± S.E. of mean)					
		TC	TG	TPL	αLPL	βLPL	αLP/βLP
PA nephrosis	100 ± 2	113 ± 8	93 ± 6	207 ± 18	309 ± 25	105 ± 10	2·94
PA + Atromid-S	130† ± 6	**81*** ± 9	**72*** ± 9	**130†** ± 10	213† ± 9	73*§ ± 6	2·92
PA + β-benzalbutyrate	108 ± 4	104 ± 11	**71†** ± 2	**136†** ± 9	226* ± 17	**85*** ± 3	2·67
PA + cholestyramine	111 ± 9	123 ± 15	**75** ± 13	222 ± 22	317 ± 38	104 ± 11	3·05
PA + 13,437-Su	141† ± 10	100 ± 12	86 ± 8	174 ± 24	249 ± 39	111 ± 17	**2·24**
Untreated	**75†** ± 2	**73‡** ± 3	**63†** ± 4	**139†** ± 4	**181‡** ± 6	**88*** ± 4	**2·06†**

* † ‡ Significantly different from PA nephrosis (*$P<0·05$; †$P<0·01$; ‡$P<0·001$). Values not significantly different from those in untreated animals are printed in bold type.
†Significantly below normal ($P<0·05$).
Mean ± S.E. of mean calculated from seven animals per treatment group and 16 normal animals.
Treated animals were given 105 mg/kg of PA over 9 days and sacrificed at 22 days; drugs were given for 7 days prior to sacrifice.

MATERIALS AND METHODS

Animals. Female Sprague–Dawley albino rats weighing 150 ± 25 g were allowed free access to a standard diet and water during the experimental time. Urines were collected during daily weighing. All animals were killed after stunning with a blow to the head, by rapid exsanguination from a cut throat. Liver weight was measured after rapid removal and transferance to an ice-cooled container.

Induction of nephrosis. Experimental nephrosis was induced[1, 9] by administering Puromycin aminonucleoside (PA) Sigma Co., U.S.A.) by daily subcutaneous injections for 7 days. Some of the rats received 15 mg/kg/day and were sacrificed 22 days after starting the injections (Group I). A second group received 7·5 mg/kg/day and was sacrificed 8 days after starting the injections (Group II).

Rats in Group I developed a moderate nephrosis, as shown by a significantly elevated urinary protein excretion (200–300 per cent of normal values, measured as milligrams of protein/milligrams of creatinine), a 20 per cent fall in serum albumin concentration, and significant hyperlipemia (see Table 1). Group II had minimal nephrosis with urinary protein excretion (mg/mg creatinine) equal to 150 per cent of normal. Serum albumin was decreased about 15 per cent and there was a significant mild increase in serum lipid concentrations (see Table 2).

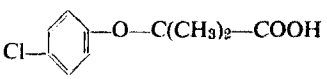
TABLE 2. EFFECT OF ATROMID-S AND D-THYROXINE ON SERUM LIPID CONCENTRATIONS IN PA-NEPHROTIC RATS AT 8 days

Serum concentrations (mg%; mean ± S.E.)	Untreated	PA Nephrosis	PA + Atromid-S	PA + D-Thyroxin
TC	**73**† ± 2	91 ± 6	65†§ ± 4	88 ± 7
TG	**63** ± 4	**59** ± 3	49§ ± 6	**60** ± 18
TPL	**139*** ± 4	162 ± 15	115†§ ± 8	177 ± 22
L	**62**‡ ± 3	92 ± 6	48‡ ± 5	n.m.
Sph	**17** ± 1	**20** ± 2	**17** ± 2	n.m.
LL	**43** ± 2	**46** ± 3	**41** ± 3	n.m.
αLPL	**181*** ± 6	210 ± 18	149*§ ± 12	239 ± 27
βLPL	**88*** ± 4	102 ± 8	**80*** ± 5	**86** ± 12

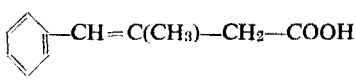
n.m. = not measured
Animals were given 45 mg/kg of PA over 7 days and sacrificed at 8 days; drugs were given for 7 days prior to sacrifice.
* † ‡ Significantly different from PA-Nephrosis (*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$). Values not significantly different from those in untreated animals are printed in bold type.
§Significantly below normal ($P < 0.05$).

Chemical determinations. Urinary creatinine was determined according to Edwards and Whyte,[26] urinary and serum proteins according to Lowry *et al.*[27] and serum albumin, globulins and alpha- and beta-lipoproteins (αLPL, β-LPL) using Cellogel electrophoresis.[10] Serum total cholesterol (TC) was measured according to Zlatkis *et al.*,[28] triglycerides (TG) separated on Florisil columns according to Blankenhorn *et al.*[29] and measured according to Van Handel and Zilversmit[30] and total phospholipids (TPL) and serum phospholipid distribution (lecithin, L.; sphingomyelin, Sph.; lysolecithin, LL) according to Rouser *et al.*[31]

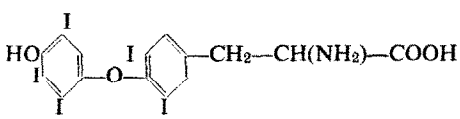
Drugs. All drugs used were given daily by gastric intubation for 7 days. Atromid-s (clofibrate, ethyl-p-chlorophenoxyisobutyrate, supplied by I.C.I., Macclesfield, England) was given as a 5% solution in olive oil in a dose of 300 mg/kg/day; Sodium betabenzalbutyrate (Istituto Biochimico Italiano, Milan, Italy) as a 0·5% solution in water, 100 mg/kg/day; cholestyramine (a strongly basic anion exchange resin, supplied by Merck, Sharp and Dohme, Rahway, U.S.A.) in a dose of 3 g/kg/day suspended in water (20 ml/kg/day); D-thyroxine (Bracco Co., Milan, Italy) in a dose of 10 mg/kg/day in a 0·4% solution in a mixture of ethanol: 0·5 N NaOH (19:1); 13,437-Su (Ciba Co., Basel, Switzerland) in a dose of 50 mg/kg/day as a 2·5% solution in 95% ethanol. Control rats received equal amounts of the diluting solutions. Chlorophenoxyisobutyrate, betabenzalbutyrate, and thyroxine have the following structures.



Chlorophenoxyisobutyrate



Betabenzalbutyrate



D-thyroxine

and 13,437-Su (Ciba) is a tetralin phenoxyacetate derivative (exact structure not revealed).

RESULTS AND COMMENTS

The rats in Group I, after puromycin aminonucleoside treatment, showed a marked increase in serum cholesterol, triglycerides, phospholipids, alpha-lipoprotein lipids and, less evidently, in beta-lipoprotein lipids (Table 1). The αLPL/βLPL ratio was also increased from 2 to 3. At the same time, the liver weight was increased in agreement with other results showing increased liver total protein content and microsomal enzyme activity.[32] Chlorophenoxyisobutyrate and β-benzalbutyrate provoked a return to normal serum levels of TG, TPL, βLPL and a decrease of αLPL. Chlorophenoxyisobutyrate, but not beta-benzalbutyrate, at the dose used, also decreased serum cholesterol concentration to normal. Cholestyramine and 13,437-Su had no clear-cut effects on lipids but 13,437-Su induced an increase in liver weight. It should be noted however that 13,437-Su is supposed to be effective primarily in male rats, while the rats considered in these experiments were female.[33, 34]

The results clearly indicate in the case of 13,437-Su and β-benzalbutyrate that the effect on liver weight is unrelated to the possible effect on serum lipids. These data are in agreement with Ruegamer *et al.*[35] who consider that the antilipemic action of chlorophenoxyisobutyrate is unrelated to its hepatomegalic and enzyme inducing

effects. The drugs chlorophenoxyisobutyrate and 13,437-Su increase liver size well above the maximal levels induced by PA nephrosis itself. Betabenzalbutyrate is remarkably free of this side effect. The induction of hepatomegaly is commonly related to induction of drug metabolising microsomal enzymes,[32] a property which is shared by chlorophenoxyisobutyrate and 13,437-Su. It is of interest that the hepatomegalic and inducing effect is additive to that of PA nephrosis. It has also been suggested on theoretical grounds by analogy with other halogenated hydrocarbons, that liver glutathione metabolism and mercapturic acid formation might well be involved in the hepatomegalic and enzyme inducing process.[9]

In rats submitted to a milder nephrotic syndrome (Group II) chlorophenoxyisobutyrate is still active in decreasing, often below normal levels, the serum concentrations of TG, TC, TPL, L, αLPL and βLPL, while D-thyroxine is not effective in the same experimental conditions, probably because of the short time of treatment. However, D-thyroxine affected protein metabolism; it significantly lowered serum total protein and particularly alpha$_1$ globulin, as well as beta and gamma globulin concentrations, and increased the alpha over beta lipoprotein and the albumin over alpha$_1$ globulin ratios.[36]

The data indicate for the first time that nephrotic hyperlipemia can be reduced or normalised by the use of chlorophenoxyisobutyrate and betabenzalbutyrate. The mode of action of these drugs is not yet clear, but the following findings appear to be important. Chlorophenoxyisobutyrate has been studied in some detail. It apparently competes with other organic acids such as thyroxine, tryptophan, free fatty acids, 17-ketosteroids and pyridoxal for binding sites to plasma albumin.[37] Circulating thyroxine and tryptophan are displaced and accumulate in the liver, causing a strictly localized "hepatic hyperthyroidism", which greatly increases the synthesis of certain protein enzymes, particularly liver mitochondrial alpha-glycerol-phosphate dehydrogenase, GPD.[35, 38, 39] At the same time, the increase in liver tryptophan content increases liver oxidized nicotinamide adenine dinucleotide coenzyme (NAD) content to 250 per cent of control values.[40] The increased liver GPD and NAD concentrations should greatly increase L-alpha-glycerolphosphate breakdown to dihydroxyacetonephosphate. This would have the effect of shunting intermediates from triglyceride and phospholipid synthesis to the glycolytic pathway. Decreased availability of free fatty acids during chlorophenoxyisobutyrate therapy would further diminish lipid synthesis.[41, 42] In addition, both chlorophenoxyisobutyrate and betabenzalbutyrate block cholesterol synthesis in rat liver slices.[43, 44] Chlorophenoxyisobutyrate appears to act at stages after the formation of acetyl-coenzyme A,[43] whereas betabenzalbutyrate acts at the earliest stage of cholesterol and fatty acid synthesis by inhibiting the activation of fatty acids by the enzyme acetate: CoA ligase.[44] In bacteria, betabenzalbutyrate markedly inhibits the formation of butyryl-AMP (an intermediate in butyryl-CoA synthesis) by a non-competitive mechanism.[45] Thus the two drugs appear to act in different ways, although the effects of betabenzalbutyrate on liver concentrations of thyroxine, tryptophan, GPD and NAD have yet to be studied.

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ADDENDUM

Recently the effects of chlorophenoxyisobutyrate (0·2% of diet) on PA nephrotic hyperlipemia and liver lipid levels in rats has been described by Connor and colleagues of Iowa City. Normal serum and liver cholesterol and triglyceride levels were maintained by the drug in mild to moderate nephrosis. In severe nephrosis (16 daily injections of PA), mean serum cholestererol and triglyceride levels rose in control rats to 276 and 462 mg per cent respectively, and values in the treated rats rose to 216 and 168 mg per cent. The antilipemic effects of the drug was thought to be due to inhibition of synthesis of lipids by the liver (J. C. HOAK, W. E. CONNOR, M. L. ARMSTRONG and E. D. WARNER, *Lab. Invest.* **19**, 370, 1968). Hypophysectomized PA—nephrotic rats also were found to have normal serum cholesterol and triglyceride concentrations (J. C. HOAK, W. E. CONNOR, and D. B. STONE, *Proc. Soc. exp. Biol. Med.* **122**, 588, 1966).