Peskar, B. M., Weiler, H. (1983) Gut 24: A480
Pinder, R. M., Brogden, R. N., Sawyer, P. R., Speight, T. M., Spencer, R., Avery, G. S. (1976) Drugs 11: 245–307
Rask-Madsen, J., Bukhave, K., Madsen, P. E. R., Bekker, C. (1983) Eur. J. Clin. Invest. 13: 351–356
Robert, A., Nezamis, J. E., Lancaster, C., Hanchar, A. J. (1979) Gastroenterol. 77: 433–443
Roberts, N. B., Walker, V., Etherington, D. J., Baron, J. H., McConnell, R. B., Taylor, W. H. (1983) Acta Gastro. Ent. Belg. 46: 448–458
Van Huis, G. A., Kramer, M. F. (1981) Gut 22: 782–787
Vapaatalo, H., Linden, I.-B., Metsa-Ketela, T., Kangasaho, M., Laustiola, K. (1978) Experientia 34: 384–385
Varró, V. (1983) Acta Physiol. Hungarica 61: 13–22
Whittle, B. J. R., Kauffman, G. L., Moncada, S. (1981) Nature 292: 472–474

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The effect of bezafibrate on hyperlipidaemia in experimental nephrotic syndrome in rats

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The effects of bezafibrate on hyperlipidaemia in experimental nephrotic syndrome in rats has been investigated. The treated group received bezafibrate 10 mg kg^{-1} p.o. daily. No significant differences in total serum cholesterol occurred, but a significant reduction in serum triglyceride ($P < 0.005$) and elevation in HDL cholesterol ($P < 0.005$) occurred. These findings may have implications for therapeutic intervention in severe hyperlipidaemia of the nephrotic syndrome in man.

The genesis and significance of the hyperlipidaemia which occurs in the nephrotic syndrome remains obscure. The changes in lipoprotein metabolism that occur are probably secondary to the hypoalbuminaemia, as an inverse relation exists between the hypoalbuminaemia and hyperlipidaemia (Baxter 1962), and infusing albumin will reduce the hyperlipidaemia (Bogdonoff et al 1961). Although a sustained elevation of serum lipids is a risk factor in arterial disease, there are conflicting opinions regarding the prevalence of arterial disease in the nephrotic syndrome (Curry & Roberts 1977; Wass et al 1979) and the role of hyperlipidaemia in its progression.

Clofibrate, a lipid lowering agent, has been used to treat essential hyperlipidaemia. However an acute muscular syndrome (myalgia) and elevations of serum creatine phosphokinase have complicated its use in treating the hyperlipidaemia of the nephrotic syndrome (Bridgman et al 1972). The myalgia seen in such patients is related to high serum drug levels consequent upon reduced plasma protein binding and renal excretion (Bridgman et al 1972).

Bezafibrate, another lipid lowering agent with different pharmacological properties to clofibrate, has been assessed with regard to its lipid lowering effect in experimental nephrotic syndrome in rats, induced by puromycin aminonucleoside.

Methods

Fifteen female Wistar rats (6 control, 9 treated), 200–275 g received a single intraperitoneal injection of puromycin aminonucleoside 70 mg kg^{-1} (Sigma Chemical Co., St Louis, USA, Lot No 109C–4009) in 1·0 ml of sterile water to induce the nephrotic syndrome (Fiegelson et al 1957; Derr et al 1968). The rats were housed in metabolic cages with free access to standard rat feed and water for 24 h before sample collection. Nine rats received a daily oral dose of bezafibrate 10 mg kg^{-1} (Boehringer Mannheim Ltd) as a suspension in 0·5% w/v methylcellulose, commencing 24 h after the administration of puromycin aminonucleoside, for the duration of the study.

Blood samples and 24 h urine samples were obtained on days 0, 7, 14 and 21. Total serum cholesterol was determined enzymatically (Cholesterol C System, Boehringer Mannheim Ltd) (Stahler et al 1977). HDL cholesterol was determined in the supernatant of serum, using a similar method, after precipitation of other lipid fractions with phosphotungstic acid and magnesium ions (Burstein et al 1970). Serum triglycerides were determined by an enzymatic colorimetric assay (Peridochrom, Boehringer Mannheim Ltd). Serum LDL were calculated by application of the Friedewald formula (Friedewald et al 1972). Serum protein was determined by the biuret method (Weichselbaum 1946), and urinary proteins were measured colorimetrically using bromocresol green (Kachmar & Grant 1976).

All values are expressed as mean ±s.e.m. Student's *t*-test was used for unpaired data.

Results

The serum proteins decreased in both groups by day 7 (control 66·5 ± 2·4 to 45·3 ± 2·6 g $litre^{-1}$, $P < 0.001$, treated group 67·0 ± 3·3 to 49·0 ± 3·5 g $litre^{-1}$, $P < 0.005$) but by week 3 had returned to near normal values (control 60·9 ± 3·5 g $litre^{-1}$, treated group 58·2 ± 2·9 g $litre^{-1}$). Urinary protein excretion rose significantly by day 7 in both groups (control 8·8 ± 1·26 to 177·6 ± 25·8 mg $24\ h^{-1}$, $P < 0.001$, treated group 17·2 ± 5·4 to 160·0 ± 37·2 mg $24\ h^{-1}$, $P < 0.001$). A similar degree of

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severity of hypoproteinaemia and proteinuria was achieved in both groups of animals. A rise in total serum cholesterol occurred but there was no significant difference between the two groups. (Fig. 1.) The serum triglyceride concentration rose in both groups, although the increase in the treated group was significantly less than that of the control group ($P < 0{\cdot}01$ at week 1, $P < 0{\cdot}005$ at week 2). At week 3 there was no significant difference in the triglyceride levels of the two groups. The fall in triglyceride of the control group at week 3 occurred simultaneously with the fall in proteinuria and rise in serum proteins, reflecting the limited time course of the induced nephrotic syndrome. A significant rise in HDL cholesterol occurred in both groups, however, the rise in the treated groups was significantly greater than that of the controls ($P < 0{\cdot}01$ at week 2, $P < 0{\cdot}005$ at week 3). No significant difference in LDL cholesterol was found during the first 2 weeks, however, by week 3, the LDL cholesterol of the treated group was significantly lower than the control group ($P < 0{\cdot}01$).

Discussion

The changes in urinary protein excretion, serum proteins and serum lipids induced by the puromycin aminonucleoside in this study are similar to those previously described (Gherardi & Calandra 1981). These changes resemble human nephrotic syndrome, but in rats an elevation in HDL cholesterol also occurs (Gherardi & Calandra 1981). This is accompanied by increased hepatic synthesis of apolipoprotein AI (Sparks et al 1981), the major structural lipoprotein of HDL. Elevation of the triglyceride fraction of the serum lipids is also seen, which may be related to defective conversion of VLDL lipoproteins and chylomicron remnants to LDL (Gherardi et al 1977). Lipolysis of VLDL and chylomicrons is mediated by lipoprotein lipase and hepatic triglyceride lipase, and during this process the cholesterol contained in these lipid fractions may be transferred to HDL_3 (Eisenberg 1982).

Bezafibrate has been shown to lower serum triglyceride and cholesterol levels significantly in normal rats (Stegmeier et al 1980) and, in hyperlipidaemic states, both in rats (Catapano et al 1982) and man (Vessby et al 1980). Administration of bezafibrate to rats with puromycin aminonucleoside-induced nephrosis resulted in a significant reduction in serum triglyceride levels, and an elevation in the HDL cholesterol. One effect of the drug is an increase in lipoprotein lipase activity in skeletal muscle (Vessby et al 1982). An increased lipolysis of the triglyceride-rich fractions and cholesterol enrichment of HDL, might account for the triglyceride-lowering capacity of bezafibrate in this animal model.

This finding has implications for treatment of the hyperlipidaemia seen in the nephrotic syndrome. Clofibrate is unsuitable for use in the nephrotic syndrome, but bezafibrate has the advantages that serum levels are approximately 30 times lower than those of clofibrate

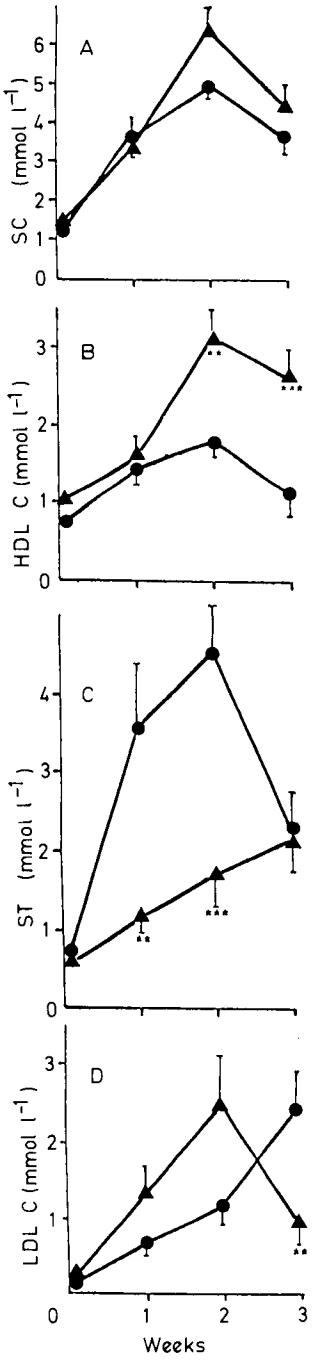

FIG. 1. Effects of bezafibrate on (a) total serum cholesterol, (b) HDL cholesterol, (c) serum triglycerides and (d) LDL cholesterol in experimental nephrotic syndrome (values: mean ± s.e.m.). Key: (●), control; (▲), treated; (**), $P < 0{\cdot}01$; (***), $P < 0{\cdot}005$.

using standard dosages (0·2 and 0·5 g three times daily, respectively) (Abshagen et al 1980). Also, the shorter half life of bezafibrate (2 h), compared with that of clofibrinic acid, the active moiety of clofibrate (10–30 h), and a higher renal clearance, make the likelihood of drug accumulation less likely (Abshagen 1982). However, caution is required when directly extrapolating to human nephrotic syndrome, as bezafibrate exhibits the same high degree of protein binding as clofibrate (Abshagen et al 1980).

REFERENCES

Abshagen, U. (1982) in: Crepaldi, G., Greten, H., Schettler, G., Baggio, G. (eds) Lipoprotein Metabolism and Therapy of Lipid Disorders. Excerpta Medica, pp 66–78

Abshagen, U., Bablok, W., Koch, K., Lang, P. D., Schmidt, H., Senn, M., Storic, H. (1980) in: Greten, Lang, Schettler (eds) Lipoproteins and Coronary Heart Disease. Gerhard Witztrock,pp 107–109

Baxter, J. H. (1962) Arch. Int. Med. 109: 149–161

Bogdonoff, D., Linhart, J., Klein, R. F., Estes, E. H. (1961) J. Clin. Invest. 40: 1024–1025

Bridgman, J. F., Rosen, S. M., Thorp, J. M. (1972) Lancet ii: 506–509

Burstein, M., Scholnick, H. R., Morfin, R. (1970) J. Lipid Res. 11: 583–595

Catapano, A. L., Trezzi, E., Roma, P. (1982) in: Crepaldi, G., Greten, H., Schettler, G., Baggio, G. (eds) Lipoprotein Metabolism and Therapy of Lipid Disorders. Excerpta medica, pp 83–87

Curry, R. C., Roberts, W. C. (1977) Am. J. Med. 63: 183–192

Derr, R. F., Loechler, D. K., Alexander, C. S., Nagasawa, H. T. (1968) J. Lab. Clin. Med. 72: 363–369

Eisenberg, S. (1982) in: Crepaldi, G., Greten, H., Schettler, G., Baggio, G. (eds) Lipoprotein Metabolism and Therapy of Lipid Disorders. Excerpta Med. Clin. pp 12–20

Fiegelson, E. B., Drake, J. W., Recant, L. (1957) J. Lab. Clin. Med. 50: 437–446

Friedewald, W. T., Levy, R. I., Fredrickson, D. S. (1972) Clin. Chem. 18: 499–502

Gherardi, E., Calandra, S. (1981) Biochim. Biophys. Acta 710: 188–196

Gherardi, E., Rota, E., Calandra, S., Genova, R., Tamborino, A. (1977) Eur. J. Clin. Invest. 7: 563–570

Kachmar, J. F., Grant, G. H. (1976) in: Tietz, N. (ed.) Fundamentals of Clinical Chemistry. W. B. Saunders

Stahler, F., Gruber, W., Stinshoff, K. (1977) Med. Lab. 30: 29–37

Sparks, E. C., Tennenburg, S. D., Marsh, J. B. (1981) Metabolism 30: 354–357

Stegmeier, K., Stork, H., Lenz, H., Leuschner, F., Liede, V. (1980) in: Greten, H., Lang, P. D., Schettler, G. (eds) Lipoproteins and Coronary Heart Disease. Gerhard Witztrock, pp 76–83

Vessby, B., Lithell, H., Hellsing, K., Ostlund-Lindqvist, A. M., Gustafsson, I., Boberg, J., Lederman, H. (1980) Atherosclerosis 37: 257–269

Vessby, B., Lithell, H., Gustafsson, S., Lederman, H. (1982) in: Crepaldi, G., Greten, H., Schettler, G., Baggio, G. (eds) Lipoprotein Metabolism and Therapy of Lipid Disorders. Excerpta Medica, pp 101–107

Wass, V. J., Jarrett, R. J., Chilvers, C., Cameron, J. S. (1979) Lancet ii: 664–667

Weichselbaum, T. E. (1946) Am. J. Clin. Path. 10: 40–49