ELSEVIER

European Journal of Pharmacology 350 (1998) 203–210

ejp

Cardioprotective effect of probucol in the atherosclerosis-prone JCR:LA-cp rat

James C. Russell [a,*], Sandra E. Graham [a], Roger M. Amy [b], Peter J. Dolphin [c]

[a] *Department of Surgery, University of Alberta, Edmonton, Alberta, Canada T6G 2S2*
[b] *British Columbia Cancer Agency, 600 West 10th Avenue, Vancouver, British Columbia, Canada V5Z 4E6*
[c] *Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7*

Received 18 December 1997; revised 17 March 1998; accepted 25 March 1998

Abstract

Probucol is an antihyperlipidemic agent with antioxidant effects and antiatherosclerotic properties in hypercholesterolemic conditions. The JCR:LA-corpulent strain of rats exhibits all aspects of the human 'metabolic syndrome' characterized by obesity, insulin resistance, hypertriglyceridemia, atherogenesis, and ischemic myocardial damage. Male rats were treated with 100 mg/kg body weight probucol from 6 to 12 weeks or from 6 to 39 weeks of age. Short-term metabolic effects were assessed at 12 weeks and both metabolic and cardiovascular effects at 39 weeks of age. Probucol treatment of corpulent male rats did not reduce plasma lipid concentrations or hyperinsulinemia. The index of severity of intimal lesions of the aortic arch was not different from that of controls, although the lesions appeared to be qualitatively more severe. There were significantly fewer adherent macrophages on the endothelial surface. The endothelial layer was unchanged and smoothly covered the vascular surface, including the intimal lesions. Notwithstanding the extensive atherosclerotic lesions, probucol-treated rats had markedly fewer ischemic myocardial lesions. The cardioprotective effect, possibly due to the antioxidant properties of probucol, appears to occur at the level of the endothelium and occurs in the presence of continuing obesity, hyperinsulinemia, hypertriglyceridemia, and atherosclerosis.

Keywords: Probucol; Atherosclerosis; Myocardial lesion; Hypertriglyceridemia; JCR:LA-cp rat

1. Introduction

Two major classes of metabolic disorders are strongly associated with the development of atherosclerosis and cardiovascular disease. These are: abnormalities of cholesterol synthesis and exchange, of which defects of the low-density lipoprotein (LDL) receptor are probably the most important; and diabetes, both type 1 (insulin-dependent) and type 2 (non-insulin-dependent). A central element of these varying disorders is an abnormal lipid metabolism, albeit of differing origins. Genetic defects of the LDL receptor are relatively uncommon. They are now well characterized and a very good animal model, the Watanabe Heritable Hyperlipidemic (WHHL) rabbit, is available (Nagano et al., 1992). Type 1 diabetes is an autoimmune insulin deficiency state with alterations of glucose and lipid metabolism. It also is a disease affecting only a small proportion of the population. In contrast, type 2 diabetes, which is an insulin-resistant, hyperinsulinemic state, is increasingly common in prosperous societies. The common initial presentation of type 2 diabetes is sometimes referred to as the 'metabolic syndrome,' and is characterized by abdominal obesity, insulin resistance with impaired glucose tolerance. Very-low-density lipoprotein (VLDL) hypertriglyceridemia is primarily evident in a hypertriglyceridemia, but the metabolism and particle structure of lipoproteins also give rise to elevated plasma concentrations of cholesterol, both free and esterified. Individuals exhibiting the metabolic syndrome are highly at risk for atherosclerosis and myocardial infarct (Steiner, 1986; Reaven, 1988).

The JCR:LA-corpulent rat is a unique animal model that exhibits all aspects of the metabolic syndrome, includ-

* Corresponding author. Tel.: +1-403-492-6359; fax: +1-403-492-4923; e-mail: jim.russell@ualberta.ca

0014-2999/98/$19.00

ing atherosclerosis and ischemic myocardial lesions (Amy et al., 1988; Russell et al., 1990a; Russell, 1995). Animals that are homozygous normal (+/+) or heterozygous for the mutant autosomal recessive cp gene (+/cp) are normal, lean rats, indistinguishable from the parent LA/N strain. Rats that are homozygous for the cp gene (cp/cp) are obese and exhibit the metabolic syndrome (Dolphin et al., 1987; Russell et al., 1987, Russell et al., 1989c, 1994; Vance and Russell, 1990). The cp mutation has recently been shown to create a stop codon in the extracelluar domain of the leptin receptor (ObR), resulting in an absence of receptors (Wu-Peng et al., 1997). The origin of the insulin resistance is not yet clear, but may be related to the role of leptin in suppressing pancreatic insulin release (Emilsson et al., 1997) or to the presence of excessive intracellular triglyceride (Russell et al., unpublished observations). Atherosclerosis and cardiovascular disease develop in cp/cp male rats from an early age, while cp/cp females are spared until an advanced age (Russell and Amy, 1986a,b). Evidence to date has been consistent with the hypothesis that hyperinsulinemia plays a critical role in the pathophysiological processes leading to atherosclerosis in the JCR:LA-cp rat (Russell et al., 1989a,b). The role of the marked VLDL hyperlipidemia is less clear. Moderate reduction in plasma lipid levels alone has no protective effect (Russell et al., 1991a), although an 80% reduction in triglycerides with accompanying lower cholesterol levels and improved insulin status, induced by MEDICA 16, is highly protective (Russell et al., 1991b, 1995). The origin of the vasculopathy is clearly multifactorial and polygenetic as even closely related strains of obese rats, while insulin resistant and hyperinsulinemic, do not develop cardiovascular complications (Amy et al., 1988; Russell et al., 1990a; Russell, 1995). One component of this genetic background appears to lie in the vascular smooth muscle cells, which are hyperplastic, proliferate abnormally in cell culture, and are hyper-responsive to cytokines, including insulin-like growth factor-1 (Absher et al., 1997).

Probucol is an agent that has both hypolipidemic and antioxidant properties, that are considered to be cardioprotective (Jackson et al., 1991). It has been shown to have antiatherogenic effects in the WHHL rabbit, supporting its use in the treatment of hypercholesterolemic states (Kita et al., 1987). Recently, probucol, but not the antioxidant α-tocopherol (or vitamin E), has been shown to markedly inhibit restenosis of human coronary arteries following angioplasty (Tardif et al., 1997), suggesting direct effects on the vessel wall. Whereas probucol has been shown to have beneficial effects on cardiovascular disease associated with hypercholesterolemia, there are no reports of its effects on cardiovascular disease associated with hyperinsulinemia and related hypertriglyceridemia. Thus, we have investigated the short- and long-term effects of probucol on metabolism and cardiovascular disease in the cp/cp rat. Our results show no protection against atherogenesis, but do demonstrate a strong cardioprotective effect.

2. Methods

2.1. Animals and experimental procedures

Male rats, +/+ and cp/cp, were bred in our breeding colony as previously described (Russell et al., 1995). They were weaned at 3 weeks of age and were maintained on wood chip bedding in polycarbonate cages at 21°C with 50% relative humidity and on a 12-h light/12-h dark cycle. They were treated with probucol added to the feed from 6 weeks of age. Food was Wayne Rodent Blox (Harlan–Sprague–Dawley, Madison, WI, USA), a grain-based diet of under 4% total lipid content. Probucol (Marion Merrell Dow, Cincinnati, OH, USA) was incorporated into powdered food so as to give a daily dose of 100 mg/kg body weight, and the food was moistened and pelleted. Food consumption and body weight were measured weekly, and the probucol concentration was adjusted accordingly. All treatments conformed with the guidelines of the Canadian Council on Animal Care and were subject to prior institutional review and approval.

The rats were bled from the tail at 12 weeks of age after an overnight fast, and the plasma glucose, insulin, and total lipid profile were determined. At 39 weeks of age, the rats were starved overnight, anesthetized with halothane in oxygen, bled from the heart, and perfusion-fixed with 1.25% glutaraldehyde and 1.85% formaldehyde, as previously described (Russell and Amy, 1986a; Russell et al., 1995). The heart and pancreas were removed and fixed further for light microscopy. The aorta was further fixed in 2.5% glutaraldehyde and prepared for electron microscopy. Plasma was processed for lipid, glucose and insulin assays.

2.2. Analytical techniques

The total lipid profile was measured in whole plasma by the technique of Kuksis et al. (1978). Glucose was assayed using a rapid glucose oxidase method (Beckman Instruments, Brea, CA, USA), and insulin by a double antibody radioimmunoassay (Kabi Pharmacia Diagnostics, Uppsala, Sweden) using rat insulin standards.

2.3. Histology

The hearts were cut into 3 blocks (base, mid-heart, and apex regions), fixed in 3.7% neutral buffered formaldehyde and processed as described previously (Russell et al., 1990a, 1995). Two adjacent sections were cut from each block and stained with hematoxylin and eosin and Masson's trichrome, respectively. The incidence of ischemic myocardial lesions in hematoxylin and eosin-stained sections was determined blindly by an experienced pathologist, with confirmation of lesions through the Masson's trichrome-stained sections. Lesion stages were identified as: Stage 1, areas of necrosis; Stage 2, areas of cell lysis with chronic inflammatory infiltration; Stage 3, nod-

ules of chronic inflammatory cell infiltration; and Stage 4, old, scarred lesions (Russell et al., 1990a). Stage 4 lesions are the most important, as they reflect the cumulative record of earlier stage lesions that were large enough to remain identifiable after the scarring and contraction of the repair process. The number of lesions in the sections from each heart was summed and the mean incidence for each group was calculated. This procedure yields a consistent index of the ischemic damage to the myocardium (Russell et al., 1993). Pancreata were fixed initially in Bouin's solution, were further fixed in formalin, and were processed conventionally with hematoxylin and eosin staining.

2.4. Electron microscopy

The aortic arch from perfusion-fixed animals was dissected free of all extraneous tissue and was split along the greater and lesser curves. The two halves of the arch, including the stumps of the great vessels, were postfixed with osmium tetroxide and uranyl acetate, dried with graded ethanol solutions, and triple-point dried from propylene oxide. The mounted segments were sputter-coated with gold, and the intimal surfaces were examined completely using a Hitachi Model S2500 scanning electron microscope. Lesions were identified and classified as: areas of adherent fibrin; raised intimal lesions; areas of adherent macrophages; or areas of de-endothelialization (Russell et al., 1995). The aortic arch, after fixation and processing, retains its in vivo morphology in two semi-rigid segments composed of compound curves. It is not possible to flatten the arch or to obtain planar images of the arch and lesioned areas that are suitable for quantitation through image analysis. Thus, all lesions were recorded photographically and each type of lesion was assigned a 'severity score,' based on areal extent and character of the lesion, for each animal. The scale used ranged from 0 to 3, with 0 representing the absence of any lesions and 3 representing the most severe involvement, as seen in cp/cp control animals.

2.5. Statistical methods

The numerical data are presented as mean ± S.E.M. (standard area of the mean). Statistical analysis was by analysis of variance and Wilcoxon's rank sum test, as appropriate, with $P < 0.05$, two-tailed, taken as significant.

3. Results

3.1. Metabolic responses

Probucol treatment was well tolerated by the cp/cp rats and at 12 weeks had no significant effect on either food consumption (39.4 ± 2.6 vs. 37.1 ± 0.9 g/day for probucol-treated and control rats, respectively) or body weight (489 ± 10 vs. 480 ± 5 g). There were, similarly, no differences in food intake or body weight between probucol-treated and control cp/cp rats at 39 weeks of age (data not shown). At 12 weeks of age, probucol-treated rats showed no difference in fasting plasma glucose levels compared to either +/+ or cp/cp controls (Table 1). Similarly, fasting plasma glucose concentrations at 39 weeks of age did not differ between either the control group or the probucol-treated group. At 12 weeks of age, probucol-treated rats had significantly lower fasting insulin levels; however, at 39 weeks of age, this effect was no longer evident, and plasma insulin levels of the treated rats were not significantly different from those of the control rats (Table 1).

Table 1
Plasma insulin and glucose concentrations in male JCR:LA-cp rats treated with probucol

	Plasma glucose (mmol/l	Plasma insulin (pmol/l)
12-week-old rats		
+/+ control	9.1 ± 0.32	215 ± 23[a]
cp/cp control	8.4 ± 0.28	1492 ± 51
cp/cp probucol-treated	8.8 ± 0.32	483 ± 87[a]
39-week-old rats		
+/+ control	7.3 ± 0.2	173 ± 28[a]
cp/cp control	7.8 ± 0.2	1009 ± 151
cp/cp probucol-treated	7.4 ± 0.6	1283 ± 206

Values are mean ± S.E.M. for samples taken from fasting animals, 10 rats in each group.
[a] $P < 0.001$ compared to cp/cp control.

Table 2 shows that the cp/cp rats treated with probucol from 6 weeks of age showed no significant lowering of any of the plasma lipids compared to the cp/cp control animals at either 12 or 39 weeks of age.

3.2. Pancreatic morphology

Examination of the pancreatic sections showed that probucol treatment had no protective effect against the development of the marked hyperplasia of the islets of Langerhans that is characteristic of the cp/cp rat (Fig. 1A). The probucol-treated rats, however, did show a frequent occurrence of smaller islets with essentially normal morphology and without the extensive fibrosis and trabecular appearance of the hyperplastic islets (Fig. 1B). Such islets were not seen in the cp/cp control rats.

3.3. Aortic arch lesions

Scanning electron microscope examination of the aortic arch of probucol-treated cp/cp rats showed that, at 39 weeks of age, all rats exhibited advanced atherosclerotic lesions. In most animals, the entire aortic arch was affected

Table 2
Effect of probucol on whole serum lipids of male JCR:LA-cp rats

	Cholesterol	Cholesteryl esters	Total cholesterol	Phospholipids	Triglycerides
12-week-old rats					
+/+ control (6)	0.50 ± 0.03	1.11 ± 0.07	1.61 ± 0.09	0.94 ± 0.05	0.02 ± 0.16
cp/cp control (10)	0.76 ± 0.12	2.89 ± 0.35	3.60 ± 0.46	2.24 ± 0.31	4.05 ± 0.48
cp/cp probucol-treated (7)	0.88 ± 0.10	2.31 ± 0.25	3.19 ± 0.35	2.63 ± 0.26	3.28 ± 0.33
39-week-old rats					
+/+ control (10)	0.31 ± 0.03	0.93 ± 0.04	1.23 ± 0.05	0.77 ± 0.05	0.24 ± 0.04
cp/cp control (10)	0.93 ± 0.09	2.77 ± 0.29	3.68 ± 0.38	2.84 ± 0.28	3.49 ± 0.36
cp/cp probucol-treated (10)	0.76 ± 0.13	2.89 ± 0.35	3.60 ± 0.46	2.24 ± 0.31	4.05 ± 0.48

Values are mmol/l, mean ± S.E.M.; number of rats in Data parentheses. Data +/+ for rats are shown for reference only; no statistical comparisons made. There were no significant differences between probucol-treated and control rats at either age.

by large, raised intimal lesions, as shown in Fig. 2. These lesions were larger and more extensive than those seen in cp/cp control rats (Fig. 2A,C). While the raised lesions were more extensive in the probucol-treated rats, the endothelial covering over the lesions was smoother and did not show the areas of desquamation or ulceration often

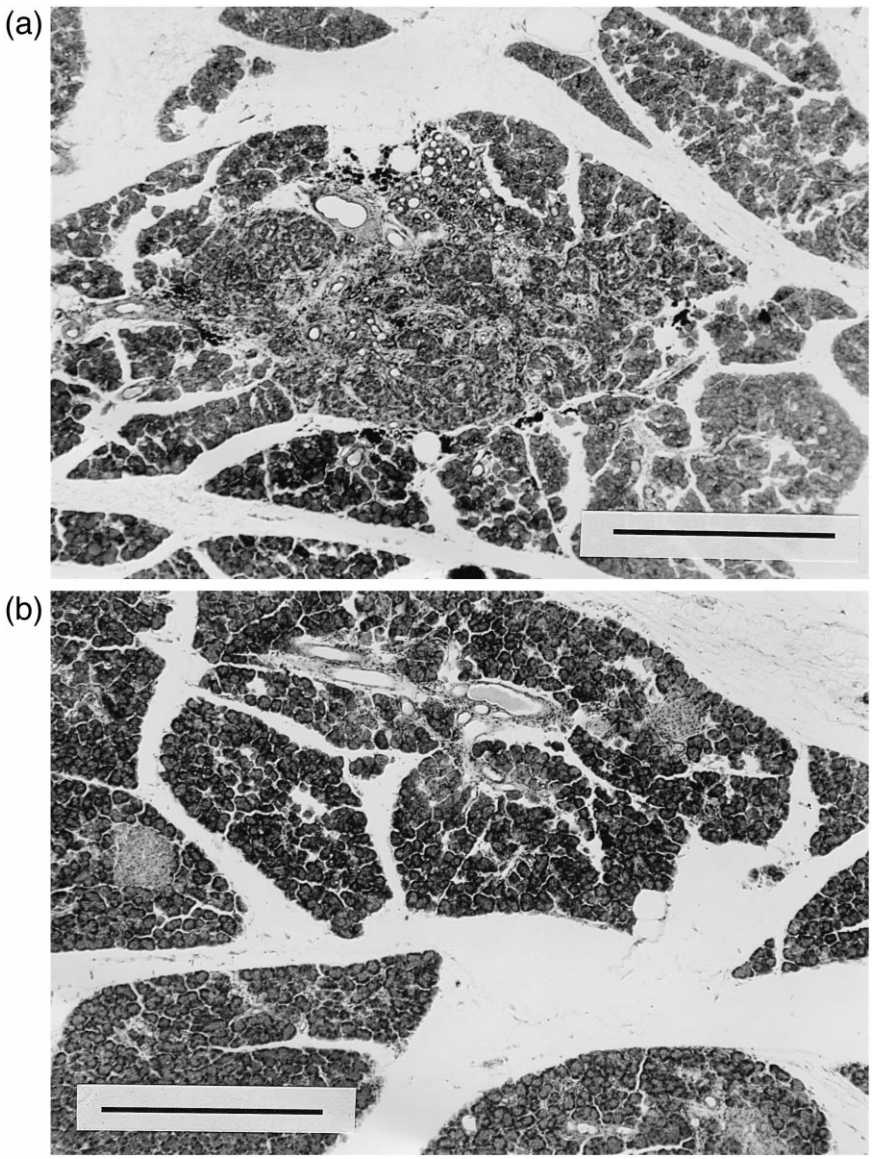

Fig. 1. Histological sections of the pancreata of 39-week-old cp/cp rats treated with probucol. (A) section with a typical, large trabecular islet with fibrosis. (B) section showing several small, morphologically normal islets of Langerhans. Hematoxylin and eosin stain, bar = 500 μm.

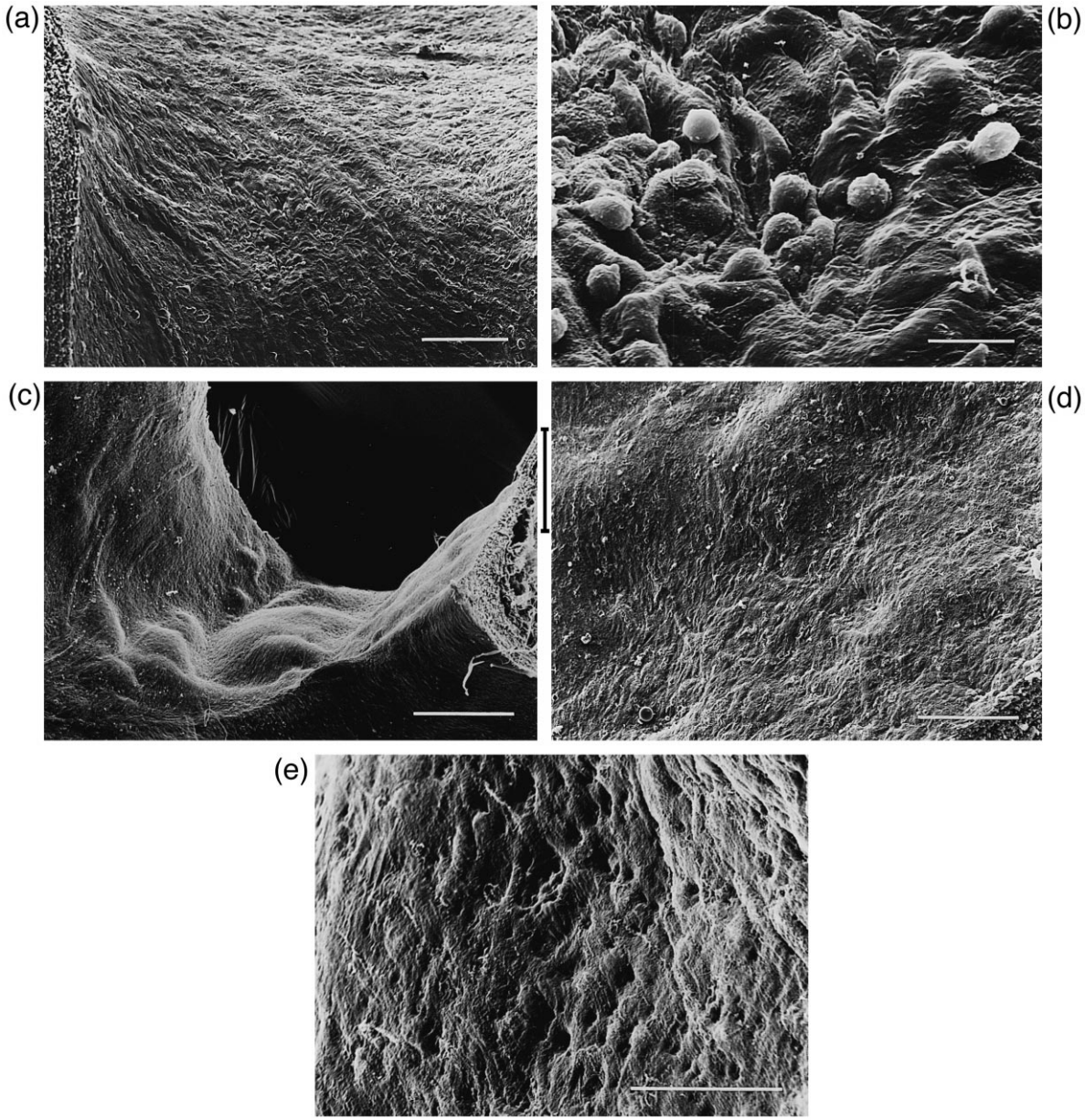

Fig. 2. Scanning electron photomicrographs of the aortic arch of male cp/cp rats at 39 weeks of age. (A) control rat, showing large raised intimal lesion; bar = 100 μm. (B) control rat, showing raised lesion with adherent macrophages; bar = 10 μm. (C) probucol-treated rat, showing severe raised intimal lesion with smooth overlying endothelium; bar = 250 μm. (D) probucol-treated rat, showing intact endothelium overlying raised lesion; bar = 100 μm. (E) probucol-treated rat, showing extensive fenestrations of the intact endothelial layer; bar = 25 μm.

seen in cp/cp control animals (Fig. 2D). Areas with clean, intact endothelium were commonly found on the surface of the aortas of probucol-treated rats that had extensive fenestrations or openings in the endothelial sheet (Fig. 2E). The severity score for raised lesions, as shown in Table 3, was not different between control and probucol-treated rats, as

Table 3
Severity of lesions of the aortic arch of male cp/cp rats

	Fibrin	Raised lesions	Macrophages	De-endothelialization
Control	0	2.75 ± 0.22	1.63 ± 0.41	0.63 ± 0.23
Probucol-treated	0.22 ± 0.21	2.78 ± 0.14	0.33 ± 0.16[a]	1.11 ± 0.10

Values are mean ± S.E.M. of the severity score in arbitrary units, determined as described in Section 2. There were 10 rats in each group.
[a] $P = 0.05$ vs. control.

Table 4
Frequency of myocardial lesions in male cp/cp rats treated with probucol

	Lesion stage			
	1	2	3	4
+ / + control	0	0	0.10 ± 0.10	0.10 ± 0.03
cp/cp control	0.33 ± 0.18	0.33 ± 0.18	0.33 ± 0.18	1.83 ± 0.40
cp/cp probucol-treated	0.20 ± 0.13	0	0	0.30 ± 0.15
Significance of difference, probucol treatment vs. cp/cp control	NS	NS	NS	$P < 0.01$

Values are mean ± S.E.M. of the frequency of lesions observed, as described in Section 2. There were 10 rats in each group. NS, not significant.

the advanced lesions seen in cp/cp control rats had previously defined the maximal severity score of 3. Probucol-treated rats had a significant reduction in adherent macrophages on the endothelial surface. Severity scores for endothelial damage, adherent macrophages, and fibrin deposition were not different between groups.

3.4. Myocardial lesions

The frequency of ischemic myocardial lesions in probucol-treated rats was found to be significantly lower than in cp/cp controls and not different from that in + / + controls (Table 4). No Stage 2 lesions (early cell lysis and chronic inflammatory infiltration) were seen, and the incidence of old, scarred lesions was significantly reduced ($P < 0.01$).

4. Discussion

4.1. Pharmacological response of the JCR:LA-cp rat

The JCR:LA-cp rat exhibits a status very close to that of the human obesity, insulin resistant, hypertriglyceridemic syndrome. The development of advanced vascular pathology and ischemic lesions makes it a particularly attractive model for the study of antiatherosclerotic and cardioprotective agents. Such a small-animal model permits both highly cost-effective studies of drug efficacy and basic mechanisms and experiments not possible with human subjects. We have examined the responses of the cp/cp rat, both metabolic and pathophysiological, to a variety of agents and have found that the rats consistently respond in a manner similar to that of humans. In the present study, the results again mirror those found clinically, but provide information not obtainable in human subjects.

4.2. Insulin and glucose metabolism

The male cp/cp rat is not frankly diabetic, and thus fasting plasma glucose concentrations did not differ between genotypes and probucol caused no change. The marked reduction in the hyperinsulinemia of probucol-treated rats at 12 weeks of age was, however, unexpected. The effect was no longer present in 39-week-old rats, suggesting that the effect is related to developmental changes in young rats between 6 and 12 weeks of age. While it is speculative, it is possible that the early improvement in insulin metabolism is due to the antioxidant properties of probucol. The protective effect may be insufficient to maintain an inhibition of excessive insulin synthesis and release or to reduce islet hyperplasia and damage as the rats age. The sub-population of relatively normal islets seen in 39-week-old rats may represent islets that have been partially protected by probucol. The protective effects seen in the islets may be related to later cardioprotective effects and are indicative of beneficial effects of probucol unrelated to lipid metabolism.

4.3. Lipid metabolism

Probucol has significant lipid-lowering properties in the presence of LDL receptor defects, such as is seen in the WHHL rabbit (Kita et al., 1987; Walldius et al., 1994). The origin of the hyperlipidemia in the cp/cp rat, in contrast, appears to lie in the marked insulin resistance and diversion of diet-derived glucose to triglyceride synthesis (Russell et al., 1989c, 1996). The triglyceride incorporated into VLDL (Vance and Russell, 1990) is then transported to the peripheral tissues, principally to the growing fat depots. The process is not expected to be inhibited by probucol, and this was confirmed in both young and old cp/cp rats (Table 2). This is in contrast to observed lipid-lowering effects of probucol in humans (Walldius et al., 1994), rabbits (Kita et al., 1987), and other strains of rats (Tada et al., 1992). However, these other subjects were lean and either more or less normolipidemic or hypercholesterolemic. The cp/cp rat, with its marked metabolic syndrome, is only mildly hypercholesterolemic, but is insulin resistant and highly hypertriglyceridemic—a quite different metabolic state. The modestly higher plasma cholesterol concentrations of the cp/cp rat are a consequence of the requirement for cholesterol and cholesterol esters to assemble and stabilize the increased flow of VLDL particles from the liver (Dolphin et al., 1987). The increased high-density lipoprotein concentrations are entirely due to flow-through from the VLDL particles (Vance and Russell, 1990). These processes, and the plasma cholesterol, could not be inhibited by probucol without

major disruption in the glucose/fatty acid metabolism of the rats, and this did not occur.

4.4. Vascular lesions

The extent and severity of the raised intimal lesions on the aortic arch of the probucol-treated male cp/cp rats are striking and the worst seen to date in this animal model. These lesions reflect the accumulation of lipid and lipid-rich cells in the intimal space (Richardson et al., 1997). The equivalence of plasma hyperlipidemia in the control and probucol-treated rats suggests that the process of VLDL particle infiltration through the endothelium would be similar in the two groups. The aortic surfaces of probucol-treated rats had significantly fewer adherent macrophages, but did frequently show fenestrations of the endothelial layer (Fig. 2E). Thus, the endothelium of the treated rats may have been more permeable to lipoprotein particles at the same time as the endothelial cells themselves were more healthy and less attractant to macrophages. Intimal macrophages appear to play a scavenger role, ingesting and oxidizing lipidemic materials. Only if overwhelmed do they become lipid-laden foam cells (Faggiotto et al., 1984). A reduced macrophage population may thus have allowed for increased accumulation of lipid and other products in the subendothelial space, leading to the observed exacerbation of the raised lesions. In such lesions, active vascular smooth muscle cells (Absher et al., 1997) migrating into the intimal space may become the dominant lipid-laden cells in the lesions. Unlike macrophages, such smooth muscle cells are unable to leave for the lumen (Faggiotto and Ross, 1984) and remove the ingested lipid.

4.5. Myocardial lesions

The reduction of the frequency of ischemic myocardial lesions is very clear and occurred in the presence of advanced atherosclerosis in the aorta. This implies that the protective mechanism must involve prevention of the secondary effects of atherosclerosis such as vasospasm and thrombus formation. The arteries of the male cp/cp rat have a defect in the nitric oxide-mediated relaxation mechanism that probably reflects both endothelial dysfunction and vascular smooth muscle cell hypercontractility (Absher et al., 1997; O'Brien et al., 1998). A role for vasospasm in the induction of the myocardial lesions is supported by the finding of cardioprotective effects by the Ca^{2+} channel antagonists, nifedipine and nisoldipine (Russell et al., 1990b, 1997). In addition, recent results show increased levels of plasminogen activator inhibitor-1 in plasma and in the arterial wall of the male cp/cp rat (Schneider et al., 1997). The resultant inhibition of tissue plasminogen activator would be expected to lead to the persistence of intravascular thrombi.

We suggest that the cardioprotection afforded by probucol is due to improvement in endothelial, and perhaps smooth muscle cell, function. This leads to both a greater integrity of the endothelial layer, with inhibition of fibrin formation, and a reduction in vasospasm. A further possibility is that probucol, perhaps through its antioxidant characteristics, affords protection against ischemia-induced myocardial damage (Tada et al., 1992).

4.6. Implications

Our results illustrate that inhibition of ischemic myocardial damage is possible even without the prevention of obesity, insulin resistance, hyperlipidemia, or even atherosclerosis. They are consistent with the finding of inhibition of restenosis in humans by Tardif et al. (1997). Our findings in an animal model of the obesity/insulin resistance/hypertriglyceridemia syndrome have potential implications for the treatment of atherosclerosis in a significant segment of the human population. The precise definition of the mechanisms involved would potentially permit the development of new, more effective agents than probucol.

Acknowledgements

This work was supported by the Heart and Stroke Foundation of Alberta and the Northwest Territories. Ming Chen, Sharon Ewoniak, Juno Brady, and Wolf Fazikas provided essential technical assistance. The probucol was a generous gift from Marion Merrell Dow.

References

Absher, P.M., Schneider, D.J., Russell, J.C., Sobel, B.E., 1997. Increased proliferation of explanted vascular smooth muscle cells: A marker presaging atherogenesis. Atherosclerosis 131, 187–194.

Amy, R.M., Dolphin, P.J., Pederson, R.A., Russell, J.C., 1988. Atherogenesis in two strains of obese rats. The fatty Zucker and LA/N-corpulent. Atherosclerosis 69, 199–209.

Dolphin, P.J., Stewart, B., Amy, R.M., Russell, J.C., 1987. Serum lipids and lipoproteins in the atherosclerosis-prone LA/N-corpulent rat. Biochim. Biophys. Acta 919, 140–148.

Emilsson, V., Liu, V.L., Cawthorne, M.A., Morton, N.M., Davenport, M., 1997. Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. Diabetes 46, 313–316.

Faggiotto, A., Ross, R., 1984. Studies of hypercholesterolemia in the nonhuman primate: II. Fatty streak conversion to fibrous cap. Arteriosclerosis 4, 341–356.

Faggiotto, A., Ross, R., Harker, L., 1984. Studies of hypercholesterolemia in the nonhuman primate: I. Changes that lead to fatty streak formation. Arteriosclerosis 4, 323–340.

Jackson, R.L., Barhart, R.C., Mar, S.J., 1991. Probucol and its mechanisms for reducing atherosclerosis. Adv. Exp. Med. Biol. 288, 367–372.

Kita, T., Nagano, Y., Yokode, M., Ishii, K., Kume, N., Ooshima, A., Yoshida, H., Kawai, C., 1987. Probucol prevents the progression of atherosclerosis in Watanabe Heritable Hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. Proc. Natl. Acad. Sci. USA 84, 5928–5931.

Kuksis, A., Myher, J.J., Geher, K., Hoffman, A.G.D., Breckenridge, W.C., Jones, G.J.L., Little, J.A., 1978. Comparative determination of plasma cholesterol and triacylglycerol levels by automated gas–liquid chromatographic and autoanalyzer techniques. J. Chromatogr. 146, 393–412.

Nagano, Y., Nakamura, T., Matsuzawa, Y., Cho, M., Veda, Y., Kita, T., 1992. Probucol and atherosclerosis in the Watanabe Heritable Hyperlipidemic rabbit: Long-term antiatherogenic effect and effects on established plaques. Atherosclerosis 29, 131–140.

O'Brien, S.F., McKendrick J.D., Radomski M.W., Davidge S.T., Russell J.C., 1998. Vascular wall reactivity in conductance and resistance arteries: Differential effects of insulin resistance. Can. J. Physiol. Pharmacol., in press.

Reaven, G.M., 1988. Role of insulin resistance in human disease. Diabetes 37, 1595–1607.

Richardson, M., Graham, S.E., DeReske, M., Dolphin, P.J., Russell, J.C., 1997. Response to cholesterol feeding by the JCR:LA-cp rat. Atherosclerosis 134, 315, (Abstract).

Russell, J.C., 1995. The atherosclerosis-prone JCR:LA-corpulent rat, In: Woodford, F.P., Davignon, J., Sniderman, A. (Eds.), Atherosclerosis X: Proceedings of the 10th International Symposium on Atherosclerosis. Elsevier, Amsterdam, pp. 121–125.

Russell, J.C., Amy, R.M., 1986a. Early atherosclerotic lesions in a susceptible rat model: The LA/N-corpulent rat. Atherosclerosis 60, 119–129.

Russell, J.C., Amy, R.M., 1986b. Myocardial and vascular lesions in the LA/N-corpulent rat. Can. J. Physiol. Pharmacol. 64, 1272–1280.

Russell, J.C., Ahuja, S.K., Manickavel, V., Rajotte, R.V., Amy, R.M., 1987. Insulin resistance and impaired glucose tolerance in the atherosclerosis-prone LA/N corpulent rat. Atherosclerosis 7, 620–626.

Russell, J.C., Amy, R.M., Graham, S., Dolphin, P.J., 1993. Effect of castration on hyperlipidemic, insulin resistant JCR:LA-corpulent rats. Atherosclerosis 100, 113–122.

Russell, J.C., Amy, R.M., Manickavel, V., Dolphin, P.J., 1989a. Effects of chronic ethanol consumption in atherosclerosis-prone JCR:LA-corpulent rat. Arteriosclerosis 9, 122–128.

Russell, J.C., Amy, R.M., Manickavel, V., Dolphin, P.J., Epling, W.F., Pierce, D., Boer, D.P., 1989b. Prevention of myocardial disease in JCR:LA-corpulent rats by running. J. Appl. Physiol. 66, 1649–1655.

Russell, J.C., Koeslag, D.G., Amy, R.M., Dolphin, P.J., 1989c. Plasma lipid secretion and clearance in hyperlipidemic JCR:LA-corpulent rats. Arteriosclerosis 9, 869–876.

Russell, J.C., Amy, R.M., Michaelis, O.E., McCune, S.M., Abraham, A.A., 1990a. Myocardial disease in the corpulent strains of rats. In: Shafrir, E. (Ed.), Frontiers in Diabetes Research: Lessons from Animal Diabetes III. Smith-Gordon, London, pp. 402–407.

Russell, J.C., Koeslag, D.G., Dolphin, P.J., Amy, R.M., 1990b. Prevention of myocardial lesions in JCR:LA-corpulent rats by nifedipine. Arteriosclerosis 10, 658–664.

Russell, J.C., Amy, R.M., Koeslag, D.G., Dolphin, P.J., 1991a. Independence of myocardial disease in the JCR:LA-corpulent rat of plasma cholesterol concentration. Clin. Invest. Med. 14, 288–295.

Russell, J.C., Dolphin, P.J., Hameed, M., Stewart, B., Koeslag, D.G., Rose-Kahn, G., Bar-Tana, J., 1991b. Hypolipidemic effect of β,β'-tetramethyl hexadecanedioic acid (MEDICA 16) in hyperlipidemic JCR:LA-corpulent rats. Arterioscler. Thromb. 11, 602–609.

Russell, J.C., Graham, S., Hameed, M., 1994. Abnormal insulin and glucose metabolism in the JCR:LA-corpulent rat. Metabolism 43, 538–543.

Russell, J.C., Amy, R.M., Graham, S.E., Dolphin, P.J., Wood, G.O., Bar-Tana, J., 1995. Inhibition of atherosclerosis and myocardial lesions in the JCR:LA-cp rat by β,β'-tetramethylhexadecanedioic acid (MEDICA 16). Arterioscler. Thromb. Vasc. Biol. 15, 918–923.

Russell, J.C., Graham, S.E., Dolphin, P.J., Brindley, D.N., 1996. Effects of benfluorex on serum triacylglycerols and insulin sensitivity in the corpulent rat. Can. J. Physiol. Pharmacol. 74, 879–886.

Russell, J.C., Dolphin, P.J., Graham, S.E., Amy, R.M., 1997. Cardioprotective and hypolipidemic effects of nisoldipine in the JCR:LA-cp rat. J. Cardiovasc. Pharmacol. 29, 586–592.

Schneider, D.J., Absher, P.M., Neimane, D., Russell, J.C., Sobel, B.E., 1997. Fibrinolysis and atherogenesis in the JCR:LA-cp rat in relation to insulin and triglyceride concentrations in blood. Diabetologia, in press.

Steiner, G., 1986. Hypertriglyceridemia and carbohydrate intolerance: Interrelations and therapeutic implications. Am. J. Cardiol. 57 (suppl), 27G–30G.

Tada, N., Mochizuki, S., Takayama, T., Yabe, H., Seki, S., Taniguchi, M., Nagano, M., 1992. Protective effect of probucol on ischemia-reperfusion arrhythmias in the rat heart. Can. J. Cardiol. 8, 975–980.

Tardif, J.-C., Coté, G., Lesperance, J., Bourassa, M., Lambert, J., Doucet, S., Bilodeau, L., Nattel, S., de Guise, P., 1997. For the Multivitamins and Probucol Study Group. Probucol and multivitamins in the prevention of restenosis after coronary angioplasty. N. Engl. J. Med. 337, 365–372.

Vance, J.E., Russell, J.C., 1990. Hypersecretion of VLDL, but not HDL, by hepatocytes from the JCR:LA-corpulent rat. J. Lipid Res. 31, 1491–1501.

Walldius, G., Erikson, U., Olsson, A.G. et al., 1994. The effect of probucol on femoral atherosclerosis: The Probucol Quantitative Regression Swedish Trial (PQRST). Am. J. Cardiol. 74, 875–883.

Wu-Peng, X.S., Chua, S.C. Jr., Okada, N., Liu, S.-M., Nicolson, M., Leibel, R.L., 1997. Phenotype of the obese Koletsky (f) rat due to Tyr763Stop mutation in the extracellular domain of the leptin receptor (Lepr). Diabetes 46, 513–518.