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Combined effect of probucol and insulin on cataracts of diabetic rats fed a high cholesterol diet

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Abstract

We investigated the effects of long-term treatment with probucol, a hypolipidemic agent with antioxidative action, insulin, or their combination on cataracts of streptozotocin-induced diabetic rats fed a high cholesterol diet. Each rat was checked for cataracts at 0, 1, 2, 4, 8, 12 and 15 weeks after streptozotocin injection. Cataracts were observed from 8 weeks in untreated hypercholesterolemic and diabetic rats and the incidence of catarats increased to 100% by 15 weeks. The incidence of cataracts in rats treated with probucol, insulin and their combination was first seen at 12, 12 and 15 weeks, respectively, and was 86%, 63% and 33%, respectively, at 15 weeks.

The preventive effects of both agents alone and their combination on the cataracts were confirmed by histopathological evaluation of eyeballs. The combined treatment with both agents markedly improved hyperglycemia, hyperlipidemia and increased serum lipid peroxide levels. These results indicate that the combined treatment with probucol and insulin is useful in preventing the development and progression of diabetic cataracts.

Keywords: Diabetes; Cataract; Hyperglycemia; Hyperlipidemia; Lipid peroxide

1. Introduction

Diabetes mellitus is a disease characterized by hyperglycemia, and its complications have been attributed to the long duration of this condition due to the abnormality of glucose metabolism (Brownlee et al., 1988; Brownlee, 1992). At the same time, hyperlipidemia has been frequently observed in insulin-dependent (Nikkila and Kekki, 1973; Nikkila and Hormila, 1978) and non-insulin-dependent diabetic patients (Reaven and Greenfield, 1981); it was observed in experimental insulin-dependent (Hirano et al., 1991; Ito et al., 2001) and non-insulin-dependent diabetic animal models (Man et al., 1997). Therefore, hyperlipidemia in addition to hyperglycemia has been also thought to be a major risk factor for the development and progression of diabetic complications such as vasospastic angina (Garcia et al., 1974), nephropathy (Ravid et al., 1998; Dominguez et al., 2000), cataract (Tsutsumi et al., 1999) and peripheral neuropathy (Gottsater et al., 1999). Recently, it has been reported that under diabetic conditions, oxygen-derived free radicals are easily produced and may play a role in the development of diabetic complications (Jakus, 2000; Gurler et al., 2000; West, 2000). Under hyperglycemic conditions, oxygen-derived free radicals are produced mainly through glycation reaction (Sakurai and Tsuchiya, 1988; Hund et al., 1991). Furthermore, it has been shown that the production of oxygen-derived free radicals is enhanced in the arteries of hypercholesterolemic rabbits (Mügge et al., 1994). These findings suggest that hyperlipidemia in addtion to hyperglycemia may play a role in the pathogenesis of diabetic complications through enhanced generation of oxygen-derived free radicals.

Probucol has been widely used clinically for the prevention of the progression of atherosclerosis, because this agent acts as a potent antioxidant (Parthasarathy et al., 1986; Regnstrom et al., 1990; Baumastark et al., 1992) in addition to having a lipid-lowering action (Yamamoto et al.,

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1986; Schwartz, 1988). There have been a few reports concerning beneficial effects of probucol on experimental diabetes mellitus. Pretreatment with probucol, for example, reportedly prevents the in vivo diabetogenic actions of alloxan in rats probably by its strong free radical scavenger action(Matsushita et al., 1989). Probucol has been also shown to prevent the beta-cell function in diabetic C57BL/KsJ-db/db mice through reduction of oxidative stress (Gorogawa et al., 2002). On the other hand, insulin has been widely used for the treatment of type 1 (insulin-dependent) diabetes mellitus as a hormone supplement therapy. From these findings, the combination of probucol and insulin is expected to be beneficial in preventing diabetic complications.

In the first half of the present experiment, we clarified that a long-term high cholesterol diet markedly increased the incidence of streptozotocin-induced insulin-dependent diabetic cataracts in rats with severe hyperlipidemia, and markedly increased serum lipid peroxide levels. This result is in agreement with that of Tsutsumi et al. (1999) who reported that the cholesterol-rich diet, instead of a standard diet, accelerated the progression of streptozotocin-induced diabetic cataracts in rats.

Therefore, the aim of the present study was to investigate the effects of long-term treatment with probucol, a hypolipidemic agent with antioxidative action, insulin or their combination on the development and progression of cataracts of streptozotocin-induced diabetic rats fed a high cholesterol diet.

2. Materials and methods

2.1. Animals

Seven-week-old male Sprague–Dawley rats (Charles River, Hino, Japan) were housed in an isolator caging system in an air-conditioned animal room at 23 ± 1 °C. All experimental procedures described were approved by the Committee for Ethics and Animal Experimentation, Nihon Bioresearch Inc.

2.2. Drugs

The drugs used were probucol (Sinlestal®, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) and insulin [Isophan Insulin (Aqueous Suspension), NPHiszilin®, Takeda Pharmaceutical Co., Ltd., Osaka, Japan).

2.3. Induction of diabetes and experimental procedure

Rats were fasted for 20 h before the experiment and then divided into groups. They were fed a standard diet before the experiment and a standard diet, high cholesterol diet or probucol-supplemented high cholesterol diet after onset of the experiment. The standard diet (pellets) consisted of 20% casein, 63.2% sucrose, 10% corn oil, 2% agar, 0.8% vitamin mixture and 4% salt mixture. The high cholesterol diet (pellets) consisted of the standard diet with 1% cholesterol (wt/wt) and 0.5% cholic acid (wt/wt) in place of an equal amount of sucrose. The probucol-supplemented diet contained 1% probucol (wt/wt) in the high cholesterol diet. These diets were produced by Oriental Yeast Co., Ltd. (Tokyo, Japan). To induce insulin-deficient diabetes, the fasted rats in five groups were injected intravenously with streptozotocin (Sigma Chemical Co., Ltd., St Louis, MO, U.S.A.) dissolved in a citrate buffer (0.1 M with pH 4.5) at a dose of 40 mg/kg. The fasted rats in two groups were injected with the equivalent volume of citrate buffer as non-diabetic normal groups. Experimental groups were the following (Table 1): (1) Standard diet-fed normal (SD-fed Nor) group, (2) high cholesterol diet-fed normal (HCD-fed Nor) group, (3) standard diet-fed diabetic (SD-fed Diab) group, (4) high cholesterol diet-fed diabetic (HCD-fed Diab) group, (5) probucol-supplemented high cholesterol diet-fed diabetic (Prob-suppl HCD-fed Diab) group, (6) high cholesterol diet-fed diabetic+insulin (HCD-fed Diab+Ins) group and (7) probucol-supplemented high cholesterol diet-fed diabetic+insulin (Prob-suppl HCD-fed Diab+Ins) group. The rats in HCD-fed Diab+Ins and Prob-suppl HCD-fed Diab+Ins groups received a daily s.c. injection (2 U/rat) of insulin at 10:00 a.m. from the day after the start of the experiment. The body weight and food consumption for 24 h of each rat were measured at 0 (before the injection of streptozotocin or citrate buffer), 1, 2, 4, 8, 12 and 15 weeks after the start of the experiment. Immediately before measurement of the weight, each rat was checked for cataracts. Blood samples were withdrawn from the cavernous sinus with a capillary under light-ether anesthesia at 9:00 a.m. for determination of serum glucose, total cholesterol, triglyceride and lipid peroxide contents. These biochemical parameters (except for lipid peroxide) were determined using Automated Chemistry Analyzer (AU 400, Olympus Optical Co., Ltd., Tokyo). Namely, serum glucose, total cholesterol and triglyceride levels were determined by glucose dehydrogenase, CODEDAOS and GPOEDAOS methods, respectively. At 8 and 15 weeks, the serum lipid peroxide was determined as the concentration of malondialdehyde, a secondary product of lipid peroxidation, by adding thiobarbituric acid in accordance with the method of Yagi (1976). At 4, 8 and 15 weeks, animals selected from each group were sacrificed by decapitation and their eyeballs were removed under light ether anesthesia for histopathological evaluation.

2.4. Histopathological evaluation of eyeballs

Eyeballs removed for evaluation were fixed in 10% neutral buffered formalin. Paraffin sections (2 μm) were

Table 1
Effects of a long-term treatment with probucol, insulin or their combination on body weight and food consumption in streptozotocin-induced diabetic rats fed a high cholesterol diet

Group	A. Body weight (g)							B. Food consumption (g)						
	0	1	2	4	8	12	15 (weeks)	0	1	2	4	8	12	15 (weeks)
SD-fed Nor	287 ± 3 (n=10)	312 ± 5 (n=10)	374 ± 8 (n=10)	415 ± 11 (n=10)	463 ± 12 (n=10)	497 ± 14 (n=10)	515 ± 13 (n=10)	22 ± 1 (n=10)	23 ± 0 (n=10)	24 ± 0 (n=10)	23 ± 1 (n=10)	22 ± 1 (n=10)	22 ± 1 (n=10)	24 ± 1 (n=10)
HCD-fed Nor	286 ± 4 (n=10)	310 ± 5 (n=10)	361 ± 5 (n=10)	403 ± 7 (n=10)	447 ± 9 (n=10)	473 ± 8 (n=10)	505 ± 10 (n=10)	24 ± 1 (n=10)	23 ± 1 (n=10)	23 ± 1 (n=10)	23 ± 1 (n=10)	23 ± 1 (n=10)	25 ± 0 (n=10)	24 ± 0 (n=10)
SD-fed Diab	283 ± 3 (n=10)	263 ± 5^{aa} (n=10)	273 ± 8^{aa} (n=10)	259 ± 11^{aa} (n=10)	248 ± 15^{aa} (n=9)	243 ± 14^{aa} (n=9)	232 ± 18^{aa} (n=9)	23 ± 1 (n=10)	37 ± 4^{a} (n=10)	47 ± 1^{aa} (n=10)	48 ± 2^{aa} (n=10)	45 ± 2^{aa} (n=9)	41 ± 2^{aa} (n=9)	39 ± 3^{aa} (n=9)
HCD-fed Diab	288 ± 3 (n=10)	266 ± 6^{aa} (n=10)	266 ± 8^{aa} (n=10)	255 ± 14^{aa} (n=8)	230 ± 21^{aa} (n=6)	237 ± 21^{aa} (n=5)	257 ± 30^{aa} (n=3)	22 ± 1 (n=10)	38 ± 3^{aa} (n=10)	57 ± 2^{aa,bb} (n=10)	55 ± 3^{aa} (n=8)	48 ± 6^{aa} (n=6)	41 ± 5^{aa} (n=5)	46 ± 9^{aa} (n=3)
Prob-suppl HCD-fed Diab	282 ± 3 (n=10)	263 ± 6 (n=10)	269 ± 6 (n=10)	263 ± 8 (n=10)	267 ± 11 (n=8)	265 ± 15 (n=8)	272 ± 21 (n=7)	20 ± 1 (n=10)	39 ± 3 (n=10)	60 ± 3 (n=10)	61 ± 2 (n=10)	56 ± 3 (n=8)	52 ± 3 (n=8)	51 ± 3 (n=7)
HCD-fed Diab+Ins	281 ± 3 (n=10)	253 ± 6 (n=10)	272 ± 7 (n=10)	271 ± 10 (n=10)	295 ± 11^{cc} (n=10)	299 ± 16 (n=8)	293 ± 17 (n=8)	18 ± 1 (n=10)	28 ± 3^{c} (n=10)	44 ± 2^{c} (n=10)	44 ± 2^{cc} (n=10)	41 ± 3 (n=10)	34 ± 1 (n=8)	30 ± 2 (n=8)
Prob-suppl HCD-fed Diab+Ins	281 ± 6 (n=10)	251 ± 8 (n=10)	272 ± 8 (n=10)	272 ± 10 (n=10)	291 ± 12^{c} (n=10)	283 ± 13 (n=10)	283 ± 11 (n=9)	19 ± 2 (n=10)	30 ± 2^{c} (n=10)	38 ± 1^{cc} (n=10)	41 ± 2^{cc} (n=10)	39 ± 2 (n=10)	37 ± 2 (n=10)	35 ± 2 (n=9)

Each value represents the mean ± S.E. n indicates the number of rats used.
[a] $P<0.05$, as compared with the SD-fed Nor group (Dunnett's test).
[aa] $P<0.01$, as compared with the SD-fed Nor group (Dunnett's test).
[bb] $P<0.01$, as compared with the SD-fed Diab group (Student's t-test).
[c] $P<0.05$, as compared with the HCD-fed Diab group (Dunnett's test).
[cc] $P<0.01$, as compared with the HCD-fed Diab group (Dunnett's test).

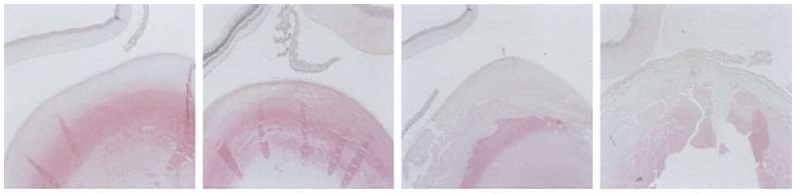

Fig. 1. Representative photomicrographs of eyeballs from normal and streptozotocin-induced diabetic rats with cataracts. HE stain ×25. The severity of histopathological changes (disarrangement of lens fiber) in eyeballs (0: normal, 1: mild, 2: moderate, 3: severe).

stained with hematoxylin and eosin (HE) for light microscopy. The degree of histopathological changes (disarrangement of lens fiber) in eyeballs of each animal was scored as follows. Normal: score 0, mild: score 1, moderate: score 2 and severe: score 3. Representative micrographs of the degree of histopathological changes in eyeballs are shown in Fig. 1. The average score of each group was calculated for the histopathological evaluation.

2.5. Statistical analysis

Results obtained were expressed as the mean ± S.E. The data, except for the mortality rate and the incidence rate of cataracts, were analyzed for statistical significance by Dunnett's test for multiple comparison or Student's *t*-test for comparison between the two groups. The statistical analysis for the mortality rate and the incidence rate of the cataracts was conducted using Fisher's exact test.

3. Results

3.1. Body weight and food consumption

The average body weight of rats in all groups at the onset of the experiment was 282–288 g (Table 1A). The body weight of the SD-fed Nor and HCD-fed Nor groups increased by 228 g and 219 g, respectively, by 15 weeks when the experiment was terminated (Table 1A). There was no significant difference in the weight gain between these two groups. On the other hand, the body weight of the SD-fed Diab and HCD-fed Diab (control) groups decreased be 51 g and 31 g, respectively, by 15 weeks.

The body weight of the HCD-fed Diab+Ins and Prob-suppl HCD-fed Diab+Ins groups increased by 12 g and 2 g, respectively, by 15 weeks after streptozotocin administration, although those of the Prob-suppl HCD-fed Diab group decreased by 10 g. Thus, daily treatment with insulin or the combination of probucol and insulin suppressed the loss of the body weight caused by streptozotocin-induced diabetes mellitus.

The food consumption of the SD-fed Nor and HCD-fed Nor groups was 22–24 g/24 h and 23–25 g/24 h, respectively, throughout the observation period of 1–15 weeks (Table 1B), while that of the SD-fed Diab and HCD-fed Diab (control) groups was 1.6–2.1 times and 1.7–2.4 times, respectively, significantly greater than that of the SD-fed Nor group throughout 1–15 weeks.

The animals in the HCD-fed Diab+Ins and Prob-suppl HCD-fed Diab+Ins groups consumed less food than those in the HCD-fed Diab (control) group, although there was no apparent difference between the HCD-fed Diab and Prob-suppl HCD-fed Diab.

In Table 1A and B, the decrease in the number (*n*) of the animals isdue to the increase in the number of dead animals.

3.2. Non-fasting serum glucose, total cholesterol, triglyceride and lipid peroxide levels

The non-fasting serum glucose levels of the SD-fed Nor and HCD-fed Nor groups were 122–153 mg/dl and 107–149 mg/dl, respectively, throughout the observation period of 1–15 weeks (Table 2). The serum glucose levels of the SD-fed Diab and HCD-fed Diab (control) groups rose markedly from 1 week after streptozotocin administration (1 week: 567 ± 29 mg/dl and 584 ± 18 mg/dl, respectively), and almost the same high levels were maintained up to 15 weeks. No significant difference in the serum glucose levels was seen between the SD-fed Nor and HCD-fed Nor groups or between the SD-fed Diab and HCD-fed Diab groups.

The serum glucose levels of the HCD-fed Diab+Ins and Prob-suppl HCD-fed Diab+Ins groups were significantly lower than those of the HCD-fed Diab (control) group from 2 weeks (8 weeks: HCD-fed Diab, 634 ± 42 mg/dl vs. HCD-fed Diab+Ins, 308 ± 21 mg/dl, $P<0.01$; Prob-suppl HCD-fed Diab+Ins, 272 ± 19 mg/dl, $P<0.01$).

The non-fasting serum total cholesterol levels of the SD-fed Nor, HCD-fed Nor, SD-fed Diab and HCD-fed Diab groups were 64–73 mg/dl, 132–365 mg/dl, 134–345 mg/dl and 2735–6756 mg/dl, respectively, throughout the observation period of 1–15 weeks after streptozotocin administration (Table 3A). The total cholesterol levels of the HCD-fed Nor and SD-fed Diab groups were 1.9–5.7 times and 2.1–4.8 times, respectively, higher than those of the SD-fed Nor group throughout this period, while the total cholesterol levels of the HCD-fed Diab group were 16.0–29.0 times higher than those of the SD-fed Diab group. Thus, the serum total cholesterol levels of streptozotocin-induced diabetic rats were markedly increased by high cholesterol diet feeding.

The total cholesterol levels of the Prob-suppl HCD-fed Diab, HCD-fed Diab+Ins and Prob-suppl HCD-fed

Table 2
Effects of a long-term treatment with probucol, insulin or their combination on serum glucose in streptozotocin-induced diabetic rats fed a high cholesterol diet

Group	Serum glucose						
	0	1	2	4	8	12	15 (weeks)
SD-fed Nor	140 ± 5	133 ± 2	122 ± 2	129 ± 4	138 ± 3	131 ± 3	153 ± 3
	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)
HCD-fed Nor	140 ± 1	107 ± 1	117 ± 3	131 ± 4	110 ± 2	116 ± 2	149 ± 3
	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)
SD-fed Diab	127 ± 2	567 ± 29^{aa}	588 ± 20^{aa}	562 ± 19^{aa}	552 ± 28 ^{aa}	531 ± 33^{aa}	487 ± 55^{aa}
	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 9)	(*n* = 9)	(*n* = 9)
HCD-fed Diab	131 ± 2	584 ± 18^{aa}	663 ± 25^{aa}	570 ± 107^{aa}	634 ± 42^{aa}	540 ± 51^{aa}	491 ± 27^{aa}
	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 8)	(*n* = 6)	(*n* = 5)	(*n* = 3)
Prob-suppl	130 ± 4	582 ± 28	595 ± 24	566 ± 32	624 ± 33	569 ± 30	474 ± 20
HCD-fed Diab	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 8)	(*n* = 8)	(*n* = 7)
HCD-fed	124 ± 2	546 ± 21	456 ± 21^{cc}	406 ± 17^{cc}	308 ± 21^{cc}	277 ± 14^{cc}	298 ± 19^{cc}
Diab+Ins	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 8)	(*n* = 8)
Prob-suppl	133 ± 4	534 ± 18	429 ± 21^{cc}	381 ± 23^{cc}	272 ± 19^{cc}	305 ± 22^{cc}	278 ± 22^{cc}
HCD-fed Diab+Ins	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 9)

Each value represents the mean ± S.E. *n* indicates the number of rats used.
^{aa} $P<0.01$, as compared with the SD-fed Nor group (Dunnett's test).
^{cc} $P<0.01$, as compared with the HCD-fed Diab group (Dunnett's test).

Diab+Ins groups were significantly lower than those of the HCD-fed Diab (control) group throughout 1–15 weeks (12 weeks: HCD-fed Diab, 5892 ± 502 mg/dl vs. Prob-suppl HCD-fed Diab, 3466 ± 932 mg/dl, $P<0.05$; HCD-fed Diab+Ins, 944 ± 121 mg/dl, $P<0.01$; Prob-suppl HCD-fed Diab+Ins, 1758 ± 284 mg/dl, $P<0.05$). Thus, daily treatment with probucol, insulin or the combination of both agents markedly suppressed hypercholesterolemia in the HCD-fed Diab rats.

The non-fasting serum triglyceride levels of the SD-fed Nor, HCD-fed Nor, SD-fed Diab and HCD-fed Diab groups were 89–194 mg/dl, 98–184 mg/dl, 668–2108 mg/dl and 993–3637 mg/dl throughout the experimental period of 1–15 weeks (Table 3B). Thus, the rats of the SD-fed Diab and HCD-fed Diab (control) groups exhibited hypertriglyceridemia. The serum triglyceride levels of the HCD-fed Diab were 1.8 times and 1.7 times, respectively, higher than those of the SD-fed Diab at 8 and 12 weeks but there was no significant difference in the triglyceride levels between both groups.

The triglyceride levels of the Prob-suppl HCD-fed Diab, HCD-fed Diab+Ins and Prob-suppl HCD-fed Diab+Ins groups were significantly lower than those of the HCD-fed Diab (control) group throughout 2–12 weeks after streptozotocin administration (12 weeks: HCD-fed Diab, 3637 ± 899 mg/dl vs. the Prob-suppl HCD-fed Diab, 1850 ± 290 mg/dl, $P<0.05$; HCD-fed Diab+Ins, 218 ± 40 mg/dl, $P<0.01$; Prob-suppl HCD-fed Diab+Ins, 234 ± 33 mg/dl, $P<0.01$).

The non-fasting serum lipid peroxide levels of the SD-fed Nor, HCD-fed Nor and SD-fed Diab groups at 8 and 15 weeks after streptozotocin administration were 2.6–3.9 nmol/ml, and there were no significant differences among the three groups (Table 4). On the other hand, the lipid peroxide levels of the HCD-fed Diab (control) group were 3.8 times and 6.4 times, respectively, higher than those of the respective SD-fed Nor group at 8 and 15 weeks. Thus, the serum lipid peroxide levels of streptozotocin-induced diabetic rats were markedly increased by high cholesterol diet feeding.

The lipid peroxide levels of the Prob-suppl HCD-fed Diab, HCD-fed Diab+Ins and Prob-suppl HCD-fed Diab+Ins groups were maintained at almost normal levels at both weeks measured. Thus, the increase in the serum lipid peroxide levels of diabetic rats induced by high cholesterol diet feeding was completely suppressed by daily treatment with probucol, insulin or the combination of both agents.

3.3. Mortality rate and the incidence rate of cataracts

No animals died in the SD-fed Nor or HCD-fed Nor group during the 15-week observation period after the start of the experiment (Table 5A). The mortality of animals in the SD-fed Diab group began from 8 weeks after streptozotocin administration and the mortality rate was 10% throughout 8–15 weeks. The mortality rate in the HCD-fed Diab (control) group was 20% at 4 weeks, then continued to increase reaching 70% by 15 weeks.

The death of animals in the Prob-suppl HCD-fed Diab, HCD-fed Diab+Ins and Prob-suppl HCD-fed Diab+Ins groups was first observed at 8, 12 and 15 weeks, respectively, with a mortality rate of 30%, 20% and 10%, respectively, at 15 weeks.

The diabetic cataracts in the SD-fed Diab group appeared at 12 and 15 weeks after streptozotocin administration, with an incidence rate of 56% (Table 5B). The cataracts in the HCD-fed Diab (control) group appeared at 8 weeks, earlier than in the SD-fed Diab group, with an incidence rate of 17% and the incidence of cataracts increased to 80% and 100%, respectively, at 12 and 15 weeks.

Table 3
Effects of a long-term treatment with probucol, insulin or their combination on serum total cholesterol and triglyceride levels in streptozotocin-induced diabetic rats fed a high cholesterol diet

Group	A. Serum total cholesterol (mg/dl)							B. Serum triglyceride (mg/dl)						
	0	1	2	4	8	12	15 (weeks)	0	1	2	4	8	12	15 (weeks)
SD-fed Nor	72 ± 2 (n = 10)	64 ± 3 (n = 10)	66 ± 3 (n = 10)	72 ± 4 (n = 10)	71 ± 5 (n = 10)	73 ± 4 (n = 10)	71 ± 4 (n = 10)	108 ± 8 (n = 10)	138 ± 11 (n = 10)	171 ± 16 (n = 10)	177 ± 20 (n = 10)	194 ± 16 (n = 10)	136 ± 13 (n = 10)	89 ± 9 (n = 10)
HCD-fed Nor	79 ± 3 (n = 10)	365 ± 55 (n = 10)	255 ± 22 (n = 10)	202 ± 12 (n = 10)	174 ± 13 (n = 10)	179 ± 11 (n = 10)	132 ± 7 (n = 10)	147 ± 18 (n = 10)	143 ± 7 (n = 10)	156 ± 12 (n = 10)	145 ± 11 (n = 10)	184 ± 7 (n = 10)	161 ± 15 (n = 10)	98 ± 7 (n = 10)
SD-fed Diab	74 ± 3 (n = 10)	134 ± 17 (n = 10)	193 ± 29 (n = 10)	345 ± 71 (n = 10)	323 ± 82 (n = 9)	277 ± 55 (n = 9)	324 ± 114 (n = 9)	73 ± 8 (n = 10)	668 ± 150 (n = 10)	986 ± 198[aa] (n = 10)	1118 ± 176[a] (n = 10)	1648 ± 316[aa] (n = 9)	2108 ± 498[aa] (n = 9)	1145 ± 424[aa] (n = 9)
HCD-fed Diab	66 ± 2 (n = 10)	2735 ± 404[aa,bb] (n = 10)	5599 ± 561[aa,bb] (n = 10)	6133 ± 715[aa,bb] (n = 8)	6756 ± 682[aa,bb] (n = 6)	5892 ± 502[aa,bb] (n = 5)	5178 ± 1342[aa] (n = 3)	90 ± 11 (n = 10)	993 ± 310[aa] (n = 10)	1082 ± 140[aa] (n = 10)	1964 ± 457[aa] (n = 8)	2911 ± 611[aa] (n = 6)	3637 ± 899[aa] (n = 5)	1651 ± 492[aa] (n = 3)
Prob-suppl HCD-fed Diab	64 ± 4 (n = 10)	1571 ± 359[c] (n = 10)	2684 ± 641[cc] (n = 10)	4012 ± 780[c] (n = 10)	3684 ± 1019[cc] (n = 8)	3466 ± 932[c] (n = 8)	2387 ± 925[c] (n = 7)	95 ± 15 (n = 10)	606 ± 210 (n = 10)	484 ± 129[c] (n = 10)	704 ± 225 (n = 10)	969 ± 490[cc] (n = 8)	1850 ± 290[c] (n = 8)	1149 ± 687 (n = 7)
HCD-fed Diab+Ins	60 ± 3 (n = 10)	559 ± 69[cc] (n = 10)	634 ± 77[cc] (n = 10)	1148 ± 190[cc] (n = 10)	807 ± 163[cc] (n = 10)	944 ± 121[cc] (n = 8)	1084 ± 186[c] (n = 8)	92 ± 13 (n = 10)	531 ± 105 (n = 10)	353 ± 73[cc] (n = 10)	311 ± 57[cc] (n = 10)	131 ± 19[cc] (n = 10)	218 ± 40[cc] (n = 8)	248 ± 38 (n = 8)
Prob-suppl HCD-fed Diab +Ins	66 ± 4 (n = 10)	584 ± 56[cc] (n = 10)	886 ± 145[cc] (n = 10)	1431 ± 153[cc] (n = 10)	1189 ± 202[cc] (n = 10)	1758 ± 284[c] (n = 10)	1306 ± 249[c] (n = 9)	82 ± 11 (n = 10)	663 ± 127 (n = 10)	389 ± 60[c] (n = 10)	360 ± 56[c] (n = 10)	144 ± 21[cc] (n = 10)	234 ± 33[cc] (n = 10)	220 ± 32 (n = 9)

Each value represents the mean ± S.E. n indicates the number of rats used.

[a] $P<0.05$, as compared with the SD-fed Nor group (Dunnett's test).

[aa] $P<0.01$, as compared with the SD-fed Nor group (Dunnett's test).

[bb] $P<0.01$, as compared with the SD-fed Diab group (Student's t-test).

[c] $P<0.05$, as compared with the HCD-fed Diab group (Dunnett's test).

[cc] $P<0.01$, as compared with the HCD-fed Diab group (Dunnett's test).

Table 4
Effects of a long-term treatment with probucol, insulin or their combination on serum lipid peroxide levels in streptozotocin-induced diabetic rats fed a high cholesterol diet

Group	Serum lipid peroxide (nmol/ml)	
	8	15 (weeks)
SD-fed Nor	2.6 ± 0.1 (n=10)	2.6 ± 0.1 (n=10)
HCD-fed Nor	2.8 ± 0.1 (n=10)	2.6 ± 0.1 (n=10)
SD-fed Diab	3.1 ± 0.1 (n=9)	3.9 ± 0.2 (n=9)
HCD-fed Diab	10.0 ± 0.9^{aa,bb} (n=6)	16.7 ± 1.7^{aa,bb} (n=3)
Prob-suppl HCD-fed Diab	3.4 ± 0.2^{cc} (n=8)	3.5 ± 0.2^{cc} (n=7)
HCD-fed Diab+Ins	3.7 ± 0.2^{cc} (n=10)	4.8 ± 0.5^{cc} (n=8)
Prob-suppl HCD-fed Diab+Ins	3.0 ± 0.1^{cc} (n=10)	3.3 ± 0.2^{cc} (n=9)

Each value represents the mean ± S.E. n indicates the number of rats used.
[aa] P<0.01, as compared with the SD-fed Nor group (Dunnett's test).
[bb] P<0.01, as compared with the SD-fed Diab group (Student's t-test).
[cc] P<0.01, as compared with the HCD-fed Diab group (Dunnett's test).

In contrast to this, the incidence of cataracts in the the Prob-suppl HCD-fed Diab, HCD-fed Diab+Ins and Prob-suppl HCD-fed Diab+Ins groups were first seen at 12, 12 and 15 weeks, and with incidence rates of 50%, 25% and 0%, respectively, at 12 weeks and 86%, 63% and 33%, respectively, at 15 weeks. Thus, the incidence rate of cataracts and the mortality rate in streptozotocin-induced diabetic animals were markedly increased by the high cholesterol diet feeding. However, long-term treatment with probucol, insulin or the combination of both agents markedly restricted the increase in both parameters. Combined treatment with both agents was the most effective.

3.4. Histopathology of eyeballs

When histopathological changes in eyeballs for cataracts were evaluated at 4, 8 and 15 weeks after streptozotocin treatment, the disarrangement of lens fiber in eyeballs was observed at all weeks in animals evaluated in the HCD-fed Diab group (control) but only at 15 weeks in animals of the SD-fed Diab group (Table 6).

The severity of the disarrangement of lens fiber in the Prob-suppl HCD-fed Diab, HCD-fed Diab+Ins and Prob-suppl HCD-fed Diab+Ins groups significantly decreased by 33%, 47% and 67%, respectively, compared to that of the HCD-fed Diab (control) group, at 15 weeks.

4. Discussion

The present study indicates that a long-term high cholesterol diet for streptozotocin-induced diabetic rats not only precipitates the onset of cataracts, but also increases the incidence of this complication as well as the mortality rate. Furthermore, the results demonstrate that the long-term combined treatment with probucol, a hypolipidemic agent with antioxidative action, and insulin synergistically prevents the development and progression of cataracts in streptozotocin-induced diabetic rats fed a high cholesterol diet.

Diabetic cataracts have been believed to be caused by hyperglycemia. Kinoshita et al. (1979) have shown that hyperglycemia activates aldose reductase and promotes the formation of sorbitol from glucose, which leads to the onset of cataracts.

However, as mentioned in the Introduction, hyperlipidemia is often observed in insulin-dependent or insulin-independent diabetes mellitus in humans and experimental animals, and hyperlipidemia in addition to hyperglycemia has been thought to be a major risk factor for the development and progression of diabetic complications, including cataracts. Tsutsumi et al. (1999) suggested that development of cataracts in streptozotocin-induced diabetic rats fed a cholesterol-rich diet may be accelerated by hyperlipidemia and low high-density lipoprotein (HDL)cho-

Table 5
Effects of long-term treatment with probucol, insulin or their combination on the mortality rate and incidence rate of cataracts in streptozotocin-induced diabetic rats fed a high cholesterol diet

Group	A. Mortality rate (%)							B. Incidence rate of cataracts (%)						
	0	1	2	4	8	12	15 (weeks)	0	1	2	4	8	12	15 (weeks)
SD-fed Nor	0	0	0	0	0	0	0	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)
HCD-fed Nor	0	0	0	0	0	0	0	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)
SD-fed Diab	0	0	0	0	10	10	10	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=9)	56^{a} (n=9)	56^{a} (n=9)
HCD-fed Diab	0	0	0	20	40	50^{a}	70^{aa,b}	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=8)	17 (n=6)	80^{aa} (n=5)	100^{aa} (n=3)
Prob-suppl HCD-fed Diab	0	0	0	0	20	20	30	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=8)	50 (n=8)	86 (n=7)
HCD-fed Diab+Ins	0	0	0	0	0	20	20	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	25 (n=8)	63 (n=8)
Prob-suppl HCD-fed Diab+Ins	0	0	0	0	0	0^{c}	10^{c}	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0 (n=10)	0^{cc} (n=10)	33 (n=9)

n indicates the number of rats used.
Mortality rate (%)=(number of dead rats/number of rats used) × 100.
Incidence rate of cataracts (%)=(number of rats with cataracts/number of living rats) × 100.
[a] P<0.05, as compared with the SD-fed Nor group (Fisher's test).
[aa] P<0.01, as compared with the SD-fed Nor group (Fisher's test).
[b] P<0.05, as compared with the SD-fed Diab group (Fisher's test).
[c] P<0.05, as compared with the HCD-fed Diab group (Fisher's test).
[cc] P<0.01, as compared with the HCD-fed Diab group (Fisher's test).

Table 6
Effects of a long-term treatment with probucol, insulin or their combination on histopathological changes in eyeballs in streptozotocin-induced diabetic rats fed a high cholesterol diet

Group	Histopathological changes in eyeballs		
	4	8	15 (weeks)
SD-fed Nor	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
HCD-fed Nor	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SD-fed Diab	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0^{aa}
HCD-fed Diab	2.0 ± 0.0^{aa,bb}	2.2 ± 0.2^{aa,bb}	3.0 ± 0.0^{aa}
Prob-suppl HCD-fed Diab	1.2 ± 0.2^{c}	2.0 ± 0.0	2.0 ± 0.0
HCD-fed Diab+Ins	1.2 ± 0.5	1.8 ± 0.2	1.6 ± 0.2^{c}
Prob-suppl HCD-fed Diab+Ins	0.8 ± 0.2^{c}	1.0 ± 0.0^{c}	1.0 ± 0.0^{cc}

Each value represents the mean ± S.E. of 5 rats.
^{aa} $P<0.01$, as compared with the SD-fed Nor group (Dunnett's test).
^{bb} $P<0.01$, as compared with the SD-fed Diab group (Student's t-test).
^{c} $P<0.05$, as compared with the HCD-fed Diab group (Dunnett's test).
^{cc} $P<0.01$, as compared with the HCD-fed Diab group (Dunnett's test).

lesterol. In the present experiment, in streptozotocin-induced diabetic rats (unlike normal rats), a high cholesterol diet markedly elevated the non-fasting serum total cholesterol levels, compared to a standard diet, without effect on non-fasting serum glucose levels. Therefore, it is conceivable from our results that the acceleration of the development of diabetic cataracts by a high cholesterol diet may be due to severe hypercholesterolemia. In this experiment, we determined serum total cholesterol content, but did not identify separate amounts of low-density lipoprotein (LDL) cholesterol and HDL cholesterol. However, it is thought that severe hypercholesterolemia in diabetic rats fed a high cholesterol diet may be due to the marked increase in non-HDL cholesterol such as LDL cholesterol, because there is a negative correlation between HDL cholesterol content and the incidence of cataracts in diabetic rats fed a cholesterol-rich diet (Tsutsumi et al., 1999).

It has been shown that streptozotocin-induced diabetic rats fed a high cholesterol diet become markedly hyperlipidemic (Mochizuki et al., 1999) and that the small intestinal acyl-coenzyme A: cholesterol acetyltransferase activities in streptozotocin-induced diabetic rats are markedly higher than activities seen in normal rats (Kusunoki et al., 2000). Therefore, it is suggested from the above findings that marked hypercholesterolemia, observed in diabetic rats fed a high cholesterol diet in our present experiment, may be due to hyperabsorption of cholesterol from the small intestine via high cholesterol acetyltransferase activities.

Currently, oxygen-derived free radicals and lipid peroxide, easily formed in the diabetic state, are thought to play an important role in the development of diabetic complications (Armstrong and Al-Awadi, 1991; Baynes, 1991). Under diabetic conditions, oxygen-derived free radicals are produced mainly through glycation reaction (Sakurai and Tsuchiya, 1988; Hund et al., 1991). Furthermore, it has been shown that the generation of oxygen-derived free radicals is enhanced in the arteries of hypercholesterolemic rabbits (Mügge et al., 1994) and in diabetic rat glomeruli incubated with native and oxidized low-density lipoproteins (Chen et al., 2000). These findings suggest that the enhanced production of oxygen-derived free radicals in the diabetic state may be due to hyperlipidemia in addition to hyperglycemia. In the present experiment, in streptozotocin-induced diabetic rats, a high cholesterol diet resulted in a marked elevation of serum lipid peroxide levels, when these levels were measured at 8 and 15 weeks after streptozotocin treatment, although a standard diet feeding showed no apparent elevation. When each rat was checked for cataracts at 0, 1, 2, 4, 8, 12 and 15 weeks after streptozotocin treatment, cataracts were observed from 8 weeks in streptozotocin-induced diabetic rats fed a high cholesterol diet and from 12 weeks in the diabetic rats fed a standard diet; the incidence at 15 weeks was 100% in the former and 56% in the latter. The mortality rate of the diabetic rats fed a high cholesterol diet began to increase from 4 weeks and reached 70% at 15 weeks, although the rate of the diabetic rats fed a standard diet was only 10% even at 15 weeks.

It is postulated from the results obtained by our present experiment and findings mentioned above that hyperglycemia, hyperlipidemia and increased oxidative stress are major risk factors for the development and progression of diabetic cataracts with the increased mortality rate. In the present experiment, treatment with probucol, insulin or their combination markedly suppressed the onset and incidence of cataracts in the diabetic rats fed a high cholesterol diet. Specifically, when the incidence of cataracts was compared at 12 (15) weeks, the incidence in rats treated with probucol, insulin and their combination was 50% (86%), 25% (63%) and 0% (33%), respectively, compared with 80% (100%) of untreated control. The preventive effects of both agents and their combination on the incidence of cataracts were in good agreement with those obtained by histopathological evaluation of eyeballs at 15 weeks. In addition, the mortality rates were markedly suppressed by treatment with the two agents and their combination. Thus, the combined treatment with probucol and insulin was the most effective in preventing the diabetic cataracts and death. Treatment with probucol, insulin and their combination markedly improved hyper-

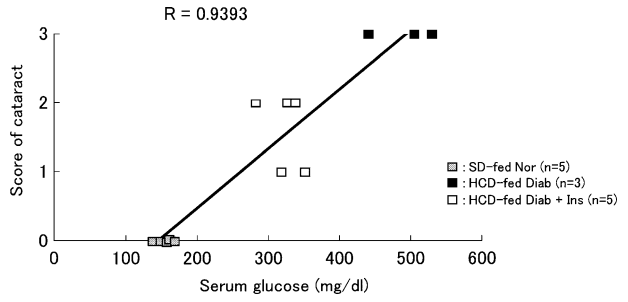

Fig. 2. Relationship between the decrease in serum glucose and the prevention of eye damage by long-term treatment with insulin in diabetic rats fed a high cholesterol diet 15 weeks after streptozotocin injection. n indicates the number of rats used.

cholesterolemia, hypertriglyceridemia and increased serum lipid peroxide levels in streptozotocin-induced diabetic rats fed a high cholesterol diet. In addition, treatment with insulin and the combination of probucol and insulin markedly improved hyperglycemia, although probucol alone had no effect on hyperglycemia. The improving action of insulin on hyperlipidemia and increased lipid peroxidation may be a secondary action caused by its hypoglycemic action.

Recently, Sia et al. (2002) have reported that probucol markedly improves post-myocardial infarction in rats, and its beneficial effects may be associated with reduced cardiac fibrosis, oxidative stress and expression of pro-inflammatory cytokines such as interleukin (IL)-1beta and IL-6. Therefore, it is postulated that the pro-inflammatory cytokines-reducing action of probucol may also be associated with its preventive action against the development and progression of diabetic cataracts.

Intensive insulin therapy may cause a transient worsening of retinopathy in patients with diabetes through increased retinal vascular endothelial growth-factor gene expression (Lu et al., 1999). In this connection, Su et al. (2000) reported that the progression of retinopathy was not stopped, despite sustained recovery to normoglycemia in a long-term study of streptozotocin-induced diabetic rats. In the present experiment, blood glucose control by insulin (2 U/rat s.c.) was not sufficient. Treatment with insulin alone prevented the development and progression of diabetic cataracts. There was a clear relationship between the decrease in serum glucose and the prevention of eye damage by insulin (Fig. 2). This result strongly supports the idea that blood glucose control by insulin may be very important for the prevention of the development and progression of cataracts of insulin-dependent diabetes mellitus.

In summary, it is concluded from these results that the combined treatment with probucol and insulin is useful in preventing the development and progression of diabetic cataracts, and that the preventive action of the combined treatment with both agents may be mainly due to hypoglycemic, hypolipidemic and free-radical-scavenging actions. However, further experiments are needed to clarify the mechanism of the combination of both agents.

References

Armstrong, D., Al-Awadi, F., 1991. Lipid peroxidation and retinopathy in streptozotocin-induced diabetes. Free Radic. Biol. Med. 11, 433–436.

Baumastark, M.W., Aristegui, R., Zoller, T., Frey, I., Berg, A., Keul, J., 1992. Probucol, incorporated into LDL particles in vivo, inhibits generation of lipid peroxides more effectively than endogenous antioxidants alone. Clin. Biochem. 25, 395–397.

Baynes, J.W., 1991. Role of oxidative stress in development of complications in diabetes. Diabetes 40, 405–412.

Brownlee, M., 1992. Glycation products and the pathogenesis of diabetic complications. Diabetes Care 15, 1835–1843.

Brownlee, M., Cerami, A., Vlassara, H., 1988. Advanced glycosylation endproducts in tissue and the biochemical basis of diabetic complications. N. Engl. J. Med. 318, 1315–1321.

Chen, H.C., Tan, M.S., Guh, J.Y., Tsai, H.H., Lai, Y.L., 2000. Native and oxidized low-density lipoproteins enhance superoxide production from diabetic rat glomeruli. Kidney Blood Press. Res. 23, 133–137.

Dominguez, J.H., Tang, N., Xu, W., Evan, E.P., Siakotos, A.N., Agarwal, R., Walsh, J., Deeg, M., Pratt, J.H., March, K.L., Weiss, V.M., Baynes, M.F., Peterson, J.W., 2000. Studies of renal injury III: lipid-induced nephropathy in type II diabetes. Kidney Int. 57, 92–104.

Garcia, M.J., McNamara, P.M., Gordon, T., Kannel, W.B., 1974. Morbidity and mortality in diabetics in the Framingham population. Diabetes 23, 105–111.

Gorogawa, S., Kajimoto, Y., Umayahara, Y., Kaneto, H., Watada, H., Kuroda, A., Kawamori, D., Yasuda, T., Matsuhisa, M., Yamasaki, Y., Hori, M., 2002. Probucol preserves pancreatic beta-cell function through reduction of oxidative stress in type 2 diabetes. Diabetes Res. Clin. Pract. 57, 1–10.

Gottsater, A., Ahmed, M., Fernlund, P., Sundkvist, G., 1999. Autonomic europathy in type 2 diabetic patients is associated with hyperinsulinanemia and hypertriglyceridaemia. Diabet. Med. 16, 49–54.

Gurler, B., Vural, H., Yilmaz, N., Satici, A., Aksoy, N., 2000. The role of oxidative stress in diabetic retinopathy. Eye 14 (Pt. 5), 730–735.

Hirano, T., Mamo, J.C.L., Takeuchi, H., Nagano, S., Takahashi, T., 1991. Correlation of insulin deficiency and hypertriglyceridemia in diabetic rats. Diabetes Res. Clin. Pract. 12, 173–180.

Hund, J.V., Smith, C.C., Wolff, S.P., 1991. Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes 39, 1420–1424.

Ito, M., Kondo, Y., Nakatani, A., Hayashi, K., Naruse, A., 2001. Characterization of low dose streptozotocin-induced progressive diabetes in mice. Environ. Toxicol. Pharmacol. 9, 71–78.

Jakus, V., 2000. The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. Bratisl. Lek. L. 101, 541–551.

Kinoshita, J.H., Fukushi, S., Kador, P., Merola, L.O., 1979. Aldose reductase in diabetic complications of the eye. Metabolism 28, 462–469.

Kusunoki, J., Aragane, K., Kitamine, T., Kozono, H., Kano, K., Fujinami, K., Kojima, K., Chiwata, T., Sekine, Y., 2000. Postprandial hyperlipidemia in streptozotocin-induced diabetic rats is due to abnormal increase in intestinal acyl coenzyme A: cholesterol acetyltransferase activity. Arterioscler. Thromb. Vasc. Biol. 20, 171–178.

Lu, M., Amano, S., Miyamoto, K., Garland, R., Keough, K., Qin, W., Adamis, A.P., 1999. Insulin-induced vascular endothelial growth factor expression in retina. Investig. Ophthalmol. Vis. Sci. 40, 3281–3286.

Man, Z.-W., Zhu, M., Noma, Y., Toide, K., Sato, T., Asahi, Y., Hirashima, T., Mori, S., Kawano, K., Mizuno, A., Sano, T., Shima, K., 1997. Impaired β-cell function and deposition of fat droplets in the pancreas as a consequence of hypertriglyceridemia in OLETF rat, a model of spontaneous NIDDM. Diabetes 46, 1718–1724.

Matsushita, M., Yoshino, G., Iwai, M., Matsuba, K., Morita, M., Iwatani, I., Yoshida, M., Kazumi, T., Baba, S., 1989. Protective effect of probucol on alloxan diabetes in rats. Diabetes Res. Clin. Pract. 7, 313–316.

Mochizuki, H., Takido, J., Oda, H., Yokogoshi, H., 1999. Improving effect of dietary taurine on marked hypercholesterolemia induced by a high-cholesterol diet in streptozotocin-induced diabetic rats. Biosci. Biotechnol. Biochem. 63, 1984–1987.

Mügge, A., Brandes, R.P., Böger, R.H., Dwenger, A., Bodr-Böger, S., Frölich, J.C., Lichtlen, P.R., 1994. Vascular release of superoxide radicals is enhanced in hypercholesterolemic rabbits. J. Cardiovasc. Pharmacol. 24, 994–998.

Nikkila, E.A., Hormila, P., 1978. Serum lipid and lipoproteins in insulin-treated diabetes. Demonstration of increased high density lipoprotein concentrations. Diabetes 27, 1078–1085.

Nikkila, E.A., Kekki, M., 1973. Plasma triglyceride transport kinetics in diabetes mellitus. Metabolism 22, 1–22.

Parthasarathy, S., Young, S.G., Witzum, J.L., Pittman, R.C., Steinberg, D., 1986. Probucol inhibits oxidative modification of low density lipoprotein. J. Clin. Invest. 77, 641–644.

Ravid, M., Brosh, D., Ravid-Safran, D., Levy, Z., Rachmani, R., 1998. Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. Arch. Intern. Med. 158, 998–1004.

Reaven, G.M., Greenfield, M.S., 1981. Diabetic hypertriglyceridemia. Evidence for three clinical syndromes. Diabetes 30 (Suppl. 2), 66–75.

Regnstrom, J., Walldius, D., Carlson, L.A., Nilsson, J., 1990. Effect of probucol treatment on the susceptibility of low density lipoprotein isolated from hypercholesterolemic patients to become oxidatively modified in vitro. Atherosclerosis 82, 43–51.

Sakurai, T., Tsuchiya, S., 1988. Superoxide production from nonenzymatically glycation protein. FEBS Lett. 236, 406–410.

Schwartz, C.J., 1988. The probucol experience: a review of the past and a look at the future. Am. J. Cardiol. 62, 1B–5B.

Sia, Y.T., Perker, T.G., Liu, P., Tsoporis, J.N., Adam, A., Rouleau, J.L., 2002. Improved post-myocardial infarction survival with probucol in rats: effects on left ventricular function, morphology, cardiac oxidative stress and cytokine expression. J. Am. Coll. Cardiol. 39, 148–156.

Su, E.N., Alder, V.A., Yu, D.Y., Yu, P.K., Cringle, S.J., Yogesan, K., 2000. Continued recovery to normoglycemia in a long-term study of streptozotocin-induced diabetes in rats. Graefe Arch. Clin. Exp. Ophthalmol. 238, 163–173.

Tsutsumi, K., Inoue, Y., Yoshida, C., 1999. Acceleration of Development of diabetic cataract by hyperlipidemia and low high-density lipoprotein in rats. Biol. Pharm. Bull. 22, 37–41.

West, I.C., 2000. Radicals and oxidative stress in diabetes. Diabet. Med. 17, 171–180.

Yagi, K., 1976. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem. Med. 15, 212–216.

Yamamoto, A., Matsuzawa, Y., Yokoyama, S., Funahashi, T., Yamamura, T., Kishino, B., 1986. Effects of probucol on xanthomata regression in familiar hypercholesterolemia. Am. J. Cardiol. 57, 29H–35H.