Effect of cerivastatin sodium, a new inhibitor of HMG-CoA reductase, on plasma lipid levels, progression of atherosclerosis, and the lesional composition in the plaques of WHHL rabbits

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1 The aim of this study was to examine whether cerivastatin sodium, a new inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, affects the lesional composition of spontaneously developed atherosclerosis due to hypercholesterolaemia and delays progression of the lesions.

2 We administered cerivastatin to 2-month-old WHHL rabbits, a low-density lipoprotein receptor-deficient animal model, at a dose of 0.6 mg kg⁻¹ day⁻¹ for 32 weeks. We examined the plasma lipid levels, the severity of atherosclerosis, and composition of atherosclerotic lesions. Lesional composition was determined using immunohistostaining for macrophages and smooth muscle cells, and Azan-Mallory staining for collagen fibres and extracellular lipid deposits.

3 Compared to the control group, the plasma cholesterol levels were decreased in the treated group by 39% (12.7 ± 0.6 mmol L⁻¹ versus 20.9 ± 1.0 mmol L⁻¹, *P* < 0.001). Atherosclerosis was suppressed by about 37% as measured by the thickness of the aortic lesions (158 ± 13 µm versus 250 ± 15 µm, *P* < 0.001), and by 28% as measured by coronary stenosis (62.7 ± 11.4 versus 86.9 ± 12.2, *P* < 0.05). In the cerivastatin group, regarding the per cent areas of lesional components in the lesion area, the macrophages (21.0 ± 1.5% versus 27.9 ± 1.9%, *P* < 0.01) and extracellular lipid deposits (3.2 ± 0.4% versus 5.1 ± 0.4%, *P* < 0.001) were decreased in the aortic lesions, and the per cent area of macrophages in the coronary lesions was also decreased (4.9 ± 1.4% versus 11.6 ± 2.4%, *P* < 0.05). The per cent area of smooth muscle cells and collagen fibres did not significantly decrease.

4 These results indicate that cerivastatin contributed to the plaque stabilization and delayed progression of early atherosclerosis in young WHHL rabbits, in addition to the potent hypolipidemic effects.

Keywords: Cerivastatin; HMG-CoA reductase inhibitor; plasma cholesterol; atherosclerosis; coronary stenosis; macrophage; smooth muscle cell; WHHL rabbit

Abbreviations: AUC, area under concentration curve; EDTA, ethylenediaminetetraacetic acid; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HPLC, high-pressure liquid chromatography; LCX, left circumflex artery; LMT, main trunk of left coronary artery; RCA, right coronary artery; RCO, origin portion of right coronary artery

Introduction

Clinical trials have demonstrated that inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase prevent development of coronary events, in addition to having a hypolipidemic effect (Scandinavian Simvastatin Survival Study Group, 1994; Shepherd *et al.*, 1995; Downs *et al.*, 1998; Rosenson & Tangney, 1998). Vaughan *et al.* (1996) reported that HMG-CoA reductase inhibitors do more than just lower cholesterol regarding prevention of coronary events.

We have previously shown that an inhibitor of HMG-CoA reductase, pravastatin sodium, reduces plasma cholesterol levels, and suppresses atherosclerosis in WHHL rabbits, a low-density lipoprotein receptor-deficient animal model (Watanabe *et al.*, 1988; Shiomi *et al.*, 1990; 1995; 1998). In our recent study using mature WHHL rabbits that suffered from established atherosclerosis, pravastatin stabilized atherosclerotic plaques (Shiomi *et al.*, 1995). WHHL rabbits are a useful animal model for examination of antiatherosclerotic effects of drugs, since atherosclerosis is developed due to spontaneous hypercholesterolemia (Watanabe, 1980; Shiomi *et al.*, 1992).

Recently, it was reported that cerivastatin sodium (cerivastatin, BAY w 6228), a newly synthesized inhibitor of

HMG-CoA reductase, potently inhibits the activity of HMG-CoA reductase in the microgram range (Angerbauer *et al.*, 1994; Bischoff *et al.*, 1997). In a study of inhibition of cholesterol synthesis in rat hepatocytes, the dose of cerivastatin causing 50% inhibition was less than one-hundredth of the required dose of lovastatin (Bischoff *et al.*, 1997). In addition, cerivastatin did not affect the blood pressure and heart rate in dogs or the hematological parameters of rats (Dr H. Bischoff, Bayer AG, Germany, personal communication). On the other hand, *in vitro* studies demonstrated that although pravastatin has a weak effect on inhibition of smooth muscle cell growth, cerivastatin caused a potent inhibition of proliferation of human smooth muscle cells (Negre-Aminou *et al.*, 1997; Corsini *et al.*, 1996). Igarashi *et al.* (1997a,b) also reported that cerivastatin suppressed neointimal thickening by attenuating intimal smooth muscle cell proliferation and infiltration of macrophages in balloon-injured rabbit models. In addition, Wada *et al.* (1996) reported that the plasma tissue factor levels and tissue factor pathway inhibitor levels were decreased in 21 Japanese patients treated with cerivastatin, although the other coagulation parameters did not change.

The inhibitory effect on smooth muscle cell proliferation varies among various HMG-CoA reductase inhibitors. In our previous observations, pravastatin sodium, an HMG-CoA

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reductase inhibitor which shows a low inhibitory effect on smooth muscle cell proliferation *in vitro* (Corsini *et al.*, 1996) caused no decrease in the smooth muscle cell content in the atheromatous plaques of WHHL rabbits. On the other hand, fluvastatin sodium, an inhibitor of HMG-CoA reductase which potently inhibits smooth muscle cell proliferation *in vitro* (Corsini *et al.*, 1996) reduced the smooth muscle cell content in the atheromatous plaque of WHHL rabbits (Shiomi *et al.*, 1998). Several researchers have discussed the conclusion that inhibition of smooth muscle cell proliferation is unfavourable for the prevention of coronary events (Van der Wal *et al.*, 1994; Davies, 1995; Libby, 1995; Weissberg *et al.*, 1996). Therefore, we considered it important to examine whether cerivastatin affects the lesional composition of atherosclerosis, especially smooth muscle cells.

In this study, we examined whether cerivastatin affects lesional composition, especially smooth muscle cells, in the spontaneously developed atheromatous plaques due to hypercholesterolaemia, and confirmed the preventive effect on atherosclerosis. In addition, we compared the effects of cerivastatin on the lesional composition of atherosclerosis with those of other HMG-CoA reductase inhibitors previously studied.

Methods

Reagents

Cerivastatin was supplied by Bayer Yakuhin, Ltd. (Osaka, Japan). HHF35, a monoclonal antibody specific for muscle actin, was purchased from Enzo Diagnostics, Inc. (NY, U.S.A.), RAM-11, a monoclonal antibody specific for rabbit macrophages, was purchased from Dako A/S (Glostrup, Denmark), and avidin-biotinylated enzyme complex (ABC) kits was purchased from Vector Laboratories Inc. (CA, U.S.A.).

Animals

In order to measure plasma cerivastatin concentration, we used 12 normal male Japanese white rabbits aged 3 months (Kitayama Labes Co., Ltd., Ina, Nagano Prefecture, Japan). To examine the antiatherogenic effects of cerivastatin, we used 20 WHHL rabbits aged 2 months, which were bred at Kobe University and had a high incidence of severe coronary atherosclerosis (Shiomi *et al.*, 1992). These rabbits were divided into control group (five females and five males) and cerivastatin group (five females and five males) so as to make the plasma lipid level, body weight, and litter distribution uniform. The rabbits in the cerivastatin group were subcutaneously injected with cerivastatin solution (1 mg ml^{-1} in saline) at a dose of $0.6 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 32 weeks. Since plasma drug concentration does not increase in rabbits when cerivastatin is given orally, we administered the drug to rabbits by subcutaneous injection. The control rabbits were injected subcutaneously with saline. Rabbits were housed individually in metal cages in a room maintained at a constant temperature and humidity, and were fed 100 g of laboratory diet (RC4, Oriental Yeast Co., Tokyo, Japan) per day. Water was given *ad libitum*. All aspects of animal experimentation and care were conducted according to the Guidelines for Animal Experimentation of the Kobe University School of Medicine, the Law Concerning the Protection and Control of Animals (Law No. 105, 1973), and Standards Relating to the Care and

Management, etc. of Experimental Animals (Notification No. 6, 1980).

Plasma lipid measurement

Blood samples were taken from the marginal ear vein after overnight fasting and plasma was obtained by addition of sodium-EDTA and centrifugation. Plasma total cholesterol, triglyceride and phospholipid levels were measured by enzymatic methods.

Preparation of histological sections

At the end of 32 weeks of drug administration, rabbits were anaesthetized by intravenous injection of sodium pentobarbital (25 mg kg^{-1}) and perfused with lactated Ringer's solution. After the perfusion, the aortas were excised, and then the coronary arteries were perfused with Bouin's fixative at a constant pressure of 100 mmHg. The aortas were divided into aortic arch, thoracic aorta, and abdominal aorta. The thoracic aortas and hearts were immersed in Bouin's fixative for at least 24 h. The remainders of the aortas were frozen and used for other purposes. After immersion-fixation, aortas were longitudinally cut open and the surface area of the intima and the lesion were determined with a colour image analyzer, SP-500 (Olympus, Ltd., Osaka, Japan). Aortic atherosclerosis was histologically examined at five locations: the regions beneath the first, third, fifth, seventh and ninth orifices of the intercostal artery. The segments in each portion were embedded in paraffin, and ten sections, $4 \mu\text{m}$ thickness, were cut serially from each segment.

Coronary atherosclerosis was examined in the main trunk of the left coronary artery (LMT, four segments), the origin portion of the right coronary artery (RCO, four segments), the left circumflex artery (LCX, 30 segments), and the right coronary artery (RCA, 20 segments). The hearts were divided into six blocks, and the blocks were embedded in paraffin. The blocks containing LMT or RCO were sectioned at $200 \mu\text{m}$ intervals and the other blocks were sectioned at $500 \mu\text{m}$ intervals. A total of 58 segments from each heart were examined and ten sections, $4 \mu\text{m}$ in thickness, were cut serially from each segment.

One section of each segment was stained with elastic van Gieson's stain. Sections from each aortic segment and from the coronary segments showing the most severe stenosis in the LMT, RCO and LCX were stained with monoclonal antibodies, HHF35 and RAM-11, and with Azan-Mallory's stain. Immunostaining was carried out with a Vectastain ABC kit according to the manufacturer's instructions.

Quantitative analysis of atherosclerosis and lesional composition

All parameters for atherosclerotic lesions were measured with a colour image analyzer. The degree of atherosclerosis was evaluated by the per cent of lesion area in the surface of intima (the surface area of lesions/the surface area of the whole intima) and the thickness of atherosclerotic lesions (area of intimal lesions/length of external elastic lamina) in the thoracic aortas (Shiomi *et al.*, 1990), and by the score of stenosis in the coronary arteries (Shiomi *et al.*, 1995). Intimal lesion area was estimated using sections stained with elastic van Gieson's stain. We measured coronary stenosis in 58 segments per heart and calculated the coronary stenosis score of each segment. The coronary score was graded as follows: no lesion, 0 points; stenosis of 10% or less narrowing, 1

point; stenosis of 10–20% narrowing, 2 points; stenosis of 20–30% narrowing, 3 points; stenosis of 30–40% narrowing, 4 points; stenosis of 40–50% narrowing, 5 points; stenosis of 50–60% narrowing, 6 points; stenosis of 60–70% narrowing 7 points; stenosis of 70–80% narrowing, 8 points; stenosis of 80–90% narrowing, 9 points; stenosis of over 90% narrowing, 10 points. These points were summed for each portion, and the total points for each rabbit were also counted.

Lesional components were quantitatively evaluated with the colour image analyzer at a magnification of $\times 56$ to $\times 280$, and the lesion area and the area of each lesional component was measured according to the method reported previously (Shiomi *et al.*, 1994). We defined cells stained with HHF35 as smooth muscle cells and cells stained with RAM-11 as macrophages. In sections treated with Azan-Mallory's stain, fibres stained with cobalt blue were defined as collagen, and extracellular vacuoles and lacunae as extracellular lipid deposits. The per cent area of each lesional component was calculated by dividing the area of each lesional component by the lesion area and multiplying by 100%.

Evaluation of xanthoma

Xanthomas were observed macroscopically on the digital joints in each leg after the skin had been removed. The severity of xanthoma was graded as follows: no lesions (–), slight lesion (+) showing diffuse granular lesions, moderate lesion (++) showing one massive lesion with or without diffuse granular ones, and severe lesion (+++) showing more than one massive lesion with or without diffuse granular ones (Shiomi *et al.*, 1990).

Plasma concentration of cerivastatin

The plasma concentration of cerivastatin was measured after a single administration of the drug. Normal Japanese white rabbits were divided into four groups. Three rabbits were given cerivastatin orally at a dose of 0.15 mg kg^{-1} , and three rabbits each in the other three groups were subcutaneously injected with the drug at a dose of 0.15 mg kg^{-1} , 0.3 mg kg^{-1} or 0.6 mg kg^{-1} . Blood samples were obtained from the marginal ear vein of each rabbit at 0.5, 1.0, 2.0, 4.0 and 6.0 h after drug injection. The plasma cerivastatin concentration was determined using HPLC equipped with a Lichrospher 100 RP-18 column according to the method of Krol *et al.* (1993) with slight modification. We analysed T max, C max, and the area under the concentration curve (AUC) from 0–6 h.

Statistical analysis

Data are presented as means $\pm$ s.e.mean. Statistical analysis was carried out by the Student's *t*-test or Aspin-Welch's *t*-test for the means of plasma lipid levels, Mann-Whitney U-test for

the means of atherosclerosis, Fischer's exact probability test for incidences of xanthoma, and Wilcoxon's rank sum test for xanthoma severity.

Results

There were no findings of myopathy or hepatic disorders in any rabbits. In the atherosclerosis prevention study, one rabbit in the control group died suddenly at the 30th week of treatment. Autopsy of this rabbit showed no sign of infection or any other abnormality.

Plasma cerivastatin concentration

In preliminary experiments, plasma cholesterol levels did not decrease in WHHL rabbits administered cerivastatin orally at a dose of 0.15 mg kg^{-1} . Accordingly, we examined plasma cerivastatin concentrations in normal rabbits. Table 1 shows the plasma concentrations of cerivastatin. C max and AUC were remarkably higher in the subcutaneous injection group than in the oral administration group at the same dose. These data show that oral administration of cerivastatin is not suitable for rabbits. With subcutaneous injection, C max and AUC increased dose-dependently.

Plasma lipid levels

Table 2 shows plasma lipid levels of WHHL rabbits treated with cerivastatin. At the start of this experiment, plasma lipid levels were similar in the cerivastatin and control groups. At the end of the experiment, the total cholesterol levels of the cerivastatin group were significantly decreased, by 39%, compared to the control group. Although the phospholipid levels were also significantly decreased, by 33%, the decrease in triglyceride levels of the cerivastatin group was not significant. In the control group, the plasma lipid levels were decreased with progression of the study. In general, the plasma lipid levels of WHHL rabbits are decreased with ageing due to an age-associated decrease in VLDL secretion from the liver (unpublished results).

Table 1 Plasma cerivastatin concentration in normal rabbits after a single administration

	Dose (mg kg^{-1})	T max (h)	C max (ng ml^{-1})	AUC _{0–6} (ng ml^{-1})
Oral administration	0.15	0.5	1.9 ± 0.4	3.1 ± 1.3
Subcutaneous administration	0.15	0.5	60 ± 11	159 ± 36
	0.30	0.5	117 ± 5	175 ± 8
	0.60	0.5	276 ± 26	405 ± 60

Data are presented as means $\pm$ s.e.mean ($n=3$). AUC_{0–6} means area under concentration curve from 0–6 h after drug administration.

Table 2 Plasma lipid levels of WHHL rabbits

	Total cholesterol (mmol L^{-1})		Triglyceride (mmol L^{-1})		Phospholipid (mmol L^{-1})	
	Start	End	Start	End	Start	End
Control ($n=9$)	30.2 ± 0.8	20.9 ± 1.0	3.4 ± 0.5	2.6 ± 0.3	7.2 ± 0.2	5.8 ± 0.3
Cerivastatin ($n=10$)	30.5 ± 0.5	12.7 ± 0.6	3.5 ± 0.3	2.0 ± 0.1	7.3 ± 0.1	3.9 ± 0.1
		$P < 0.001$				$P < 0.001$

Data are represented as means $\pm$ s.e.mean. Statistical analysis was carried out with Student's *t*-test or Aspin-Welch's *t*-test.

Table 3 Atherosclerosis of the thoracic aorta of WHHL rabbits

	Orifice 1	Lesion thickness at each orifice of the intercostal artery (μm)					Per cent lesion area in the intima surface (%)
		Orifice 3	Orifice 5	Orifice 7	Orifice 9	Mean	
Control ($n=9$)	241 $\pm$ 40	283 $\pm$ 23	275 $\pm$ 36	248 $\pm$ 32	205 $\pm$ 34	250 $\pm$ 15	67.7 $\pm$ 6.8
Cerivastatin ($n=10$)	162 $\pm$ 25	186 $\pm$ 28	173 $\pm$ 38	156 $\pm$ 30	115 $\pm$ 22	158 $\pm$ 13	43.4 $\pm$ 8.2
		$P<0.05$			$P<0.05$	$P<0.001$	

Data are represented as means $\pm$ s.e.mean. Statistical analysis was carried out with the Mann-Whitney U-test. Lesional thickening was evaluated using cross sections prepared beneath orifice No. 1, No. 3, No. 5, No. 7 and No. 9. The mean value was calculated using all the data ($n=45$ in the control group and $n=50$ in the cerivastatin group). [Per cent lesion area in the intimal surface] = [the surface area of lesions]/[the surface area of intima of longitudinally cut-opened aorta] $\times$ 100.

Severity of aortic and coronary atherosclerosis

Table 3 shows measurements of the extent of atherosclerotic lesions of the thoracic aorta. The lesion thickness of the cerivastatin group was significantly lower at the third and ninth orifices of the intercostal artery, and the mean value of all portions was also significantly decreased, by 37%, compared to the control group ($158 \pm 13 \mu\text{m}$ versus $250 \pm 15 \mu\text{m}$, $P<0.001$). The per cent lesion area in the surface of the intima that was calculated by dividing the surface area of lesions by the surface area of the whole intima was decreased by 37%; these were not significantly different. Figure 1 shows the coronary stenosis in control and treated rabbits. We examined 58 sections per heart. The coronary stenosis score of the cerivastatin group was significantly decreased, by 28% in the total heart (62.7 ± 11.4 versus 86.9 ± 12.2 , $P<0.05$), compared to the control group. The average of the most severe stenosis in each portion was $44.9 \pm 4.5\%$ ($n=40$) in the cerivastatin group and $50.9 \pm 4.4\%$ ($n=36$) in the control group; these were not significantly different.

Lesional composition

Figure 2 shows the lesional composition of atherosclerotic plaques of the thoracic aorta. Although the per cent areas of collagen and smooth muscle cell were not altered, those of macrophages and extracellular lipid deposits were significantly decreased, by 25% (21.0 ± 1.5 versus 27.9 ± 1.9 , $P<0.01$) and 37% (3.2 ± 0.4 versus 5.1 ± 0.4 , $P<0.01$), respectively, in the cerivastatin group compared to the control group. Typical findings are shown in Figure 3. The control rabbits showed accumulation of large foam cells and extracellular lipid deposits in the atheromatous plaques, while the area occupied by macrophages and extracellular lipid deposits were smaller in the cerivastatin-treated rabbits. In addition, the atherosclerotic lesions were not as thick in the cerivastatin group as in the control group. Although the frequency of disappearance of internal elastic lamina, accumulation of cholesteryl crystals, and calcification did not decrease, the frequency of fragmentation of the internal elastic lamina was significantly decreased in the cerivastatin group (76% versus 96%, $P<0.01$).

Figure 4 shows the lesional composition of the coronary atherosclerosis. The per cent area of macrophages in the cerivastatin group was significantly decreased, by 58%, compared to the control group (4.9 ± 1.4 versus 11.6 ± 2.4 , $P<0.05$). Other lesional components tended to be decreased by the cerivastatin treatment. Typical findings are shown in Figure 5. The frequency of fragmentation and disappearance of the internal elastic lamina were significantly decreased in the cerivastatin group (19% versus 48%, $P<0.05$ and 7% versus 33%, $P<0.05$, respectively).

Figure 1 Coronary atherosclerosis of WHHL rabbits treated with cerivastatin sodium. Statistical analysis was carried out with the Mann-Whitney U-test. Bars indicate means $\pm$ s.e.mean. LMT, the main stem of left coronary artery; RCO, the origin portion of right coronary artery; LCX, left circumflex artery; RCA, right coronary artery. For calculation of coronary stenosis score see Methods.

Figure 2 Lesional composition of aortic atherosclerosis of WHHL rabbits treated with cerivastatin sodium. Statistical analysis was carried out with the Mann-Whitney U-test. Bars indicate means $\pm$ s.e.mean. [per cent area of lesional composition] = [area of each lesional component]/[lesion area] $\times$ 100.

Xanthoma

Table 4 summarizes findings about the xanthoma of WHHL rabbits. The incidence of xanthoma in the cerivastatin group was significantly lower in the hind legs and total legs than in the control group. The distribution of severity of xanthoma shows that the degree of xanthoma in the cerivastatin group was significantly less than in the control group ($P<0.001$). Although the frequency of degree of severity over (+) was 61% in the control group, it was only 3% in the cerivastatin group.

Figure 3 Cross-sections of the thoracic aortas of WHHL rabbits treated with cerivastatin sodium. Sections were immunohistochemically stained with HHF35, a monoclonal antibody specific for muscle actin, or RAM-11, a monoclonal antibody specific for rabbit macrophages. The positive staining shows as black, and vacuoles and lacunae show extracellular lipid deposits. (a) Control; HHF35 staining. (b) Cerivastatin; HHF35 staining. (c) Control; RAM-11 staining. (d) Cerivastatin; RAM-11 staining. Small arrows indicate smooth muscle cells, large arrows indicate macrophages, arrowheads indicate extracellular lipid deposits, and asterisks indicate arterial media. Bars indicate 100 μm.

Discussion

In the present study, cerivastatin decreased the plasma total cholesterol levels and suppressed progression of the early atherosclerosis and xanthoma in young WHHL rabbits. In

Figure 4 Lesional composition of coronary atherosclerosis of WHHL rabbits treated with cerivastatin sodium. Statistical analysis was carried out with the Mann-Whitney U-test. Bars indicate means $\pm$ s.e.mean. [per cent area of lesional composition] = [area of each lesional component]/[lesion area] $\times$ 100.

addition, we showed a decrease in the per cent areas of macrophages and extracellular lipid deposits and no decrease in the per cent areas of smooth muscle cells and collagen fibres in the early atherosclerotic lesions of young WHHL rabbits treated with cerivastatin.

In a similar study using fluvastatin sodium, another HMG-CoA reductase, while the per cent areas of smooth muscle cells, collagen fibres, and extracellular lipid deposits were decreased, the per cent area of macrophages was increased in the early atherosclerotic lesions of young WHHL rabbits (Shiomi *et al.*, 1998). Both cerivastatin and fluvastatin show inhibitory effects on proliferation of smooth muscle cells in *in vitro* studies (Corsini *et al.*, 1996; Negre-Aminou *et al.*, 1997; Bellosta *et al.*, 1998) and *in vivo* studies using normocholesterolaemic rabbits which had neointimal thickening of arteries caused by injury (Soma *et al.*, 1993; Bandoh *et al.*, 1996; Igarashi *et al.*, 1997b). However, in our studies using young WHHL rabbits, fluvastatin caused a decrease in the per cent area of smooth muscle cells in the atherosclerotic lesions whereas cerivastatin did not, despite the similar hypolipidemic effects of the two drugs. On the other hand, pravastatin, which shows little inhibitory effect on smooth muscle cell proliferation *in vitro* (Soma *et al.*, 1993; Corsini *et al.*, 1996; Bellosta *et al.*, 1998) decreased the per cent area of macrophages and extracellular lipid deposits, but did not affect smooth muscle cells in the established lesions of mature WHHL rabbits (Shiomi *et al.*,

Figure 5 Cross-sections of the left circumflex arteries of WHHL rabbits treated with cerivastatin sodium. Sections were immunohistochemically stained with HHF35, a monoclonal antibody specific for muscle actin, or RAM-11, a monoclonal antibody specific for rabbit macrophages. The positive staining shows as black, and vacuoles and lacunae show extracellular lipid deposits. (a) Control; staining with HHF35. (b) Cerivastatin; staining with HHF35. (c) Control; staining with RAM-11. (d) Cerivastatin; staining with RAM-11. Small arrows indicate smooth muscle cells, large arrows indicate macrophages, arrowheads indicate extracellular lipid deposits, and asterisks indicate arterial media. Bars indicate 100 μ m.

Table 4 Xanthoma of WHHL rabbits

	Forelegs	Incidence (%)		Total	—	Distribution of severity (%)		
		Hind legs				+	++	+++
Control	100 (18/18)	100 (18/18)	100 (36/36)	0 (0/36)	39 (14/36)	44 (16/36)	17 (6/36)	
Cerivastatin	100 (20/20)	70 (14/20)	85 (34/40)	15 (6/40)	83 (33/40)	3 (1/40)	0 (0/40)	
		$P < 0.05$	$P < 0.05$					

Values in parentheses are represented as (xanthoma-positive legs)/(examined legs). The xanthoma of the treated group was significantly milder than that of the control group by Wilcoxon's rank sum test ($P < 0.001$). Statistical analysis of the incidence was carried out with Fisher's exact probability test. For distribution of severity see Methods.

1995). These results suggest that the effects of various HMG-CoA reductase inhibitors on the composition of atherosclerotic lesions differ, despite the similar hypolipidemic effects. Although we have no data to explain these inconsistencies, each HMG-CoA reductase inhibitor may have yet unknown properties. Rosenson and Tangney (1998) also reported that the properties of various HMG-CoA reductase inhibitors differ. They reviewed the antiatherosclerotic properties of various HMG-CoA reductase inhibitors, especially lovastatin, pravastatin and simvastatin. In their review, the effects of different HMG-CoA reductase inhibitors varied regarding endothelial function, inflammatory responses, antioxidant capacity, fibrinogen levels, blood and plasma viscosity, levels of plasminogen activator inhibitor, and Lp(a) levels.

Since smooth muscle cells regulate synthesis of intercostal collagens, no change or an increase in smooth muscle cell content in the atheromatous plaques should render a plaque less liable to disruption than would a decrease in smooth muscle cells. Libby (1995) and Weissberg *et al.* (1996) have reviewed evidence that intimal smooth muscle cells protect plaques from rupturing and render unstable, potentially lethal lesions more stable. Moreover, Davies (1995) and Van der Wal *et al.* (1994) showed that regions of the fibrous cap of plaques that have ruptured contain relatively few smooth muscle cells. Therefore, the lack of decrease of smooth muscle cells and collagen fibres in the atheromatous plaques suggests that cerivastatin did not make the plaques prone to disruption in young WHHL rabbits.

On the other hand, *in vitro* studies (Negre-Aminou *et al.*, 1997; Corsini *et al.*, 1996; Igarashi *et al.*, 1997b) and an *in vivo* study using balloon-injured arteries of normocholesterolaemic rabbits (Igarashi *et al.*, 1997a,b) showed that cerivastatin inhibited proliferation of smooth muscle cells. Regarding the disagreement on inhibition of smooth muscle cell proliferation between our present results and previous reports, we think that the mechanism regulating smooth muscle cell proliferation in spontaneously developed atherosclerosis may be different from those in injured arteries or culture conditions, and cerivastatin may effectively inhibit smooth muscle cell proliferation in culture conditions or injured arteries. Alternatively, the stimulation of smooth muscle cell proliferation may be more potent in culture conditions and injured arteries than in spontaneously developed atherosclerosis due to hypercholesterolaemia. Cerivastatin may inhibit smooth muscle cell proliferation more effectively in response to strong proliferative stimuli than in response to weak proliferative stimuli. In a previous study, we observed that the content of smooth muscle cells in the atherosclerotic lesions was low in WHHL rabbits aged about 10 months (Shiomi *et al.*, 1994). Therefore, the inhibitory effect of cerivastatin on smooth muscle cell proliferation may not have been observed in this study.

In our previous study using mature WHHL rabbits given pravastatin from 10 to 22 months of age, pravastatin prevented

decreases in collagen fibres and smooth muscle cells in the established atherosclerotic lesions, despite decreases in collagen and smooth muscle cells in the lesions of the placebo group (Shiomi *et al.*, 1995). In the present study, however, the per cent areas of collagen fibres and smooth muscle cells in the early atheromatous plaques did not decrease significantly in the cerivastatin group, compared to the control groups. This disagreement regarding the effects of HMG-CoA reductase inhibitors on collagen and smooth muscle cells may be due to the difference in the stage of atherosclerosis in the two studies. The present study used young rabbits which had lesions of early to transitional stages (Shiomi *et al.*, 1994), while mature WHHL rabbits which had lesions of transitional to advanced stages (Shiomi *et al.*, 1994) were used in the previous study.

Our results showed that cerivastatin reduced the atheromatous core, macrophages and extracellular lipid deposits in the centre of the lesion. These results are similar to those of previous studies using mature WHHL rabbits which suffered from established atherosclerosis treated with pravastatin alone (Shiomi *et al.*, 1995) and in combination with cholestyramine (Shiomi *et al.*, 1990). Davies *et al.* (1993) reported that unstable plaques had large lipid cores, and Galis *et al.* (1995) reported that macrophages secreted matrixdegradation proteinases, and collagen fibres might be degraded. Therefore, reduction of atheromatous cores in the lesions is considered favourable with respect to stabilization of the plaque.

Although it was reported that the plasma concentration of cerivastatin was increased by an oral administration in humans (Krol *et al.*, 1993) and various animals (Steinke *et al.*, 1996), an extremely low plasma drug concentration and no decrease in the plasma lipid levels were observed when cerivastatin was given to WHHL rabbits orally. Therefore, we administered cerivastatin to rabbits by subcutaneous injection in this study. By subcutaneous injection of cerivastatin, we achieved reduction of plasma cholesterol levels and suppression of atherosclerosis and xanthoma. We consider that an adequate plasma drug level led to the large effect on reduction of plasma cholesterol level and preventing atherosclerosis.

In conclusion, this study showed that cerivastatin suppressed progression of early atherosclerosis and qualitatively ameliorated and stabilized the atheromatous plaques, in addition to exerting potent hypolipidemic effects. Furthermore, our results suggest that the effects of various HMG-CoA reductase inhibitors on atherosclerotic lesions are somewhat different.

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