SUPPRESSION OF ATHEROSCLEROSIS IN CHOLESTEROLEMIC RABBITS BY DIMETHYL SULFOXIDE

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Introduction

In 1968 Herzmann reported that 1% dimethyl sulfoxide (DMSO) in the drinking water of cockerels fed cholesterol reduced the accompanying hypercholesterolemia by 50%.[1] We reasoned that if DMSO is an agent that decreases lipid concentration, it may reduce hypercholesterolemia and artherosclerosis in rabbits fed cholesterol. While our studies were in progress Kedar and Sohar reported that DMSO slows cholesterol-induced atherosclerosis in rabbits without affecting plasma cholesterol levels.[2] Our studies support and extend those findings and suggest that DMSO retards the development of atherosclerosis by suppressing the uptake and accumulation of cholesterol by tissues and cells.

Methods

Hypercholesterolemia and atherosclerosis were induced in 24 male, New Zealand white rabbits, weighing 2–3 kg, by feeding them 0.5% cholesterol in their diet. Half of the animals received 2% DMSO in their drinking water. At 2-week intervals plasma cholesterol and triglycerides were measured by standard autoanalyzer II techniques.[3] Tissue cholesterol was analyzed by the method of Abell *et al.*[4] Atherosclerosis in the thoracic aorta was scored by drawing images of the luminal surface on paper and calculating the area involved by planimetry. Cultures of dermal fibroblasts were derived from explants of newborn foreskin and grown to confluency in McCoy's medium supplemented with 20% human serum and antibiotics. Cells between the third and eighth subculture were used in these studies. Human low density lipoproteins (LDL) were iodinated with ^{125}I by the iodine monochloride technique of MacFarlane.[5]

Results and Discussion

No significant differences were observed in the body weights of the control and DMSO-treated rabbits (4.2 ± 0.2 versus 4.1 ± 0.2, respectively). DMSO treatment did not delay the onset or reduce the severity of the dietary-induced hypercholesterolemia. The mean plasma cholesterol of all rabbits rose from less than 100 mg/dl about 1300 mg/dl after four weeks and remained elevated throughout the cholesterol feeding. Plasma triglycerides were not affected by the cholesterol feeding or by DMSO administration. Gross inspection of the thoracic aortas showed that DMSO treatment reduced the severity of the cholesterol-

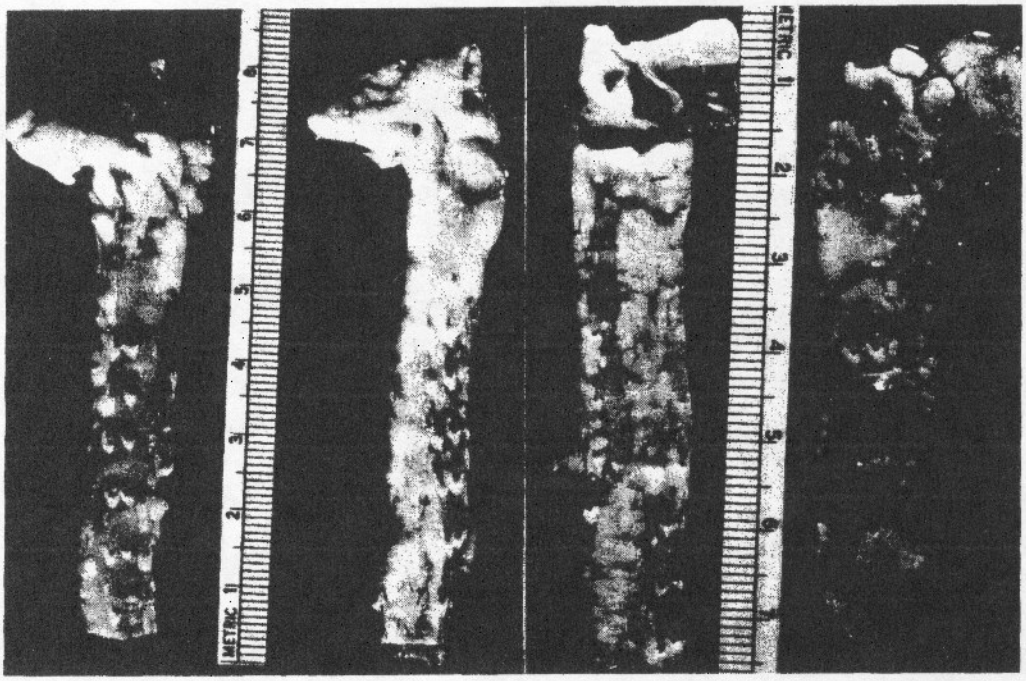

FIGURE 1. Representative thoracic aortas of New Zealand white rabbits fed 0.5% cholesterol and 2% dimethyl sulfoxide for 12 weeks.

induced atherosclerosis 30–40% (FIGURE 1 and TABLE 1). Because the DMSO-treated rabbits appeared to have accumulated less fatty material in thier visceral tissues, several tissues were analyzed for total cholesterol (TABLE 2). As expected, the control animals accumulated a considerable amount of cholesterol in their aorta, skin, and visceral tissues. By comparison, in the DMSO-treated rabbits the cholesterol content of six and the nine tissues analyzed was reduced. The cholesterol content of the thoracic and abdominal aorta was reduced significantly by 50% despite a plasma cholesterol of about 1500 mg/dl.

In order to determine the mechanism of action of DMSO in reducing the accumulation of plasma cholesterol by tissues *in vivo*, the uptake and accumulation of LDL-cholesterol by cultured dermal fibroblasts *in vitro* was investigated.

TABLE 1

INHIBITION OF GROSSLY VISIBLE ATHEROSCLEROTIC LESIONS BY DIMETHYL SULFOXIDE IN RABBITS FED CHOLESTEROL

	Atherosclerotic Involvement					
	12 wks 0.5% Cholesterol			12 wks 0.5% Cholesterol + 12 wks Normal Diet		
Treatment	Mild (<20%)	Moderate (20–50%)	Severe (>50%)	Mild (<20%)	Moderate (20–50%)	Severe (>50%)
Group I (Control)	+	++	+++	+	++	+++
Group II (DMSO)	++	+++	+	++	+++	+

Each + represents one animal.

TABLE 2

CHOLESTEROL CONTENT OF TISSUES FROM RABBITS FED CHOLESTEROL AND DIMETHYL SULFOXIDE FOR 12 WEEKS

	mg Cholesterol per g Dry Weight ± S.E.M.			
Tissue	Untreated Controls	Group I 0.5% Cholesterol	Group II 0.5% Cholesterol + 2% DMSO	Percent Reduction
Thoracic aorta	2 ± 2	59 ± 11	28 ± 6	53*
Abdominal aorta	4 ± 1	28 ± 3	15 ± 1	45*
Heart	7 ± 1	16 ± 1	17 ± 1	–
Lung	20 ± 3	52 ± 6	41 ± 6	22
Liver	10 ± 1	128 ± 12	92 ± 5	28
Spleen	11 ± 2	104 ± 11	66 ± 10	37
Kidney	11 ± 1	40 ± 3	34 ± 4	15
Upper jejunum	13 ± 4	21 ± 3	23 ± 3	–
Skin	6 ± 1	30 ± 8	20 ± 5	33
Plasma cholesterol	60 ± 10	1570 ± 225	1490 ± 156	

Statistical analysis was by Student's *t*-test. Each value is the mean ± S.E.M.
*$p < 0.04$.

Confluent fibroblasts were preincubated for 48 h with lipoprotein-deficient serum (to maximize LDL receptors) and then incubated with [^{125}I]LDL (30 μg protein/ml) for 3 h at 37°C.[6] The medium was removed, the cells were dissociated with 0.05% trypsin-EDTA to release receptor-bound [^{125}I]LDL. Radioactivity in trichloroacetic acid (TCA)-soluble fraction of medium is assumed to represent degraded LDL, that in the TCA-precipitable portion of the cell trypsinate is taken as bound LDL and the radioactivity in the TCA-precipitable, nonlipid-extractable fraction of the cells represents internalized LDL. TABLE 3 shows that DMSO in the culture medium significantly reduced the binding of LDL to the cell surface receptors by about 35%. The amount of LDL that was subsequently internalized and degraded was also reduced by 38 and 26%, respectively.

The results of this study indicate that DMSO retards the development of dietary cholesterol-induced atherosclerosis in rabbits and suppresses the accumulation of cholesterol in tissues despite severe hypercholesterolemia. While the mechanism of action of DMSO in suppressing atherosclerosis is not known, our *in*

TABLE 3

EFFECT OF DMSO ON THE BINDING, INTERNALIZATION, AND DEGRADATION OF [^{125}I]LDL BY HUMAN SKIN FIBROBLASTS

	Nanogram [^{125}I]LDL/mg Cell Protein ± SEM		
	Binding	Internalization	Degradation
Control	188 ± 11	160 ± 4	584 ± 59
2% DMSO	122 ± 7	99 ± 9	432 ± 23
% Reduction	35	38	26

Cells were preincubated for 48 h with 10%, lipoprotein-deficient serum to maximize LDL receptors.

Cells were incubated for 3 h with [^{125}I]LDL equivalent to 30 μg LDL protein/ml medium.

vitro studies suggest that one of its antiatherogenic effects is related to its capacity to reduce the binding, uptake, and degradation of plasma LDL by tissues and cells.

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