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## Short Communication

# THE NEW ORAL HYPOGLYCEMIC AGENT, CS-045, INHIBITS THE LIPID PEROXIDATION OF HUMAN PLASMA LOW DENSITY LIPOPROTEIN *IN VITRO*

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**Abstract**—Several lines of evidence have revealed that the oxidative modification of low-density lipoprotein (LDL) is probably associated with the genesis of the atherosclerotic region. CS-045 is a new (thiazolidine) class of oral hypoglycemic agent which has a hindered phenol in the side chain (an analogue of α-tocopherol). The present results indicate that CS-045 had a relatively high antioxidative potency in inhibiting the lipid peroxidation of human plasma LDL *in vitro* induced by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) compared with that of α-tocopherol. These findings suggest that CS-045 may be useful in preventing the progression of atherosclerosis in diabetic patients.

*Key words:* CS-045; α-tocopherol; low-density lipoprotein; lipid peroxidation

The prevalence of macrovascular disease is markedly increased among individuals with diabetes mellitus. Its major clinical manifestations are consequences of atherosclerosis of coronary arteries, cerebral arteries, and large arteries of the lower extremities. This atherosclerotic vascular disease is the major cause of mortality and significant morbidity in diabetes [1].

Recent evidence suggests that the oxidative modification of LDL‡ plays an important role in atherogenesis [2]. Oxidized LDL may promote atherogenesis through its cytotoxicity to endothelial cells, its chemotactic effect on monocytes, its inhibitory effect on macrophage mobility, and its uptake by the macrophage scavenger receptor mechanism, resulting in a stimulation of cholesterol esterification and, hence, foam cell formation [2]. Oxidative stress is considered to be increased in diabetic patients [1]. If oxidized LDL is crucial to atherogenesis, the potential role of antioxidants in the prevention of the oxidative modification of LDL assumes great importance.

A class of compounds has recently been identified that appears to work by either mimicking or enhancing insulin action without any effects on β-cell insulin secretion [3]. CS-045-((±)-5-[4-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl-methoxy)-)benzyl]-2,4-thiazolidinedione) is an example of this new (thizolidine) class of oral hypoglycemic agents [3]. CS-045 has a hindered phenol similar to α-tocopherol in structure (Fig. 1). This agent has already been shown to improve hyperglycemia and hypertriglyceridemia in patients with non-insulin-dependent diabetes mellitus [4]. However, the effect of CS-045 on the oxidative modification of LDL have yet to be examined. The present study was undertaken to compare the effects of CS-045 and α-tocopherol on lipid peroxidation of human plasma LDL *in vitro*.

### *Materials and Methods*

*Materials.* CS-045 was provided by Sankyo Company Ltd (Tokyo, Japan). 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) was from Wako Chemical Co. (Tokyo, Japan). α-tocopherol and other chemicals were purchased from Sigma Co. (St. Louis, U.S.A.).

*Preparation LDL and* in vitro *lipid peroxidation.* LDL (d 1.019–1.063 g/ml) was isolated by ultracentrifugation from normal human plasma collected in EDTA (1 mg/ml) [5]. The isolated LDL was dialyzed against phosphate-buffered saline, pH 7.4 (PBS), stored at 4°C under $N_2$, and used within a week. Protein concentration was determined by Lowry's method [6], using bovine serum albumin (BSA) as a standard. LDL (0.1 mg protein/ml) was incubated with 2 mM AAPH in PBS at 37°C for 0–24 hr. CS-045 and α-tocopherol were dissolved in PBS containing 2% dimethylformamide (DMFA) and 0.1% BSA as vehicle. The extent of lipid peroxidation was estimated in thiobarbituric acid-reacting substances (TBS-RS) according to the method previously described [7]. Briefly, 1 ml of LDL sample was mixed with 10 μl of 10% t-butylated hydroxytoluene, 10 μl of 1 mM $CuSO_4$, 1 ml of 10% trichloroacetic acid, and 1 ml of 0.67% thiobarbituric acid. After heating at 100°C for 15 min, insoluble materials were removed by centrifugation at 3,000 rpm for 10 min. The reaction product was assayed spectrophotometrically by reading absorbance at 535 nm. TBA-RS (malondialdehyde, MDA) concentration was calculated using an extinction coefficient of $1.56 \times 10^5$ $M^{-1}$ $cm^{-1}$.

### *Results and Discussion*

It has been well established that AAPH generates free radicals thermally at a constant rate of 37°C and induces oxidative

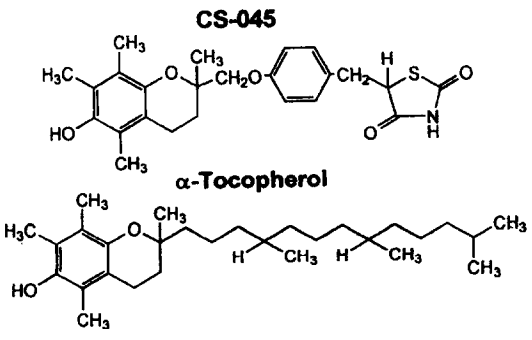


Fig. 1. The structures of CS-045 and α-tocopherol.

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‡ Abbreviations: LDL, low density lipoprotein; AAPH, 2,2′-azobis(2-amidinopropane)dihydrochloride; TBA-RS, thiobarbituric acid-reactive substance; MDA, malondialdehyde.

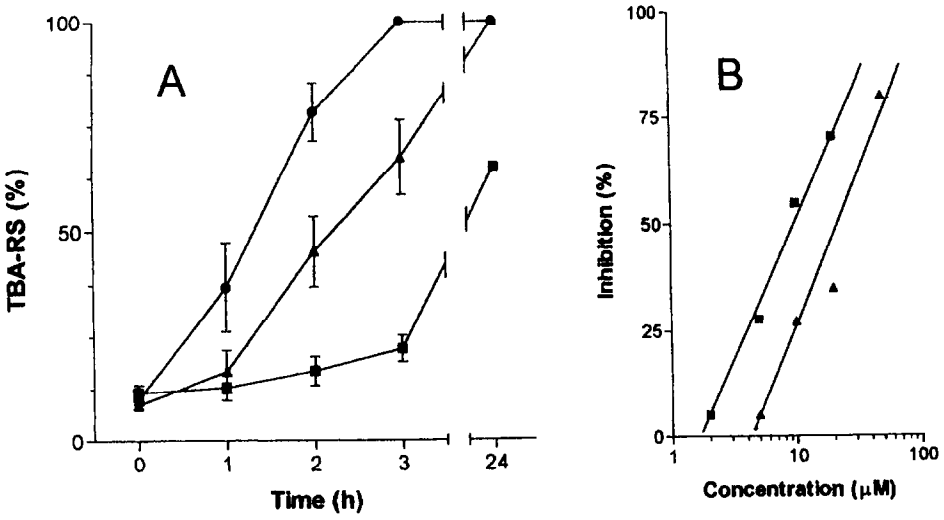


Fig. 2. Inhibition of LDL lipid peroxidation by α-tocopherol and CS-045. LDL (0.1 mg protein/ml) was subjected to oxidation with 2 mM AAPH in PBS in the absence (●) and presence (▲) of α-tocopherol or (■) CS-045. Panel A: TBA-RS contents were expressed as a percent of maximum TBA-RS content observed at 3 hr of incubation with 2 mM AAPH and vehicle alone. Data are presented as the mean ± *SE* of 5 independent experiments. Panel B: After 3 hr of incubation of LDL with 2 mM AAPH and the various concentrations of α-tocopherol (5–50 μM) or CS-045 (2–20 μM), TBA-RS contents in the reaction mixture were measured. Inhibition (%) = $(TBA\text{-}RS_{vehicle} - TBA\text{-}RS_{antioxidant})/(TBA\text{-}RS_{vehicle}) \times 100$ (%).

modifications of human plasma LDL *in vitro* [8, 9, 10]. Oxidative modification of LDL was assessed by its enrichment in lipid peroxides as evidenced by an increase in TBA-RS. The basal TBA-RS content of LDL (0 time) was 0.183 nmol MDA/mg protein. After the addition of 2 mM AAPH, TBA-RS content gradually increased and reached a maximum (22.9 nmol MDA/mg protein) at 3 hr. TBA-RS content did not further increase until 24 hr. In the presence of 20 μM α-tocopherol, lipid peroxidation of LDL was completely suppressed during the first hour, and the TBA-RS content was 65% of the vehicle alone at 3 hr of incubation. This inhibitory effect was not observed at 24 hr of incubation. On the other hand, 20 μM CS-045 inhibited AAPH-induced lipid peroxidation of almost completely during the first 3 hr of incubation (10% of the vehicle alone). This inhibition by CS-045 was still observed even at 24 hr of incubation (65% of the vehicle alone).

Panel B of Fig. 2 shows inhibitory potencies of CS-045 and α-tocopherol on AAPH-induced lipid peroxidation of LDL. LDL was incubated with 2 mM AAPH and various concentrations of α-tocopherol (5–50 μM) or CS-045 (2–20 μM), and TBA-RS was measured at 3 hr of incubation. Under this experimental condition, both α-tocopherol and CS-045 inhibited AAPH-induced LDL peroxidation in a dose-dependent manner. The 50% inhibitory concentrations of CS-045 and α-tocopherol were 10 μM and 25 μM, respectively.

A previous report proposed an antioxidative effect for CS-045, which has a hindered phenol and is similar to α-tocopherol in structure, by estimating rat liver microsomal lipid peroxidation [3]. In the present study, we demonstrated that CS-045 exhibited an antioxidative effect on the oxidative modification of human plasma LDL, and that its antioxidative potency was significantly higher than that of α-tocopherol. Peroxy radicals derived from AAPH abstract hydrogen atoms from polyunsaturated fatty acids in LDL, and initiate the chain reaction [9, 10]. The hindered phenol of α-tocopherol acts as a chain-breaking antioxidant, whereas the major function of the phytyl side chain is to retain the molecule in LDL [9, 10]. Although the mechanism of enhanced antioxidative activity of CS-045 is not clear, the thiazolidine ring of CS-045 may affect the stability of the molecule and the affinity for LDL.

Probucol, a hypocholesterolemic drug whose the structure is similar to the antioxidant butylated hydroxytoluene, is known to reduce lesion formation in atherosclerosis-prone hypercholesterolemic rabbits [11, 12]. This suggests that the antioxidant is effective in preventing the progression of atherosclerosis. A large body of evidence has revealed that LDL oxidation might be accelerated in diabetes [1, 13–16]. The processes of protein glycation can generate reactive oxygen species [13]. Hypertriglyceridemia may be related to increased production of superoxide by monocytes [14]. In addition, the appearance of small and dense LDL particles in diabetes, which are more susceptible than larger particles to oxidative modification, may also promote LDL oxidation [15, 16]. If oxidized LDL were able to play an important role in atherogenesis, antioxidative drugs would be useful in preventing angiopathy in diabetes.

The final goal of the treatment of diabetes is to prevent diabetic complications characterized by microangiopathy and atherosclerosis produced by several factors such as hyperglycemia and dyslipidemia. The present results clearly indicate that CS-045, known to improve hyperglycemia and hypertriglycridemia, exercises its antioxidative effect with relatively high potency. This suggests that the use of CS-045 may help prevent the progression of angiopathy in diabetic patients. Further studies are required to clarify the antioxidative effect of CS-045 *in vivo*.

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