ELSEVIER

European Journal of Pharmacology 383 (1999) 373–379

ejp

www.elsevier.nl/locate/ejphar

SKP-450 inhibits migration and DNA synthesis stimulated by oxidized low density lipoprotein in smooth muscle cells

Hyun Hee Kim [a], Sung-Eun Yoo [b], Won Suk Lee [a], Byung Yong Rhim [a], Ki Whan Hong [a, c, *]

[a] *Department of Pharmacology, College of Medicine, Pusan National University, Pusan 602-739, South Korea*
[b] *Korea Research Institute of Chemical Technology, Daejeon, South Korea*
[c] *Center for Biofunctional Molecules, Pohang University of Science and Technology, Pohang 790-600, South Korea*

Received 15 February 1999; received in revised form 26 July 1999; accepted 30 July 1999

Abstract

This study was carried out to examine the inhibitory effects of SKP-450 (2-[2″(1″,3″-dioxolone)-2-methyl]-4-(2′-oxo-1′-pyrrolidinyl)-6-nitro-2*H*-1-benzopyran), a potassium channel opener, on the proliferation and migration stimulated by oxidized low density lipoprotein (LDL) of cultured smooth muscle cells of Wistar Kyoto rat aorta. SKP-450 (10^{-7} and 10^{-6} M) as well as probucol (10^{-7}–10^{-5} M) reduced the production of thiobarbituric acid reactive substances from LDL submitted to $CuSO_4$ (10 μM). The increased [^{3}H]thymidine incorporation and migration (chemotactic and wound-edge) of the cultured smooth muscle cells in association with increased production of platelet-derived growth factor (PDGF)-BB-like immunoreactivity stimulated by oxidized LDL were significantly reduced by SKP-450 (10^{-7}–10^{-6} M). Inhibition by SKP-450 of the oxidized LDL-stimulated [^{3}H]thymidine incorporation was antagonized by iberiotoxin (10^{-7} M), but not by glibenclamide (10^{-6} M), suggestive of mediation of Ca^{2+}-activated K^+ channel opening in the action of SKP-450. Taken together, SKP-450 inhibited the proliferation and migration of the smooth muscle cells as well as PDGF production stimulated by oxidized LDL, accompanying with its antiperoxidative action.

Keywords: SKP-450; Antiperoxidation; LDL (low density lipoprotein), oxidized; Migration; Proliferation; PDGF (platelet-derived growth factor)

1. Introduction

Elevated plasma concentrations of LDL are associated with accelerated atherogenesis (Golstein and Brown, 1977). The oxidized LDL which is detected in atherosclerotic lesions (Stringer et al., 1989) has been described to be a key step in the initiation of early atherosclerotic lesion (Henrickson et al., 1981). Steinberg et al. (1989) have proposed the lipid-infiltration hypothesis, in that the initiating event in the development of fatty streak is an oxidative modification of LDL that markedly increases its uptake in the arterial intima. Some properties of oxidized LDL are potentially implicated in the atherosclerotic lesion generation: chemoattractant migration and proliferation of smooth muscle cells, chemotactic for monocytes, and foam cell formation, stimulation of monocyte-endothelial cell interaction, and induction of expression of growth factors (Steinbrecher et al., 1990; Ross, 1995). Among these, smooth muscle cells accumulation in the intima is considered as one of key events in the formation and progression of lesion of atherosclerosis (Clowes and Schwartz, 1985; Ylä-Herttuala et al., 1989; Autio et al., 1990; Ross, 1995).

In cultured cells, oxidized LDL greatly stimulates proliferation and migration of smooth muscle cells from media to the intima via abolishing the physiological inhibitory control of normal endothelium, leading to increase in its atherogenic potential (Steinberg et al., 1989). Ylä-Herttuala et al. (1989) and Autio et al. (1990) have shown that oxidized LDL in the intima exerts chemotactic role for medial smooth muscle cells, contributing to plaque formation and atherogenesis, but native LDL has little significant activity. Thus, the oxidative modification of LDL can be an important event in atherogenesis and it participates in inflammatory tissue injury (Li et al., 1993).

On the other hand, K^+ channel opener, cromakalim, has been described to possess vasorelaxant and antihyper-

* Corresponding author. Department of Pharmacology, College of Medicine, Pusan National University, 10 Ami-Dong 1-Ga, Seo-Gu, Pusan 602-739, South Korea. Tel.: +82-51-240-7727, 7726; fax: +82-51-244-1036.

E-mail address: kwhong@hyowon.pusan.ac.kr (K.W. Hong)

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00552-X

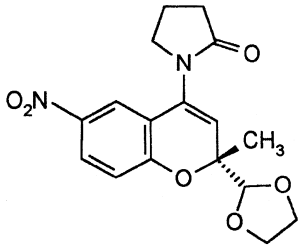

Fig. 1. Chemical structure of (−)-enantiomer SKP-450 (2-[2″(1″,3″-dioxolone)-2-methyl]-4-(2′-oxo-1′-pyrrolidinyl)-6-nitro-2*H*-1-benzopyran).

tensive actions by opening of the K^+ channels, thereby leading to hyperpolarization of the plasma membrane and consequently inhibiting activation of the voltage-dependent Ca^{2+} channels (Hamilton et al., 1986). Recently, Hong et al. (1998) have documented the pharmacological actions of SKP-450 (2-[2″(1″,3″-dioxolone)-2-methyl]-4-(2′-oxo-1′-pyrrolidinyl)-6-nitro-2*H*-1-benzopyran) (Fig. 1), a K^+ channel opener, in which SKP-450 exerts a higher potency than does levcromakalim in vasorelaxation and vasodepressor effects. To our knowledge, the effects of K^+ channel opener on the oxidative modification of LDL and oxidized LDL-induced migration and proliferation of smooth muscle cells were not reported.

Thus, it was aimed (1) to determine whether SKP-450 exerts inhibitory effect on the copper-induced LDL peroxidation, (2) to examine the inhibitory effects of SKP-450 on the oxidized LDL-stimulated DNA synthesis and migration of cultured smooth muscle cells, and (3) to confirm the inhibitory effect of SKP-450 on the PDGF-BB production stimulated by the oxidized LDL.

2. Materials and methods

2.1. Cell cultures

Rat aortic smooth muscle cells were obtained by primary explant culture method described by Sung et al. (1994). Male adult Wistar Kyoto rats (180–200 g) were anesthetized with sodium pentobarbital (100 mg/kg, i.p.). Thoracic aortic segment isolated was placed in Dulbecco's modified eagle's medium (DMEM) containing gentamicin (50 μg/ml). Endothelium was removed by rubbing the intimal surface with a wooden stick. The segments (5×5 mm^2) of aorta were placed intimal surface down into 6-well plates. DMEM containing 20% fetal bovine serum including gentamicin (50 μg/ml) was added over the segments, and muscle cells were allowed to grow out from the aorta for 7–9 days. After smooth muscle cells reach confluence, they were harvested by brief trypsinization and grown in 6-well plates (seeded with 2×10^5 cells per well). The subcultured cells of passage 3 were used. The smooth muscle cells were estimated to be > 95% in purity by cell morphology and by the immunoexpression of myosin. The viability of the cells was > 93% by exclusion test with 0.2% trypan blue.

2.2. Oxidation of LDL

Human LDL (Sigma) was dissolved in distilled water at a concentration of 1 mg/ml. LDL was dialyzed against three changes of phosphate-buffered solution before oxidation to remove EDTA at 4°C for 18 h. The LDL (500 μg/ml) was oxidized by exposure to 10 μM $CuSO_4$ for 18 h at room temperature and dialyzed again against three changes of phosphate-buffered solution at 4°C for 24 h. When the extent of lipid peroxidation was estimated for thiobarbituric acid reactive substance assay using 1,1,3,3-tetramethoxypropane (Sigma) as a standard agent, the oxidative component increased to approximately 70% of the native LDL when analyzed by high performance liquid chromatography (HPLC system, Waters).

The result of thiobarbituric acid reactive substances was expressed as nmol of malondialdehyde equivalents per mg protein, respectively. The average degree of oxidation for native LDL and oxidized LDL was 2.71 ± 0.4 and 27.75 ± 0.2 nmol malondialdehyde equivalents per mg protein, respectively.

2.3. DNA synthesis

Rat aortic smooth muscle cells were grown in 24-well plate to near confluence (3 days) in DMEM containing either 10% FBS. They were made quiescent by substituting DMEM-FBS solution with DMEM containing insulin (5 μg/ml), transferrin (5 μg/ml), and sodium selenite (5 ng/ml) for 48 h. SKP-450 was added 15 min before addition of oxidized LDL (50 μg/ml) for 24-h incubation. DNA synthesis was assessed by measuring the incorporation of [^{3}H]thymidine (1 μCi/ml) into trichloroacetic acid-insoluble fraction for 4 h. Cells were washed with DMEM and treated with 0.25 ml of 0.2 N NaOH for 30 min followed by addition of 1 ml of trichloroacetic acid (15%) for at least 2 h. The samples were filtered through under vacuum by using GF/B Whatman glass microfiber filters (Whatman, Clifton, NJ). After washing the filters 3 times with 2 ml of 5% trichloroacetic acid, the radioactivity was counted by Liquid Scintillation Analyzer (Model A2100, TRI-CARB 2100TR, Packard).

2.4. Chemotactic migration

Chemotatic migration of cultured smooth muscle cells was assayed by using microchemotactic chamber and polycarbonate filters with pores of 8 μm in diameter (Transwell, Corning Costa, Cambridge). Cultured cells were trypsinized and suspended at a concentration of 5×10^5 cells/ml supplemented with 0.4% fetal bovine serum. A volume (50 μl) of cell suspension was placed in the upper chamber, and 100 μl of medium containing either oxidized

LDL or native LDL (50 μg/ml, each) was placed in the lower chamber. The cell suspension was incubated at 37°C under 5% CO_2 in air for 18 h. After incubation, cells on the upper side of the filter were scraped off. The smooth muscle cells that had directly migrated to the lower side of the filter were trypsinized. The cells were stained with Wright-Giemsa solution (Sigma) and counted with the hemocytometer for quantification of chemotactic migration. Migration activity was calculated as the ratio of migrated cells (to low side of filter) to the remained cells in the upper side of the filter, and expressed in percentage.

2.5. Wound-edge migration

The wound-edge migration of cells was determined by razor wound method as described by Bürk (1973). Confluent cell culture plates were wounded with razor by pressing gently through the cell sheet into the plastic well to mark the start line, and then the razor blade was drawn through the monolayer to make clear on one side. The medium was replaced with 1 ml of DMEM with 10% fetal bovine serum containing either native LDL or oxidized LDL (50 μg/ml, each) in the absence and presence of different concentrations of SKP-450. Cell migration was allowed for up to 24 h, and then terminated fixing with ethanol and stained with Wright-Giemsa solution. To evaluate wound-edge migrations, two randomly chosen fields in each well were photographed and the number of cells that randomly migrated from the start line was counted by a person blinded with respect to the identity of the experimental treatments, and the mean values were obtained.

2.6. PDGF-BB-like immunoreactivity assay

Cultured cells were suspended at a concentration of 5×10^5 cells/ml supplemented with 10% fetal bovine serum in the well for 48 h. Then, the wells were treated with native or oxidized LDL without and with SKP-450 (10^{-7} and 10^{-6} M) for 24 h. Thereafter, the cell-conditioned medium collected was centrifuged at $8000 \times g$ for 5 min to remove the detached cell debris, and incubated (at 37°C for 2 h) on the microplates precoated with anti-human PDGF-BB antibodies (2.5 μg/ml, each). By adding the substrate solution of 100 μM of *n*-nitrophenyl phosphate, the color was developed in proportion to the amount of antibody-bound PDGF-BB. The absorbance was read at 405 nm using Microplate Scanning Spectrophotometer (Power Wave X340, Bio-Tek Instruments, Winooski, VT). The concentration of PDGF-BB-like immunoreactivity was expressed as nanogram per well.

2.7. Drugs

SKP-450 (2-[2″(1″,3″-dioxolone)-2-methyl]-4-(2′-oxo-1′-pyrrolidinyl)-6-nitro-2*H*-1-benzopyran) (Fig. 1) was generously donated from the Korea Research Institute of Chemical Technology (Daejeon, Korea) and dissolved in dimethylsulfoxide as a 10 mM stock solution. Glibenclamide (Sigma) was sonicated in 1 ml of NaOH (0.1 N) and diluted with 5% glucose to make a 10 mM stock solution. Probucol and 2′,7′-dichlorofluorescin diacetate (Sigma), nifedipine and iberiotoxin (Research Biochemicals International) were used.

2.8. Statistics

All data are expressed as means ± S.E.M. Statistical differences between groups were determined by paired or unpaired Student's *t*-test or analysis of variance. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Antioxidant action

Application of $CuSO_4$ (10 μM) caused a time-dependent increase in thiobarbituric acid reactive substances production as shown in inset of Fig. 2, and reached a plateau at 18 h. Thus, to see the effect of SKP-450 on its production, LDL was oxidized for 18 h in the presence of SKP-450. In this study, SKP-450 as well as probucol reduced the thiobarbituric acid reactive substance production from LDL submitted to $CuSO_4$ (10 μM) in a concentration-dependent manner. The antioxidant activity of SKP-450 (10^{-7} and 10^{-6} M) was similar to probucol (10^{-7} and 10^{-6} M). Free radical generation was further determined by using 2′,7′-dichlorofluorescein method (Bass et al., 1983). The increased free radicals generated in the presence of $CuSO_4$ (10 μM) were significantly scavenged

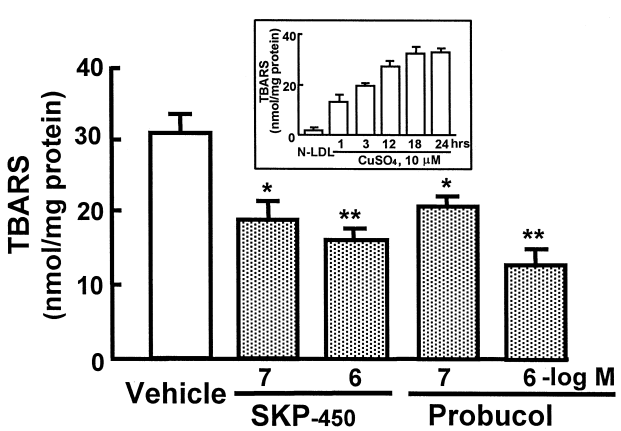

Fig. 2. Inhibitory effects of SKP-450 and probucol on the thiobarbituric acid reactive substance levels produced when native LDL (N-LDL) was exposed to $CuSO_4$ (10 μM). Inset: Time course effect of $CuSO_4$ on the thiobarbituric acid reactive substance formation. Values are means ± S.E.M. of 4 experiments in duplicate. *, $P < 0.05$; **, $P < 0.01$ vs. vehicle.

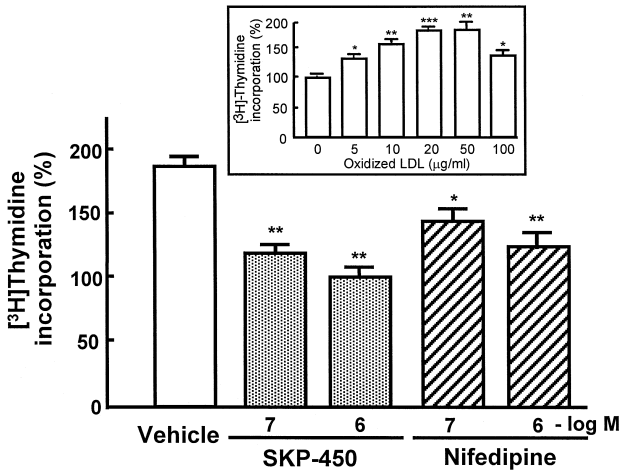

Fig. 3. Effects SKP-450 and nifedipine on the [^{3}H]thymidine incorporation induced by oxidized LDL in cultured smooth muscle cells from rat thoracic aorta. Inset: Concentration-dependent responses of [^{3}H]thymidine incorporation to oxidized LDL (5–50 μg/ml). Values are means ± S.E.M. of 5 to 7 experiments in duplicate. Basal level: 2546.3 ± 21.3 cpm ($n = 7$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ vs. control (Inset) or vehicle.

by probucol (10^{-6}–10^{-5} M), but not by SKP-450 (10^{-7} and 10^{-6} M) (data not shown).

3.2. DNA synthesis

The basal [^{3}H]thymidine incorporation was 2546.3 ± 21.3 cpm ($n = 7$) in the rat aortic smooth muscle cells. Oxidized LDL caused a concentration-dependent increase in [^{3}H]thymidine incorporation from concentration of 5 to 50 μg/ml of oxidized LDL, and the increase in [^{3}H] incorporation was reduced by 100 μg/ml of oxidized LDL (Inset of Fig. 3).

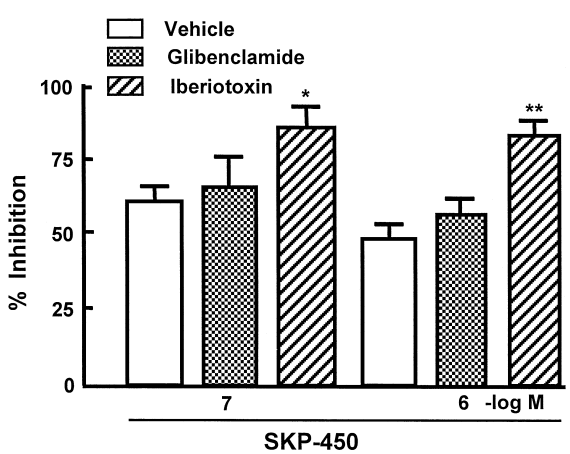

Fig. 4. Reverse by iberiotoxin (Ca^{2+}-activated K^+ channel blocker), but not by glibenclamide (ATP-sensitive K^+ channel blocker), of SKP-450-inhibited [^{3}H]thymidine incorporation. [^{3}H]Thymidine incorporation was stimulated by oxidized LDL (50 μg/ml) in cultured smooth muscle cells. The oxidized LDL-stimulated [^{3}H]thymidine incorporation was 221.3 ± 17.9% ($n = 6$) in the absence of SKP-450. Values are means ± S.E.M. of 6 experiments in duplicate. *, $P < 0.05$; **, $P < 0.01$ vs. vehicle.

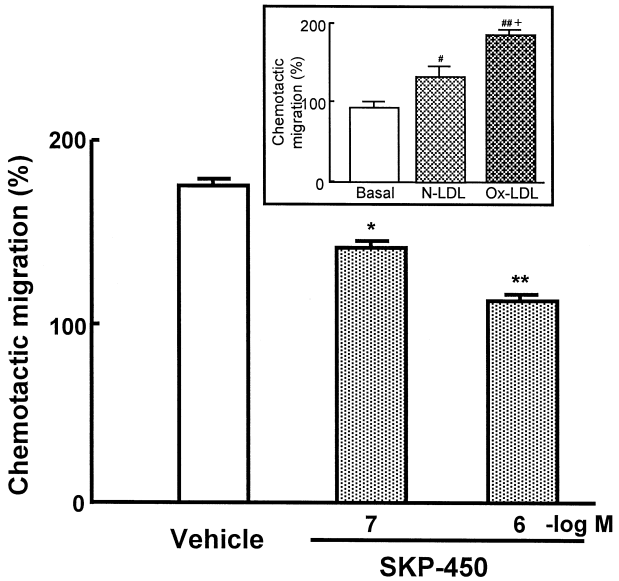

Fig. 5. Inhibitory effect of SKP-450 on the oxidized LDL (Ox-LDL)-stimulated chemotactic migration of cultured smooth muscle cells. The oxidized LDL (50 μg/ml) was added below the filter. Inset: Comparison of the chemotactic migration induced by Ox-LDL (176.0 ± 3.6%) with those of basal level and by native LDL (N-LDL). Values are means ± S.E.M. of 4 experiments in duplicate. #, $P < 0.05$; ##, $P < 0.01$ vs. basal. †, $P < 0.01$ vs. N-LDL. *, $P < 0.05$; **, $P < 0.01$ vs. vehicle.

Oxidized LDL (50 μg/ml) caused a significant increase in [^{3}H]thymidine incorporation by 182.6 ± 12.2% ($P < 0.01$) over the basal level. This increased [^{3}H] incorporation was reduced by 10^{-7} and 10^{-6} M of SKP-450 to 130.6 ± 13.1% and 123.5 ± 12.4% ($P < 0.01$, each), respectively. Pretreatment with 10^{-7} and 10^{-6} M of nifedipine exerted a similar inhibition (Fig. 3).

On the other hand, inhibition by SKP-450 (10^{-7} and 10^{-6} M) of the oxidized LDL-stimulated [^{3}H]-incorporation was significantly reversed by iberiotoxin (10^{-7} M), Ca^{2+}-activated K^+ channel blocker, but not by gliben-

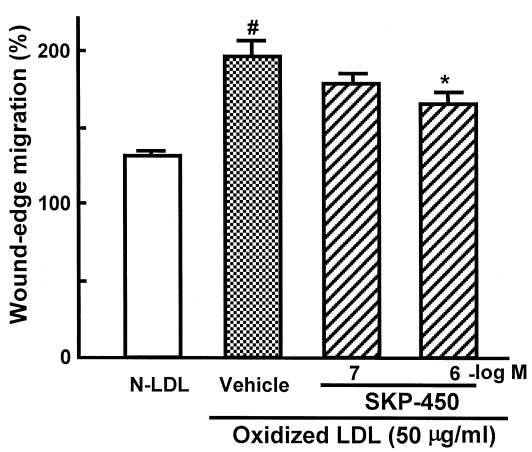

Fig. 6. Effect of SKP-450 on the wound-edge migration which was stimulated by oxidized LDL (50 μg/ml) of the cultured smooth muscle cells. Values are means ± S.E.M. of 4 experiments in duplicate. #, $P < 0.05$ vs. native LDL (N-LDL); *, $P < 0.05$ vs. vehicle.

clamide (10^{-6} M), ATP-sensitive K^+ channel blocker (Fig. 4).

3.3. Smooth muscle cell migration

As shown in Fig. 5, both oxidized LDL and native LDL (50 μg/ml, each) significantly stimulated the chemotactic migration by $176.0 \pm 3.6\%$ ($P < 0.01$) and $130.0 \pm 5.2\%$ ($P < 0.05$) over the basal level, respectively. However, oxidized LDL exerted a higher potency than native LDL ($P < 0.05$). In the presence of SKP-450 (10^{-7} and 10^{-6} M), the increased migration stimulated by oxidized LDL was significantly suppressed to $142.0 \pm 4.2\%$ ($P < 0.05$) and $116.2 \pm 3.3\%$ ($P < 0.01$), respectively.

Otherwise, oxidized LDL (50 μg/ml) significantly enhanced the wound-edge migration in comparison to native LDL. This increased migration was suppressed by 10^{-6} M of SKP-450, but in less degree, when compared with its effect on chemotactic migration (Fig. 6).

3.4. PDGF-BB-like immunoreactivity production

The basal release of PDGF-like immunoreactivity was 39.3 ± 3.5 ng/well equivalent to PDGF-BB. Oxidized LDL (5–50 μg/ml) caused an increase in PDGF-BB-like immunoreactivity production in a concentration-dependent manner (Inset of Fig. 7). Its production in the presence of oxidized LDL (50 μg/ml) was 136.4 ± 8.8 ng/well ($P <$ 0.001). Oxidized LDL-stimulated PDGF-BB-like immunoreactivity production was significantly larger than the value induced by native LDL. Pretreatment with SKP-450, 10^{-7} and 10^{-6} M, markedly inhibited the PDGF-BB-like immunoreactivity production to 81.0 ± 4.1 ng/well ($P <$ 0.05) and 69.1 ± 8.1 ng/well ($P < 0.01$), respectively (Fig. 7).

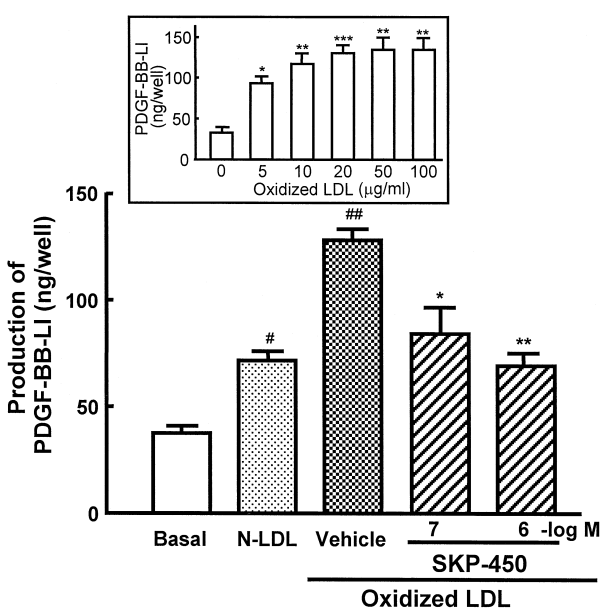

Fig. 7. Effect of SKP-450 on the oxidized LDL-stimulated production of PDGF-BB-like immunoreactivity (PDGF-BB-like immunoreactivity, 136.4 ± 8.8 ng/well) of cultured smooth muscle cells. Inset: Concentration-dependent increase in PDGF-BB-like immunoreactivity production in response to oxidized LDL (5–100 μg/ml). SKP-450 significantly inhibited PDGF-BB-like immunoreactivity production stimulated by oxidized LDL (50 μg/ml). Values are means ± S.E.M. of 5 experiments in duplicate. #, $P < 0.01$; ##, $P < 0.001$ vs. basal. *, $P < 0.05$; **, $P <$ 0.01; ***, $P < 0.001$ vs. control (Inset) or vehicle

4. Discussion

In the present study, we demonstrate that SKP-450, a potassium channel opener, has an antiatherogenic effect by inhibiting (1) the copper-induced oxidative modification of LDL, (2) oxidized LDL-induced migration and [^{3}H]-thymidine incorporation, and (3) oxidized LDL-induced PDGF-BB-like immunoreactivity production from smooth muscle cells of rat aorta.

SKP-450 as an orally active ATP-sensitive K^+ channel opener exerts highly potent antihypertensive and vasorelaxant activities (Hong et al., 1998), and cardioprotective effects in isolated rat hearts in comparison with lemakalim (Lee et al., 1998). In the present study, SKP-450 protected LDL from oxidative modification induced by copper ions as did probucol, a known antioxidant. Henry (1991) documented the antiperoxidative action of Ca^{2+} antagonists such as nifedipine, verapamil, and diltiazem. Weisser et al. (1992) also emphasized the importance of oxidized LDL-induced increase in $[Ca^{2+}]_i$ as a mechanism for its atherogenic potential. Rojstaczer and Triggle (1995) have further described that, in the copper-induced oxidation system, Ca^{2+} antagonists have antioxidant effects with the following order of potency: felodipine > nifedipine > amlodipine > verapamil > diltiazem. Is the antiperoxidative action of dihydropyridine derivatives related with activation of L-type voltage-operated calcium channel? At present time, it is not clear how Ca^{2+} antagonist exerts its antioxidant effect. Free radical scavenging effect was not identified with SKP-450, which was distinct from the effect of probucol (Parthasarathy et al. 1986). The alterations in cytoplasmic calcium have been associated with lipid peroxidation in some cell species (Li et al., 1993). Breugnot et al. (1991) have speculated that the structural modification of the LDL particle may be occurred by insertion of the hydrophobic agent, leading to decreased susceptibility of the lipids to oxidation. Steinberg et al. (1989) have further described that whatever the details of the initiating steps are, once the LDL contains fatty acid lipid peroxides, there follows (especially in the presence of ions) a rapid propagation that amplifies dramatically the number of free radicals and leads to extensive fragmentation of the fatty acid chains. Based on these reports, it is likely that SKP-450 operates most effectively in lipid-rich plasma membrane to suppress the propagation (or initiation) of lipid peroxidation because of its high lipophilicity.

There are growing evidences that accumulation of smooth muscle cells is a combination of proliferation and directed migration of arterial smooth muscle cells from the media into the intima (Ferns et al., 1991), where oxidized LDL has a role for a chemoattractant to smooth muscle cells (Autio et al., 1990). Intracellular calcium has a role of important signaling system involved in the atherogenic process such as hypertrophy, cell migration and proliferation, and cell damage (Weinstein and Heider, 1989; Weisser et al., 1992). Lichtlen et al. (1990) have provided clinical evidence that nifedipine retards the angiographically demonstrated progression of lesions of coronary artery in patients with coronary artery disease.

In the present study, increased [^{3}H]thymidine incorporation as well as chemotactic and wound-edge migrations induced by oxidized LDL were significantly reduced by SKP-450. Furthermore, the increase in [^{3}H]thymidine incorporation by oxidized LDL was associated with increase in PDGF-BB-like immunoreactivity formation, which was also inhibited by SKP-450. As a regulator of migration and proliferation of smooth muscle cells, the production of PDGF was postulated to be implicated as a potent mitogen or a chemotactic factor (Grotendorst et al., 1981; Nilsson et al., 1985). With these reports, it is suggested that inhibition of PDGF-BB by SKP-450 is closely related with inhibition of proliferation and migration stimulated by oxidized LDL. Mogami and Kojima (1993) reported that nickel and reduction of extracellular Ca^{2+} blocked the PDGF-induced Ca^{2+} entry under the premise that the stimulation of calcium entry is prerequisite for initiation of DNA synthesis induced by PDGF in vascular smooth muscle cells. Recently, Steifen et al. (1998) documented their in vitro observations with amlodipine, a Ca^{2+} channel blocker, where its inhibitory effect on cell proliferation might not be related to blockade of voltage-dependent Ca^{2+} channels but to interference with mitogenic signaling system. Overall, the increased PDGF-like immunoreactivity production by oxidized LDL is likely to be involved in the proliferation and migration of aortic smooth muscle cells, and SKP-450 strongly inhibits PDGF-like immunoreactivity production.

In the present study, the inhibition by SKP-450 of the [^{3}H]thymidine incorporation stimulated by oxidized LDL was antagonized by iberiotoxin, but not by glibenclamide, suggesting that some inhibitory effects of SKP-450 are mediated by activation of Ca^{2+}-activated K^+ channels, but not by ATP-sensitive K^+ channels.

Taken together, it is summarized that (1) SKP-450 elicits strong vasorelaxation and hypotension (Hong et al., 1998), (2) it reduces peroxidative modification of LDL, and 3) it potently inhibits PDGF-BB-like immunoreactivity production accompanying with inhibition of proliferation and migration of smooth muscle cells. Further in vivo study will be required to elucidate the effect of SKP-450 on the development of neointima formation in the balloon-injured carotid artery of rat.

Acknowledgements

The Korea Research Institute of Chemical Technology, Daejeon, Korea, generously donated SKP-450. This study was supported by funds from the Korea Science and Engineering Foundation (G-7) and in part by funds from Center for Biofunctional Molecules, Pohang University of Science and Technology, Pohang Korea. We are grateful to Dr. Jonathan Kaskin for the critical English reading of the manuscript.

References

Autio, I., Jaakkola, O., Solakivi, T., Nikkari, T., 1990. Oxidized low-density lipoprotein is chemotactic for arterial smooth muscle cells in culture. FEBS Lett. 277, 247–249.

Bass, D.A., Parce, J.W., Dechatelet, L.R., Szejda, P., Seeds, M.C., Thomas, M., 1983. Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation. J. Immunol. 130, 1910–1917.

Breugnot, C., Maziere, C., Auclair, M., Mora, L., Ronveaux, M.F., Salmon, S., Santus, R., Morliere, P., Lenaers, A., Maziere, J.C., 1991. Calcium antagonists prevent monocyte and endothelial cell-induced modification of low density lipoproteins. Free Radical Res. Commun. 15, 91–100.

Bürk, R.R., 1973. A factor from a transformed cell line that affects cell migration. Proc. Natl. Acad. Sci. USA 70, 369–372.

Clowes, A.W., Schwartz, S.M., 1985. Significance of quiescent smooth muscle migration in the injured rat carotid artery. Circ. Res. 56, 139–245.

Ferns, G.A.A., Raines, E.W., Sprugel, K., Motani, A.S., Reidy, M.A., Ross, R., 1991. Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. Science 253, 1129–1132.

Golstein, J.L., Brown, M.S., 1977. The low-density lipoprotein pathway and its relation to atherosclerosis. Annu. Rev. Biochem. 46, 897–930.

Grotendorst, G.R., Seppä, H.E.J., Kleinman, H.K., Martin, G.R., 1981. Attachment of smooth muscle cells to collagen and their migration toward platelet-derived growth factor. Proc. Natl. Acad. Sci. USA 78, 3669–3672.

Hamilton, T.C., Weir, S.W., Weston, A.H., 1986. Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. Br. J. Pharmacol. 88, 103–111.

Henrickson, T., Mahoney, E.M., Steinberg, D., 1981. Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: recognition by receptor for acetylated low density lipoproteins. Proc. Natl. Acad. Sci. USA 78, 6499–6503.

Henry, P.D., 1991. Antiperoxidative actions of calcium antagonists and atherogenesis. J. Cardiovasc. Pharmacol. 18 (Suppl. 1), S6–S10.

Hong, K.W., Shin, H.S., Kim, C.D., Shin, Y.W., Yoo, S.-E., 1998. Pharmacological profiles of SKP-450 and its family, a K^+ channel opener, in comparison with levcromakalim. Pharmacol. Res. 38, 191–197.

Lee, B.H., Yoo, S.-E., Shin, H.S., 1998. Hemodynamic profile of SKP-450, a new potassium-channel activator. J. Cardiovasc. Pharmacol. 31, 85–94.

Li, Q., Tallant, A., Cathcart, M.K., 1993. Dual Ca^{2+} requirement for optimal lipid peroxidation of low density lipoprotein by activated human monocytes. J. Clin. Invest. 91, 1499–1506.

Lichtlen, P.R., Hugenholtz, P.G., Rafflenbeul, W., Hecker, H., Jost, S., Deckers, J.W., 1990. Retardation of angiographic progression of coronary artery disease by nifedipine. Lancet 335, 1109–1113.

Mogami, H., Kojima, I., 1993. Stimulation of calcium entry is prerequisite for DNA synthesis induced by platelet-derived growth factor in vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 196, 650–658.

Nilsson, J., Sjölund, M., Palmberg, L., Von Euler, A.M., Jonzon, B., Thyberg, J., 1985. The calcium antagonist nifedipine inhibits arterial smooth muscle cell proliferation. Atherosclerosis 58, 109–122.

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

Rojstaczer, N., Triggle, D.J., 1995. Structure-function relationships of calcium antagonists. Effect of oxidative modification of low density lipoprotein. Biochem. Pharmacol. 51, 141–150.

Ross, R., 1995. Cell biology of atherosclerosis. Annu. Rev. Physiol. 57, 791–804.

Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., Witztum, J.L., 1989. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. N. Engl. J. Med. 320, 915–924.

Steinbrecher, U.P., Zhang, H., Lougheed, M., 1990. Role of oxidatively modified LDL in atherosclerosis. Free Radical Biol. Med. 9, 155–168.

Steifen, O., Gogusev, J., Zhu, D.-L., Iouzalen, L., Herembert, T., Drueke, T.B., Marche, P., 1998. Amlodipine inhibition of serum-, thrombin-, or fibroblast growth factor-induced vascular smooth-muscle cell proliferation. J. Cardiovasc. Pharmacol. 31, 786–793.

Stringer, M.D., Gorog, P.G., Freeman, A., Kakkar, V.V., 1989. Lipid peroxides and atherosclerosis. Br. Med. J. 298, 281–284.

Sung, C.P., Arleth, A.J., Storer, B.L., Ohlstein, E.H., 1994. Angiotensin type I receptors mediate smooth muscle proliferation and endothelin biosynthesis in rat vascular smooth muscle. J. Pharmacol. Exp. Ther. 271, 429–437.

Weinstein, D.B., Heider, J.G., 1989. Antiatherogenic properties of calcium antagonists. Am. J. Med. 86 (Suppl. 4A), 27–32.

Weisser, B., Locher, R., Mengden, T., Vetter, W., 1992. Oxidation of low density lipoprotein enhances its potential to increase intracellular free calcium concentration in vascular smooth muscle cells. Arterioscler. Thromb. 12, 231–236.

Ylä-Herttuala, S., Palinski, W., Rosenfeld, M.E., Parthasarathy, S., Carew, T.E., Butler, S., Witztum, J.L., Steiberg, D., 1989. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J. Clin. Invest. 84, 1086–1095.