チアゾリジン誘導体薬とジヒドロビリジン系カルシウム拮抗薬の抗酸化作用

水重克文

ANTIOXIDATIVE ACTIVITIES OF THIAZOLIDINEDIONES AND DIHYDROPYRIDINE-TYPE CALCIUM ANTAGONISTS

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Abstract Oxidative stress is involved in the initiation and progression of atherosclerosis. Oxygen free radicals either on cell wall membrane or on LDL particles facilitate lipid peroxidation non-enzymatically in several clinical conditions related to oxidative stress such as aging, smoking, coronary ischemia-reperfusion and diabetes mellitus. We examined the antioxidant activities of thiazolidinediones (insulin sensitizer) and their effect on cardiovascular function in type 2 diabetic model rats, and also those of dihydropyridines (commonly used anti-anginal and antihypertensive agents) in cultured human endothelial cells. In the pre-diabetic or early diabetic stage, hyperinsulinemia or insulin resistance is thought to be closely associated with oxidative stress. We administered pioglitazone or troglitazone, and measured 8-iso-prostaglandin F$_2$α and malondi aldehyde-thiobarbituricacid. This study demonstrated impairment of the antioxidant system and an accumulation of collagen in the myocardium or aortic wall, and confirmed the antioxidant effects of thiazolidinedione compounds.

In the present review, we introduce the role of transforming growth factor (TGF)-β-mediating cardiovascular complications and the effects of thiazolidinediones on cardiovascular function. We also examined the effects of dihydropyridine-type calcium antagonists on lipid peroxidation in cultured human arterial endothelial cells. Endothelial cells were exposed to 1mM H$_2$O$_2$, and then treated with nifedipine, amiodipine or azelnidipine (a newly developed dihydropyridine). The antioxidant activity was evaluated by measuring 8-iso-prostaglandin F$_2$α concentration. Azelnidipine exhibits potent antioxidant activities, which we also introduced in this review.

(Key Words: diabetes mellitus, atherosclerosis, myocardial fibrosis, oxidative stress, isoprostanes, insulin-resistance, thiazolidinedione, dihydropyridine)

Introduction

As a consequence of gaining the ability to utilize oxygen, species that have adapted to living out of water are susceptible to oxidative stress injury. Today, oxidative stress is known to be involved in many diseases. In several clinical conditions related to oxidative stress, a series of bioactive prostaglandin (PG) F$_2$-like compounds, known as isoprostanes, has been discovered$^{[1]}$. These eicosanoids are produced from arachidonic acid via a non-enzymatic process of lipid
peroxidation catalyzed by oxygen free radicals
either on cell membranes or on LDL particles\(^1\). In particular, 8-iso-PGF\(_{2\alpha}\) is of importance in
inducing vasoconstriction\(^2\) and stimulating vascular
smooth muscle cell proliferation\(^3\). In diabetes
mellitus (DM), considerable controversy surrounds
the precise mechanism by which hyperglycemia
contributes to the development of atherosclerosis.
In this review, we summarize the role of oxidative
stress in the initiation and progression of
atherosclerosis in DM, and its prevention with
thiazolidinediones and dihydropyridine-type calcium
antagonists.

**Oxidative Stress and Isoprostane**

Isoprostane, a new member of the eicosanoids
produced via a free radical-generated and cyclo-
oxigenase-independent process\(^1\), was first
discovered by Morrow et al. in 1990. This
prostaglandin F\(_2\alpha\)-like substance is detected in
pooled sera from normal human beings when
stored for several months at \(-20^\circ\)C\(^5\). During storage,
even at such low temperature, its concentration
increased by several hundred fold. By means of
biochemical analyses, Morrow et al. found that a
ring structure is formed in situ in arachidonic acid
at the sn-2 position of the phospholipid in the lipid
bilayer of cytoplasmic membrane. This is processed
through free radical-mediated lipid peroxidation
independent of cyclooxygenases, and a PGF\(_2\alpha\)-like
substance is cleaved, probably by phospholipase A\(_2\)
to produce the free form\(^6\). The eicosanoids produced
via this free radical-mediated pathway were
named "isoprostanes"\(^1\). 8-iso-PGF\(_{2\alpha}\). The first F\(_2\alpha\)-
isoprostane to be discovered, is particularly
abundant in vivo. Many reports have shown that
it is an effective and sensitive marker of oxidative
stress in vivo and has many biological functions.
Renal arterial vasoconstriction may be caused via
putative specific isoprostane receptors, partially
homologous to but distinct from thromboxane
receptors in cultured rat aortic smooth muscle cells\(^5\).
In these cells, 8-iso-PGF\(_{2\alpha}\) also stimulated their
proliferation through activation of the mitogen-
activated protein kinase (MAP-kinase) cascade\(^7\).

Heterologous tissue distribution of putative isoprostane
receptors was observed, since binding studies showed
specific binding in cultured bovine aortic endothe-
Dial cells\(^8\) but not in cultured rat mesangial cells\(^9\).
In the former cells, 8-iso-PGF\(_{2\alpha}\) proliferation was
partially mediated through the production of
endothelin\(^10\).

Since we initially reported the atherogenic
property of isoprostane, many others have shown
its involvement in human atherosclerotic disorder.
The marked increase of urinary 8-iso-PGF\(_{2\alpha}\) secretion
in smoking\(^11-15\) is believed to be closely related both
to oxidative stress and to cardiovascular diseases\(^16\).

The abundance of 8-iso-PGF\(_{2\alpha}\) in atherosclerotic
plaques of endoarterectomized carotid artery specimens
was measured using biochemical and immunohistochemical
methods\(^17\). Davi et al\(^18\) found the increase of
urinary 8-iso-PGF\(_{2\alpha}\) excretion to be independent of
cyclooxygenases in hypercholesterolemic patients,
which was inversely related to plasma vitamin E
concentration. They also reported that urinary
8-iso-PGF\(_{2\alpha}\) excretion was greater in diabetic patients
than in healthy controls, a feature which reduced
in parallel with improved metabolic control\(^19\). Pratico et a
\(^20\) showed that in apolipoprotein E deficient mice
the extension of atherosclerosis of the aortic wall
is closely related to its 8-iso-PGF\(_{2\alpha}\) content, and
that vitamin E improved atherosclerosis as well as
decreased 8-iso-PGF\(_{2\alpha}\) content.

**Diabetes Mellitus, Oxidative Stress and Thiazolidinediones**

Although a number of equally tenable hypoth-
eses, including the advanced glycation end product
hypothesis\(^21-22\) or the oxidative stress hypothesis\(^23-29\),
have been suggested as the mechanisms of atherosclerosis
in DM, none are definitive due to the complexity
of diabetic pathophysiology. Additionally, in the
process of atherosclerosis, many factors are
reported to be involved\(^29\), including the presence
of vasoactive substances such as angiotensin II\(^29\),
mechanical stretches\(^29\), growth factors, and cytokines\(^29\).
In particular, transforming growth factor-\(\beta\)
(TGF-\(\beta\)) is considered an important factor\(^29\), and
its action has been reported to involve oxidative
stress\(^29,30\).
Numerous epidemiological studies have shown that dietary intake of vitamin E (α-tocopherol) is inversely correlated to the risk of cardiovascular disease. However, randomized intervention trials have failed to prove the cardiovascular benefit of treatment with this anti-oxidative vitamin. On the other hand, troglitazone, a thiazolidinedione possessing a similar molecular structure of α-tocopherol (Fig. 1), was developed as an antidiabetic drug to improve peripheral insulin sensitivity and hyperinsulinemia both in diabetic animal models and in patients with type 2 DM.

The antidiabetic effect of pioglitazone, the second thiazolidinedione, depends on the presence of insulin. Pioglitazone decreases insulin resistance in the periphery and liver, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Thiazolidinediones are potent and highly selective agonists for peroxisome proliferator-activated receptor (PPAR) γ. PPAR receptors are found in tissues that are important for insulin action, such as adipose tissue, skeletal muscle, and hepatic parenchymal cells. Activation of PPARγ nuclear receptors modulates the transcription of a number of insulin-responsive genes involved in the control of glucose and lipid metabolism. Okuno et al., examined the effects of troglitazone on the white adipose tissues of an obese animal model (obese Zucker rat) and demonstrated an increase in the number of small adipocytes and a decrease in the number of large adipocytes. Small adipocytes take up more glucose than large adipocytes at submaximal levels of insulin and are more sensitive to the antilipolytic action of insulin. The authors suggested that this is the primary action of troglitazone and an important mechanism of the normalization of tumor necrosis factor (TNF). α levels and lipid metabolism. In DM animal models, pioglitazone similarly reduced the hyperglycemia, hyperinsulinemia and hypertriglyceridemia that are characteristic for insulin-resistant states, such as type 2 DM. The metabolic changes produced by thiazolidinediones result in increased responsiveness of insulin-dependent tissues. Since thiazolidinediones enhance the effects of circulating insulin, they do not decrease blood glucose in animal models that lack endogenous insulin.

**Fig. 1 Structures of thiazolidinediones and α-tocopherol**

Impairment of Aortic Distensibility in Type 2 Diabetes Mellitus and the Effects of Thiazolidinediones

Antioxidant treatment, as well as correcting insulin sensitivity, improved histopathological changes of the aorta in type 2 DM rats. This improvement was associated with a decrease in plasma and tissue levels of 8-iso-PGF₂α, a lipid peroxidation product of arachidonic acid metabolism, thus suggesting its contribution to arteriosclerosis by initiating the pathogenic process in the vascular system. We used Otsuka Long-Evans Tokushima Fatty (OLETF) rats as model animals of type 2 diabetes and Long-Evans Tokushima Otsuka (LETO) rats as non-diabetic control animals. The OLETF rat manifests mild obesity, normoglycemia with insulin resistance and enhanced gene expression of collagen before 15 weeks of age. These clinical and pathological features resemble human pre-diabetes. On the basis of oral glucose tolerance testing in our study, the OLETF rats were pre-diabetic, and insulin resistance occurred at 10 to 20 weeks of age. At 30 weeks of age, the OLETF rats showed minor abnormalities in fasting blood glucose levels (112±12 mg/dl) and elevation...
of 2-hour blood glucose (294±41 mg/dl) and insulin (7,967±1,436 pg/ml) levels. At 24 and 36 weeks of age, the casual blood glucose levels were similarly elevated. This suggests that the OLETF rats had reached the stage of impaired glucose tolerance with postprandial hyperglycemia. In this condition, impairment of lipid metabolism was simultaneously apparent.

In the previous experiment, animals were randomly assigned to 3 experimental groups at 20 weeks of age: those given a standard diet, a diet supplemented with troglitazone, or a diet supplemented with vitamin E. At 36 weeks of age, treatment with either troglitazone or vitamin E markedly reduced tissue 8-iso-PGF$_2$α in the aorta (Fig. 2). Cross-sectional vessel and lumen areas of the excised aorta stained with hematoxylin-eosin were measured by tracing the adventitia-media border and intima surface, respectively, using a magnification of x20. The medial area was calculated as (vessel area) − (lumen area). At 36 weeks of age, the medial area of untreated OLETF rats was larger than that of untreated LETO rats. In OLETF rats, the medial area was significantly reduced following treatment with either troglitazone or vitamin E, and more so in the TR-treated OLETF than in the VE-treated OLETF group. The ratio of medial to vessel area calculated as (medial area) x100/(vessel area) showed a similar trend to that seen in the results of the medial area. The nuclear number of smooth muscle cells per visual field was counted using a magnification of x400. The nuclear number of smooth muscle cells per cross-sectional area was calculated as (nuclear number per visual field area) ×(medial area)/(visual field area)$^{30}$. The nuclear number of smooth muscle cells per cross-sectional area in the treated groups were smaller than in the untreated group. In particular, the number in the VE-treated OLETF rats was significantly smaller than in the TR-treated OLETF rats (Fig. 3).

Vascular smooth muscle cells have been found to have insulin receptors and to exhibit insulin-induced responses, and Law et al. have demonstrated that the proliferation and migration of cultured smooth muscle cells are inhibited by troglitazone in an arterial balloon injury model in rats. In addition to insulin-induced responses, Rao and Berk, and Kyaw et al. have shown that vascular smooth muscle cell proliferation is strongly related to active oxygen species production. Our in vivo study also demonstrated that the potent anti-oxidant activity of vitamin E effectively prevented cell proliferation, suggesting that oxidative stress was relatively important as a mechanism of cell proliferation.

Troglitazone is not only an insulin-sensitizing compound, but also a potent anti-oxidant due to its structural similarity to vitamin E, and therefore both the hyperinsulinemia and oxidative stress implicated in the development of type 2 DM are considered to be the cause of smooth muscle cell proliferation.

The transforming growth factor (TGF)-β family has been identified in mammals, where it modulates cell growth and differentiation as well
as extracellular matrix deposition and degradation. Immunohistochemical analyses of TGF-β and TGF-β receptor II were carried out, and were semi-quantitatively analyzed according to the ‘scoring’ method. Scores were as follows: 0, absent or very weak staining; 1+, staining involving 1% to 25% of smooth muscle cell and endothelial cell area; 2+, staining involving 25% to 50%; 3+, staining involving 50% to 75%; and 4+, staining involving >75%. Immunohistochemical stainings of TGF-β and TGF-β receptor II were found to be much more abundant in the endothelium and medial smooth muscle cells of the aorta in OLETF rats than in LETO rats. The immunohistochemical staining score of TGF-β decreased in the troglitazone treated groups, but the expression of TGF-β receptor II was not changed.

In the previous study, there was a significant accumulation of collagen in the aortic media of OLETF rats. Collagen accumulation may have also contributed to the increase in medial wall thickness. Gene expression of the TGF-β or enhanced gene expression of collagen may participate in the onset of tissue fibrosis by stimulating extracellular matrix synthesis. In our study, expression of TGF-β and an increase in aortic wall thickness were observed simultaneously. In addition, potent antioxidant activity decreased the expression of TGF-β, which may have decreased the stimulation of extracellular collagen synthesis. On the other hand, 8-iso-PGF₁α is formed during the reactive oxygen species-dependent peroxidation of lipid-esterified arachidonic acid, and its increase accurately indicates oxidative stress. Montero et al measured the plasma and urine concentrations of 8-iso-PGF₁α and TGF-β expression in glomeruli in streptozotocine-induced diabetic rats, and their results clearly indicate the relationship between 8-iso-PGF₁α production and TGF-β expression in vivo despite its expression in mesangial cells. Consequently, the production of 8-iso-PGF₁α due to oxidative stress induced the expression of TGF-β and also medial fibrosis of the aorta in DM rats. Both TGF-β and TGF-β receptor expression were greater in diabetic rats than in non-diabetic control animals. TGF-β expression was suppressed by either troglitazone or vitamin E, while TGF-β receptor expression was affected by neither of these two agents. This inhibition of TGF-β expression paralleled the suppression of medial proliferation by troglitazone and vitamin E.

With regard to lipid and glucose metabolism, vitamin E improved these parameters to a degree similar to that observed in the troglitazone group. Kaneto et al demonstrated in a type 2 diabetes mouse model that apoptosis induced by oxidative stress causes reduction of β-cell mass, and that
antioxidant treatment (N-acetyl-L-cysteine) suppressed apoptosis in β-cells. Although vitamins C and E were not effective in their experiment, the usefulness of antioxidants in the treatment of type 2 diabetes has been suggested. Consequently, the exact reason for the improvement of lipid and glucose metabolism in the present experiment remains unknown, but it may be due to preservation of β-cell function or the amelioration of insulin resistance through anti-oxidation by vitamin E.

Natarajan et al. have studied the formation of 8-iso-PGF₂α in porcine vascular smooth muscle cells cultured under hyperglycemic conditions, and suggested that 8-epi-PGF₂α may lead to increased vascular smooth muscle cell growth. Indeed, our in vivo study demonstrates the increase in the nuclear number of vascular smooth muscle cells following augmentation of 8-iso-PGF₂α production. The direct addition of 8-iso-PGF₂α to vascular smooth muscle cells for 24 hours leads to an increased formation of the matrix protein fibronectin, which is known to play a causative role in vascular hypertrophy and neointima formation (hypertrophic and hyperplastic effects). In our in vivo study, the concentration of 8-iso-PGF₂α in aortic tissue was reduced following treatment with both vitamin E and troglitazone, but a significant reduction in plasma 8-iso-PGF₂α was observed only after administration of vitamin E. Although the actual reason for this discrepancy is unknown, a decrease in 8-iso-PGF₂α and a reduction of medial proliferation due to anti-oxidation were accurately demonstrated.

Myocardial Dysfunction During the Prediabetic Stage

Although the principal pathophysiological feature of DM is the morphological and functional alteration of microvessels (diabetic microangiopathy)⁶⁵, the presence of myocardial dysfunction independent of coronary artery disease in DM (diabetic cardiomyopathy) has been well documented⁶⁶. Such focal changes in microvessels are insufficient to account for the diffuse myocardial degeneration with interstitial fibrosis in diabetic cardiomyopathy. We evaluated cardiac function in the type 2 diabetic rat model (OLETF rat). The deceleration time of early left ventricular diastolic filling waveform recorded by Doppler echocardiography was prolonged and its peak velocity was decreased in the pre-stage of type 2 DM. A lengthening of deceleration time and a decrease in peak velocity of early diastolic filling suggest the presence of myocardial diastolic dysfunction⁶⁶, which is interestingly related to insulin resistance. In the present study, TGF-β receptor II expression increased significantly in the left ventricle of OLETF rats, and the ratio of collagen content/dry weight of the left ventricle was significantly higher in OLETF rats at the pre-diabetic stage⁶⁶. The gene expression of this cytokine might be induced by metabolic abnormalities and participate in the onset of cardiac fibrosis by stimulating extracellular matrix synthesis.

Baynes reported that oxidative stress may be amplified by a continuing cycle of metabolic stress, tissue damage, and cell death, leading to increased free radical production and compromised free radical inhibitory and scavenger systems. We also demonstrated that the previously observed impairment of the antioxidative system was in the aortic wall during the early stage of type 2 DM, and troglitazone, belonging to the thiazolidinedione group, had the effect of an antioxidant in vivo⁶⁸. Myocardial interstitial accumulation of connective tissue and glycoproteins also developed early in experimental diabetic animals and was associated with left ventricular diastolic dysfunction⁶⁶. In the prediabetic stage, it has been suggested that hyperinsulinemia or insulin resistance are closely associated with oxidative stress⁷⁰. Increased reactive oxygen species are involved in the initiation of myocardial collagen accumulation and the development of tissue damage. Cardiac fibrosis may play a role in the progressive deterioration of cardiac hemodynamics and provide an explanation for the diastolic dysfunction.

Prevention of Myocardial Fibrosis

Pioglitazone is effective not only in the treatment of established type 2 DM and in the prevention of nonhyperglycemic insulin-resistant state deteriorat
ion, but it also has potential as a therapeutic agent to treat diabetic cardiomyopathy and to prevent diabetic heart failure in the early stage. In our previous study\(^5\), plasma triglycerides and total cholesterol were significantly reduced following 5 weeks of oral treatment with 0.01% pioglitazone-containing food from 15 weeks of age. Plasma insulin and glucose were also markedly reduced by pioglitazone treatment. Induction of oxidative stress could be evaluated by an increase in plasma concentration of lipid peroxidation product\(^6\), which was determined by measurement of malondialdehyde-thiobarbituric acid (MDA) using the TBA method. Pioglitazone decreased MDA concentration and left ventricular collagen content/dry weight at the pre-stage of type 2 DM, and collagen content/dry weight was significantly correlated with MDA concentration \((r=0.60, P=0.007)\). Left ventricular weight was decreased and interstitial fibrosis was histopathologically reduced after 5 weeks of treatment with pioglitazone at the pre-diabetic stage. With regard to the left ventricular diastolic dysfunction, the peak velocity of early transmitral inflow significantly increased and its deceleration time tended to be shortened by pioglitazone, which improved left ventricular diastolic dysfunction.

Abnormal regulation of TNF-\(\alpha\) was important in the pathogenesis of obesity-related insulin resistance\(^6\), which exacerbated oxidative stress. In the previous study, the decrease in lipid peroxidation products measured as MDA was observed following treatment with pioglitazone, and myocardial collagen content was significantly reduced\(^6\). We previously reported that TGF-\(\beta\) receptor \(\beta\) increased significantly in the left ventricle of diabetic rats, and the ratio of collagen content/dry weight of the left ventricle was greater in diabetic than in non-diabetic rats aged 15 weeks\(^6\). Although several mechanisms of cardiac dysfunction in type 2 DM have been proposed\(^6\), the gene expression of TGF-\(\beta\) or enhanced gene expression of collagen may participate in the onset of cardiac fibrosis by stimulating extracellular matrix synthesis\(^8\). Consequently, the correlative reduction in collagen content/dry weight and MDA concentration may suggest that a reduction in oxidative stress decreases the expression of TGF-\(\beta\) and stimulation of extracellular collagen synthesis\(^8\).

In addition, although Inoue et al\(^8\) demonstrated that troglitazone has a similar molecular structure to \(\alpha\)-tocopherol and has an antioxidant activity in vitro experiments, pioglitazone does not have a similar molecular structure. In the structure of pioglitazone, the aromatic phenyl ring and pyridine ring are hydroxylated, thus possibly scavenging hydroxyl radicals. Since pioglitazone does not have a structural similarity to \(\alpha\)-tocopherol (established antioxidant), its scavenging effect may be weak. The antioxidant effect of pioglitazone was also produced by indirectly increasing insulin sensitivity. Consequently, the antioxidation mechanism of pioglitazone may be different from that of troglitazone. The hypoglycemic and hypolipidemic effects of pioglitazone may have diminished the expression of TNF-\(\alpha\). Furthermore, reduction in oxidative stress may have suppressed the TGF-\(\beta\) and collagen accumulation.

### Antioxidant Effects of Dihydropyridines

Dihydropyridine-type calcium antagonists have anti-atherogenic potency independent of calcium entry blockades. Jackson et al\(^9\) demonstrated the anti-atherogenic action of nifedipine in balloon-injury model rats and showed that such action was the result of early inhibition of smooth muscle cell proliferation. Anti-oxidative activity was indicated to be a possible anti-atherogenic mechanism of dihydropyridines\(^9\). Recently, azelnidipine was developed as a dihydropyridine calcium blocker and is characterized by gradual and long-lasting antihypertensive action with low incidence of tachycardia\(^8\), a well-known complication of this drug class. Azelnidipine exhibits potent anti-oxidative activity at its clinical dosage, which may be of significant clinical benefit when combined with this long-lasting activity and mild decrease in heart rate.

We examined directly whether or not \(\text{H}_2\text{O}_2\)-induced membrane lipid peroxidation in cultured human arterial endothelial cells, and found that
1mM H₂O₂ induced approximately 130 pg/ml 8-iso-PGF₁α. In this experiment, total 8-iso-PGF₁α was significantly inhibited by α-tocopherol (p < 0.05), various concentrations of azelnidipine (p < 0.05), nifedipine (p < 0.05) and amlodipine (p < 0.05). Inhibition of 8-iso-PGF₁α by 10nM azelnidipine was greater than that by 100µM α-tocopherol (p < 0.05), 100nM nifedipine (p < 0.05) or 100nM amlodipine (p < 0.05). Arita et al. measured the plasma concentration of azelnidipine after 4-week oral administration of 8 mg once a day, and its concentration was found to be 13.0 ± 7.8 ng/ml (22.1 ± 13.3nM). Their results indicated that the clinical dose of azelnidipine maintained a plasma concentration of 10nM or greater. In our study, the concentration of 8-iso-PGF₁α was suppressed to approximately 40% of that normally observed without azelnidipine incubation. With regard to the serum concentration consistent with the clinical dosage of nifedipine and amlodipine, these were 180.7nM at maximum concentration for oral administration of 20 mg nifedipine and 10.4nM for oral administration of 10 mg amlodipine. The fluid concentration of nifedipine in our study was based on the serum concentration on oral administration. Additionally, we compared nifedipine’s antioxidant activity with that of 10nM and 100nM azelnidipine (clinical level and the same level as nifedipine, respectively). As a result, the suppression of 8-iso-PGF₁α was not significantly different between the 10nM and 100nM concentrations of azelnidipine, and the antioxidant activity of 100nM nifedipine was lower than that of azelnidipine, even at 10nM. In the case of amlodipine, antioxidant activity was low even though the concentration of the present study (100nM) was higher than that used clinically (approximately 10nM). Our results indicated that the antioxidant activity of azelnidipine was greatest among the three calcium antagonists examined and α-tocopherol. Yao et al. also examined the antioxidant activities of nine calcium antagonists in rat brain homogenates and found the relative order of antioxidant potency to be nifedipine > bnrnidipine > benidipine > nocardipine > amlodipine > nilvadipine > nitrendipine, with little inhibitory effect shown by diltiazem and verapamil. These data indicate that nifedipine is a potent antioxidant agent, but in clinical usage azelnidipine was found to be more so.

An increase in antioxidant effect between concentration of 1nM and 10nM of azelnidipine demonstrated its dose-dependent antioxidant activity. However, no significant difference in concentration of 8-iso-PGF₁α was observed between the 10nM and 100nM dosages. Mak et al. demonstrated concentration-dependent (10−40 µM) inhibitory effects of nifedipine against the mitochondrial lipid peroxidation. Although the mechanism of azelnidipine’s antioxidant effects was not examined, this difference in dose-dependency between our experiment and that of Mak et al. may be due to methodological differences, the production of hyperoxidant conditions or the cells used (ventricular myocytes or endothelial cells).

Mason et al. evaluated the neuroprotective effects of voltage-sensitive L-type Ca²⁺ channel blockers, including nifedipine, amlodipine and nimodipine, that modulate Ca²⁺ homeostasis and attenuate reactive oxygen species. In their experiments, using cerebellar granule cell preparations, amlodipine was the most potent inhibitor of neuronal apoptosis by limiting oxidative damage. They suggested that the electron-rich aromatic ring of amlodipine is highly characteristic of a “chain-breaking” antioxidant. In our study, amlodipine’s weaker antioxidant activity compared to that of nifedipine was due to the difference in experimental conditions. Nevertheless, the molecular structure of each dihydropyridines containing an aromatic ring is an important factor regulating its antioxidant activity (Fig. 4). We believe that because the molecular structure of azelnidipine contains either the hydrophobic portion or the electron-rich aromatic rings produced by oxidation of the dihydropyridine ring, which are able to trap free radicals, its potent antioxidant activity is attributable to structural characteristics.
Fig. 4 Structures of dihydropyridine-type calcium antagonists

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