

Edaravone (3-Methyl-1-Phenyl-2-Pyrazolin-5-one), A Novel Free Radical Scavenger, for Treatment of Cardiovascular Diseases

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Abstract: Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a strong novel free radical scavenger, is used for treatment of patients with acute brain infarction. Edaravone has preventive effects on myocardial injury following ischemia and reperfusion in patients with acute myocardial infarction. Antioxidant actions of edaravone include enhancement of prostacyclin production, inhibition of lipoxygenase metabolism of arachidonic acid by trapping hydroxyl radicals, inhibition of alloxan-induced lipid peroxidation, and quenching of active oxygen, leading to protection of various cells, such as endothelial cells, against damage by reactive oxygen species (ROS). Recently, we have shown that edaravone improves endothelial function through a decrease in ROS in smokers. From a clinical perspective, it is important to select an appropriate drug that is effective in improving endothelial function in patients with cardiovascular diseases. The novel free radical scavenger edaravone may represent a new therapeutic intervention for endothelial dysfunction in the setting of atherosclerosis, chronic heart failure, diabetes mellitus, or hypertension. This review focuses on clinical findings and on putative mechanisms underlying the beneficial effects of the antioxidative agent edaravone on the atherosclerotic process in patients with cardiovascular diseases.

Keywords: Cardiovascular disease, endothelial function, reactive oxygen species, free radical scavenger.

INTRODUCTION

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, Fig. 1), a strong novel free radical scavenger, has been developed by Mitsubishi-Tokyo Pharmaceuticals Inc (Tokyo, Japan). This agent has been used in patients with acute brain infarction since April 2001 in Japan [1-7]. Edaravone has been shown to prevent brain edema after ischemia and reperfusion injury in animal models [8-13] and in stroke patients [14]. Moreover, it has been shown that edaravone has preventive effects on myocardial injury following ischemia and reperfusion in the rat heart [15,16] and in patients with acute myocardial infarction [17].

It is thought that edaravone shows antioxidant actions through enhancement of prostacyclin production, inhibition of lipoxygenase metabolism of arachidonic acid by trapping hydroxyl radicals, inhibition of alloxan-induced lipid peroxidation, and quenching of active oxygen, leading to protection of various cells such as endothelial cells and myocardial cells, against damage by reactive oxygen species (ROS). In addition, edaravone improves endothelial function in smokers through an increase in nitric oxide (NO) bioavailability [18].

SOURCES AND METABOLISM OF ROS

ROS includes superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), hypochlorous acid (HOCl), NO, and peroxynitrite ($ONOO^{\cdot-}$). $O_2^{\cdot-}$, OH, and NO are

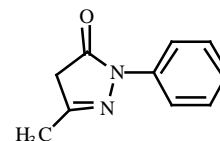


Fig. (1). Chemical structure of edaravone.

classified as free radicals that have unpaired electrons and potent ability of oxidation. H_2O_2 , HOCl, and $ONOO^{\cdot-}$ are classified as non-free radicals that also have the ability to oxidize. The sources of ROS are a variety of cell types such as vascular smooth muscle cells, endothelial cells, and mononuclear cells. Potential sources of ROS production include nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, NO synthase, mitochondrial electron transport, cyclooxygenase, glucose oxidase, and lipoxygenase (Fig. 2). These various oxidase enzymes produce superoxide. The antioxidant enzyme superoxide dismutase (SOD) rapidly dismutates superoxide to H_2O_2 . SOD has been identified as three enzymatic types: Cu/Zn SOD, Mn SOD, and extracellular SOD. Then H_2O_2 is eliminated by glutathione peroxidase (GPx) and catalase to water.

ROLE OF OXIDATIVE STRESS IN CARDIOVASCULAR DISEASES

Several lines of evidence have demonstrated that oxidative stress plays an important role in the pathogenesis and development of cardiovascular diseases, including hypertension, hypercholesterolemia, diabetes mellitus, atherosclerosis, myocardial infarction, angina pectoris, and heart failure [19-22]. The susceptibility of vascular cells to

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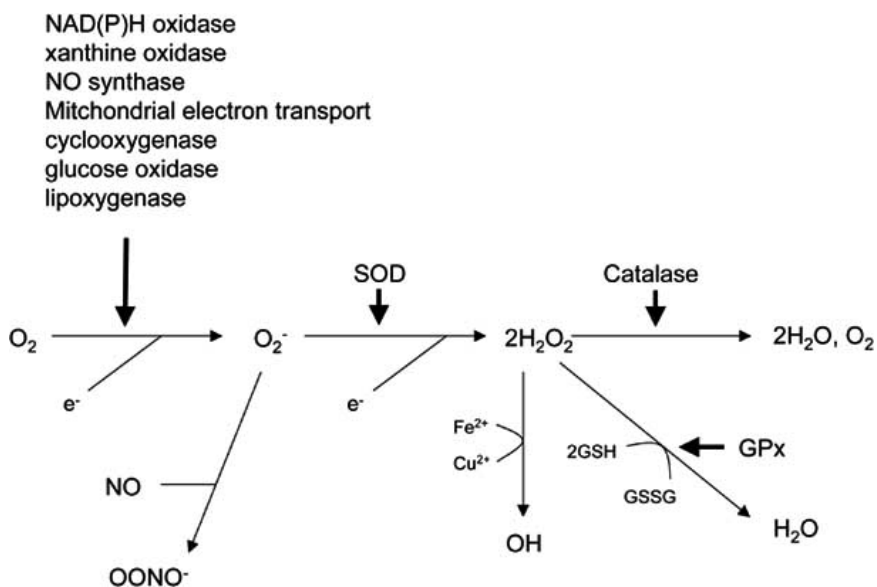


Fig. (2). Metabolism of reactive oxygen species. NAD(P)H indicates nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; O_2 , oxygen; O_2^- , superoxide anion; e^- , electron; SOD, superoxide dismutase; H_2O_2 , hydrogen peroxide; H_2O , water; $OONO^-$, peroxynitrite; OH, hydroxyl radical; GSH, glutathione; GSSG, disulfide of glutathione; GPX, glutathione peroxidase.

oxidative stress is a function of the overall balance between the degree of oxidative stress and the antioxidant defense capability (Fig. 3). Protective antioxidant mechanisms are complex and multifactorial. Antioxidant defense systems, such as SOD, GPx, and catalase scavenge ROS in the vasculature, resulting in inhibition of NO degradation. Although SOD rapidly converts superoxide to hydrogen peroxide, hydrogen peroxide per se is involved in vascular remodeling, inflammation, apoptosis, and growth of vascular smooth muscle cells as an intracellular second messenger [23]. Excess ROS, especially free radicals oxidize various molecules. The production of lipid peroxidation and protein oxidation induce overexpression of redox genes, intracellular calcium overload, and DNA fragmentation, resulting in damage of vascular smooth muscle cells, endothelial cells, or myocardial cells.

ENDOTHELIAL FUNCTION IN CARDIOVASCULAR DISEASES

The vascular endothelium is involved in the release of various vasodilators, including NO, prostaglandins, and endothelium-derived hyperpolarizing factor as well as vasoconstrictors [24,25]. NO plays an important role in the regulation of vascular tone, inhibition of platelet aggregation, and suppression of smooth muscle cell proliferation [26,27]. Impaired endothelium-dependent vasodilation has been found in the forearm, coronary, and renal vasculature in patients with cardiovascular diseases [28-43]. Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis [44]. Indeed, Perticone *et al.* [45] evaluated cardiac outcome in untreated essential hypertensive patients characterized by the three tertiles of acetylcholine (ACh)-induced vasodilation, and they found that patients with the lowest tertile of ACh-induced vasodilation had a significantly higher event ratio than did patients with moderate and high tertiles of ACh-induced vasodilation. These findings suggest that forearm endothelial

dysfunction is a marker of future cardiovascular events in patients with hypertension. In patients with coronary artery diseases, Suwaidi *et al.* [46] found that severe coronary endothelial dysfunction is associated with increased cardiovascular events. Schachinger *et al.* [47] demonstrated a link between coronary endothelial dysfunction and subsequent cardiovascular events in patients with coronary artery diseases. ACh-induced vasodilation and flow-mediated vasodilation are also useful for predicting cardiovascular events in these patients [47-49]. In patients with peripheral arterial disease also, conduit artery endothelial dysfunction assessed by flow-mediated vasodilation independently predicts long-term cardiac outcome [50]. These clinical studies have shown that endothelial function can serve as an independent predictor of cardiovascular events [51,52]. From a clinical perspective, it is important to select an appropriate intervention that is effective in improving endothelial function in patients with cardiovascular diseases.

Several investigators have reported possible mechanisms of impairment of endothelial function in cardiovascular diseases; abnormalities of shear stress, increase in the amount of endogenous endothelial NO synthase (eNOS) inhibitor asymmetrical dimethylarginine, increases in the amount of vasoconstrictors, such as angiotensin II (Ang II), endothelin-1, and norepinephrine, and inactivation of NO by ROS [53-55]. Growing evidence has shown an interaction between oxidative stress and endothelial function. Enhanced production of ROS and an attenuated antioxidant system may contribute to endothelial dysfunction in cardiovascular diseases. In other words, enhanced NO inactivation caused by excess ROS production, rather than decreased NO production, may play an important role in the impaired endothelium-dependent vasodilation in cardiovascular diseases. These findings suggest that a decrease in NO inactivation contributes to the improvement in endothelial function in patients with cardiovascular diseases.

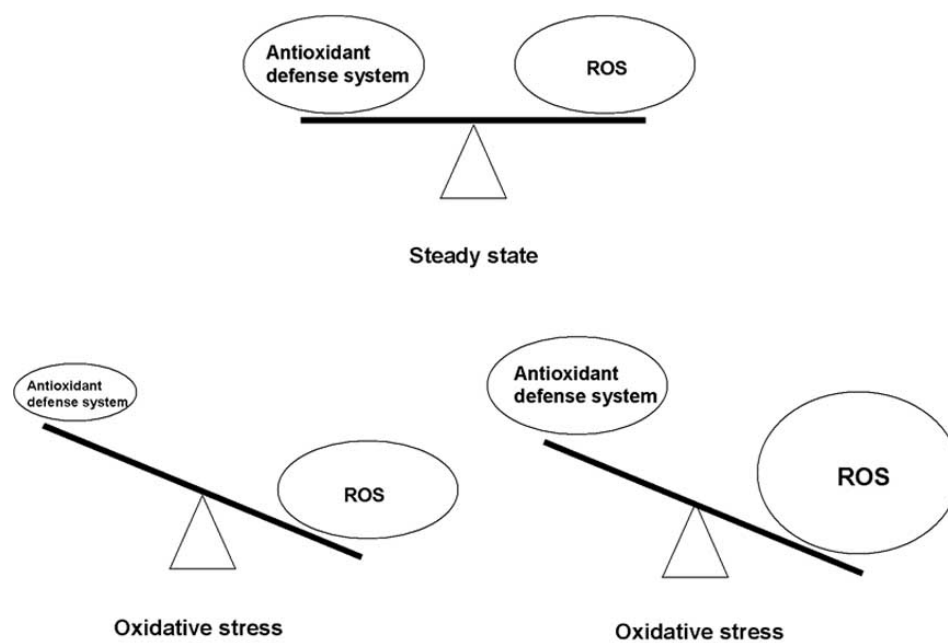


Fig. (3). Steady state and oxidative stress: a balance between the degree of oxidative stress and the antioxidant defense capability. ROS indicates reactive oxygen species.

BENEFITS OF EDARAVONE FOR CEREBROVASCULAR DISEASES

Experimental studies have shown beneficial effects of edaravone on postischemic reperfusion injury [8-13,56-62]. Edaravone has been shown to ameliorate infarct size and brain edema in embolization and transient focal, global, and hemispheric ischemia models in adult rats [8-13,56-60] and to attenuate the hypoxic-ischemia encephalopathy in neonatal rats [61]. Edaravone has been also shown to be effective in preventing cerebral artery vasospasm following subarachnoid hemorrhage in canine [62].

In Japan, edaravone was approved in April 2001 for treatment of acute brain infarction and subarachnoid hemorrhage in the acute phase [1-7]. Several investigators have reported that edaravone has beneficial effects on prevention of brain damage in patients with stroke [14,15,63]. Although the usefulness of edaravone for treatment of mild to moderate stroke in the acute phase has been established, it is unclear whether edaravone is effective against brain damage in patients with severe stroke. Data obtained from large clinical trials are needed to confirm the validity and safety of this drug.

BENEFITS OF EDARAVONE FOR CARDIOVASCULAR DISEASES

It is expected that edaravone has beneficial effects on coronary artery and myocardial cells after ischemic and postischemic myocardial injury in patients with ischemic heart diseases, including acute myocardial infarction and angina pectoris. Some animal studies using acute myocardial ischemia-reperfusion models have suggested protective effects of edaravone on myocardial damage. In 1994, Yanagisawa *et al.* [15] showed that intravenous infusion of edaravone at a dose of 3 mg/kg attenuates the loss of

myocardial creatine kinase activity from the left ventricular free wall in rats subjected to coronary artery occlusion for 10 minutes followed by reperfusion for 24 hours and reduced infarct size by approximately 50% compared with that in the control vehicle group. Minhaz *et al.* [16] reported that edaravone attenuated the myocardial necrotic area by approximately 50% in isolated reperfusion rat heart subjected to coronary artery occlusion. This beneficial effect was related to reduction in myocardial damage. Also, in rabbit hearts subjected to ischemic reperfusion, a bolus infusion of edaravone reduced the necrotic area [64]. It has been reported that edaravone at a dose of 15 mmol reduced the death of isolated adult rabbit ventricular cells by approximately 40% compared with that in the control vehicle group [65]. Oral administration of edaravone at a dose of 30 mg/kg per day for 2 weeks improved peak negative dp/dt in heart of diabetic rats but did not alter plasma glucose levels or hemodynamic parameters [66]. Recently, Tsujimoto *et al.* [67] have shown that intraperitoneal infusion of edaravone at a dose of 10 mg/kg twice daily for 7 days attenuates pressure overload-induced cardiac hypertrophy by approximately 30% in mice hearts subjected to transverse thoracic aorta constriction.

There has been only one published study on the effects of edaravone on ventricular function and myocardial damage in humans. Tsujita *et al.* [17] investigated the effects of edaravone on left ventricular function and infarct size using a randomized, placebo-controlled, open-label protocol in 80 patients with acute myocardial infarction. Intravenous administration of edaravone at a dose of 30 mg for 10 minutes before myocardial reperfusion decreased serum concentrations of creatine kinase-MB isoenzymes, a surrogate point of infarct size, and improved left ventricular ejection fraction in patients with acute myocardial infarction

compared with those in the placebo group (Fig. 4). These findings suggest that edaravone has cardioprotective effects. Recently, two compounds with free radical scavenging activity (tirilazad and ebselen) other than edaravone and one compound with free radical trapping properties (NXY-059) have been claimed to be neuroprotective agents [68]. These agents may also have a protective effect on postischemic injury in the coronary vasculature and myocardium in patients with cardiovascular diseases though a decrease in oxidative stress.

In other postischemic reperfusion models, the usefulness of edaravone for organ protection has been reported. Edaravone improves gastrocnemius and tibialis anterior muscles injury in a rat ischemic limb model [69] and prevents kidney postischemic reperfusion injury in rats [70,71] and lipopolysaccharide-induced liver damage in rats [72]. These findings suggest that edaravone may have beneficial effects on ischemia-reperfusion injury in various muscles, vessels, and tissues in different organs.

SIDE EFFECTS OF EDARAVONE

Side effects, including acute renal failure, liver dysfunction, acute allergic reaction, disseminated intravascular coagulation, thrombocytopenia, leukocytopenia and renal dysfunction, during edaravone treatment are occasionally observed by >5%, respectively [73]. Thus, edaravone can be used with a low rate of side effects. No side effects are anticipated if the dose of 30 mg. However, since death due to acute renal failure during edaravone treatment has been reported, this agent is contraindicated for patients with severe renal dysfunction. In addition, edaravone should be carefully used in elderly patients and patients with liver disease, renal disease, hematologic disease, or dehydration.

PUTATIVE MECHANISMS UNDERLYING ANTIOXIDANTS ACTIONS OF EDARAVONE

After ischemia-reperfusion, large amounts of ROS are produced from vascular smooth muscle cells, endothelial cells, mononuclear cells. It has been shown that edaravone reduces or restores the amount of ROS increased by

postischemic reperfusion and prevents impairment of the antioxidant defense system [74,75]. Scavenging ROS by edaravone may play a key role in preventing postischemic reperfusion injury in various types of cells and tissues. Previously reported antioxidant actions of edaravone include 1) enhancement of prostacyclin production [76], 2) inhibition of lipoxygenase metabolism of arachidonic acid by trapping hydroxyl radicals [76], 3) inhibition of alloxan-induced lipid peroxidation [10], and 4) quenching of reactive oxygen, leading to protection of various cells, such as endothelial cells, against damage by ROS [56]. Edaravone is metabolized to its glucuronide and sulfate conjugates in the liver and excreted rapidly in the urine. The putative mechanism underlying the antioxidant action of edaravone is as follows [77] (Fig. 5): an electron transfer from an edaravone anion to a peroxy radical yields an edaravone radical and peroxy anion, and this reaction breaks the chain oxidation of lipids. Then, edaravone peroxy radical transforms to 4,5-dione by elimination of a hydrogen atom and one electron. Finally, 2-oxo-3-(phenylhydrazono)-butanoic acid (OBP) is produced by the hydrolysis of 4,5-dione. It is thought that edaravone exists near the cell membrane or perhaps on the cell membrane. Edaravone has a low molecular weight (MW 174.2), is both lipid-soluble and water-soluble, and has good cell membrane permeability [77]. It has been confirmed that edaravone has the ability to pass through the blood-brain barrier in dogs [78].

Edaravone directly prevents hydroxyl radical-induced injury of cultured bovine aortic endothelial cells [76]. In addition, edaravone stimulates the conversion of arachidonic acid to prostacyclin and inactivates ROS, resulting in protection of endothelial cells [79]. Interestingly, edaravone induced endothelial NO synthase (eNOS) in the ischemic spinal cord in rabbits, preventing spinal cord damage [80], and it also restored the reduced expression of eNOS mRNA and protein in the rabbit artery following irradiation [81]. Yoshida *et al.* [82] have recently reported that edaravone enhances the expression of eNOS and restores the reduction in eNOS by oxidized low-density lipoprotein in endothelial cells. These findings suggest that edaravone prevents the cell

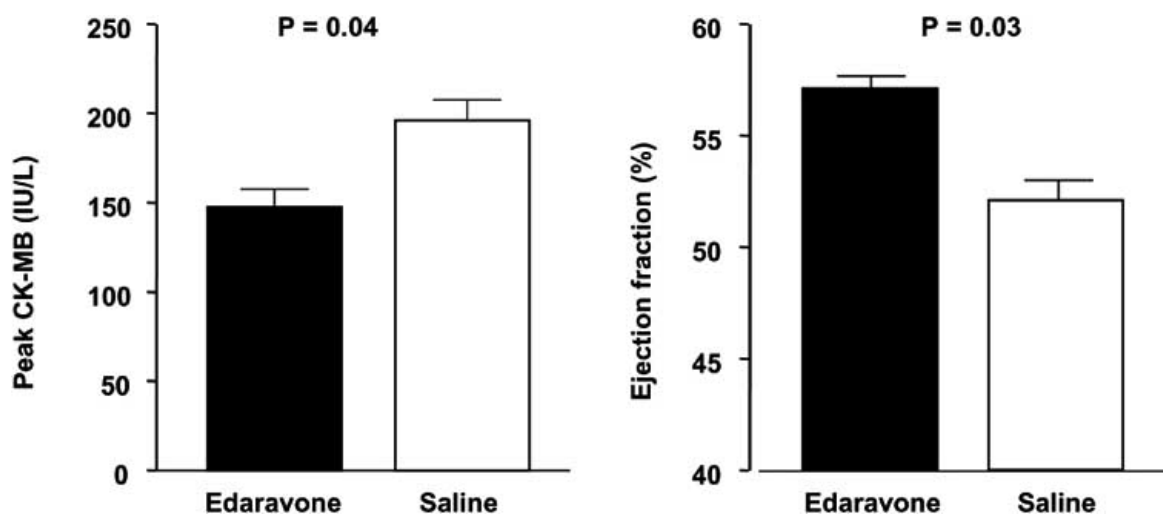


Fig. (4). Effects of edaravone on peak CK-MB and ejection fraction in patients with acute myocardial infarction (modified by reference [12]).

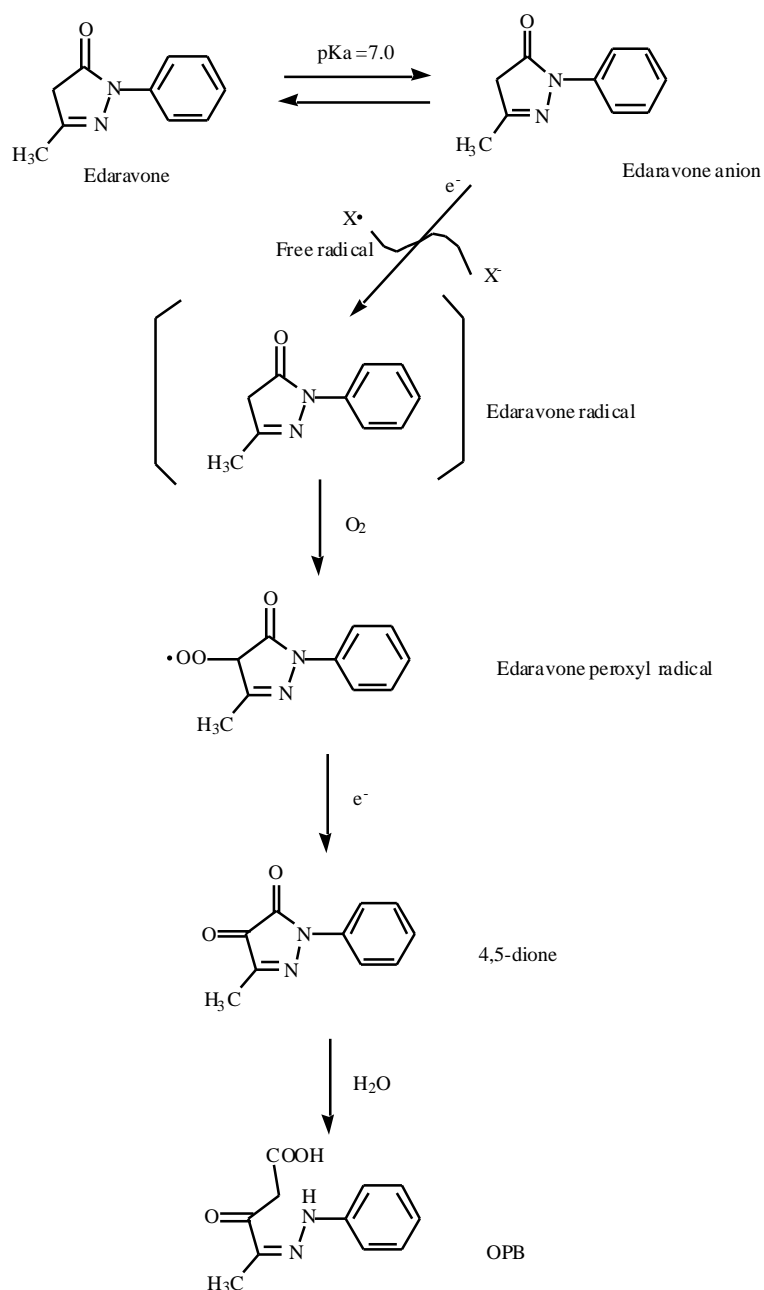


Fig. (5). Putative mechanisms of antioxidant actions of edaravone (modified by reference [5]). OPB indicates 2-oxo-3-(phenylhydrazono)-butanoic acid.

damage induced by oxidative stress through not only direct ROS scavenging effect but also restoration of reduced eNOS expression.

To investigate how endothelial function is affected by excess ROS, smokers are appropriate subjects for research. Indeed, endothelium-dependent vasodilation in forearm arteries was impaired in smokers compared with that in nonsmokers [18]. The urinary excretion of 8-hydroxy-2'-deoxyguanosine, a principal stable marker of hydroxyl radical damage to DNA, was significantly increased in smokers compared to that in nonsmokers. Recently, we have demonstrated that edaravone augments ACh-induced

vasodilation in smokers but not in nonsmokers and restores impaired endothelium-dependent vasodilation in smokers to the same level as that in nonsmokers (Fig. 6) [18]. The enhancement of forearm blood flow response to ACh by edaravone was completely abolished by an NO synthase inhibitor N^G-monomethyl-L-arginine [18]. These findings suggest that edaravone improves endothelial-dependent vasodilation in smokers through a decrease in ROS. It is well known that a balance between ambient levels of superoxide and NO release plays a critical role in the maintenance of normal endothelial function. ROS, including hydroxy radicals, directly scavenge NO and produce toxic

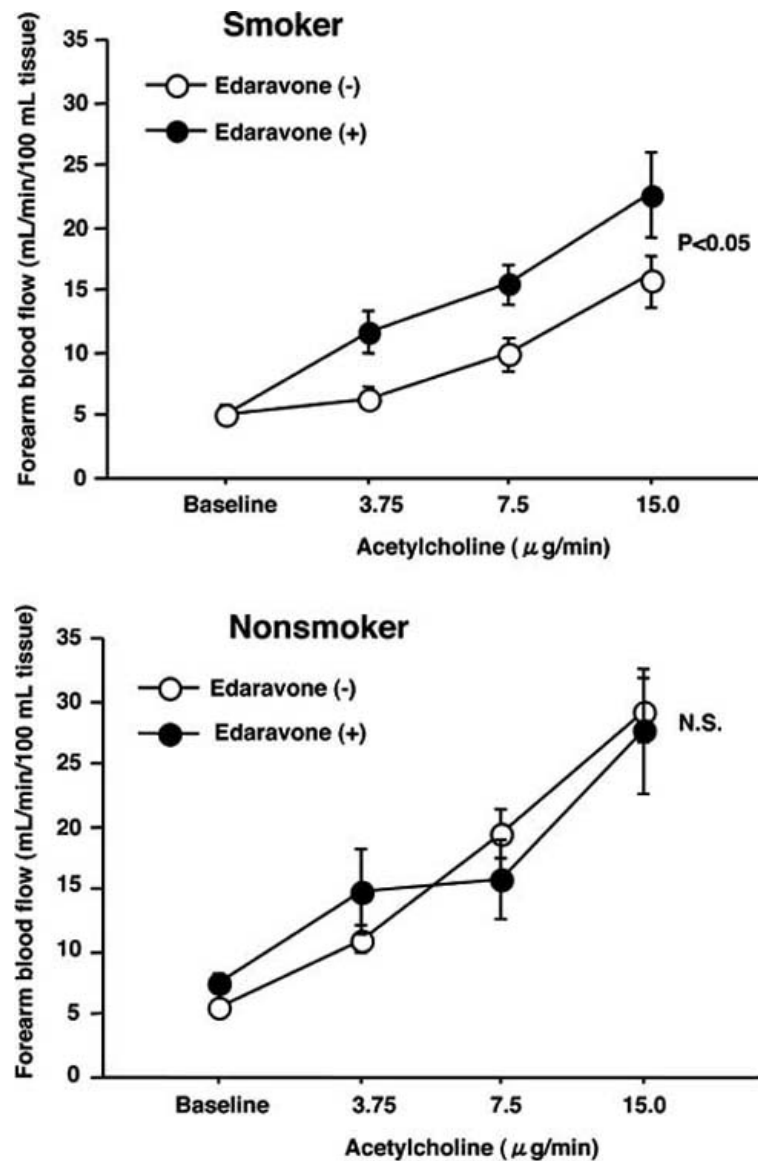


Fig. (6). Comparison of the responses of FBF to co-infusion of ACh with edaravone (●) and to infusion of ACh alone (○) in smokers and nonsmokers (modified by reference [34]).

peroxynitrite [83]. Therefore, the improvement in endothelium-dependent vasodilation by edaravone may be due to an inhibition of ROS-induced NO degradation rather than increased NO production. Recently, we have shown that inactivation of the renin-angiotensin system, particularly Ang II, by successful renal angioplasty decreases oxidative stress, resulting in improved endothelium-dependent vasodilation in patients with renovascular hypertension [43]. These findings suggest that oxidative stress may be involved in impaired NO-mediated vasodilation in humans.

Fig. (7) shows the putative mechanisms by which edaravone improves endothelial function in patients with cardiovascular diseases. The novel free radical scavenger edaravone may represent a new therapeutic intervention for endothelial dysfunction in the setting of atherosclerosis,

chronic heart failure, diabetes mellitus, or hypertension through its free radical scavenging and antioxidant actions.

CURRENT & FUTURE DEVELOPMENTS

In this review, we indicated the possibility that edaravone has beneficial effects on not only myocardial and vascular injury following ischemia and reperfusion in patients with acute myocardial infarction, but also in atherosclerosis in the chronic phase. Recently, it has been reported that edaravone can be used for the various diseases such as amyotrophic lateral sclerosis [84] and mitochondrial myopathy [85]. Due to the lack of clinical studies using edaravone, it remains unclear whether edaravone treatment is beneficial for patients who have excess oxidative stress and whether edaravone reduces the mortality rate of these patients. Controlled studies using a large population of patients are needed to determine the effects of edaravone on endothelial

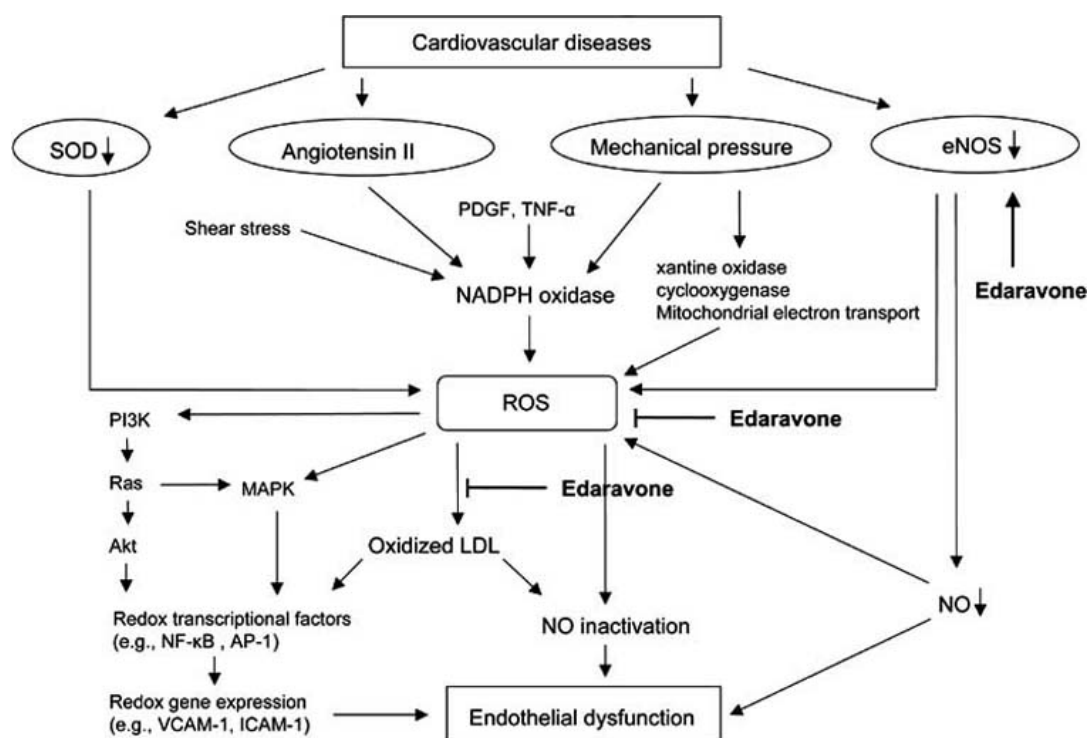


Fig. (7). Putative mechanisms of edaravone-induced improvement in endothelial dysfunction in patients with cardiovascular diseases. ROS indicates reactive oxygen species; eNOS, endothelial nitric oxide synthase; PDGF, platelet derived growth factor; TNF-alpha, tumor necrosis factor-alpha; NADPH, nicotinamide adenine dinucleotide phosphate; PI3K, phosphatidylinositol-3-kinase; MAPK, mitogen-activated protein kinase; LDL, low density lipoprotein; NF-kappa B, nuclear factor-kappa B; AP-1, activator protein-1; NO, nitric oxide; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1.

cells, vascular smooth muscle cells, and myocardial cells after ischemic and postischemic myocardial injury. It is expected that edaravone will be useful for treatment of various diseases in which oxidative stress may be involved in the pathogenesis.

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