Hypertension and Oxidative Stress

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Yasunobu HIRATA and Hiroshi SATONAKA

Department of Cardiovascular Medicine, University of Tokyo

Abstract: It is well established that oxidant stress increases in patients with hypertension. Production of reactive oxygen species (ROS) increases, while antioxidants such as superoxide dismutase (SOD) and vitamin C decrease in this condition. Important sources of ROS are vascular wall. The major stimuli are mechanical stretch on the vascular wall and activation of the renin-angiotensin system. In particular, angiotensin II activates NADPH/NADH oxidase of the vascular smooth muscle cells, resulting in release of ROS. In fact, SOD normalizes blood pressure in some forms of hypertension. Oxidative stress promotes atherosclerosis through various mechanisms. Among them, the interaction between ROS and endothelium-derived nitric oxide (NO) is the most important. ROS traps NO and in turn diminishes the antiarteriosclerotic effects of NO. The effects of antihypertensives like angiotensin converting enzyme (ACE) inhibitor and Ca channel blocker are in part due to antioxidant activity. ACE inhibitors increase NO availability by reducing angiotensin II production and bradykinin degradation. Antihypertensive therapy taking this perspective into account would most likely be effective to prevent complications of hypertension.

Key words: Reactive oxygen species; Nitric oxide; Endothelium; Angiotensin

Introduction

There is accumulating evidence that oxidative stress plays a role in the progression of hypertension. This article will discuss the mechanism of elevated oxidative stress in hypertension and antihypertensive treatments in this regard.

Oxidative Stress in Hypertension

There are numerous reports that oxidative stress is increased in patients with hypertension. Though direct measurement of *in-vivo* reactive oxygen species (ROS) involves difficulties, it has been shown that serum lipid peroxides or ROS released from isolated vessels are increased in essential hypertensive patients or hypertensive animal models. It has also been reported that such antioxidants as vitamin E, glutathione peroxidase, or superoxide dismutase (SOD) are decreased in essential hypertensives. Nakazono *et al.*[1] have observed that

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administration of SOD, which eliminates ROS, to spontaneously hypertensive rats (SHR) reduces blood pressure remarkably. On the other hand, the same amount of SOD administered to normotensive control rats did not change blood pressure, suggesting that there are relative increases of ROS in SHR and this contributes to hypertension.

It is known that low density lipoprotein (LDL) is easily oxidized in hypertensives. Oxidative stress, measured on the basis of native LDL oxidization as a measure, is increased to a greater extent in 'non-dippers', whose blood pressure does not decrease during the night, than dippers. Because end organ damages are more progressed in non-dippers, oxidative stress most likely is correlated with the severity of the disease. As LDL oxidization progresses, hypertension deteriorates due to atherosclerosis.

Angiotensin II and Oxidative Stress

Though plasma renin activity in essential hypertensive subjects or SHR is not necessarily elevated, their blood pressure is effectively lowered by angiotensin converting enzyme (ACE) inhibitors. This is believed to be due to renin-angiotensin system activation in the cardiovascular tissues. Harrison *et al.*[2] have found by measuring the amount of ROS released from rat vessel walls that angiotensin II (AII) administration markedly increases ROS. It has also been shown that this effect is suppressed by AII receptor antagonists.

Among the numerous sources from which ROS are released, NADPH/NADH oxidase is the most important. Since administration of AII to vessels after endothelial denudation does not make a significant difference to the ROS production, it is believed that ROS are released mainly by NADPH/NADH oxidase in vascular smooth muscle cells. AII enhances release of ROS, but norepinephrine does not exert this effect. While administration of SOD to rats rendered hypertensive through AII infusion lowers blood pressure, this effect is not observed in hypertensive rats induced by norepinephrine infusion or in normotensive rats. Furthermore, SOD improves endothelium-dependent vasodilatory response in AII-induced hypertensive rats. These findings strongly suggest that ROS are involved in AII-induced blood pressure elevation or vascular damage.[3]

On the other hand, there remains the question whether oxidative stress is milder in so-called low-renin hypertension. Harrison *et al.*[4] have also found that ROS formation in the aorta of deoxycorticosterone acetate (DOCA)-salt-induced hypertensive rats was about four times as much compared with normotensives. Though plasma renin activity in DOCA-salt rats is known to be extremely low, this is not necessarily the case with the tissue renin-angiotensin system. Administration of AII receptor antagonists, however, made no difference to blood pressure or endothelial function. While SOD administration did not lower blood pressure, it improved endothelium-dependent vasodilatory response.

It has been shown that mechanical stretch to vessel wall induces ROS release. This suggests the possibility that high blood pressure itself increases ROS independent of renin-angiogensin system activity, thus probably attenuating the effect of nitric oxide (NO). But the lack of responsiveness of blood pressure to SOD administration suggests that oxidative stress does not directly contribute to blood pressure elevation in low-renin hypertension so much as in AII-dependent hypertension.

ROS and NO

Although ROS thus generated oxidize a variety of substances, the most important mechanism in blood pressure regulation is ROS' reaction with endothelium-released NO and the ensuing inactivation of NO. Numerous anti-oxidative mechanisms exist in the body against these ROS. SOD, the most important among them, immediately tries to eliminate ROS. Since, however, ROS react with NO three times faster

than SOD, a strongly oxidative substance called peroxinitrite is formed out of vessel wall-released ROS and NO.

NO released from endothelial cells dilates the vessel by increasing intracellular cyclic GMP concentration of vascular smooth muscle cells. Effects of NO on the vessel wall also include inhibition of platelet aggregation or of white blood cell adhesion to endothelial cells, and also, as a result, suppressed excretion of cell proliferative or migration stimulatory factors from these blood cells. NO also directly inhibits cell proliferation or LDL oxidation. As NO production decreases, therefore, it is expected that vasoconstriction will occur and thus promote progression of atherosclerosis. In fact, NO synthase inhibitor administration to animals elicits blood pressure increase and marked damage to the vessel. In humans also, from the fact that infusion of this inhibitor into the brachial artery causes vasoconstriction, it is suggested that NO is constantly released and exerts antihypertensive or antisclerotic effects.

It is known that endothelium-dependent vasodilatory response via endothelial NO release induced by acetylcholine or other substances is attenuated in essential hypertension since early stages. On the other hand, endothelium-independent vasodilation which is mediated by direct actions of sodium nitroprusside or other agents on smooth muscle cells is kept intact until later stages. NO reduction that accompanies hypertension, considering the fact that it is at least partially reversed by appropriate antihypertensive treatment, is believed to be due to endothelial damage caused by hypertension. Possibility remains, nonetheless, of congenital NO reduction for example as a result of NO synthase gene abnormalities. In any case, NO reduction leads to vascular damage deterioration due to hypertension.

Though NO captures ROS and neutralizes their cytotoxicity, cytoprotective capabilities of endothelium-derived NO are lost at the same time. Furthermore, while peroxinitrite, which is generated out of reaction between NO and ROS, shows a vasodilatory effect at lower concentrations like NO, it affects activities of numerous enzymes at higher concentrations through its strong oxidative property for example by turning tyrosine residues into nitrotyrosines.

NO synthase itself has capabilities to generate ROS, especially when its substrates for NO formation, such as L-arginine, or its cofactor tetrahydrobiopterine (BH4) are in short supply. Though much is not yet understood about the roles of BH4 in essential hypertension, its supplementation is known to improve endothelial function in hyperlipidemia or in rats with ischemic acute renal failure.[5)]

Oxidative Stress and Antihypertensive Therapy

1. Ca channel blockers

Ca channel blockers are known to possess not only antihypertensive, but also organ-protective properties against ischemia in the brain, heart, or kidneys. They are also antiatherosclerotic. Though many of dihydropyridine Ca blockers have been shown to exert antioxidative effects on isolated cardiomyocyte membrane or LDL, these effects do not necessarily correlate with the degree of vasodilatory capacities. Since, furthermore, optical isomers incapable of antagonizing L-type Ca channels also show similar antioxidant effects, these capabilities are believed due to chemical properties of dihydropyridine structure.

Capabilities to improve or protect endothelial functions have come to attract attention recently as a measure of usefulness of antihypertensive agents on the ground that endothelial dysfunction precedes not only hypertension but also atherosclerosis of various etiologies such as hyperlipidemia, diabetes mellitus, or smoking and subsequently causes proliferation or migration of vascular smooth muscle cells. Endothelium-protective capabilities of Ca blockers do not seem strong, but evidence of their usefulness is accumulating. For example, in a study of hypertensive subjects,

endothelium-dependent dilatory response to agents such as acetylcholine in the brachial artery, which had been attenuated in hypertensives compared with healthy subjects, improved after administration of Ca blockers along with blood pressure reduction.[6)]

As a possible mechanism, it has been hypothesized that dihydropyridine Ca blockers such as nifedipine directly release NO from endothelium. NO release usually occurs through activation of NO synthase induced by increases in endothelial intracellular Ca concentration. In view of the fact that L-type Ca channels do not exist on endothelial cells, it is difficult to expect a mechanism based on intracellular Ca increase. Amlodipine also facilitates NO release from the canine coronary artery microvasculature through a mechanism mediated by bradykinin B2 receptor stimulation. It is also important that antioxidant properties of Ca blockers suppress trap of NO by ROS and that Ca blocker-induced vasodilation increases shear stress which stimulates NO synthase expression.

Most of Ca blockers are lipophilic and have affinity to cell membrane. Especially amlodipine shows antioxidant actions by binding to the membrane. Thus there is much experimental evidence that Ca antagonists are antioxidant. Though several studies suggest that Ca blockers reduce blood pressure as well as oxidative stress and enhance antioxidant capabilities in humans, it remains to be investigated how much this antioxidant effect contributes to blood pressure reduction.

2. ACE inhibitors

As discussed above, ROS generation is increased in hypertension due to AII-induced activation of NADPH/NADH oxidase in vascular smooth muscle cells. It is expected, therefore, that ROS will be reduced by decreasing the effects of AII. ACE inhibitors not only decrease AII production but also inhibit the degeneration of bradykinin and thus induce NO release from endothelial cells by stimulating their B2 receptors.

According to previous studies, ACE inhibitors are the most endothelium-protective among antihypertensive agents whether in humans or animals.[7)] Large-scale clinical trials on patients with atherosclerosis have also demonstrated that cardiovascular complications are reduced by long-term ACE inhibitor administration,[8)] making ACE inhibitors the recommended medication for hypertensive patients particularly prone to atherosclerosis. Similar effects are expected with AII receptor blockers which have become available recently in Japan though clinical experience or evidence is scarce yet.

3. β-adrenergic blockers

Some of the vasodilatory β-adrenergic blockers possess antioxidant capacities. Carvedilol, whose role as a therapeutic agent against heart failure now being established, is believed to owe its usefulness at least in part to its antioxidant properties. This effect, which has also been demonstrated in humans, decreases generation of ROS derived from granulocytes or monocytes in healthy subjects.[9)] In hypertensives, carvedilol elicits suppression of LDL oxidization along with blood pressure reduction.[10)] Experiments with animals have shown that carvedilol exerts stronger endothelium-protective effects than other β-adrenergic blockers without antioxidant capabilities in stroke-prone SHR. Celiprolol also has similar antioxidant capabilities probably based on endothelial NO release.[11)]

4. Vitamin E

Vitamin E (main ingredient: α-tocopherol) also exerts antioxidant effects. Reports are numerous that its administration improves endothelium-dependent vasodilatory responsiveness. There has been a lot of controversy, nonetheless, about its usefulness. Several previous clinical trials have reported that vitamin E has reduced occurrence of cardiovascular complications. According to the HOPE study, which has been completed recently, however, 400 IU of vitamin E administered daily to

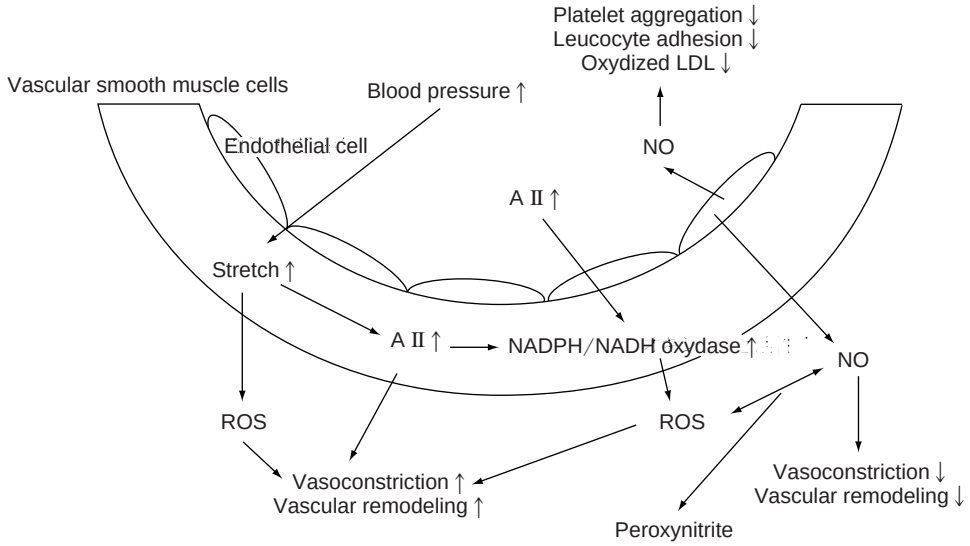

Fig. 1 Oxydarive stress and nitric oxide (NO) in hypertension. ROS: reactive oxygen species, AII: angiotensin II

patients with high risks for cardiovascular diseases over a period of 4.5 years on average did not make any significant difference to the occurrence of cardiovascular events.[12)]

5. Vitamin C

Vitamin C strongly inhibits oxidization of lipids, especially of LDL. Oral administration or intra-arterial infusion of vitamin C has been shown to improve endothelium-dependent vasodilatory responsiveness in patients with not only hypertension but also with ischemic heart disease, hyperlipidemia, or in smoking subjects. It has also been reported that there is a negative correlation between vitamin C and blood pressure. This is believed to be a result of free radical elimination by vitamin C, or of increased vitamin C consumption due to hypertension-induced oxidative stress elevation. It has been reported that 500 mg of vitamin C administered daily to hypertensive subjects 60 to 80 years of age for three months reduced only the systolic blood pressure by 2 mmHg.

Lembo *et al.*[13)] have shown that while vasoconstrictive responsiveness to norepinephrine is enhanced in essential hypertensive patients, vitamin C administration reduced this responsiveness only in hypertensives. Furthermore, since administration of NO synthase inhibitor together with vitamin C eliminated this effect, it has been suggested that norepinephrine-induced NO release is attenuated by ROS in hypertensives. Since serum vitamin C concentrations in the range of millimoles are required to elicit acute effects,[14)] question remains whether these effects can be expected by ordinary oral dosages. Duffy *et al.*[15)] have reported, however, that though 2-gram bolus oral intake of vitamin C did not make any difference to blood pressure, 500 mg taken daily for one month lowered the mean pressure by 10 mmHg. This remains to be investigated in large-scale studies in the future.

Conclusion

ROS are increased in hypertension in response to vessel stimulation by mechanical stretch or AII. Reaction of ROS with endothelium-released NO inhibits vasodilatory or antisclerotic effects of NO and thus can exacerbate the disease (Fig. 1). An increasing number of agents with antioxidant capabilities becoming available in recent years, antihypertensive

therapy taking this perspective into account would most likely be effective to prevent complications of hypertension.

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