Comparison of *In vitro* Antioxidant Activity of Olmesartan and Amlodipine

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ABSTRACT

Introduction: Free radical oxidative stress has been implicated in the pathology of a wide variety of clinical disorders. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by them. Angiotensin II receptor blockers used in the treatment of hypertension, have also been reported to protect organs such as kidney and heart. Althoug, the mechanisms of these protective effects are not fully understood, it is generally thought that their antioxidant effects likely play a role. Amlodipine, a calcium channel blocker, seems to exert atheroprotective effects through its antioxidant properties related to its chemical structure and independent of its calcium channel-blocking effect. Though research has proved that olmesartan and amlodipine exhibit antioxidant activity independent of their antihypertensive effects, there are not many *in vitro* studies to analyse which drug is a better antioxidant. **Objective**: This *in vitro* study was done to compare the antioxidant activity of olmesartan and amlodipine. **Materials and Methods:** In this study, we demonstrated the antioxidant activities of 10 mg/ml stock solutions of olmesartan and amlodipine *in vitro.* DPPH (1,1 Diphenyl 2-picryl hydrazide) and Nitric oxide free radical scavenging assays were done. **Results:** Olmesartan showed significant (49.48%) and consistent free radical scavenging activity by DPPH and Nitric oxide radical scavenging assays. Free radical scavenging activity of amlodipine was more significant (56.89%) than olmesartan but was inconsistent and non-concentration dependent. **Conclusion:** Hence this *in vitro* study has proved that olmesartan has better antioxidant activity than amlodipine.

Key words: Free radicals, Oxidative stress, Olmesartan, Amlodipine, DPPH, Nitric oxide.

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INTRODUCTION

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases.[1,2] It has become clear that oxidative stress, particularly at early stage of disease, is related to slight disturbances of oxidation–reduction potentials localized to selected compartments within the cell, rather than changes in the overall redox status of the cell. Such disturbances in redox signalling within vascular cells play important roles in the pathogenesis of numerous cardiovascular diseases,[3] including atherosclerosis, hypertension, heart failure and diabetic vascular dysfunction.[4,5]

Antioxidants are agents which scavenge the free radicals and prevent the damage caused by them. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA. They play an important role in various fields such

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as medicine (to treat cancer, cardiovascular disorders, and chronic inflammations), cosmetics (anti-ageing process), food industries (food preservative) and others.[2]

Recent studies have shown that clinically available drugs like the statins, ACE inhibitors and angiotensin receptor antagonists inhibit the vascular NADPH oxidases and reduce vascular reactive oxygen species production which might explain some of their beneficial effects.

Angiotensin II type 1 receptor blockers, which inhibit the renin-angiotensin system, are used in the treatment of hypertension. In addition to their ability to lower blood pressure, these compounds have also been reported to protect organs, such as kidney and heart. Although the mechanisms of these protective effects are not fully understood, it is generally thought that their antioxidant effects likely play a role. RAS antagonists may exert a renal protective effect that is independent of its antihypertensive effect, and may be involved in reducing of the levels of oxidative stress.[6] Angiotensin receptor blockers improved endothelium-dependent coronary dilation in hypertensive patients independent of BP reduction. These beneficial effects on coronary vasomotion might be a result of the antioxidant properties of Angiotensin receptor blockers.

Recent studies have shown that commonly used Angiotensin receptor blocker olmesartan possesses antioxidant effects which might be independent of its antihypertensive effects.[7]

Calcium channel blockers, which are antihypertensive and antianginal drugs, may also exert antioxidant and cytoprotective effect against free radical mediated vascular injury. Amlodipine is a long acting calcium antagonist with vascular selectivity. Its dihydropyridine ring reduces the free radicals to nonreactive forms.[8] Amlodipine decreases plasma levels of vasoconstrictor, thromboxane A, elevates vasodilators like prostacyclin, Nitric Oxide (NO), increases aortic tissue cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP). It increases NO production in pulmonary circulation in patients of essential hypertension, probably through a mechanism involving bradykinin. It is known to up regulate the activity of Superoxide Dismutase (SOD).[9] Animal studies with cholesterol fed rabbits showed that amlodipine reduced aortic cholesterol accumulation, blood and aortic lipid peroxidation, malondialdehyde (MDA) levels, increased SOD in blood and aortic tissue and suppressed catalase.[10]

Though research has proved that olmesartan and amlodipine exhibit antioxidant activity independent of their antihypertensive effects, there are not many *in vitro* studies to analyse which drug is a better antioxidant.

Hence this *in vitro* study was done to compare the antioxidant pharmacological activity of olmesartan and amlodipine.

MATERIALS AND METHODS

Samples Preparation

10 mg/ml stock solutions of olmesartan and amlodipine were prepared with ethanol (60 μM).

Determination of antioxidant activity

DPPH radical scavenging assay

DPPH radical scavenging activity was done using the method of Yohozowa *et al.* The reagents required were 1,1 Diphenyl 2-picryl hydrazide (DPPH) and Ethanol (60 μM). The reaction mixture containing 1 ml of DPPH solution (200 μM in ethanol) and serial dilutions (100 μg to 1000 μg) of the sample drugs olmesartan and amlodipine were shaken and incubated separately in dark for 20 min at room temperature. The resultant absorbance was recorded at 517 nm using spectrophotometer. Ascorbic acid was used as reference standard. Control sample was prepared by mixing 1.9 ml of DPPH and 0.1 ml of solvent. The percentage scavenging of DPPH free radical by olmesartan and amlodipine were calculated using the formula:

$$\% \text{ inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{\text{Absorbance of control}} X\ 100$$

Nitric oxide radical scavenging Assay

The nitric oxide radical scavenging activity was done using the method of Alderson *et al.* 3 ml of reaction mixture containing sodium nitroprusside (10 mM in phosphate buffered saline) and serial dilutions (10 μg to 1000 μg) of the sample drugs olmesartan and amlodipine were incubated separately at 37°C for 4 h. Aqueous solution of Sodium Nitroprusside spontaneously generates Nitric Oxide (NO) at physiological pH, which interacts with oxygen to produce Nitrite ions which act as free radicals.

This was estimated by using Griess reagent and the absorbance was read at 546 nm using colorimeter. Griess reagent contains 1% Sulphanilamide, 2% Phosphoric acid and 0.1% Naphthyl ethylene diamine dihydrochloride in 100 ml of distilled water. Control sample was prepared by mixing 1 ml of solvent +2 ml of Sodium Nitro Prusside+0.5 ml of Griess reagent.

The percentage scavenging of nitric oxide free radical by olmesartan and amlodipine were calculated using the formula:

$$\% \text{ inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{\text{Absorbance of control}} X\ 100$$

RESULTS

DPPH free radical scavenging assay of olmesartan showed a gradual increase in free radical scavenging activity in a concentration dependent manner upto 800 µg. Amlodipine though showed more significant free radical scavenging activity upto 800 µg concentration it was not concentration dependent. Then there was a slight decrease in percentage inhibition for both olmesartan and amlodipine at maximal drug concentration of 1000 µg (Table 1).

Comparison of Free radical scavenging activity of olmesartan and amlodipine by DPPH assay is depicted (Figure 1).

Olmesartan exhibited good free radical scavenging activity with nitric oxide at lower concentrations (10 & 50 µg) and also at higher concentrations (400, 800, 1000 µg). The radical scavenging activity was insignificant at 100 & 200 µg drug concentrations. Similarly amlodipine exhibited good free radical scavenging activity with nitric oxide at lower concentrations (10 & 50 µg) and also at higher concentrations (800, 1000 µg). The radical scavenging activity was insignificant at 200 & 400 µg drug concentrations (Table 2).

Comparison of Free radical scavenging activity of olmesartan and amlodipine by Nitric Oxide assay is depicted (Figure 2).

DISCUSSION

Free radical oxidative stress has been implicated in the pathology of a wide variety of clinical disorders.[1]Numerous physiological and biochemical processes in the human body may produce oxygen centred free radicals and other reactive oxygen species as by-products. Overproduction of such free radicals can cause oxidative damage to biomolecules, eventually leading to many chronic diseases such as cancer, diabetes, ageing etc.[11]

Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by many other mechanisms and thus prevent disease.[1]

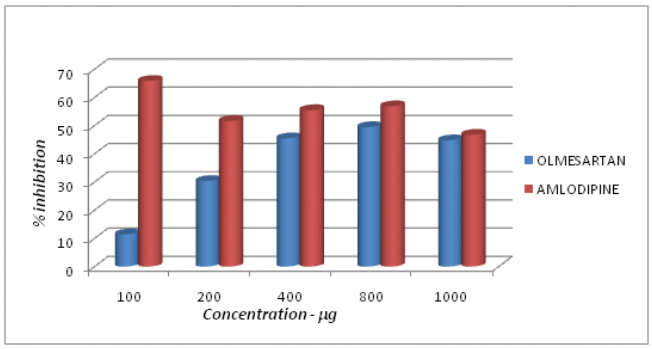

Figure 1: Free radical scavenging activity by DPPH assay

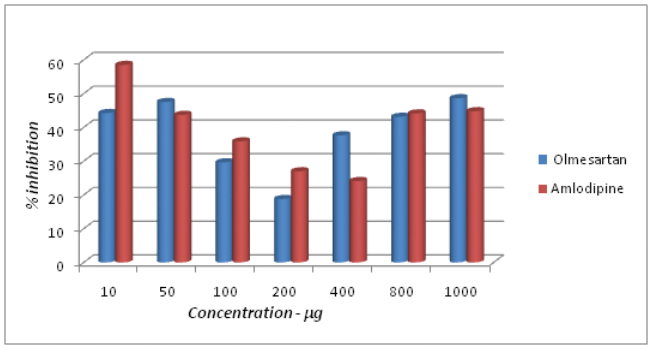

Figure 2: Free radical scavenging activity by Nitric oxide assay

Table 1: DPPH free radical scavenging assay

Concentration	Percentage Inhibition	
	Olmesartan	Amlodipine
100	11.45	65.85
200	30.35	51.68
400	45.49	55.51
800	49.48	56.89
1000	44.79	46.77

Table 2: Nitric oxide free radical scavenging assay

Concentration	Percentage Inhibition	
	Olmesartan	Amlodipin
10	44.25	58.50
50	47.52	43.70
100	29.64	35.84
200	18.79	26.99
400	37.65	24.12
800	43.13	44.14
1000	48.65	44.79

DPPH assay is considered a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants, since the radical compound is stable and need not be generated.[12,13]

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. Excess concentration of nitric oxide is implicated in the cytotoxic effects observed in various disorders like AIDS, cancer, Alzheimer's and arthritis. Oxygen reacts with the excess NO to generate nitrite and peroxy nitrite anions, which act as free radicals.[1]

Miyata *et al*. reported that the antioxidant effects of olmesartan were greater than any other ARBs in an *in vitro* study. A recent *in vitro* study has reported that clinical concentrations of olmesartan exert antioxidant properties. This was further confirmed in a clinical trial for hemodialysis patients as well.[7]

Amlodipine, a unique third-generation dihydropyridine-type calcium channel blocker, seems to exert atheroprotective effects through its antioxidant properties related to its chemical structure and independent of its calcium channel-blocking effect.[14,15]

Olmesartan but not amlodipine improved endothelium-dependent coronary dilation & forearm dilation in hypertensive patients independent of BP reduction. These beneficial effects on coronary & forearm vasomotion might be via an antioxidant property of ARBs.[16,17]

In this study, olmesartan an angiotensin receptor antagonist showed significant and consistent free radical scavenging activity by DPPH and Nitric oxide radical scavenging assays.Free radical scavenging activity of calcium channel blocker amlodipine was more significant than olmesartan but was inconsistent and non-concentration dependent.

Our *in vitro* study proved that Angiotensin receptor blocker olmesartan has better antioxidant activity than calcium channel blocker amlodipine. Therefore olmesartan which is an antihypertensive drug may be effective also as an antioxidant in a wide variety of disease conditions caused by oxidative stress.

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