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Research Article

Assessment of *In vitro* pharmacological activity of Olmesartan by analytical techniques

T. Rajathilagam*, S. Seethalakshmi

Department of Pharmacology, ESIC Medical College, Chennai, Tamil Nadu, India

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***Correspondence to:**
T. Rajathilagam,
Email: rajathilagamtr@yahoo.co.in

ABSTRACT

Background: 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 has 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. Hence, this *in vitro* study was done to demonstrate the antioxidant pharmacological activity of a commonly used Angiotensin receptor blocker Olmesartan.
Methods: In this study, we demonstrated the antioxidant pharmacological activity of Olmesartan *in vitro* by 1,1 Diphenyl 2-picryl hydrazide (DPPH) and nitric oxide (NO) free radical scavenging assays.
Results: Olmesartan showed significant free radical scavenging activity by DPPH and NO radical scavenging assays.
Conclusion: Hence, Olmesartan, which is an antihypertensive drug, may be effective also as an antioxidant in a wide variety of disease conditions caused by oxidative stress.

Keywords: 1,1 Diphenyl 2-picryl hydrazide, Free radicals, *In vitro*, Oxidative stress, Olmesartan, Nitric oxide

INTRODUCTION

Free radicals of different forms are constantly generated for the 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 the 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 signaling 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 as medical field (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 reduction of vascular reactive oxygen species production 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] In fact, contrary to CCB, Angiotensin II Type 1 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 II Type 1 receptor blockers.

Recent studies have shown that Olmesartan possesses antioxidant effects which might be independent on its antihypertensive effects.[7]

Hence, this *in vitro* study was done to demonstrate the antioxidant pharmacological activity of Olmesartan.

METHODS

Sample preparation

10 mg/ml stock solution of Olmesartan was prepared with ethanol (60 µM).

Determination of antioxidant activity

1,1 Diphenyl 2-picryl hydrazide (DPPH) radical scavenging assay

DPPH radical scavenging activity was done using the method of Yohozowa et al. The reagents required were DPPH and Ethanol (60 µM). The reaction mixture containing 1 ml of DPPH solution (200 µM in ethanol) and serial dilutions (100-1000 µg) of the sample drug Olmesartan was shaken and incubated in dark for 20 min at room temperature. The resultant absorbance was recorded at 517 nm using a spectrophotometer. A 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 was calculated using the formula

% inhibition = (Absorbance of control - Absorbance of test sample/Absorbance of control) × 100

Nitric oxide (NO) radical scavenging assay

The NO radical scavenging activity was done using the method of Alderson et al. 3 ml of a reaction mixture containing sodium nitroprusside (10 mM in phosphate buffered saline) and serial dilutions (10-1000 µg) of the sample drug Olmesartan were incubated at 37°C for 4 hours. An aqueous solution of sodium nitroprusside spontaneously generates 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% sulfanilamide, 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in 100 ml of distilled water. Control sample was prepared by mixing 1 ml of solvent + 2 ml of sodium nitroprusside + 0.5 ml of Griess reagent.

The percentage scavenging of NO free radical by Olmesartan was calculated using the formula,

% inhibition = (Absorbance of control - Absorbance of test sample/Absorbance of control) × 100

RESULTS

DPPH free radical scavenging assay of Olmesartan showed a gradual increase in free radical scavenging activity in a concentration dependent manner up to 800 µg. Then there was a slight decrease in percentage inhibition at a maximal drug concentration of 1000 µg (Table 1).

Olmesartan exhibited good free radical scavenging activity with NO at lower concentrations (10 and 50 µg) and also at higher concentrations (400, 800, 1000 µg). The radical scavenging activity was insignificant at 100 and 200 µg drug concentrations (Table 2).

Comparison of Free radical scavenging activity of Olmesartan by DPPH and NO assays is depicted (Figure 1).

DISCUSSION

Free radical oxidative stress has been implicated in the pathology of a wide variety of clinical disorders.[1] Numerous

Table 1: DPPH free radical scavenging assay.

S. No	Concentration (µg/ml)	Percentage inhibition
1	100	11.45
2	200	30.35
3	400	45.49
4	800	49.48
5	1000	44.79

DPPH: 1,1 Diphenyl 2-picryl hydrazide

Table 2: NO free radical scavenging assay.

S. No	Concentration (µg/ml)	Percentage inhibition
1	10	44.25
2	50	47.52
3	100	29.64
4	200	18.79
5	400	37.65
6	800	43.13
7	1000	48.65

NO: Nitric oxide

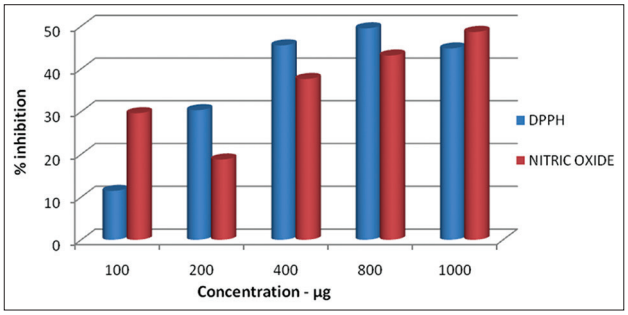

Figure 1: Free radical scavenging activity of Olmesartan.

physiological and biochemical processes in the human body may produce oxygen-centered 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, and ageing.[8]

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]

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.[9,10]

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

In this study, Olmesartan an angiotensin receptor antagonist showed significant free radical scavenging activity by DPPH and NO radical scavenging assays.

From this study, we concluded that Olmesartan has free radical scavenging pharmacological activity *in vitro*. Hence, 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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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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