INHIBITION OF ANGIOTENSIN CONVERTING ENZYME ACTIVITY BY REDUCED GLUTATHIONE: A DOSE DEPENDENT INVITRO STUDY.

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ABSTRACT: The Angiotensin converting enzyme (ACE) is a dipeptidyl carboxypeptidase and plays an important role in the regulation of blood pressure. Several potent inhibitors of this enzyme have been reported to be active antihypertensive agents. Sulfhydryl (SH) group containing ACE inhibitors used as a antihypertensive agents. Reduced glutathione (GSH) as antioxidant play an important role in reducing the blood pressure. Several recent studies have shown that reduced glutathione enhance nitric oxide pathway and increases the bioavailability of nitric oxide resulting in vasodilatation. In this study reduced glutathione and oxidized glutathione (GS-SG) were investigated for inhibition against ACE using Hip-His-Leu (HHL) as substrate. The inhibition of ACE by different concentrations of reduced glutathione was much more than that of oxidized glutathione. The inhibition of ACE by reduced glutathione ranges from 12.5% to 60%. Oxidized glutathione shows less than 5% of inhibition. This study shows that apart from the antioxidant role, reduced glutathione inhibits ACE activity which plays a crucial role in the regulation of blood pressure.

Keywords: Angiotensin converting enzyme (ACE), Reduced glutathione (GSH)

INTRODUCTION

Angiotensin converting enzyme is a glycoprotein peptidyldipeptide hydrolase (EC 3.4.15.1) that cleaves histidylleucine dipeptide from angiotensin I, a relatively inactive dipeptide. The latter is converted to the potent vasoconstrictor, angiotension II. ACE also inactivates bradykinin and plays an important role in the regulation blood pressure (Erdos, 1990). Several potent inhibitors of this enzyme have been reported to be active antihypertensive agents (Skidgel, 1993).

Reduced glutathione (GSH), the most abundant low molecular weight thiol in cells and tissues, participates in various physiological processes including synthesis of DNA and proteins (Meister, 1985)**,** regulation of enzyme activity and of both inter and intracellular transport system (Deneke son and Fan burg)**.** The main role of GSH is based on its ability to protect the cells against oxidative damage caused by free radicals (Jhone et al., 1995). By providing a reducing milieu to cells, GSH also serves as an important substrate for the reductive detoxification of reactive intermediates such as hydrogen peroxide or hydro peroxides (Meister, 1992)**.** Reduced glutathione (GSH) plays an important role in the regulation of blood pressure by improving the endothelial function by increasing the bioavailability of nitric oxide which acts as an antioxidant (Nisratola. D. Vaziri, 2000).

Peptides derived from Soybean and Casein (Maruyamma et al., 1987, Khmura et al., 1990 and Marnyamma et al., 1989) shows inhibitory properties of ACE. Endogenous peptides like carnosine, homocarnosine and anserine (How et al., 2003) shows dose dependent ACE inhibitory activities. Sulphydryl group containing antihypertensive drugs were also used as ACE inhibitors and GSH possesses sulphydryl group which stimulates for this study. An attempt was made to identify the ACE inhibitory effect of reduced glutathione (GSH) and oxidized glutathione (GS-SG). This communication reports the presence of inhibitory property of reduced glutathione towards ACE.

MATERIALS AND METHODS

Reduced glutathione and oxidized glutathione were obtained from Sigma Aldrich. Angiotensin converting enzyme was prepared by using a modification method used by Cushman and cheng (Cushman and Cheung, 1971). Albino rats of both sexes were used in these experiments.

After the animals were decapitated, the lungs were isolated and washed with ice-cold 100mM borate buffer, pH 8.3, containing 50mM KCl and froze until further use. One g of lung tissue was diced and homogenized in 10ml of the same ice-cold buffer using motor driven Teflon/glass homogenizer at 4^0 C. The homogenate was centrifuged at 20000g for 20 min at 4^0 C. The supernatant was collected and dialyzed for 12 h, against 20 volumes of the same buffer at 4^0 C to remove endogenous low molecular weight inhibitors. The dialyzed supernatant was used as the enzyme source for Angiotensin converting enzyme. Protein content of the supernatant was measured by the method of Lowry (Lowry et al., 1951) using bovine serum Albumin as standard. Reduced and oxidized glutathione solutions were prepared of following concentration: 3, 6,9,12, 15, 18 & 21µmoles/L respectively.

ACE activity was measure by modification of the method described by Schnaith (Schnaith et al., 1994). Using hippuryl-L-histidyl-L-leucine (HHL) as substrate. The reaction mixture contained 0.2 ml of 5mM HHL prepared in 200mM borate buffer,pH 8.3, containing 1000mM KCl and lung extract. Lung extract in a volume of 50 µl was added to initiate the reaction was stopped by adding 2ml of 100mM N-(2-hydroxy ethyl)-piperazine-N'-2-ethane sulfonic acid (HEPES),pH 9.0, containing 2.5mM EDTA, 1ml of 136mM cyanuric chloride prepared in1,4-dioxane was added to the reaction mixture and mixed vigorously by vortex mixing for 15s. The absorbance of the yellow colour that developed was measured at 405nm.The specificity of the reaction for ACE was tested by adding 10 µl of 10µM captopril to the incubation buffer. Under the assay conditions captopril blocked 99% of ACE activity.

One unit of ACE was defined as the amount of enzyme catalyzing the release of 1nmole of hippuric acid from HHL per minute at 37^0 C. Under the assay conditions 50 µl of lung extracts (200-240 µg protein) released 330 nmoles of hippuric acid equivalent to an absorbance of 0.4 fm HHL.

ACE inhibitory activity of glutathione was measured by using suitable aliquots of reduced and oxidized glutathione in the assay system. Reduction in ACE activity was the measure of inhibition. One unit of inhibitory was the amount that suppressed enzyme activity by one unit.

RESULTS

The different concentrations of reduced and oxidized glutathione are incubated with ACE reaction mixture. The data on inhibition of ACE activity by different concentration of glutathione are summarized in table-1. 3µmol concentration of reduced glutathione shows 12.5 % of reduction in the ACE activity with the inhibitory units of 25. 6µmol concentration of GSH shows 37 % of inhibition in ACE activity having 75 units of inhibitory activity. 9µmol concentration shows 50 % of ACE inhibition with 100 ACE inhibitory units. ACE activity was inhibited by 55 % by the 12 µmol concentration of GSH having 110 inhibitory units. 15, 18 and 21µmol GSH concentration has similar inhibitory units of 113 with 60 % reduction in ACE activity. Even further increase in GSH concentration shows not more than 60 % of inhibition in ACE activity.

Table-1: Inhibition of Angiotensin converting enzyme activity by reduced and oxidized glutathione of different concentrations.

Concentration in µmol/l	Reduced glutathione (Inhibitory units/ml)	Oxidized glutathione (Inhibitory units/ml)
3	25.00 ± 1.56	1.2 ± 0.21
6	75.00 ± 1.29	3.5 ± 0.12
9	100.50 ± 1.10	5.0 ± 0.22
12	110.50 ± 1.38	5.5. ± 0.13
15	112.50 ± 1.23	6.0 ± 0.18
18	112.85 ± 1.38	6.1 ± 0.21
21	113.50 ± 0.94	6.0 ± 0.23

Data are mean ± SD of 20 experiments

Various concentrations of oxidized glutathione with ACE reaction mixture did not show significant inhibition of ACE activity. The maximum inhibition of ACE by GS-SG is less than 5%. Figure-1 & 2 shows the inhibitory units and percentage of inhibition of GSH and GS-SG.

Reduction in ACE activity by 50 % to 60 % is achieved by the concentration of 9 to 21µmol/L of GSH, whereas no significant inhibition of ACE by oxidized glutathione.

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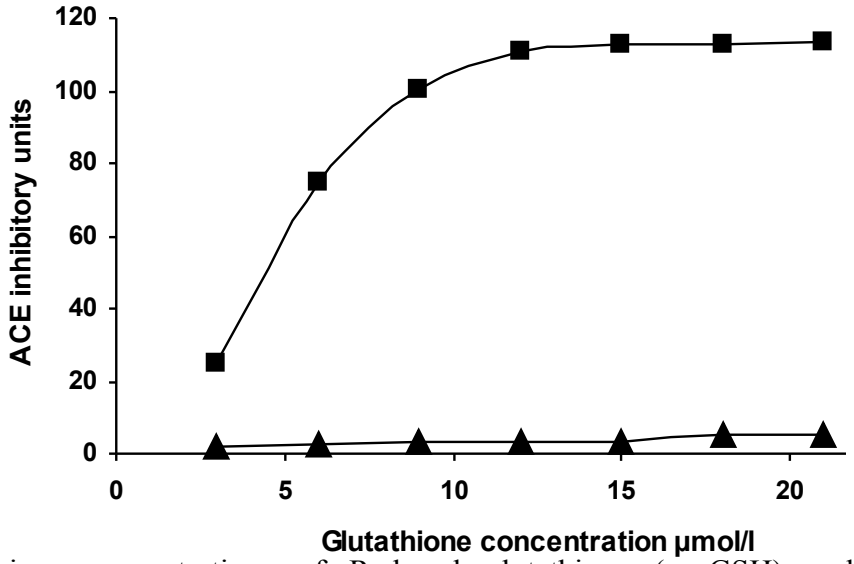

Figure-1: Various concentrations of Reduced glutathione (■ GSH) and Oxidized glutathione (▲ GS-SG) with their respective inhibitory units of ACE

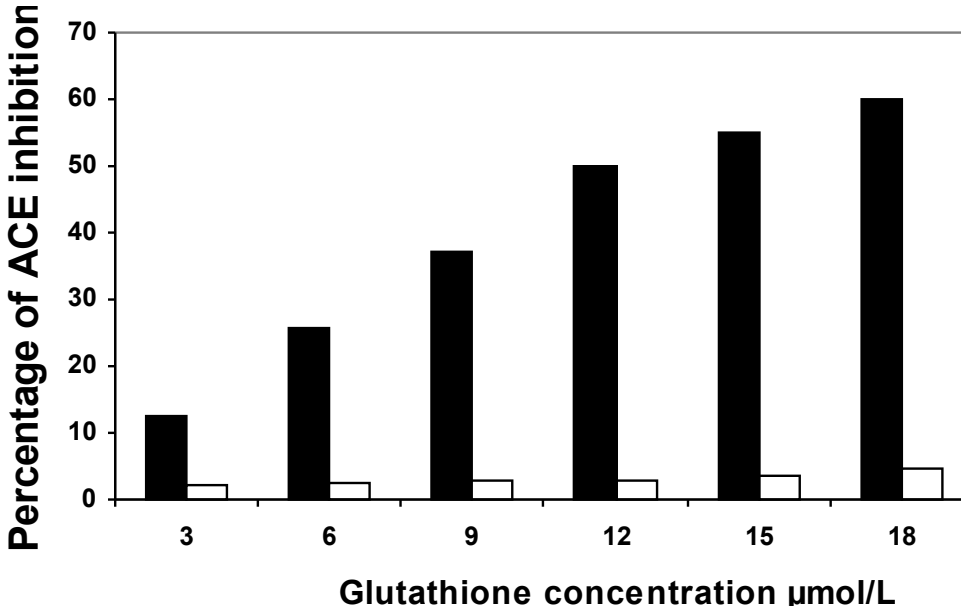

Figure- 2: Percentage of Inhibition of ACE by various concentrations of reduced glutathione (■ GSH) and Oxidized glutathione (□ GS-SG).

DISCUSSION AND CONCLUSION

Reduced glutathione plays a key role in the free radical scavenging system. Depletion of reduced glutathione in normal rats results in severe hypertension (Nisratola. D. Vaziri, 2000) Moreover various studies demonstrate reduced glutathione promotes vasodilatation of blood vessels by stabilizing nitric oxide to form less reactive S-nitrosoglutathione and protects cells against oxidant attack (Jung Dae park, 2002 and Clancy et al., 1994). Angiotensin converting enzyme plays a significant role in the regulation of blood pressure. In hypertension individuals the ACE activity inhibition decreases the blood pressure.

In the present study ACE inhibition by reduced glutathione is more significant when compared to oxidized glutathione. More than 50% of ACE inhibition was achieved by reduced glutathione at the concentration of 9 µmole and above. The inhibitory units are proportionate to the reduced glutathione concentrations but not for oxidized glutathione. Free sulphydryl (SH) group is present in reduced glutathione, where as it is absent in oxidized glutathione. The above findings suggest that the sulphydryl (SH) involves in the inhibition of ACE.

According to Jung Dave Park & Domg H. Kim (Ol'ga Pechanove et al., 1999), Sulphydryl group present in the cystein derivatives form a strong interaction with the Zinc ion at the active site of carboxypeptidase enzymes. Angiotensin converting enzyme is a zinc containing carboxy peptidase. This study exposes the reduced glutathione role in hypertension by inhibiting the ACE activity which may be due to free sulphydryl group.

Our findings show that apart from the antioxidant role, glutathione inhibits ACE activity, which plays crucial role in the regulation of blood pressure. However further investigations are required to unveil the exact mechanism of inhibition.

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