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DUAL ROLE OF S-NITROSOCAPTOPRIL AS AN INHIBITOR OF ANGIOTENSIN-CONVERTING ENZYME AND A NITROSO GROUP CARRIER

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SUMMARY: The S-nitroso derivatives of captopril can act as an inhibitor of an angiotensin-converting enzyme in the presence of thiol such as glutathione. S-Nitrosocaptopril also rapidly transfers its nitroso moiety to a heme protein, which is presumably the responsible mechanism for the activation of guanylate cyclase. These results suggest that S-nitrosocaptopril may serve as an effective hypotensive agent.

Infusion of sodium nitroprusside, SNP, commonly is used to reduce arterial blood pressure because of the rapid onset and termination effect of this drug (1). At the present time there is a considerable evidence supporting the mechanism that the hypotensive effect of SNP is mediated through activation of guanylate cyclase (EC 4.6.1.2) via the production of nitric oxide (2). It has been known that the physiological degradation of SNP results in the increase of blood cyanide levels. The risk of cyanide intoxication limits the amount of SNP that may be safely administered (3). There have been reports which show that captopril (D-3-mercapto-2-methylpropanoyl-L-proline) can reduce the dose of SNP required to produce delibrate hypotension (4, 5). Captopril acts as a competitive inhibitor of an angiotensin-converting enzyme (ACE; EC 3.4.15.1), which plays a critical role in blood pressure regulation by converting the inactive peptide angiotensin I to the vasoactive peptide angiotensin II. Thus it is a sysmetic hypotensive drug that functions by decreasing peripheral vascular resistance in essential hypertension (6).

It has been known that thiols react readily with nitrovasodilators such as nitric oxide (NO) gas, glyceryl trinitrate, SNP, and sodium nitrite to form S-nitrosothiols (7). Recently, S-nitrosothiols have been proposed as common biologically active intermediates for the various nitrovasodilators as well as a possible precursor of endothelium-derived NO (8) or even endothelium-derived relaxing factor itself (9). It has been shown that S-nitrosothiols activate guanylate cyclase and subsequently reduce blood pressure (10). Because captopril contains free sulfhydryl group it can be readily converted to a related S-nitrosothiol by nitrosating agents. The present study reveals that S-nitrosocaptopril inhibits ACE in the presence of glutathione (GSH) and it acts as a nitroso group carrier. These results indicate that S-nitrosocaptopril may be an active hypotensive agent.

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MATERIALS AND METHODS

Captopril, ACE, GSH, hemoglobin, sodium dithionite, 2-furanacryloyl-L-phenylalnylglycylglycine (FAPGG) were purchased from Sigma. Sodium nitrite was from Aldrich Chemicals. S-Nitrosocaptopril was prepared according to the method of Saville (11). The method was slightly modified by the dropwise addition of HCl to a solution containing eqimolar amount of captopril and sodium nitrite until pH 1.5 was obtained. After standing for 5 min at 23° C, the red S-nitrosocaptopril solution was neutralized with NaOH. S-Nitrosocaptopril displays dual absorption maxima at 546 nm and 330 nm with absorptivities of 19.8 and 998 $M^{-1}cm^{-1}$, respectively. The homolytic decomposition of S-nitrosothiols was followed spectrophotometrically by a decrease in absorbance at the absorption maxima. Activities of ACE were measured with FAPGG, 5 x 10^{-5} M, as substrate in 0.05 M Tris, pH 7.5, containing 0.3 M NaCl according to Holmquist *et al.* (12). The decrease in absorbance at 328 nm was monitored and initial velocities were measured during the first 10% of hydrolysis. The denitrosation of S-nitrosocaptopril by GSH was followed by HPLC immediately after mixing two reactants at pH 7.4 and 23° C. Deoxyhemoglobin, which was prepared by the method of Craven *et al.* (13), and S-nitrosocaptopril in 0.05 M phosphate, pH 7.4 were equlibrated separately for 10 min at 0° C with a continuous flow of nitrogen in a septum-capped vial, and then the S-nitrosocaptopril solution was transferred to deoxyhemoglobin solution by a gas-tight Hamilton syringe. Visible spectra for the mixture was followed in the region of 350-650 nm.

RESULTS AND DISCUSSION

Contrary to previous reports on the stability of S-nitrosothiols (10, 14), S-nitrosocaptopril is considerably more stable than several other S-nitrosothiols under physiological conditions. S-Nitrosocaptopril decomposes with a second order rate constant, k_2, of 9 x 10^{-5} $M^{-1}sec^{-1}$ at 37° C and pH 7.4. In contrast, other S-nitrosothiols such as S-nitrosocysteine, S-nitroso-N-acetyl-DL-penicillamine exhibit k_2 of 0.114 and 0.012 $M^{-1}sec^{-1}$, respectively.

The addition of 0.01-0.1 μM S-nitrosocaptopril to ACE did not significantly inhibit ACE whereas 0.1 μM captopril exhibits more than 90% of ACE activity as shown in Fig. 1. Even with 100 μM of S-nitrosocaptopril, only 58% of inhibition was observed. When the same reaction was performed in the presence of 5 μM GSH, the concentration dependent inhibition similar to captopril was observed as shown in Fig. 1. A control experiment with 5 μM GSH did not produce a noticeable inhibition of ACE. The sulfhydryl group of captopril has been known to be critical in its inhibitory role. Its strength of binding to ACE (Ki = 1.7 nM) is derived largely from the interaction of its sulfhydryl group with the zinc atom at the active site of the enzyme (15). Therefore it is plausible to assum that replacement of the sulfhydryl group of captopril by a sodium nitrite will not yield an effective inhibitor. Inactivation of ACE by S-nitrosocaptopril in the presence of GSH appears to be the result of a denitrosation by GSH which may convert S-nitrosocaptopril to captopril. It has been shown that S-nitrosoglutathione rapidly exchanges the NO moiety with thiols to give S-nitrosothiol and GSH. In general, S-nitrosothiols are nonselective nitrosating agents which act by transferring their nitroso

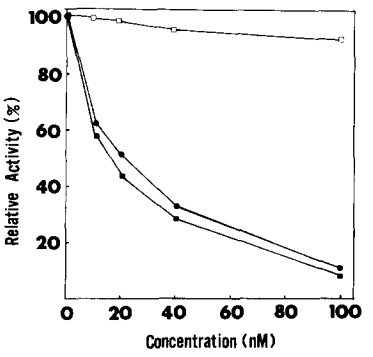

Fig. 1. Effects of S-nitrosocaptopril (- □ -), S-nitrosocaptopril in the presence of GSH (- ● -), and captopril (- ■ -) on the activity of ACE. The assay mixture contained 1.6 x 10^{-8} M ACE, 5 x 10^{-5} M FAPGG, 0.3 M NaCl in 0.05 M Tris, pH 7.5. The reaction was started by addition of ACE. Results are expressed means of at least three separate experiments that showed minor variation from each other.

group directly to NO acceptors such as thiols, sulfhydryl groups in protein (16). A similar denitrosation was observed with S-nitrosocaptopril and GSH by HPLC (data not shown). This reaction is extremely fast and reaches equilibrium instantaneously. Because the degree of transnitrosation depended on the concentration of the thiols, it can be assumed that administered S-nitrosocaptopril may rapidly react with thiol-containing compounds under physiological conditions, probably mainly with GSH, which is one of the most abundant thiol compounds in an *in vivo* system, to give an active inhibitor captopril.

The heme moiety of reduced hemoproteins, including soluble guanylate cyclase, have a high affinity for NO and react rapidly with NO to generate the NO-heme or NO-heme adduct of the corresponding hemoprotein (17). NO ultimately stimulates guanylate cyclase by the formation of NO-heme complex at the active site of enzyme. Direct evidence for a role of the NO-heme complex in the activation of guanylate cyclase was obtained from studies with preformed nitrosyl-hemoglobin (NO-Hb), generated by reaction of NO and hemoglobin (18). Therefore, the formation of NO-Hb adduct has been used as an indicative reaction for the nitrovasodilators resulting the activation of guanylate cyclase. NO-Hb possesses a distinct absorbance in the Soret region that can be easily distinguished from the absorbance of related hemoglobin adducts such as oxyhemoglobin, carboxyhemoglobin, or methemoglobin. Fig. 2 shows the visible spectra for 50 μM deoxyHb, 50 μM of deoxyHb with 400 μM S-nitrosocaptopril at pH 7.4 and 23° C in the region of 350-650 nm. The hemoglobin derivatives display a characteristic shift in the Soret absorbance maxima from 430 nm for deoxyHb to 418 nm, which is the characteristic peak for NO-Hb. The reaction occured almost instantaneously. S-Nitrosothiols, which activate guanylate cyclase such as S-nitrosoglutathione, S-nitrosocysteine, S-nitroso-N-acetyl-DL-penicillamine, also induced same reaction. These results indicate that S-nitrosocaptopril can inhibit ACE by denitrosation induced by GSH, and that S-nitrosocaptopril can activate guanylate cyclase by transfer of the NO moiety to

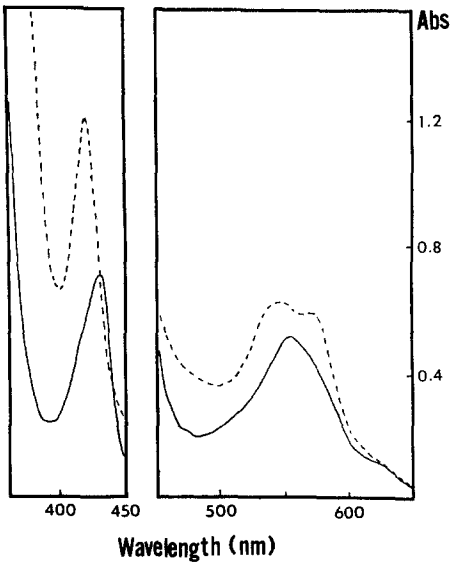

Fig. 2. VIS spectra of 50 μM deoxyHb (——) and 50 μM deoxyHb with 400 μM S-nitrosocaptopril at 23° C in 0.05 M phosphate, pH 7.4 (-----). For 350-450 nm range, samples were diluted 10 times.

heme. These two reactions could occur simultaneously. Since ACE and guanylate cyclase play an important role in the regulation of blood pressure S-nitrosocaptopril could have therapeutic value in place of SNP and would eliminate the side effect and risks associated with the latter's metabolic degradation of cyanide.

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