Vol. 158, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 16, 1989 Pages 52-57

DIRECT SCAVENGING OF FREE RADICALS BY CAPTOPRIL, AN ANGIOTENSIN CONVERTING ENZYME INHIBITOR

Debasis Bagchi, Renuka Prasad and Dipak K. Das*

Cardiovascular Division, University of Connecticut School of Medicine, Farmington, CT 06032

Received November 7, 1988

Captopril, an angiotensin converting enzyme (ACE) inhibitor, was hypothesized to be a potential scavenger of free radicals because of the presence of a thiol group. The scavenging action of captopril was examined against superoxide anion (O_2^-), hydroxyl radical ($OH^\cdot$), hypohalite radical (HOCL) either generated biochemically, or derived from activated polymorphonuclear leukocytes (PMN). Our results indicate that captopril is an extremely potent free radical scavenger, scavenging power being as effective as superoxide dismutase (SOD) against O_2^-, or dimethylthiourea against $OH^\cdot$, but better than allopurinol against $OCL^\cdot$ plus HOCL. Free radical scavenging action of captopril against PMN-derived free radical is equivalent to the combined effects of SOD, catalase and allopurinol. © 1989 Academic Press, Inc.

Captopril, an angiotensin converting enzyme (ACE) inhibitor has been found to reduce hypertension (1), to possess antiinflammatory property (2), and more recently, to provide protection against reperfusion arrhythmias (3). While the first two properties are shared by other ACE inhibitors (1,2), the last property is unique for captopril. This drug also possesses immunosuppressant activity which makes it an ideal drug like D-penicillamine, used for treating rheumatoid arthritis (4). In this respect, captopril shares a common feature with penicillamine, both possessing a thiol group, a property not shared by other ACE inhibitors.

The fact that captopril, but not other ACE inhibitors such as enalapril and HOE 498, do not demonstrate a cardioprotective effect by reducing reperfusion arrhythmias (3), suggests that the former drug may possess some unique property not shared by other ACE inhibitors. Recently, thiol containing antiarrhythmic drugs such as penicillamine have been found to scavenge hypohalite radicals ($OCL^\cdot$) and hypochlorous acid (HOCL) formed via myeloperoxidase- $CL^\cdot$ -H_2O_2 system (5). Interestingly, the presence of HOCL and $OCL^\cdot$ has also been indicated in the mediation of myocardial ischemic reperfusion injury (6,7). We,

* To whom reprint requests should be addressed.

0006-291X/89 $1.50
Copyright © 1989 by Academic Press, Inc.
All rights of reproduction in any form reserved.

therefore, hypothesized that the cardioprotective effects of captopril may be due to its scavenging action against HOCL and $OCL^{\cdot}$. To test this hypothesis, we chemically generated $OCL^{\cdot}$ and HOCL as well as other oxygen-derived free radicals such as superoxide anion (O_2^-) and hydroxyl radicals ($OH^{\cdot}$) and examined the possible scavenging action of captopril. Our results indicate that captopril is an extremely potent scavenger of all the free radicals examined including O_2^-, $OH^{\cdot}$, $OCL^{\cdot}$ and HOCL.

METHODS

Various free radicals were generated from biochemical reactions using known generating systems (8). O_2^- was generated from the well-known reaction of hypoxanthane with XO, while $OH^{\cdot}$ was generated by further adding $FeCl_3$ and EDTA to the O_2^- generating system. Hypohalite and HOCL were generated from NaOCL by adding a weak acid. PMNs were obtained from rabbits and activated with fMLP as described elsewhere (9).

Generation of free radicals were examined by three different methods. Generation of O_2^- was examined by cytochrome C reduction assay method (10). $OH^{\cdot}$ was examined after trapping the radical with sodium salicylate using a high pressure liquid chromatography (11). O_2^-, $OH^{\cdot}$ as well as HOCL plus $OCL^{\cdot}$ were also examined after adding luminal to them and monitoring the chemiluminescent response with a luminometer (8).

To study the free radical scavenging property of captopril, captopril was simultaneously added to the free radical generating system. To compare the scavenging role of captopril with known free radical scavengers, SOD (for O_2^-), DMTU (for $OH^{\cdot}$), and allopurinol (for $OCL^{\cdot}$ plus HOCL) replaced the captopril.

RESULTS

Scavenging of Free Radicals by Captopril

The O_2^- generated by the action of XO on hypoxanthine was progressively scavenged with increasing concentrations of captopril (Fig. 1A). Although the scavenging action of captopril on O_2^- was observed in both the methods, the concentration required to cause 50% scavenging varied significantly between the methods. Thus, 250 μM and 1 mM captopril was required to scavenge 50% of O_2^- by luminol-chemiluminescence and 50% of O_2^- by cytochrome C reduction methods, respectively. The $OH^{\cdot}$ radical generated by the action of Fe^{3+} on O_2^- when captured as the hydroxylated derivative of salicylic acid, the HPLC method yielded two peaks (Fig. 2). Both the peak responses increased with the increased generation of $OH^{\cdot}$ (data not shown) and were equally decreased by captopril. Similar attenuation of both the peaks were also observed with DMTU (data not shown). In this method of measurement, 25 μM captopril scavenged 50% of the generated $OH^{\cdot}$. In contrast to O_2^- scavenging, the captopril concentration required to scavenge 50% of $OH^{\cdot}$ did not significantly differ between HPLC and chemiluminescent methods (Fig. 1B). However, it is interesting to note that the concentration of captopril required to

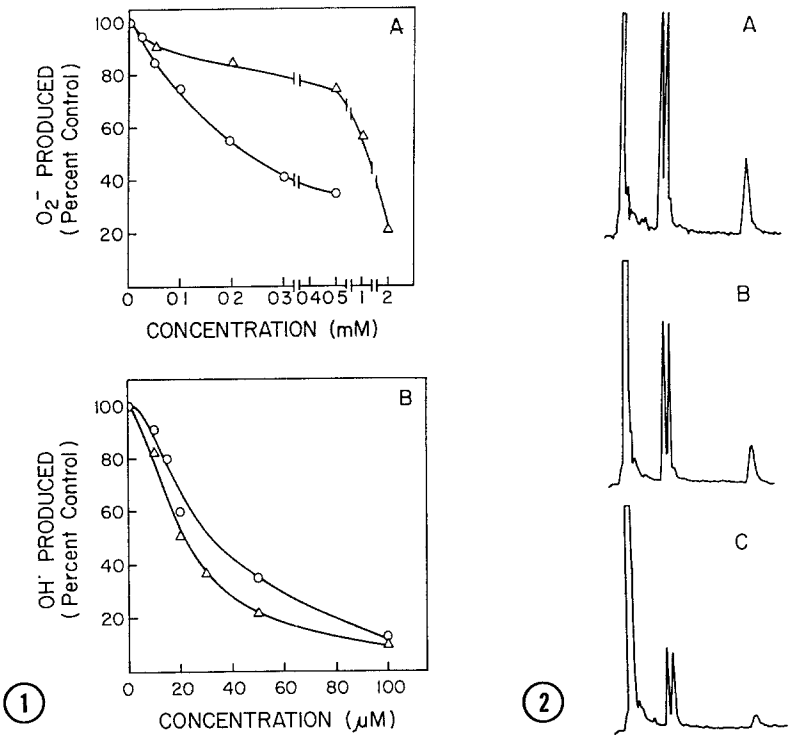

Figure 1. Captopril scavenging of O_2^- and OH· as a function of its concentration (A) measured by cytochrome C reduction (Δ-Δ) and luminol-chemiluminescent (o-o) methods, (B) OH· measured by HPLC (Δ-Δ) and luminol-chemiluminescent (o-o) methods.

Figure 2. Effect of captopril on the peak response of hydroxylated products of salicylic acid on HPLC. The concentration of captopril were (A) 0 μM, (B) 20 μM and (C) 100 μM.

scavenge OH·radical is only 10% of that required to scavenge O_2^- (Fig. 1A and 1B). Like OH·, OCL·plus HOCL was also scavenged by captopril at lower concentrations (Fig. 3C). Thus, 50% of OCL·and HOCL were scavenged by 25 μM captopril.

Comparison of Free Radical Scavenging Properties of Captopril with Known Scavengers

In order to further confirm the free radical scavenging actions of captopril, we compared the O_2^--scavenging action of captopril with SOD using cytochrome C reduction assay method, OH· scavenging action with DMTU using HPLC, and OCL· plus HOCL scavenging action with allopurinol using luminometer (Figure 3). At each dose tested, captopril scavenged O_2^- as effectively as SOD and scavenged OH· as effectively as DMTU. On the other hand, captopril was a better scavenger of OCL· and HOCL than allopurinol.

Effects of Captopril on the Free Radicals Generated by Activated PMN

Since activated PMN has been indicated in the mediation of ischemic reperfusion injury in mammalian heart, we studied the scavenging properties of

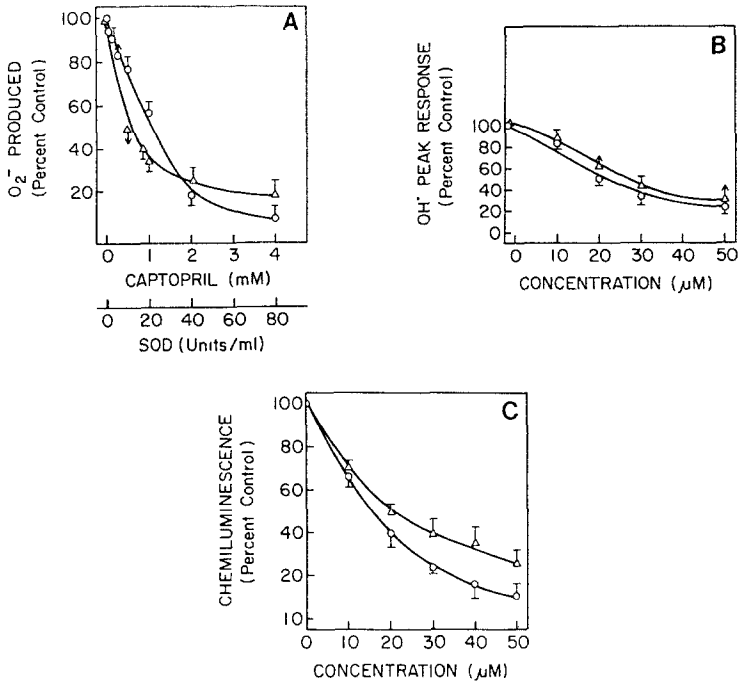

Figure 3. Comparison of free radical scavenging capacities of captopril with those of O_2^-, OH· and OCL· scavengers.
A) Increasing amounts of captopril (Δ-Δ) or SOD (o-o) were added to O_2^- generating system and O_2^- was measured by the method of cytochrome C reduction.
B) Increasing concentration of allopurinol (Δ-Δ) or DMTU (o-o) were added to OH· generating system and the OH· was measured by HPLC method.
C) Increasing amounts of allopurinol (Δ-Δ) or captopril (o-o) were added to OCL· + HOCL generating chemiluminescent method was employed to measure the free radical.

captopril on the free radicals generated by activated PMN. PMN was activated with fMLP and the chemiluminescent response was monitored as a function of duration of free radical generation (Figure 4). Captopril inhibited the chemiluminescent response significantly at each time point. We also compared the effects of captopril with other free radical scavenging systems. As shown in the Figure, not only captopril inhibited the activated PMN-mediated chemiluminescent response better than any other scavenger tested, it also inhibited the response to the same degree compared to that obtained by the combined treatments of SOD, catalase and allopurinol.

DISCUSSION

The results of our study clearly indicates that captopril, an ACE inhibitor, is also a potent free radical scavenger. While it works as a O_2^- scavenger like SOD, it appears to be as potent as DMTU in scavenging OH·, and a better OCL· plus HOCL scavenger compared to allopurinol. Captopril also

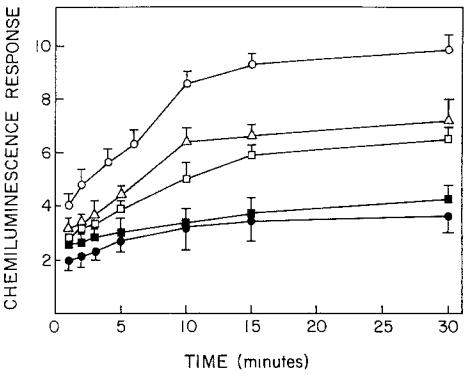

Figure 4. Effect of captopril and various free radical scavengers on the free radicals generated by activated PMNs. The activated PMNs (1 X 10^6 cells) were incubated with none (o-o), 1 μM allopurinol (Δ-Δ), or SOD (8 u/ml) and catalase (5 u/ml) (□-□) or SOD, catalase, and allopurinol (■-■) or 25 μM captopril (●-●). 0.5 x 10^{-7}M fMLP was used to activate PMNs.

scavenged the free radicals generated from the activated PMN to the same extent compared to the combined effects of SOD, catalase and allopurinol. Since SOD, DMTU and allopurinol are well known scavengers of O_2^-, $OH^\cdot$ and HOCL, respectively (8,6), this study suggests that captopril can be equally or even more effective than other known free radical scavengers in removing oxygen-derived free radicals as well as HOCL.

Oxygen-derived free radicals have been indicated in the mediation of myocardial ischemic-reperfusion injury (12). Recent studies also suggested that free radicals may be generated from the activated PMN via H_2O_2-myeloperoxidase-CL·system during the reperfusion of ischemic myocardium (6). Accordingly, SOD and catalase (6) as well as allopurinol (6,8) haven been found to moderate the reperfusion injury presumably by scavenging the free radicals generated in the reperfused heart. Recently, captopril has also demonstrated cardioprotective effect by reducing reperfusion arrhythmias (3). It is, therefore, reasonable to speculate that captopril moderated the reperfusion arrhythmias by scavenging the free radicals, and not by virtue of its anti-ACE properties. Indeed, other ACE-inhibitors including enalapril or teprotide did not demonstrate any cardioprotective ability although they inhibited ACE successfully (3).

The free-radical scavenging action of captopril is believed to be due to the presence of the SH-group in its structure. Thiol-containing drugs are known to scavenge hypohalite radical and inhibit its formation by myeloperoxide from PMNs (5). It was suggested that competition of thiol-containing drugs for substrate-binding sites of myeloperoxidase may be the possible mechanism of these drugs for scavenging HOCL and OCL· . While our study did not examine this possibility, it conclusively demonstrated that captopril removed O_2^-,

$OH^{\cdot}$ and $OCL^{\cdot}$ plus HOCL by directly scavenging the free radicals from the system.

ACKNOWLEDGEMENTS: This study was supported by USPHS NIH HL 22559, HL 33889 and HL 34360. We are grateful to Alexandra Pisko for her skillful secretarial assistance.

REFERENCES

1. Ondetti, M.A., Rubin, B., and Cushman, D.W. (1977) Science, 196, 441-444.
2. Martin, M.F.R., McKenna, F., Bird, H.A., Surrall, K.E., Dixon, J.S., and Wright, V. (1984) Lancet 1, 1325-1328.
3. vanGilst, W.H., deGraeff, P.A., Wesseling, H., and deLangen, C.D.J. (1986) J. Cardiovasc. Pharmacol. 8, 722-728.
4. Fantone, J.C., Schrier, D., and Weingarten, B. (1982) J. Clin. Invest. 69, 1207-1211.
5. Cuperus, R.A., Muijsers, A.O. and Wever, R. (1985) Arthr. Rheumat. 28, 1228-1233.
6. Das, D.K., Bagchi, D., Engelman, R.M., Subramanian, R., Prasad, R., Jones, R., Cordis, G. and Otani, H. (In press). In Medical Biochemical and Chemical Aspects of Free Radicals Elsevier Biomedical, New York.
7. Lucchesi, B.R., and Mullane, K.M. (1986) Ann. Rev. Pharmacol. Toxicol. 26, 201-224.
8. Das, D.K., Engelman, R.M., Clement, R., Otani, H., Prasad, M.R., and Rao, P.S. (1987) Biochem. Biophys. Res. Commun. 148, 314-319.
9. Grisham, M.B., Engerson, T.D., McCord, J.M., and Jones, H.P. (1985) J. Immunol. Methods 82, 315-320.
10. Das, D.K., and Neogi, A. (1984) Clin. Physiol. Biochem. 2, 32-38.
11. Luber, J.M., Rao, P.S., and Rujikarn, N. (In press) J. Chromatogr.
12. Das, D.K., Engelman, R.M., Rousou, J.A., Breyer, R.H., Otani, H., and Lemeshow, S. (1986). Basic Res. Cardiol. 81, 155-166.