J. Pharm. Pharmacol. 1995, 47: 59–61
Received May 27, 1994
Accepted September 6, 1994

Captopril Induces Iron Release From Ferritin and Oxidative Stress

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Abstract

Captopril has been reported to possess reducing and iron-binding properties, which could favour iron delocalization from ferritin and oxidative stress.

In the present paper, we have found that the drug was effectively capable of inducing a significant mobilization of ferritin iron, which was apparently superoxide anion-independent. Once released from ferritin as a result of captopril action, iron became free in the reduced form and could induce oxidant damage, as evaluated by deoxyribose-oxidative degradation. This phenomenon was not antagonized by the reported oxygen radical-scavenging properties of the drug.

These data indicate that captopril is not always an antioxidant drug, and suggest that it may act as a pro-oxidant in the presence of ferritin in-vivo.

Tissue oxidative stress is dependent on the presence and availability of free catalytic transition metals, such as iron (Halliwell & Gutteridge 1989, 1990). Free iron is not readily available under physiological conditions, but its possible source is the storage protein ferritin (Halliwell & Gutteridge 1990; Reif 1992). Iron is bound to ferritin as iron (III), and it can undergo mobilization as a result of reduction to iron (II) (Reif 1992); chelating substances also favour ferritin iron delocalization (Reif 1992). Ferritin iron has been proved to be released by superoxide anion (Biemond et al 1984; Reif 1992), redox-cycling xenobiotics (Winterbourn et al 1991; Reif 1992) and reductants of physiological significance, such as reduced flavins, nitric oxide, ascorbic acid and the thiols glutathione and cysteine (Sirivech et al 1974; Reif & Simmons 1990; Reif 1992).

Captopril (1-(3-mercapto-2-methyl-1-oxopropyl)-L-proline) is an angiotensin-converting enzyme (ACE) inhibitor largely used for the treatment of hypertension and congestive heart failure. Experimental studies have indicated that some beneficial effects of captopril may also be due to antioxidant mechanisms, apparently related to its reducing sulphydryl group (Westlin & Mullane 1988; Bagchi et al 1989). However, this biochemical feature, and the iron-binding properties of captopril (Campbell & Hasinoff 1991; Weglicki & Mak 1992), could also be responsible for drug pro-oxidant activity, as a result of ferritin iron delocalization. The present study, therefore, was designed to investigate the potential pro-oxidant capacity of captopril due to iron release from ferritin.

Materials and Methods

Materials

Reagents were obtained from Sigma Chemical Co, St Louis, MO. Ferritin (Sigma type I) was from horse spleen, and superoxide dismutase (SOD) from bovine erythrocytes. Ferritin was purified from potential loosely (i.e. non-specifically) bound iron by dialysis against 1 mM EDTA and Chelex 100 resin ($10\,g\,L^{-1}$), followed by extensive dialysis against double-distilled water containing Chelex 100 resin (Boyer & McCleary 1987). Ferritin iron content, determined essentially as reported by Drysdale & Munro (1965) with 2 mM ferene S as the iron colorimetric detector (Artiss et al 1981), was estimated to be about $3{\cdot}65\,mM\,mg^{-1}$ ferritin. The water used in the study was deionized, glass double-distilled and Chelex 100 resin-treated. Experiments were carried out in plastic or acid-washed glassware.

Ferritin iron release and ferritin iron-mediated oxidative stress

To detect iron (II) release from ferritin with specific colorimetric detectors, such as ferene S (Artiss et al 1981), iron (II) must not undergo oxidative processes, which can be due to the presence of phosphates (Cohen 1985). Thus, physiological saline rather than a phosphate buffer was used for specific purposes (Thomas & Aust 1986). Experimental tubes, with a final volume of 1·0 mL physiological saline, contained $200\,\mu g\,mL^{-1}$ ferritin, $200\,\mu M$ ferene S and captopril at 130, 250 and $430\,\mu M$ final concentrations. In this regard, it is interesting to note that a ferritin excess over captopril should be present in the blood environment in-vivo, considering mean serum ferritin and captopril levels in man of about 200 and $100\,ng\,mL^{-1}$, respectively (Cody 1990; Salonen et al 1992). Even though such low ferritin and captopril concentrations cannot be used in-vitro, due to sensitivity problems in the spectrophotometric approach usually followed to detect ferritin iron release (Biemond et al 1984; Thomas & Aust 1986; Boyer & McCleary 1987; Winterbourn et al 1991; Reif 1992), it could be relevant to use an appropriate ferritin : drug ratio to mimic in-vivo conditions. In our experiments, therefore, an excess of ferritin over captopril was used, with a ferritin : captopril ratio of about 2 : 1 for the highest captopril concentration.

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Reaction mixtures were incubated for 30 min at 37°C. Absorbance values at 594 nm, due to the ferene S-iron (II) complex formation, were recorded on a double beam Varian DMS 200 spectrophotometer against appropriate ferritin and ferene S-containing blanks. Amounts of iron released from ferritin were expressed as μM, using for calculation a molar extinction coefficient of $3{\cdot}55 \times 10^4$ mol^{-1} cm^{-1} (Artiss et al 1981).

The effects of 130, 250 and 430 μM of the SH-lacking ACE-inhibitor ramiprilat, and those of SOD at a concentration known to scavenge superoxide anion and antagonize superoxide reductive capacity (i.e. 25 units mL^{-1} (Tien et al 1982)), were also investigated in other experiments.

When the iron bound to ferritin as Fe (III) undergoes reductive delocalization processes and becomes free and available as Fe (II), biomolecule oxidant damage can be triggered by iron-related radicals (Halliwell & Gutteridge 1990; Reif 1992). To investigate whether captopril-induced ferritin iron mobilization may be a biologically relevant phenomenon, captopril and ferritin were incubated with deoxyribose, which is readily oxidizable by iron-related radicals (Halliwell & Gutteridge 1989, 1990). For this specific study, a phosphate buffer was used, since phosphates are present intracellularly and in extracellular fluids in-vivo, and they can favour iron (II) auto-oxidation with oxidizing radical generation (Flitter et al 1983; Cohen 1985). Reaction mixtures contained 40 mM potassium phosphate buffer, pH 7·4, 4 mM deoxyribose and 200 μg mL^{-1} ferritin, with and without 130, 250 and 430 μM captopril; incubation was for 30 min at 37°C. To each millilitre of reaction mixture, 1·0 mL 0·6% thiobarbituric acid (TBA) aqueous solution and 1·0 mL 2·5% trichloroacetic acid were added, followed by 30 min heating at 95°C. After cooling, the pink chromogen was extracted with *n*-butanol and read spectrophotometrically at 532 nm. For these experiments, controls consisted of captopril alone, deoxyribose alone, ferritin alone, captopril plus deoxyribose, captopril plus ferritin, and deoxyribose plus ferritin. Specific results were expressed as nmol TBA-reactants (mg deoxyribose)$^{-1}$, using for calculations a molar extinction coefficient of $1{\cdot}54 \times 10^5$ mol^{-1} cm^{-1}.

Statistics

Data were calculated as means ± s.d. of five different experiments. Drug effects were studied by the one-way analysis of variance, followed by the Bonferroni's test (Glantz 1987). $P < 0{\cdot}05$ was regarded as statistically significant.

Results and Discussion

As depicted in Fig. 1, captopril was capable of inducing a significant iron release from ferritin. Indeed, 4·2 ± 0·08, 7·8 ± 0·13 and 11·5 ± 0·3 μM of iron was mobilized from ferritin by 130, 250 and 430 μM of captopril, respectively, after 30 min incubation (130 vs 250 μM, $P < 0{\cdot}05$; 130 and 250 vs 430 μM, $P < 0{\cdot}05$); these values correspond to about 0·5, 1 and 1·5% of the total ferritin iron, respectively. Captopril, therefore, possesses an efficiency comparable with or even higher than that reported for cytotoxic xenobiotics, such as quinones, anthracyclines, bipyridyls and nitroaromatics (Winterbourn et al 1991), or for activated human neutrophils (Biemond et al 1984) and nitric oxide (Reif & Simmons 1990) in-vitro. SOD failed to affect significantly iron mobilization induced by captopril, ruling out superoxide involvement. This basically agrees with the report of Tien et al (1982), showing that thiol-dependent cytochrome c reduction is unaffected by SOD and is directly mediated by the thiol reducing capacity.

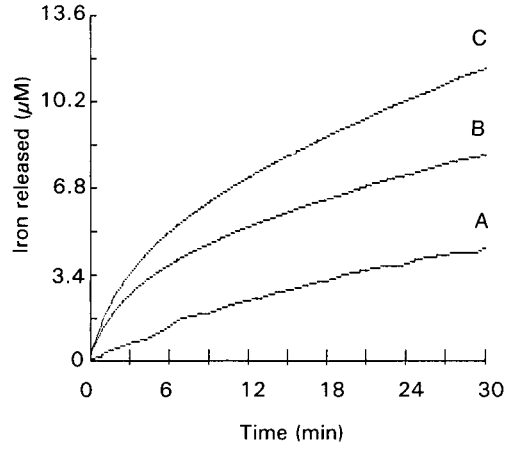

FIG. 1. The time-course of formation of Fe^{2+}-ferene S complex (followed at 594 nm and 37°C for 30 min in physiological saline, containing 200 μM ferene S), as a result of the interaction of ferritin (200 μg mL^{-1}) with 130 (A), 250 (B) and 430 (C) μM captopril. Iron release from ferritin alone in physiological saline was negligible.

Ramiprilat was ineffective at promoting ferritin iron release, indicating that captopril-mediated iron mobilization is independent of ACE inhibitory activity and is essentially related to the drug thiol group.

When captopril, ferritin and deoxyribose were incubated for 30 min at 37°C, 0·95 ± 0·2, 2·02 ± 0·4 and 3·6 ± 0·3 nmol TBA-reactants (mg deoxyribose)$^{-1}$ were generated in the presence of 130, 250 and 430 μM captopril, respectively (130 vs 250 μM, $P < 0{\cdot}05$; 130 and 250 vs 430 μM, $P < 0{\cdot}05$). On the other hand, in control experiments, captopril deoxyribose or ferritin alone, as well as captopril plus deoxyribose and captopril plus ferritin, gave virtually undetectable TBA-reactants. Conversely, ferritin plus deoxyribose resulted in some TBA-reactant generation, apparently as a consequence of the reaction occurring during the acidic heating phase of the test; this procedure conceivably induces ferritin denaturation and oxidizing-iron mobilization (Monteiro et al 1991). Accordingly, in the absence of captopril, ferritin addition to deoxyribose immediately before heating with thiobarbituric and trichloroacetic acids gave reactant values similar to those observed after incubating deoxyribose with ferritin for 30 min at 37°C (data not shown). The present data show that once released from ferritin as a consequence of captopril action, iron becomes free and readily available to trigger oxidant damage. It must be stressed that this phenomenon was not inhibited by the antioxidant properties of captopril, thus suggesting that, in the presence of ferritin, the drug may effectively act as a pro-oxidant in-vivo.

It is intriguing to reconcile our findings, showing a ferritin iron-related pro-oxidant capacity of captopril, with literature data concerning drug antioxidant properties. However, it is possible that the antioxidant effects of

captopril observed in cell systems, such as in the heart, may be indirect and related to enhanced captopril-mediated prostacyclin production (Swartz et al 1980; Werns & Lucchesi 1989). Recent studies have shown that captopril affords protection towards free radical-induced endothelial cell injury and reperfusion arrhythmias (which are radical-mediated (Kloner et al 1989)) specifically through prostacyclin-dependent mechanisms (van Gilst et al 1986; Liao & Chen 1992). In this regard, there is evidence that prostacyclin reduces tissue free-radical generation (Takeuchi et al 1992) and the overflow of catecholamines (which are recognized radical-generating molecules (Halliwell & Gutteridge 1989)) in the ischaemic myocardium (van Gilst et al 1986). Prostacyclin also antagonizes white blood cell oxidant generation (Simpson et al 1987), which might further result in decreased oxidative stress in-vivo. In some experimental models, this indirect antioxidant capacity of captopril could be prevalent over both its ferritin-related pro-oxidant properties and direct radical-scavenging effects (these latter, however, are very unlikely in man, because of the low therapeutic concentrations of captopril (Cody 1990)). On the other hand, although caution is needed before extrapolating our findings to the in-vivo setting, it could be hypothesized that some side-effects of captopril, especially on kidney function, may be a consequence of its specific pro-oxidant properties; indeed free radicals and oxidative stress have been emphasized in the pathophysiology of glomerular dysfunction and kidney diseases (Iwasaki 1990). In any case, our study shows that captopril cannot be regarded simply as an antioxidant drug, but that it readily acts as a pro-oxidant in the presence of ferritin; this aspect should be taken into account in future experimental and clinical investigations.

Acknowledgements

Thanks are due to Dr Franca Daniele for her assistance in preparing the manuscript.

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