Captopril has no significant scavenging antioxidant activity in human plasma in vitro or in vivo

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1 Captopril has been reported to possess hydroxyl radical (OH·) and hypochlorous acid (HOCl) scavenging effects, which could contribute to its therapeutic activity in the clinical setting.

2 The objective of the present study was to determine whether therapeutically achievable captopril concentrations could augment antioxidant properties of human plasma and protect it against OH·- and HOCl-driven oxidant injury in vitro. Possible drug influences on systemic oxidative stress status in vivo were also investigated in subjects taking 50 mg captopril orally by measuring plasma and red blood cell peroxidation, as well as plasma protein thiols.

3 The results show that captopril is incapable of enhancing antioxidant properties of human plasma, of protecting it against specific oxidative attack and of decreasing systemic oxidant load in vivo.

4 The present data, therefore, do not support the contention of a beneficial action of captopril through systemic antiradical-antioxidant effects in human beings.

Keywords captopril, antioxidant, oxidative stress, hydroxyl radical, hypochlorous acid

Introduction

Experimental investigations have suggested that captopril may exert some beneficial effects in the clinical setting as a result of its thiol group-related antioxidant properties [1–3]. In this regard, captopril has been shown to scavenge efficiently only hydroxyl radical (OH·) and hypochlorous acid (HOCl) [3]. It must be noted, however, that high captopril concentrations and aqueous buffers in the absence of biomolecules present in the blood environment in vivo have usually been used to study captopril antioxidant effects in vitro. These biomolecules, such as albumin, can readily react with OH· and HOCl [4, 5]. Thus, it seems relevant for an appropriate evaluation of captopril antioxidant activity to investigate whether the drug is capable of enhancing antioxidant properties of human plasma, as well as of protecting endogenous biomolecules against oxidant aggression and of decreasing systemic oxidant burden in humans. These issues have specifically been addressed in the present paper.

Methods

Captopril added to human plasma

Reagents were from Sigma Aldrich s.r.l., Milano, Italy. Heparinized plasma was obtained from seven male healthy adults (35–45 years old).

We at first investigated whether captopril, added to human plasma at therapeutically relevant concentrations, could augment antioxidant properties of plasma itself against OH· and HOCl-induced oxidant injury. Regarding OH·, it is known that this oxygen radical is also generated via Fenton chemistry reactions in the presence of EDTA-complexed iron, H2O2 and ascorbate as the reducing agent [3–7]. Once produced, OH· can damage oxidatively the sugar deoxyribose (DOR), an integral component of DNA [4, 5], producing aldehydic substances capable of reacting with thiobarbituric acid (TBA) under acidic conditions [3–7]. Biological or pharmacological compounds able to scavenge efficiently OH· can counteract OH·-dependent DOR oxidation and TBA-reactants (TBA-R) formation [3–7]. Reaction mixtures (1.0 ml) contained 10 mmol l−1 potassium
phosphate buffer, pH 7.4, 2.8 mmol l\(^{-1}\) DOR, 10 \(\mu\)mol l\(^{-1}\) Fe(III) (pre-mixed with 50 \(\mu\)mol l\(^{-1}\) EDTA), 0.7 mmol l\(^{-1}\) \(\text{H}_2\text{O}_2\), and 50 \(\mu\)mol l\(^{-1}\) ascorbic acid, with and without 50 \(\mu\)l of plasma containing or not 10, 50 and 100 \(\mu\)mol l\(^{-1}\) captopril (final drug concentrations were therefore 0.5, 2.5 and 5 \(\mu\)mol l\(^{-1}\)). After 30 min incubation at 37\(^\circ\)C, 10 \(\mu\)l l\(^{-1}\) of 25% trichloroacetic acid (TCA), 1.0 ml l\(^{-1}\) of 0.6% TBA aqueous solution and 10 \(\mu\)l of 5% butylated hydroxytoluene (BHT) in ethanol were added, followed by 15 min heating at 95\(^\circ\)C. After cooling, the red chromogen, yield of OH\(^\cdot\)-induced DOR oxidation [3–7], was extracted with n-butanol and read spectrophotometrically at 532 nm against an appropriate blank. Results were calculated as nmol TBA-R/\(\mu\)mol DOR, using a molar extinction coefficient of 154,000.

Antioxidant effects of control and captopril-containing plasma samples against HOCI were evaluated testing their capability to inhibit HOCI-induced 5-thio-2-nitrobenzoic acid (TNB) oxidation [8]. In this context, it is known that the highest oxidant capacity of HOCI is explicated against sulphhydril groups [5, 8, 9], the thiol compound TNB, therefore, is readily oxidized by HOCI, this phenomenon being inhibitable by HOCI scavengers [8]. TNB was prepared through reduction of 5, 5-dithiobis(2-nitrobenzoic acid) (DTNB) with 2-mercaptoethanol [8], and its concentrations were calculated using a molar extinction coefficient of 13,600 at 412 nm [8]. Reaction mixtures (1.0 ml) contained 10 mmol l\(^{-1}\) potassium phosphate buffer, pH 7.4, 80 \(\mu\)mol l\(^{-1}\) TNB, 50 \(\mu\)l of plasma with or without captopril and 33 \(\mu\)mol l\(^{-1}\) HOCI. After 15 min incubation at 25\(^\circ\)C, absorbance values at 412 \(\mu\)m were read spectrophotometrically, using appropriate plasma- and captopril-containing blanks. The concentrations of NaOCl-derived HOCI were calculated by the TNB oxidation method of Thomas et al. [8], or using a molar extinction coefficient of 100 at 235 nm [4, 7].

**Captopril present in the blood after drug administration in humans**

In a second set of experiments, we investigated whether captopril, once present in the blood environment after drug administration in humans, could lower systemic oxidant load in vivo and protect plasma against OH\(^\cdot\)- driven peroxidation and HOCI-induced oxidation of protein thiols (P-SH). Five healthy subjects (31–43 years old) of our institutional medical and technical staff were studied twice at a 48 h interval. In the first day, after plasma and erythrocyte collection for specific biochemical tests in basal conditions, placebo tablets were administered orally in the morning in fasting conditions and, after 1 h, blood was collected again to repeat the assays. In the second day, the same experimental approach was followed, except that 50 mg captopril tablets (Capoten, Bristol-Myers Squibb S.p.A., Roma, Italy) were administered by mouth; after 1 h, plasma free captopril levels were also measured essentially as previously reported [10]. In this regard, it must be noted that plasma peak concentrations of free captopril are reached after 1.0 h from drug intake in humans, followed by their rapid and marked decrement [10, 11].

Systemic oxidative stress status before and after captopril intake was evaluated assessing erythrocyte and plasma peroxidation, as well as plasma P-SH levels. Erythrocyte peroxidation was assayed basically as reported by Clot et al. [12], after reaction of 1.0 ml l\(^{-1}\) of the protein-free supernatant resulting from 20\% TCA-treated erythrocytes with 1.0 ml l\(^{-1}\) of 0.6% TBA aqueous solution. After 30 min heating in the presence of 15 \(\mu\)l of 5% BHT in ethanol, the chromogen was extracted with n-butanol and read at 532 nm against an appropriate blank. Notably, this assay gives results strictly related to those of malondialdehyde measurement by a direct h.p.l.c. method [12], and it has been pointed out as a valuable index of oxidative stress in vivo [12]. Plasma lipid peroxidation was studied through the assay of TBA-R, fluorescent damage products of lipid peroxidation (FDPL) and conjugated dienes (CD). For TBA-R, 0.5 ml l\(^{-1}\) aliquots of plasma were added to 0.5 ml l\(^{-1}\) of 2.5% TCA, 20 \(\mu\)l of 5% BHT in ethanol, 0.1 ml l\(^{-1}\) of 8.1% sodium dodecyl sulphate and 0.5 ml l\(^{-1}\) of 0.6% TBA aqueous solution, followed by 30 min heating at 95\(^\circ\)C. The chromogen was then extracted with n-butanol and read at 532 nm against an appropriate blank. FDPL were assessed according to Dillard & Tappel [13], with some modifications. Plasma lipids were extracted with 6.0 ml l\(^{-1}\) of chloroform/methanol (2:1, vol/vol), followed by 2 min vortex mixing and bi-distilled water addition. After a brief centrifugation, the chloroform phase was dried under a flow of argon gas. The residue was resuspended in cyclohexane and subjected to fluorometric study at 360/430 nm excitation/emission, as well as to ultraviolet spectrophotometric study at 233 nm to assay CD [14]. Plasma P-SH represent adequate indicators of oxidative damage in vivo [4, 5, 15, 16], since P-SH are oxidized and consumed by oxidant species [4, 5, 15, 16]. P-SH were measured spectrophotometrically at 412 nm reacting 50 \(\mu\)l of plasma with 0.25 mmol l\(^{-1}\) DTNB (Ellman’s reagent) in 0.2 mol l\(^{-1}\) potassium phosphate buffer, pH 8.5, plus 2.0 mmol l\(^{-1}\) EDTA [16]. Oxidant-stimulated plasma peroxidation was induced using a copper/H\(_2\text{O}_2\)/OH\(^\cdot\) generating system [4, 5], since copper is more active than iron at triggering peroxidation of plasma lipids [17]. The prooxidant system contained 80 mmol l\(^{-1}\) CuCl\(_2\) and 0.55 mmol l\(^{-1}\) H\(_2\text{O}_2\), with incubation for 180 min at 37\(^\circ\)C. TBA-R, FDPL and CD were then measured, as described above. Regarding HOCI-mediated plasma oxidant damage, 105 \(\mu\)mol l\(^{-1}\) HOCI were added to plasma samples obtained before and after drug intake, followed by 30 min incubation at 25\(^\circ\)C. Since HOCI oxidizes preferentially thiol groups [5, 8, 9], the loss of plasma P-SH induced by HOCI was measured spectrophotometrically at 412 nm as described above.

**Statistics**

Data were calculated as means±s.d. The effects of captopril added to plasma were evaluated by the

Results

As shown in Table 1, captopril added to human plasma was incapable of enhancing antioxidant properties of plasma itself against either OH⁻ or HOCl.

Plasma drug concentrations were 1.54 ± 0.38 μmol l⁻¹ after 1.0 h from 50 mg captopril tablet intake. At this time, systolic and diastolic blood pressure values fell from 127 ± 5 and 81 ± 5 mmHg to 113 ± 4.5 and 69 ± 7.2 mmHg, respectively (both P<0.001, paired Student’s t-test). Since the biochemical data observed in the first ‘placebo day’ were similar to those of the second ‘drug day’, and neither placebo nor captopril affected baseline biochemical data, we will report only the results obtained with captopril administration. Notably, the levels of plasma peroxidation were not significantly different before and after captopril intake (0.85 ± 0.1 vs 0.87 ± 0.09 nmol TBA-R ml⁻¹ of plasma for TBA-R, 24 ± 3.1 vs 24.5 ± 3.5 units of relative fluorescence ml⁻¹ of plasma for FDPL, and 1.65 ± 0.2 vs 1.7 ± 0.25 absorbance units at 233 nm ml⁻¹ of plasma for CD), and neither were those of erythrocyte peroxidation (0.56 ± 0.13 vs 0.58 ± 0.12 nmol TBA-R ml⁻¹ of packed cells) and of plasma P-SH (432.5 ± 27 vs 428 ± 25.5 nmol ml⁻¹ of plasma). Captopril intake, therefore, did not result in decreased systemic oxidative burden in vivo at drug peak concentrations. Table 2 shows that the copper/H₂O₂ oxidant system induced a significant increase of plasma TBA-R (P<0.001), FDPL (P<0.001) and CD (P<0.025) with respect to the basal values; comparable lipid peroxidation levels, however, were observed in the pre- and post-captopril period plasma samples (P=NS, Table 2). When plasma was challenged with HOCl, its P-SH content underwent a significant decrement (P<0.001), which was about −17 and −18% in the plasma obtained before and after captopril intake, respectively (P=NS, Table 2).

Discussion

The present study shows that captopril, at therapeutic concentrations, does not enhance antioxidant properties of human plasma nor lower systemic oxidant load in vivo or protect endogenous biomolecules against oxidative damage. A possible concern potentially arising from our data may be related to the physiological relevance of the oxidants OH⁻ and HOCl used and of their concentrations. It could be indeed speculated that some captopril antioxidant effects might have been seen with milder degrees of oxidant attack. The aforementioned oxidants have been selected in light of the unambiguous experimental evidence that captopril can react with and scavenge only OH⁻ and HOCl [3], which are the most toxic and relevant oxidizing species [4, 5]. It should be noted that the concentrations of HOCl used in the study appear effectively ‘physiologically’ relevant, considering that activated neutrophils can generate even more than 100 μmol l⁻¹ HOCl [19]. Regarding OH⁻, instead, its in vitro levels are to date unknown. In our first set of experiments, however, the concentration of OH⁻-generating prooxidants was half that usually used in vitro to assess OH⁻ scavenging capacity of various drugs [3–7], while in the second set oxidant concentrations were the lowest possible to induce a clearly detectable plasma peroxidation. On the other hand, in the second set of experiments putative antioxidant effects of captopril in humans were evaluated also in the absence of any ‘artificial’ oxidative challenge. In fact, in this specific approach plasma and erythrocyte oxidant load was really physiological, because it was simply related to the intrinsic radical generation, as opposed to the endogenous antioxidants, of human beings before and after captopril intake. In any event, basic conceptual aspects do not point to an effective scavenging antioxidant activity of captopril in the blood environment in vivo, whatever could be the degree of oxidant load.
operative in various conditions. Blood contains indeed strong antioxidant defences, resulting in a high anti-
oxidant capacity [4, 5]. For example, albumin reaches mean concentrations of about 650 μmol l\(^{-1}\) in human plasma, whereas peak plasma levels of captopril are 0.5–1.5 μmol l\(^{-1}\) in humans after administration of usual drug doses of 25–50 mg [10, 11]. Moreover, albumin readily reacts with HOCl [4, 5, 9], and is characterized by a second order rate constant for the reaction with OH\(^-\) of 2.3 × 10\(^7\) mol\(^{-1}\) s\(^{-1}\) [5], which is near seven-
fold higher than that of captopril, i.e. 3.6 × 10\(^6\) mol\(^{-1}\) s\(^{-1}\) [3]. Thus, OH\(^-\) and HOCl react with 650 μmol l\(^{-1}\) albumin and not with 0.5–1.5 μmol l\(^{-1}\) captopril (this was exactly the case in our second experimental set). Similar considerations may be done for other physiologi-
cal plasma antioxidants, such as glucose, ascorbate and uric acid, which, though so diluted, had higherstrong antioxidant defences, resulting in a high anti-
oxidant capacity, besides a mere problem of antioxidantOH.

Table 2 Effects of 50 mg captopril intake on oxidant-induced plasma peroxidation and P-SH oxidation

<table>
<thead>
<tr>
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<th>Pre-captopril</th>
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<th>Post-captopril</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Cu(^{2+})/HOCl</td>
<td>Basal</td>
<td>Cu(^{2+})/HOCl</td>
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<tr>
<td>TBA-R</td>
<td>0.85 ± 0.1</td>
<td>1.29 ± 0.1</td>
<td>0.87 ± 0.1</td>
<td>1.32 ± 0.1</td>
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<tr>
<td>FDPL</td>
<td>24 ± 3.1</td>
<td>33.5 ± 0.3</td>
<td>24.5 ± 3.5</td>
<td>34.7 ± 4.1</td>
</tr>
<tr>
<td>CD</td>
<td>1.65 ± 0.2</td>
<td>2.15 ± 0.3</td>
<td>1.7 ± 0.4</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>P-SH</td>
<td>432.5 ± 27</td>
<td>358 ± 20.7</td>
<td>428 ± 25.5</td>
<td>351.8 ± 23</td>
</tr>
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</table>

Plasma and erythrocyte thiobarbituric acid reactants (TBA-R) are expressed as nmol TBA-R ml\(^{-1}\) of plasma and mmol TBA-R ml\(^{-1}\) of packed cells, respectively. Plasma fluorescent damage products of lipid peroxidation (FDPL) and conjugated dienes (CD) are given as units of relative fluorescence mg\(^{-1}\) of plasma and absorbance units at 233 nm ml\(^{-1}\) of plasma, respectively. Plasma P-SH are expressed as nmol P-SH ml\(^{-1}\) of plasma. See Methods section for further explanations. Means ± s.d. of five experiments performed on different plasma samples of five subjects before and 1.0 h after the intake of 50 mg captopril tablets (*P < 0.025, and †P < 0.01 vs Basal (unpaired Student’s t-test); ‡P = NS vs the respective values of the pre-captopril period (paired Student’s t-test).

Thus, for concentration and kinetic problems, captopril is not an effective scavengingantioxidant in the blood environment.

Experimental studies may suggest that captopril could result in some ‘indirect-type’ antioxidant effects in vivo, potentially due to decreased oxidant generation by neutrophils [22] and enhanced production of prostacy-
clin [23], which has antioxidant properties [24]. However, our data show that indicators of systemic oxidative stress, such as plasma lipoperoxidation and P-SH, as well as oxidant-driven plasma peroxidation and P-SH oxidation, are unaffected by captopril intake at peak drug concentrations, pointing to no significant antioxidant effect of captopril in vivo. Moreover, captop-
ril intake does not affect erythrocyte peroxidation, suggesting that also cell systems may not benefit from a putative drug antioxidant activity in vivo. In this context, it has been shown that the concentrations of free captopril remain virtually unchanged after drug incubation with erythrocytes [25], indicating that theoxidant species reacted preferentially with the endogen-
ous antioxidants, which, although so diluted, had higher concentrations than captopril itself. Moreover, plasma is endowed with several antioxidant defences, which specifically work in a synergic way to cope with oxidant injury. For example, transferrin binds-inactivates cata-
ytic iron, while albumin scavenges OH\(^-\) arising from iron-mediated reactions [4, 5]. This synergism of action appears relevant and amplifies the endogenous anti-
oxidant capacity, besides a mere problem of antioxidant concentration. Captopril is characterized not only by very low therapeutic levels [10, 11], but is also a poor thiol donor [21], which does not contribute to plasma antioxidant properties. Thus, for concentration and kinetic problems, captopril is not an effective scavenging antioxidant in the blood environment.
Captopril-a potential free radical scavenger: inhibition of in vivo drug antioxidant activity in humans. In this regard, Sobotka et al. [29] reported that captopril, at mean doses of about 50 mg day$^{-1}$, reduced the output of pentane (a lipid peroxidation index) in patients with congestive heart failure, apparently showing drug antioxidant effects in vivo. These authors, however, have measured breath pentane via a gas chromatographic technique, using a Chromosorb column and a flame ionization detector [29]. This method has been shown to be subjected to poor reliable [30, 31]. Even if further studies may be advisable to investigate whether captopril, alone or in association with other drugs usually used in patients with congestive heart failure, could improve various oxidative stress indices in this clinical setting, our data indicate that systemic antiradical-antioxidant effects of captopril are not feasible in humans.

References


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