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Captopril: a free radical scavenger

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The use of captopril in heart failure and hypertension is becoming increasingly accepted. Captopril has a sulphydryl group in its molecular structure. We wondered if this might confer free radical scavenging activity on the drug and have investigated this in an *in vitro* system. Results show that captopril is a free radical scavenger and we suggest that this action might be relevant in its use in heart failure and other vascular diseases.

Keywords captopril free radical angiotensin converting enzyme inhibitor

Introduction

Cardiac failure is defined as a clinical state resulting from the inability of the heart to provide sufficient blood for tissue metabolic needs (Breckenridge, 1988). The clinical syndrome has long been recognised but its management remains one of the major problems in clinical cardiology. Angiotensin converting enzyme (ACE) inhibitors are used routinely in the management of severe cardiac failure and it has been supposed that their beneficial effects are related to a decrease in angiotensin II. ACE inhibitors also have several other possible modes of action, for example, inhibiting the inactivation of bradykinin (Breckenridge, 1988).

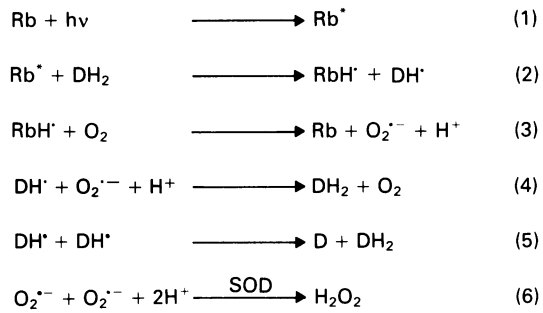
In animal models cardiac failure can be induced by pretreatment with the cytotoxic agent adriamycin (Gervasi *et al.*, 1986). It is thought that part of the mechanism responsible for the development of cardiac failure is damage to the heart by free radical (FR) generation. Free radicals are highly reactive species and can induce a wide variety of tissue damage, for example, they can inactivate enzymes, inhibit DNA synthesis and most importantly damage cell membranes by attacking the membrane proteins involved in the transport of ions and by inducing oxidation of membrane polyunsaturated fatty acids (Simpson & Lucchesi, 1987). The most widely recognised enzymatic defences against free radical reactions in the body are the enzymes superoxide dismutase and catalase (Shlafer *et al.*, 1982). However, it is becoming increasingly obvious that other scavengers such as glutathione and vitamins E and C play important roles (Ortolani *et al.*, 1987). Indeed, glutathione in addition to scavenging radicals reduces disulphides.

Captopril is an ACE inhibitor which is a derivative of the amino-acid proline. It contains a sulphydryl group and we considered that it was likely to be a free radical scavenger. This would be a further action of this drug which may be relevant to its use in heart failure.

The aim of this study was to investigate the scavenging activity of captopril *in vitro*.

Method

The technique that was used to assess the scavenging ability of captopril was that described by Misra & Fridovich (1977). In this assay free radicals are generated by photo-oxidation of dianisidine sensitised by riboflavin. The photo-oxidation of dianisidine involves a complex series of free radical chain reactions involving superoxide ion ($O_2^{\cdot -}$) as the propagating species (Figure 1). A general free radical scavenging compound has an inhibitory effect on this reaction leading to a decrease in the oxidised dianisidine measurable by a u.v./visible spectrophotometer. In contrast any compound which specifically scavenges $O_2^{\cdot -}$ will remove the $O_2^{\cdot -}$ from steps 3 and 4, thus increasing the amount of oxidised dianisidine and hence will have an augmentary effect on the assay. This assay can thus be used to determine whether a compound is a general free radical scavenger or a scavenger specific for $O_2^{\cdot -}$. A substance with no free radical scavenging activity will have no



Rb – riboflavin
hv – energy of photon of light
Rb^* – excited riboflavin
DH_2 – o-dianisidine
$O_2^{\cdot -}$ – superoxide anion
D – product formed by photo-oxidation measured at 460 nm.

Figure 1 Photo-oxidation of *o*-dianisidine.

effect on the assay. This assay has been validated in other studies (McNeil *et al.*, 1981; Chopra *et al.*, 1988).

Materials

Riboflavin and *o*-dianisidine were purchased from Sigma Chemicals Ltd. Riboflavin solution (1.3×10^{-5} M) was prepared in 0.01 M potassium phosphate buffer pH 7.5 and *o*-dianisidine solution (10^{-2} M) was prepared in ethanol. Captopril (Squibb & Sons, Inc.) was dissolved in ethanol.

Illumination for the photochemical reaction was provided by a pair of parallel 10 watt white fluorescent tubes mounted 6 inches apart in an aluminium foil-lined open ended box. These tubes provide a constant source of wide-band radiation.

Assay procedure

The procedure for the assay was as follows: 60 μl of ethanol was added to 0.88 ml of riboflavin solution. Thereafter 60 μl of *o*-dianisidine solution was added. Absorbance of light was measured at 460 nm using a Pye Unicam PU 8600 u.v./visible spectrophotometer. The cuvette was then transferred to the illumination box, illuminated for 4 min and the absorbance was remeasured at 460 nm. The change in absorbance at 460 nm after 4 min illumination of this ethanol control served as the control reading and was referred to as zero percent inhibition on the assay.

60 μl of captopril solution was then added to 0.88 ml of riboflavin solution followed by 60 μl of *o*-dianisidine and the measurements were carried out as above. Measurements were repeated on eight occasions at different final concentrations of the drug, 0.5, 1.0, 2.5, 5.0 and 15.0 μg ml^{-1} (i.e. 2.28×10^{-6} M, 4.5×10^{-6} M, 1.14×10^{-5} M, $2.2. \times 10^{-5}$ M, and 6.9×10^{-5} M) Percentage inhibition on the assay was calculated at each concentration. The intra-assay variation for this assay is 4.3%.

Analysis of the data was carried out using non-parametric statistics (Mann-Whitney U-test) comparing the control samples with those containing captopril.

Results

Figure 2 shows the percentage inhibition of the assay by increasing doses of captopril. The higher the concentration of captopril the greater the inhibition (all $P < 0.001$, Mann-Whitney).

Discussion

In this study we have shown that captopril has free radical scavenging activity *in vitro*. Glutathione, a well recognised scavenger (McNeil *et al.*, 1981) obtains its activity from the presence of a sulphydryl group. Captopril also has a sulphydryl group in its molecule and this may also be the reason for its activity in this experiment. By no means all captopril is bound to ACE. Much of it is carried as adsorbed

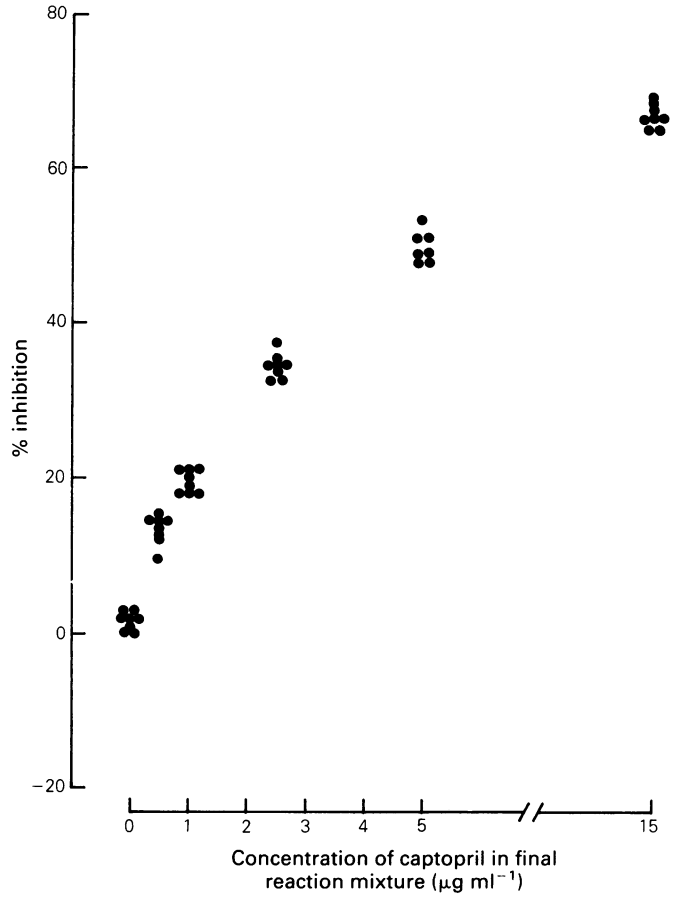


Figure 2 Percentage inhibition on the assay by captopril.

captopril or as disulphides with endogenous sulphydryl-containing compounds such as glutathione and plasma proteins (Heel *et al.*, 1980). These disulphides can serve as depot forms of the drug. The drug can therefore function as recyclable antioxidant agent.

Free radicals are known to be prothrombotic. They can do this directly by inducing oxidation of polyunsaturated fatty acids (Laghi Pasini *et al.*, 1984), and by damaging endothelium (McCord, 1985). They can also cause thrombosis indirectly by interacting with the arachidonic acid cascade leading to a selective increase in the vasoconstrictor platelet aggregant thromboxane A_2 (Gutteridge, 1976). We have already shown that free radical pathology exists in ischaemic heart disease and peripheral vascular disease (Belch *et al.*, 1988). There is much evidence for free radical mediated damage in hypoxic reperfusion injury (McCord, 1985; Simpson & Lucchesi, 1987; Belch *et al.*, 1988), and this may be relevant to the role of captopril as a free radical scavenger. Other workers have noted abnormalities in diabetic microangiopathy (Jennings *et al.*, 1987). Hommel *et al.* (1986) have shown that captopril reduces albuminuria in diabetics who do not have hypertension. If the kidney microvascular disease of diabetes is augmented by free radicals, then captopril may produce its beneficial response by its action as a free radical scavenger.

In conclusion we have delineated a further mechanism of action of captopril, that is as a free radical scavenger and studies are currently underway to examine its action *ex vivo*.

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