Influence of ACE inhibitors on free radicals and reperfusion injury: pharmacological curiosity or therapeutic hope?

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- 1 The currently available evidence shows that thiol containing ACE inhibitors are free radical (FR) scavengers *in vitro*; in particular the OH[•] radical is effectively scavenged by these compounds. There is also good evidence that, *in vivo*, ACE inhibitors can preserve myocardial contractile function following a period of reversible ischaemia (by directly protecting myocytes and/or preserving coronary flow through protection of endothelial cells). These *in vivo* benefits are probably also due to FR scavenging, mainly due to the presence of a thiol group but, also as a consequence of augmented prostanoid production.
- 2 Use of more relevant animal models and testing of a range of doses of ACE inhibitors might be undertaken before clinical investigation is considered. Because of its nature, however, the existence and importance of reperfusion injury in man will only be proven or disproven by pharmacological intervention. One option is to compare a thiol containing agent with absent or minimal haemodynamic effects with placebo as an adjunct to thrombolysis. This is the simplest approach. An alternative approach is to conduct a comparative study of a –SH containing ACE inhibitor and a non –SH containing ACE inhibitor given prior to thrombolysis. There are many problems with either approach. The lack of reliable measures of FR activity in man and difficulty in measuring accurately left ventricular function post-myocardial infarction means that mortality is likely to be the only reliable end-point in such a study. As the mortality rates in recent thrombolysis trials have been relatively low, large numbers of patients would probably need to be recruited for such a study to have the power to differentiate reliably between drugs (a power calculation is difficult, in any case, as the magnitude of any benefit of anti-FR intervention is uncertain).
- 3 Therefore, although the available evidence suggests that the anti-FR effect of –SH ACE inhibitors is more than just a pharmacological curiosity, the challenge of proving therapeutic benefit in a clinical trial is a daunting one.

Keywords ACE inhibitors free radicals reperfusion injury

Introduction

Preservation of functioning cardiac muscle at the time of, and following, myocardial infarction (MI) is a major therapeutic goal. β -adrenoceptor antagonists, nitrates and thrombolytic agents are of established benefit in this respect and the focus of attention has recently shifted to angiotensin converting enzyme (ACE) inhibitors. Following encouraging experimental studies, there is now clinical evidence that these agents, given in the subacute or convalescent phase, favourably influence ventricular structure and function post-MI (Pfeffer *et al.*, 1988; Sharpe *et al.*, 1988). Major trials are now underway,

or planned, to determine whether the potential clinical benefits (reduced incidence of heart failure and death) of these effects can be realised.

It is also possible that ACE inhibitors may be of benefit in the *acute* phase of MI. The apparently salutary effects of ACE inhibitors on myocardial infarct size, coronary blood flow, systemic haemodynamics and neuroendocrine activation in experimental models have all been reviewed in detail (Ambrosioni & Borghi, 1989; Dargie & Ray, 1989).

More recently, with the widespread use of thrombo-

lytic therapy, another potential role for ACE inhibitors, that of limiting 'reperfusion injury', has also been suggested (Westlin & Mullane, 1988). The aim of this review is to assess whether there is sufficient evidence to justify clinical evaluation of ACE inhibitors, in this respect, as an adjunct to reperfusion therapy.

Reperfusion injury and free radicals

To talk about 'reperfusion injury' may seem paradoxical when faced with ever increasing evidence of benefit from thrombolytic therapy. Reperfusion obviously prevents the cell necrosis that would undoubtedly occur without reflow. However, there is evidence that cell injury or death, distinct from any cellular damage induced by the preceding period of ischaemia, may be induced by reperfusion itself (Bolli *et al.*, 1989; Kloner *et al.*, 1989). Therefore treatments that prevent the latter damage might permit greater myocardial recovery than seen with reperfusion therapy alone.

What causes reperfusion injury?

There are believed to be several types of experimental reperfusion injury and these have been the subject of recent reviews elsewhere (Editorial, 1989; Kloner *et al.*, 1989a,b; Opie, 1989). There are probably multiple mechanisms involved in reperfusion injury and the relative importance of these is almost certainly different for each of the adverse manifestations of reflow. Reversible contractile dysfunction ('stunning') is the best characterised, experimentally, of these injuries and there is persuasive evidence that free radicals (FR) are involved in its pathogenesis (Bolli *et al.*, 1989; Kloner *et al.*, 1989). The FR involved appear to be generated within the first few minutes of reperfusion, probably in the extracellular fluid or within the vascular wall (Bolli *et al.*, 1989). A

human counterpart of stunning has recently been described (Kloner *et al.*, 1989; Patel *et al.*, 1988).

What are free radicals?

Free radicals (FR) are short lived cytotoxic chemicals produced by a number of normal and abnormal cellular metabolic and enzymatic pathways; FR are also generated by activated neutrophils (Dormandy, 1989; Southorn & Powis, 1988). The sequence of ischaemia (by priming tissues) followed by reperfusion (by re-admitting oxygen) appears to particularly favour excessive (oxygen derived) FR production while also depressing cellular anti-FR ('antioxidant') defences. Recent experimental studies have suggested that certain ACE inhibitors can remove or 'scavenge' FR. What is the nature of this evidence and is it merely a pharmacological curiosity or the basis for therapeutic hope?

ACE inhibitors and FR scavenging

In vitro evidence

As with the major intracellular FR scavenger glutathione, the presence of a sulphhydryl or thiol ($-SH$) group in the structure of a compound confers FR scavenging properties upon it (Southern & Powis, 1988). Consequently the prototype $-SH$ containing ACE inhibitor, captopril, has been examined for FR scavenging activity.

Superoxide anion-radical Westlin & Mullane (1988) reported that captopril was a superoxide anion-radical ($O_2^{\cdot-}$) scavenger. Using the purine-xanthine oxidase method, these authors found that captopril, but not teprotide or enalaprilat, caused a dose dependent decrease in the amount of $O_2^{\cdot-}$ detected spectrophotometrically by reduction of ferricytochrome C (the IC_{50} was ≈ 0.25 mM). Bagchi *et al.* (1989b) also reported that $O_2^{\cdot-}$, generated through the reaction of hypoxanthine with xanthine oxidase, and detected by ferricytochrome C, was scavenged by captopril with an IC_{50} of 1 mM. These findings are difficult to interpret for several reasons. Firstly, captopril, like other thiols, may itself reduce ferricytochrome C (Aruoma *et al.*, 1989; Bolli *et al.*, 1989; Kukreja *et al.*, 1990; Mehta *et al.*, 1990). Secondly, captopril may interact with certain purines used in the xanthine oxidase reaction (Egan *et al.*, 1988). Thirdly, if captopril does scavenge $O_2^{\cdot-}$ then the $-SH$ group should no longer be measurable in the reduced form; Egan *et al.* (1988) were unable to show any loss of reduced $-SH$ when captopril (up to 5×10^{-4} M) was exposed to $O_2^{\cdot-}$ *in vitro*. We found that captopril (and other $-SH$ containing ACE inhibitors) preferentially interacts with radicals other than $O_2^{\cdot-}$ (Chopra *et al.*, 1989, 1990; Figure 1). Similarly, Pi & Chen (1989) found that captopril (up to 360 μ M) did not scavenge $O_2^{\cdot-}$ produced by buffer electrolysis. Kukreja *et al.* (1990) and Mehta *et al.* (1990) were also unable to show scavenging by captopril (up to 100 μ M) of $O_2^{\cdot-}$ generated by xanthine plus xanthine oxidase (detected by ferricytochrome C) or auto-oxidation of dihydrofumarate (detected by reduction of nitro-blue tetrazolium). The former workers also demonstrated

Figure 1 Free radical (FR) scavenging at drug concentration 4×10^{-5} M by $-SH$ ACE inhibitors (captopril, epicaptopril, zofenopril), non $-SH$ ACE inhibitors (enalaprilat, quinaprilat, perindoprilat) and the endogenous $-SH$ compound glutathione. FR scavenging is expressed as % inhibition of an assay generating FR through photo-oxidation of dianisidine sensitised by riboflavin (see Chopra *et al.*, 1989).

that captopril can inhibit the auto-oxidation of adrenaline (epinephrine) to adrenochrome at both pH 7.8 and 10.2. At pH 7.8 adrenaline auto-oxidation is $O_2^{\cdot-}$ independent and superoxide dismutase insensitive; at pH 10.2 adrenaline auto-oxidation is $O_2^{\cdot-}$ dependent and inhibited by SOD. Rather than specifically scavenging $O_2^{\cdot-}$ captopril may, therefore, simply act as a non-specific antioxidant, reducing one or more intermediates in the oxidation of adrenaline to adrenochrome (Westlin & Mullane (1988) also used this reaction to suggest that captopril could scavenge $O_2^{\cdot-}$).

In summary, the currently available evidence suggests that captopril does not scavenge $O_2^{\cdot-}$ except, perhaps, at very high (mM) concentrations. This is in keeping with evidence that other thiol compounds scavenge $O_2^{\cdot-}$ with a very low rate constant, if at all (Aruoma *et al.*, 1989; Bolli *et al.*, 1989). Further work using other biochemical detectors or EPR-spin trapping would be helpful in clarifying the effect of ACE inhibitors on $O_2^{\cdot-}$.

Other radicals

The hydroxyl radical ($OH^{\cdot}$) The hydroxyl radical ($OH^{\cdot}$) is believed to be the most toxic oxygen derived FR and is a likely culprit in reperfusion injury. Bagchi *et al.* (1989a) have examined the ability of captopril to scavenge $OH^{\cdot}$. The standard reduction mixture hypoxanthine/xanthine oxidase/ferric chloride was used to generate $OH^{\cdot}$ and the radical was detected by high performance liquid chromatography (h.p.l.c.) measurement of the hydroxylate derivative of salicylic acid. Captopril decreased production of this metabolite with an IC_{50} of 25 μM , an effect equipotent to that of the known $OH^{\cdot}$ scavengers allopurinol and dimethylthiourea. Using similar protocols, the same group also employed h.p.l.c. to detect 5,5-dimethylpyrroline-*N*-oxide (DMPO) – $OH^{\cdot}$ adducts and found these to be significantly reduced in the presence of captopril; the IC_{50} was 20 μM (Bagchi *et al.*, 1989a). Non –SH ACE inhibitor controls were not studied. In a preliminary report, Mak *et al.* (1990) have also shown that captopril can scavenge $OH^{\cdot}$. This group used the technique of spin trapping followed by electron paramagnetic resonance (EPR) detection to measure DMPO – $OH^{\cdot}$ adducts formed after $OH^{\cdot}$ generation with dihydrofumarate and ferric ions. Captopril and zofenopril, at concentrations of 10 and 50 μM , reduced DMPO – $OH^{\cdot}$ formation by 20% and 50% respectively; similar scavenging was reported with an alternative $OH^{\cdot}$ generating system (hydrogen peroxide and ferrous ions). Neither enalaprilat nor lisinopril (non –SH ACE inhibitors) scavenged $OH^{\cdot}$.

Other organic radicals We have also found that captopril, unlike enalaprilat, can scavenge other FR in a dose dependent fashion (Chopra *et al.*, 1989; Figure 1). Other –SH compounds and –SH containing ACE inhibitors exhibit similar activity whereas non –SH ACE inhibitors are inert, like enalaprilat (McMurray *et al.*, 1990).

In summary, it appears that, in contradistinction to the case with $O_2^{\cdot-}$, –SH containing ACE inhibitors can effectively scavenge other FR *in vitro* at drug concentrations in the low micromolar range.

It should be emphasised that most of these reports have concerned only chemical reactions *in vitro*.

Polymorphonuclear leucocyte activity

Neutrophils (PMN), as a source of FR and other oxidants, may have a role to play in myocardial injury. Westlin & Mullane (1988) originally reported that captopril scavenged $O_2^{\cdot-}$ produced by isolated PMN stimulated by 4 β -phorbol-12-myristate-13-acetate (PMA) at an IC_{50} of 15 μM . Unfortunately, the $O_2^{\cdot-}$ detector system used was ferricytochrome C which means that any results must be interpreted with caution, as alluded to earlier. Kukreja *et al.* (1990), aware of these limitations, were unable to reproduce the findings of Westlin & Mullane (1988). Bagchi *et al.* (1989a,b) used a different detector system, chemiluminescence, which probably measures production of a variety of radical and other toxic metabolites of oxygen. These authors found that captopril 25 μM inhibited the chemiluminescent response to PMN stimulated by *N*-formyl-L-methionyl-L-leucyl-L-phenyl-alanine (FMLP). Captopril was more potent in this respect than the standard antioxidants allopurinol, superoxide dismutase (SOD) and catalase alone, or in combination; non –SH ACE inhibitor controls were not tested. These findings were attributed to $O_2^{\cdot-}$ scavenging by captopril.

Egan *et al.* (1988), however, found that captopril dose dependently inhibited NADPH oxidase of PMN with an IC_{50} of 3×10^{-4} M. This membrane based enzyme system oxidises NADPH to $NADPH^+$, reducing O_2 to $O_2^{\cdot-}$ in the process. These observations obviously offer an alternative explanation for the findings of Bagchi *et al.* (1989b). Again, non –SH control drugs were not tested, but this enzyme system is known to be inhibited by thiols (Cross, 1990).

While these results seem encouraging it must be remembered that the existing literature suggests that other –SH agents actually enhance neutrophil adhesiveness, chemotaxis, phagocytosis and degranulation (Mistos *et al.* 1986; Patrone *et al.*, 1981). It should also be noted that in all the studies cited above isolated PMN were studied. Important cell-cell interactions determine PMN activity *in vivo* and the effect of a drug on isolated cells will not necessarily be the same in the intact organism. *In vivo*, ACE inhibition *per se*, as opposed to possession of a –SH group, may also influence white cell behaviour (Farber *et al.*, 1990).

Finally, it would also be of interest to know if ACE inhibitors influence the activity of myeloperoxidase, an enzyme that enables the PMN to produce the powerful oxidant HOCl (which may act as a precursor of the $OC\dot{C}l^{\cdot}$ radical) [see below].

Other reactive oxygen species

Hypochlorous acid (HOCl) Bagchi *et al.* (1989b) have also examined the effect of captopril on chemiluminescent detection of $HOCl/OC\dot{C}l^{\cdot}$ produced by adding weak acid to NaOCl. Captopril appeared to scavenge $HOCl/OC\dot{C}l^{\cdot}$ with an IC_{50} of 25 μM . It is not clear from this report whether the primary effect of captopril is on HOCl rather than the $OC\dot{C}l^{\cdot}$ radical, though we have found that captopril does scavenge HOCl (unpublished observations). Non –SH controls

have not been used though other thiol containing drugs such as *N*-acetylcysteine (NAC) and mercaptopropionylglycine (MPG) are also effective scavengers of HOCl at concentrations between 10 and 100 μM (Aruoma *et al.*, 1989; Cuperus *et al.*, 1985).

Hydrogen peroxide (H_2O_2) This is an important biological oxidant, in its own right, and a potential precursor of $\text{OH}^\cdot$. We have recently found that captopril can scavenge H_2O_2 (unpublished observations).

In vivo experiments

Does the *in vitro* evidence that $-\text{SH}$ containing ACE inhibitors scavenge FR translate into myocardial protection *in vivo*?

As discussed above reversible myocardial mechanical dysfunction, or 'stunning', is probably, at least in part, due to FR injury. If certain ACE inhibitors effectively remove FR then their administration should result in decreased biochemical evidence of FR activity and decreased contractile disturbance.

Whole animal studies Westlin & Mullane (1988) first showed that this might be the case. Captopril 5 mg kg^{-1} (apparently administered by systemic intravenous bolus injection), given 15 min before coronary artery occlusion in dogs, led to a significantly greater (to 65% of pre-occlusion value) recovery of contractility in the ischaemic segment following reperfusion than did placebo (10% of pre-occlusion value).

Enalaprilat (1.6 mg kg^{-1}) resulted in a significantly worse recovery of function than placebo (-30% of pre-occlusion value). More importantly, captopril 5 mg kg^{-1} i.v., 2 min prior to reperfusion, also led to a greater recovery of contractility. SQ14 534, an isomer of captopril with one hundredth of its ACE inhibiting activity, at a dose of 5 mg kg^{-1} i.v., was equally efficacious in restoring mechanical function when given according to the same protocol.

Przyklenk & Kloner (1989) and Przyklenk *et al.* (1989) have also shown that zofenopril (SQ26703) 5 mg kg^{-1} , by i.v. bolus injection 1 min prior to reperfusion, results in improved recovery of contractility in the canine model.

These findings are clearly encouraging but do leave certain questions unresolved. Westlin & Mullane (1988) found that there were no significant differences in systemic haemodynamics between captopril and the non $-\text{SH}$ ACE inhibitors they investigated; no haemodynamic measurements were reported for SQ14534. Similarly, Przyklenk & Kloner (1989) and Przyklenk *et al.* (1989) did not report the effect of zofenopril on haemodynamic parameters. Such measurements for each agent are clearly important as improvement in the haemodynamic determinants of myocardial oxygen demand might improve myocardial function as suggested by the beneficial effects of other vasodilators given post-reperfusion (Przyklenk & Kloner, 1987); however, the similar haemodynamic effects of captopril and enalapril do suggest that a non-haemodynamic mechanism accounts for the beneficial effects of $-\text{SH}$ compounds.

Equally important is assessment of area of myocardium made ischaemic; this was reported to be similar for each drug group in the study of Westlin & Mullane (1988) but was not commented upon in the preliminary report of Przyklenk & Kloner (1989) and Przyklenk *et al.* (1989). A third haemodynamic measure of some importance is coronary blood flow. It has been suggested that $-\text{SH}$ ACE inhibitors may increase coronary blood flow more than non $-\text{SH}$ agents in this class (Van Gilst *et al.*, 1988). A differential effect of this type could account for the above findings. While these areas require clarification before specific benefit can be attributed to $-\text{SH}$ containing ACE inhibitors it is worth noting that other thiols can preserve myocardial contractility in comparable protocols and this benefit cannot be attributed to any systemic or coronary haemodynamic effect (Bolli *et al.*, 1987, 1989). Similarly, while the cited studies of captopril and zofenopril do not contain either indirect (e.g. from biochemical markers such as malondialdehyde) or direct (e.g. from spin trapping studies) evidence of an anti-free radical effect of these drugs, a recent report shows convincingly that the protective effect of another thiol, MPG, in the canine model, is associated with effective scavenging of FR and that penetration of myocytes is probably not essential for this action (Bolli *et al.*, 1989).

Studies in isolated hearts

Other authors have used less clinically relevant models to examine the antioxidant effect of ACE inhibitors. Pi & Chen (1989) used a rat isolated working heart model. Free radicals were generated by electrolysis of the perfusate leading to biochemical damage (increased myocardial thiobarbituric acid reactive substances/decreased myocardial SOD), decreased coronary flow and mechanical dysfunction; the culprit radical in this model appears to be $\text{OH}^\cdot$. Captopril afforded dose dependent protection from all three types of injury; 90 $\mu\text{mol l}^{-1}$ was the minimal effective concentration. Captopril was more effective than dimethylsulphoxide (DMSO), a known $\text{OH}^\cdot$ scavenger. Another $-\text{SH}$ agent, S8, offered the same degree of protection as captopril. Importantly, however, Pi & Chen (1989) showed that hearts removed from rats given ramipril (a non $-\text{SH}$ ACE inhibitor) 1 mg kg^{-1} intraperitoneally 1 h before sacrifice, and then perfused with ramiprilat 12.5 $\mu\text{mol l}^{-1}$, also exhibited less biochemical and functional injury. The protective effect of ramipril, however, was abolished by co-infusion of indomethacin; by contrast that of captopril was only partially attenuated. Iloprost, 30 n mol l^{-1} , a stable synthetic analogue of epoprostenol (PGI_2) and *in vitro* FR scavenger, also showed significant protective effects in this model. These experiments are noteworthy for several reasons. Firstly biochemical markers of FR activity/damage were made simultaneously with functional assessments. Interventions that improved contractility also lessened the biochemical injury strengthening the case that increased FR activity is the cause of mechanical dysfunction. Secondly, however, the restoration of mechanical function was also associated with greater coronary flow in these experiments. It is not clear, therefore, whether FR

activity is the direct cause of contractile dysfunction or whether contractile dysfunction is a consequence of a primary effect of FR on the coronary vasculature. Thirdly, these experiments also suggest that *in vivo*, as opposed to *in vitro*, ACE inhibitors may exhibit anti-FR activity through stimulation of prostaglandin production and not merely as a consequence of possessing a -SH group. There are however important caveats to this last conclusion. Ramipril pre-treatment was given prior to removal of the heart and its exposure to increased FR activity. Furthermore ACE inhibitors do not necessarily share the same ability to stimulate prostaglandin production; nevertheless the same group has also shown that pretreatment with enalapril ameliorates ischaemia-reperfusion induced myocardial dysfunction in subsequently rat isolated hearts (Li & Chen, 1987).

Bagchi *et al.* (1989a) also used the rat isolated heart to study the effect of captopril on FR activity. Langendorff preparations were perfused for 15 min, in the presence or absence of 50 μM captopril, before 60 min of ischaemia was induced; the heart was then reperfused for a further 60 minutes. It should be noted that the duration of ischaemia in this protocol is sufficient to induce myocardial necrosis and that captopril was given prior to ischaemia. DMPO was included in the perfusate and DMPO -OH $\cdot$ adducts, detected by h.p.l.c., were increased by ischaemia and further following reperfusion. Captopril inhibited DMPO adduct formation during all stages of the protocol. Compared with placebo, captopril also significantly reduced malondialdehyde and arachidonic acid levels, whereas it augmented 6-keto-prostaglandin $\text{F}_{1\alpha}$ and thromboxane B_2 (TxB_2) (breakdown products of arachidonic acid) concentrations. These observations also show that captopril has significant effects on arachidonic acid metabolism/prostaglandin production which could, in part, account for inhibition of FR production or FR scavenging by captopril. Indices of contractility were not measured in this study though coronary blood flow was reported to be augmented by captopril (numerical values not given).

Non -SH ACE inhibitors may also exert antioxidant effects *in vivo*, as opposed to *in vitro*, in ways other than augmenting prostaglandin synthesis e.g. by binding transition metals.

Studies in isolated cells/tissues

Mak *et al.* (1990) have examined the effect of a number of ACE inhibitors on FR mediated damage to cultured endothelial cells. These workers found that the OH $\cdot$ generating system, dihydrofumarate plus ferric ions, caused an increase in lipid peroxidation (malondialdehyde [MDA] production) and cell death (trypan blue exclusion). Preincubation with the -SH ACE inhibitors captopril and zofenopril caused dose dependent (10–200 μM) inhibition (15–60%) of MDA formation. Loss of cell viability was also prevented (IC_{50} 25 μM . DMPO-OH $\cdot$ adduct formation was inhibited by these drugs (see above). Enalaprilat and lisinopril were ineffective. Whether endothelial cells are likely to be the site of any postulated FR attack *in vivo* is uncertain and no similar study has been conducted with other cell types such as myocytes (Buderus *et al.*, 1989).

Outstanding problems

Is free radical scavenging really the mechanism of the beneficial action of -SH ACE inhibitors?

Ischaemia-reperfusion generates FR and scavenging of these FR is associated with evidence of biochemical and mechanical protection. This does not, however, prove that FR are causally and directly involved in reperfusion injury. It remains a possibility, however remote, that FR generation is an epiphenomenon and FR scavenging a secondary and irrelevant action of these drugs in preventing reperfusion injury. It is conceivable, for example, that -SH agents improve contractility by restoring β -adrenoceptor function or act by restoring key cellular thiols to a more reduced state (Ceconi *et al.*, 1990; Haenen *et al.*, 1989).

Problems with the drugs

The specific benefit, if any, of ACE inhibitors in the setting of ischaemia/reperfusion seems to depend mainly on the presence of a -SH group in the drug molecule and, to a lesser extent, on augmented prostanoid production; the neuroendocrine and haemodynamic effects of these drugs may also be helpful in this situation. While these multiple actions may, theoretically, make ACE inhibitors a particularly attractive therapeutic option they make experimental design and assessment difficult. For example, the use of a simple thiol, such as NAC, with little if any haemodynamic effect, would be preferable, from an experimental point of view, in studying the effect of an anti-FR intervention in clinical ischaemia/reperfusion.

Problems with dose

The *in vitro* studies and experiments in isolated hearts, cited above, suggest that the protective concentration of captopril is somewhere between 20 and 100 μM . Both these types of study are, however, open to the criticism of having employed protocols likely to generate very high levels of FR, possibly much higher than would be obtained during reperfusion *in vivo*. This dilemma is, however, not resolved by the evidence from studies in whole animals. Both captopril and zofenopril were given in high dose (5 mg kg^{-1}) and drug concentrations were not measured in blood or tissue. Similar work with other thiols is, however, of interest. Bolli *et al.* (1989) obtained mechanical protection with MPG in a similar canine model at a calculated concentration of approximately 152 μM in the coronary blood (Bolli *et al.*, 1989). This group also showed comparable protection with dimethylthiourea at concentrations of 4–9 mM, obtained prior to ischaemia (Bolli *et al.*, 1987). More recently Qui *et al.* (1990) showed that contractility in isolated working rat hearts could be preserved with 8 μM N-acetylcysteine (NAC) in the perfusate; NAC was, however, added prior to ischaemia. Similarly, Ceconi *et al.* (1990) have shown that the -SH agent 1,2-dimercaptopropanol at a concentration of 1 $\mu\text{mol l}^{-1}$, given prior to ischaemia, preserved contractile function of isolated rabbit hearts. This enormous variation in protective concentrations, while *in vitro* most thiol compounds exhibit similar FR

scavenging potency, suggests that dose response studies are badly needed to help interpret the possible therapeutic relevance of FR scavenging in ischaemia/reperfusion. Low micromolar concentrations are at the upper end of the therapeutic range. Much larger concentrations are a potential problem. Though high plasma levels might only be needed for a relatively short period for anti-FR effect (Bolli *et al.*, 1989), they could result in two types of complication. Firstly, high concentrations are likely to increase the duration of any significant hypotension that resulted from inhibition of angiotensin converting enzyme. The second potential problem is of thiol toxicity. Though this may occur with chronic dosing it is less likely to be important where high concentrations are only required in the short term (a loading dose of 150 mg kg⁻¹ of NAC is routinely given over 15 min for paracetamol poisoning and thiol toxicity is not a significant problem).

Problems with the model

The canine model of ischaemia followed by reperfusion bears little resemblance to the clinical situation of successful coronary thrombolysis. In the former a snare is tightened to occlude a normal coronary artery. A brief period (usually 15 min) of ischaemia follows which is insufficient to cause myocardial necrosis. Rapid reperfusion is then induced by loosening the coronary snare. The animals are anaesthetised and are often treated with anti-arrhythmic drugs. In the clinical case multivessel coronary disease and variable collateralisation are frequently present. Myocardial necrosis has occurred. Reperfusion, if it occurs, may be intermittent and grad-

ual; subsequent coronary run-off may be very poor due to significant residual stenosis.

In the animal model the FR generation related to stunning occurs as a discrete burst within the first few minutes of reperfusion and it is difficult to extrapolate such a finding to the usual circumstances in man (Bolli *et al.*, 1989). It is therefore unfortunate that no more clinically relevant model has been used to study ACE inhibitors. Forman *et al.* (1988) have, however, taken steps in this direction using the thiol agent NAC. These workers employed a canine model where coronary artery occlusion was prolonged (1 h instead of 15 min) to ensure myocardial necrosis. NAC (150 mg kg⁻¹ loading, 50 mg kg⁻¹ h⁻¹ maintenance) was given after 30 min of ischaemia but before reperfusion. After one hour the coronary snare was gradually released and 30,000 units of intracoronary streptokinase given. At one hour and three hours post reperfusion contractile function within the 'at risk zone' was significantly better in the animals that received NAC; coronary flow was not different from the placebo group. Infarct size was similar in the two treatment groups. This study is therefore important in suggesting that a substantial myocardial functional benefit can be obtained with a thiol compound in a model much more comparable to the clinical situation. Further evolution of animal models should be pursued since it is possible to experimentally occlude coronary arteries by inducing thrombosis and to re-open these in a more gradual fashion with thrombolytic agents.

Dr McMurray is supported by the British Heart Foundation. We are grateful to Dr H. J. Dargie, Dr G. McInnes and Professor W. E. Smith for their advice.

References

- Ambrosioni, E. & Borghi, C. (1989). Potential use of ACE inhibitors after acute myocardial infarction. *J. cardiovasc. Pharmac.*, **14** (Suppl. 9); 592-594.
- Aruoma, O. I., Halliwell, B., Hoey, B. M. & Butler, J. (1989). The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypochlorous acid. *Free Rad. Biol. Med.* (1989a), **6**, 593-597.
- Bagchi, D., Iyengar, J., Stockwell, P. & Das, D. K. (1989a). Enhanced prostaglandin production in the ischemic reperfused myocardium by captopril linked with its free radical scavenging action. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **38**, 145-150.
- Bagchi, D., Prasad, R. & Das, D. K. (1989b). Direct scavenging of free radicals by captopril, an angiotensin converting enzyme inhibitor. *Biochem. Biophys. Res. Comm.*, **158**, 52-57.
- Bolli, R., Jeroudi, M. O., Patel, B. S., Aruoma, O. I., Halliwell, B., Lai, E. K., McCay, P. B. (1989). Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. *Circulation*, **65**, 607-622.
- Bolli, R., Xi Xhu, W., Hartley, C. J., Michael, L. H., Repine, J. E., Hess, M. L., Kukreja, R. C. & Roberts, R. (1987). Attenuation of dysfunction in the post-ischemic 'stunned' myocardium by dimethylthiourea. *Circulation*, **76**, 458-468.
- Buderus, S., Siegmund, B., Spahr, R., Krutzfeldt, A. & Piper, H. M. (1989). Resistance of endothelial cells to anoxia-reoxygenation in isolated guinea pig hearts. *Am. J. Physiol.*, **257**, H488-493.
- Ceconi, C., Curello, S., Cargnoni, A., Boffa, G. M. & Ferrari, R. (1990). Antioxidant protection against damage during cardiac ischaemia and reperfusion: effect of dimercaptopropanol. *Cardioscience*, **1**, 191-198.
- Chopra, M., McMurray, J., Dargie, H. J. & Smith, W. E. (1990). Free radical scavenging: a potentially beneficial action of thiol-containing angiotensin converting enzyme inhibitors? *Biochem. Soc. Trans.*, **18**, 1184-1185.
- Chopra, M., Scott, N., McMurray, J., McLay, J., Bridges, A., Smith, W. E. & Belch, J. J. F. (1989). Captopril: a free radical scavenger. *Br. J. clin. Pharmac.*, **27**, 396-399.
- Cross, A. R. (1990). Inhibitors of the leukocyte superoxide generating oxidase: mechanisms of action and methods for their elucidation. *Free Rad. Biol. Med.*, **8**, 71-93.
- Cuperus, R. A., Muijsers, A. U. & Wever, R. (1985). Antiarthritic drugs containing thiol groups scavenge hypochlorite and inhibit its formation by myeloperoxidase from human leukocytes. *Arth. Rheum.*, **28**, 1228-1233.
- Dargie, H. J. & Ray, S. G. (1989). The effects of angiotensin converting enzyme inhibition on coronary blood flow and infarct size limitation. *J. human Hypertension*, **3** (Suppl. 1), 101-106.
- Dormandy, T. L. (1989). Free radical pathology and medicine. *J. Roy. Coll. Phys. (Lond.)*, **23**, 221-227.
- Editorial (1989). Reperfusion injury after thrombolytic therapy for acute myocardial infarction. *Lancet*, **ii**, 655-658.
- Egan, T. M., Minta, J. O., Scrimgeour, K. G. & Cooper, J. D. (1988). Captopril — a potential free radical scavenger: in-

- hibition of PMN NADPH oxidase. *Clin. invest. Med.*, **11**, 351–356.
- Farber, H. W., Center, D. M., Rounds, S. & Danilov, S. M. (1990). Components of the angiotensin system cause release of a neutrophil chemo attractant from cultured bovine and human endothelial cells. *Eur. Heart J.*, **11** (Suppl. B), 1–4.
- Forman, M. B., Puett, D. W., Cates, C. U., McCroskey, D. E., Beckman, J. K., Greene, H. L. & Virmani, R. (1988). Glutathione redox pathway and reperfusion injury: effect of n-acetylcysteine on infarct size and ventricular function. *Circulation*, **78**, 202–213.
- Haenen, G. R. N. M., Plug, H. J. M., Vermeulen, N. P. E., Timmerman, H. & Bast, A. (1989). Contribution of 4-hydroxy-2,3-trans-nonenal to the reduction of β -adrenoceptor function in the heart by oxidative stress. *Life Sci.*, **45**, 71–76.
- Kloner, R. A., Przyklenk, K. & Patel, B. (1989a). Altered myocardial states: The stunned and hibernating myocardium. *Am. J. Med.*, **86**, (Suppl 1A): 14–22.
- Kloner, R. A., Przyklenk, K. & Whittaker, P. (1989b). Deleterious effects of oxygen radicals in ischaemia/reperfusion: resolved and unresolved issues. *Circulation*, **80**, 1115–1127.
- Kukreja, R. C., Kontos, H. A. & Hess, M. L. (1990). Captopril and enalaprilat do not scavenge the superoxide anion. *Am. J. Cardiol.*, **65**, 241–271.
- Li, K. & Chen, X. (1987). Protective effects of captopril and enalapril on myocardial ischaemia and reperfusion damage of rat. *J. mol. cell. Cardiol.*, **19**, 909–915.
- McMurray, J., Chopra, M., Smith, W. E. & Dargie, H. J. (1990). Free radical scavenging: a potentially beneficial action of captopril. *Eur. Heart J.*, **11** (Suppl.), P287.
- Mak, I. T., Freedman, A. M., Dickens, B. F. & Weglicki, W. B. (1990). Protective effect of sulphydryl containing angiotensin converting enzyme inhibitors against free radical injury in endothelial cells. *Biochem. Pharmacol.*, **40**, 2169–2175.
- Mehta, J. L., Nicolini, F. A. & Lawson, D. L. (1990). Sulphydryl group in angiotensin converting enzyme inhibitors and superoxide radical formation. *J. cardiovasc. Pharmacol.*, **16**, 847–849.
- Mistos, S. E., Fantone, J. C., Gallagher, K. P., Walden, K. M., Simpson, P. J., Abrams, G. D., Schork, M. A. & Lucchesi, B. R. (1986). Canine myocardial reperfusion injury: protection by a free radical scavenger, N-2-mercaptopropionyl glycine. *J. cardiovasc. Pharmacol.*, **8**, 978–988.
- Opie, L. H. (1989). Reperfusion injury and its pharmacological modification. *Circulation*, **80**, 1049–1062.
- Patel, B., Kloner, R. A., Przyklenk, K. & Braunwald, E. (1988). Post ischemic myocardial 'stunning': A clinically relevant phenomenon. *Ann. int. Med.*, **108**, 626–628.
- Patrone, F., Dallegri, F., Maggi, A. M., Rapetto, C. & Lanzi, G. (1981). Stimulation of neutrophil chemotaxis, adhesiveness, phagocytosis and hexose monophosphate shunt activity by n-(2-mercapt-propionyl) glycine. *Res. exp. Med. (Berl.)*, **178**, 257–262.
- Pfeffer, M. A., Gerfussio, A. L., Vaughan, D. E., Parisi, A. F. & Braunwald, E. (1988). Effect of captopril on progressive ventricular dilatation after anterior myocardial infarction. *New Engl. J. Med.*, **319**, 80–86.
- Pi, X. J. & Chen, X. (1989). Captopril and ramiprilat protect against free radical injury in isolated working hearts. *J. mol. cell. Cardiol.*, **21**, 1261–1271.
- Przyklenk, K. & Kloner, R. A. (1987). Acute effects of hydralazine and enalapril on contractile function of post-ischaemic 'stunned' myocardium. *Am. J. Cardiol.*, **60**, 934–936.
- Przyklenk, K. & Kloner, R. A. (1989). Relationships between structure and effects of ACE inhibitors: comparative effects in myocardial ischaemic/reperfusion injury. *Br. J. clin. Pharmacol.*, **28**, 1675–1755.
- Przyklenk, K., Whittaker, P. & Kloner, R. A. (1989). Zofenopril, a newly developed sulphydryl-containing converting enzyme inhibitor, enhances contractile function of 'stunned' myocardium. In *Current advances in ACE inhibition*, eds MacGregor, G. A. & Sever, P. S. pp 279–282. Edinburgh: Churchill Livingstone.
- Qui, Y., Bernier, M. & Hears, D. J. (1990). The influence of n-acetylcysteine on cardiac function and rhythm disorders during ischaemia and reperfusion. *Cardioscience*, **1**, 65–74.
- Sharpe, D. N., Murphy, J., Smith, H. & Hannan, S. (1988). Treatment of patients with symptomless left ventricular dysfunction after myocardial infarction. *Lancet*, **i**, 255–259.
- Southorn, P. A. & Powis, G. (1988). Free radicals in medicine. I. Chemical nature and biological reactions. II. *Mayo clin. Proc.*, **63**, 381–???
- Van Gilst, W. H., Scholtens, E., deGraeff, P. A., Langen, C. D. J. & Wesseling, H. (1988). Differential influences of angiotensin converting enzyme inhibitors on the coronary circulation. *Circulation*, **77** (Suppl. I), I-24–I-29.
- Westline, W. & Mullane, K. (1988). Does captopril attenuate reperfusion-induced myocardial dysfunction by scavenging free radicals? *Circulation*, **77** (Suppl. I) I-30–I-39.

(Received 12 September 1990,
accepted 31 October 1990)