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Effect of trimetazidine on membrane damage induced by oxygen free radicals in human red cells

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The effect of trimetazidine, 1–(2, 3, 4 trimethoxybenzyl)piperazine di-hydrochloride, on membrane damage induced by oxygen free radicals in red cells was studied in seven healthy volunteers after oral administration. Red cells collected prior to and after a 7 day treatment period with trimetazidine were incubated in the presence of phenazine methosulphate (an intracellular oxygen free radical generator) and diethyldithiocarbamate (a Cu-Zn superoxide dismutase inhibitor). The loss of intracellular K^+ induced by oxygen free radicals and the membrane content of peroxidated lipids were significantly reduced in red cells collected after the period of treatment. These results indicate a potent antioxidant activity of trimetazidine which could explain its cardioprotective role during ischaemic and reperfusion phases in which oxygen free radicals are generated and probably implicated in the genesis of cardiac cell injury.

Keywords antioxidant erythrocytes superoxide lipid peroxidation potassium

Introduction

Highly reactive molecules, called oxygen free radicals, are generated *in vivo* as by-products of normal metabolism.

During acute myocardium infarction and the subsequent reperfusion phase, an important generation of oxygen free radicals occurs and protective enzymes are overwhelmed (superoxide dismutase, catalase, glutathione peroxidase) (Rao *et al.*, 1981; Guarnieri *et al.*, 1980). This is followed by membrane lipid peroxidation (Guarnieri *et al.*, 1980) and loss of intracellular K^+ attributed to an increase in membrane permeability to K^+ ions (Vleugels & Carmeliet, 1976; Coraboeuf *et al.*, 1976).

Recently, we were able to reproduce these events in human red cells incubated with phenazine methosulphate (PMS) (Maridonneau *et al.*, 1983a) which produced superoxide anions ($O_2^{\cdot -}$) and other active oxygen species (Nishikimi *et al.*, 1972) capable of inducing lipid peroxidation. In PMS-treated erythrocytes, a loss of intracellular K^+ consecutive to the free radical generation was observed and enhanced by diethyldithiocarbamate (DDC, an inhibitor of the Cu-Zn superoxide dismutase). This K^+ loss is essentially due to an increase in passive K^+ permeability resulting from membrane lipid peroxidation (Maridonneau *et al.*, 1983b).

The present study was undertaken to determine the effect of an antianginal drug, trimetazidine (TMZ), 1–(2, 3, 4 trimethoxybenzyl)piperazine, dihydrochloride, on damage caused by free radicals in red cell membranes. We postulated that one of the possible mechanisms of action of this cardioprotective drug was by the scavenging of oxygen free radicals.

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Methods

Seven healthy donors, five men and two women in an age range from 23–35 years, were treated with consent for 7 days at therapeutic doses of TMZ (40 mg day^{-1} for a body weight less than 60 kg and 60 mg day^{-1} for a body weight greater than 60 kg). The plasma steady state level of this drug (40 mg day^{-1}) after 48 h is at the following concentration: 17.86 ± 5.83 ng ml^{-1} (about 5×10^{-8} M) (Royer *et al.*, 1983). These volunteers were not receiving any other treatment.

Venous blood collected in heparinized tubes, was centrifuged at 1750 *g* for 10 min at 4°C and the plasma and the buffy-coat were removed. Cells were then washed four times with cold iso-osmotic $MgCl_2$.

Passive K^+ permeability was measured as previously described by Cusi & Garay (1981). Briefly, cells were incubated in a magnesium sucrose medium containing 0.1 mM ouabain and 0.02 mM bumetanide, inhibitors of the Na^+, K^+ pump and the Na^+, K^+ co-transport system respectively. In these conditions, the residual K^+ efflux, measured in the supernatant by flame photometry represents the passive K^+ permeability.

Intracellular K^+ was measured after haemolysis of cells which were transfered to distilled water. Membranes were centrifuged and K^+ was measured in the supernatant by flame photometry.

The technique for measuring malondialdehyde (MDA) in human erythrocytes was published elsewhere (Maridonneau *et al.*, 1983a). Briefly, cells were incubated for 2 h at 37°C in a magnesium-sucrose medium in the absence and in the presence of 2 mM PMS + 1 mM DDC (final concentrations). Cells were then thoroughly washed and haemolysed. Membranes were incubated in presence of thiobarbituric acid (TBA) and the chromophoric product resulting from the reaction between MDA and TBA was measured at 532 nm.

In each experiment, superoxide dismutase was inhibited by preincubating cells for 30 min with 1mM DDC as previously described (Maridonneau *et al.*, 1983a). We ensured that DDC was not able to affect K^+ permeability and lipid peroxidation since PMS was not added to the cell suspension.

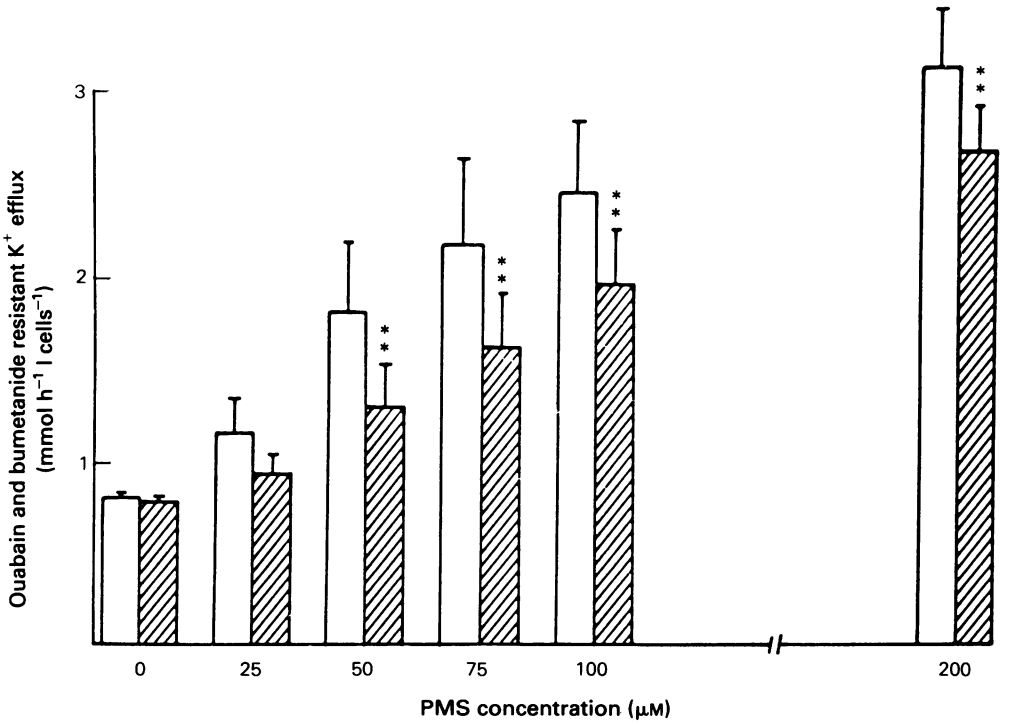

Figure 1 Effect of TMZ on the (PMS, DDC)-dependent increase in passive K^+ permeability: Ouabain and bumetanide resistant K^+ efflux stimulated by various PMS concentrations and 1 mM DDC was measured prior to (□) and after (▨) 1 week's treatment with TMZ in red cells from seven healthy subjects. Results are expressed as mean ± s.e. mean. Treatment means were compared to no-treatment means using a paired Student's *t*-test. ** $P < 0.05$.

Results

The effect of PMS + DDC was compared between control red cells and red cells collected from the same donors after a 7 day period of treatment with TMZ (TMZ-cells). In these conditions, TMZ-cells were less sensitive to oxygen free radical damage as shown by a significant decrease in the K^+ loss ($P < 0.05$) except for 25 μM PMS which poorly stimulated the K^+ efflux (Figure 1).

We excluded a possible membrane effect of TMZ treatment because the rate constants of K^+ permeability (PK)

$$PK = \frac{\text{passive } K^+ \text{ efflux (mmol } h^{-1} \text{ l cells}^{-1})}{\text{intracellular } K^+ \text{ concentration (mmol l cells}^{-1})}$$

were very similar in non-treated cells and TMZ-cells incubated without PMS and DDC: (mean ± s.d.) $9.23 \times 10^{-3} \pm 5 \times 10^{-4}$ h^{-1} and $9.24 \times 10^{-3} \pm 4 \times 10^{-4}$ h^{-1} respectively.

Nevertheless, we were not able to diminish the (PMS, DDC)-dependent K^+ loss when cells were treated *in vitro* by TMZ in a concentration range from 0.02 to 0.5 mM. In this range of concentrations, no change of the basal passive K^+ efflux was caused by TMZ further indicating that TMZ did not act directly on K^+ permeability.

Membrane content of MDA, a marker of lipid peroxidation, did not differ in red cells prior to and after treatment of volunteers with TMZ: (μmol × 1 $cells^{-1}$) 8.83 ± 3.93 and 9.15 ± 4.12 respectively. The MDA content, about four-fold enhanced in presence of PMS + DDC, was significantly decreased in TMZ-cells (Figure 2).

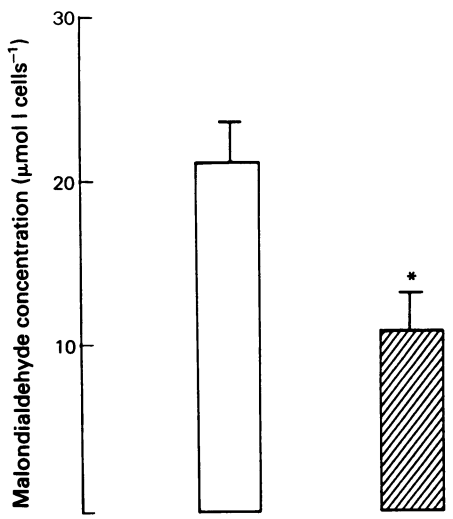

Figure 2 Effect of TMZ on the MDA content in (PMS, DDC)-treated red cells: red cells collected prior to (□) and after (▨) 1 week's treatment with TMZ from seven volunteers were incubated for 2 h at 37 °C with 2 mM PMS and 1 mM DDC. Membrane content in MDA was measured at 532 nm using a TBA assay. Values are expressed as mean ± s.e. mean. Treatment mean was compared to no-treatment mean using a paired Student's *t*-test. * $P < 0.001$.

Discussion

Recent studies have demonstrated the involvement of endogenously generated oxygen free radicals in the pathophysiology of ischaemia-induced tissue damage. It was possible to enhance the recovery of myocardial function with antioxidant drugs or enzymes able to scavenge free radicals during experimental heart ischaemia and reperfusion phase (Hess *et al.*, 1983; Schlafer *et al.*, 1982).

Thus, an oxygen free radical scavenger action of TMZ could partially explain the previously reported cardioprotective effect of this drug (Cahn *et al.*, 1982; Yanagisawa *et al.*, 1978). Indeed, two deleterious effects of oxygen free radicals, loss of intracellular K^+ and lipid peroxidation, were considerably decreased in red cells collected from volunteers treated by TMZ.

Nevertheless, this effect was not reproduced when TMZ was added *in vitro* to red cell suspensions indicating its inability, in these experimental conditions, to scavenge free radicals. This hypothesis was further suggested by the absence of an oxygen free radical scavenger effect of TMZ investigated in a cell-free reaction medium as described by Nishikimi *et al.* (1972) (data not shown).

These results suggest that TMZ becomes a potent antioxidant molecule when it is metabolized. Two metabolites of TMZ were identified in plasma after oral administration of [^{14}C]-TMZ to healthy volunteers (Oulsnam *et al.*, 1984). The effect of these metabolites in the red cell model has yet to be studied.

In conclusion, the administration of TMZ at therapeutic doses efficiently protects human red cells against free radical-induced membrane damage. This antioxidant activity presently reported in red cells could represent the mechanism of action of TMZ or its metabolites at the cardiac level and thus partially explain the cardioprotective effect of this drug observed during ischaemic heart disease (Cahn *et al.*, 1982; Yanagisawa *et al.*, 1978).

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