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Mercaptodextran – a new copper chelator and scavenger of oxygen radicals

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ABSTRACT The therapy of copper poisoning with mercaptodextran inhibits the copper–induced haemolysis, whereas 2,3– dimercaptopropanesulfonic acid (DMPS) may accelerate such haemolysis. Some aspects of the mechanisms of these effects were investigated. The possible generation of activated oxygen species during the interaction of Cu^{++} and chelating thiols was studied using a chemoluminiscent method detecting oxygen radicals. It was found that incubation of DMPS with copper ions or erythrocyte membranes was accompanied by generation of oxygen radicals. Mercaptodextran added to similar suspensions did not lead to oxygen radical production. And unlike DMPS, mercaptodextran acted as a scavenger of radicals generated by the xanthine oxidase/acetaldehyde system. The different ability of the chelating thiols to cope with free radicals may explain their different potentials to protect against copper–induced haemolysis. Our results also indicate that mercaptodextran may be a useful therapeutic agent in cases of haemolytic crisis in Wilson's disease.

KEY WORDS copper; sulfhydryl compounds; dextrans; chelating agents; superoxide; free radicals; luminescence; erythrocyte membrane

Despite their high affinity for heavy metals, the use of thiols for treatment of metal poisonings is limited because of their toxicity. The exact mechanisms of the toxic effects of thiols is not fully evaluated. There are evidences that some thiols are able to produce activated oxygen, especially superoxide radicals (O_2^-), during their autoxidation[1]. Superoxide may further participate in reactions in which more reactive oxygen species such as HO· are produced[2,3]. It has been demonstrated that these activated oxygen species may attack and damage almost all cell constituentes[2,3], and therefore it seems reasonable to speculate if the toxicity of thiols in part may be related to free radical production.

This side effect is particularly undesirable when treating poisoning with copper, since this metal itself may induce oxygen radical production. Recently a new thiol antidote, mercaptodextran (D–SH) possessing high affinity for heavy metals and low toxicity, has been synthesized[4]. It has been shown that mercaptodextran is particularly efficient in protecting against copper–induced haemolysis[5]. In the context of the above–mentioned proposal, it was of interest to investigate the ability of D–SH to produce activated oxygen species, also in the presence of copper ions, and to compare it with 2,3–dimercapto–propanesulfonic acid (DMPS), a widely used antidote of low molecular weight. The aim of the present work was to investgate the possibility for generation of activated oxygen at different conditions by these thiols, and also to study their posssible O_2^-–scavenging activity.

MATERIALS AND METHODS

Luminol (5–amino–2,3–dihydro–1,4–phtalazinedione) was obtained from Koch–Light Ltd, England. DMPS was purchased from Johnson & Johnson, USA. Xanthine

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oxidase (EC 1.2.3.2) was a product of Fluka AG, Switzerland. All other reagents were of the highest quality commercially available, and water was glass distilled. Superoxide dismutase (EC 1.15.1.1) 2800 U / mg, estimated according to Maral *et al*[6], was prepared from bovine erythrocytes by the method of McCord & Fridovich [7]. White erythrocyte membranes were prepared from human red blood cells by the method of Steck [8]. Superoxide was produced by the xanthine oxidase system using acetaldehyde as the substrate. The concentration of the enzyme was 1 mU / ml and that of acetaldehyde 1 mmol / L.

The production of activated oxygen was tested by registration of luminol-dependent chemiluminescence [9]. The sample cuvette contained 0.1 mmol / L luminol and the thiol compound to be tested in 2 ml of 5 mmol / L phosphate buffer, pH 7.4. In all experiments the concentrations of the thiols to be compared were equimolar with respect to SH-groups. At least 3 experiments with 3 –5 replicates were performed. The figures present typical chemiluminescence response of one measurement.

RESULTS

Metal-free aqueous solutions of D-SH or DMPS did not cause any detectable chemiluminescence (CL), indicating that the thiols tested did not produce activated oxygen at the conditions of our experiments Fig 1 shows the effect of adding $FeCl_3$ or $CuCl_2$ (1 – 50 μmol / L) to the solutions of D-SH or DMPS. Neither Fe^{3+} nor Cu^{2+} were able to induce any measurable chemiluminescence (CL) in the D-SH solution. Similar results were obtained for the effect of Fe^{3+} on the CL of DMPS (data not shown). However, the addition of $CuCl_2$ to the DMPS containing cuvette caused concentration-dependent changes of the CL. Increasing the $CuCl_2$ concentration from 1 to 10 μmol / L caused a progressive increase in the CL response. Upon further increase of the $CuCl_2$ concentration, above 10 μmol / L, a concetration-dependent inhibition of the CL was observed. Addition of superoxide dismutase (SOD) (10 U / ml) completely inhibited the Cu^{2+}-induced CL of DMPS. When the enzyme was inactivated by heating, the inhibitory effect of SOD was completely abolished. Our results indicate that the interaction of DMPS with Cu^{2+} may give rise to O_2^--generation. The decreased CL observed at $CuCl_2$ concentrations above 10 μmol / L may be explained from a O_2^--scavenging effect of appropriate concentration of copper.

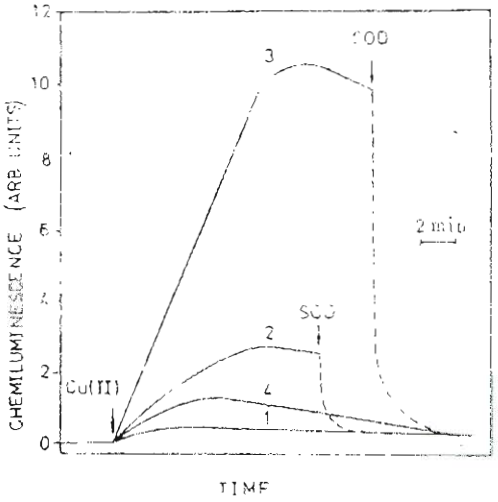

Fig 1. Luminol-dependent chemiluminescence induced by mercaptodextran (D-SH) or DMPS, in the presence of copper at 37°C. The sample cuvette contained 0.1 mmol / L of luminol in 2 ml of phosphate buffer, pH 7.4 with the following additions:

- D-SH and $CuCl_2$ 1 – 50 μmol / L – **curve 1**
- DMPS and $CuCl_2$ 1 μmol / L – **curve 2**
- DMPS and $CuCl_2$ 10 μmol / L – **curve 3**
- DMPS and $CuCl_2$ 20 μmol / L – **curve 4**

The concentration of D-SH and DMPS was 0.25 mmol / L with respect to SH groups and that of SOD was 10 U / ml.

The possible interaction of the thiol antidotes DMPS and D-SH with blood cells was tested in a model system containing isolated erythrocyte membranes. Addition of DMPS to

these membrane suspensions induced CL (Fig 2). The intensity of the CL response strongly depended on the DMPS concentration. Thus, increasing the DMPS concentration up to 0.5 mmol / L (Fig 2) increased the light emission, whereas higher concentrations tended to inhibit the CL response. At a DMPS concentration of 2.5 mmol / L the CL response was completely abolished. It is interesting to note that in the latter case the presence of 1 μmol / L $CuCl_2$ drastically increased the light emission. Addition of SOD inhibited the CL response, suggesting that O_2^- is somehow produced as a result of the DMPS–erythrocyte membrane interactions.

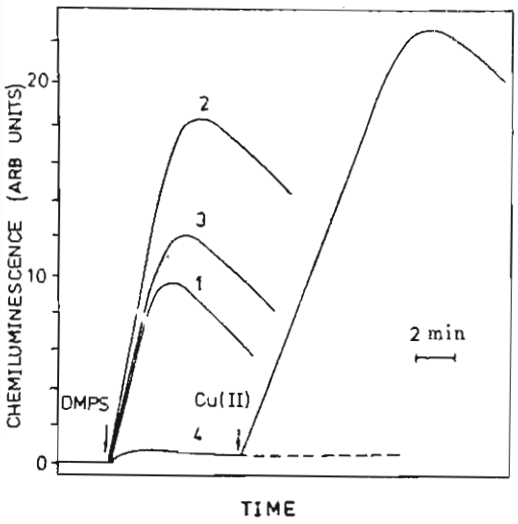

Fig 2. Luminol–dependent chemiluminescence as a result of thiol compound – erythrocyte membrane interaction. The sample cuvette contained luminol 0.1 mmol / L, and 10^8 erythrocyte membrane ml, and DMPS

– 0.25 mmol / L – **curve 1**
– 0.50 mmol / L – **curve 2**
– 1.00 mmol / L – **curve 3**
– 2.50 mmol / L – **curve 4**

in 2 ml of phosphate buffer, pH 7.4. The concentration of $CuCl_2$ was 1 μmol / L, temperature 37℃

D–SH in the presence of erythrocyte membranes did not induce any measurable CL (data not given). Furthemore, addition of $CuCl_2$ at different concentrations had no effect in this case. These results indicate that the D–SH–membrane interactions do not produce activated oxygen, even in the presence of Cu^{2+}.

As it has been demonstrated previously that the Cu^{2+} erythrocyte membrane interactions can produce O_2^-[10,11], it is tempting to propose that the lack of CL in presence of D–SH is associated with an ability of D–SH to scavenge O_2^-.

To test this proposal, a system in which O_2^- was produced enzymatically (xanthine oxidase / acetaldehyde) was used. The main results obtained in these experiments are presented in Fig 3. It was found that D–SH at concentrations higher than 0.25 mmol / L competely inhibited the CL in this case. In contrast, the native (not thiolated) dextran had no effect on the CL. This fact strongly suggests that the SH groups of D–SH are responsible

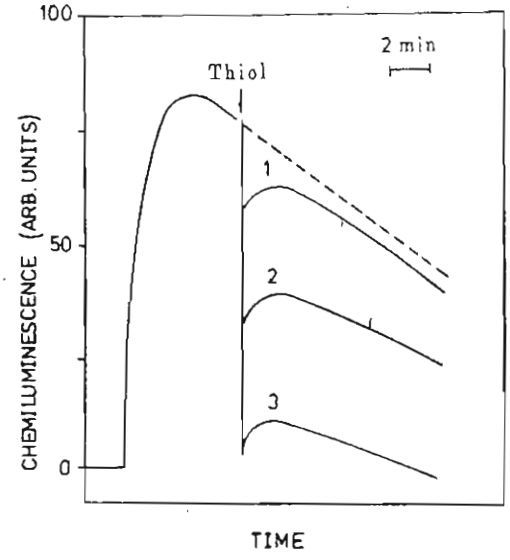

Fig 3. Inhibition of xanthine oxidase – acetaldehyde induced luminol–depedent chemiluminescence by DMPS and D–SH. The sample cuvette contained xanthine oxidase 1 mU / ml and acetaldehyde 1 mmol / L in phosphate buffer pH 7.4 with:

DMPS – 0.25 mmol / L – **curve 1**
DMPS – 0.50 mmol / L – **curve 2**
D–SH – 0.25 mmol / L – **curve 3**

for its scavenging activity towards O_2^-. But as the inhibitions on the CL might be due to direct interaction between D–SH and xanthine oxidase, leading to inactivition of the enzyme, an additional experiment was carried out, in which D–SH and xanthine oxidase were separated by a dialysis membrane using the two compartment cuvette described earlier[12]. Also in this case complete inhibition of the CL by D–SH was observed.

A reasonable interpretation of these results is that D–SH really acts as a strong O_2^-–scavenger.

When the possible O_2^-– scavenging activity of DMPS was tested, it was found that quenching of the CL could be achieved, but at much higher thiol concentrations than for D–SH. As seen from Fig 3 DMPS at concentrations 0.25 mmol / L only slightly decreased the xanthine oxidase / acetadehyde induced CL. This result indicate that the activity of DMPS to scavenge O_2^- is significantly lower than that of D–SH.

DISCUSSION

The results obtained in this study indicate that DMPS and D–SH differ considerably with respect to their ability to generate and scavenge activated oxygen. Thus, it was found that DMPS produced O_2^- by interacting with copper or erythrocyte membranes, while D–SH did not produce O_2^- under the same conditions. Furthermore, even at low concentrations D–SH completely inhibited the O_2^-–induced luminol–dependent CL, an effect that appears to be due to the ability of D–SH to scavenge O_2^-.

The mechanism of the DMPS–erythrocyte membrane interaction causing luminol–dependent CL is not clear. It appears likely that some of the erythrocyte membrane constituents are able to catalyse the oxidation of SH groups of DMPS with concomitant O_2^-–release. It has been clearly demonstrated that increased O_2^- production in biological systems may give rise to deleterious changes including lipid peroxidation[2]. Consequently, our finding that the DMPS–erythrocyte membrane interaction causes activated oxygen generation may provide a biochemical explanation of side effects of this chelator. The fact that the activated oxygen is generated in close proximity to the membranes may make it more harmful than radicals produced in the cytosol, for several reasons: Firstly, O_2^- is significantly more soluble in nonpolar than in polar environment, which may cause its accumulation in the lipid bilayer of the membrane. And secondly, O_2^- generated close to the membrane is expected to escape from enzymatic dismutation.

It has been suggested previously that the DMPS treatment involves risk of side effects owing to accidental oxygen radical production[13]. Thus, we observed that addition of DMPS in combination with $CuSO_4$ increased the hemolysis of human erythrocytes suspended *in vitro*, whereas D–SH as well as penicillamine and dimercaptosuccic acid protected the cells against copper–induced lysis[5,14]. It is consistent with these reports that the interaction of D–SH with membranes in the present paper did not lead to generation of activated oxygen, even in the presence of transition metal ions. A practical results from these studies is that D–SH[5] as well as dimercaptosuccinic acid[15] appear to be promising therapeutic alternatives to penicillamine in cases of copper–induced hemolytic crisis, eg, in Wilson's disease.

Another main conclusion to be drawn from the present data is that the differences of the toxicity of thiol antidotes may be associated, at least in part, with their ability to generate or scavenge free radicals; and the search for new antidotes should take into account their behaviour with respect to free radicals.

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Selective cytotoxicity against human tumor cells by an anti–gastric cancer monoclonal antibody–mitomycin C conjugate

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ABSTRACT An anti–gastric cancer monoclonal antibody, MGb_2, was chosen to prepare MGb_2–mitomycin C (MMC) conjugate. Four to five molecules of MMC were introduced into each molecule of antibody with the antibody activity well retained. The conjugate showed a highly selective cytotoxicity upon human gastric cancer cells KATO–Ⅲ. In the 48–h exposure test, the cytotoxic effect of MGb_2–MMC upon target cells was similar to that of free MMC, but much greater than that of normal mouse immunoglobulin–MMC conjugate. Instead, the MGb_2–MMC showed a statistically less cytotoxic effect upon non–target cells. Imaging and biodistribution studies indicated that the MGb_2 was still well localized in tumor tissue after its conjugation with MMC.

KEY WORDS monoclonal antibodies; mitomycins; immunologic cytotoxicity; cultured tumor cells; stomach neoplasms

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Mitomycin C (MMC), an antibiotic, has