Free Radical Research, Volume 37 Number 7 (July 2003), pp. 699–703

Taylor & Francis
healthsciences

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/26/11
For personal use only.

Inhibition of Photohemolysis Induced by *m*-Chloroperbenzoic Acid by Metal Complexes with SOD-mimetic Activity

ABDEL EL-AZIZ F. ABDEL EL-AZIZ[a] and IBRAHIM H. EL-SAYED[b,*]

[a]*Biochemistry Division, Chemistry Department, Faculty of Science, Mansoura University, Egypt;* [b]*Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute, Minofya University, Sadat City, Egypt*

Accepted by Professor M.A. Marletta

(Received 15 June 2002; In revised form 29 November 2002)

Red blood cells (RBCs) are probably the most common target through the damaging action of reactive oxygen species on the cells. The photohemolysis activity of *m*-chloroperbenzoic acid (CPBA) was concentration- and exposure time-dependent. Twenty minutes photo exposure time and 200 μm of CPBA concentration were optimum to study the effect of generated superoxide ($O_2^{\bullet-}$) and hydroxyl ($^{\bullet}OH$) radicals on RBCs. RBCs lysis photosensitized by CPBA was investigated in the presence of $[(VL_2O)(VL_2H_2O)]Cl_6$, $[MnL_2O]_2Cl_4 2H_2O$, $[FeL_2Cl_2]Cl\,H_2O$, $[CoL_2Cl_2]_4H_2O$ or $[ZnL_2Cl_2]H_2O$ respectively, where L is 2-methylaminopyridine, with SOD-mimetic activities with the aim of ascertaining their protective activity towards the photo induced cell damage. The decrease of photolytic activity caused by these complexes was concentration-dependent and the maximum percentage of protective activity was 75, 70, 68, 57 or 24% for $[(VL_2O)(VL_2H_2O)]Cl_6$, $[MnL_2O]_2Cl_4\ 2H_2O$, $[FeL_2Cl_2]Cl\,H_2O$, $[CoL_2Cl_2]_4H_2O$ or $[ZnL_2Cl_2]H_2O$ complex respectively, against the cell irradiated without addition of metal complexes. The comparison between the decrease of photolytic activity caused by these complexes and their SOD-mimetic activity of these metal complexes showed an appreciable correlation.

Keywords: Photohemolysis; SOD-mimetic; Protection; Free radicals

INTRODUCTION

Reactive oxygen species (ROS) are well known to be involved in some human diseases. ROS such as superoxide($O_2^{\bullet-}$) and hydroxyl radicals ($^{\bullet}OH$) formed continuously as a result of biochemical reactions and can cause a significant oxidative damage.[1,2] The significance of $O_2^{\bullet-}$ has been demonstrated to be crucial in paraquat toxicity,[3] reperfusion injury in organ transplantation[4] and hyperoxygenation pulmonary toxicity in animals.[5] $O_2^{\bullet-\bullet}$ and $^{\bullet}OH$ can induce single-strand breaks in DNA and its involvement has been implicated in tumor promotion.[6] Oxidative damage of DNA, lipids and proteins in the human body is generally considered to be an important factor in carcinogenesis and may also play a role in cutaneous photosensitization.[7]

Biological membranes, particularly in mitochondria and erythrocytes are considered a critical target for cell damage by photosensitization.[8] Erythrocytes belong to be the most common target in studying damaging action of oxygen radicals. The role of oxygen free radicals in erythrocyte lysis has been suggested for some time.[9] It has been postulated that either membrane oxidation or hemoglobin denaturation occurs in the destruction of red blood cells. In addition, vascular damage and subsequent tumor cell anoxia are the indirect mechanisms leading to tumor necrosis by photosensitization.[10] Some evidence indicated that the ROS plays an important role in the photolysis of human erythrocytes sensitized by photosensitization with protoporphrin IX and ketoporfen.[11]

*Corresponding author. Tel.: +2049601265. Fax: +2049601268. E-mail: ibrahimelsayed@yahoo.com

ISSN 1071-5762 print/ISSN 1029-2470 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/1071576031000088274

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/26/11
For personal use only.

The postulated defense role of antioxidants against the activities of free radicals in the body has become an area of much recent interest to both medical professionals and the general public alike. Superoxide dismutase (SOD) is one of the enzymes which protects aerobic organisms from oxygen toxicity so, when erythrocytes are loaded with SOD, catalase and glutathione peroxidase they were shown to be more resistance to photohemolysis.[9] A major problem with the use of SOD is its high molecular weight, which limits its diffusibility into cells. The use of low molecular weight complexes which exhibit SOD-mimetic activity avoids some of the practical limitations of using the enzyme itself. The primary advantage of complexes of this type concerns diffusability into the cell and cell organelles. One such compound, copper(II) complexes of diisopropylasalicylate $[(Cu)_2(disps)_4]$ has SOD-mimetic and was reported it to protect against oxidative damage.[12] Also, the manganese (IV) complex of desferioxamine (MnDf) has been reported to be a very efficient SOD-mimetic.[13]

In the present study, we studied the behavior of CPBA as a photosensitizer in the photolysis of human red blood cells. Furthermore, this work was extended to test the protective effect of some metal complexes of 2-methylaminopyridine such as $[(VL_2O)(VL_2H_2O)]Cl_6$, $[MnL_2O]_2Cl_4\ 2H_2O$, $[FeL_2Cl_2]Cl\ H_2O$, $[CoL_2Cl_2]_4H_2O$ or $[ZnL_2Cl_2]H_2O$, where L is 2-methylaminopyridine showed SOD-mimetic activity against RBCs lysis photosensitized by CPBA.

MATERIALS AND METHODS

Chemicals

m-Chloroperbenzoic acid was supplied by Merck. All other chemicals of analytical grade were purchased from Sigma. Metal complexes of 2-methylaminopyridine such as $[(VL_2O)(VL_2H_2O)Cl_6$, $[MnL_2O]_2Cl_4\ 2H_2O$, $[FeL_2Cl_2]Cl\ H_2O$, $[CoL_2Cl_2]4H_2O$ and $[ZnL_2Cl_2]H_2O$ were synthesized as mentioned in our previous work.[14]

Determination of SOD-mimetic Activity

SOD-mimetic activity of the investigated complexes were examined according to the method of Nihiskimi *et al*.[15] The assay relies on the ability of the enzyme to inhibit the phenazine methosulphase (PMS) mediated reduction or nitroblue tetrazolium (NBT) dye to blue formazan. The reaction mixture was contained in a final volume of 3.0 ml. It was composed of 0.5 ml of 0.3 mM NBT, 0.5 ml of NADH, 1.8 ml of 0.1 mM sodium pyrophosphate buffer pH 8.3, 0.1 ml of 16.6, 33.3, 66.6 or 100 μM of $[VL_2O)(VL_2H_2O)]Cl_6$, $[MnL_2O]_2Cl_4\ 2H_2O$, $[FeL_2Cl_2]Cl\ H_2O$, $[CoL_2Cl_2]_4H_2O$ or $[ZnL_2Cl_2]H_2O$ complex and 0.1 ml of 0.093 mM PMS and the increase in absorbance at 560 nm was recorded using spectrophotometer for five minutes.

Irradiation Conditions

Red blood cells were irradiated using a photochemical reactor equipped with a 1000 W tungsten halogen lamp (Lohuis R 75) with a 500–550 nm emission range which was supported in an ethanol cooled quartz tube at 20°C in 3 ml quartz of 1 cm light path and photohemolysis followed.

Photohemolysis Assay

Human erythrocytes were obtained from healthy volunteers by centrifugation at 3000 rpm of freshly drawn, heparinzed blood. The erythrocytes were washed three times with 0.01 M phosphate-buffered saline (PBS), pH 7.4. The packed red blood cells were suspended in PBS at concentration 1×10^6 cells/ml.

The photohemolysis experiment was carried out in a total volume of 3.0 ml 0.01 M PBS pH 7.4 containing 10^6 RBC cells/ml and 20, 50, 100 or 200 CPBA as photosensitizer. The photohemolysis was carried out again by irradiated the tubes for 5, 10 and 20 min, samples containing a total volume 3.0 ml 0.01 M PBS pH 7.4 containing 10^6 RBC cells/ml and 200 μM CPBA as photosensitizer. The hemolysis data were recorded by measuring the increase of absorbance of released hemoglobin at 540 nm.

Inhibition of Photohemolysis by SOD-mimetic Complexes

RBC cells (10^6 ml) were suspended in 3 ml total volume 0.01 M PBS (pH 7.4) containing 200 μM *m*-chloroperbenzoic acid. Before irradiation, the samples were incubated with 5, 25, 50 or 100 μM $[(VL_2O)(VL_2H_2O)]Cl_6$, $[MnL_2O]_2Cl_4\ 2H_2O$, $[FeL_2Cl_2]Cl\ H_2O$, $[CoL_2Cl_2]_4H_2O$, or $[ZnL_2Cl_2]H_2O$ complexes and then all sample tubes were irradiated for 20 min and hemoglobin released was measured at 540 nm.

Data Analysis

Data were expressed as mean ± SD and analyzed statistically using unpaired independent student's *t*-test. To compare experimental groups against the control group, a one-way ANOVA was performed. The 0.05 level of probability was used as a measure of the statistical significance.

RIGHTSLINK

TABLE I Photohemolysis of RBC's induced by different concentrations of CPBA and irradiated for 20 min

	Concentrations of CPBA (μM)			
	20	50	100	200
Mean ± SD*	0.095 ± 0.001	0.153 ± 0.007	0.201 ± 0.013	0.415 ± 0.20
% Hemolysis	23%	37%	48%	94%

*Six tubes photohemolysis assay for each concentration of CPBA. % Hemolysis = (Mean of O.D. test/Mean of O.D. control) × 100.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/26/11
For personal use only.

RESULTS

It is noticed from Table I that the hemolytic activities of CPBA were 32, 37, 48 or 94% in samples incubated with 20, 50, 100 or 200 μM CPBA respectively, compared with untreated control samples.

Time course-dependent manner of the photohemolysis induced by 200 μM CPBA is shown in Table II. The samples irradiated for 5, 10 and 20 min produced 25, 56 and 97% photohemolysis respectively, compared with unirradiated control.

Thus, the photohemolysis activity of CPBA oxidative products was concentration- and exposure time-dependent. Twenty minutes photo exposure time and 200 μM CPBA concentration were optimum to study the effect of generated superoxide (O_2^-) and hydroxyl ($^\bullet OH$) radicals on RBCs.

Figure 1 shows the percentage of inhibition of NBT reduction at different concentrations of $[(VL_2O)(VL_2H_2O)]Cl_6$, $[MnL_2O]_2Cl_4\ 2H_2O$, $[FeL_2Cl_2]Cl\ H_2O$, $[CoL_2Cl_2]_4H_2O$ and $[ZnL_2Cl_2]H_2O$. Comparison between these data clearly shows that the vanadium and manganese complexes are the highest SOD-mimetic activities while zinc complex have relatively less activity.

Figure 2 shows the photoprotective action of $[(VL_2O)(VL_2H_2O)]Cl_6$, $[MnL_2O]_2Cl_4\ 2H_2O$, $[FeL_2Cl_2]Cl\ H_2O$, $[CoL_2Cl_2]_4H_2O$ and $[ZnL_2Cl_2]H_2O$ complexes against the cell damage induced by CPBA. The investigated complexes protect RBCs integrity in a dose dependent way (Fig. 2). The comparison between the decrease of photolytic activity caused by these complexes and their SOD-mimetic activity of these metal complexes showed an appreciable correlation.

DISCUSSION

The oxidation of membrane lipids has been implicated as the primary events in oxidative damage, the inhibition of oxidative hemolysis of RBCs induced by free radicals has been studied previously.[16,17] Different efforts have been made to increase the protection of RBCs against photohemolysis by enrichment of RBCs with SOD. It has been found that water-soluble chain breaking antioxidant such as ascorbic acid, uric acid and chromanol suppressed the hemolysis in a dose dependent mode.[9] However, no lysis was observed when RBCs were incubated in the dark either with CPBA alone or irradiated without addition of CPBA, and this confirms the role of oxygen radicals, generated in the presence of CPBA, which seem to play a significant role in photohemolytic activity. The photohemolysis induced by CPBA may be attributed to the activity of the generated oxygen radicals ($^\bullet OH$ or O_2^-) through the photosensitization reaction.[18,19] The production of $^\bullet OH$ *in vitro* through a photosensitization process occurred by interaction of O_2^- and H_2O_2 to yield the potent hydroxyl radical oxidant:

$$O_2^- + H_2O_2 \rightarrow O_2 + {}^\bullet OH + {}^- OH$$

It is now known that this reaction does not occur spontaneously but must be catalyzed by the action of transition metals (Me) such as copper or iron:[20]

$$O_2^- + Me^{2+} \rightarrow Me^{1+} + O_2$$

$$H_2O_2 + Me^{1+} \rightarrow Me^{2+} + {}^\bullet OH + {}^- OH$$

The photogeneration of free radicals by CPBA during photosensitization processes produces not only $O_2^{\bullet -}$ or $^\bullet OH$ but also singlet oxygen. Singlet

TABLE II Time course-dependent manner of the photohemolysis of RBCs induced by 200 μM CPBA

	5 min	10 min	20 min
*Mean ± SD	0.105 ± 0.005	0.231 ± 0.15	0.429 ± 0.20
% Hemolysis	25%	56%	97%

*Six tubes of photohemolysis assay at each time. % Hemolysis = (Mean of O.D. test/Mean of O.D. control) × 100.

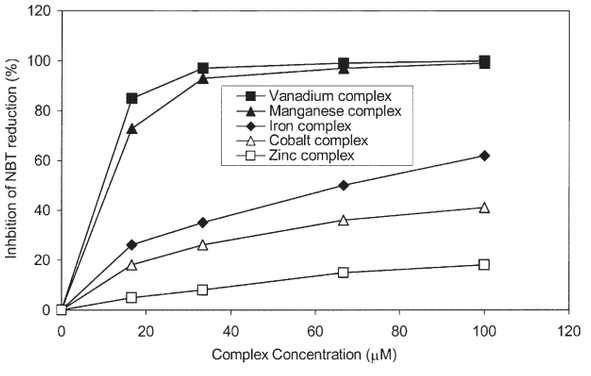

FIGURE 1 SOD-mimetic activity of different metal complexes of 2-methylaminopyridine.

RIGHTSLINK

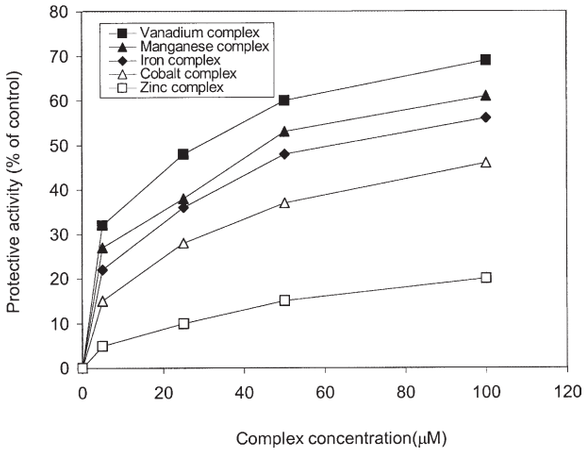

FIGURE 2 Percentage of protective activity of metal complexes against the photohemolysis induced by 200 μM CPBA.

oxygen was produced from H_2O_2 and O_2 through electron transfer in aqueous solution in presence of iron (produced in excess with hemolysis of RBCs)[21,22] or as a secondary production due to the superoxide anion production.[12]

Two different approaches have been employed to inhibit RBC's hemolysis, one reinforcing the protective systems within, the other protecting the outer part of RBCs by the inhibition of membrane damage.[9] Free radicals are involved in normal biochemical process like oxidation-reduction and cellular metabolism; however, they also mediate many disease processes.[23] The participation of oxygen free radicals in the lysis of red cells is important in situations of some intravascular hemolysis.[24] To increase the antioxidant properties of erythrocytes different efforts have been made to enrich RBCs with SOD.[9]

The addition of external SOD may be incapable of significant protection against $O_2^{\bullet -}$ owing to its molecular characteristics which reduced its availability at the site of superoxide generation,[7,25] from the point of view these results were suggested that these metal complexes may have scavenging activities toward oxygen radicals generated through photodegradation of CPBA. These metal complexes with SOD-mimetic activity have a highly protective effort on RBC's hemolysis and are highly effective compared to natural SOD since they are inexpensive, stable at room temperature and not changed with time. Our metal complexes with SOD-mimetic activity protect the RBCs against photohemolysis at a concentration range lower than that employed in the toxicity studies.[14]

In conclusion, our results suggest that $[(VL_2O)(VL_2H_2O)]Cl_6$, $[MnL_2O]_2Cl_4\ 2H_2O$, $[FeL_2Cl_2]Cl\ H_2O$, $[CoL_2Cl_2]_4H_2O$, or $[ZnL_2Cl_2]H_2O$ mimicking SOD complexes may be useful in preventive or therapeutic interventions where biological compartments are subjected to oxidative stress.

References

[1] Jacob, R.A. and Burii, B.J. (1996) "Oxidative damage and defense", *Am. J. Clin. Nutr.* **63**, 985S–990S.
[2] Mates, J.M. (2000) "Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology", *Toxicology* **153**, 83–104.
[3] Krall, J., Bagley, A.C., Mullenbach, G.T., Halliwell, R.A. and Lynch, R.F. (1988) "Superoxide mediates the toxicity of paraquat for cultures mammalian cells", *J. Biol. Chem.* **263**, 1910–1914.
[4] Zweier, J.L., Rayburn, B.K., Flaherty, J.T. and Weisfeldt, M.L. (1988) "Recombinant superoxide dismutase reduces oxygen free radicals concentrations in reperfused myocardium", *J. Clin. Investig.* **80**, 1628–1734.
[5] Crapo, J.D. and Tierney, D.F. (1974) "Superoxide dismutase and pulmonary toxicity", *Am. J. Physiol.* **226**, 1401–1407.
[6] Burdon, R.H., Alliangana, D. and Gill, V. (1995) "Control of cell proliferation by reactive oxygen species", *Free Radic. Res.* **23**, 471–486.
[7] Ortel, B., Gage, W. and Hasan, T. (1990) "Investigations of a manganese-containing mimic of superoxide dismutase in riboflavin phototoxicity in human cells *in vitro*", *J. Photochem. Photobiol. B: Biol.* **7**, 261–276.
[8] Salet, G. and Moreno, G. (1990) "Photosensitization of mitochondria. Molecular and cellular aspects", *J. Photochem. Photobiol.* **5**, 133–150.
[9] Condorelli, G., Costanzo, L.L., Guidi, G., Giuffrida, S., Rizzabelli, E. and Vecchio, G. (1994) "Inhibition of photohemolysis with sod-like activity", *J. Inorg. Biochem.* **54**, 257–265.
[10] Fritsch, C., Becker-Wegerich, P.M., Schulte, K.W., Lehmann, P., Ruzicka, T. and Goerz, G. (1996) "Treatment of large superficial basal cell carcinoma of the breast. Combination by photodynamic therapy and surgery controlled by photodynamic diagnosis", *Hautarzt* **47**, 517–521.
[11] Castanzo, L.L., DeGuidi, G., Condrelli, G., Cambria, A. and Fama, G. (1989) "Molecular mechanism of drug photosensitization II. Photohemolysis sensitized by ketoprofen", *Photochem. Photobiol.* **50**, 359–365.
[12] Athar, M., Mukhtar, H., Elmets, C.A., Zaim, M.T., Lloyd, J.R. and Bichers, D.R. (1998) "*In situ* evidence for the involvement of superoxide anions in cataneous porphyrin photosensitization", *Biochem. Biophys. Res. Commun.* **151**(3), 1054–1059.
[13] Darr, D., Zarilla, K.A. and Fridovich, I. (2000) "A mimetic of superoxide dismutase activity based upon desferrioxamine B and manganese (IV)", *Arch. Biochem. Biophys.* **258**, 351–355.
[14] El-Naggar, M.M., "Protective action of some Cu(II) complexes against photohemolysis induced by *m*-chloroperbenzoic acid", *J. Inorg. Biochem.* **65**, 263–266.
[15] Nishikimi, M., Roa, N.A., Yogi, K., "The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen", *Biochem. Biophys. Res. Commun.* **46**, 849–854.
[16] Kong, S. and Davison, A.J. (1981) "The relative effectiveness of hydroxyl radical, hydrogen peroxide, superoxide ion and reducing free radicals in causing damage to biomembranes. A study of radiation damage to erythrocyte ghosts using selective free radical scavengers", *Biochem. Biophys. Acta* **640**, 313–325.
[17] Halliwell, B. and Gutteridge, J.M.C. (1984) "Oxygen toxicity, oxygen radicals, transition metals and disease", *Biochem. J.* **219**, 1–14.
[18] EL-Naggar, M.M., El-Waseef, A.M., El-Halafawy, A.H. and El-Sayed, I.H. (1998) "Antitumor activity of vanadium[IV], manganese[IV], Iron[III], cobalt[II] and copper[II] complexes of 2-methylaminopyridine", *Cancer Lett.* **133**, 71–76.
[19] Abou-Seif, M.A.M. and El-Gendy, E.M.E. (1998) "Photolysis and membrane lipid peroxidation of human erythrocytes by m-chloroperbenzoic acid", *Clin. Chem. Acta* **277**, 1–11.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/26/11
For personal use only.

RIGHTSLINK

[20] Wilson, J.X. (1997) "Antioxidant defense of the brain: a role of astrcytes", *Can. J. Physiol. Pharmacol.* **75**, 1149–1163.
[21] Del Mastro, R.F. (1980) "An approach of free radicals in medicine and biology", *Acta Physiol. Scand.* **492**, 153–168.
[22] Bus, J.S., Aust, S.D. and Gibson, J.E. (1997) "Superoxide and singlet oxygen catalyzed lipid peroxidation as a possible mechanism for paraquat (methyl viologen) toxicity", *Biochem. Biophys. Res. Commun.* **58**, 749–755.
[23] Aruoma, O.L. (1996) "Characterization of drugs as antioxidant prophylactics", *Free Radic. Med.* **20**(5), 675–705.
[24] Lefer, A.M. and Lefer, D.J. (1993) "Pharamacology of the endothelim in ischemia and circulatory shock", *Ann. Rev. Pharmacol. Toxicol.* **33**, 71–90.
[25] Cuzzocrea, C., Mazzon, E., Dugo, L., Caputi, A.P., Aston, K., Reliy, D.P. and Salvemini, D. (2001) "Protective effects of a new stable, highly active SOD mimetic, M40401 in splanchnic artery occlusion and reperfusion", *Br. J. Pharmcol.* **132**, 19–29.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/26/11
For personal use only.

RIGHTSLINK