J. King Saud Univ., Vol. 9, *Science* (1), pp. 43-47 (A.H. 1417/1997)

Comparative Action of Hydroxyl Radical Scavengers on Mercury Chloride-Induced Haemolysis in Human and Sheep Erythrocytes

M. A. El-Missiry* and M. A. Ismail
Biology Department, King Faisal University
P.O. Box 1759, Al-Hassa, Saudi Arabia

(Received 23/11/1994; accepted for publication 16/6/1996)

Abstract. Human and sheep erythrocytes were treated with 0.05 mM mercuric chloride *in vitro* in the presence of two hydroxyl, (OH) radical scavengers: ethanol and ethylene glycol. Both cell types were protected from mercuric chloride induced haemolysis, presumably as a result of OH scavenging.

Introduction

Ethanol, ethylene glycol and dimethyl sulphoxide are efficient scavengers of OH radicals and have demonstrated protective effects in several aqueous systems [1]. In a model membrane system where peroxidation of unsaturated lipids is the predominant form of damage, ethanol and formate are protective, indicting that the hydroxyl radical plays a major role in the initiation of peroxidation of unsaturated acyl chain [2].

Among several hypotheses, put forward to explain the mechanism of mercury toxicity, is the one involving the generation of free radicals such as superoxide radical (O_2), hydroxyl radical (HO) and hydrogen peroxide (H_2O_2) [3]. Radical mediated lipid peroxidation plays a crucial role in many physiological and pathological process such as inflammation, aging, carcinogenesis, drug reaction and drug toxicity and defence against protozoa [4, pp. 1-8].

*Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt.

The aim of the present investigation was to study the action of hydroxyl scavengers, ethyl alcohol and ethylene glycol on mercury chloride-induced haemolysis in human and sheep erythrocytes.

Materials and Methods

Blood samples were collected from normal human donors and sheep, into heparinized tubes, and used in the same day. All operations were performed at 25°C. Prior to each experiment, blood cells were washed four times with 145 mM NaCl and 10 mM phosphate buffer, pH 7.4 and the buffy coats were removed after each centrifugation. Erythrocytes were suspended in the same buffer at 10% haematocrit level.

Erythrocyte suspensions were treated with mercuric chloride ($HgCl_2$) at a concentration of 0.05 mM in the presence and absence of the hydroxyl scavengers, ethanol (Et OH) and ethylene glycol. Two concentrations were used in this experiment, 10^{-2} M and 10^{-3} M for each scavenger. At the end of the incubation period of 6 hr, 1 ml aliquot was centrifuged and the liberated haemoglobin was converted into methaemoglobin [1] and determined spectrophotometrically at 540 nm. Percent of haemolysis was calculated from the optical absorbance of treated sample at 540 nm and compared with the optical absorbance of 100% haemolysis.

Results

Haemolysis at 6 hr after mercuric chloride treatment was studied at the two concentrations of ethanol and ethylene glycol. The data are presented in the following table. It is evident that sheep erythrocytes treated with 0.05 mM mercuric chloride in the absence of OH scavengers underwent higher haemolysis than human cells under the same conditions.

Table. Percent haemolysis of human and sheep erythrocytes 6 hours following 0.05 mM mercuric chloride treatment in presence and absence of ethanol and ethylene glycol. Mean values ± S.D. of 4 samples are presented

Treatment	Human erythrocytes	Sheep erythrocytes
Control	8.00 ± 1.2	7.97 ± 1.7
$Hg\ Cl_2$	40.50 ± 2.9*	50.83 ± 4.1*
Ethanol		
10^{-2} M	24.00 ± 1.1*	8.30 ± 0.8^{NS}
10^{-3} M	23.90 ± 1.2*	6.08 ± 1.9^{NS}
Ethylene glycol		
10^{-2} M	16.60 ± 1.2*	14.00 ± 0.9*
10^{-3} M	17.00 ± 1.1*	13.00 ± 0.7*

* Significant $P < 0.001$; NS Insignificant $P > 0.05$

It was observed, also, that human and sheep erythrocytes were efficiently protected from haemolysis by 10^{-2} M and 10^{-3} M of ethyl alcohol and ethylene glycol. There was no marked difference in the protection efficiency between the two concentrations used of each scavenger for both types of cells.

However, it was recorded that the proteciton impact of ethylene glycol was more than ethyl alcohol in case of human erythrocytes. On the other hand, the protective effect of ethanol was more pronounced than ethylene glycol in case of sheep cells.

Although a marked protection was obtained for sheep and human erythrocytes treated with $HgCl_2$ in the presence of the OH radical scavengers, there was species difference. Sheep erythrocytes were more protected than human cells.

Discussion

The protection by ethanol and ethylene glycol against $HgCl_2$ induced haemolysis, in both human and sheep erythrocytes, indicates that the bulk of the membrane damage leading to rupture of RBCs may be cased by hydroxyl radicals. This is in accordance with the finding of other workers [1]. The protection was observed when the scavengers were added before mercurial treatment.

The protective influence against haemolysis was manifested at scavenger concentration of 10^{-2} and 10^{-3} M which are lower than that used in fixation of cells and tissues. In a previous work, a protective effect was obtained for human and bovine RBCs irradiated in the presence of 1, 10^{-3} and 10^{-5} M ethanol and was presumed to result from scavenging of OH [1]. These findings suggest that the effect of ethanol was due to the scavenging of OH and not its fixative properties in cell membrane.

There were species differences demonstrated in the protection of sheep RBCs, as compared with human cells, against metal toxicity. The sheep cells were more protected than human red cells. Kurata *et al.* [2] found that there is species difference in the levels of antioxidant parameters in erythrocytes of sheep and human. They recorded that superoxide dismutase, glutathione peroxidase and glutathione are higher in sheep than human erythrocytes, despite the fact that the specific metabolic rates are similar in both types of cells.

Another possibility for the observed species difference is that human RBCs were less efficient in scavenging radicals produced in the erythrocyte membranes [1] as compared with ovine cells. Therefore, scavenging of OH radicals by OH radical scavengers might give the chance to the antioxidant systems in the sheep red cells to display their function and show a protective impact.

Another possible explanation of the results may be found in the particular composition of sheep erythrocyte membranes. It was recorded that the composition of the cellular membranes differs among the various animal species [5]. A higher content of

sphingomyelin (SPH) and lower content of phosphatedylcholine have been described in erythrocytes from ruminant species, as compared to other animal species. This is particularly evident in the sheep red blood cells, where the content of SPH is very low [6]. In the light of this information, the composition of the erythrocyte membrane phospholipids might determine the penetration of the reagent into the cell, and probably causing different oxidation rates [7].

References

[1] Miller, G.G. and Raleigh, J.A. "Action of Some Hydroxy Radical Scavengers on Radiation-induced Haemolysis." *Int. J. Radiat. Biol.*, 43 (1983), 411-419.

[2] Kurata, M., Suzuki, M. and Agar, N.S. "Antioxidant Systems and Erythrocyte Life Span in Mammals." *Comp. Biochem. Physio.*, 106B (1993), 477-487.

[3] Di Guiseppi, J. and Fridovich, I. "The Toxicity of Molecular Oxygen." *CRC Crit. Rev. Toxicol.*, 12 (1984), 315-342.

[4] Sies, H. "Oxidative Stress: Introductory Remarks." In: Sies, H. (Ed.). *Oxidative Stress*. London: Academic Press, 1985.

[5] Dawson, R.M.C., Hemington, N. and Lindsay, D.B. "The Phospholipids of Erythrocyte Ghosts of Various Species." *Biochem. J.*, 77 (1960), 226-230.

[6] Engen, R.L. and Clark, C.L. "High Performance Liquid Chromatography Determination of Erythrocyte Membrane Phospholipid Composition in Several Animal Speciesl." *Am. J. Vet. Res.*, 51 (1990), 577-580.

[7] Avellini, L., Spaterna, A., Rebold, G.P. and Gaiti, A. "Defence Mechanisms against Free Radical-Induced Damage in Sheep, Cattle and Dog Erythrocytes." *Comp. Biochem. Physiol.*, 106 B (1993), 391-394.

تأثير بعض قانصات أيونات الهيدروكسيل الُحرّة على تحلل خلايا الدم الحمراء للإنسان والغنم بكلوريد الزئبق

محمد عمرو المسيري و محمد عاطف اسماعيل

العنوان الحالي : قسم الأحياء، جامعة الملك فيصل، الأحساء، ص. ب ١٧٩٥، المملكة العربية السعودية.*

(استلم في ٢٠ جمادى الثانية ١٤١٥هـ؛ قبل للنشر في ٣٠ محرم ١٤١٧هـ)

ملخص البحث. تمّت دراسة تحلل خلايا الدم الحمراء للإنسان والغنم تحت تأثير كلوريد الزئبق في وجود وغياب قانصات أيونات الهيدروكسيل الحُرّة، مثل الكحول الإيثيلي والجليكول الإيثيلي.

وجد أن هذه القانصات قد عملت على الحماية ضد التحلل الدموي الناجم عن كلوريد الزئبقيك. ولقد فسرت هذه الحماية على أن تكون نتيجة اقتناص أيونات الهيدروكسيل الحُرّة المسببة لتحلل خلايا الدم الحمراء.

---

* والعنوان الدائم: قسم علم الحيوان، كلية العلوم، جامعة المنصورة، المنصورة، جمهورية مصر العربية.