STUDIES ON THE ANTIPEROXIDE AND ANTIHAEMOLYTIC ACTION OF POLYAMINES USING HUMAN ERYTHROCYTES

Chethana Chetan, Ashok Prabhu, Vivian D'Souza and Shrinivas B. Rao*

Department of Biochemistry, Kasturba Medical College, Mangalore. 575 001

ABSTRACT

Blood RBC's were used to study the role of polyamines, putrescine, spermidine and spermine individually as antioxidants against the peroxidative action of H_2O_2 which leads to haemolysis of erythrocytes. Results indicated that putrescine did not act as antioxidant at all concentrations used in our studies. However, spermidine and spermine suppressed peroxidative changes in RBC's due to the action of H_2O_2 and this suppression was greater with higher concentration of these individual polyamines used, separately. Further, all the three polyamines decreased haemolysis of erythrocytes at all concentrations studied. The action of putrescine to suppress haemolysis of RBC's without showing antioxidant property hypothetically suggests that these properties of respective polyamines may have independent mechanism of action.

KEY WORDS : Putrescine, spermidine, spermine, lipid peroxidation, erythrocytes, haemolysis

INTRODUCTION

The polyamines, putrescine, spermidine and spermine are ubiquitous naturally occurring organic cations required by cells for differentiation and proliferation (1,2). Cells unable to synthesize these polyamines exhibit drastically defective cytoskeleton (3, 4). Polyamines also show antioxidant action (5) and inhibit lipid peroxidation (6). Peroxides formed due to free radicals generated in biological systems lead to cell damage and tissue destruction (7). The harmful free radicals formed in biological systems can be destroyed by antioxidants (8). The aim of the present work was to evaluate the role of polyamines as antioxidants using human blood erythrocytes with and without exposure to H_2O_2 (9) and measure the toxic peroxides formed as malondialdehyde which lead to haemolysis of RBC's.

MATERIALS AND METHODS

Heparinised aseptic disposable syringes were

used to collect 10ml of blood by venepuncture from normal healthy volunteers who were post-graduate students, from both sexes, in the 20-25 yrs age group from our department. Blood was immediately centrifuged at 3000g for 10 mins and the sedimented RBC's were separated and washed thrice with cold physiological saline. The cell pellet was then suspended in equal volume of saline phosphate isoosmotic buffer, pH 7.4 to give a cell suspension of 50%. Aliquots of this cell suspension were incubated at 37 °C in the absence and presence of 6% H_2O_2 and various concentrations of individual polyamines separately and lipid peroxidation measured as malondialdehyde by the method of Stocks and Dormandy (10). RBC haemolysis was measured by the method of Kartha and Krishnamurthy (9) and total haemoglobin by the cyanmethaemoglobin method (11). All chemicals used were of reagent grade. The polyamines were purchased from Sigma chemicals, USA.

***Author for correspondence :**

RESULTS AND DISCUSSION

The present investigation indicates the lack of antioxidant property for putrescine (Table 1) both in the presence and absence of H_2O_2 . However, the higher polyamines, spermidine (Table 2) and spermine

(Table 3) showed statistically significant effect in suppressing lipid peroxidation within the concentration range of 0.3 to 1.2 mM of respective polyamines used in this study. Further, this suppressive action of these two polyamines was concentration dependent with higher concentration

Table 1. Effect of putrescine on lipid peroxide values in the presence and absence of H_2O_2 .

Putrescine mM	n mol.malondialdehyde/gm Hb			
	Without H_2O_2		With H_2O_2	
	0hr	1hr	0hr	1hr
0.0	4.4 ± 0.16	4.8 ± 0.22	7.1 ± 0.48	41.6 ± 3.7
0.3	4.4 ± 0.16	5.1 ± 0.26	7.2 ± 0.50	42.4 ± 3.7
0.6	4.4 ± 0.15	5.6 ± 0.24	7.3 ± 0.53	42.7 ± 3.7
0.9	4.4 ± 0.16	5.7 ± 0.23	7.3 ± 0.53	43.0 ± 3.7
1.2	4.5 ± 0.15	5.7 ± 0.23	7.3 ± 0.56	43.1 ± 3.7

- Values are mean ± SE of 10 separate experiments
- Putrescine showed no statistically significant antioxidant action when erythrocytes were incubated in the presence and absence of H_2O_2 .

Table 2. Effect of spermidine on lipid peroxide values in the presence and absence of H_2O_2 .

Spermidine mM	n mol.malondialdehyde/gm Hb			
	Without H_2O_2		With H_2O_2	
	0hr	1hr	0hr	1hr
0.0	4.2 ± 0.30	4.8 ± 0.25	7.1 ± 0.37	49.0 ± 4.0
0.3	4.2 ± 0.30	*4.5 ± 0.26	7.1 ± 0.46	*46.4 ± 4.0
0.6	4.2 ± 0.30	*4.3 ± 0.26	7.0 ± 0.45	*44.2 ± 3.9
0.9	4.2 ± 0.30	*4.3 ± 0.24	7.0 ± 0.45	*42.1 ± 4.0
1.2	4.2 ± 0.30	*4.2 ± 0.23	6.9 ± 0.46	*39.7 ± 4.0

- Values are mean ± SE of 10 separate experiments
- Spermidine showed statistically significant (* $P \leq 0.001$) antioxidant activity when erythrocytes were incubated for 1hr with and without H_2O_2 .

Table 3. Effect of spermine on lipid peroxide values in the presence and absence of H_2O_2

Spermine mM	nmol. malondialdehyde/gm Hb			
	Without H_2O_2		With H_2O_2	
	0hr	1hr	0hr	1hr
0.0	4.3±0.21	4.8±0.19	7.7 ± 0.42	43.2 ± 3.7
0.3	4.3±0.20	*4.7±0.17	7.8 ± 0.41	*41.0 ± 3.7
0.6	4.3±0.20	*4.6±0.19	7.7 ± 0.42	*38.9 ± 3.6
0.9	4.3±0.20	*4.4±0.21	7.8 ± 0.41	*37.0 ± 3.6
1.2	4.3±0.20	*4.3±0.22	7.9 ± 0.41	*34.8 ± 3.7

a) Values are mean ± SE of 10 separate experiments

b) Spermine showed statistically significant (* $p \leq 0.001$) antioxidant activity when erythrocytes were incubated for 1hr with and without H_2O_2

showing greater suppression of peroxide formation. Extensive work done by other investigators using liver microsomes (12), phospholipid vesicles (13), rat lung (14), brain (15) and cardiac tissue (16) has indicated conflicting and controversial results with regard to the antioxidant property for all three polyamines. Moreover, Das and Nair (17) observed that RBC's from fresh blood did not contain significant amount of malondialdehyde due to the endogenous polyamines present in blood which blocked lipid peroxidation. Further, Matcovics et.al. (15) reported antiperoxide action using all three polyamines. Subsequently no information is published on this subject to the best of our knowledge. However, our work demonstrated lack of antioxidant property for putrescine upto a concentration of 1.2 mM while the other two polyamines showed antioxidant effect at all concentrations studied from 0.3 to 1.2mM. Whether this is related to putrescine concentration, used in our studies is unclear particularly in the context that concentration dependent antiperoxide action of polyamines has been reported (6,17).

Further, our studies indicated that in the non-oxidative state, when H_2O_2 was not used, there was no RBC haemolysis for both 0 hr and 1hr time points (separate data not presented). However, in the

presence of H_2O_2 though no haemolysis was observed at 0 hr there was statistically significant haemolysis at 1 hr time point (Table 4). Moreover, this haemolysis was significantly suppressed by all the three polyamines in the concentration range of 0.3 to 1.2 mM used separately and was concentration dependent with greater suppression of haemolysis at higher levels of each of the polyamines (Table 4). Studies by other workers (18) indicated that all the three polyamines putrescine, spermidine and spermine independently suppressed RBC haemolysis at concentration greater than 1mM which they explained as due to the property of polyamines to suppress lipid peroxidation. However, results of the present study clearly indicate the inability of putrescine to suppress lipid peroxidation in the concentration range of 0.3 to 1.2mM (Table1) while at the same concentration it showed antihaemolytic effect (Table 4). These results suggest that the mechanism of action of putrescine as an antihaemolytic agent has no relationship with its failure to suppress peroxidative changes in RBC's and seems to be an independent mechanism. Hypothetically, whether a similar conclusion can be drawn for the higher polyamines spermidine and spermine with regard to their antiperoxide and antihaemolytic action which may be by mechanisms

Table 4. Effect of each polyamine an erythrocyte haemolysis when incubated for 1hr with H₂O₂.

Polyamines mM	% haemolysis		
	Putrescine	Spermidine	Spermine
0.0	5.1± 0.48	3.3 ±0.64	4.7± 0.44
0.3	4.3 ±0.64	2.7 ±0.58	3.9 ±0.33
0.6	*3.5± 0.72	*2.4 ±0.46	*3.6± 0.27
0.9	*3.2± 0.69	*2.1± 0.54	*3.3 ±0.38
1.2	*3.1± 0.69	*1.7 ±0.62	*2.8 ±0.34

- a) Values are mean±SE of 6 separate experiments
- b) No haemolysis was observed in the absence of H₂O₂ and at 0hr time point in the presence of H₂O₂.
- c) Increase in polyamine concentration decreased % haemolysis which was statistically significant (*P ≤ 0.001).

independent of each other is unclear at present and needs further research.

REFERENCES

1. Heby, O. (1986) Putrescine, spermidine and spermine. *News Physiol. Sci.* 1, 12-15.
2. Pegg, A.E. and Mc Cann, P.P. (1982) Polyamine metabolism and function. *Am. J. Physiol.* 243, C212-C221.
3. Allen, J.C. (1983) Biochemistry of the polyamines. *Cell Biochem. Funct.* 1, 131- 140.
4. Tabor, C.W. and Tabor, H. (1984). Polyamines. *Annu. Rev. Biochem.* 53, 749- 790.
5. Kafy, A.M.L. and Lewis, D.A. (1988) Antioxidant effects of exogenous polyamines in damage of lysosomes inflicted by xanthine oxidase or stimulated polymorphonuclear leucocytes. *Agents Action* 24, 145- 151.
6. Tadoline, B. (1988) Polyamine inhibition of lipid peroxidation, the influence on iron oxidation in the presence of compounds mimicking phospholipid polar heads. *Biochem. J.* 249, 33-36.
7. Southern, P.A. and Powis, G. (1988) Free radicals in Medicine: Chemical nature and biologic reactions. *Mayo Clin. Proc.* 63, 381- 389.
8. Halliwell, B. and Chirico, S. (1993) Lipid peroxidation, its mechanism measurement and significance. *J. Clin. Nutri.* 57, 715- 725.
9. Kartha, V.N.R and Krishnamurthy, S. (1978). Effects of hypervitaminosis A on haemolysis and lipid peroxidation in the rat. *J. Lipid Res.* 19, 332-335.
10. Stocks, J. and Dormandy, T.L. (1971) The oxidation of human red cell lipids induced by H₂O₂. *Br. J. Haematol.* 20, 95-98.
11. Varley, H. (1988) Haemoglobin and related compounds. *Practical Clinical Biochem.* 4th Indian ed., CBS Publ., Delhi p. 585- 586.

12. Kitada, M., Igarashi, K., Hirose, S. and Kitagawa, H. (1979) Inhibition by polyamines of lipid peroxide formation in rat liver microsomes. *Biochem. Biophys. Res. Comm.* 87, 388- 394.
13. Tadolini, B., Cabrini, L., Landi, L., Varani, E. and Pasquali, P. (1984) Polyamines binding to phospholipid vesicles and inhibition of lipid peroxidation. *Biochem. Biophys. Res. Comm.* 122, 550- 555.
14. Khanna, Y.P., Taneja, S.K., Raj, H.G.S. and Venkatasubramanian, T.A. (1982) Polyamines modify paraquat induced changes in pulmonary superoxide dismutase and lipid peroxidation. *Res. Comm. Chem. Pathol. Pharmacol.* 35, 337- 340.
15. Matcovics, B., Kecskemeti, V., Varga, S.I., Novak, Z. and Kertesz, Z. (1993) Antioxidant properties of di and polyamines. *Comp. Biochem. Physiol.* 104, 475- 479.
16. Das, S.K. and Nair, R.C. (1980) Superoxide dismutase, glutathione peroxidase, catalase and lipid peroxidation of normal and sickled erythrocytes. *Br. J. Haematol.* 44, 87- 92.
17. Gupta, K.P. and Mehrotra, N.K. (1993) Ornithine decarboxylase inactivation by putrescine : involvement of lipid peroxidation. *Biochem. Mol. Biol. Intl.* 30, 697-704.
18. Varga, S.I., Novak, Z., Pataki, L., Patocskai, M. and Matcovics, B. (1992) The influence of antioxidants on the oxidative stress of red blood cells. *Clin. Chim. Acta.* 205, 241- 244.