Biochemistry (Moscow), Vol. 67, No. 8, 2002, pp. 945-948. Translated from Biokhimiya, Vol. 67, No. 8, 2002, pp. 1137-1141.

Protective Role of Vitamin E on Mefenamic Acid-Induced Alterations in Erythrocytes

I. Ahmad* and M. Suhail

Department of Biochemistry, University of Allahabad, Allahabad 211002, UP, India;
E-mail: ahmadimtiaz@hotmail.com or ssuhail@sancharnet.in

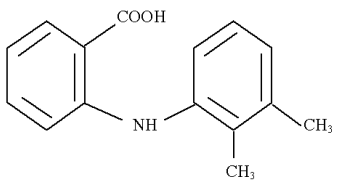
Received November 8, 2001
Revision received January 14, 2002

Abstract—Erythrocyte osmotic fragility (O.F.), acetylcholinesterase (AChE) activity, and the level of malonyl dialdehyde (MDA) of control, mefenamic acid treated, and mefenamic acid with vitamin E treated rats were investigated. Administration of mefenamic acid to albino rats brought about a significant increase in the osmotic fragility of red cells and a significant ($p < 0.01$) decrease in the activity of AChE. We have also observed increased red cell level of MDA and decreased cholesterol (Chl), hemoglobin (Hb), and reduced glutathione (GSH) content. Supplementation of vitamin E to the mefenamic acid treated rats restored the O.F., AChE activity, level of MDA, and Chl, Hb, and GSH content almost to normal. These observations suggest that mefenamic acid causes functional impairment of red cell membrane, while vitamin E shows its protective role in maintaining normal red cell functions.

Key words: vitamin E, mefenamic acid, erythrocyte, osmotic fragility, glutathione, acetylcholinesterase, cholesterol, hemoglobin, malonyl dialdehyde

Vitamin E has been known to behave as a biological antioxidant and preserves membrane integrity [1]. Earlier we have described the role of vitamin E in protecting erythrocyte reduced glutathione (GSH) from the oxidative effect of metamizol [2]. It is generally accepted that the biological activity of vitamin E relates to its ability to prevent the oxidation of unsaturated fats in cell membranes and studies suggest that vitamin E has a membrane stabilizing effect [3]. Vitamin E also maintains essential sulfhydryl (SH) groups of membrane proteins [4]. A review summarizing the membrane stabilizing effect of vitamin E has been presented by Shiro [5].

Certain non-steroidal anti-inflammatory analgesic and anti-malarial drugs have been reported to cause oxidative stress and damage to red cells [6, 7]. The potential serious side effects seen after mefenamic acid treatment are hemolytic anemia [8] and central nervous system (CNS) toxicity [9]. The chemical structural formula of mefenamic acid ($C_{15}H_{15}NO_2$) is as follows:



* To whom correspondence should be addressed.

Red cell osmotic fragility is useful in clinical detection of hypochromic and congenital hemolytic anemia [10] and is related to cellular deformability, an important aspect of red cell function. Acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7; AChE) occurs both in the central nervous system as well as in the erythrocytes of mammals in detergent soluble forms [11]. The significance of erythrocyte AChE is not clear, but this enzyme is being studied in erythrocytes since many of its properties are similar to the enzyme in brain tissue [12] and is regarded as a valid model system for the brain enzyme [13]. Studies have also been directed towards the possible role of AChE in cation transport and membrane rigidity [14].

We undertook the present work to study the role of vitamin E in protecting against the alterations that could take place in the red cell and its membrane with mefenamic acid treatment. Important parameters such as red cell osmotic fragility, AChE activity, cholesterol, Hb, GSH contents, and the level of malonyl dialdehyde (MDA) were studied.

MATERIALS AND METHODS

5,5′-Dithiobis-(2-nitrobenzoic acid) and Tris-HCl (Trizma chloride) were purchased from Sigma (USA).

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Acetylthiocholine iodide was purchased from Fluka (Switzerland). All reagents were of the highest purity available.

Albino rats (Charles Foster strain) were procured from the Laboratory Animal Division, Central Drug Research Institute, Lucknow, India. Young male rats (200-250 g) were maintained on commercial rat diet (Lipton India Ltd., India) under standard hygienic conditions [15]. Rats were divided into three groups. Group 1 rats (control) were kept on the standard diet and water *ad libitum*, group 2 rats were given mefenamic acid per orally four times a day (total dose 16.7 mg/kg body weight), while group 3 rats were given mefenamic acid plus vitamin E (α-tocopherol acetate) *per os* (6.7 mg/kg body weight).

After five days of continuous drug treatment, blood was collected on the 6th day from the caudal vein in heparin (10 IU/ml) for osmotic fragility determinations and citrate–dextrose for AChE assays and malonyl dialdehyde measurements, kept at 4°C. The osmotic fragility was determined following the method of Dacie and Lewis [16].

The blood samples were centrifuged at 1000*g* for 15 min. The isolated red cells were washed 4-5 times with 0.154 M NaCl to remove plasma and buffy coat. Acetylcholinesterase activity of red cells was assayed following the method of Beutler [17] based on the procedure of Ellman et al. [18]. Hemoglobin was estimated by the ferricyanide/cyanide method as described by Beutler [17].

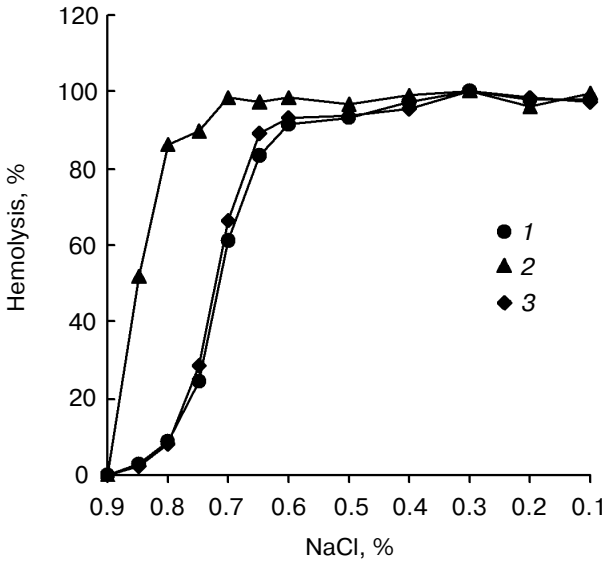
Cholesterol and reduced glutathione contents were estimated by the method of Zak et al. [19] and Beutler et al. [20], respectively. The level of red cell malonyl dialdehyde (MDA) was determined by the thiobarbituric acid reactivity of MDA according to the method of Stocks and Dormandy [21].

The statistical significance of the difference between mean values was determined by Student's *t*-test.

RESULTS

The red cell osmotic fragility (O.F.) profiles of group 1, 2, and 3 rats obtained by plotting percent hemolysis against the corresponding NaCl concentration in the range of 0.9 to 0.1% are shown in the figure. The erythrocyte O.F. of mefenamic acid treated rats was increased, while rats treated with mefenamic acid plus vitamin E showed an almost normal O.F. profile as compare to control. The NaCl concentration of 50% hemolysis is taken as a measure of mean erythrocyte fragility (MEF) [22]. The MEF of group 1, 2, and 3 rats are 0.715, 0.853, and 0.723, respectively (figure), which shows an increase in the MEF of group 2 (mefenamic acid treated) rats.

The red cell acetylcholinesterase (AChE) activity and malonyl dialdehyde (MDA), cholesterol (Chl), hemoglobin (Hb), and reduced glutathione (GSH) contents of group 1, 2, and 3 rats are presented in the table. There is a significant ($p < 0.01$) decrease in red cell AChE activity, cholesterol ($p < 0.001$), Hb ($p < 0.01$), and GSH ($p < 0.001$) contents and a significant ($p < 0.01$) increase in the level of MDA in rats given mefenamic acid. However, the activity of AchE, level of MDA and other parameters returned almost to normal in rats treated with mefenamic acid plus vitamin E.



Osmotic fragility (O.F.) profiles of erythrocytes of control (*1*), mefenamic acid treated (*2*), and mefenamic acid plus vitamin E treated rats (*3*)

DISCUSSION

The increase in red cell osmotic fragility (O.F.) and malonyl dialdehyde (MDA) level, while decreased AChE activity, and cholesterol, Hb, and GSH content reflects that mefenamic acid causes alteration in red cell membrane functions and its fluidity. The decreased membrane cholesterol content and increased MDA level of rats treated with mefenamic acid might have increased the osmotic fragility of red cells, which increased the cell lysis. Increased erythrocyte osmotic fragility is of clinical significance because it has been linked to several diseases that results in hemolytic anemia [23]. Disruption of human erythrocyte membrane structure with mefenamic acid has already been observed by Sigeru et al. [24].

Red cell AChE is a sulfhydryl (SH)-bearing enzyme [25] and studies have been directed towards the possible role of AChE in cation transport and membrane rigidity [14]. GSH serves as a reductant of membrane protein disulfides, in addition to averting membrane thiol oxidation [26]. Therefore, fall of GSH may be associated with

Red cell acetylcholinesterase activity and malonyl dialdehyde (MDA), membrane cholesterol, hemoglobin (Hb), and reduced glutathione (GSH) levels of control, mefenamic acid treated, and mefenamic acid plus vitamin E treated rats

Parameter studied	Experimental conditions/animal group		
	Control (group 1)	Mefenamic acid (group 2)	Mefenamic acid plus vitamin E (group 3)
Acetylcholinesterase (IU/g Hb)*	17.54 ± 2.27	10.98 ± 1.61[a]	20.08 ± 0.57
MDA (μmole/ml packed cells)	0.0029 ± 0.0011	0.0038 ± 0.0020[b]	0.0031 ± 0.0008
Cholesterol (μg/mg protein)	187.40 ± 5.40	164.50 ± 6.50[c]	179.80 ± 6.20
Hb (g/100 ml erythrocytes)	14.83 ± 0.76	9.83 ± 0.29[d]	14.66 ± 0.76
GSH (mg/g Hb)	1.656 ± 0.157	0.968 ± 0.066[e]	1.639 ± 0.132
GSH (mg/100 ml erythrocytes)	24.88 ± 1.03	9.51 ± 0.52[f]	24.11 ± 3.23

Note: Values are means of 3-4 experiments ± SD. Significance of differences compared to control: [a]$p < 0.01$; [b]$p < 0.01$; [c]$p < 0.001$; [d]$p < 0.01$; [e]$p < 0.001$; [f]$p < 0.001$.

* Enzyme activity is expressed in terms of international units (IU; μmol acetylthiocholine iodide hydrolyzed per minute) per g of hemoglobin at 37°C.

the protection of membrane SH groups as there is a direct link between thiol status of the cell membrane and cellular GSH. Depletion of GSH in the intact erythrocytes results in rapid oxidation of large amounts of hemoglobin (Hb) to methemoglobin (metHb) [27]. Decreased red cell AChE correlates well with the reported central nervous system toxicity of mefenamic acid [9]. In the present study vitamin E showed its protective role on AChE activity, in agreement with the observation of Davies et al. [4] that vitamin E maintains essential SH groups of the membrane protein and prevent oxidation of SH groups during lipid peroxidation [28]. It is also possible that vitamin E alters membrane fluidity in such a way that the activity of AChE increases as a result of the vertical displacement of membrane enzymes exposing more active sites [29].

Evidence is gradually accumulating for the importance of vitamin E in maintaining normal cell membrane structure and function. The importance of vitamin E as an antioxidant providing protection against membrane damage has been well documented [30, 31]. We have earlier reported that vitamin E restored the activity of red cell AChE of rats treated with metamizol [32]. Vitamin E also maintains membrane fluidity, a significant aspect of red cell function and lack of it may lead to decreased fluidity of the membrane [33], which may result in decreased erythrocyte life-span. Vitamin E is a powerful free radical scavenger, which inhibits the autocatalytic process of lipid peroxidation of membrane fatty acids [34]. In the present work the red cell osmotic fragility, AChE activity, level of MDA, and cholesterol, Hb, and GSH contents are almost like control values in case of vitamin E plus drug treated rats.

Regardless of the exact mechanism(s) by which mefenamic acid produces its effect on red cell membrane, these findings clearly shows the functional impairment of red cell membrane as expressed by alterations in the osmotic fragility, level of malonyl dialdehyde, AChE activity, and cholesterol, Hb, and GSH contents and protection by vitamin E. Our study demonstrating the protective role of vitamin E on red cell membrane alterations induced by mefenamic acid could therefore be of clinical relevance.

Author I. Ahmad is grateful to the Council of Scientific and Industrial Research (CSIR), Ministry of Science and Technology, Government of India, New Delhi, India, for providing Post-Doctoral Research Associate Fellowship.

REFERENCES

1. Stoyanovski, D., Kagan, V., and Packer, L. (1989) *Biochem. Biophys. Res. Commun.*, **160**, 834-838.
2. Ahmad, I., and Suhail, M. (1993) *Oxid. Commun. (Bulgaria)*, **16**, 273-275.
3. Diplock, A. T. (1983) *Ciba Fdn.*, **101**, 45-55.
4. Davies, K. J. A., Sevanian, A., Muakassab-Kelly, S. F., and Hochstein, P. (1986) *Biochem. J.*, **235**, 747-750.
5. Shiru, U. (1989) *Vitaminy*, **63**, 75-85.
6. Zalusky, R. (1970) *Bull. N. Y. Acad. Med.*, **46**, 427-431.
7. Ahmad, I., and Suhail, M. (1998) *Ind. J. Chem. Tech.*, **5**, 107-108.
8. Jackson, J. M., Quinlan, J., and Goatcher, P. (1970) *Br. Med. J.*, **2**, 297-298.
9. Goodman, L. S., and Gilman, L. A. (1985) in *The Pharmacological Basis of Therapeutics*, Macmillan Publishing Co. Inc., New York, pp. 704-706.

10. U. S. Dept. of the Army TM8-227-4 (1963) in *Laboratory Procedures in Clinical Hematology*, US Govt. Printing office, Washington, DC, pp. 427-430.
11. Massoulie, J., and Bon, S. (1982) *Ann. Rev. Neurosci.*, **5**, 57-106.
12. Sorenson, K., Gentinetta, R., and Brodbeck, U. (1982) *J. Neurochem.*, **30**, 1050-1060.
13. Ott, P. (1985) *Biochim. Biophys. Acta*, **822**, 375-392.
14. Henstis, W. H., and McConnel, H. M. (1974) *Biochem. Biophys. Res. Commun.*, **57**, 726-730.
15. Mitruka, B. M., Rawnsley, H. M., and Vadehra, D. V. (1976) in *Animals for Medical Research: Models for the Study of Human Disease*, John Willey & Sons, pp. 591-594.
16. Dacie, J. V., and Lewis, S. M. (1984) in *Practical Haematology*, Churchill-Livingstone Inc., New York, pp. 152-156.
17. Beutler, E. (1984) in *Red Cell Metabolism: A Manual of Biochemical Methods*, Grune and Stratton Inc., New York.
18. Ellman, G. I., Courtney, K. D., Andres, V., Jr., and Featherstone, R. M. (1961) *Biochem. Pharmacol.*, **7**, 88-95.
29. Zak, B., Zlatkis, A., and Boyle, A. J. (1953) *J. Lab. Clin. Med.*, **41**, 486-492.
20. Beutler, E., Duron, O., and Kelly, B. M. (1963) *J. Lab. Clin. Med.*, **61**, 882-890.
21. Stocks, J., and Dormandy, T. L. (1971) *Br. J. Haematol.*, **20**, 95-111.
22. Good, W. (1971) *Exp. Physiol. Biochem.*, **4**, 163-168.
23. Rice-Evans, C. A., and Dunn, M. J. (1982) *Trends Biochem. Sci.*, **7**, 282-286.
24. Sigeru, O., Hatsumi, A., and Magobei, Y. (1989) *Fukuoka Daigaku Yakagaku Kiyo* (Japan), **13**, 25-31.
25. Zirkle, L. G., Jr., Mengel, C. E., Bulter, S. A., and Fuson, R. (1965) *Proc. Soc. Exp. Biol.*, **119**, 833-840.
26. Kosower, N. S., Zipswer, Y., and Faltin, Z. (1982) *Biochim. Biophys. Acta*, **691**, 345-352.
27. Awasthi, Y. C., Garg, H. S., Dao, D. D., Partridge, C. A., and Srivastava, S. K. (1981) *Blood*, **58**, 733-739.
28. Meister, A. (1981) *Trends Biochem. Sci.*, **7**, 231-237.
39. Shinitzky, M., and Souroujan, S. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4428-4431.
30. McCay, P. B., and King, M. M. (1980) in *Vitamin E: A Comprehensive Treatise*, Dekkar, New York, pp. 289-317.
31. Farrel, P., Bieri, J. G., Frantantoni, J. F., Wood, R. E., and Disant'Agree, P. A. (1977) *J. Clin. Invest.*, **60**, 233-241.
32. Suhail, M., and Ahmad, I. (1992) *Bioved*, **3**, 95-98.
33. LeBel, C. P., Odunze, I. N., Adams, J. D., and Bondy, S. C. (1989) *Biochem. Biophys. Res. Commun.*, **163**, 860-866.
34. Rose, C. S., and Gyorgy, P. (1952) *Am. J. Physiol.*, **168**, 414-418.