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HUMAN RED CELLS ENHANCE THE FORMATION OF 5-LIPOXYGENASE-DERIVED PRODUCTS BY NEUTROPHILS

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Upon activation, human neutrophils generate 5-lipoxygenase products which are involved in inflammation as well as other physiological and pathophysiological processes. We have examined the influence of red cells on the generation of lipoxygenase-derived products by neutrophils utilizing high pressure liquid chromatography system which permitted quantitation of 5-HETE, leukotriene B_4 (and its isomers) and the omega oxidation products of leukotriene B_4 (20-hydroxyleukotriene B_4, 20-carboxyleukotriene B_4) within the same sample. Co-incubation of red cells with neutrophils (50:1, red cells:neutrophils) resulted in a 722 percent increase in 5-hydroxyeicosatetraenoic acid production and a slight increase in leukotriene B_4 and its omega oxidation products which were not accompanied by increases in 15-hydroxyeicosatetraenoic acid production. The role of the sulfhydryl status of the red cell and its ability to scavenge hydrogen peroxide were assessed in relationship to the interaction of red cells on the neutrophil-derived lipoxygenase products. Together, these findings indicate that red cells can regulate the levels of lipid-derived mediators produced by neutrophils. Moreover, they suggest that red cell-neutrophil interactions may be of importance in inflammatory reactions.

KEY WORDS: Eicosanoids, blood cells.

ABBREVIATIONS: HPETE (hydroperoxyeicosatetraenoic acid); HETE (hydroxyeicosatetraenoic acid); LO (lipoxygenase); LTA_4 (leukotriene A_4, 5S-trans-5(6)-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid); LTB_4 (leukotriene B_4, 5S,12R-dehydroxy-6,14-cis-8,11-trans-dihydroxyeicosatetraenoic acid); 20-OH-LTB_4 (20-hydroxyleukotriene B_4); 20-COOH-LTB_4 (20-carboxyleukotriene B_4); PBS (phosphate buffered saline); NEM (N-ethylmaleimide); AT (3-amino-1,2,4-triazole).

INTRODUCTION

Red cells possess a variety of mechanisms for coping with oxidative stress generated by xenobiotics.[1] Recent biological evidence indicates that the red cell can protect other cells from an oxidative insult.[2-4] The level of this protection is related to scavenging of hydrogen peroxide by red cells by either catalase or glutathione-dependent metabolism. Intracellular superoxide dismutase is not efficient in removing extracellularly generated superoxide, probably a reflection of the limited capacity of superoxide to traverse the intact red cell membrane. In contrast, under the same experimental conditions red cells efficiently removed hydrogen peroxide generated in the extracellular environment, particularly by catalase. The removal of hydrogen peroxide by red cells can also lead to decreased formation of hydroxyl radical and white blood cell-myeloperoxidase-mediated hypochlorous acid.[5]

In addition to generating active oxygen species, human neutrophils can generate lipoxygenase products of arachidonic acid which also serve as mediators of inflamma-

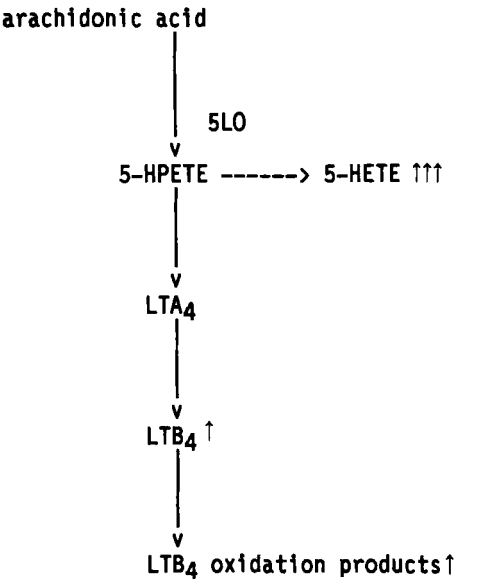

FIGURE 1 Lipoxygenase pathway in human neutrophils. Arrows represent increases found in red cell-neutrophil interaction (see Table 1).

tion.[6] Leukotrienes are generated by initial oxygenation of arachidonic acid via 5-lipoxygenase (Figure 1), while interactions between 5- and 15-lipoxygenase can lead to the formation of lipoxins. Lipoxygenase products of arachidonic acid display a diverse array of biological activities which are of interest in inflammation, hemostasis and reperfusion injury.[6] Therefore, a possible role for red cells in the formation and actions of lipoxygenase products is of particular interest.

Although mature human red cells are devoid of lipoxygenases they can transform exogenous LTA_4 to LTB_4 suggesting that red cells can amplify the level of LTB_4 at sites of neutrophil activation.[7] When co-incubated *in vitro*, human red cells and neutrophils generated elevated levels of LTB_4, providing further evidence that human red cells can transform neutrophil-derived LTA_4 to LTB_4.[8] Thus to date, red cells can influence the levels of neutrophil-derived mediators by at least two pathways, one involving the scavenging of reduced products of oxygen and another involving the transcellular metabolism of LTA_4 leading to increased levels of LTB_4.

To further understand the nature of red cell-neutrophil interactions we have monitored the levels of both 5- and 15-lipoxygenase products generated upon co-incubation of these cells. We present evidence that red cells can selectively enhance the levels of the 5-lipoxygenase but not 15-lipoxygenase-derived products of human neutrophils.

MATERIALS AND METHODS

Adult fresh human blood was drawn into heparin. A small portion was used to prepare red cells. This portion was washed three times with Dulbecco's PBS. The

remaining blood was used for the isolation of neutrophils by dextran sedimentation followed by Ficoll-Hyaque gradient centrifugation.[9] Cell suspensions containing 98 ± 1 percent neutrophils were warmed at 37°C for 5 minutes in Dulbecco's PBS prior to co-incubation with red cells. Co-incubations of red cells and neutrophils, or neutrophils alone, were exposed to A-23187 (2.5 μM) for 20 min, at 37°C. Incubations were terminated by addition of ethanol (2 vol). Lipoxygenase products were extracted and quantitated as described.[10] Briefly, the lipoxygenase products were separated by reverse phase high pressure liquid chromatography utilizing a LKB dual-pump system equipped with an Altex Ultrasphere-ODS (4.6 mm × 25 cm) column, injector and solvent controller (LKB, Brommer, Sweden) and a photodiode array rapid spectral detector linked to an AT&T PC6300. Post run high pressure liquid chromatography analyses were performed with a 2140–202 Wavescan program and Nelson Analytical 3000 series chromatography data system. PGB_2 was used as an internal standard.

RESULTS

Co-incubation of red cells with neutrophils at a 50:1 ratio of red cells to neutrophils resulted in a 722 percent increase in 5-HETE and a slight increase in LTB_4 and its omega oxidation products without a coincident increase in 15-HETE over that seen with neutrophils alone (Table 1). To determine if the scavenging of hydrogen peroxide by the red cell plays a role in the lipoxygenase pathways of neutrophils, red cells were pretreated with AT and hydrogen peroxide to irreversibly inactivate catalase. When these red cells were co-incubated with neutrophils a 173 percent increase in 5-HETE production was observed when compared to the co-incubation of red cells with neutrophils. When catalase was added to neutrophils without red cells present 5-HETE levels showed essentially no change when compared to its production by neutrophils incubated in the absence of catalase. To determine if red cells might influence 5-lipoxygenase activity through its glutathione sulfhydryls, red cells were preincubated with NEM. When NEM-pretreated red cells were coincubated with

TABLE 1
Neutrophil-derived lipoxygenase products.

	Percentage changes				
	5-HETE	15-HETE	LTB_4	20-OH LTB_4	20-COOH LTB_4
RBC*	722	nc	3	50	17
CAT*	13	nc	−23	2	12
AT+	173	−25	−39	−33	−3
NEM+	−65	nc	−23	−45	−45

* Compared to lipoxygenase products produced by neutrophils alone.
+ compared to lipoxygenase products produced in co-incubation of neutrophils and red cells.
Red cell/neutrophil ratio 50:1 (neutrophils-20 × 10^6 cells/ml); Incubations at 37°C for 20 minutes; Cells were stimulated with A_{23187} (2.5 μM); In experiments with catalase, 1500 units/ml of catalase was added to neutrophils; AT-treated red cells (2 percent hematocrit) pretreated with 0.05 M AT and 4.5. mM hydrogen peroxide for one hour at 37°C and washed three times with Dulbecco's PBS before using in co-incubations; NEM-pretreated red cells were made by incubation of red cells (25 percent hematocrit) with 2.5 μM NEM/ml in Dulbecco's PBS, incubated 1/2 hour at 37°C and then washed three times with Dulbecco's PBS before using in co-incubations; Recovery of 5-HETE is 3.44 ng. in neutrophils. Each value is the mean percentage change of two samples from a set of representative experiments.

neutrophils 5-HETE production decreased to 65 percent of the level of that observed with co-incubation of red cells and neutrophils.

DISCUSSION

The presence of platelets, endothelial cells or red blood cells may change both the levels and profile of eicosanoids generated by human neutrophils.[8,11,12] The change in lipoxygenase products can occur by the capacity of the other cell type to metabolize neutrophil-derived lipoxygenase products. In previous studies on neutrophil-red cell interactions it was observed that red cells are capable of metabolizing the neutrophil-derived product LTA_4 to LTB_4, perhaps by means of red cell epoxide hydrolase activity.[7,8] In our experiments we observed that red cells not only interact with neutrophils to enhance LTB_4 levels but that they can regulate the activity of the entire 5-lipoxygenase pathway in a manner that leads to an enhancement of 5-HETE production. The action of the red cell on neutrophils appears to be selective since the levels of 15-HETE and lipoxins were unaffected.

When red cell catalase activity is irreversibly inactivated a further increase occurred in the formation of 5-HETE. No apparent change was observed with catalase exposed to neutrophils in the absence of red cells, suggesting that the peroxidatic activity of catalase in red cells may play a role in the activation of the 5-lipoxygenase pathway. The specific reductant responsible for this activity is not apparent from these experiments.

The 5-lipoxygenase activity of neutrophils could be regulated by the glutathione status of the neutrophil. Under conditions of oxidative stress, there was an enhancement of 5-lipoxygenase activity.[13] The experiments with NEM-pretreated red cells and neutrophils showed decreased formation of 5-lipoxygenase products and therefore suggest that reduced sulfhydryls in red cells may contribute to the stimulation of 5-lipoxygenase pathway of the neutrophil.

Neutrophil-derived lipoxygenase products may play a significant role in myocardial damage in the reperfusion of the myocardium following alteration in blood flow to cardiac muscle. Neutrophils accumulate in areas of ischemic myocardial injury and produce inflammatory mediators.[14,15] Since red cells are generally a component of the inflammatory response, their influence on the inflammatory response could be significant, especially if they enhance production of 5-lipoxygenase pathway products such as 5-HETE and LTB_4, which can serve as chemotactic stimuli. One could thus envision further inflammatory response due to the neutrophil-red cell interaction and a worsening of myocardial damage. The modification of 5-lipoxygenase activity in the neutrophil by red cells could represent an important contribution to the inflammatory response in reperfusion injury.

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References

1. Stern, A. Red cell oxidative damage In Oxidative Stress, H. Sies, editor, Academic Press, Orlando, FL 331–349, (1985).
2. Van Asbeck, B., Hoidal, S., Vercellotti, G.M., Schwartz, B.A., Moldow, C.F. and Jacob, H.S. *Science,* **227**, 756–759, (1985).
3. Toth, K.M., Clifford, D.P., Berger, E.M., White, C.W. and Repine, J.E. *J. Clin. Invest.*, **74**, 292–295, (1985).
4. Agar, N.S., Sadrazadeh, S.M.H., Hallaway, P.E. and Eaton, J.W. *J. Clin. Invest.*, **77**, 319–321, (1986).
5. Winterbourn, C.C. and Stern, A. *J. Clin. Invest.*, **80**, 1486–1491, (1987).
6. Samuelsson, B., Dahlin, S-E., Lindgren, J.A., Rouzer, C.A. and Serhan, C.N. *Science,* **237**, 1171–1176, (1987).
7. Fitzpatrick, F., Liggett, W., McGee, J., Bunting, S., Morton, D. and Samuelsson, B. *J. Biol. Chem.*, **259**, 11403–11407, (1984).
8. McGee, J.E. and Fitzpatrick, F.A. *Proc. Nat. Acad. Sci. (USA),* **83**, 1349–1353, (1986).
9. Boyum, A. *Scand. J. Clin. Lab. Invest.*, 21, *Suppl.*, **97**, 77–89, (1968).
10. Serhan, C.N. *Biochim. Biophys. Acta*, (1989), in press.
11. Marcus, C.N., Rutherford, L.E., Korchak, H.M. and Weissmann, G. *Biochem. Biophys. Res. Commun.*, **109**, 130–137, (1982).
12. Feinmark, S.J. and Cannon. P.J. *J. Biol. Chem.*, **261**, 16466–16472, (1986).
13. Hatzelmann, A. and Ullrich, V. *Eur. J. Biochem.*, **169**, 175–184, (1987).
14. Jolly, S.R., Kane, W.J., Hook, B.G., Abrams, G.D., Kundel, S.C. and Lucchesi, B.R. *Am. Heart J.*, **112**, 682–690, (1986).
15. Simpson, P.J., Fantone, J.C. and Lucchesi, B/R. Myocardial Ischemia and Reperfusion Injury: Oxygen radicals and the role of the neutrophil. In Oxygen Radicals and Tissue Injury, Federation of American Society for Experimental Biology, Bethesda, MD pp. 63–77, (1988).

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