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PRO-HEMOLYTIC EFFECT OF ALDEHYDIC PRODUCTS OF LIPID PEROXIDATION

G. POLI,[†] F. BIASI, E. CHIARPOTTO, R. CARINI, G. CECCHINI, U. RAMENGHI[‡] and M.U. DIANZANI.

Institute of General Pathology, University of Torino, Corso Raffaello 30, 10125 Torino, Italy
[‡] *Institute of Paediatrics, University of Torino, Piazza Polonia 94, 10125 Torino, Italy*

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In order to evaluate the pro-hemolytic action exerted by different classes of biogenic aldehydes, normal red cells obtained from human beings of both sexes were incubated at 37°C under iso or hypo-osmotic conditions in the presence of hydroxyalkenals or alkanals, in a concentration compatible with those actually recovered during red cell lipid peroxidation. None of the tested aldehydes showed a direct hemolytic effect, i.e. red cell lysis in iso-osmotic conditions. Conversely, almost all assayed alkanals and hydroxyalkenals exibited a pre-lytic damage of human erythrocytes, as detected in the red cells suspended in hypo-osmotic medium. The highest pro-hemolytic effect was displayed by hexanal, nonanal, 2-nonenal and 4-hydroxynonenal.

KEY WORDS: Aldehydes, lipid peroxidation, hemolysis, 4-hydroxynonenal, hexanal.

INTRODUCTION

An increasing number of experimental observations suggests the possible involvement of oxidative stress among the mechanisms of damage eventually leading to red cell lysis.[1-3] Actually, the erythrocyte is in principle one of the cell types most susceptible to pro-oxidant stimuli, due to its high percent content of arachidonic acid[4] and in general of polyunsaturated fatty acids. Consistently, in several hemolytic anemias the occurrence of biochemical events able to overhelm the red cell antioxidant defenses has been described.[1,3]

In relation to the kind of lipid peroxidation products possibly involved in the erythrocyte damage, several years ago filtrates obtained from peroxidising liver microsomes have been shown to produce pre-lytic damage in rat erythrocytes, detected in terms of an increased susceptibility to osmotic fragility tests.[5] More recently, the analysis of the pre-lytic action of the different fractions obtained by thin layer chromatography separation of the above mentioned microsomal filtrates allowed to demonstrate that 4-hydroxynonenal, aldehydic product of lipid peroxidation[6] exerts pro-hemolitic effect at micromolar concentration at least in the case of rat red cells.[7]

Since numerous are the aldehydic products proved to originate not only in rat liver membranes[8,9] but also in human red cells[10,11] undergoing oxidative stress, the pre-lytic effect of different classes of biogenic carbonyls has been evaluated using human

† To whom to address correspondence.

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TABLE I
Decreased resistance to osmotic stress by red cells incubated at 37°C for 180 min under hypotonic conditions in the presence of different aldehydes[a]

Aldehyde addition		% Hemolysis[b]
None		31 ± 3
Propanal	1 mM	42 ± 4
Butanal	1 mM	42 ± 2
Pentanal	1 mM	43 ± 4
Hexanal	0.5 mM	40 ± 5
Hexanal	1 mM	57 ± 2
Eptanal	1 mM	41 ± 2
Octanal	1 mM	42 ± 3
Nonanal	0.5 mM	47 ± 6
Nonanal	1 mM	72 ± 10
Propanal + Butanal + Pentanal + Hexanal (1:1:1:1)	1 mM	43 ± 3
2-Nonenal	0.5 mM	45 ± 1
2-Nonenal	1 mM	62 ± 9
4-OH-Hexenal	0.5 mM	53 ± 22
4-OH-Hexenal	1 mM	63 ± 18
4-OH-Nonenal	0.5 mM	45 ± 7
4-OH-Nonenal	1 mM	68 ± 14

[a] See Methods section for details on the osmotic fragility test employed.
[b] Values are means ± SD of 3–4 experiments and are expressed as percent of total hemolysis obtained with identical red cells aliquots but resuspended in distilled water.

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erythrocytes and the corresponding results are the subject of the present report. The concentration of the tested carbonyls was compatible with that actually recovered during red cell lipid peroxidation.[10,11]

MATERIALS AND METHODS

Human red cells were obtained from adult healthy subjects of both sexes. After centrifugation of the blood samples (500 g × 10 min), plasma was discarded and red cells washed two times in NaCl 0.9%, then resuspended in saline to make a 20% (v/v) cell concentration. The erythrocyte suspensions so obtained were kept over night at 4°C. To perform erythrocyte incubations under normo- or hypo-osmotic conditions 0.2 ml cell aliquots were brought up to 2 ml with 0.05 M Na-phosphate buffer (pH 6.6) + 0.09 M NaCl or with the only buffer respectively.

Cell incubation was then carried out at 37°C for 30, 60, 90, 150 and 180 min, in the presence or in the absence of defined aldehydes at the final concentration of 0.5–1 mM. At the end, the different samples were suitably diluted with isotonic phosphate buffer (1:4, v/v) and centrifuged at 500 g × 10 min.

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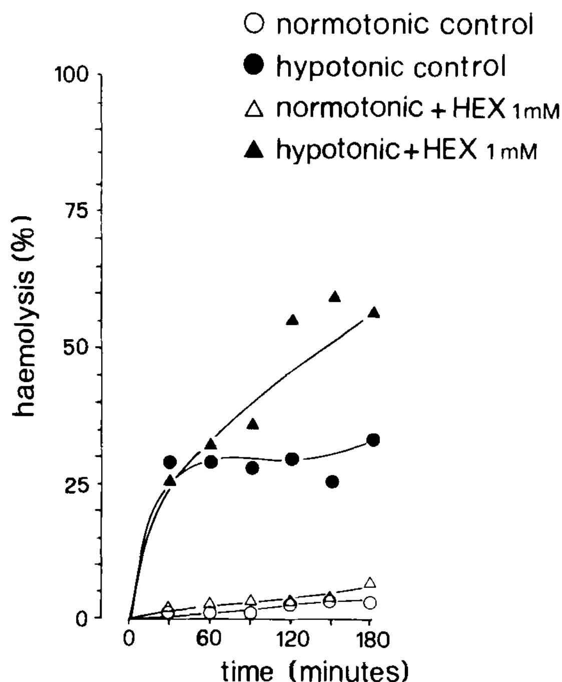

FIGURE 1 Time course incubation of human red cell suspensions (20%, v/v) in normotonic or hypotonic medium (see Methods for details). Where employed, hexanal was added to the cell suspension at time zero.

Percent hemolysis was determined by measuring the optical density at 542 mm of the supernatant fractions, and comparing it to the absorbance of erythrocyte suspensions hemolysed in distilled water, value assumed as 100%. The purity and the concentration of the synthetic aldehydes used was routinely checked by h.p.l.c.

RESULTS AND DISCUSSION

Two important points have to be once again stressed examining the present results: (i) all the carbonyl compounds employed in this study have been demonstrated to originate during peroxidative breakdown of rat liver membranes[8,9] and human erythrocyte membranes,[10,11] i.e. they are biogenic; (ii) the '*in vitro*' oxidative stress induced by t-butyl-hydroperoxide in normal human red cells leads to the accumulation of 1–1.5 mM of unpolar carbonyls, whose about 50% are alkanals and 2-alkenals, 30% hydroxyalkenals and 20% osazones;[11] in other words the range of aldehyde concentration used takes into account such a finding.

None of the tested aldehydes showed a direct hemolytic effect, i.e. no red cell lysis was evident under normotonic conditions. Conversely, several assayed aldehydes exhibited a consistent pre-lytic damage detectable when red cells were incubated under hypotonic conditions (corresponding to the osmotic pressure given by 0.35–0.4% NaCl). The most effective compounds in the class of alkanals, 2-alkenals were hexanal (HEX) and 2-nonenal; their action was similar to that of 4-hydroxy-

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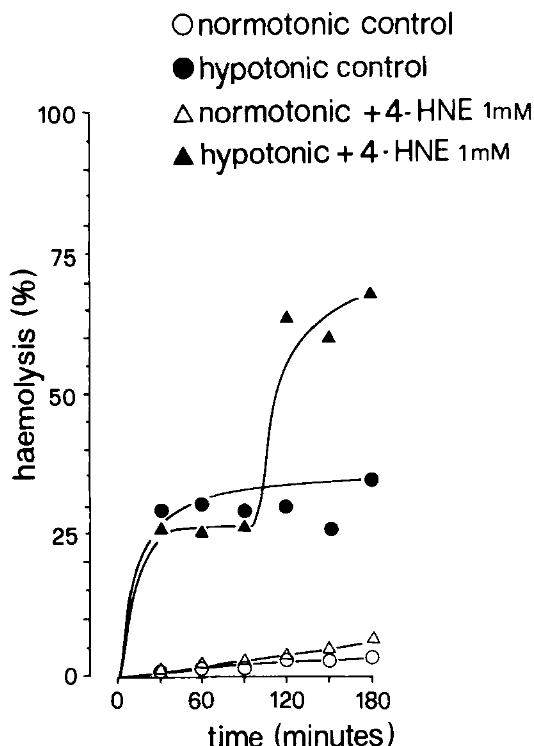

FIGURE 2 Time course incubation of human red cell suspensions (20%, v/v) in normotonic or hypotonic medium (see Methods). Where employed 4-hydroxynonenal was added to the cell suspension at time zero.

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nonenal (4HNE) and 4-hydroxyhexenal (Table I). It is noteworthy that the mixture of different alkanals to reach 1 mM final concentration in the erythrocyte suspension gave results similar to those obtained with the single compounds (Table I). This last finding better mimics the hematological conditions in which the total amount of alkanals can reach the millimolar range. In Figs. 1 and 2 is reported the time course of the effect exerted by HEX and 4-HNE, respectively, on normotonic and hypotonic red cell suspensions. The pro-hemolytic action of the examined aldehydes appears significantly evident after a lag period of about two hours.

Such a latency could be interpreted as the time necessary for hypothetical mechanisms of lysis to accomplish their action. The monitoring of the reduced glutathione content in the erythrocytes during the incubation with HEX and 4-HNE seems to exclude a major role of the tripeptide in inducing the observed effect. In fact, while the pro-hemolytic action of the two carbonyls is quite similar, only 4-HNE is able to relevantly reduce the GSH content of red cells (by 70–80%), not modified at all by the treatment with hexanal (data not shown).

In conclusion, since the exposure of human normal red cells to strong pro-oxidant stimuli can lead to millimolar steady-state concentrations of aldehydic compounds, the demonstration of their pro-hemolytic effect within this range could be of some interest. It is essential in any case to evaluate if aldehydes produced during minimal

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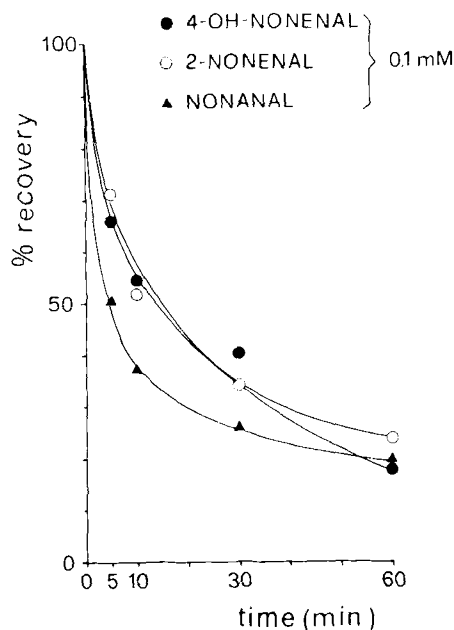

FIGURE 3 Disapperance of different carbonyls externally added to normal red cell 20% suspension in NaCl 0.9%. Recovery was determined by h.p.l.c. as described elsewhere.[9]

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or medium but recurrent oxidative stress can accumulate in the red cells; carbonyl metabolism in human erythrocytes should be investigated. As reported in Fig. 3, the half-time for disappearance of nonanal externally added to red cell suspensions (20%, v/v in saline) is five minutes, while that of 2-nonenal and 4-hydroxynonenal is ten minutes. The erythrocyte metabolism of the other aldehydes is under study.

In relation to the increased susceptibility of aldehyde-treated red cells to the osmotic stress, preliminary experiments suggest that it occurs through a decreased membrane fluidity due to aldehyde covalent binding to membrane components. Actually, the ability of some alkanals and alkenals to cross-link with erythrocyte membrane protein has been recently demonstrated,[12] also the association of lipid peroxidation and polymerisation of membrane proteins with erythrocyte aging.[13] Finally, the spontaneous or metabolic partial transformation of aldehydes into the corresponding free fatty acids must be considered with regard to hemolysis, due to the detergent activity of the latter class of substances.

Acknowledgment

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