Lack of Correlation Between TBARS Production and PUFA Degradation During Incubation of Membrane Erythrocytes in an OH• (Fe2+/H2O2) Generator System

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ABSTRACT

We investigated the effects of an OH• (Fe2+/H2O2) generator system on erythrocyte membrane, particularly the time-course of lipid peroxidation as estimated by measurement of conjugated dienes, thiobarbituric reactive substances (TBARS), lipofuscin-like pigments, and α-tocopherol. Polyunsaturated fatty acids (PUFAs), especially arachidonic acid (20:4 ω 6) and docosahexenoic acid (22:6 ω 3), were also measured. Erythrocyte membranes were suspended in phosphate buffer containing Fe2+ (200 µM) and H2O2 (1.42 mM), and incubated in a shaking water bath at 37°C. Initially, there was an increase in TBARS and lipofuscin-like pigments, two well-known end products of PUFA oxidative degradation, whereas PUFAs remained unchanged (incubation time: 1 h). After two or more hours of incubation, marked lipid peroxidation was noted, with the appearance of conjugated dienes and a decrease of PUFAs, indicating that lipid peroxidation had occurred after a lag phase during which TBARS were not produced from PUFAs. This suggests that another OH• target was involved.

Index Entries: Erythrocytes; lipid peroxidation; hydroxyl radicals; polyunsaturated fatty acids.

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INTRODUCTION

In several circumstances during oxidative stress, we studied the occurrence of thiobarbituric reactive substances (TBARS) in relation with other more specific signs of lipid peroxidation, i.e., appearance of conjugated dienes and disappearance of polyunsaturated fatty acids (PUFAs). The results suggested a time lag between these different parameters. To investigate this process, we used the well-known oxidative stress model by incubating erythrocyte membranes in the presence of a system generating OH• radicals. Over the course of 24 h, TBARS, conjugated dienes, lipofuscin-like pigments, α-tocopherol, and PUFAs were observed simultaneously.

METHODS

Human erythrocyte membranes were prepared as described by Dodge et al. (1), using a 5 mM phosphate buffer, pH 7.4, for the final washing. The protein concentration of the preparation was around 5 mg/mL. Membranes (750 µL) were suspended in 5 mM phosphate buffer, pH 7.4, containing 200 µM FeSO₄ and 1.42 mM H₂O₂, and then incubated. FeSO₄ and H₂O₂ were omitted in controls. Incubation was performed in a shaking water bath at 37°C. At the end of the incubation period, butylated hydroxytoluene (42 µM) and EDTA (21 mM) were added.

TBARS were measured according to the method of Wilbur et al. (2), as modified by Sawas and Gilbert (3), using 500 µL of incubation medium. Lipids were extracted from 250 µL of incubation medium in chloroform/methanol (1:2) according to the procedure of Bligh and Dyer (4).

For dienes detection, final lipid extracts were evaporated under a stream of nitrogen and redissolved in 3 mL hexane. The scan was performed from 200 to 300 nm, and the second derivative spectrum was calculated according to Corongiu and Milia (5). Conjugated dienes corresponded to a minimum peak at 233–235 nm. Results are expressed in arbitrary units as d²A/dλ², where A = absorbance.

Lipofuscin-like pigments were determined in the Bligh and Dyer extract by spectrofluorometric assay according to Bidlack and Tappel (6). Results are expressed as a percentage of fluorescence vs controls.

Fatty acids were analyzed by gas chromatography as previously described (7). Results are expressed as percentages of total fatty acids.

α-Tocopherol was assayed from 500 µL of membrane suspension, after saponification and extraction, by liquid chromatographic separation and fluorometric detection, as described by Hatam and Kayden (8) and Mino et al. (9).