Agents and Actions, vol. 22, 3/4 (1987) 0065-4299/87/040355-02 $ 1.50+0.20/0
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Antioxidant defence and protection of cell membranes from lipid peroxidation

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Peroxidation of polyunsatured lipids and free radical formation cause damage to cellular components, particularly to biological membranes which develop marked fragility. Animal tissues are defended against this damage by their antioxidant capability. Previous results have shown that erythrocytes and platelets are very rich in glutathione peroxidase (GSH-Px) a selenium-dependent enzyme which increases in conditions of Se dietary supplementation. Aortas taken from Se-treated rats synthesized more prostacyclin-like activity in comparison to controls [1] and production was inhibited by aminotriazole, a peroxidase inhibitor [2]. Conditions of low antioxidant defence of plasma, as occurs in vitamin E-deficient rats, resulted in high thromboxane B_2 (TxB_2) production in serum, unbalanced ratio between TxB_2 and 6-Keto-$PGF_{1\alpha}$, and reduced "prostacyclin-stimulating factor" [3]. We investigated the type of tissue toxicity deriving from chronic deficiency of either vit. E or selenium and evaluated the reliability of peripheral markers of tissue toxicity in these conditions [4]. Heart and kidney malondialdehyde (MDA), a typical product of lipid peroxidation, was significantly increased in vit. E and Se-deficient rats. The iron-binding capacity of plasma was reduced in Se-deficient and increased in Se-supplemented animals. In erythrocytes, the resistance to osmotic haemolysis was low in vit. E and Se-deficient but high in Se-supplemented animals, a condition of high GSH-Px activity. Platelet MDA formation induced by arachidonic acid (AA) raised both in Se- and in vit. E-deficient groups and may be regarded as a peripheral marker of reduced antioxidant defence at tissue level. We investigated the influence of the extracellular antioxidant potential in the mechanisms of stimulus-response coupling in platelets. The interaction of platelets with physiological activating agents such as thrombin, platelet-activating factor (PAF), vasopressin and ADP, is followed by a rapid increase in cytosolic Ca^{2+} concentration, accompanied by functional responses, namely shape change, aggregation and secretion.

The rise in cytosolic Ca^{2+} is largely due to an extracellular Ca^{2+} influx and, to a lesser extent, to release from the dense tubular system. It has been shown that agonists act on platelets by activating a phosphodiesterase which specifically splits polyphosphoinositides, generating the second messengers diacylglycerol responsible for the activation of protein kinase C and inositol 1,4,5-triphosphate, inducing release of Ca^{2+} from intracellular stores. The rise of cytosolic Ca^{2+} and the activation of protein kinase C jointly cooperate to induce platelet aggregation and release secretary granules. We showed that a variety of antioxidant agents such as butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), nordihydroguaiaretic acid (NDGA) and the one-electron donor 1,1′-dimethylferrocene inhibit increase of cytosolic Ca^{2+}, as monitored by quin 2 acetoxymethylester fluorescence, induced by the physiological agonists thrombin, vasopressin and platelet-activating factor in aspirinated human

platelets [5]. These antioxidants also inhibit shape change, aggregation and ATP secretion. Cytosolic Ca^{2+} increase originating from intracellular stores in the presence of EGTA was also inhibited by antioxidants. It is suggested that some still unknown free radical-dependent pathways are involved in the mechanism of platelet activation. Our results show that the modulation of the oxidant-antioxidant balance *in vivo* and *in vitro* may strongly influence many physiological cellular functions.

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