Biological and Biochemical Responses of Skin Fibroblast to Oxidative Stress Induced by Fe³⁺-Ascorbate

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In inflammation, various kinds of inflammatory cells are mobilized and some of them produce and secrete oxidative molecules such as oxygen radicals and hypochlorous acid. Oxidative molecules thus formed begin to peroxide lipids and proteins and deteriorate their functions. However, the effects of these oxidants on biological and biochemical behavior of living cells are poorly understood. Therefore, cultured fibroblast was studied. Oxidative stress was chemically induced by Fe³⁺-ascorbate.

Fibroblasts obtained from normal skin of a healthy man aged 62 were cultured in Eagle MEM supplemented with 10% fetal bovine serum and antibiotics in an atmosphere with 5% CO₂ at 37 °C. The experiment was started when fibroblasts became confluent in Petri dishes with 6-cm diameter and the medium was changed into serum-free, phenol red-free MEM. As an oxidative reagent, FeCl₃ (0.2 mM) and L-ascorbic acid (0.1 mM) were added to the culture and lipid peroxide secreted into the medium was measured according to the method of Naito and Yamanaka [1]. Briefly, culture supernatant was acidified with HCl, mixed with 0.67% 2-thiobarbituric acid (TBA) solution and heated at 95 °C for 30 min. After cooling, methanol/n-butanol (15:85, by volume) was added, shaken vigorously and the absorbance of butanol layer (535 nm) was measured spectrophotometrically with Du-50, Beckman Co. Lipid peroxide level was expressed in terms of nanomoles of malondialdehyde. During this experiment, cell viability was well kept. Lipid peroxide level in the media was gradually increased by the addition of Fe³⁺-ascorbate (0.45 nmol/10⁶ cells at 12 h, 0.60 nmol/ 10⁶ cells at 24 h, 0.51 nmol/10⁶ cells at 48 h, 0.90 nmol/10⁶ cells at 72 h) as compared with the control (no such additives) (0.15 nmol/10⁶ cells at 0 time, 0.27 nmol/10⁶ cells at 12 h, 0.46 nmol/10⁶ cells at 24 h, 0.27 nmol/10⁶ cells at 48 h, 0.42 nmol/10⁶ cells at 72 h). Addition of antioxidants, glutathione (100 μ M) and DL- α -tocopherol (2 mM)

to Fe³⁺-ascorbate system effectively inhibited lipid peroxidation (46% inhibition by the former and 32% by the latter). Along with lipid peroxidation, glutathione peroxidase activity in fibroblast (postnuclear supernatant), measured by the method of Paglia and Valentine, was elevated in Fe³⁺-ascorbate system (260 mU/mg protein at 24 h, 530 mU/mg at 48 h, 270 mU/mg at 72 h) as compared with that in the control system (100 mU/mg protein at 24 h, 230 mU/mg at 48 h, 90 mU/mg at 72 h). Morphologically, fibroblasts became thin in parallel with the progress of lipid peroxidation. Proteins in postnuclear supernatant of fibroblast were examined by SDS-poly-acrylamide gel electrophoresis, which revealed a synthesis of a high molecular weight protein (above 200 kd) in the Fe³⁺-ascorbate system but not in the control system. Since it is known that lipid peroxide and its secondary oxidative products often produce complexes with proteins or amino acids, e.g. lipofuscin, the high molecular weight protein may be a cross-linked one produced by such conjugation. The molecular size may suggest that it is of collagen origin. The behavior of fibroblasts as described above, some beneficial and others harmful, could modify the course of inflammation in vivo and may finally contribute to the settlement of inflammation, causing fibrosis in the last stage.

On the subject of fibrosis, more detailed studies are expected.

References

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