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Superoxide Dismutase Content in Human Epidermis and Squamous Cell Epithelioma*

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The role of superoxide dismutase (SOD) in the protection of the cell against the toxicity of reactive oxygen intermediates is well established [5]. Indeed, the enzyme provides defense not only against superoxide radicals (O_2^-) by catalyzing the reaction

$$2\,O_2^- + 2\,H^+ \longrightarrow H_2O_2 + O_2$$

but also against hydroxyl radicals ($OH^\cdot$) which may be generated by the Haber-Weiss reaction (6)

$$O_2^- + H_2O_2 \longrightarrow O_2 + OH^- + OH^\cdot .$$

In this respect, also the potential toxicity of H_2O_2, as a generator of $OH^\cdot$ radicals in the Haber-Weiss reaction, is minimized by SOD which keeps the concentration of O_2^- low.

A study of the SOD content in the epidermis and epidermal tumors appears of great interest for various reasons. Free radicals may be generated in the skin during biological oxidations and as a consequence of photochemical reactions. Aging of the skin and formation of skin tumors is undoubtedly related to the damaging effect of radiation through the formation of oxygen radicals. Moreover, a relationship has been suggested between radiation sensitivity and SOD content of human tumors [14] which implies a possible diagnostic tool available to the clinician on a routine basis.

After the first observation of a lack of mitochondrial (manganese) SOD in two fast-growing experimental tumors (Morris hepatoma 3924A and Ehrlich ascites tumor cells) published by Dionisi et al. [4], other reports confirmed these data and also showed that tumor cells are poor in the cytosolic (copper and zinc) enzyme [3, 10, 12, 13, 15]. The current point of view is that tumors exhibit usually, but not

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Abbreviation: SOD = superoxide dismutase

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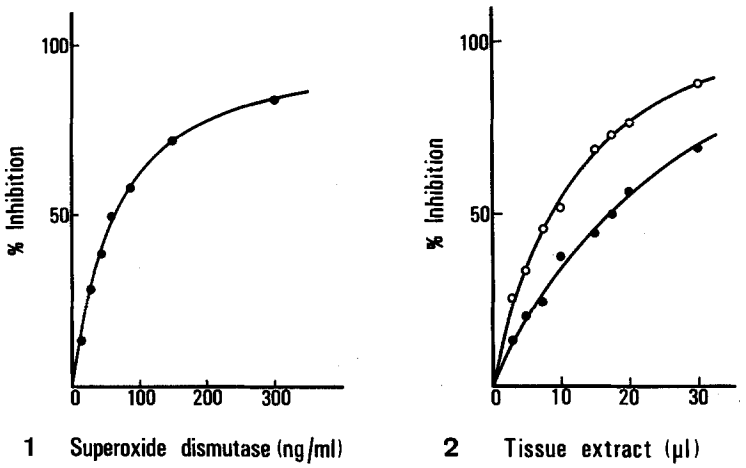

Fig. 1. Calibration curve obtained with SOD purified from bovine blood cells. The percent inhibition of autoxidation of epinephrine by SOD is given as a function of the enzyme concentration. Autoxidation of epinephrine to adrenochrome was followed at 480 – 575 nm, using a dual-wavelength/split-beam Aminco-Chance spectrophotometer, in a reaction mixture containing 50 mM sodium carbonate, 0.1 mM EDTA, and 0.4 mM epinephrine (pH 10.2). Temperature, 30° C. Fifty percent inhibition was attained by 65 ng per ml of SOD

Fig. 2. Comparison between two inhibition curves of adrenochrome formation by tissue extracts prepared from human epidermis (○——○) and squamous cell epithelioma (●——●). For preparation of tissue extracts and the assay method, see text and legend to Fig. 1. Superoxide dismutase was extracted by 1.4 g of epidermis and 0.97 g of epithelioma and dissolved in appropriate amounts of double-distilled water to give the same dilution. Fifty percent inhibition was achieved by 8.75 and 17.5 µl of epidermis and tumor extracts, respectively

always, a lowered Cu-Zn SOD activity and always a loss of Mn SOD activity (see [11] for review). Furthermore, it has recently been observed that in Morris hepatomas with different growth rates the decrease in Cu-Zn SOD is correlated to the growth rate, the lowest activities being associated with the fastest growth rates [1, 2].

The present work is a preliminary study on the determination of Cu-Zn SOD content in a human epidermal tumor, the squamous cell epithelioma. The extraction procedure of McCord and Fridovich [8] and the epinephrine autoxidation method [9] appeared to be suitable for the assay of the enzyme content in small specimens of tissue (as little as 0.7 g). From a comparison with a standard curve of purified bovine blood SOD it was calculated that the amount of enzyme in the tumor is about 65% of that present in extracts prepared with the same procedure from normal human epidermis.

During surgical operations thin slices of epidermis, weighing 1.5 – 2.0 g, were obtained from nonexposed areas (thighs) of humans between 40 and 60 years of age. Squamous cell epithelioma specimens taken from various sites varied in weight from 0.7 – 2.5 g. Both the tissues were cooled in ice-cold 0.15 M NaCl. Tumors were dissected and the adhering foreign tissue, the necrotic and hemorrhagic areas were removed. After two washings in 0.15 M NaCl, the tissues were finely minced

with scissors and homogenized with an Ultra-Turrax (Janke and Kunkel KG) in 10 volumes of 0.05 M K_2HPO_4, 0.1 mM EDTA, pH 7.8 for eight periods of 15 s each, at intervals of 30 s. The rest of the procedure for extraction of cytosolic SOD was essentially that reported by McCord and Fridovich [8] with the omission of the DE 32 column [7], as suggested by Sykes et al. [14]. The enzyme was assayed by testing the inhibition of increasing volumes of tissue extract on the autoxidation of epinephrine to adrenochrome, according to the method of Misra and Fridovich [9]. The 50% inhibition obtained from such curves was compared with that obtained from a standard curve for bovine blood SOD (Fig. 1).

The results obtained with extracts from a sample of epidermis and of a squamous cell epithelioma are shown in Fig. 2. It appears evident from the comparison of the two curves that it is necessary to add to the assay system a volume of tumor extract equal to about twice that of epidermal tissue extract to obtain in both cases 50% inhibition of adrenochrome formation. Indeed, the calculated values of SOD content in the experiment reported in Fig. 2 were 13.3 and 26.4 μg/g of tumor and epidermis, respectively. A series of three experiments of this type for each tissue gave a mean value of SOD equal to 16.0 ± 2.2 μg/g for the epithelioma compared to 24.2 ± 1.1 μg/g for the epidermis.

Although the present data do not yet allow a comprehensive discussion, they are already indicative of significant differences in the content of cytosolic SOD between an epidermal tumor, the squamous cell epithelioma, and its corresponding normal tissue. Such results integrated by those from other skin tumors, e.g., basal cell epithelioma and melanoma, might provide interesting information on a possible relationship between SOD content, growth rate, and radiation sensitivity which, among other aspects, could be useful in the therapeutic approach to epidermal tumors.

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