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Superoxide dismutase activity in larynx cancer and cervical lymph node metastases

Aktywność dysmutazy ponadtlenkowej w raku krtani i przerzutach do węzłów chłonnych szyi

INTRODUCTION

There is evidence that cellular prooxidant states can promote cells to neoplastic growth. Prooxidant states, i.e. is increased concentration of active oxygen and organic peroxides can be caused by different classes of agents including hyperbaric oxygen, radiation, xenobiotic metabolites, Fenton-type reagents. They cause mutations, chromosomal aberrations, cytotoxity, carcinogenesis and cellular degeneration related to aging.

In carcinogenesis active oxygen appears to play a role mostly in the promotion phase during which gene expression of initiated cells is modulated by affecting genes that regulate cell differentiation and growth. Active oxygen is known to induce chromosomal aberration with high efficiency and could play a role in progression [3].

Prooxidant states can be prevented or supressed by the enzymes of the cellular antioxidant defense and low molecular weight scavenger molecules, and many antioxidants are antipromotors and anticarcinogens [18, 20].

The three major antioxidant enzymes — superoxide dismutase, glutathione peroxidase and catalase — are unique in cofactor requirements and cellular location.

Superoxide dismutase (SOD) is among the most active scavengers of reactve oxygen species providing defense against the cellular oxidative stress [10]. Superoxide dismutases catalyze the dismutation of O_2^- to hydrogen peroxide and oxygen. Three isoforms of SOD have been identified in mammals, two of which are intracellular.

CuZn SOD in human fibroblasts, hepatoma cells and yeast cells is predominantly a peroxisomal enzyme [10].

Mn SOD in eucaryotic cells is strictly a mitochondrial enzyme located in the inner membrane and synthesized by nuclear genes. Overexpression of human manganese containing Mn SOD activity has been demonstrated to supress malignancy in human melanoma and breast carcinoma cells *in vitro* and *in vivo* [15]. A diminished level of Mn SOD activity occurs in a wide variety of tumor cells. The lowered activity is generally a consequence of lower amounts of enzyme proteins and its mRNA [22].

Extracellular superoxide dismutase (EC-SOD) contains copper and zinc and may be associated with endothelial cell surfaces as a protective antioxidant "layer" over the cells [8].

The study was undertaken to measure and compare the activities of intracellular SOD in cancerous and noncancerous human laryngeal tissues and metastatic lymph nodes.

MATERIALS AND METHODS

15 human primary larynx squamous cell carcinoma and 8 cervical lymph node metastatic carcinoma were used in these experiments. As control material macroscopically normal mucosa from the lingual surface of the epiglottis was used. The control mucosa was at least 1.5 cm away from tumor margin. All tissues were obtained after surgery — total laryngectomy and total neck dissection in patients 44–68 year of age at the Department of Otolaryngology, Medical University of Lublin. Freshly removed tumor tissues and normal mucosa were used immediately or stored at –75°C until assayed. Tissues were homogenized in 10 mM Tris- HCl buffer containing 0.3 M sucrose, 0.5 mM PMSF, pH 7.5. The homogenates were centrifuged at 2.500 g for 10 min. Measurments of enzymatic activities were performed in the supernatants.

Total intracellular SOD activity was measured by a modification of the method of Randox Laboratories using RANSOD–assay. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazon dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction at an absorption wavelength of 505 nm.

SOD activity was expressed in terms of units per miligram of protein, where 1 unit is defined as the amount of enzyme required to inhibit the rate of I.N.T. reduction by 42% under the above condition. Total protein concentration of the samples was determined by the Lowry protein assay [14]. Comparative analysis was performed using the Mann–Whitney test. P value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The comparison of total (Cu/Zn- and Mn-)SOD activity in human larynx squamous cell carcinoma, in metastases and in control-normal mucosa is shown in Table 1.

Table 1. Comparison of total SOD activity in homogenates of primary larynx squamous cell carcinoma, cervical lymph nodes metastases and control larynx mucosa. Results are the averages ±SD of 15, 8 and 10 experiments. Statistical significance $P < 0.05$ when compared with control (Mann–Whitney test).

Tissue	SOD activity U/mg of protein
Larynx squamous cell carcinoma N = 15	140.51 ± 38.44 $p < 0.05$
Metastases N = 8	110.15 ± 55.79
Control larynx mucosa N = 10	101.12 ± 26.47

Our data demonstrated that the activity of SOD is significantly higher in larynx squamous cell carcinoma (140.51 ± 38.44 U/mg of protein) in comparison to control mucosa from lingual surface of epiglottis (101.12 ± 26.47 U/mg of protein). P less than 0.05.

Additionally, our studies revealed that mean activity of total SOD in metastatic cervical lymph node carcinoma is higher as compared to SOD in control tissues (110.15 ± 55.79 U/mg of protein). There were no statistical differences between mean parameters in metastases and normal tissues, but the activity of superoxide dismutase in metastases ranged from 54.69 U/mg to 197.66 U/mg of protein. This discrepancy may relate to the high heterogenity these tissues.

Our findings were comparable with the data established by other authors. Durak et al. [4] found higher CuZnSOD and catalase activities in cancerous laryngeal tissues than in corresponding noncancerous one. They also found [2] that total SOD activity in pre and post operative serum is higher in patients with squamous cell laryngeal cancer.

The activities of total superoxide dismutase were found higher in other cancerous tissues — in malignant meningiomas [13], breast cancer [17], human colorectal cancer [1] compared with those of noncancerous one.

In colorectal cancer tissue SOD activity increased with the progression of stage and changed with the depth of invasion [19].

In lung cancer [7, 12], thyroid cancer [6], malignant skin tumors [9], bladder cancer [5] the SOD activities were lower than those in adjacent normal tissues.

The study indicates that antioxidant defence system is altered in cancerous tissues.

Many anticancer drugs have been shown to produce reactive oxygen species, destructive for carcinoma cells or inactivating the antioxidant enzyme and cancer cells low in SOD activity may offer a theoretical base for radiation therapy and chemotherapy [21, 23].

Results of Yamaguchi revealed that intracellular SOD may play a central role in protecting cancer cells against reactive oxygen species generated by anticancer drugs and radiation [24].

In experimental liver metastases in CDF 1 mice the administration of recombinant human superoxide dismutase significantly increased the number of metastatic nodules. Catalase had no significant effect [16].

It is hypothesized that mitochondrial Mn SOD by scavenging oxygen radicals induced by cytokins, some cytotoxic drugs and ionising radiation is protective and promotes survival of tumor cells from lethal effects of these treatments [11].

In our observation in 2 patients with extremely high SOD activity in larynx cancer tissues (192.97 U/mg and 184.37 U/mg) after surgery and radiotherapy was recurrence of neoplastic disease.

The present study, along with the evidence provided by others, supports the view that the activities antioxidant enzymes in biological samples may be used as nonspecific prognostic markers and suggest that elucidate of the antioxidant system in cancer tissue can provide us with a better strategy for cancer treatment.

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STRESZCZENIE

Reaktywne formy tlenu generowane są w organizmach żywych zarówno w procesach fizjologicznych jak również w stanach patologicznych. Reagują one w sposób niespecyficzny z różnymi składnikami komórki prowadząc do ich modyfikacji i uszkodzenia. Wykazano, że reaktywne formy tlenu mogą być promotorami i progresorami w procesach karcynogenezy.

Nowotwory charakteryzują się zmianą aktywności enzymów uczestniczących w ochronie antyoksydacyjnej i często wykazują obniżoną aktywność CuZn SOD i Mn SOD. Wysoka aktywność dysmutaz nadtlenkowych może przyczyniać się do zwiększonej agresywności i proliferacji nowotworu.

Przeprowadzono badania aktywnosci dysmutazy nadtlenkowej w komórkach raka płaskonabłonkowego krtani, przerzutach do węzłów chłonnych szyi i prawidłowej błonie śluzowej krtani. Wykazano istotną statystycznie, wyższą aktywność dysmutazy nadtlenkowej w raku krtani w porównaniu z tkanką kontrolną. Wyższą aktywność SOD, w porównaniu z kontrolną tkanką obserwowano w przerzutach do węzłów chłonnych. U 2 pacjentów z bardzo wysokimi aktywnościami SOD w guzach nowotworowych zanotowano niepowodzenie w leczeniu.