Study of Total Lipid Peroxidation and Antioxidant Activity in Pulmonary and Mediastinal Malignant and Benign Tumor Tissue

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Total lipid peroxidation and antioxidant activity were studied in pulmonary and mediastinal malignant and benign neoplasms. In malignant tumors total lipid peroxidation increased and antioxidant activity decreased; the intensity of these shifts depended on histological characteristics and degree of malignancy: the most pronounced changes were observed in thymoma and adenocarcinoma tissues and minimum changes were found in bronchoalveolar carcinoma tissue.

Key Words: *pulmonary and mediastinal malignant and benign neoplasms; total lipid peroxidation; antioxidant activity*

Taking into account the key role of DNA damage in carcinogenesis, we can admit that agents reacting with DNA and chemically modifying it can act as carcinogens. Many studies demonstrated that DNA damage in radiation and chemical carcinogenesis is paralleled by the formation of highly reactive oxygen forms, initiating and promoting the tumor process [5,8,9]. Free radicals initiate LPO in cell membranes, which leads to their damage. In tumor cells activity of antioxidant enzymes decreases, which leads to accumulation of free radicals and stimulates their destructive effects. It should be noted that many anticancer drugs produce antioxidant effects; in turn, the anticarcinogen effect of antioxidants was demonstrated on various models of chemical carcinogenesis [10,13].

The presence of the system LPO/antioxidant activity was detected in lung tissue membranes [1]. It is therefore important to evaluate the state of LPO and antioxidant activity (AOA) in lung tumors of different degree of malignancy and to compare the findings with the corresponding values in lung parenchyma adjacent to the tumor and in benign lung tumors. This became the object of our study. We found no published data on this subject. Only two papers present the data on LPO level in non-small cell lung cancer and lung parenchyma removed with the tumor during surgery. Total LPO and total AOA in different types of malignant tumors of the lungs and mediastinum were not measured and the findings were not compared with the respective values in benign tumors of the lungs [14,15].

MATERIALS AND METHODS

We analyzed material obtained during surgery (tissues of malignant and benign tumors of the lungs and mediastinum).

We examined 16 specimens of pulmonary and mediastinal malignant tumor tissue (3 thymomas, 3 bronchoalveolar cancer (BAC), 3 adenocarcinomas, and 7 specimens of squamous-cell carcinoma) and 10 specimens of benign tumors (3 hamartomas, 2 tuberculomas, 3 chondromas, 1 neurinoma, and 1 retrosternal goiter). The patients were: 10 men and 6 women

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aged 50-70 years with malignant tumors and 5 men and 5 women aged 47-72 years with benign tumors.

Tissue specimens were frozen and stored at -20°C. Before the study 10% homogenates were prepared: tissues were cut into small fragments and homogenized in normal saline on cold in a Potter—Eveilleme homogenizer, after which the samples were centrifuged at 1500 rpm for 15 min. Total LPO and AOA were measured in the supernatant.

LPO in tissues was evaluated by the modified method of L. C. Trost and K. B. Wallace based on prooxidant properties of myoglobin [11]. Myoglobin (Fe^{2+}) stimulates LPO due to capacity to bind molecular oxygen with the formation of 6-oxymyoglobin, superoxide and hydroxyl radicals of unsaturated fatty acids. Superoxide radical, in turn, attacks double bonds of polyunsaturated fatty acids with the formation of TBA-reactive compounds. Arachidonic acid containing four double bonds was used as the substrate and target for $O_2^{\bullet-}$ and other radicals. In addition, oxidized hemoglobin reacting with H_2O_2 forms hem-bound protein (Mb-H adduct) capable of catalyzing oxidase reactions even in the presence of a potent reducer $O_2^{\bullet-}$ (superoxide) [12]. One more advantage of this method is that myoglobin-stimulated LPO does not depend on SOD, catalase, and other antioxidants. We used TBA test as LPO indicator and recorded LPO by changes in the content of TBA-reactive substances at λ=532 nm in the presence of butylated hydroxytoluene, which, according to M. Borhan *et al.*, increases the sensitivity and intensity of hydroperoxide color formation [7].

The data were estimated using the formula proposed by I. A. Volchegorskii *et al.* [2]. AOA was evaluated as described previously [3]. Both parameters were expressed in percents.

All studies were repeated and the results were statistically processed using Student's test.

RESULTS

The level of LPO in the majority of malignant tumors was higher than in benign neoplasms (Table 1). The intensity of LPO depended on histological characteristics and degree of tumor malignancy.

The highest level of LPO was detected in thymoma; it was slightly lower in adenocarcinoma and squamous-cell carcinoma, while in BAR tissue the level of LPO was even lower than in benign neoplasms (Table 1).

In a special series of experiments we measured LPO in squamous-cell lung carcinoma and lung parenchyma at a distance of 3-4 cm from the tumor (borderline tissue) obtained during surgery from 5 patients. The intensity of LPO in lung parenchyma adjacent to the tumor and containing no atypical cells was markedly lower (by more than 70%) than in the tumor (52.10±13.64 and 196.64±35.75%, respectively) and considerably higher (by 74.7%) than in benign tumors.

The study of AOA showed (Table 1) negative values of AOA in the majority of malignant tumors in comparison with the control (suspension of yolk lipoproteins), which was due to predominance of LPO completely suppressing AOA in malignant tumors. Like in LPO measurements, the studied parameters clearly depended on histological characteristics and malignancy of the tumor: LPO most markedly exceeded AOA in thymoma and adenocarcinoma characterized by high rate of metastasizing and drug resistance, while in squamous-cell carcinoma LPO exceeded AOA to a lesser extent. In BAR tissue AOA was positive, but 61.6% lower than in benign tumors. It should be noted that AOA values in BAR tissue varied within a great range, but in all cases were positive and lower than in benign tumors.

Hence, we revealed a relationship between LPO and AOA, on the one side, and histology and malignancy of pulmonary and mediastinal tumors, on the other.

The maximum LPO intensity was found in thymoma, an intensely growing tumor characterized by extremely high activity of γ-glutamyl transferase (GGT, EC 2.3.2.40). This enzyme provides material for intense growth of tumor cells: GGT activity in thymoma tissue increased 70-fold in comparison with its level in intact thymus) [4].

Adenocarcinoma is characterized by high malignancy [6]. It actively metastasizes and is characterized by resistance to chemotherapy. Our study showed the most pronounced suppression of AOA in this tumor.

BAR does not destroy lung tissue during its growth, but only compresses it; in addition, it is sensitive to drug therapy. The intensity of LPO in this tumor is low (even lower than in benign tumors), but AOA is 2.6 times lower than in benign tumors [6].

It is noteworthy that LPO level in lung tissue sites at a distance of 3-4 cm from the malignant tumors and containing no atypical cells was lower than in tumors, but 74% higher than in benign tumors. This is in line

TABLE 1. LPO and Total AOA in Pulmonary and Mediastinal Benign Neoplasms and Malignant Tumors (*M*±*m*, %)

Tissue	LPO	AOA
Malignant tumors		
thymoma	333.86±61.91	-61.93±16.46
squamous-cell carcinoma	169.98±38.80	-33.86±19.74
adenocarcinoma	166.67±22.11	-69.66±20.28
BAR	18.93±2.44	13.54±6.86
Benign tumors	29.82±8.46	35.23±10.00

with our previous findings [4] on changes in the glutathione metabolism enzymes in these tissues, similar to those in cancer cells, but these changes are less pronounced than in malignant tumor tissue. Hence, our present results confirm that tissues adjacent to the tumor are predisposed to the development of the tumor process before the appearance of morphological changes, and this should be taken into consideration when determining the volume of surgical intervention.

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