Paramagnetic Changes in Pulmonary Tumors*

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ESR, Pulmonary Tumors, Ascorbic Acid, Spin Concentration

Electron spin resonance studies on healthy and tumerous human lung samples have been conducted in order to determine possible differences in free radical concentration and shape of the spectra between the different sections of the lung. It could be shown that in healthy lung tissue the signal caused by the semidehydroascorbate (SDA) radical is not prominent because of the prevailing high partial oxygen pressure. On formation of a tumor, the spin concentration increases, possibly due to the higher metabolic rate; here, the SDA peak is also more pronounced which indicates alterations in the interaction between cell constituents and ascorbic acid. Within the tumor, the spin concentration is considerably reduced which is probably caused by a still higher concentration of ascorbic acid. Addition of ascorbic acid to the different lung specimens enhanced the just described effect while oxidizing substances, such as H_2O_2, reversed it.

Introduction

There are fewer free radicals in tumors than in comparable normal tissues according to most studies on this subject [1–7]. However, a few investigators reported an increase in the level of free radical concentration above that of healthy tissue in an early stage of cancer followed by a decrease [8, 9]. Because the latter observation was made only with lyophilized samples, changes in the level of free radicals in rats treated with either a carcinogen or having been injected with tumor cells was investigated simultaneously in both, lyophilized and non-lyophilized samples [10, 11]. Similar trends in both types of preparations could be observed in this investigation in the case of 7,12-dimethylbenzanthracene induced breast tumors.

In a series of studies on patients with acute lymphatic leukemia and in model systems we could identify the π-electron type radical with axial symmetry located at about $g = 2.005$ as being caused by ascorbic acid added to either blood constituents or to copper-containing proteins [12–14]. It could be shown that the low field peak is produced by the semidehydroascorbate (SDA) radical while the peak located slightly upfield appears to be caused by a protein radical [14]. Furthermore, we observed a characteristic change in spin concentration with increasing concentrations of ascorbic acid [12]. Thus, the early rise as well as the subsequent decrease in spin concentration in neoplastic tissues may be the result of alterations in the interaction of tumor cells with ascorbic acid.

The following electron spin resonance (ESR) experiments were conducted in order to determine systematically 1) the presence of the same species of radicals in other types of tissues, such as human lung and 2) to examine differences in free radical concentration between healthy and tumorous pulmonary tissues. Because determination of free radical changes during the development of the lung tumors could obviously not be made, samples of different sections of the lung and tumor tissue were used under the assumption that they might reflect free radical concentrations in the development of a neoplasm.

Materials and Methods

The tissue samples were obtained immediately following surgical removal from different parts of the lung of eleven patients with bronchial cancer. In each case, a sample was taken from the center of the

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tumor, the periphery of the tumor, normal lung abutting on the tumor, normal lung about 6 cm away from the tumor, and normal pulmonary tissue from another lobe in cases of pneumonectomy specimens. All of these tissue samples were examined histologically; a small part of each of the samples (a few g at the most) of the different areas was immediately lyophilized. In several instances, different concentrations of ascorbic acid or H_2O_2 were also added to the samples before lyophilization. Obviously, the latter experiments will give qualitative results only.

All patients were of male sex but of different age. Surgical treatment consisted either of lobectomy or pneumonectomy. Nine of the tumors were of pulmonary origin; 6 were epidermoid carcinomas, 2 adenocarcinomas, and one a pulmonary carcinoid. The remaining two tumors were metastatic, one of osseous and the second of intestinal origin. In addition, lung tissue of patients with silicosis, thoracic trauma, bronchiekstases, chronic tuberculosis, and Hodgkin's disease was also used for additional control purposes. In each instance, the tissue samples were also studied histologically.

Ascorbic acid and H_2O_2 were purchased from Merck, Darmstadt, Germany.

The ESR spectra were obtained with a Varian E-9, 100-kHz modulation X-band spectrometer. A DPPH (diphenylpicrylhydrazil) standard ($g = 2.0036$) was used as a reference for marking resonance positions. The modulation amplitude was 0.2 mT and the microwave power 5 mW for all samples investigated. The spectra were recorded at different sensitivities marked at the left-hand side of each spectrum. All measurements were carried out at room temperature. The relative spin concentration was obtained by double integration of the spectra by means of a planimeter.

Results and Discussion

ESR spectra of lyophilized lung tissue are shown in Fig. 1. As can be seen, in the healthy tissue the SDA radical peak (at about $g = 2.005$) is less pronounced than the protein radical peak (spectrum ①). This might be due to the fact that the partial oxygen pressure in the lung is relatively high. Since the SDA radical is strongly influenced by oxygen, its peak will be relatively small because it is transformed into the diamagnetic dehydroascorbate.

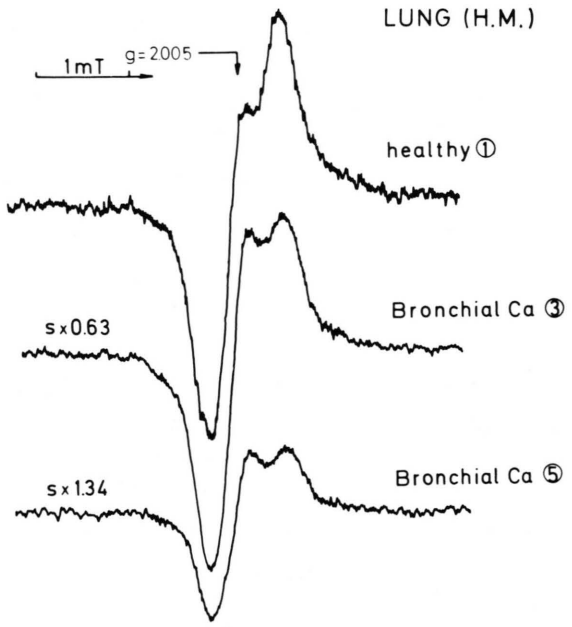

Fig. 1. ESR spectra of different lung tissues of a male patient (H. M.) with bronchial carcinoma: ① healthy tissue, ③ tissue from the surface of the tumor, ⑤ tissue from the center of the tumor. $s \triangleq$ rel. sensitivities.

It is interesting to note that the shape of the spectrum and the spin concentration of the peripheral rim of the tumor are different (spectrum ③). The SDA peak is more pronounced and the spin concentration enlarged. A gradual change in shape and spin concentration from healthy lung tissue to the surface of the tumor was observed in most instances. From there on, towards the center of the tumor, only a decrease in spin concentration was found (s. spectrum ⑤). These differences in spin concentration are summarized in Table I.

The change in spin concentration and shape of the spectra suggests an increase in concentration of ascorbic acid towards the center of the tumor. Relevant investigations are under way.

It should be pointed out that this pattern in ESR response is obtained only in the early stage of tumor growth. In a more advanced state, a "healthy" response pattern is seen neither in the histologically normal tissue samples of the same nor in an adjacent normal lung lobe of the pneumonectomy specimens. The surprising finding that the spin concentration is the highest at the surface of a tumor cannot be explained yet. It might be due to the high metabolic rate of this region.

Table I: Rel. spin concentrations of the different lung specimens shown in Fig. 1.

	tumor center ⑤	just outside of tumor ③	far away from tumor ①
Spin conc. (rel. units)	13 ± 2	41 ± 7	26 ± 4

In one instance, a primary pulmonary tumor had metastasized to a hilar lymph node; thus, the ESR spectra of a healthy lymph node and that with the metastasis were recorded. Although in both cases the SDA peak is well developed (a spectrum similar to ③, Fig. 1), the spin concentration of the tumor containing node is only about 1/5 that of the healthy lymph node.

In additional experiments, 0.2 ml of ascorbic acid solution (1 to 100 mM) were added *in vitro* to about 5 g of the different lung specimens. Since it is unknown how much of the added vitamin C actually interacted with the lung tissue, such an experiment can only provide qualitative data. This investigation was conducted because we had previously observed that the spectra shown in Fig. 1 are due to an ascorbic acid-copper protein interaction. Thus, spectral modification of lung tissue through addition of ascorbic acid could provide insight into this interaction. Interpretation of the observed effect showed two patterns of response, namely that of 1) healthy tissue and 2) tissue from samples extending from the surface of the tumor to the center of the tumor. In the first case, the spin concentration increases up to the highest concentration of ascorbic acid used. Moreover, the SDA peak is already well pronounced after the addition of 1 to 5 mM of ascorbic acid. In the second case, the spin concentration increases first with increasing vitamin C concentration of up to about 10 mM followed by a decrease at larger ascorbic acid concentrations.

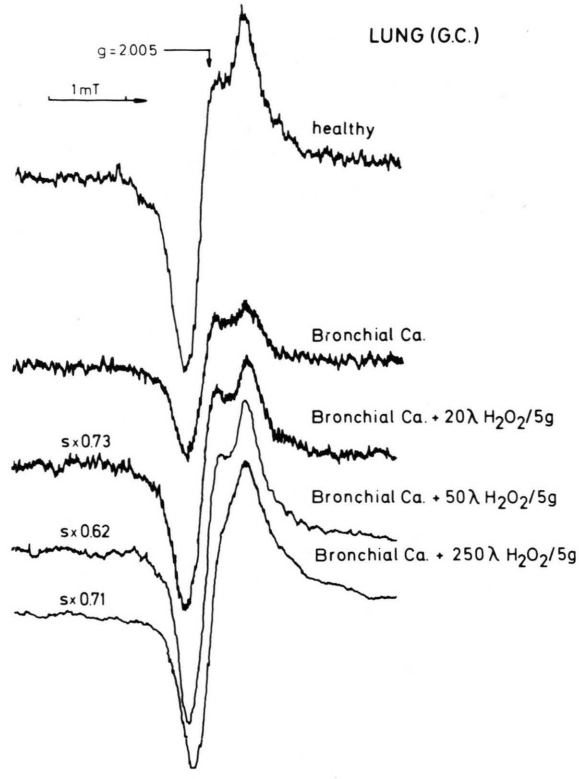

Fig. 2. ESR spectra of different lung tissues of a female patient (G. C.) with bronchial carcinoma: healthy tissue and tissue from the center of the tumor treated with different concentrations of H_2O_2 (1 $\lambda \triangleq$ 1 µl). $s \triangleq$ rel. sensitivities.

These results permit one to conclude that in the case of human lung cancer the metabolism of ascorbic acid is, as in other types of neoplasia, modified and that the concentration of ascorbic acid increases towards the center of a tumor. Thus, as has also been pointed out previously, oxidizing substances should reverse the observed effect [12, 13, 15]. As can be seen in Fig. 2, increasing concentrations of H_2O_2 increase the spin concentration of the cancerous sample and change concomitantly the shape of the spectrum. When, in this special case, 50 µl of H_2O_2 were added *in vitro* to 5 g of cancerous lung tissue, the resulting spectrum resembled that of healthy tissue (s. Fig. 2). A further increase in H_2O_2 suppresses almost completely the SDA peak. It should be emphasized again, that these values are of qualitative nature only.

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