INVESTIGATION OF ANTIRADICAL ACTIVITY AND CONCENTRATION OF SULFHYDRYL GROUPS IN NORMAL AND TUMOR TISSUES

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The research of Émanuél' and his followers [1, 4] has demonstrated the essential role of free-radical reactions in malignant transformation. In particular, changes in free-radical processes (FRP) and the level of antioxidative activity (AOA) in different tissues under the influence of chemical carcinogens and in developing tumors have been well studied [2]. The intensity of FRP is controlled by a system of biological antioxidants, the presence of which determines the antioxidative potential of the tissues. The concept of AOA of biological antioxidants also implies their ability to interact with free radicals (FR). This ability determines the antiradical activity (ARA) of biological antioxidants [1]. Among the bioantioxidants an important place is occupied by sulfhydryl compounds, such as glutathione, cysteine, etc. [2]. Some workers consider that glutathione can inactivate FR *in vivo* [6, 9], although, according to others, sulfur-containing biological antioxidants react mainly with hydroperoxides [3].

The object of the present investigation was to study ARA and the content of sulfhydryl groups (SH groups) in normal and the corresponding tumor tissues. By comparing the results, the degree to which ARA is maintained by the presence of sulfhydryl compounds could be judged.

EXPERIMENTAL METHOD

Experiments were carried out on 58 Syrian hamsters, 44 BO_6 rats, and 39 Wistar rats. Rhabdomyoblastomas were induced in some of the hamsters by a single intramuscular injection of 7,12-dimethylbenz(a)anthracene in a dose of 1 mg per animal. Liver tumors were induced by diethylnitrosamine. The carcinogen was injected intraperitoneally into the rats in a dose of 80 mg/kg once a week for 10 weeks. Neoplasms of the stomach were induced in rats by administration of a single dose of 250 mg/kg N-methyl-N'-nitro-N-nitrosoguanidine via gastric tube. Tumors of the kidneys were obtained after a single intraperitoneal injection of dimethylnitrosamine in a dose of 60 mg/kg. Neoplasms of the intestine were induced in rats with 1,2-dimethylhydrazine. The carcinogen was injected subcutaneously once a week for 5 months in a dose of 21 mg/kg.

Animals with tumors and also control animals were killed by cervical dislocation. In some rats the liver was perfused by injecting cold physiological saline through the superior vena cava. The isolated tissues were homogenized in 0.25 M sucrose solution in a glass Potter's homogenizer, yielding a 10% homogenate. The content of SH groups in ARA was determined in the cytosol fraction obtained after centrifugation of the homogenate for 60 min at 105,000g. The homogenate was processed at 4°C. ARA was determined by Glavind's method [7] and the concentration of SH groups by Ellman's method [5]. By Glavind's method it is possible to express ARA in microequivalents of SH groups per milligram protein, so that the results obtained by the two methods could be compared. The protein content in the samples was determined by Lowry's method [8]. Some of the tumor tissues were fixed in 10% formalin solution and subjected to standard histological treatment. Sections were stained with hematoxylin and eosin. The results were subjected to statistical analysis by Student's t test.

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TABLE 1. ARA and Content of SH Groups in Normal and Tumor Tissues (M ± m)

Test object	Normal tissues: number of animals in group	ARA, μeq SH groups/mg protein	free SH groups, μmoles/mg protein	Test object	Tumor tissue: number of animals in group	ARA, μeq SH groups/mg protein	free SH groups, μmoles/mg protein
Thigh muscles	14	1,039±0,027	1,049±0,027	Rhabdomyoblastoma	22	2,608±0,054	1,256±0,048
Liver	13	1,485±0,083	1,638±0,090	Hemangioendothelioma	12	1,391±0,066	1,029±0,088
				Hepatocellular carcinoma	3	3,353±0,084	2,643±0,168
Forestomach	12*	1,120	1,530	Tumors of stomach	4	1,360±0,072	0,982±0,050
Glandular part of stomach	12	1,310±0,191	1,476±0,072				
Kidney	8	1,973±0,093	2,222±0,082	Tumors of kidneys	11	2,415±0,085	2,085±0,111
Large intestine	5	1,140±0,026	1,178±0,027	Tumors of large intestine	5	2,298±0,147	0,946±0,070

*Pooled data for 12 animals.

EXPERIMENTAL RESULTS

The results obtained in the course of the investigation are summarized in Table 1. ARA of individual normal tissues varied from 1.039 to 1.970 μeq SH groups/mg protein. The highest values of this parameter were obtained for the kidneys and liver. Otherwise the values of ARA were approximately equal. The highest concentration of SH groups also was recorded in the kidneys. Correlation was found between values reflecting ARA of the tissues and their content of SH groups. Comparison of the averaged results showed that in all normal tissues tested the concentration of SH groups was higher than the corresponding ARA, expressed in μeq SH groups/mg protein. Consequently, the difference between ARA and the concentration of SH groups was always a negative value.

In intestinal tumors, rhabdomyoblastomas, and hepatocellular carcinomas ARA was increased from 21 to 125.8% ($P < 0.01$) compared with that in the corresponding normal tissues. In the other cases no significant changes were observed in this value. In the rhabdomyoblastomas and hepatocellular carcinomas a statistically significant increase also was recorded in the content of SH groups. The content of SH groups in the remaining tumor tissues was lower than in the corresponding normal tissues. In all the tumors studied ARA, expressed in μeq SH groups/mg protein, was higher than the concentration of SH groups. Consequently, the difference between these values in the neoplasms was always a positive value.

Comparison of these values suggests that ARA in the normal tissues studied was determined mainly by sulfhydryl compounds. A proportion, although small, of these substances do not possess antiradical properties, in agreement with the opinions of other workers [3]. By contrast, in the tissues of the tumors studied ARA was formed both by sulfhydryl compounds and by other substances not containing SH groups. This may probably be connected with an increase in the concentration of tocopherols [1].

The results of this investigation are evidence that the body contains sulfhydryl compounds capable of interacting actively with FR *in vitro*. The role of these compounds as FR inhibitors is particularly great in normal tissues. By contrast, the tumor tissues which were studied also contain other substances with antiradical properties.

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