Lipid Peroxidation in Malignant Tumors of Human Kidneys

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The intensity of ascorbate-dependent free radical oxidation of endogenous lipids in malignant tumor tissue of human kidneys was studied. The LPO induction period was markedly increased in renal-cell carcinoma and malignant mesenchymal tumors compared to normal renal cortex. The content of α-tocopherol in cancer tissue lipids was considerably (7-fold) increased compared to that in normal renal cortex, which explains high antioxidant activity of these carcinomas. A less pronounced increase in the content of α-tocopherol in lipids of malignant mesenchymal tumors compared to the cortex (2-fold) can also contribute to their LPO resistance.

Key Words: *malignant human renal tumors, lipid peroxidation; a-tocopherol*

Previously we revealed increased content of α-tocopherol in human renal-cell carcinoma (RCC) compared to adjacent renal cortex not involved in tumor process (considered as normal) [5,12]. The highest content of this natural antioxidant was found in light-cell carcinomas, where its concentration 5-30-fold (average 16-fold) surpassed the normal. These results suggest that activity of lipid peroxidation (LPO) processes in RCC tissue decreased. On the other hand, some authorities consider that LPO processes in human RCC are more active than in intact renal tissue [7]. According to these data, antioxidant potential of tumor tissue in patients with light-cell carcinoma is lower and the content of secondary LPO product (malonic dialdehyde — MDA) in this tumor is higher than in intact renal tissue.

These contradictory data prompted us to investigate the intensity of ascorbate-induced LPO in renal tumor tissue, in order to obtain a more complete picture of free-radical oxidation of endogenous polyunsaturated fatty acids compared to MDA assay only. In parallel, the contents of α-tocopherol and total lipids were measured in the studied tissues in order to evaluate the content of lipid-soluble antioxidant in these tissues.

MATERIALS AND METHODS

The kidneys of 10 patients (6 men and 4 women aged 34-65 years) with malignant tumors were examined. Immediately after removal the kidneys were transported into laboratory in cold containers. Specimens of tumor tissue were collected from sites without necrotic foci, cicatrices, or cysts; specimens of the renal cortex were taken at the maximum distance from tumor nodes. In two cases the tumors almost completely replaced the renal tissue, and therefore no cortical specimens were collected. Specimens for α-tocopherol and total lipid assays were kept in sealed containers at -80°C.

The intensity of ascorbate-dependent LPO was measured [4] immediately after the kidney was delivered into the laboratory. Tumor tissue and normal cortex were homogenized in cold 66 mM phosphate buffer (pH 5.9, 15 mg tissue/ml buffer) in a Potter type homogenizer. The homogenates were incubated for 60 min at 37°C with constant stirring and aeration. LPO was initiated with ascorbic acid (0.5 mM). Aliquots for measurement of secondary LPO products were col-

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lected before incubation (initial level) and at 10-15-min intervals after addition of ascorbate. Secondary LPO products were measured in the reaction with thiobarbituric acid (TBA) [6] and denoted as TBA-reactive substances (TBARS). Optical density was measured at 532 nm on a Spectromom spectrophotometer. The initial TBARS absorption was distracted from the optical density of the subsequent samples and kinetic curves reflecting the relationship between optical density and duration of incubation were plotted. The intensity of ascorbate-dependent LPO was characterized by the length of induction period, which was found by drawing a perpendicular to the time axis from the point on the curve after which the concentration of TBARs linearly increased. The content of total protein in homogenates was measured by the Biuret method [2].

For measuring α-tocopherol, homogenates from defrosted tissues in normal saline were prepared (1:5 w/v). After saponification of lipids and hexane extraction α-tocopherol was measured on a Hitachi-650-10 S spectrofluorimeter at 292 nm excitation and 325 nm emission wave lengths [14]. Total lipids were measured by gravimetry. Tissues were fragmented during freezing in liquid nitrogen and lipids were extracted by the Folch method [10].

Tumor samples for histological analysis were fixed in neutral formalin buffered by Lilly method and in Carnoy liquid and embedded in paraffin. The sections were stained with hematoxylin and eosin, Sudan III, and toluidine blue.

The results were statistically processed using paired Wilcoxon's and Student's *t* tests.

Biochemical data were analyzed with consideration for renal tumor histogenesis. Group 1 consisted of 6 kidneys with epithelial tumors: light-cell carcinoma of differentiation degree II in 1 case and mixed-cell carcinoma with predominance of light cells in 5 cases. Group 2 included 4 kidneys with malignant mesenchymal tumors (MMT), 3 of which were identified as angiomyoliposarcopma and one as angiomyofibrosarcoma. In group 1, the cortical matter of the same kidney served as the control for RCC. In group 2, the renal tissue could not be obtained in two cases because it was almost completely replaced with tumor, and therefore cortical matter of the contralateral kidneys served as the control; the data were united with the parameters recorded for the cortex of 6 kidneys with RCC (a total of 8 samples). The control data differed negligibly, and therefore were pooled.

RESULTS

The intensity of ascorbate-dependent LPO in RCC tissue was lower than in normal renal cortex (Fig. 1, *a*). The latency of ascorbate-dependent LPO for normal cortex was 26.2±1.6 min, while in experiments with tumor tissue no TBARS accumulation in incubation medium was detected 60 min after LPO induction. After 60-min incubation the contents of TBARS (regarded as MDA) for normal renal cortex and tumors were 594±116 and 35.2±16.7 nmol/g wet weight, respectively ($p<0.01$), or 8.05±2.10 and 0.70±0.39 nmol/mg protein. These data indicate that RCC tissue is resistant to LPO initiated by ascorbate in endogenous polyunsaturated fatty acids. This resistance probably results from a 4.5-60 times higher concentration of LPO inhibitor α-tocopherol in tumor tissue, (20 times, on average; Table 1). The content of total lipids was also increased in RCC tissue, but the concentration of the antioxidant in per mg lipids was 7-fold higher than in

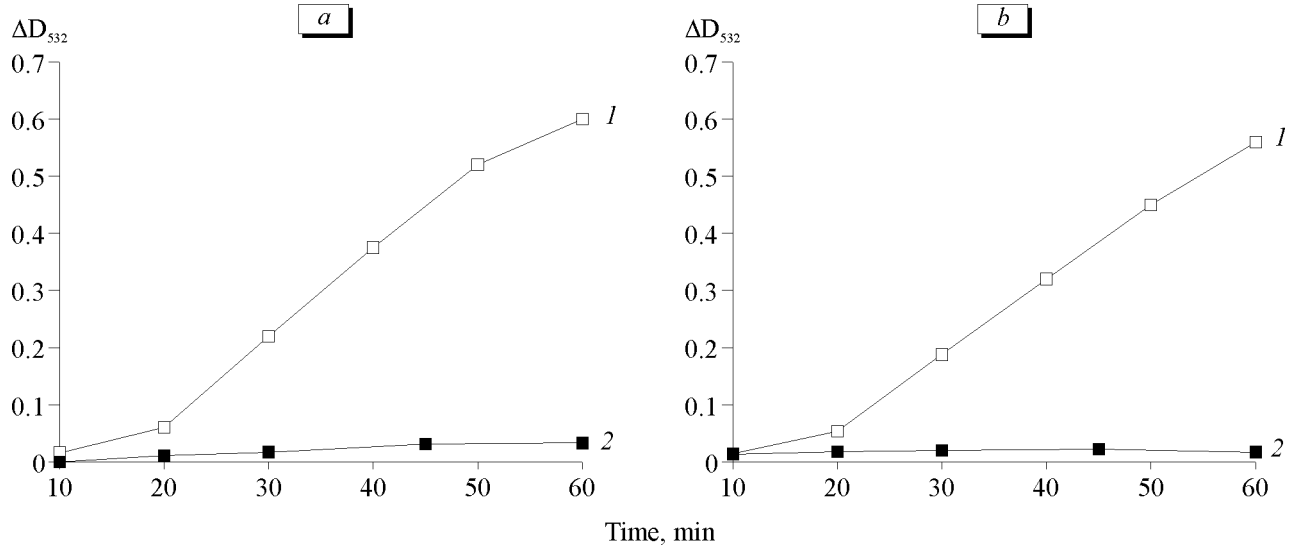

Fig. 1. Kinetics of ascorbate-dependent LPO in human renal-cell carcinoma (*a*) and malignant mesenchymal renal tumors (*b*). *1*) tumor tissue; *2*) normal renal cortex. Ordinates: changes in optical density.

TABLE 1. Content of α-Tocopherol, Total Lipids, and Protein in Normal Renal Cortex and Tumor Tissue from Patients with RCC and MMT (*M*±*m*)

Parameter	RCC		MMT	
	cortex (*n*=6)	tumor (*n*=6)	cortex (*n*=8)	tumor (*n*=4)
α-Tocopherol				
μg/g tissue	9.6±0.6	199.0±76.8*	10.30±0.62	38.7±16.9
	(8.0-11.5)	(45-518)	(8.0-13.2)	(12.2-64.5)
μg/g lipids	0.36±0.03	2.54±0.52*	0.37±0.02	0.82±0.18***
	(0.24-0.44)	(1.18-4.28)	(0.24-0.45)	(0.35-1.03)
Total lipids, mg/g tissue	26.9±1.5	75.5±25.4**	28.20±1.37	68.6±46.5
	(23.4-32.9)	(38-185)	(23.4-34.6)	(13-185)
Protein, mg/g tissue	77.7±11.7	75.2±12.4	82.8±9.5	65.0±13.5
	(60-130)	(42-115)	(60.2-130.0)	(45-96)

Note. *p<0.001, **p<0.01, ***p<0.05 compared to the cortex (paired Wilcoxon test and Student's t test for RCC and MMT, respectively).

the cortex. The concentration of α-tocopherol in RCC lipids far surpassed the corresponding parameter in the cortex and in most normal tissues. In the liver it was 0.4 μg/mg lipids, in fatty tissue 0.15 μg/mg, in muscles 0.5 μg/mg, and the highest in the testes and pituitary: 1.0-1.2 μg/mg, which corresponded to the lowest values in RCC lipids [9].

The intensity of ascorbate-dependent LPO in MMT was also much lower than in the cortex and did not differ from that in RCC tissue (Fig. 1, *b*). TBARS production (taken for MDA) during 60 min of incubation in MMT homogenates and in the control cortical matter was 25.7±17.2 and 554.8±87.3 nmol/g wet tissue (p<0.01) or 0.50±0.41 and 8.40± 2.14 nmol/mg protein, respectively. The content of α-tocopherol in MMT tissue notably increased in only two of 4 samples (63.5 and 64.5 μg/g wet tissue), while in two other samples it was close to that in the cortex (14.6 and 12.2 μg/g). In the two former cases the level of total lipids was also high (61.4 and 185 mg/g wet tissue, respectively), while in the two latter tumors it was lower than in the control (15.4 and 13.0 mg/g, respectively). Fluctuations in the concentrations of α-tocopherol and total lipids in MMT tissue can reflect their heterologous structure which included (in various ratios) fibrous, vascular, muscle, and fatty components, no signs of malignancy being detected in some of them at histological analysis. None the less, the mean concentration of α-tocopherol in MMT per mg lipids was 2 times higher than in the cortex and in only one tumor it was the same as in the control.

Hence, low activity of ascorbate-dependent LPO in MMT and in RCC could be caused by increased concentration of α-tocopherol in lipids, though this increase was not so high as in tumor tissue and was not observed in all samples. It is noteworthy that in one MMT with α-tocopherol level the same as in the control, the activity of LPO was also very low. It seems that MMT resistance to LPO processes is determined not only by high α-tocopherol concentration, but by other factors as well, *e.g.* by low content of polyunsaturated fatty acids (the main LPO substrate), high activity of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, catalase, *etc.*), increased rigidity and low permeability of cell membranes, *etc.* [8].

The detected low liability of tumorous kidneys to LPO is in line with published reports about the resistance of some experimental tumors (mouse and rat hepatomas and sarcomas, mouse mammary adenocarcinoma) to free radical oxidation [3,8].

Published data on the activity of LPO in human malignant tumors are scanty and ambiguous. Intensification of ascorbate- and NADPH-induced LPO was observed in malignant tumors of the liver in patients in comparison with hepatic tissue of normal controls, the concentration of α-tocopherol being markedly decreased in cancer tissue [1]. The content of polyunsaturated fatty acids was high and the level of MDA tended to increase in breast cancer tissue in comparison with nontumor tissue [11]. As for the data on LPO activation in human renal light-cell cancer tissue [7], we consider that the methodological aspects of this work do not meet the requirements of its goal. For example, when measuring the antioxidant potential, the authors used cod-liver oil as the substrate for LPO initiated by a superoxide radical, but it was probably not alone, as polyunsaturated acids of tissue homogenates could oxidize during incubation. Therefore, it is more correct to evaluate the antioxidant potential by the time of induction of free radical oxidation of proper polyunsaturated lipids. It is noteworthy that our results are in line with a recent report in which the

authors, based on the results of immunomorphological studies of the kidneys from RCC patients, conclude that LPO is less intensive in tumor tissue, because RCC cells contain less proteins modified by one of secondary products of LPO, 4-hydroxy-2-nonenal than the cells of intact renal tubules or the kidneys of subjects without renal carcinoma [13].

Hence, our experiments demonstrated high resistance of malignant renal tumors (RCC and MMT) to ascorbate-induced LPO of endogenous polyunsaturated fatty acids in comparison with the cortical matter not involved in the tumor process. This resistance can be partially determined by high level of α-tocopherol, especially in RCC tissue.

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