EFFECT OF THE ANTIOXIDANT PHENOSAN ON PHYSICOCHEMICAL PROPERTIES OF RAT KIDNEY CELL MEMBRANES DURING CARCINOGENESIS INDUCED BY NITROSODIMETHYLAMINE

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Tumor cells differ considerably from normal cells in the structural organization and function of their cell membranes [1]. Chemical carcinogenesis is known to be accompanied by lipid dedifferentiation [7], by a disturbance of oxidative reactions in membrane lipids [6], and also by changes in microviscosity and orderliness of the lipid bilayer of membranes [9]. Meanwhile there is evidence of the inhibitory action of synthetic antioxidants on this type of carcinogenesis [10]. The mechanism of the anticarcinogenic effect of antioxidants has not been finally studied. It has been shown that the efficacy of action of antioxidants is determined by their ability to act on the structure of membranes and to inhibit lipid peroxidation (LPO) [2].

The aim of this investigation was to study the physicochemical properties of the lipid bilayer and LPO in kidney cell membranes during carcinogenesis induced by nitrosodimethylamine (NDMA), with the additional administration of the antioxidant phenosan.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing initially 140-150 g, kept on an ordinary diet. The animals as a whole were divided into four groups. Rats of group 1 received the carcinogen NDMA, rats of group 2 received the carcinogen plus the oxidant phenosan-1K, animals of group 3 received phenosan-1K alone, and group 4 consisted of intact animals.

NDMA was administered perorally by means of a tube in a dose of 10 mg/kg body weight daily for 6 doses. The antioxidant phenosan-1K (synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR) was administered in a dose of 30 mg/kg perorally through a tube in aqueous solution twice — 48 h and 24 h before the carcinogen was given, and then 7 times, at intervals of 5 days, in the course of 1 month after administration of the carcinogen.

The investigation was conducted in the early (1 month) and later (3 months) periods of carcinogenesis. Membranes of mitochondria [5] and microsomes [3] of the renal cortex of the rats were obtained by differential centrifugation.

To study the structural state of the lipid bilayer of the cell membranes two spin probes were used [4]: the doxyl derivative of stearic acid with a nitroxide ring attached to the 5th carbon atom (5-doxyl stearate; from "Sigma," USA), and 2,2,6,6-tetramethyl-4-palmitoylhydroxypiperidine-1-oxyl (15C), synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR.

The spin probes were incorporated into the membranes as follows: an alcoholic solution of the probes (5 mmoles/liter) was added to membrane suspensions containing of the order of 3 mg protein to 1 ml. The final concentration of the probes in the suspensions was 100 μmoles/liter, or 0.5% of the mass of lipid. Incorporation was carried out at 25°C in a shaker for 15 min. In the dilutions used (not above 1:500) the ethanol of the original solutions of the spin probes did not affect the parameters of EPR spectra in the membranes. Measurements were made with a "Varian E4" radiospectrometer (USA) in quartz cuvettes at 25°C; the volume of the samples was of the order of 50 μl.

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TABLE 1. Results of Investigation of Membranes by Probe 15C at Different Periods of Carcinogenesis (M ± m)

Experimental conditions	$\tau_c \cdot 10^{-9}$, sec			
	Mitochondria		Microsomes	
	1 month	3 months	1 month	3 months
NDMA	1,32±0,06* (7)	1,17±0,06* (6)	1,14±0,05* (6)	0,96±0,04* (6)
NDMA + phenosan	0,99±0,05** (7)	0,85±0,04** (6)	0,88±0,04** (7)	0,77±0,03** (6)
Phenosan	0,48±0,04* (7)	0,55±0,03* (6)	0,42±0,02* (7)	0,44±0,03* (6)
Healthy rats	0,8±0,02 (7)	0,79±0,02 (6)	0,65±0,03 (6)	0,63±0,02 (6)

Legend. Here and in Tables 2 and 3: *p < 0.01 compared with healthy rats; **p < 0.01 compared with NDMA. Number of animals in group shown in parentheses.

TABLE 2. Results of Investigation of Membranes by Probe 5-Doxyl Stearate at Different Periods of Carcinogenesis (M ± m)

Experimental conditions	S			
	Mitochondria		Microsomes	
	1 month	3 months	1 month	3 months
NDMA	0,71±0,01* (7)	0,7±0,01* (6)	0,68±0,01* (6)	0,64±0,02* (6)
NDMA + phenosan	0,67±0,01** (7)	0,63±0,02** (6)	0,62±0,01** (7)	0,58±0,01** (6)
Phenosan	0,55±0,02* (7)	0,55±0,01* (6)	0,52±0,01* (6)	0,53±0,01 (6)
Healthy rats	0,62±0,01 (7)	0,61±0,01 (6)	0,56±0,01 (6)	0,55±0,01 (6)

TABLE 3. LPO in Membranes of Rat Renal Cortex at Different Periods of Carcinogenesis (M ± m)

Experimental conditions	Level of malonic dialdehyde, nmole/mg protein			
	Mitochondria		Microsomes	
	1 month	3 months	1 month	3 months
NDMA	1,09±0,54* (13)	0,83±0,063 (10)	1,22±0,038* (12)	1,12±0,12 (10)
NDMA + phenosan	0,68±0,066 (12)	0,75±0,053 (9)	1,05±0,098 (13)	0,99±0,089 (10)
Phenosan	0,65±0,063 (13)	0,8±0,053 (10)	0,94±0,069 (11)	1,12±0,15 (9)
Healthy rats	0,89±0,066 (14)	0,79±0,065 (10)	0,99±0,078 (12)	1,16±0,058 (10)

The LPO level was estimated as the quantity of malonic dialdehyde [8]. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

As the investigations showed, structural changes in subcellular membranes were observed even in the early stages of carcinogenesis (after 1 month). They consisted of an increase in the rotary correlation time of the 15C spin probe, i.e., an increase in microviscosity of the membranes (Table 1), and also an increase in the orderliness of the lipid bilayer of the membranes (the S parameter of the spin probe 5-doxyl stearate; Table 2). Changes in both mitochondrial and microsomal membranes were observed to be in the same direction.

Investigation of LPO revealed a higher malonic dialdehyde level in the mitochondria and microsomes of the renal cortex of the rats in the early stages of carcinogenesis (1 month; Table 3). Thus activation of LPO in the early periods of carcinogenesis was accompanied by increased microviscosity and orderliness of the lipid bilayer of the membranes.

In the later stages of carcinogenesis (3 months) changes in the microviscosity and orderliness of the lipid bilayer of the membranes were preserved (Tables 1 and 2) and LPO in these membranes was at a lower level than in the early periods (Table 3). We know that LPO is considerably inhibited in tumor cells [6]. The tendency for the LPO level to fall in the later stages of carcinogenesis compared with the early stages, which was observed, evidently reflected the beginning of the stage of tumor progression.

Administration of the antioxidant phenosan-1K prevented activation of LPO (Table 3) and helped to restore the normal physicochemical properties of the membranes (Tables 1 and 2). For instance, a decrease was observed in the rotary correlation time (τC) of the 15C spin probe and of the parameter or orderliness (S) of the lipid bilayer (spin probe 5-doxyl stearate) of the mitochondrial and microsomal membranes of the renal cortex of rats receiving phenosan-1K as well as the carcinogen, compared with these parameters in rats receiving the carcinogen alone (Tables 1 and 2).

Administration of the antioxidant phenosan-1K also promoted inhibition of tumor growth. Tumors of the kidneys developed in 56% of cases 5 months after the beginning of administration of the carcinogen, whereas in the group of animals receiving phenosan-1K in addition to the carcinogen, they developed in only 15% of cases ($p < 0.01$).

It can be postulated on the basis of these data that inhibition of tumor growth, induced by a chemical carcinogen, by means of this phenolic antioxidant is mediated through the modifying action of phenosan on oxidative and structural properties of the lipid phase of the cell membranes.

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