METABOLISM OF LIPID PEROXIDES DURING CHEMICAL CARCINOGENESIS

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The content of lecithins and cephalins and the activity of enzymic and nonenzymic systems of phospholipid peroxidation in the microsomes and mitochondria of the rat liver were sharply changed after injection of 3,4-benzpyrene. Significant changes take place in the content of lipid peroxides and activity of enzymes utilizing lipid peroxides (glutathione peroxidase, glutathione reductase) in the rat liver during carcinogenesis. Accumulation of lipid peroxides in the rat liver during carcinogenesis was shown to be connected with disturbances of balance between the systems generating and detoxicating lipid peroxides. The absence of lipid peroxides in the tumors can be attributed to high activity of protective enzyme systems and it reflects adaptive mechanisms aimed at maintaining a high background of proliferating cells in the tumor.

KEY WORDS: carcinogenesis; lipid peroxides; phospholipids; glutathione peroxidase; glutathione reductase.

Unsaturated acyl groups of membrane phospholipids under aerobic conditions are readily oxidized by a free-radical mechanism under the influence of enzyme systems with the formation of the corresponding hydroperoxides [2, 3]. Peroxides of lipids or their oxidative destruction products can exert a harmful systemic action on the cell [1, 6]. Peroxidation of lipids (POL) is regulated in vivo through the participation of enzymes utilizing lipid peroxides (GSH-peroxidase, GSSG-reductase) [4]. It was shown previously that free-radical peroxidation of polyene lipids in the host tissues may be an important stage in the pathogenesis of malignant growth [2, 3, 5].

It was accordingly decided to study some aspects of the metabolism of lipid peroxides during chemical carcinogenesis.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 120-150 g were each given a subcutaneous injection of 5 mg 3,4-benzpyrene (3,4-BP) in 0.5 ml oxidized olive oil or an equal dose of anthracene, a noncarcinogenic hydrocarbon (control) [8]. The subcellular organelles were isolated from the liver as described previously [3, 8]. The phospholipid content was determined after isolation of the corresponding fraction by thin-layer chromatography on silica-gel [9] and the content of lipid peroxides was determined by iodometric titration with amperometric recording of the end point [7]. Activity of the system of enzymic NADPH-dependent peroxidation (NDP) of phospholipids was characterized by the initial rate of accumulation of malonic dialdehyde [2]. Activity of the system of nonenzymic, ascorbate-dependent peroxidation (ADP) of lipids (ascorbate without exogenous Fe^{2+}) was characterized by the reciprocal of the induction period (the time taken to reach $\Delta D_{532} = 0.2$). The results of measurements of activity of the POL systems were expressed as the experiment/control ratio. Activity of GSH-peroxidase and GSSG-reductase was determined in the supernatant (750 g, 10 min) of the homogenate of perfused tissue in the presence of 0.1% Triton X-100 [4].

EXPERIMENTAL RESULTS

In the course of carcinogenesis significant changes took place in the content of the most readily peroxidized [13] fractions of phospholipids – lecithins and cephalins – in the microsomes and mitochondria of the rat

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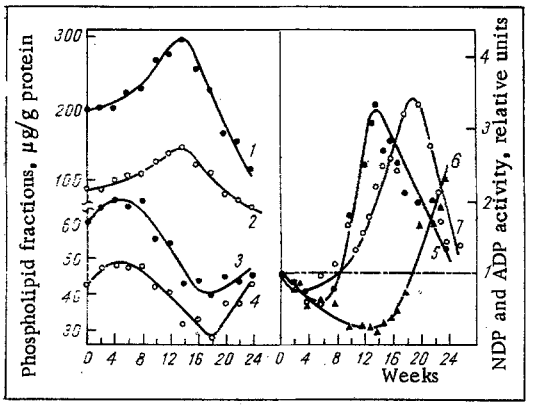

Fig. 1. Changes in content of licithins (1, 3) and cephalins (2, 4) and in activity of NDP (5) and ADP (6, 7) in rat liver microsomes (1, 2, 5, 6) and mitochondria (3, 4, 7) after injection of 3,4-BP.

liver during carcinogenesis (Fig. 1). After injection of 3,4-BP the content of lecithins and cephalins in the microsomes of the rat liver increased (Fig. 1) to reach a maximum in the 14th week of carcinogenesis (the time of appearance of the tumors). Development of the tumor was accompanied by a sharp decrease in the content of lecithins and cephalins in the liver microsomes of the tumor-bearing rats, and in the terminal stage of growth of the tumor it was below the initial level.

NDP activity in the rat liver microsomes fell very slightly during the first weeks after injection of 3,4-BP, but rose sharply after the 10th week of carcinogenesis, to reach a maximum at the 14th week of carcinogenesis (Fig. 1). In the period of growth of the tumor (after the 14th week) NDP activity fell, but even in the terminal stage it was higher than the liver NDP activity of the control animals. ADP activity in the liver microsomes changed antibatically relative to the change in NDP activity in the same membranes (Fig. 1).

In the stage of carcinogenesis when NDP activity in the liver microsomes was maximal (from the 10th through the 18th weeks) ADP activity in the same subcellular particles was thus inhibited, i.e., POL in the liver microsomes thus evidently takes place during carcinogenesis entirely through the action of the enzyme system, whereas nonenzymic oxidation of phospholipids is unlikely during this period.

Activation of NDP in the liver microsomes during carcinogenesis was accompanied by a marked increase in the content of lipid peroxides in the liver and by accumulation of lysophosphatides and, in particular, of lysolecithins, which are formed during peroxidation of the corresponding phospholipids and also in model systems [13], in the liver microsomes (Fig. 2). The appearance of hydrophilic peroxide groups in polyunsaturated acyl groups of phospholipids must lead to a change in the conformational properties of the microsomal membranes [1-5] and to destruction of cytochrome P_{450} [12]. A change in NDP activity in the liver microsomes during carcinogenesis in fact induces an antibatic change in activity of the microsomal system of xenobiotic oxidation [8], and under these circumstances the content of cytochrome P_{450} in the liver microsomes of rats with tumors falls progressively [8].

The content of lecithins and cephalins in the rat liver mitochondria fell from the 8th through the 18th week of carcinogenesis (Fig. 1). ADP activity in the mitochondria, on the other hand, rose sharply in this same period of carcinogenesis, but fell in the terminal stage of tumor growth (Fig. 1). The character of the change in NDP activity in the microsomes and ADP activity in the mitochondria of the liver during carcinogenesis was similar, but the maximum on the curve of changes in ADP activity in the mitochondria was shifted by 5-6 weeks to the right along the time axis relative to the maximum of the change in NDP activity in the microsomes. This phenomenon may possibly reflect induction of nonenzymic POL in the liver mitochondria during carcinogenesis by radical products of microsomal lipid oxidation. The development of free-radical reactions in microsomal phospholipids under the influence of NADPH-dependent dioxygenase in vitro may be accompanied by co-oxidation of lipids of other membrane structures which do not contain POL enzyme systems, probably on account of "leakage" of intensively formed radical products [10, 14]. The possibility likewise cannot be ruled out that increased solubilization of microsomal flavoproteins during oxidation of phospholipids by NADPH-dependent dioxygenase may enable them to play a role in the catalysis of POL in the hyaloplasm and other membranes, notably in the mitochondria [11].

TABLE 1. Activity of GSH-Peroxidase and GSSG-Reductase and Phospholipid Content in Liver and in Sarcoma Induced by 3,4-BP (M ± m)

Tissue	GSH-peroxidase, units/mg protein	GSSG-reductase, units/mg protein	Phospholipids, mg/g tissue
Liver of intact rats (26)	37,0±1,0	9,0±0,1	25,2±1,6
Tumor:			
16 weeks (4)	46,7±3,5*	3,4±0,2*	5,1±0,2*
20 weeks (3)	69,8±2,2*	22,0±0,4*	5,5±1,1*
24 weeks (5)	67,4±1,2*	19,6±0,4*	5,0±0,7*

Legend. Number of experiments given in parentheses; *) $P < 0.05$.

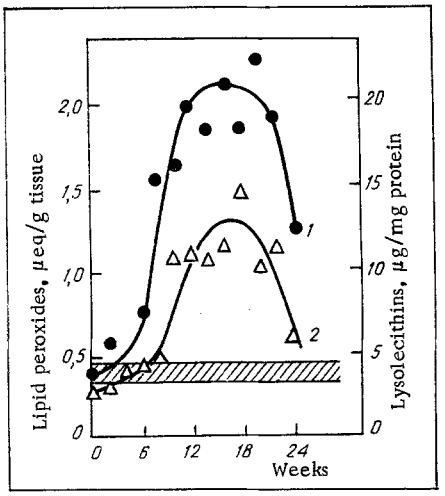

Fig. 2

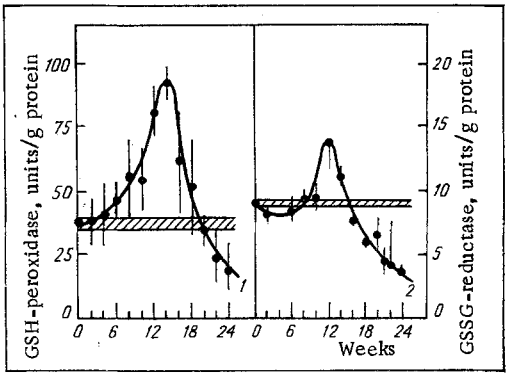

Fig. 3

Fig. 2. Changes in content of lipid peroxides in liver (1) and lysolecithins in liver microsomes (2) of rats after injection of 3,4-BP.

Fig. 3. Changes in GSH-peroxidase (1) and GSSG-reductase activity (2) in rat liver after injection of 3,4-BP.

Activity of GSH-peroxidase and GSSG-reductase in the rat liver increased after injection of 3,4-BP to reach a maximum in the 12th-14th weeks of carcinogenesis (Fig. 3), it fell during the period of tumor growth, and in the terminal stage was below the control level (Fig. 3). Activation of GSH-peroxidase in the rat liver during carcinogenesis evidently reflects the inductive synthesis of this enzyme through accumulation of the substrate, namely lipid peroxides (Fig. 2). Administration of hydroperoxides of arachidonic acid into intact mice in fact causes a marked increase in liver GSH-peroxidase activity [5]. Nevertheless, although from the 8th through the 18th week of carcinogenesis the liver GSH-peroxidase level rose (Fig. 3), the real content of lipid peroxides in the liver in this period also increased considerably (Fig. 2), evidence of an imbalance in the action of the systems for generation and inactivation of lipid peroxides in the tissues during carcinogenesis.

Tumor cells could not successfully reproduce if an increase in POL in the host led to intensification of free-radical oxidation of lipids in the tumor itself. The virtually complete absence of POL products in tumors [2, 3, 5, 7] indicates the existence of molecular mechanisms protecting the actively proliferating tumor cells against the cytostatic action of lipid peroxides. The absence of lipid peroxides in the tumor cannot be explained by an increase in the content of lipid antioxidants and it is probably due to the extremely high activity of protective enzyme systems in the tumor [2-5]. It will be clear from Table 1 that in the sarcoma induced by 3,4-BP also, activity of GSH-peroxidase and GSSG-reductase in the tumor was comparable with or much higher than the activity of these enzymes in the liver (a tissue with a high level of activity) in intact rats.

The phospholipid content in the tumor was much lower than in the liver (Table 1). Detoxication of peroxides of phospholipids in the tumor (if they are formed) thus takes place much more effectively than in the liver, for in the tumor GSH-peroxidase activity expressed per unit potential substrate of free-radical oxidation is considerably higher than in the liver. Consequently, the high resistance of tumors to POL, determined by the increased level of protective enzyme systems, probably reflects adaptive mechanisms maintaining a high pool of proliferating cells in the tumor.

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ROLE OF VITAMIN A IN CHEMICAL CARCINOGENESIS OF THE MAMMARY GLAND

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The effect of feeding rats with large doses of vitamin A on the concentration of the polycyclic hydrocarbon 7,12-dimethylbenz(a)anthracene (DMBA) and its metabolites in various organs and in the blood and also on the rate of metabolism in the liver of rats after intravenous injection of the carcinogen were studied. In hypervitaminosis A the quantity of DMBA and its metabolites was found to be considerably reduced in all the organs tested and in the blood. The rate of DMBA metabolism in the liver of the animals increased with an increase in the dose of vitamin A.

KEY WORDS: hypervitaminosis A; 7,12-dimethylbenz(a)anthracene; metabolism; carcinogenesis of the mammary gland.

During the last decade the anticarcinogenic activity of vitamin A has been studied in many countries. It has been shown that vitamin A has a prophylactic action and, in some precancerous states, a therapeutic action also in experimental animals. Preliminary feeding with large doses of vitamin A protects animals against induction of cancer in them by various chemical carcinogens [2]. The anticarcinogenic action of vitamin A in such cases may be due to modification of metabolism of hydrocarbons in the body [9] and to a change in the level of the carcinogen or of its carcinogenic metabolite in the target organ. Some workers consider that the concentration of a carcinogen in the target organ is an important factor for the induction of tumors by polycyclic hydrocarbons [4].

The object of this investigation was to study the concentration of a carcinogen and its metabolites in certain organs and in the target organ during induction of mammary gland cancer in rats receiving large doses of vitamin A.

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