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SUSCEPTIBILITY AND RESISTANCE OF RAT LIVER TISSUE TO OXIDATIVE DAMAGE DURING DIETHYLNITROSAMINE CARCINOGENESIS

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There exist many observations indicating that the peroxidative breakdown of membrane lipids is significantly decreased in most highly dedifferentiated hepatomas (1,2). The extent of such a decrease seems well related to the dedifferentiation degree (3).

A further contribution to these studies should be the analysis of both the lipid peroxidation basal levels and the response to oxidative stress in the liver tissue at different stages of its neoplastic transformation.

The present report deals with the monitoring of lipid peroxidation in homogenates from normal liver, hyperplastic nodules and hepatomas before and after treatment with ascorbate or adenosindiphosphate(ADP)-iron complex.

MATERIALS AND METHODS

Male Wistar rats, weighing 100-120 g at the beginning of the experiment, were placed under diethylnitrosamine (DEN) hepatocarcinogenesis treatment following the procedure described by Solt et al. (4). Homogenates from different tissues(20% w/v in 0.25 M sucrose) were diluted to 4% (w/v) with 0.10 M KCl and 0.033 M Tris-HCl buffer (pH 7.4). These homogenates were incubated for 60 min at 37°C in a shaking water bath (100 strokes/min). The reaction mixture contained: 0.6 ml homogenates; 500 μM ascorbic acid or 250 μM - 2 μM ADP/Fe^{2+} complex; Tris-KCl (as above reported) to a final volume of 6 ml. For ADP/Fe^{2+} treated samples 4.1 mM glucose-6-phosphate, 0.19 U/ml glucose-6-phosphate dehydrogenase and 0.164 mM NADP were also added. Control mixture contained only Tris-KCl. The reaction was started by the addition of the different homogenates. Before and at the end of incubation, lipid peroxidation levels were checked in terms of production of total unpolar carbonyls according to Esterbauer et al. (5).

RESULTS AND DISCUSSION

The monitoring of peroxidative processes has been demonstrated to be more reliable and complete when it accounts for the production not only of malonaldehyde but also of the great variety of other carbonyl compounds demonstrated to originate from membrane lipid breakdown (5).

The basal (not stimulated) production of total non polar aldehydes in homogenates from nodules and hepatoma was similar to that evidenced in the normal liver. On the contrary, the susceptibility of nodules and tumours to oxidative stress, again measured in terms of unpolar carbonyls, was markedly reduced as to the control or even absent (see table 1). The stimula-

tion of lipid peroxidation by ascorbate is due to its oxidation in air and redox coupling with metal ions. The lack of prooxidant effect of ascorbate in the liver tissue under carcinogenic treatment at the stage of hyperplastic reversible nodules is probably related to disturbances of the oxidative metabolism. The lower efficiency of ADP-iron complex in stimulating peroxidative reactions in nodules and tumours is well related with the observed parallel decrease of cytochrome P-450 dependent enzymatic activities (6). In addition, a reduced availability of polyunsaturated fatty acids in the nodular tissue in comparison with normal liver seems to play a relevant role in the attenuation of the effect of the two prooxidant treatments used.

TABLE 1. TOTAL NON POLAR CARBONYLS PRODUCED BY HOMOGENATES FROM NORMAL LIVER, HYPERPLASTIC NODULES AND HEPATOMA, AFTER 60 MIN INCUBATION AT 37 °C.*

	CARBONYL GROUPS (nanomoles/mg protein)		
EXP. SAMPLES	TREATMENT		
	NONE	500μM ASCORBATE	250μM-2μM ADP-IRON
NORMAL LIVER	2.2 ± 0.7	6.2 ± 1.2§	11.7 ± 2.4§
NODULES	4.3 ± 1.1	3.5 ± 1.3▼	7.2 ± 1.6θ
HEPATOMA	3.2 ± 0.3	4.8 ± 2.1▼	5.7 ± 0.7θ

* - The values are means ± S.D. of 4-5 experiments in duplicate, after time zero subtraction.
§ - By Student's t-test the difference between treated and untreated samples is significant at $P < 0.01$
θ - By Student's t-test the difference between treated and untreated samples is significant at $P < 0.05$
▼ - No significant difference between treated and untreated samples ($P < 0.05$).

In conclusion, a strong resistance to oxidative stress is evident in the liver tissue since the early stages of the DEN-induced carcinogenic process and it can be considered as a biochemical marker of dedifferentiation.

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