MICROSOMAL ENZYME ACTIVITY AND LIPID PEROXIDATION IN THE LIVER DAMAGED BY GALACTOSAMINE

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The important role of the cytochrome P450-containing mono-oxygenase system in the detoxicating function of the liver and in maintenance of the constancy of the chemical internal milieu of the body is well known [2, 5, 8]. It has been shown that in liver disease the process of biotransformation of xenobiotics and endogenous substrates is disturbed [6, 14]; estimation of the activity of enzymes of microsomal oxidation can thus be effectively used in hepatology [9, 13].

Disturbance of the structure and permeability of cell membranes, including membranes of the endoplasmic reticulum, is connected with activation of lipid peroxidation (LPO) processes, leading to necrobiosis and cytolytic damage of the cells [4].

It was accordingly decided to undertake a combined study of reactions of microsomal oxidation, LPO, and protein biosynthesis, which are dependent on the structure and function of endoplasmic reticulum membranes, for they are associated with the mechanism of injury of the hepatocyte. In the investigation described below, using the comparatively new galactosamine model of liver damage, a simultaneous study was made of enzymes of the mono-oxygenase mixed-function system and of LPO.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 150-220g. The animals were divided into three groups: a control group of intact rats (group 1) and two experimental. The animals of group 2 received a single injection of galactosamine in a dose of 100 mg/100 g body weight intraperitoneally [10]. Rats of group 3 received two intraperitoneal injections of barbital sodium in a dose of 10 mg/100 g, once daily, and 24 h after the second injection of barbital sodium they received galactosamine as in group 2. The control for group 3 consisted of the animals of group 2. The rats were decapitated and blood plasma was used to record the thromboelastogram. The liver was homogenized with 1.15% KCl solution. The homogenate was centrifuged at 10,000g for 15 min to obtain the microsome-cytosol fraction, which was used to study enzymes of microsomal oxidation and LPO. Cytochromes P450 and b_5 were determined by a spectrophotometric method [1, 14], using the coefficient of molar extinction [12]. NADH-ferricyanide reductase activity was determined by the decrease in $K_3Fe(CN)_6$ [3]. LPO was studied in experiments *in vitro* after incubation of the test fraction at 37°C for 30 min with galactosamine in a final concentration of 100 mM, on the basis of malonic dialdehyde accumulation [4]. The color of the reaction was determined as described in [1]. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

A fall in the total liver protein content and in protein of the microsome-cytosol fraction was found 24 h after injection of galactosamine (group 2). The body weight of the rats was reduced at the same time (Fig. 1). In the animals of group 3, receiving barbital sodium, an inducer of microsomal enzymes, and galactosamine, the weight of the liver and the body weight of the rats were increased.

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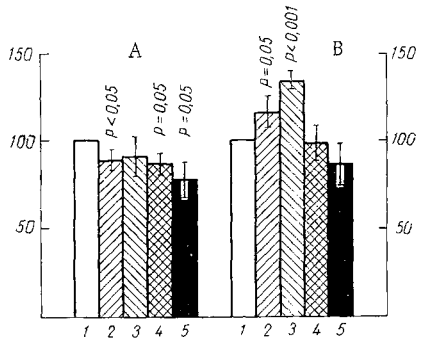

Fig. 1. Effect of galactosamine (A), and galactosamine and barbital sodium (B) on protein concentration and weight of liver and on body weight of rats 24 h after their injection (in % of control). 1) Control, 2) weight of rats (in g), 3) weight of liver (in g), 4) liver protein (in mg/g tissue), 5) protein of microsome-cytosol fraction (in mg/g tissue).

The comparative study of cytochrome P450 showed a marked increase in its concentration (24%) in the galactosamine-damaged liver when calculated per milligram protein, whereas if calculated per gram of liver there was a tendency for this parameter to fall, evidence of the unreliability of these units (Fig. 2). These results agree with data in the literature [7] and they probably reflect the strength of the mono-oxygenase system in the presence of liver damage induced by galactosamine, which has a high biotransformation level.

The results of the study of cytochrome b_5 showed a marked increase in its concentration in the liver damaged by galactosamine (group 2). No significant changes were found after injection of barbital sodium and galactosamine.

Investigation of electron transport in the cycle of hydroxylation reactions revealed an increase in NADH-ferricyanide reductase activity under the influence of glactosamine (group 2). NADH-ferricyanide reductase and cytochrome b_5 are adjacent links in the same

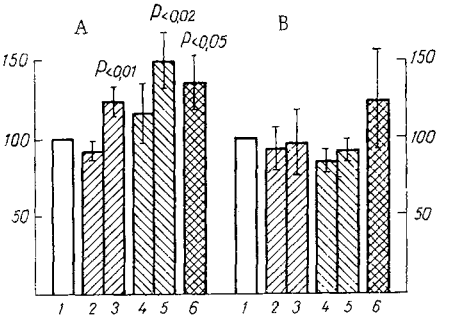

Fig. 2. Concentrations of cytochromes P450 and b_5 and NADH-ferricyanide reductase activity of liver 24 h after injection of galactosamine (A) and of galactosamine and barbital sodium (B) (in % of control). 1) Control, 2) cytochrome P450 (in nmoles/g tissue), 3) cytochrome P450 (in nmoles/mg protein), 4) cytochrome b_5 (in nmoles/g tissue), 5) cytochrome b_5 (in nmoles/mg protein/min), 6) NADH-ferricyanide reductase (nmoles/mg protein/min).

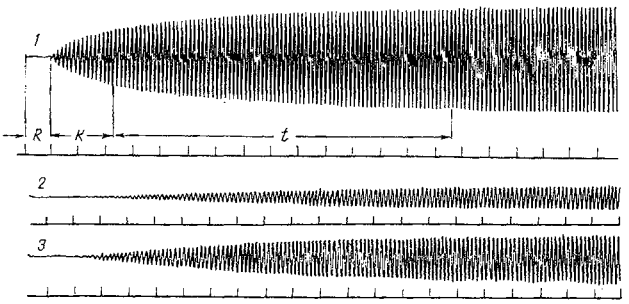

Fig. 3. Thromboelastograms of intact rat (1) and of rats receiving galactosamine (2) and barbital sodium and galactosamine (3). R) Reaction time, K) clot formation time, t) coagulation constant.

transport chain for electrons and protons. It can be tentatively suggested that the shift of these enzymes in the same direction was due to their linking in a common detoxicating mechanism, responsible for the formation of the hydroxylated substrates in the final stage.

In experiments *in vitro* galactosamine sharply activates enzymic LPO reactions (1.400 ± 0.030 instead of 0.364 ± 0.015 nmoles/mg protein/min; $P < 0.001$), which are linked with membranes of the endoplasmic reticulum. Ascorbate-dependent LPO was activated much less strongly (0.666 ± 0.024 instead of 0.545 ± 0.020 nmoles/mg protein/min; $P < 0.01$). It can be postulated that activation of enzymic LPO reactions leads to disturbance of the structure and function of endoplasmic reticulum membranes and it may be the key mechanism in the hepatotoxic manifestation of galactosamine.

Thromboelastography (Fig. 3) revealed a marked hemorrhagic syndrome after injection of galactosamine (group 2), which can be interpreted as a disturbance of synthesis of thrombogenic factors (primarily the prothrombin complex), and this evidently is connected with the fall in the total liver protein concentration mentioned previously. Preliminary injection of barbital sodium caused appreciable recovery of the clotting function of the blood, as shown by shortening of the reaction time (R) and the clot formation time (K) and an increase in the maximal amplitude.

Analysis of the results suggests that in galactosamine-induced liver damage the synthesis of exportable proteins for use in other tissues (thrombogenic factors, according to the data of these experiments) is disturbed. Meanwhile induction of or an increase in the activity of microsomal oxidative enzymes is probably a compensatory mechanism controlling biotransformation enzymes and it is in harmony with the scheme proposed in [11] for regulation of cytochrome P450 synthesis.

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