Protective Effect of Enterosgel on Rat Liver Lysosomes during Cytostatic Treatment

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Polychemotherapy with a complex of cytostatics (cyclophosphamide, doxorubicin, vincristine, prednisolone) induces progressive damage to hepatocyte membranes, which manifested in labilization of lysosomes and activation of lysosomal enzymes and serum transaminases. Enterosgel stabilized liver lysosomes and reduced manifestation of hepatocyte cytolysis.

Key Words: *enterosgel; cytostatics; polychemotherapy; liver lysosomes; membrane damage*

Enterosorption is now widely used in experimental therapy of diseases associated with endogenous intoxication [4]. Comparative study of adsorbents showed high efficiency of polymethylsiloxane hydrogel, enterosgel with globular porous matrix structure and active detoxifying effect [8].

An important problem of antitumor drug therapy is to reduce drug toxicity towards nontumor parenchymatous cells. Biotransformation of cytostatics in the liver yields toxic metabolites, which damage hepatocyte membranes [5]. Antitumor drugs can interact with lysosomes (doxorubicin, cyclophosphamide) [11,14], impair vesicular transport, induce autophagia (vincristine) [15], and can accumulate in lysosomes (doxorubicin) [9]. The cytotoxicity of anthracyclines, *e.g.* doxorubicin, depends on the state of the lysosomal system and increases after injection of lysosomotropic amine chloroquine reducing pH gradient across the lysosomal membrane [12].

We investigated the state of hepatocyte membranes during polychemotherapy (PCT) and experimental treatment with enterosgel.

MATERIALS AND METHODS

Experiments were carried out on 100 female Wistar rats (180-200 g). The complex of antitumor drugs included cyclophosphamide (Olain Plant), doxorubicin (Bryntsalov A), vincristine, and prednisolone (both from Gedeon Richter). The drugs were injected intraperitoneally in single doses equal to $^1/_5$ LD_{50} (21, 2.1, 0.14, and 2.1 mg/kg, respectively) [3]. Enterosgel (SILMA) was administered a single daily dose of 1 g/kg into the stomach for 7 days, the treatment was started 24 h after PCT. The studies were carried out 7, 14, and 21 days after PCT. Intact animals served as controls.

The rats were decapitated under light ether narcosis. The liver was perfused with cold 0.25 M sucrose solution containing 1 mM EDTA (pH 7.4). Liver homogenate (20%) was prepared in the same solution and placed on ice. Total activity of lysosomal enzymes was evaluated after lysosome destruction in the presence of 0.15% Triton X-100. Free activities of lysosomal enzymes were measured in the presence of 0.25 M sucrose (10-min incubation). Activity of β-galactosidase was measured as described previously [2] with 4-nitrophenyl-β-D-galactopyranoside as the substrate, activity of acid phosphatase (AP) was assayed with 2-glycerophosphate as the substrate. Inorganic phosphate was evaluated by a one-step method using a mixture of ammonium molybdate with Twin-80 [7]. Activity of N-acetyl-β,D-glucosaminidase (N-AG) was evaluated with 4-nitrophenyl-N-acetyl-β,D-glucosaminidine for substrate [2], activity of acid RNase by a modified method [10] with high-polymeric RNA as the substrate. Free enzyme activities in liver homogenate were expressed in percent of total activity.

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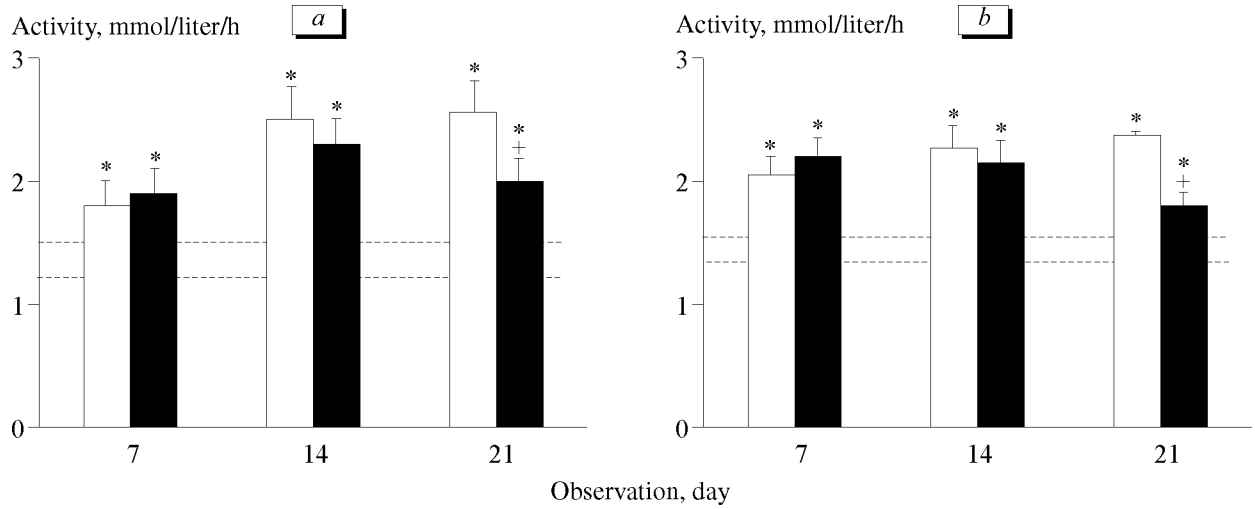

Fig. 1. Serum activities of alanine (*a*) and aspartate aminotransferases (*b*) in rats after polychemotherapy without (light bars) and with correction with enterosgel (dark bars). Here and in Figs. 2 and 3: dotted line shows the range of control values; $p<0.05$ compared to *control, +polychemotherapy without correction by the day of examination.

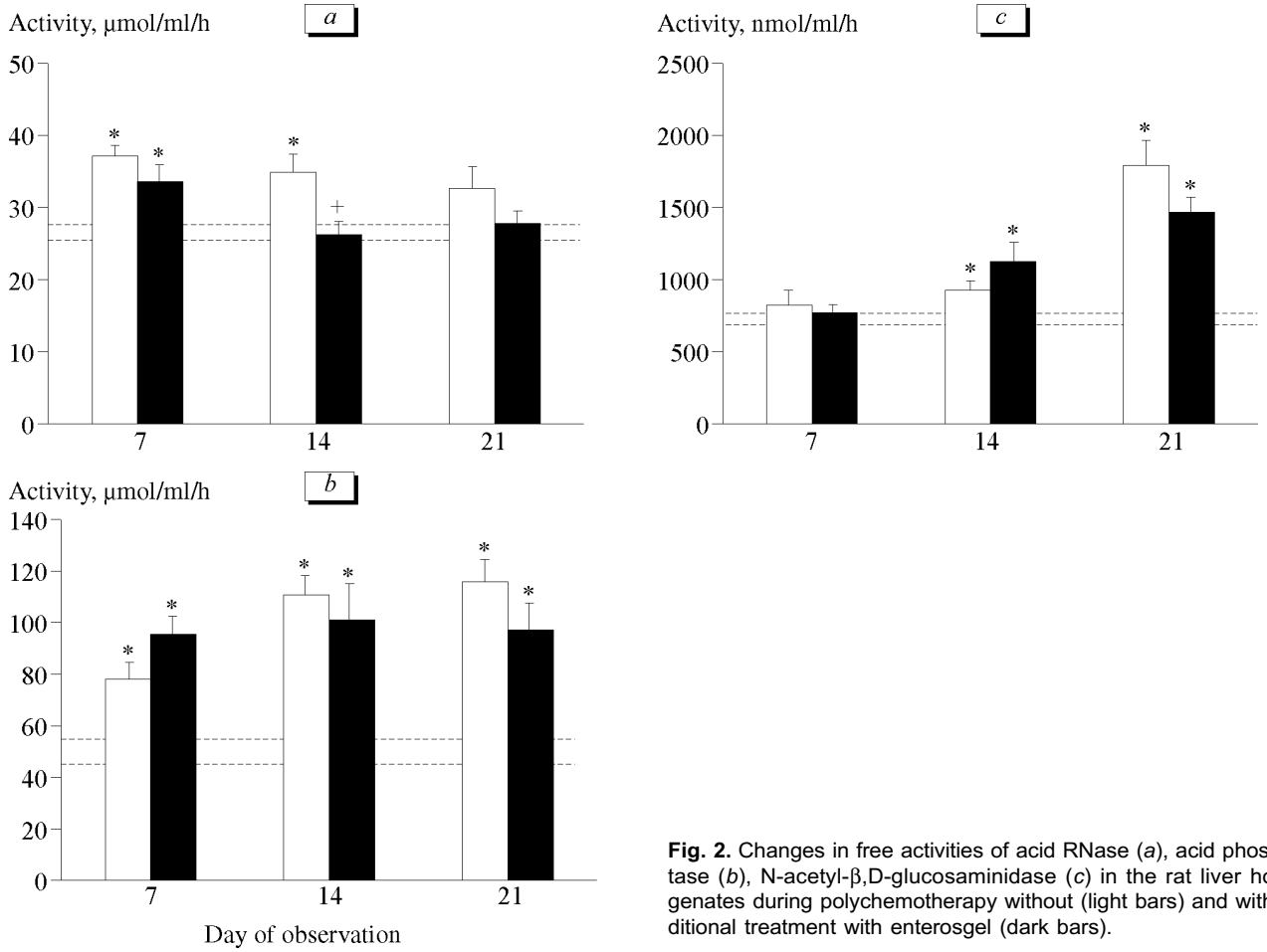

Fig. 2. Changes in free activities of acid RNase (*a*), acid phosphatase (*b*), N-acetyl-β,D-glucosaminidase (*c*) in the rat liver homogenates during polychemotherapy without (light bars) and with additional treatment with enterosgel (dark bars).

Serum aspartate- and alanine aminotransferase activities (AST and ALT, respectively, from Novotransaminase, Vektor Best) were measured.

The significance of differences was evaluated using Student's *t* test.

RESULTS

Polychemotherapy increased the serum level of hepatocyte damage markers (cytosol ALT and AST transaminases) (Fig. 1). Transaminase activity progressive-

ly increased in the course of observation: ALT activity increased 1.3-fold after 7 days and 1.8-1.9-fold after 14 and 21 days, AST activity at these terms 1.4- and 1.6-fold surpassed the control.

A triad of signs is used for detecting damage to the cell lysosomal system: increase of free and unsedimentable activity of lysosomal enzymes in liver homogenate and their appearance in the serum in high quantities. In our study free activity of lysosomal enzymes in liver homogenate from intact animals was 16-18% (Fig. 2), while cytostatic treatment increased this parameter as early as after 7 days. At later terms free enzyme activities continued to increase: AP and N-AG increased 2.2- and 2-fold, respectively. The maximum increase in free activity was observed for acid RNase (2.4 times, 41.8%) and β-galactosidase (2.9 times, 52.8%).

Polychemotherapy increased also serum activities of lysosomal enzymes. Activities of all enzymes (except acid RNase) increased over the course of observation (Fig. 3). AP activity increased 1.5-fold on day 7, 2.2-fold on day 14, and 2.3-fold on day 21 after cytostatic treatment. N-AG activity peaked (increased 2.5 times) on day 21.

The detected changes in free and serum activities of lysosomal enzymes and transaminases attested to *in vivo* hepatocyte damage involving lysosomes caused by polychemotherapy. These shifts can be partially explained by necrotic and/or apoptotic death of hepatocytes [13] initiated by multiple cytotoxic effects of drug therapy and by impairment of the barrier function of cell membranes due to LPO activation [6].

Damage to lysosomal membrane was paralleled by an increase in intralysosomal pH, which, as was mentioned above, is a factor increasing antitumor cytotoxicity of anthracyclines. Therefore labilization of lysosomes in this case not only reflects membrane damage, but can potentiate PCT cytotoxicity for hepatocytes.

Enterosgel stabilized liver lysosomes in rats during PCT. Adsorbent reduced free activity of lysosomal enzymes in liver homogenate (Fig. 2). The pro-

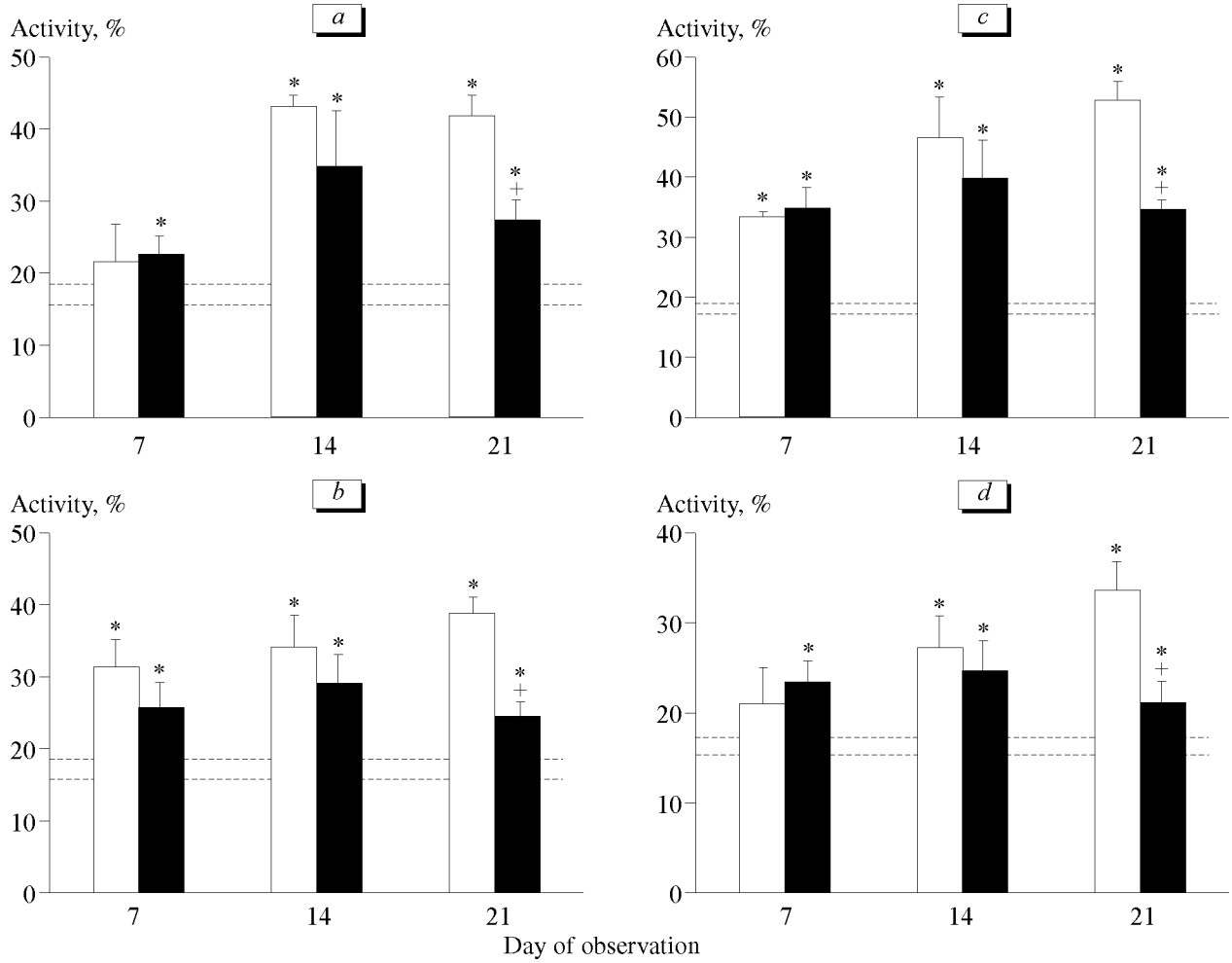

Fig. 3. Changes in free activities of acid RNase (*a*), acid phosphatase (*b*), β-galactosidase (*c*), and N-acetyl-β,D-glucosaminidase (*d*) in the sera of rats during polychemotherapy without (light bars) and with additional treatment with enterosgel (dark bars).

tective effect of enterosgel was observed at the early terms of treatment, but on days 7 and 14 the differences were insugnificant. Free activities of β-galactosidase, AP, N-AG, and acid RNase decreased 1.5-1.6-fold on day 21 in comparison with that in the PCT group without correction, but did not reach the control level.

Treatment with enterosgel prevented the increase in serum activities of lysosomal enzymes (Fig. 3). Activity of acid RNase was 1.2-1.3 times lower than in the PCT group, and on days 14 and 21 it did not differ from the control. Serum activities of AP and N-AG in animals treated with the adsorbent virtually did not differ from those in the PCT group on days 7 and 14, but tended to decrease on day 21.

Serum transaminase activities in animals treated with enterosgel tended to decrease on days 7 and 14 (Fig. 1). On day 21 activities of ALT and AST decreased significantly (1.2 and 1.3 times, respectively), but remained above the control. In general, enterosgel improved the state of hepatocyte membranes damaged by PCT. At the same time, there are histological data that enterosgel did not attenuate the main cytotoxic effect of PCT towards the target cells [1].

Hence, enterosgel effectively removed the negative aftereffects of PCT on hepatocytes. A possible mechanism of this effect is intestinal adsorption of toxic products forming during PCT, primarily enterotoxin, medium molecules, lipid peroxides, e.g. MDA [8]. These results prompt more detailed investigation of the therapeutic effect of enterosgel and open new vistas for its clinical application.

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