Relationship between Parameters of Lipid Peroxidation during Obstructive Jaundice and after Bile Flow Restoration

L. B. Dudnik, A. N. Tsupko, M. A. Shupik, G. G. Akhaladze*, E. I. Galperin*, L. V. Platonova*, E. A. Pantaz, and A. V. Alessenko

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Restoration of bile flow after 9-day cholestasis in rat liver normalized the content of lipid peroxidation products. The removal of the cholestatic factor after 12-day cholestasis was not followed by recovery of these parameters. We showed that measurement of serum concentration of lipid peroxidation products in patients with cholelithiasis during the preoperative period holds promise for selection of the optimum time for surgical treatment and prediction of the risk of postoperative complications.

Key Words: *lipid peroxidation; cholelithiasis; common bile duct ligation; liver; blood serum*

Cholestasis is often followed by liver damage. This clinical state is usually observed in cholelithiasis (CLT) or hepatoduodenal tumors due to mechanical obstruction of bile flow. Damage to the hepatic parenchyma during cholestasis is associated with toxic effects of bile components and mechanical pressure of dilated biliary ducts. However, removal of the cholestatic factor does not necessarily normalize liver function [11,12]. It is important to evaluate the period and type of surgical treatment and to develop new methods for the prediction and prevention of postoperative complications.

Oxidative stress induces death of liver cells [6]. Cholestasis is accompanied by accumulation of lipid peroxidation (LPO) products in the liver. Hence, free radical oxidation of membrane lipids plays a major role in hepatocyte death [7,9]. However, little is known about the intensity and role of LPO in liver damage after restoration of bile flow.

E. M. Emanuel' Institute of Biochemical Physics, Russian Academy of Sciences; *I. M. Sechenov Moscow Medical Academy, Moscow. ***Address for correspondence:*** ibd@sky.chph.ras.ru. L. B. Dudnik

Here we studied LPO in rat liver after the removal of the cholestatic factor (ligation of the common bile duct). Besides this, we evaluated whether parameters of LPO in the blood of patients may be used to predict postoperative complications after surgical treatment for cholestasis.

MATERIALS AND METHODS

Subhepatic cholestasis in Wistar rats was induced by double ligation of the extrapancreatic segment in the common bile duct. The duct was dissected. A sealed polyethylene catheter was introduced into the proximal end of the common bile duct. Laparotomy was performed under brietal anesthesia (50 mg/kg intravenously). The duration of cholestasis was 9 and 12 days. Blood flow was restored by cutting off the end of an intraductal catheter. Liver samples were studied 3-18 days after bile flow restoration. The liver of intact and sham-operated animals (narcosis, laparotomy, and manipulations on the common bile duct without ligation and dissection) served as the control.

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Lipids were extracted from liver homogenate [1]. Lipid concentration was measured gravimetrically.

Blood serum samples (n=48) from CLT patients during the preoperative and postoperative periods were presented by G. V. Pupelis. The reference groups included healthy donors (n=41) and patients operated for postoperative hernia (n=9). The intensity of LPO in serum samples from patients and liver samples from experimental animals was evaluated by the concentration of conjugated dienes and ketodienes. The electron spectra of lipid solutions in a methanol—hexane mixture were recorded on a Beckman spectrophotometer. Molar absorption coefficients for conjugated dienes and ketodienes were 21,000 and 23,000 liter/mol/cm, respectively. The concentration of LPO products was calculated per mg lipids [4].

The results were analyzed statistically. The differences were significant at $p \leq 0.05$.

RESULTS

Cholestatic damage to the liver caused by common bile duct ligation in rats is accompanied by phasic changes in the concentration of LPO products: this parameter decreases during at the early terms of the experiment, but sharply increases at the peak of the disease [4]. Phasic changes in LPO intensity probably reflect the effect of bile components, which are accumulated in the liver during cholestasis. During the early period of cholestasis, the rate of accumulation of bilirubin (formed at a constant rate during hemoglobin degradation in aging erythrocytes [10]) surpasses the rate of bile acid accumulation in the liver due to specific features of bile acid metabolism. Bilirubin exhibits antioxidant properties [1,2,14], while bile acids can stimulate LPO [7,12]. Bile acids can significantly increase LPO

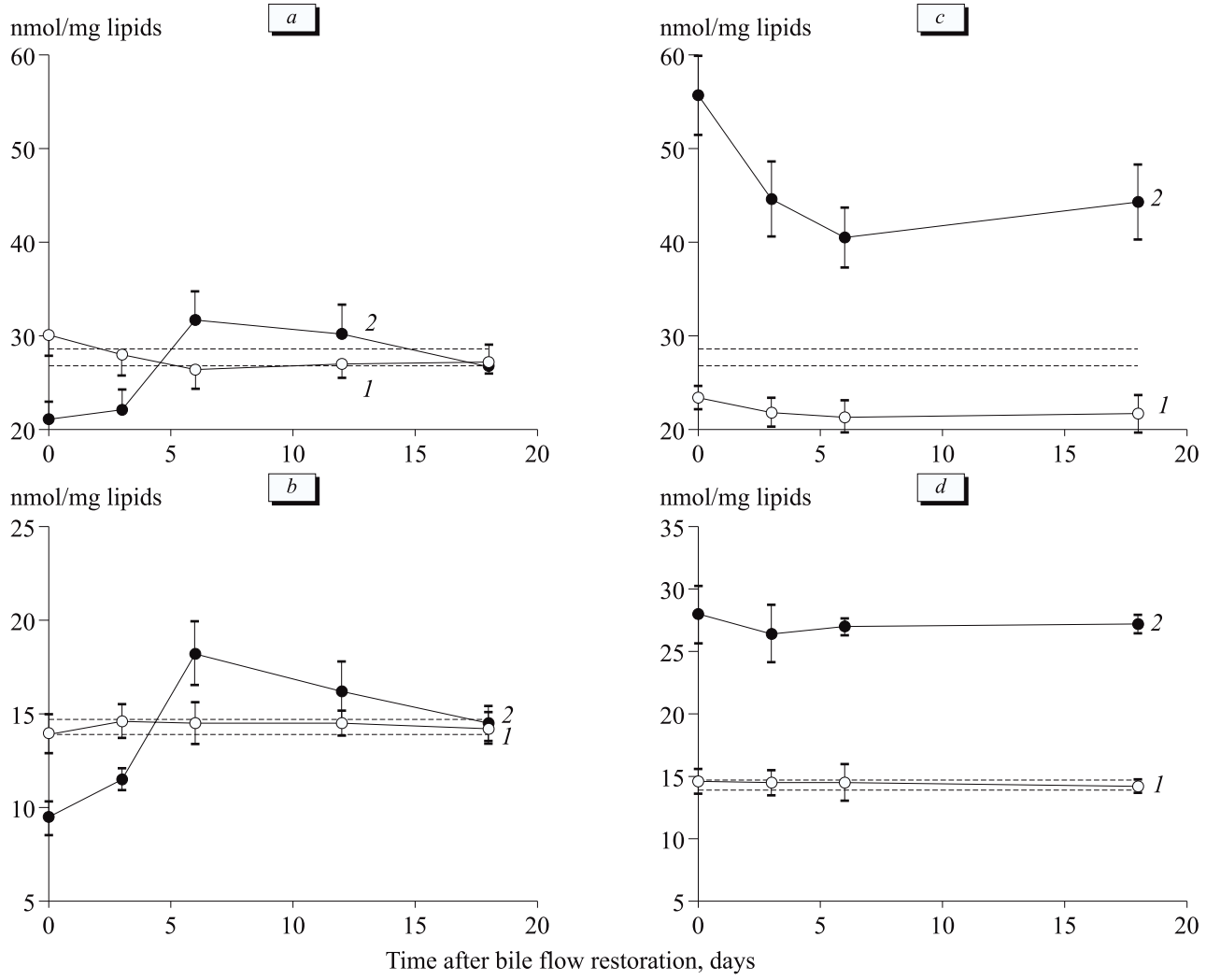

Fig. 1. Concentration of conjugated dienes (*a*, *c*) and ketodienes (*b*, *d*) in the liver of rats with bile flow restoration after 9-day (*a*, *b*) and 12-day cholestasis (*c*, *d*) due to common bile duct ligation. Sham operation (*1*); subhepatic cholestasis and further restoration of bile flow (*2*). Values between dotted lines: intact control.

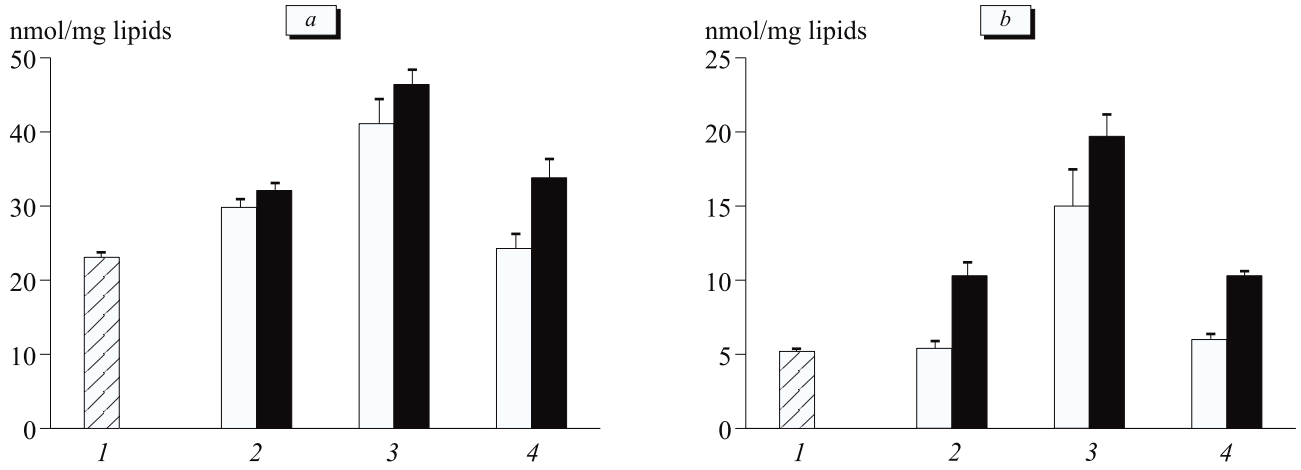

Fig. 2. Serum concentration of conjugated dienes (*a*) and ketodienes (*b*) in patients with CLT. Healthy donors (*1*); favorable course of the postoperative period (*2*); complicated course of the postoperative period (*3*); herniotomy (*4*). Light bars, preoperative period; dark bars, postoperative period.

and cause massive death of hepatocytes during prolonged cholestasis. It can be hypothesized that free radical oxidation of membrane lipids after bile flow restoration depends on the rate of this process in the preoperative period. Therefore, we measured the concentration of LPO products during bile flow restoration in rat liver in the periods corresponding to a decrease (9-day cholestasis) or increase in the test parameters (12-day cholestasis).

Bile flow restoration on day 9 after common bile duct ligation led to an increase in the concentration of LPO products (conjugated dienes and ketodienes) in rat liver. After 3 days, the content of LPO products remained 1.3-fold lower than in sham-operated animals (Fig. 1, *a*, *b*), then these parameters progressively increased and on day 6 exceeded those in sham-operated control animals (by 1.1-1.2 times). The concentration of conjugated dienes and ketodienes in treated rats progressively decreased in the follow-up period and did not differ from that in intact animals (day 18; Fig. 1, *a*, *b*).

The concentration of LPO products in rats with 12-day cholestasis increased by more than 2 times compared to intact control (Fig. 1, *c*, *d*). Bile flow restoration in rats of this group did not normalize the concentration of conjugated dienes and ketodienes. On day 18 after removal of the ligature, the concentration of LPO products 1.6-fold surpassed the control.

Thus, the intensity of LPO after bile flow restoration depends on the concentration of LPO products in the liver during the previous period. Bile flow restoration in rats against the background of reduced LPO parameters led to a slight increase in the test parameters to a value observed in intact liver. At later stages of cholestasis characterized by accumulation of LPO products, removal of the cholestatic factor was not followed by normalization of the test parameters.

Subhepatic jaundice due to CLT and hepatoduodenal tumors is one of the most common forms of cholestasis in clinical practice. Surgical treatment is indicated in these diseases, but serious postoperative complications are often observed in these patients. The most serious complication is liver failure, which can cause death. Hence, patients with obstructive jaundice are sometimes subjected to a two-stage surgery: temporal bile diversion (stage 1) and then removal of the factor causing bile flow obstruction (stage 2). We hypothesized that simple method of measuring serum concentration of LPO products in patients during the preoperative period is of significant prognostic value.

For evaluation of the effect of surgical treatment for CLT on LPO, parameters of LPO in the serum from preoperative and postoperative patients were compared to those in herniotomy patients without biliary disorders. Favorable course of the postoperative period in the majority of CLT patients was accompanied by a moderate increase in LPO. These changes were probably related to stress response, effect of narcosis, and surgical trauma (Fig. 2). The recovery of patients was followed by a decrease in LPO, which did not differ from that in healthy donors. In some patients, significant increase in LPO was accompanied by unfavorable course of the postoperative period. Retrospective study showed that parameters of LPO in these patients were elevated during the preoperative period (Fig. 2). Hence, high serum concentration of LPO products in CLT patients can be considered as an

unfavorable prognostic factor. This parameter should be used as an additional criterion for selection of the period and type of surgical treatment.

Our results indicate that LPO plays a role in the pathogenesis of liver failure during bile flow restoration at the late stage of cholestasis. The intensity of LPO after surgical treatment for cholestasis depends on the concentration of LPO products in the liver during the preoperative period. Measurement of serum concentration of LPO products in surgical patients with obstructive jaundice can be used not only for selection of the therapeutic procedure and optimal time of surgical treatment, but also for prediction of the course of the postoperative period. Antioxidant drugs should be prescribed during the preoperative and early postoperative period to reduce the risk of postoperative complications.

REFERENCES

1. A. F. Blyuger, L. B. Dudnik, A. Ya. Maiore, and I. E. Mieze, *Byull. Eksp. Biol. Med.*, **99**, No. 2, 166-169 (1985).
2. L. B. Dudnik, A. N. Tsyupko, A. V. Khrenov, and A. V. Alesenko, *Biokhimiya*, **66**, No. 9, 1252-1262 (2001).
3. L. B. Dudnik, A. N. Tsyupko, L. N. Shingarova, *et al.*, *Izv. Ros. Akad. Nauk. Ser. Biol.*, No. 6, 650-658 (2005).
4. I. D. Stal'naya and T. G. Garishvili, *Modern Biochemical Methods* [in Russian], Moscow (1977), pp. 63-64.
5. E. G. Bligh and W. J. Dyer, *Can. J. Biochem.*, **37**, No. 8, 911-923 (1959).
6. H. Kamata and H. Hirata, *Cell. Signal.*, **11**, No. 1, 1-14 (1999).
7. G. Leonarduzzi, M. Parola, G. Muzio, *et al.*, *Biochem. Biophys. Res. Commun.*, **214**, No. 2, 669-675 (1995).
8. P. Ljubuncic, B. Fuhrman, J. Oiknine, *et al.*, *Gut*, **39**, No. 3, 475-478 (1996).
9. M. Orellana, R. Rodrigo, L. Thielemann, and V. Guajardo, *Comp. Biochem. Physiol. Toxicol. Pharmacol.*, **126**, No. 2, 105-111 (2000).
10. M. Parola, G. Leonarduzzi, G. Robino, *et al.*, *Free Radic. Biol. Med.*, **20**, No. 3, 351-359 (1996).
11. T. Patel and G. J. Gores, *Hepatology*, **21**, No. 6, 1725-1741 (1995).
12. C. M. Rodrigues and C. M. Steer, *J. Hepatol.*, **32**, No. 1, 135-141 (2000).
13. R. J. Sokol, M. Devereaux, R. A. Khandwala, and K. O'Brien, *Hepatology*, **17**, No. 5, 869-881 (1993).
14. R. Stocker and B. N. Ames, *Proc. Natl. Acad. Sci. USA*, **84**, No. 22, 8130-8134 (1987).