Thermostable Hepatocyte Growth Factor and Energy Metabolism in Rats after Partial Hepatectomy

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The relationship between energy metabolism in the remaining liver tissue after 60% hepatectomy and the activity of low-molecular-weight thermostable hepatocyte growth factor was studied in rats. The energy status of the liver was markedly reduced 6 h and to a greater degree 12 h after the operation, judging from the levels of ATP, ADP, AMP, and energy potential. The energy status improved (energy potential increased to 95% of the initial level) 24-72 h after the operation. This coincided with a decrease in hexokinase and phosphofructokinase activities and an increase in glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase activities, indicating suppressed glycolysis and activation of the Krebs' cycle. High activity of low-molecular-weight thermostable hepatocyte growth factor was detected 24-72 h after resection of the liver (with maximum activity after 48 h). The activity of the hepatocyte growth factor increased if the range of energy potential surpassed the level sufficient for maintaining protein production, which is an energy-consuming process, but lower than the normal level.

Key Words: *partial hepatectomy; energy status; glycolysis, pentose monophosphate cycle, and Krebs' cycle enzymes; hepatocyte growth factor*

The hepatotropic growth factor (HGF) regulates liver regeneration after partial hepatectomy (PHE) [12]. The role of HGF in regeneration, tumor transformation, and diseases of the liver and other organs has been extensively studied [10,11,14]. The expression of HGF and its receptor, the product of *c*-met oncogene, has been detected in cultured hepatocytes and various tissues [12].

The level of HGF in the liver and serum increased after PHE, in experimental toxic hepatitis, liver involvement in ischemia or injury, and in patients after removal of hepatoma and with metastatic cancer of the liver [14]. HGF prevents postoperative damage to the liver [12]. EGF produced by genetically modified fibroblasts induces regeneration of rat liver in endotoxemia and poisoning with CCl_4 [11]. The same effect was observed after infusion of recombinant HGF after PHE in humans with cirrhosis of the liver [10]. Alteration of serum HGF level is regarded as an indicator of liver regeneration [12].

Extensive resection leads to reduction in the energy status of the liver, which may be caused by decreased ability of hepatocytes to utilize glucose, microcirculation disorders in the remaining part of the liver, leading to ischemia, and decreased the rate of oxidative phosphorylation in hepatocytic mitochondria [12,13]. The activity of hexokinase, the key enzyme of glycolysis, decreased and that of glucose-6-phosphatase, the key enzyme of gluconeogenesis, increased after 67% PHE in rats. This might be due to the necessity of maintaining a constant glucose level in the blood by the liver [8].

We assessed EGF production in liver cells under conditions of decreased energy status of liver tissue during the first days after 60% resection and analyzed

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the activities of enzymes involved in the carbohydrate-energy metabolism.

MATERIALS AND METHODS

Experiments were carried out on Wistar rats weighing 200-250 g. Resection of the liver (60%) was carried out as described previously [9]: the median, right, and right lateral lobes of the liver were removed. The animals were decapitated under ether narcosis after 6 (n=8), 12 (n=8), 24 (n=10), 48 (n=8), and 72 h (n=5). The remaining part of the liver was washed in 0.9% NaCl and immediately put on the ice. Control groups consisted of 11 intact and 3 sham-operated rats.

Adenine nucleotides were measured routinely in liver homogenates [6], the energy potential was estimated using Atkinson's formula [5]: (ATP+$^1/_2$ADP)/(ATP+ADP+AMP). The activities of hexokinase (EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and isocitrate dehydrogenase (EC 1.1.1.42) in liver homogenate were measured by the kinetic method [3,8].

For isolation of HGF, the liver was immediately frozen at -20°C. The low-molecular-weight component of the HGF fraction (lHGF), which is characterized by high hepatotropic activity [1], was used. The fraction containing lHGF was isolated, and RNA and DNA contents were measured in liver tissue as described previously [1]. High hepatotropic growth-stimulating activity of lHGF fraction, detected *in vivo* in rats with 30% PHE [1], and the data on stimulation of DNA production in cultured rat neurinoma cells NGUK1 [2] prompted us to use these cells for testing the growth-stimulating activity of HGF preparations. The choice of these cells is justified by high-level expression of HGF and its receptor genes in the cerebral glial cells [15].

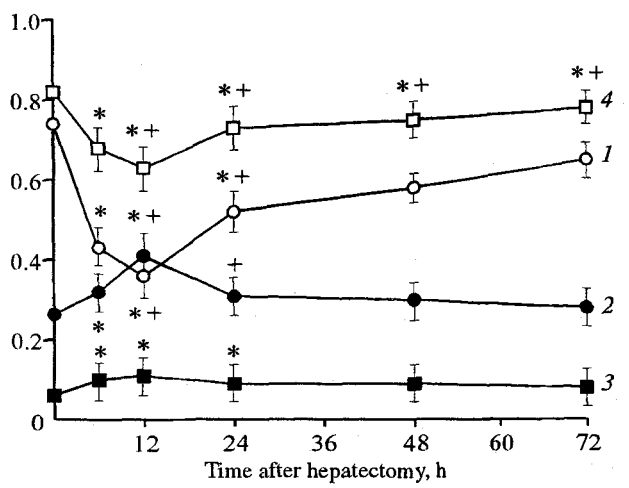

Fig. 1. Concentration of adenine nucleotides and energy potential (EP) in rat liver tissue after 60% hepatectomy. Ordinate: concentrations of adenine nucleotides (μmol/g wet tissue) and EP values. Here and on Fig. 2: p<0.01: *$vs.$ the initial level, +$vs.$ the previous value.

The growth-stimulating activity of HGF preparations was evaluated by stimulation of DNA production in NGUK1 cells, which after reaching confluency were synchronized for 72 h with 0.5% fetal calf serum in RPMI-1640. HGF preparations were added to the cells in a concentration of 5 μg protein/ml medium. The level of DNA production was evaluated from incorporation of ^{14}C-thymidine (specific activity 56 μCi/mmol) and calculated in cpm/10^6 cells.

The results were processed using Student's test and coefficient of correlation.

RESULTS

Six hours after 60% PHE, the content of ATP in liver cells decreased by 40% and the levels of ADP and AMP increased by 24 and 77%, respectively (Fig. 1). The minimal level of ARP (48% of the initial level) was observed 12 h after PHE and was paralleled by a simultaneous increase in the content of ADP and AMP. Energy potential decreased by 16% 6 h after PHE and by 23% after 12 h. Twenty-four hours after PHE, ATP level increased by 44% in comparison with 12 h postoperation, and the levels of ADP and AMP decreased by 25 and 17%, respectively. The energy potential increased by 14% after 24 h in comparison with 12 h postoperation. On days 2-3 after PHE, ATP levels increased by 13 and 12% in comparison with the previous term. The levels of ADP and AMP decreased on the third day by 7 and 17%, respectively. Starting from 24 h postoperation, energy potential was increasing and by 72 h it increased by 23% in comparison with the minimum level.

Therefore, energy deficit was observed 12 h post-resection. During the next two days the energy status of hepatocytes gradually recovered and reached 95% of the initial level 72 h after PHE. Similar data were obtained previously in studies of the energy status of rabbit liver cells on days 1-3 after 70% PHE [13].

Six and twelve hours after PHE, the activity of hexokinase decreased by 50 and 68%, respectively (Fig. 2, *1*) in comparison with the initial level. Later the level of hexokinase increased negligibly. Changes in the activity of this enzyme correlated with changes in ATP level (r=0.733). The activity of phosphofructokinase (Fig. 2, *2*) was stable throughout the entire observation period. This suggests that glycolysis is suppressed on days 1-3 after PHE. The activity of the key enzyme of the pentose monophosphate pathway glucose-6-phosphate dehydrogenase (Fig. 2, *3*) increased by 36% 12 h after PHE and then decreased to 51% of the normal level during a 1-3-day period. Activation of the pentose monophosphate pathway of glucose utilization during the first 24 h after PHE may be a compensatory mechanism providing an increase

in the production of D-ribose-5-phosphate which is required for ATP production [4]. A significant increase in the activity of isocitrate dehydrogenase by 40% for 1-3 days after PHE with the maximum 6 h postresection can be regarded as an indicator of the Krebs' cycle hyperfunction (Fig. 2, *4*). Changes in the activities of glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase were in inverse correlation with changes in ATP level (r=-0.545 and -0.508, respectively).

Thus, the removal of 60% liver parenchyma results in suppression of the activities of the key enzymes of glycolysis with parallel increase in the activity of the Krebs' cycle enzyme and a decrease in the hepatocyte energy status. These data confirm the inability of the glycolysis enzymes to support the hepatocyte energy status during the first days after PHE and activation of β-oxidation of fatty acids which are more beneficial energy substrates than glucose [4].

By the 6th h postoperation, the activity of IHGF in the liver (103.6±8.0%) was virtually the same as in the control, as well as that in the liver of sham-operated animals (93.5±10.3% of the normal level). Twenty-four hours after PHE the activity of IHGF increased, by the 48th h reached the maximum, and after 72 h decreased without reaching the initial level (Table 1).

High level of the HGF mRNA expression in the liver was observed during the first hour after 70% PHE, after which the production of HGF mRNA dropped below the control values by 4-8 h postoperation [7]. It seems that soon after PHE, the HGF mRNA produced in liver cells could not participate in the production of the growth factor because of low energy status of liver tissue. The appearance of active IHGF 24 h after PHE in our experiments may be associated with a certain energy status attained by this time and required for the maintenance of the regeneration processes in liver cells. Therefore, the activity of HGF increased in the range of the energy potential values surpassing the level sufficient for maintaining energy-consuming protein production but lower than normal. Our results indicate that the hepatocyte energy status after HPE directly affects the regeneration capacity of the remaining part of the liver.

TABLE 1. Growth-Stimulating Activity of IHGF Preparations from Rat Liver (According to ^{14}C-Thymidine Incorporation in DNA of NGUK1 Cells)

Time after HPE, h	Number of animals	Activity of IHGF, % of control[1]
6	4	103.6±8.0
24	6	219.3±16.6*
48	6	294.2±38.3*
72	5	224.4±26.4*

Note. [1]Mean arithmetic of the control was 350,000 spm×10^6 cells, *p<0.05 *vs.* the control.

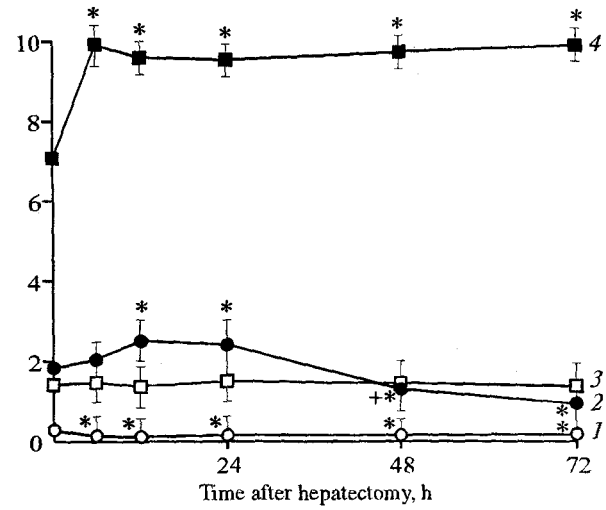

Fig. 2. Activities of hexokinase (*1*), phosphofructokinase (*2*), glucose-6-phosphate dehydrogenase (*3*), and isocitrate dehydrogenase (*4*) in rat liver normally and after 60% PHE. Ordinates: levels of activity, μmol/min×g wet liver tissue.

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