Effects of heparin on hepatic regeneration and function after partial hepatectomy in rats

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Abstract

AIM To investigate the effects of heparin on the regenerative rate and serum alanine aminotransferase level in partial-hepatectomized (two-thirds) rats.

METHOD Three different doses of heparin (100, 500 or 1000 U/kg) were given after partial hepatectomy through the tail vein at a 12h interval for 2 or 3 days. After drug treatment, rats were killed and the remnant livers were weighed for the assessment of regenerative rate. Blood samples were also collected to measure serum alanine aminotransferase levels.

RESULTS Heparin given in the present dosages for 2 or 3 days neither stimulated the regeneration of the liver nor improved the hepatic function as indicated by an insignificant change both on remnant liver weight and serum alanine aminotransferase activity as compared with the control.

CONCLUSION Heparin alone has no beneficial effects on the regeneration of livers and improvement of hepatic function in hepatectomized rats.

INTRODUCTION

It is known that liver regenerates quickly in response to tissue damage. Hepatocyte growth factor (HGF) has been implicated in the regulation of liver growth after partial hepatectomy[1,2]. It has been reported that heparin is a potent inducer of HGF production in various types of cells, including human embryonic lung and skin dermal fibroblasts, and promyelocytic leukemic and umbilical vein endothelial cells as well[3]. Furthermore, it was also demonstrated that heparin, when given to partially hepatectomized rats, could increase the plasma HGF level and stimulate DNA synthesis in hepatocytes of the remnant livers during the early stage of liver regeneration. However, this indirect indicator of tissue growth in the early phase of liver regeneration cannot extrapolate and ascertain whether heparin is a true hepatotrophic factor for liver regeneration at the later stage of the regenerative process. Also, there was no clear indication that heparin can indeed restore the liver function along with its regenerative capacity.

The purpose of this study is to investigate the effects of heparin on liver regeneration and function by measuring the regenerative rate and serum alanine aminotransferase level, respectively in partially hepatectomized rats. The results of this study could be useful for assessing the therapeutic implication of heparin as a hepatotrophic agent in man.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (180 g - 200 g) purchased from the Laboratory Animal Unit, the University of Hong Kong, were reared on a standard laboratory diet (Ralston Purina Co., USA), and given tap water. They were kept in a room where the temperature (22°C ± 1°C), humidity (65% - 70%), and day:night cycle (12:12 light:dark) were controlled.

Drug

Heparin (Sigma, St. Louise, MO, USA; sodium salt, 174 USP units/mg) was prepared in 0.9% w/ v NaCl (British Drug House, UK) solution (normal saline) in a concentration series of 50, 250, and 500U/mL for intravenous injection. Different doses of heparin 100, 500 and 1000U/kg, i.e. 0.2 mL/100 g body weight were given, respectively. Similar volume of normal saline was
injected through the same route as the control. 

Partial hepatectomy, drug treatment and hepatic regeneration assessment
Partial (two-thirds) hepatectomy was performed by excision of the median and left hepatic lobes according to the method of Higgins and Anderson[4] under pentobarbitone anaesthesia. The heparin at doses of 100, 500, and 1000U/kg or its vehicle (normal saline) were administered intravenously through tail vein every 12h starting 6h after operation for 2 (4 injections) or 3 (7 injections) days. The rats were killed 3h after the last dose. Blood samples were collected for the measurement of serum alanine aminotransferase level. At the time of killing, the remnant livers were removed and weighed to assess the hepatic regeneration. The hepatic regenerative rate in each postoperative rat was calculated from the wet weight of the remaining liver divided by the estimated preoperative weight of the whole liver times 100[5].

Measurement of serum alanine aminotransferase level
The serum alanine aminotransferase activity was measured by the method used in our laboratory with modifications[6]. Lactate dehydrogenase (LDH), L-alanine, reduced nicotinamide adenine dinucleotide (NADH) and α-ketoglutarate were prepared at concentrations of 20U/mL, 0.8M, 2mM and 0.1mM, respectively. Serum (0.1mL) was added to 0.1mL NADH, 0.1mL alanine, 0.1mL α-ketoglutarate and 0.5mL of 0.2M phosphate buffer mixture. The final solution was incubated at 37°C for 1min. LDH in 0.1mL was then added and the absorbance of the mixture was measured at 340nm for 3min with a spectrophotometer (Beckman, DU 650, USA), using mixture without LDH as the blank. The rate of decrease in absorbance was determined and the amount of alanine aminotransferase present was calculated with a formula. Serum alanine aminotransferase levels were expressed as U/mL.

Statistical analysis
The results were expressed as mean ±S.E. (x±s) and statistical analysis was performed with an analysis of variance (ANOVA) followed by a Dunnett t test. A value of P<0.05 was considered to be statistically significant.

RESULTS
Liver regeneration
The remaining liver tissue regenerated along with the time after partial hepatectomy. Two or three days after hepatectomy the regenerative rates were 51% or 84%, respectively in the vehicle treatment groups. Heparin had no significant effect on hepatic regeneration. The regenerative rates in all three doses of heparin treated for 2 or 3 days, except for the highest dose in the 3 days group, remained similar to those of the control. The highest dose of heparin treated for 3 days, however, significantly attenuated the liver regeneration when compared with control group (Figure 1).

Serum alanine aminotransferase level
Serum alanine aminotransferase activity was significantly increased after partial hepatectomy at day 2 when compared with the enzyme level of normal rats. These levels seemed to be recovered back to normal level at the 3rd day after operation. Heparin at all three doses treated for 2 or 3 days did not significantly affect the serum alanine aminotransferase activity in partially hepatectomized animals (Figure 2).
DISCUSSION
The present study showed that partial hepatectomy stimulated the remnant liver to regenerate in a time-dependent manner which was comparable with the previous study[5]. Liver regeneration is a compensative mechanism to restore the organ function after injury or related diseases. After partial hepatectomy, liver mass doubled within 48h and completely recovered in 7-10 days. This process was relatively well synchronized with the peaks of DNA synthesis and mitosis occurring at approximately 24 and 30h, respectively[4,3]. It has been reported that heparin could stimulate endogenous HGF production and consequently enhance DNA synthesis in hepatocytes in vivo after hepatic injury. The latter effect was significant within 36h after hepatectomy, but the same effect was not examined thereafter. More importantly, the actual liver regeneration and function had not been assessed[8].

Although the early phase of liver regeneration is important for liver growth, the later stage of liver development and maturation of hepatocytes is the final determinant for a normal functional liver. The present study determined the effects of heparin on liver regeneration and its function 48 and 72h after hepatectomy. Our results demonstrated that heparin at all doses accelerated no hepatic regeneration. The highest dose instead significantly retarded liver regeneration in the 3rd day. This finding suggested that higher dose and longer period of heparin treatment might be harmful rather than beneficial to liver regeneration.

Increased liver regeneration is reflected by improvement of hepatic functions. Serum alanine aminotransferase level is one of the indicators for hepatic functions in clinical practice. In the present study, partial hepatectomy induced a significant increase in serum enzyme activity and tended to recover along with the natural liver regeneration at the 3rd day after hepatectomy. These findings were in accord with the previous study[5]. Our present data also indicated that heparin could not accelerate the recovery of alanine aminotransferase level as liver regenerated on the 2nd and 3rd after hepatectomy. Although heparin could stimulate DNA synthesis of hepatocytes at the first day after partial hepatectomy[8], it could not improve the deteriorated action on the liver due to hepatectomy beyond that period of time. Taken together, heparin is not an ideal hepatotrophic drug when it is used alone in liver injury or related diseases.

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REFERENCES
1 Michalopoulos GK. Liver regeneration: molecular mechanisms of growth control. FASEB J, 1990;4:176-187
2 Zarneger R, De Frances MC, Kost DP, Lindroos P, Michalopoulos GK. Expression of hepatocyte growth factor mRNA in regenerating rat liver after partial hepatectomy. Biochem Biophys Res Commun, 1991;177:559-565
3 Matsumoto K, Tajima H, Okazaki H, Nakamura T, Heparin as an inducer of hepatocyte growth factor. J Biochem, 1993;114:820-826
4 Higgins GM, Anderson RM. Experimental pathology of the liver: restoration of the liver of the white rat following partial surgical removal. Arch Pathol, 1931;12:186-202
5 Lee SD, Wang JY, Cho CH, Wu JC, Lu RH, Lai KH, Tsai YT, Lo KJ. Effects of H2-receptor antagonists on the rat liver after partial hepatectomy or carbon tetrachloride-induced hepatic injury. Scand J Gastroenterol, 1986;21:984-990
6 Woo PCY, Kaan SK, Cho CH. Evidence for potential application of zinc as an antidote to acetaminophen-induced hepatotoxicity. Eur J Pharmacol, 1995;293:217-224
7 Grisham JW. A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver: autoradiography with thymidine-H-. Cancer Res, 1962;22:842-849
8 Matsumoto K, Nakamura T. Heparin functions as a hepatotrophic factor by inducing production of hepatocyte growth factor. Biochem Biophys Res Commun, 1996;227:455-461

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