Significance of Activation of Reticuloendothelial Function after Hepatectomy in Cirrhotic Rats

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NAKAGAWA, K., OUCHI, K., MATSUBARA, S. and SUZUKI, M. Significance of Activation of Reticuloendothelial Function after Hepatectomy in Cirrhotic Rats. Tohoku J. exp. Med., 1988, 155 (1), 11-21 — Effect of hepatectomy on the prognosis of cirrhotic rats prepared by oral administration of thioacetamide was studied from the standpoint of the reticuloendothelial function and energy metabolism of the liver. OK-432, a streptococcal preparation, was used to activate reticuloendothelial functions. Administration of OK-432 to cirrhotic rats prior to 70% hepatectomy significantly prevented the elevation of serum GOT, GPT and LDH, the prolongation of blood coagulation and the decrease of serum complement level. Hepatic ATP synthesis and RNA content were significantly increased by the use of OK-432. These findings suggest that activation of reticuloendothelial functions at the time of massive hepatectomy in cirrhotic rats may diminish hepatic injury, maintain serum complement level, and improve protein synthesis of the liver. ——— massive hepatectomy; reticuloendothelial function; liver cirrhosis; thioacetamide

The postoperative period of patients subjected to hepatectomy due to hepatocellular carcinoma associated with liver cirrhosis is frequently complicated with the episodes of multiple organ failure (MOF) such as liver failure, renal failure, respiratory failure and disseminated intravascular coagulation (DIC). It has been pointed out that deterioration in the clearance of toxic substances, due to a decreased reticuloendothelial function, is involved in the pathogenesis of these surgical complications. In order to investigate the significance of the activation of this function before massive hepatectomy, we performed 70% hepatectomy in cirrhotic rats that had been treated with OK-432 (Chugai Pharmaceutical Co., Tokyo) to activate the reticuloendothelial function and measured liver function tests, total hemolytic complement (CH50), hepatic energy metabolism and liver regeneration capacity.

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Materials and Methods

Male Spraque-Dawley rats weighing 130 to 160 g were used in the present experiments. Cirrhosis was induced by oral administration of 0.03% thioacetamide as drinking water for 5 months (Fig. 1). The animals were divided into two groups: the OK-432 group, which received an intraperitoneal injection of OK-432 at a dose of 25 KE dissolved in 2 ml of saline 48 and 24 hr prior to hepatectomy to activate the reticuloendothelial function, and the control group without injection. Under ether anesthesia, the rats underwent 70% hepatectomy according to the standard method described by Higgins and Anderson (1931) and studies of liver and reticuloendothelial function were performed.

Determination of the reticuloendothelial function

Twenty four hr after laparotomy or hepatectomy, the reticuloendothelial function was determined according to the method of Ota (1969). $^{59}$Fe-chondroitin sulfate iron colloidal particle (specific radioactivity: 8.4 μCi/Fe 4 mg/chondroitin sulfate 20 mg/ml) was given by a rapid intravenous injection at a volume of 0.5 ml/kg body weight. Blood samples (0.1 ml) were drawn via the femoral artery prior to and 5 and 20 min after injection, and the radioactivity contents were counted in a scintillation counter. The phagocytic index K was calculated according to the method of Halpern et al. (1953) using the following equation.

![Fig. 1. Macroscopic and microscopic findings of the liver treated by oral intake of 0.03% thioacetamide for 5 months.](image)
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K = \frac{\log C_1 - \log C_2}{T_2 - T_1}
\]

\(C_1\) : radioactivity (cpm) per 0.1 ml of blood at \(T_1\)
\(C_2\) : radioactivity (cpm) per 0.1 ml of blood at \(T_2\)

Twenty min following the injection of \(^{59}\)Fe-chondroitin sulfate iron colloidal particle, the body was perfused with viviperfusion (Copp and Greenberg 1946) and homogenates of the liver, spleen and lung were prepared. After radioactivity measurement, \(^{59}\)Fe uptake by each organ was calculated.

Liver function tests and titration of serum complement

Prior to and 12, 24, 36 and 48 hr after hepatectomy, blood samples were periodically drawn from the rats and used for liver function tests and determination of hemolytic activity of complement (CH50). GOT and GPT were determined by the Reitman-Frankel-Momose modification method, LDH by the PMS-nitro TB modification method, and hepa-plastin test was performed according to the method of Owren and Strandli (1969). Hemolytic activity of complement was measured according to Mayer (1961).

Determination of the mitochondrial respiratory function

As described above, the rat livers were excised and mitochondrial fractions were obtained according to the method of Hogeboom and Schneider (1948). The mitochondrial respiratory function was examined by an oxygen consumption meter (Oxygraph, Model 5/6, Gilson, Bal, France) using succinate as the substrate. From the oxygen consumption curve obtained, oxygen consumption in state 3 and adenosine diphosphate/oxygen ratio (P/O) were calculated, and the rate of ATP synthesis was obtained (Koyama et al. 1982).

Determination of RNA and DNA synthesis and content

At 11, 23, 35 and 47 hr after hepatectomy, rats were given as intraperitoneal administration of \(^3\)H-Orotic acid (20 \(\mu\)Ci/100 g). One hr later, the liver was excised, and RNA and DNA were extracted according to the Schmidt-Thannhauser-Schneider method (Schneider 1946). Radioactivities in RNA and DNA fractions were determined, and the rates of RNA and DNA synthesis were calculated using the values of specific radioactivities. The RNA and DNA contents were quantified by the orcinol and diphenylamine method, respectively.

All data were expressed as means±S.D. and the statistical significance of difference was evaluated according to Student’s \(t\)-test. Differences were judged to be significant when \(p\) values were less than 0.05.

RESULTS

Reticuloendothelial function

As shown in Fig. 2, the phagocytic index \(K\) in the cirrhotic rat was reduced to about one third of the normal rat value after laparotomy. When 70% hepatectomy was performed, the phagocytic index \(K\) of both the cirrhotic and normal rats were about one half of those after laparotomy. However, the reticuloendothelial function of the cirrhotic rat was markedly elevated by the administration of OK-432, and the phagocytic index \(K\) in cases of laparotomy was 2.4-fold higher than the non-treated group. Even 24 hr after hepatectomy, the phagocytic index \(K\) of OK-432-treated rats was 2.3-fold of the non-treated group.

As shown in Fig. 3, \(^{59}\)Fe uptake of the cirrhotic liver was lower than that of the normal liver after laparotomy, but uptakes to the spleen and lung were
Fig. 2. Phagocytic index K after laparotomy and 70% hepatectomy. Data points are means ± S.D. Experimental numbers are in parentheses. ○, laparotomy group; ●, hepatectomy group. **p < 0.01.

Fig. 3. $^{59}$Fe uptake in liver, spleen and lung after laparotomy and 70% hepatectomy. Data points are means ± S.D. Experimental numbers are in parentheses. ○, laparotomy group; ●, hepatectomy group. *p < 0.05, **p < 0.01.
markedly increased. $^{59}$Fe uptake to the liver, spleen and lung in the cirrhotic rat after the administration of OK-432 was slightly higher than the non-treated group, and a similar tendency was also seen after heptectomy, but there was no significant difference.

**Liver function tests and serum complement**

As shown in Fig. 4, GOT, GPT and LDH did not show any significant differences between normal and cirrhotic rats. After heptectomy of cirrhotic livers, these serum enzymes showed marked increases. GPT and LDH reached their peaks after 12 hr and GOT after 24 hr, then the values gradually decreased. Administration of OK-432 significantly inhibited increases in GOT, GPT and LDH in comparison with the control group.

Hepaplastin test values of cirrhotic rats were almost one half of normal rats as shown in Fig. 5. After heptectomy, cirrhotic rats showed a gradual decrease of their hepaplastin values, but after 72 hr they were recovered to the pre-operative levels. The degree of the decrease was less in OK-432 treatment, and from 6 to 36 hr after heptectomy the decrease was significantly inhibited comparing with the control group.

As shown in Fig. 6, the hemolytic activity of complement (CH$_{50}$) was significantly low in cirrhotic rats than in normal rats, and there was a further decrease after 70% heptectomy. However, the reduction of hemolytic activity of complement after heptectomy was prevented by the administration of OK-432.
Fig. 5. Changes of hepaplastin test after 70% hepatectomy in cirrhotic rats. Data points are means ± s.d. Experimental numbers are in parentheses. •, control group; ●, OK-432 group. *p < 0.05, **p < 0.01 against corresponding values in the control group.

Fig. 6. Changes of the hemolytic activity of complement (CH$_{50}$) after 70% hepatectomy in cirrhotic rats. Data points are means ± s.d. Experimental numbers are in parentheses. •, control group; ●, OK-432 group. *p < 0.05 against corresponding value in the control group.
and the CH$_{50}$ value after 12 hr showed a significant difference from the control group.

**Mitochondrial respiratory function**

As shown in Fig. 7, ATP synthesis in the liver mitochondria was somewhat lower in cirrhotic rats than in normal rats. Hepatectomy caused an exacerbation of ATP synthesis in cirrhotic liver and this exacerbation was even more marked
Fig. 8 shows the changes of hepatic RNA synthesis and content after hepatectomy in cirrhotic rats, and Fig. 9 shows changes of DNA. RNA synthesis was markedly exacerbated 12 hr after hepatectomy, and this exacerbation tended to be even greater when OK-432 was administered. The RNA content in the liver gradually increased after hepatectomy, and the increase was especially significant after 24 and 36 hr in the OK-432 group when compared with the control group.

DNA synthesis showed a maximum value 24 hr after hepatectomy and thereafter gradually decreased. DNA content decreased markedly after hepatectomy and gradually recovered thereafter. Both DNA synthesis and content were higher in the OK-432 group than in the control group, but there was no significant difference.

**DISCUSSION**

Hepatectomy performed in cases with liver cirrhosis is oftenly associated with various complications, and the postoperative course is sometimes eventful (Iwatsuki et al. 1983). It has been pointed out that the pathogenesis of MOF associated with hepatectomy involves deteriorated phagocytic capacity of the reticuloendothelial system throughout the body, particularly of the Kupffer cells. The decreased clearance of toxic substances in blood, such as many toxic metabolites, antigens, bacteria and endotoxin, due to impaired function of the reticuloendothelial system, contributes to the onset of MOF (Nakagawa et al. 1986). To clarify the significance of reticuloendothelial activation after hepatectomy in cirrhotic
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patients, various studies have been performed on rats with experimentally induced liver cirrhosis. Carbon tetrachloride was generally used for preparation of the cirrhotic liver in rats, but this method is complicated since frequent intraperitoneal injections are required. We induced cirrhotic model by administering thioacetamide orally ad libitum (Brodehl 1961). After 5 months of oral thioacetamide administration, irregularities were seen macroscopically on the liver surface and interlobular fibrosis was observed histologically. Pseudolobules with a nodular structure which were basically the same as those of cirrhotic patients were formed and there was a marked reduction of liver parenchyma.

OK-432 was selected as the reticuloendothelial activator because of its easy availability and safety in clinical practice. This agent is a biological drug prepared by co-culture of Streptococcus hemolytics Su strain with penicillin G in a given condition and lyophilization. It is now widely used as an immunopotentiator to treat cancer. We have confirmed both experimentally and clinically that OK-432 not only acts as an immunopotentiator but also strongly activates the reticuloendothelial function (Nakagawa et al. 1987a, b). Furthermore, we have reported that this drug markedly inhibits the increase of blood endotoxin after hepatectomy in cirrhotic patients. The present study using cirrhotic rats also confirmed that OK-432 tended to increase $^{59}$Fe uptake to the liver, spleen and lung as well as significantly increased phagocytic index K.

GOT, GPT and LDH are considered to appear and increase in blood during liver injury, such as hepatolysis or increased hepatocellular permeability (Sekas and Cook 1979). In this experiment, there were marked increases in GOT, GPT and LDH after hepatectomy, but these increases were significantly inhibited by the administration of OK-432. By activation of the reticuloendothelial function, the liver injury appeared to be suppressed and serum enzyme levels were decreased. However, the reticuloendothelial function also treats serum enzymes, and the significant decrease in GOT and LDH often appears to be due to increases in this treatment capacity.

To study the protein synthesis in the residual liver, coagulation factors which have a rapid turnover were measured. Among the exogenous coagulation factors, factors II, V, VII and X are produced in the liver, and it has been reported that these factors decrease at the time of various liver diseases or hepatectomy. Hepaplastin test measures factors II, VII and X and clearly reflects liver parenchymal cell disorders. In this cirrhotic model, the hepaplastin test values were significantly lower than those of the normal liver and the decrease was even more marked after hepatectomy. However, the administration of OK-432 significantly inhibited this decrease, and it appeared that the disturbance of the protein synthesis in the residual liver was alleviated.

The liver and the reticuloendothelial system produce complement components and it has therefore been reported that complement levels are low due to decreased production in cirrhotic patients (Wyke et al. 1983). In the authors'
The residual liver after hepatectomy requires a large amount of energy to synthesize nucleic acids and proteins necessary to handle the increased metabolic load and regeneration of hepatic cells, and most of their energy is supplied by ATP, which is produced in the hepatic mitochondria (Ozawa et al. 1982). Acceleration of the ATP synthesis is essential to provide this energy, but the ATP synthesis is reduced in the cirrhotic liver and it is not sufficiently stimulated in the residual liver after hepatectomy in cirrhotic patients when compared with hepatectomy in noncirrhotic patients. In the present experiment, the ATP synthesis of the residual liver tended to be promoted by the administration of OK-432. As described previously, the mechanism is considered to be the elimination of toxic substances by the promotion of phagocytic activity which has beneficial action on liver cells.

Following exacerbation of energy metabolism in the residual liver after hepatectomy, synthesis of nucleic acids is also promoted (Hecht and Potter 1956). In this experiment, the RNA synthesis tended to increase slightly 12 hr after OK-432 administration, and there were significant increases in the RNA content after 24 and 36 hr.

It is concluded that the capacity of the residual liver to treat toxic substances after hepatectomy in cirrhotic rats was promoted by the administration of OK-432, and that the residual liver dysfunction was alleviated. Prevention of the postoperative trend for low complemeten titers by OK-432 might indicate that the agent potentiate the defence mechanism against infections. It is also suggested that OK-432 has beneficial effects on energy metabolism and liver regeneration.

References


