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Effect of Picibanil (OK 432) on the Scavenging Effect of Free Radicals Produced during Liver Regeneration in the Rat

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Abstract

We administered a biological response modifier Picibanil (OK-432), attenuated Streptococcus pyogenes, via the dorsal vein of the penis after 70% hepatectomy in rats, and clarified the scavenging effect of Picibanil on free radicals generated in the regenerating liver. A group of 5 rats was intravenously administered with 25 KE/kg of OK-432 after hepatectomy, while the control group was given saline after hepatectomy. Serum levels of aspartate aminotransferase and alanine aminotransferase and the value of thiobarbituric acid-reactive substances in serum and hepatic tissue after hepatectomy were serially measured, and these values were significantly lower in Picibanil treated animals than in control animals. Free radical production in the regenerating liver was also measured by electron spin resonance spectrometry, and OK-432 injection significantly reduced free radical production. These results suggested that OK-432 reduced hepatocellular damage in regenerating liver by inhibiting lipid peroxidation.

KEYWORDS: Picibanil, free radicals, hepatectomy, liver damage

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We administered a biological response modifier Picibanil (OK-432), attenuated *Streptococcus pyogenes*, via the dorsal vein of the penis after 70% hepatectomy in rats, and clarified the scavenging effect of Picibanil on free radicals generated in the regenerating liver. A group of 5 rats was intravenously administered with 25 KE/kg of OK-432 after hepatectomy, while the control group was given saline after hepatectomy. Serum levels of aspartate aminotransferase and alanine aminotransferase and the value of thiobarbituric acid-reactive substances in serum and hepatic tissue after hepatectomy were serially measured, and these values were significantly lower in Picibanil treated animals than in control animals. Free radical production in the regenerating liver was also measured by electron spin resonance spectrometry, and OK-432 injection significantly reduced free radical production. These results suggested that OK-432 reduced hepatocellular damage in regenerating liver by inhibiting lipid peroxidation.

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Among the several radical species which have been proved to be cytotoxic, particular attention has been focused in recent years on the partially reduced forms of dioxygen. The combination of these substances, particularly in the presence of Fe^{2+} ions, generates the highly reactive hydroxyl radical or carbon-centered radicals, which are widely believed to cause molecular damage (1-6). The most important cell targets of these free radicals are nucleic acids, proteins and membrane lipids. During the process of lipid peroxidation, unsaturated lipids are mainly affected, resulting in cholesterol and fatty acid oxidation, lipid cross-linking, cell and organelle permeability changes. Molecular damage can also occur on small molecules with antioxidant activity, which are membrane components such as tocopherols, β-carotene and coenzyme-Q, and cytosolic components such as glutathione, ascorbic acid and uric acid. This leads to their decreased availability and to an enhancement of the free radical toxicity.

Iwagaki *et al.* reported that Picibanil (OK-432) has a scavenging effect on free radicals *in vitro* (7). Picibanil is a killed streptococcal preparation that increases reticuloendothelial system activity and has been described as a biological response modifier (8-10). Previous reports have revealed that lipid peroxidation by free radicals account for much of the liver injury during regeneration after hepatectomy (11). This study was conducted to investigate the scavenging effect of Picibanil on free radicals generated in the regenerating liver *in vivo*.

Materials and Methods

Operative procedures and experimental designs. Male Wistar rats weighing 250-300 g were used in the experiments. Laparotomy was performed with animals under mild ether anesthesia, and rats underwent extended right hepatectomy, which excised approximately 70% of the liver, according to the method of Higgins and Anderson (12). Five rats were administered with 25 KE/kg of Picibanil immediately after hepatectomy via the dorsal vein of the penis, while a control group of 5 rats were administered with physiological saline after hepatectomy. Serial blood samples were taken from the inferior vena cava to measure the serum values of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and thiobarbituric acid reactive substances (TBARS). Additionally, 10 ml of 4°C physiological saline was flushed through the portal vein after ligation of the proper hepatic artery, and then the liver was removed to measure hepatic

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TBARS and hepatic free radicals also for a period of 3 days after hepatectomy.

Measurement of serum AST, ALT and TBARS. Serum levels of AST and ALT were measured by the NADH-UV rate method and the serum level of TBARS was measured by a fluorescent method using thiobarbituric acid (13).

TBARS analysis in liver tissue. Liver tissues were homogenized with 10 volumes of 0.1 M sodium phosphate buffer (pH 7.4). To 0.1 ml homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% sodium acetate buffer (pH 3.5), 1.5 ml of 0.8% sodium thiobarbituric acid, and 0.7 ml of distilled water were added, and the mixture was incubated at 100°C in a water bath for 60 min. The TBARS were extracted with a mixture of n-butanol: pyridine (15: 1, v/v), and the fluorescence was measured (excitation at 515 nm; emmission, 532 nm). Tetramethoxypropane was used as the standard (13).

Free radicals analysis of liver tissues. Using homogenates of liver tissues, free radicals (hydroxyl radicals, carbon-centered radicals, hydrogen radicals) were analyzed by electron spin resonance (ESR) spectrometry (JES-FEIXG, JEOL, Tokyo, Japan), and manganese oxide was used as an internal standard (14). One hundred microliters of 1 mM $FeSO_4$ and 1 mM DETAPAC, 50 μl of 1 M H_2O_2, 50 μl of sample, and 10 μl of DMPO were mixed for 10 s and then placed in the ESR flat cell. The DMPO-OH, -C and -H spin adducts were measured after 60 s exactly.

Statistical analysis. Results are shown as mean ± standard deviation (SD) and statistical comparison for AST, ALT, TBARS and free radicals analyses were made with two-tailed unpaired student's t- test. A P value less than 0.05 was considered significant.

Results

Changes of serum levels of AST and ALT. The mean ± SD level of AST was 170 ± 48 IU/l before hepatectomy. It peaked at 1427 ± 362 IU/l by 16 h after hepatectomy in the control group, while in the Picibanil group the peak level of 861 ± 638 was seen 8 h after hepatectomy and it declined thereafter. The mean level of ALT was 37 ± 5 IU/l before hepatectomy, and it increased to the peak of 828 ± 249 IU/l by 16 h after hepatectomy in the control group. In contrast, the peak level of 445 ± 74 IU/l was seen 8 h after hepatectomy in the Picibanil group. These values fell subsequently as shown in Fig. 1. In addition, serum levels of AST and ALT were significantly lower in the Picibanil group compared to the control group.

Changes of serum and hepatic TBARS. The serum level of TBARS was 3.6 ± 0.4 nmol/ml before hepatectomy. It remained almost unchanged after hepatectomy in the control group, while it decreased on

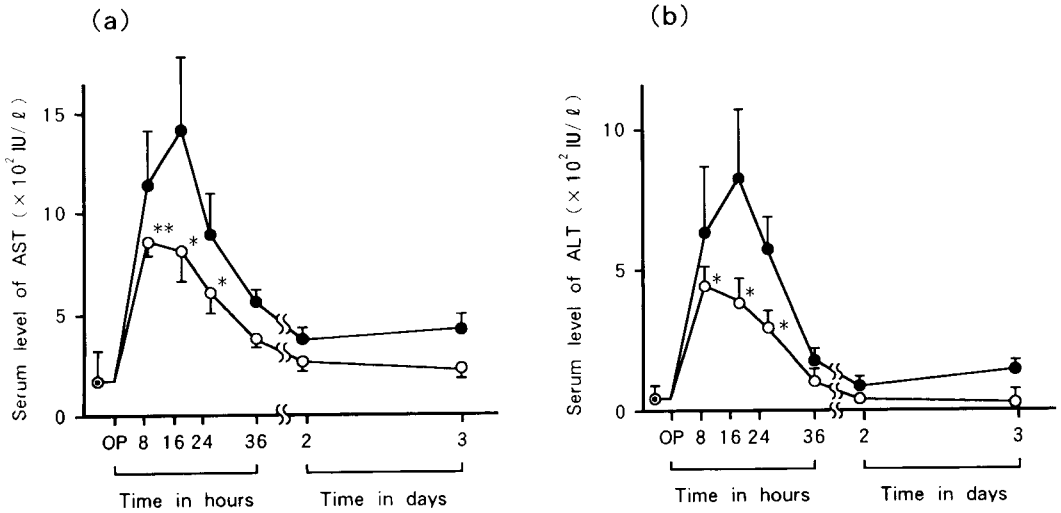

Fig. 1 Changes in serum level of aspartate aminotransferase (AST) (**a**) and alanine aminotransferase (ALT) (**b**) after hepatectomy with (○—○) and without (●—●) administration of Picibanil. $*P < 0.05$, $**P < 0.01$

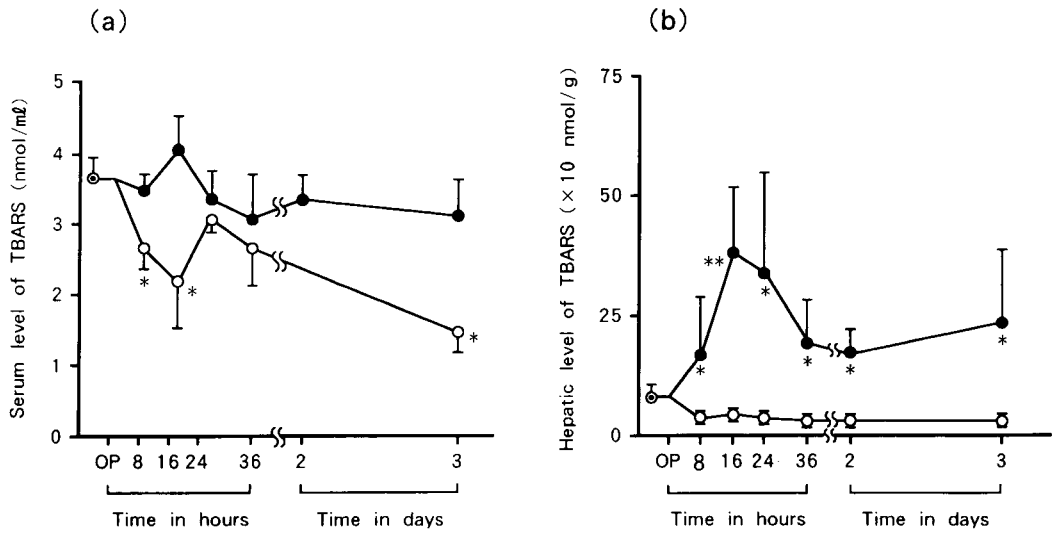

Fig. 2 Changes in serum (**a**) and hepatic (**b**) level of thiobarbituric acid reative substances (TBARS) after hepatectomy with (○—○) and without (●—●) administration of Picibanil. $*P < 0.05$, $**P < 0.01$

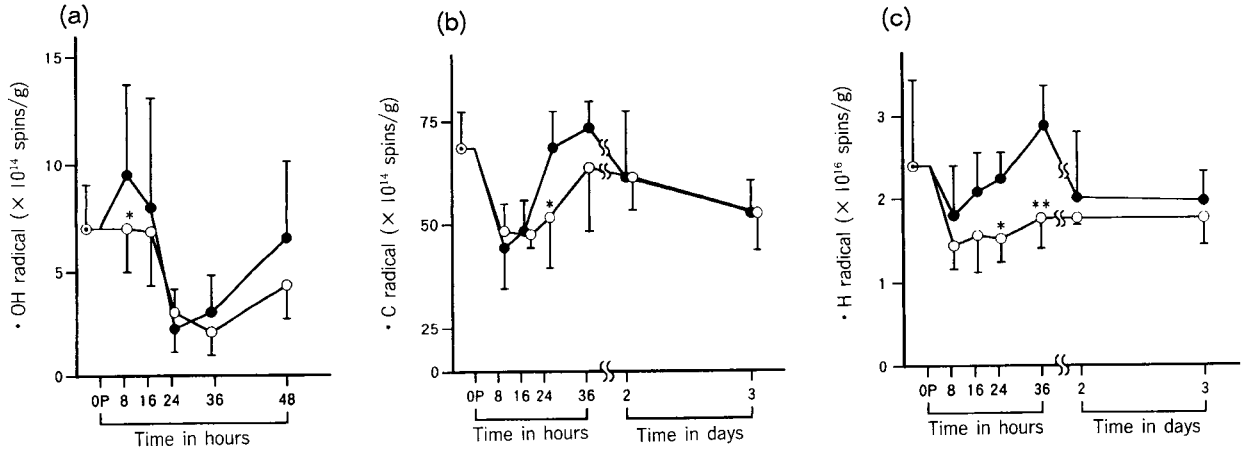

Fig. 3 Changes in hydroxyl (**a**), carbon-centered (**b**) and hydrogen (**c**) radicals production in liver tissue after hepatectomy with (○—○) and without (●—●) administration of Picibanil. $*P < 0.05$, $**P < 0.01$

day 2 and thereafter in the Picibanil group. The serum level of TBARS was significantly lower in the Picibanil group compared to the control group. The level of hepatic TBARS was 73 ± 30 nmol/g before hepatectomy and it increased to the peak of 379 ± 148 nmol/g by 16h postoperatively in the control group, and then declined. In the Picibanil group, the level of hepatic TBARS remained almost unchanged after hepatectomy. The level of hepatic TBARS was significantly lower in the Picibanil group compared to the control group (Fig. 2).

Changes of free radicals in hepatic tissue. Hydroxyl radicals in the control group reached the peak value of 1 ± 0.4 ($\times 10^{15}$) spins/g at 8h after hepatectomy and then decreased thereafter. Hydroxyl radicals generated in the Picibanil group did not increase immediately after hepatectomy and showed a decrease at 36h postoperatively, and there was a significant difference at 8h between the two groups. Carbon-centered and hydrogen radicals in the control group decreased immediately after hepatectomy and then increased to the peak of 7 ± 0.6 and

3 ± 0.4 ($\times 10^{15}$) spins/g, respectively at 36h after hepatectomy and decreased thereafter. These values were always higher than those of the Picibanil group. Both levels of carbon-centered and hydrogen radicals in the Picibanil group were significantly lower compared to the control group at 24h after hepatectomy (Fig. 3).

Discussions

Aerobic metabolism is important in the liver, therefore, lipid peroxides and free radicals are readily produced in the microsomes and mitochondria (15, 16), and antioxidant mechanisms to scavenge free radicals generated are also well-developed in the liver (17). However, when antioxidant activities are suppressed by liver injury such as hepatectomy, its homeostasis is destroyed and lipid peroxidation results in the production of a large amount of free radicals followed by damages to the microsomal and mitochondrial membranes (18).

Our present research demonstrated that the level of hepatic TBARS after hepatectomy increased with serum level of AST and ALT in the control group, suggesting that hepatic lipid peroxidation is involved in hepatocellular damage in the regenerating liver. In addition, the finding that serum levels of AST, ALT and TBARS were significantly lower in the Picibanil group compared to the control group suggests that Picibanil has a suppressive effect on lipid peroxidation in the regenerating liver. Iwagaki *et al.* previously reported that Picibanil has a scavenging effect on free radicals *in vitro* (7). Our present research confirmed that Picibanil has also an antioxidant ability *in vivo*.

In vivo, lipid peroxidation by free radicals occurs as a chain reaction initiated by hydroxyl radicals or hydroperoxyl radicals. Once the radical chain reaction starts, lipids are turned into carbon-centered radicals such as peroxyl radicals or alkoxyl radicals, and the carbon-centered radicals subsequently react with lipids (19). In the present research, hydroxyl radicals were significantly lower in the Picibanil group compared to the control group at an early period (8h) after hepatectomy. Moreover, the levels of carbon-centered radicals and hydrogen radicals were significantly lower in the Picibanil group compared to the control group at a late postoperative period (24-36h).

In terms of the free-radical chain reaction, the lower level of hydroxyl radicals in the Picibanil group at an early postoperative period suggests that Picibanil suppressed the initiation of the free-radical chain reaction, resulting in lower production of carbon-centered radicals and hydrogen radicals at a late period after hepatectomy. Ueda *et al.* have reported that lipid peroxide levels are increased and that protective factors like vitamine E are markedly decreased in the regenerating phase of rat liver after hepatectomy (11). We demonstrated that a biological response modifier Picibanil, an attenuated *Streptococcus pyogens* preparation, had a scavenging effect on free radicals generated in the regenerating rat liver and reduced hepatectomy (11). We demonstrated that a biological might enhance liver regeneration and improve survival after hepatectomy.

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