Effect of Bifemelane Hydrochloride on an Injury of the Liver Caused by Ischemia-Reperfusion in Rats

Takayuki NAGAI, Toru EGASHIRA and Yasumitsu YAMANAKA
Department of Pharmacology, Medical College of Oita, 1-1, Idaigaoka, Hazama-cho, Oita 879-56, Japan
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Abstract—In the liver ischemia-reperfusion model, the lipid peroxide level increased during ischemic periods, while a greater increase was observed during reflow periods. The increase in the cytochrome \( b_5 \) content was observed during ischemia and reflow periods. On the contrary, the cytochrome P-450 content remained unchanged during ischemic periods, but decreased during reflow periods. Bifemelane suppressed the elevation of the lipid peroxide level, the cytochrome \( b_5 \) content and the decrease in cytochrome P-450 content during the period of reperfusion.

Drug induced liver cell injury is thought to be caused by peroxidative processes through formation of free radical intermediates. Xenobiotics such as carbon tetrachloride \( (\text{CCl}_4) \) caused lipid peroxidation in hepatocyte membranes. \( \text{CCl}_4 \) hepatotoxicity is believed to be mainly attributable to highly reactive intermediates such as \( \cdot \text{CCl}_3 \) radicals (1). It is also well-known that the administration of carbon tetrachloride decreases the content of cytochrome P-450 in liver microsomes (2). In the liver ischemia-reperfusion model, fatty acid radicals may have been produced during ischemia. Lipid peroxide formation may have been induced from a chain reaction of superoxides caused by a large amount of oxygen inflow during reperfusion. However, we were not able to find any reports concerning the liver microsomal cytochrome contents. Recently, it has been reported that bifemelane (BF) diminishes the increase in lipid peroxide production during the development of cerebral ischemia (3). It has been reported that BF also displays antianoxic, EEG-activating and memory retrieval effects in laboratory research animals (4-6). These investigations prompted us to investigate the beneficial effect of BF against liver injuries caused by the production of lipid peroxides. The present study was therefore designed to investigate the effect of BF on an injury of the liver caused by ischemia-reperfusion, estimating lipid peroxides and microsomal cytochrome P-450 and \( b_5 \) contents.

Male Wistar rats weighing 300-350 g were used in all experiments. Bifemelane (BF, Eisai Co., Ltd., Tokyo) was given 10 mg/kg, p.o., daily for 7 days before treatment of ischemia-reperfusion.

To prepare the ischemia-reperfusion rats, the abdomen was opened through a midline incision under light ether anesthesia, and the left portal vein and hepatic artery were occluded with a microvessel clip. This technique enabled us to avoid portal stasis, which may lead to fatal hemodynamic instability (7). The abdomen was closed, and the animals were allowed to awaken. After 90 min of liver ischemia, the vascular clip was released, and the right lateral and caudate lobes were removed, leaving only the ischemic left lateral and median lobes behind. After 60 min of reperfusion, the rats were sacrificed. In this study, after 90 min of ischemia or 60 min of reperfusion, the rats were sacrificed and the lipid peroxide levels and contents of P-450 and \( b_5 \) were estimated.

Livers were removed, weighed and homogenized in 10 vol. 1.15% KCl containing 1 mM EDTA (pH 7.4). The homogenates were centrifuged at \( 10,000 \times g \) for 20 min. The supernatants were again centrifuged at \( 105,000 \times g \) for 60 min. Pellets were resuspended in 10 mM phosphate buffer containing 1.15% KCl and 1 mM EDTA, pH 7.4.
Table 1. Effect of bifemelane administration on the level of liver tissue malondialdehyde (MDA) in rats treated with ischemia-reperfusion

<table>
<thead>
<tr>
<th>Condition</th>
<th>Saline</th>
<th>Bifemelane</th>
</tr>
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<tbody>
<tr>
<td>No-treat</td>
<td>2.35±0.42</td>
<td>2.27±0.34</td>
</tr>
<tr>
<td>Ischemia</td>
<td>5.14±0.79</td>
<td>5.02±0.59</td>
</tr>
<tr>
<td>Ischemia+reflow</td>
<td>7.43±0.83</td>
<td>5.17±0.36*</td>
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Rats were given bifemelane, 10 mg/kg, for 7 days before treatment of ischemia-reperfusion. Rats were subjected to hepatic ischemia for 90 min and subsequent reperfusion for 60 min. Each value is the mean ± S.E. of 6 rats. *P<0.05, compared with the saline treated ischemia-reflow group (Student's t-test).

Cytochrome P-450 and b5 were determined by the method of Omura and Sato from the CO difference spectrum of dithionite-reduced microsomes and from the difference spectrum between dithionite-reduced and oxidized microsomes, respectively (8). The quantitative estimation of lipid peroxide levels in the liver homogenate was made according to the procedure of Ohkawa et al. (9), and lipid peroxides formed were expressed as nmol of malonic dialdehyde (MDA) formed per mg protein. The protein concentration was determined by the method of Lowry et al. (10) using bovine serum albumin as a standard.

As shown in Table 1, the level of MDA in the liver tissue increased about 2-fold that of control during the 90-min ischemic periods, but when reflow of hepatic blood was allowed after 90 min of liver ischemia, a greater increase in the MDA level was observed 60 min after reflow. When 10 mg/kg BF was administered for 7 days, this elevation of the MDA level in the tissue induced by ischemia-reperfusion was suppressed, but the BF-treatment did not affect the increase of MDA level during the ischemic periods.

In the case of the liver injury induced by ischemia-reperfusion, cytochrome P-450 content also remained unchanged during the ischemic period and was remarkably decreased during the reflow periods. Treatment with 10 mg/kg BF for 7 days suppressed this decreased state significantly. The content of cytochrome b5 increased during the ischemic periods, followed by a greater increase at 60 min after reflow of hepatic blood. BF treatment suppressed this elevated level of cytochrome b5 during the reflow periods (Fig. 1).

Previous reports have presented evidence showing that pretreatment of ischemic rats with α-tocopherol and coenzyme Q10 could completely reverse impaired mitochondrial function by suppressing an increase in lipid peroxides after reperfusion (11, 12). These results suggest that cellular damage in hepatic
ischemia is in part due to lipid peroxidation, especially during reperfusion. The suppressive effect on lipid peroxidation by BF suggests that it has a protective effect against liver damage, but another action such as the radical-trapping ability or membrane stabilizing action may also play an important role in protecting the fragile membrane. However, the increase of MDA level in liver tissue was also observed during ischemia, while BF did not suppress this elevation of lipid peroxides. This phenomenon may differ from that induced by ischemia-reperfusion. It seems likely that this elevation of MDA level in part depends on the destruction of liver cells by the long period of ischemia (90 min).

Oxygen-derived free radicals play a major role in ischemia-reperfusion injury of the liver. The earliest detectable lesions occur after the onset of ischemia and appear to be reversible. In addition, it is well-known that the changes occur in the centrilobular regions and consist of a dilation of the endoplasmic reticulum, swelling of the mitochondria, and cell membrane destruction (13). Thus, it is considered that cytochrome P-450 or b5 contents decrease in proportion to the degree of cell injury. In this study, however, b5 content increased in contrast with the ischemia-reperfusion model. Moreover, BF also suppressed the decrease of P-450 content or the increase of b5 content in the hepatic injury induced by ischemia-reperfusion.

We have not come across any direct evidence to support the former results. The major cause for the increase of b5 content in this experiment may be attributed to the difference in the assay conditions of b5 content, because we have determined the content of b5 using dithionite reduced microsomes photometrically. Further investigation is necessary to obtain more information about the mechanisms.

References