Effects of MET-88, a γ-Butyrobetaine Hydroxylase Inhibitor, on Tissue Carnitine and Lipid Levels in Rats

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MET-88, 3-(2,2,2-trimethylhydrazinium) propionate, suppresses carnitine synthesis by inhibiting (γ-butyrobetaine hydroxylase. The purpose of this study was to clarify the effects of suppression of carnitine synthesis on carnitine and lipid contents in tissues. MET-88 (50, 100, 200 or 400 mg/kg/d) was administered orally to male SD rats for 10, 30 or 60 d. Total carnitine and lipid (triglycerides, non-esterified fatty acids) contents were measured in heart and liver. In both tissues, treatment with MET-88 dose-dependently decreased total carnitine levels, and the reduction reached the plateau state after 30 d at each dose. MET-88 had no effect on lipid content in the heart, but increased the lipid content in the liver at the highest doses. Treatment with MET-88 at 400 mg/kg for 60 d resulted in no pathologic findings in the histological study, and also had no effect on parameters of liver function such as glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase as judged from the results of blood biochemical analysis.

We concluded that long-term treatment with MET-88 decreased the carnitine content to a constant level in both heart and liver, but had no effect on lipid contents in the heart, although it affected lipid metabolism in the liver.

Key words MET-88; carnitine; fatty acid; γ-butyrobetaine hydroxylase inhibitor

MET-88 (3-(2,2,2-trimethylhydrazinium) propionate) was synthesized as an inhibitor of γ-butyrobetaine hydroxylase, the enzyme that catalyzes the synthesis of carnitine from γ-butyrobetaine (Fig. 1).13 Recently, Kuwajima et al. showed that MET-88 also inhibited reabsorption of carnitine in the kidney.15 These findings indicate that MET-88 could decrease carnitine levels. It was also demonstrated that MET-88 had a cardioprotective effect on myocardial derangement induced by ischemia, hypoxia, or isoproterenol.13−15 The inhibition of carnitine synthesis reduces the accumulation of harmful metabolites, such as long-chain acylcarnitine, in the impaired myocardium, and this reduction may cause the cardioprotective effect. As carnitine is an essential cofactor that plays an important role in fatty acid metabolism, the inhibition of carnitine synthesis results in the suppression of fatty acid oxidation, which is the predominant energy production system in the heart.14 Tsoko et al. suggested that half of the normal carnitine content was sufficient to allow for normal fatty acid oxidation.15 However, it is unknown to what extent MET-88 would decrease the myocardial carnitine level. Therefore, the present study was designed to clarify the effect of MET-88 on carnitine and lipid contents in the heart and liver in normal rats.

MATERIALS AND METHODS

Treatment with MET-88 Male Sprague-Dawley rats, 8 weeks old (Clea, Tokyo, Japan), were used in the present study. The animals were housed under a 12-h/12-h daily light/dark cycle with food and water provided ad libitum. MET-88 was supplied by the Institute of Organic Synthesis (Riga, Latvia), and dissolved in distilled water just prior to use. Rats were orally administered vehicle (control) or MET-88 at a dose of 50, 100, 200 or 400 mg/kg once a day for 10, 30 or 60 d, and fasted for 12 h before dissection. Twenty-four hours after the last administration, blood was taken from the abdominal aorta of ether anesthetized animals with a heparinized syringe. The heart and liver were then perfused with ice-cold saline from the abdominal aorta and removed. The blood was immediately centrifuged, and the plasma was stored at −80 °C until analysis could be performed. The tissues were frozen with liquid nitrogen and stored at −80 °C until analyzed.

Biochemical Analysis Each tissue was homogenized in 0.6 M perchloric acid. The concentrations of carnitine in the homogenate were measured radiometrically according to the method of Fujisawa et al.10 To measure lipid contents, we extracted lipid from the homogenate with Folch’s solution. Triglyceride and non-esterified fatty acids (NEFA) were measured by enzymatic procedures using a commercial kit (triglycerides: Determiner TG-555, Kyowa Medex, Tokyo, Japan; NEFA: NEFA-c test, Wako, Osaka, Japan). Blood biochemical analysis was performed with an automatic analyzer (Hitachi 736 automatic analyzer, Hitachi, Tokyo), using commercial kits (glutamic-oxaloacetic transaminase [GOT] and glutamic-pyruvic transaminase [GPT], Transaminase-HR; total bilirubin, bilirubin-HR; creatinine, Creatinine-HR, Wako, Osaka, Japan).

Histological Analysis The tissues were fixed in 10% phosphate-buffered formalin. Frozen sections of the tissues were stained with Sudan III, and paraffin sections were stained with hematoxylin-eosin (H.E.).

Statistical Analysis All values were expressed as means±S.E.M. Statistical analysis was performed by one-way analysis of variance, with Dunnett’s test for multiple comparisons. Differences were considered statistically significant at p<0.05.

RESULTS AND DISCUSSION

MET-88, 3-(2,2,2-trimethylhydrazinium) propionate, suppresses carnitine synthesis by inhibiting γ-butyrobetaine hy-
droxylase (Fig. 1). In the present study, we examined to what extent MET-88 would decrease the total carnitine level in the heart and liver. All rats were healthy throughout the experimental period, and there were no significant differences in body weight among experimental groups (data not shown).

Figure 2 shows changes in total carnitine levels in the heart and liver following treatment with MET-88. In the heart, MET-88 dose-dependently decreased the total carnitine level at each time point. The total carnitine level reached nearly its minimum value by treatment with MET-88 for 30 d at each dose, and the levels remained constant thereafter. In addition to the heart, the liver also showed a similar pattern of dose-dependent reduction in the total carnitine level following treatment with MET-88. Also, the acyl carnitine level changed similarly as total carnitine level in the both tissues. It has clearly been shown that the last step of carnitine synthesis occurs in the liver, and so carnitine must be transported from the liver to each tissue via the blood circulation. We suggest that the cardiac carnitine level reduced by MET-88 is occurs primarily as a result of inhibition of carnitine synthesis in the liver, as the reductions were similar in both heart and liver at the respective doses and treatment periods. As carnitine is an essential cofactor that plays an important role in fatty acid metabolism, the inhibition of carnitine synthesis results in the suppression of fatty acid oxidation, which is the predominant energy production system in the heart. However, in the present study, no accumulation of triglycerides was observed in the heart (Fig. 3). This suggests that the heart could still utilize fatty acids as an energy source, although we actually need to measure fatty acid oxidation. Tsoko et al. suggested that half of the normal carnitine content was sufficient to allow for normal fatty acid oxidation. This suggestion may be supported by the observations that the $K_m$ for palmitoyltransferase and the level of carnitine required for maximal stimulation of fatty acid oxidation in isolated mitochondria were substantially lower than the in vivo carnitine concentration of the heart. Previous studies demonstrated that treatment with MET-88 at 100 mg/kg for 10 d had cardioprotective effects on impaired myocardium induced by ischemia, hypoxia or isoproterenol, although it had no effects on cardiac function in the intact myocardium. The present study showed that the effective dose decreased the cardiac carnitine level to about 50, 40, or 40% of the control level by treatment for 10, 30, or 60 d, respectively. Morris et al. indicated that a 60% reduction in cardiac carnitine content did not affect cardiac function in intact myocardium in rats. Also, Simi et al. reported that carnitine-depleted rats were as capable of completing and adapting to an endurance training program as controls. These findings suggest that cardiac functions were maintained and lipid metabolism could proceed in the heart even in the presence of only a very low level of carnitine.

In contrast to the heart, the triglyceride level in the liver was increased by treatment with MET-88 at the highest doses.
at all times examined. Treatment with MET-88 at 400 mg/kg for 60 d induced a 2.5-fold increase in the triglyceride level in the liver. The content of NEFA in the liver was also increased by treatment for 30 or 60 d with MET-88 at doses of 200 mg/kg and over. These findings suggest that the reduction in carnitine level might more greatly affect fatty acid metabolism in the liver than in the heart. As shown in Fig. 4(A), treatment with MET-88 at 400 mg/kg for 60 d resulted in considerable lipid deposition in the liver. The liver in rats treated with MET-88 at doses of over 200 mg/kg for 30 or 60 d showed similar findings (data not shown). All lipid droplets were small and were mainly present in peripheral hepatic lobules, differing from their distribution in intoxicated fatty liver caused by drug-induced hepatopathy. No lipid droplets were observed in the heart (data not shown). As shown in Fig. 4(B), no pathologic findings were observed in the liver. Based on the blood biochemical analysis, MET-88 also had no effect on parameters of liver function such as GOT, GPT or total bilirubin, or of kidney function such as creatinine level (Table 1). Although treatment with MET-88 at the highest dose induced an accumulation of lipid in the liver, it appeared that such an accumulation was no toxic effect in view of results of the histological study and blood biochemical analysis.

In conclusion, treatment with MET-88 dose-dependently

<table>
<thead>
<tr>
<th>Group</th>
<th>GOP (IU/l)</th>
<th>GPT (IU/l)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57±1</td>
<td>26±1</td>
<td>0.16±0.01</td>
<td>0.52±0.02</td>
</tr>
<tr>
<td>MET-88</td>
<td>57±3</td>
<td>26±1</td>
<td>0.16±0.01</td>
<td>0.52±0.02</td>
</tr>
<tr>
<td>(mg/kg/d)</td>
<td>100</td>
<td>54±1</td>
<td>25±1</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>60±2</td>
<td>25±2</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>59±2</td>
<td>27±1</td>
<td>0.17±0.01</td>
</tr>
</tbody>
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Table 1. Blood Biochemical Parameters in Rats Treated with MET-88 for 60 d

Rats were orally given vehicle (control) or MET-88 at a dose of 50, 100, 200, or 400 mg/kg/d for 60 d. Blood biochemical parameters were measured as described in the Materials and Methods section. Data are expressed as the mean±S.E.M. of values from 10 samples.

decreased the total carnitine level in both heart and liver, and the reduction led to a plateau after treatment for 30 days at each dose. Although MET-88 affected the lipid contents in the liver, it had no effect on lipid contents in the heart, suggesting that lipid metabolism in the heart could proceed under conditions of a decreased carnitine level.

REFERENCES AND NOTES


