Metformin Protects Against Carbon Tetrachloride Hepatotoxicity in Mice

Michel K.T. Poon, Po-Yee Chiu, Duncan H.F. Mak, and Kam-Ming Ko*

Department of Biochemistry, The Hong Kong University of Science & Technology, Clear Water Bay, Hong Kong, China

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Abstract. In the present study, the hepatoprotective effect of metformin (Met), a dimethylbiguanide anti-hyperglycemic, was examined in a mouse model of liver damage induced by chronic repeated administration of carbon tetrachloride (CCl₄) (5 μl/kg, twice a week for 12 weeks). Met, when given orally in drinking water at an estimated daily dose of 25 or 50 mg/kg for 10 weeks starting 2 weeks after CCl₄ challenge, protected against CCl₄ hepatotoxicity. The results indicate that the hepatoprotection afforded by Met treatment at a dose of 25 mg/kg against CCl₄ toxicity may at least in part be mediated by the enhancement of mitochondrial glutathione redox status.

Keywords: metformin, carbon tetrachloride, glutathione
initiation of CCl₄ challenge when liver damage had developed, and the effect of Met treatment on the progression of liver damage was then examined. Twenty-four hours after the last dosing of CCl₄, heparinized blood samples were drawn from ether-anesthetized animals by cardiac puncture, and liver tissue samples were obtained for biochemical analysis. Plasma alanine aminotransferase (ALT) activity was measured using an assay kit from Sigma (St. Louis, MO, USA). Mitochondrial fractions were prepared from liver homogenates by differential centrifugation, and measurements of GSH and oxidized glutathione (GSSG) levels used the enzymatic method of Griffith (7). The values were used for estimating the GSH/GSSG ratio, an index of glutathione redox status. The glutathione reductase (GR) activity was determined as described by Godin and Garnett (8). Protein concentrations of mitochondrial fractions were determined using a protein assay kit (Bio-Rad, Hercules, CA, USA). Data were analyzed by one-way ANOVA followed by Duncan’s multiple range test to detect the inter-group difference. Differences are considered to be significant when P<0.05.

As shown in Fig. 1, chronic CCl₄ treatment (5 µl/kg, twice per week for 12 weeks, p.o.) caused hepatic damage, as evidenced by a 13-fold increase in plasma ALT activity. CCl₄ is metabolized into the trichloromethyl radical and other oxidant species, resulting in the disruption of structural and functional integrity in the liver (9). The extent of CCl₄-induced hepatocellular damage can therefore be quantitated by the ALT leakage from the liver into the circulating blood. Met treatment (25 or 50 mg/kg per day for 10 weeks, p.o.) significantly decreased the plasma ALT activity (by 67 and 51%, respectively) in CCl₄-intoxicated mice, indicating hepatoprotective action against CCl₄ toxicity. The decrease in plasma ALT activity was unlikely due to the direct inhibition of the enzyme activity by Met because Met treatment did not seem to affect the plasma ALT activity in non-CCl₄ animals (Fig. 1). Paradoxically, a lesser degree of hepatoprotection was observed at a higher dosage of Met. This might possibly be due to a hepatotoxic effect produced by Met at the supra-clinical dosage, which in turn, reduced the extent of protection against CCl₄ hepatotoxicity.

Chronic CCl₄ intoxication did not produce a detectable change in hepatic mitochondrial GSH level, but it significantly increased the GSSG level (by 152%), resulting in the impairment in glutathione redox status, as evidenced by the decline in GSH/GSSG ratio (Table 1). The mitochondrial GR activity was significantly decreased (by 19%) in CCl₄-intoxicated mice. Met treatment (25 or 50 mg/kg), while decreasing the GSSG level, slightly enhanced the mitochondrial glutathione redox status in non-CCl₄-treated mice. The hepatoprotection afforded by Met treatment at a lower dosage (i.e., 25 mg/kg) against CCl₄ hepatotoxicity was associated with a significant enhancement of mitochondrial glutathione redox status resulting from a notable decrease in GSSG level. In contrast, Met treatment at a higher dosage of 50 mg/kg neither decreased the GSSG level nor enhanced the glutathione redox status in CCl₄-intoxicated animals (Table 1). On the other hand, the hepatoprotection afforded by Met treatment at both dosages was associated with significant increases in mitochondrial GR activity over the non-CCl₄ control level, with the value being higher (64%) than the CCl₄ control at a dose of 50 mg/kg. Reactive oxidant species arising from CCl₄ metabolism, particularly those generated from mitochondria (10), can deplete mitochondrial GSH level and inactivate the GR activity (11) as well as impair the hepatic GSH regeneration capacity (12) in rodents. GSH plays a pivotal role in mitochondrial antioxidant defense and the depletion of mitochondrial GSH was found to increase the susceptibility of hepatic tissue to free radical-mediated damage caused by xenobiotic metabolism (13). Presumably, Met treatment can increase the resistance of the liver to CCl₄-induced oxidative damage by enhancing the mitochondrial glutathione redox status. In this regard, the hepatoprotection afforded by schisandrin B, a dibenzocyclooctadiene derivative isolated from Fructus Schisandrae, against acute CCl₄...
toxicity has been shown to be mainly due to the enhancement of mitochondrial glutathione status (14). The inability of a higher dosage of Met to enhance mitochondrial glutathione redox status in CCl\textsubscript{4}-intoxicated mice, in spite of the relatively high GR activity, suggests that the availability of NADPH is limiting in the GR-catalyzed reaction. Since mitochondria cannot synthesize GSH (6), the impairment in the functioning of the GR-catalyzed reaction may compromise mitochondrial glutathione redox status, particularly under oxidative stress conditions. The hepatoprotection afforded by Met treatment at 50 mg/kg per day, despite the failure in maintaining mitochondrial glutathione redox status, may be related to its inhibitory action on superoxide radical production, as observed in platelets isolated from diabetic patients (15).

In conclusion, the results indicate that Met treatment protects against hepatotoxicity induced by chronic repeated administration of CCl\textsubscript{4} in mice. The hepatoprotective mechanism may, at least in part, be mediated by the enhancement of mitochondrial glutathione redox status, particularly under oxidative stress conditions.

Table 1. Effect of metformin treatment on hepatic mitochondrial glutathione redox status in control and CCl\textsubscript{4}-intoxicated mice

<table>
<thead>
<tr>
<th></th>
<th>GSH (nmol/mg protein)</th>
<th>GSSG (nmol/mg protein)</th>
<th>GSH/GSSG</th>
<th>GR Activity (mU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-CCl\textsubscript{4}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>5.51 ± 1.45</td>
<td>1.26 ± 0.17</td>
<td>4.15 ± 1.52</td>
<td>12.5 ± 2.01</td>
</tr>
<tr>
<td>Met-25</td>
<td>5.47 ± 1.13</td>
<td>1.12 ± 0.32</td>
<td>5.19 ± 1.49</td>
<td>11.9 ± 0.86</td>
</tr>
<tr>
<td>Met-50</td>
<td>5.59 ± 0.32</td>
<td>0.88 ± 0.15</td>
<td>6.51 ± 1.37</td>
<td>11.5 ± 1.03</td>
</tr>
<tr>
<td>CCl\textsubscript{4}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>5.10 ± 3.89</td>
<td>3.18 ± 3.06\textsuperscript{a}</td>
<td>3.21 ± 2.99</td>
<td>10.1 ± 2.94\textsuperscript{b}</td>
</tr>
<tr>
<td>Met-25</td>
<td>6.95 ± 1.81</td>
<td>1.20 ± 0.20\textsuperscript{a}</td>
<td>5.97 ± 2.01\textsuperscript{b}</td>
<td>15.1 ± 1.22\textsuperscript{a}</td>
</tr>
<tr>
<td>Met-50</td>
<td>5.66 ± 1.94</td>
<td>2.98 ± 1.54\textsuperscript{a}</td>
<td>2.30 ± 1.18\textsuperscript{a}</td>
<td>16.6 ± 2.45\textsuperscript{a}</td>
</tr>
</tbody>
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Animals were treated as described in Fig. 1. Hepatic mitochondrial reduced glutathione (GSH) and oxidized glutathione (GSSG) levels as well as glutathione reductase (GR) activity were measured. Values given are the mean ± S.D., n = 6. *Significantly different from the Non-CCl\textsubscript{4} CON, †significantly different from the CCl\textsubscript{4} CON, ‡significantly different from the Met-25 CCl\textsubscript{4}.

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