J Pharmacol Sci **93**, 501 – 504 (2003)

Journal of Pharmacological Sciences

Short Communication

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

Received June 11, 2003; Accepted October 3, 2003

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 (CCl_4) (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 CCl_4 challenge, protected against CCl_4 hepatotoxicity. The results indicate that the hepatoprotection afforded by Met treatment at a dose of 25 mg/kg against CCl_4 toxicity may at least in part be mediated by the enhancement of mitochondrial glutathione redox status.

Keywords: metformin, carbon tetrachloride, glutathione

Metformin (Met), a dimethylbiguanide, is clinically used for the treatment of hyperglycemia in patients with type 2 diabetes mellitus (1). The antioxidant potential of Met has early been indicated by the finding that a 2-week treatment of Met at a daily dose of 50 or 60 mg/kg could increase reduced glutathione (GSH) levels in the liver and blood in normal and diabetic rats (2). Furthermore, the long-term treatment of Met has been found to reduce plasma xanthine oxidase activity and thiobarbituric acid-reactive substances level in diabetic patients, indicative of a decrease in tissue oxidative stress (3). These changes were also associated with a decrease in erythrocyte glutathione peroxidase activity (3). Despite the fact that the liver becomes more susceptible to carbon tetrachloride (CCl_4)-induced toxicity in diabetic rats (4), it is still unknown whether Met can produce any effect on the free radical-mediated hepatic damage. A preliminary study in our laboratory indicated that the short-term and high dose treatment with Met did not show notable protection against acute CCl_4 hepatotoxicity in mice (unpublished data). In the present study, we examined the effect of long-term and low dose of Met treatment on liver damage induced by chronic repeated administration of CCl_4 in mice. Given that the maintenance of mitochondrial glutathione redox status is crucial for cell survival under oxidative stress conditions (5), the effect of Met treatment on hepatic mitochondrial glutathione redox status was also examined in control and CCl_4-intoxicated mice.

Adult male Balb/c mice (23 – 25 g; Animal Care Facilities, The Hong Kong University of Science & Technology) were maintained under a 12-h dark/light cycle at about 22°C and allowed food and water ad libitum. Mice were randomly divided into the following 6 groups, with 6 animals in each: Non-CCl_4 CON, Non-CCl_4 Met-25, Non-CCl_4 Met-50, CCl_4 CON, CCl_4 Met-25, and CCl_4 Met-50. For the CCl_4 treatment group, mice were orally administered with CCl_4 at 5 μl/kg (1%, v/v, in olive oil) via an intragastric gavage tube twice a week for 12 weeks. Non-CCl_4 animals received olive oil only. The chronic CCl_4 administration was found to produce irreversible liver damage such as liver fibrosis in mice (6). Met was dissolved in double-distilled water at concentrations of 178 and 356 mg/ml for the Met-25 and Met-50 group, respectively. Starting 2 weeks after CCl_4 challenge, the drug solutions were given to animals in their drinking water for 10 weeks. While there were no apparent differences in the daily intake of water among animals in the various groups, the daily dosages of Met were estimated to be 25 and 50 mg/kg, respectively, with the former dose being close to the average clinical dosage (i.e., 1.5 g/day) (1). Control animals received double-distilled water only. The Met treatment was started two weeks after the

*Corresponding author. FAX: +852-2358-1552
E-mail: bcrko@ust.hk

initiation of CCl_4 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 CCl_4, 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 CCl_4 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. CCl_4 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 CCl_4-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 CCl_4-intoxicated mice, indicating hepatoprotective action against CCl_4 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-CCl_4 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 CCl_4 hepatotoxicity.

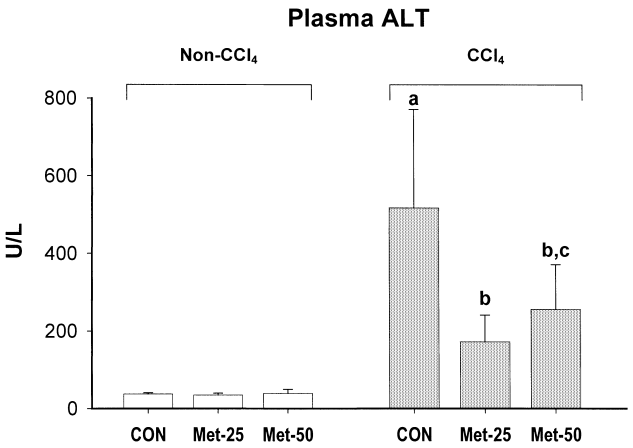

Fig. 1. Effect of metformin treatment on CCl_4 hepatotoxicity in mice. Animals were chronically treated with CCl_4 at 5 μl/kg (p.o.) twice a week for 12 weeks. Metformin (Met) was given in drinking water starting from the 3rd week, with daily dosages estimated to be 25 and 50 mg/kg for 10 weeks. Plasma alanine aminotransferases (ALT) activity was measured. Values given are the mean ± S.D., n = 6. [a]Significantly different from the Non-CCl_4 CON, [b]significantly different from the CCl_4 CON, [c]significantly different from the Met-25 CCl_4.

Chronic CCl_4 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 CCl_4-intoxicated mice. Met treatment (25 or 50 mg/kg), while decreasing the GSSG level, slightly enhanced the mitochondrial glutathione redox status in non-CCl_4-treated mice. The hepatoprotection afforded by Met treatment at a lower dosage (i.e., 25 mg/kg) against CCl_4 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 CCl_4-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-CCl_4 control level, with the value being higher (64%) than the CCl_4 control at a dose of 50 mg/kg. Reactive oxidant species arising from CCl_4 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 CCl_4-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 CCl_4

Table 1. Effect of metformin treatment on hepatic mitochondrial glutathione redox status in control and CCl_4-intoxicated mice

	GSH	GSSG	GSH/GSSG	GR Activity
	(nmol/mg protein)			(mU/mg protein)
Non-CCl_4				
CON	5.51 ± 1.45	1.26 ± 0.17	4.15 ± 1.52	12.5 ± 2.01
Met-25	5.47 ± 1.13	1.12 ± 0.32	5.19 ± 1.49	11.9 ± 0.86
Met-50	5.59 ± 0.32	0.88 ± 0.15	6.51 ± 1.37	11.5 ± 1.03
CCl_4				
CON	5.10 ± 3.89	3.18 ± 3.06[a]	3.21 ± 2.99	10.1 ± 2.94[a]
Met-25	6.95 ± 1.81	1.20 ± 0.20[b]	5.97 ± 2.01[b]	15.1 ± 1.22[b]
Met-50	5.66 ± 1.94	2.98 ± 1.54[c]	2.30 ± 1.18[c]	16.6 ± 2.45[b]

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. [a]Significantly different from the Non-CCl_4 CON, [b]significantly different from the CCl_4 CON, [c]significantly different from the Met-25 CCl_4.

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_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 GSH regeneration system may compromise the mitochondrial glutathione redox status, particularly in oxidative stress conditions, such as those induced by CCl_4 administration. While the effect of long-term Met treatment on the biosynthesis of NADPH remains to be determined, it is possible that the excessive increase in glucose flux into glycolysis caused by a high dosage of Met treatment may deprive the pentose phosphate shunt of glucose 6-phosphate (a common substrate for glycolysis and pentose phosphate pathway), thereby reducing the hepatic production of NADPH and impairing the glutathione redox cycle. 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_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.

Acknowledgment

Merck Santé (France) sponsored this study under a research contract agreement with the Biotechnology Research Institute, The Hong Kong University of Science & Technology.

References

1 Davidson MB, Peters AL. An overview of metformin in the treatment of type 2 diabetes mellitus. Am J Med. 1997;102:99–110.
2 Ewis SA, Abdel-Rahman. Influence of atenolol and/or metformin on glutathione and magnesium levels in diabetic rats. J Appl Toxicol. 1997;17:409–413.
3 Cosic V, Antic S, Pesic M, Tovanovic O, Kundal S, Djordjevic VB. Monotherapy with metformin: does it improve hypoxia in type 2 diabetic patients? Clin Chem Lab Med. 2001;39:818–821.
4 Watkins JB III, Sanders RA, Beck LV. The effect of long-term streptozotocin-induced diabetes on the hepatotoxicity of bromobenzene and carbon tetrachloride and hepatic biotransformation in rats. Toxicol Appl Pharmacol. 1988;93:329–338.
5 Meister A, Anderson ME. Glutathione. Annu Rev Biochem. 1983;52:711–760.
6 Simenova PP, Gallucci RM, Hulderman T, et al. The role of tumor necrosis factor-alpha in liver toxicity, inflammation, and fibrosis induced by carbon tetrachloride. Toxicol Appl Pharmacol. 2001;177:112–120.
7 Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem. 1980;106:207–212.
8 Godin DV, Garnett ME. Species-related variations in tissue antioxidant status—I. Differences in antioxidant enzyme profiles. Comp Biochem Physiol B. 1992;103:737–742.
9 Wolf CR, Harrelson WG Jr, Nastainczyk WM, Philpot RM, Kalyanaraman B, Mason RP. Metabolism of carbon tetrachloride in hepatic microsomes and reconstituted monooxygenase systems and its relationship to lipid peroxidation.

Mol Pharmacol. 1980;18:553–558.
10 Tomasi A, Albano E, Banni S, et al. Free-radical metabolism of carbon tetrachloride in rat liver mitochondria. A study of the mechanism of activation. Biochem J. 1987;246:313–317.
11 Ip SP, Ko KM. The crucial antioxidant action of schisandrin B in protecting against carbon tetrachloride hepatotoxicity in mice: a comparative study with butylated hydroxytoluene. Biochem Pharmacol. 1996;52:1687–1693.
12 Ko KM, Mak DHF, Li PC, Poon MKT, Ip SP. Enhancement of hepatic glutathione regeneration capacity by a lignan-enriched extract of Fructus Schisandrae in rats. Jpn J Pharmacol. 1995;69:439–442.
13 Shan X, Jones DP, Hashmi M, Anders MW. Selective depletion of mitochondrial glutathione concentrations by (*R,S*)-3-hydroxy-4-pentenoate potentiates oxidative cell death. Chem Res Toxicol. 1993;6:75–81.
14 Chiu PY, Tang MH, Mak DHF, Poon MKT, Ko KM. Hepatoprotective mechanism of schisandrin B: role of mitochondrial glutathione antioxidant status and heat shock proteins. Free Radic Biol Med. 2003;35:368–380.
15 Gargiulo P, Caccese D, Pignatelli P, et al. Metformin decreases platelet superoxide anion production in diabetic patients. Diabetes Metab Res Rev. 2002;18:156–159.