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Bicyclol, a synthetic dibenzocyclooctadiene derivative, decreases hepatic lipids but increases serum triglyceride level in normal and hypercholesterolaemic mice

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Abstract

Bicyclol is used for the treatment of chronic hepatitis B in China. In this study, the effects of bicyclol (100 or 300 mg kg^{-1}, p.o.) on serum and liver lipid contents were investigated in both normal and experimentally induced hypercholesterolaemic mice. Hypercholesterolaemia was induced by either oral administration of cholesterol/bile salt or feeding a diet containing lard/cholesterol. Daily administration of bicyclol for 7 days dose-dependently increased the serum triglyceride level (29–80%) but slightly decreased the hepatic total cholesterol level (12–17%) in normal mice. Co-administration of bicyclol with cholesterol/bile salt decreased the hepatic triglyceride and total cholesterol levels (7–15% and 25–31%, respectively), when compared with the drug-untreated and cholesterol/bile salt-treated group. Bicyclol treatment for 7 days decreased hepatic triglyceride (5–76%) and total cholesterol (5–48%) levels in mice fed with high-fat/cholesterol diet. In contrast, bicyclol treatment increased the serum triglyceride level (18–77%) in mice treated with cholesterol/bile salt or fed with high-fat/cholesterol diet. Bicyclol treatment also caused an increase in hepatic index of normal and hypercholesterolaemic mice (3–32%). The results indicate that bicyclol treatment can invariably decrease hepatic lipid levels and increase serum triglyceride levels in normal and hypercholesterolaemic mice.

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Introduction

Dibenzocyclooctadiene derivatives (such as schisandrins A, B, C, schisandrol A, B and schisandrers A, B) are found in the fruit of *Schisandra chinensis*, a traditional Chinese herb clinically prescribed for the treatment of hepatitis (Liu 1989). Bifendate, a synthetic intermediate of schisandrin C, and other schisandrins were found to protect against drug-induced liver injury and influence various aspects of liver function in rodents (Xie et al 1981; Ko & Mak 2004). Bifendate, which is now clinically used for the treatment of hepatitis with minimal observable side effects at the prescribed dosage, is also regarded as a positive control for exploring other hepatoprotective agents (Cui et al 2002; Guan et al 2005). Previous studies in our laboratory have shown that both bifendate and schisandrin B treatments increased serum or hepatic triglyceride levels in mice, and the hypertriglyceridaemic action was accompanied by a decrease in hepatic total cholesterol level (Pan et al 2006a, b). Moreover, bifendate treatment has been shown to attenuate hepatic steatosis in mice with hypercholesterolaemia induced by cholesterol/bile salt or high-fat diet (Pan et al 2006c).

Bicyclol (4,4′-dimethoxy-5,6,5′,6′-dimethylene-dioxy-2-hydroxymethyl-2′-carbonyl biphenyl) is a synthetic dibenzocyclooctadiene derivative used for the treatment of chronic viral hepatitis in China (Liu 2001; Yao et al 2002). Several studies have shown that bicyclol treatment protects against chemical-induced hepatotoxicity by carbon tetrachloride and paracetamol (acetaminophen), as well as immunologically induced liver damage by lipopolysaccharide and concanavalin A in rodents (Yang et al 1997; Li et al 2001, 2004; Zhao & Liu 2001; Wang & Li 2006). The hepatic lipid-lowering effect of bicyclol treatment on alcohol-induced fatty liver was also demonstrated in mice (Mo et al 2005). However,

whether or not bicyclol treatment can influence the hepatic lipid content in normal and hypercholesterolaemic mice remains unknown. In this study, we endeavoured to investigate the effects of bicyclol treatment on serum and hepatic lipid levels in normal and experimentally induced hypercholesterolaemic mice. Fenofibrate, a clinically used lipid-lowering drug, was also studied for comparison.

Materials and Methods

Chemicals and reagents

Bicyclol (in formulated tablets, certificate No. 051107) was purchased from Beijing Xiehe Pharmacy Factory (Beijing, China). Cholesterol (certificate No. 041103) and bile salt (certificate No. 000710) were obtained from Beijing Chemical Reagent Co. (Beijing, China). Fenofibrate (certificate No. 0405030) was bought from Beijing Yongkang Medical Ltd (Beijing, China). Sodium carboxymethylcellulose (CMC, certificate No. 971230) was obtained from Beijing Xudong Chemical Plant (Beijing, China). Assay kits for total cholesterol and triglyceride were bought from Zhongsheng Beikong Bio-technology and Science Inc. (Beijing, China).

Animal treatment

Male ICR mice (Grade II, certificate No. SCXK(jing)2002-0003), 18–20 g, were supplied by Vital River Lab Animal Co. Ltd (Beijing, China). All mice were maintained on a 12-h light–dark cycle (light on 0700–1900 h) at 20–21°C, with a relative humidity of 50–55%. They were allowed free access to water and food. Experiments were performed when the mice had grown up to a body weight of 24–26 g. Hypercholesterolaemia was induced by either oral administration of cholesterol/bile salt (2/0.5 g kg^{-1}; suspended in 0.5% CMC) for 3 days or feeding a high-fat diet (10% lard, w/w) containing cholesterol (1%, w/w) for 8 days. Non-hypercholesterolaemic (normal) mice were given the vehicle or normal diet only. In drug treatment groups, bicyclol or fenofibrate (suspended in 0.5% CMC) was intragastrically administered at doses of 100–300 or 100 mg kg^{-1} daily, respectively, for 3 days or 7 days in the respective animal model. Drug-untreated mice received the vehicle (0.5% CMC) at 10 mL kg^{-1} daily. Blood and liver tissue samples were obtained from ether-anaesthetized mice, which had been fasted for 6 h (0600–1200 h), and they were subjected to biochemical analysis. Hepatic index was estimated from the ratio of total liver weight to body weight. All experimental protocols were approved by the University Committee on Research Practice in Beijing University of Chinese Medicine.

Determination of total cholesterol and triglyceride levels

A small amount of blood was quickly obtained from the orbital vein without the use of syringe 24 h after the last dosing with the drug. Serum samples were prepared by centrifuging the whole blood for 8 min at 2000 *g* and then stored at –20°C until assay within 5 days. Liver tissue sample was homogenized in 9 volumes of 0.9% (w/v) NaCl solution by two 10-s bursts of a tissue disintegrator at 13 500 rev min^{-1}, and the homogenate was then centrifuged at 2000 *g* for 15 min to obtain the supernatants. Ten and forty microlitres of the hepatic supernatants were used to determine the total cholesterol and triglyceride levels, respectively.

Statistical analysis

Data were analysed by one-way analysis of variance and expressed as means ± s.e.m. Inter-group difference was detected by Dunnett's test using SPSS12.0 software. The difference was considered significant when $P < 0.05$.

Results

Effect of bicyclol treatment on serum and hepatic lipid levels in normal mice

Bicyclol treatment (100 and 300 mg kg^{-1} daily for 7 days) caused a significant increase in serum triglyceride level (29 and 80%, respectively) at 24 h after the last dosing, but it did not affect the serum total cholesterol level. While hepatic total cholesterol level was significantly reduced (by 17 and 12%), when compared with the vehicle control, the hepatic triglyceride level was not changed by bicyclol treatment (Figure 1).

Effect of bicyclol treatment on serum and hepatic lipid levels in cholesterol/bile salt-induced hypercholesterolaemic mice

Oral administration with cholesterol/bile salt (2.0/0.5 g kg^{-1} daily for 3 days) caused significant increases in serum and hepatic total cholesterol level (46% and 16%, respectively), but the serum triglyceride level decreased (by 46%). Bicyclol (100 and 300 mg kg^{-1} daily for 3 days) reduced the serum (2 and 18%, respectively) and hepatic (31 and 25%) total cholesterol, as well as hepatic triglyceride level (7 and 9%), in mice receiving cholesterol/bile salt treatment. In contrast, bicyclol treatment increased the serum triglyceride level (18–59%) in the hypercholesterolaemic mice. Fenofibrate treatment (100 mg kg^{-1} daily for 3 days) significantly decreased serum/hepatic total cholesterol (30/31%) and hepatic triglyceride (31%) levels, but did not change the serum triglyceride level in hypercholesterolaemic mice, when compared with the drug-untreated control (Figure 2).

Effect of bicyclol treatment on serum and hepatic lipid levels in high-fat diet/cholesterol-induced hypercholesterolaemic mice

Feeding mice with a high-fat diet containing 10% lard and 1% cholesterol for 8 days significantly increased serum/hepatic total cholesterol (65/68%) and hepatic triglyceride (193%) levels, but serum triglyceride level was reduced by 48%, when compared with mice fed with normal diet. Bicyclol treatment (100 and 300 mg kg^{-1} daily for 7 days) from day 1 to day 7 significantly decreased hepatic triglyceride

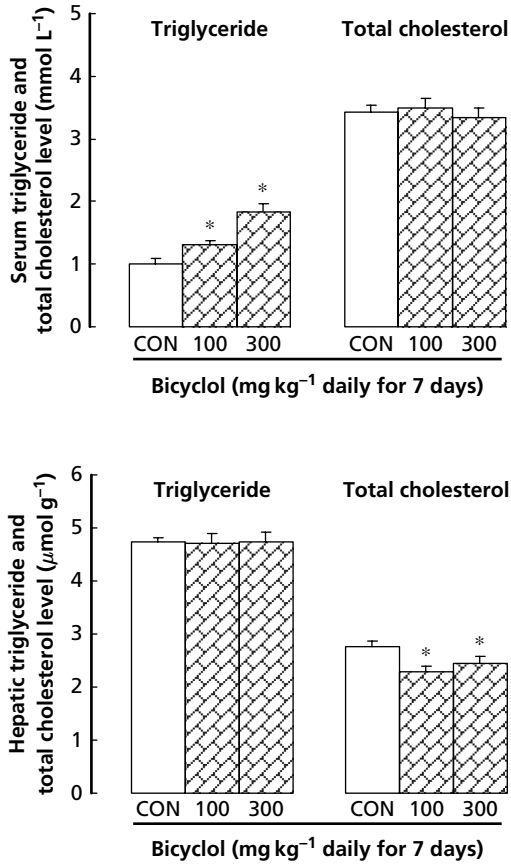

Figure 1 Effect of bicyclol treatment on serum and hepatic triglyceride and total cholesterol levels in mice. Mice were intragastrically administered with bicyclol (100 or 300 mg kg^{-1} daily, p.o.) for 7 days. Control mice (CON) received the vehicle (0.5% CMC 10 mL kg^{-1} daily, p.o.) only. Serum/hepatic triglyceride and total cholesterol levels were measured 24 h after the last dosing with bicyclol. Values given are the mean ± s.e.m., n = 10. *$P < 0.05$, compared with the CON group.

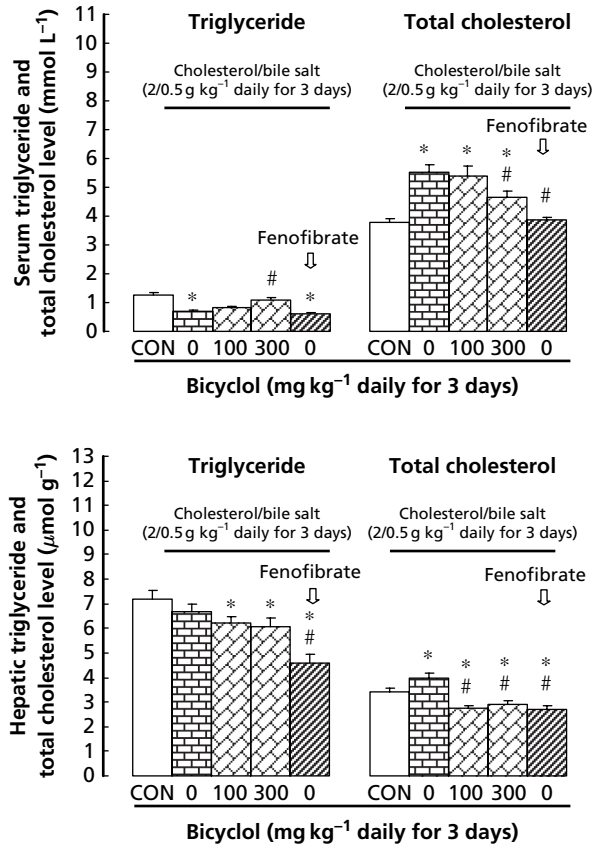

Figure 2 Effect of bicyclol treatment on serum and hepatic triglyceride and total cholesterol levels in mice orally administered with cholesterol/bile salt. Mice were intragastrically administered with cholesterol/bile salt (2.0/0.5 g kg^{-1}) for 3 days. Control mice (CON) received the vehicle only. Bicyclol (100 or 300 mg kg^{-1} daily, p.o.) or fenofibrate (100 mg kg^{-1} daily, p.o.) were co-administered for 3 days. Drug-untreated mice received the vehicle (0.5% CMC 10 mL kg^{-1} daily, p.o.) only. Serum/hepatic triglyceride and total cholesterol levels were measured 24 h after the last dosing with bicyclol or fenofibrate. Values given are the mean ± s.e.m., n = 10. *$P < 0.05$, compared with the CON group; #$P < 0.05$, compared with the drug-untreated cholesterol/bile salt group.

(57 and 67%, respectively) and total cholesterol (34 and 26%) levels, but it increased serum triglyceride levels (64–77%), when compared with the drug-untreated hypercholesterolaemic mice. Fenofibrate treatment (100 mg kg^{-1} daily for 7 days) also significantly reduced hepatic triglyceride/total cholesterol (68/46%) levels in hypercholesterolaemic mice. Serum total cholesterol level was not affected by bicyclol or fenofibrate treatment in mice fed with high-fat/cholesterol diet (Figure 3).

Effect of bicyclol treatment on hepatic index in normal and hypercholesterolaemic mice

Figure 4 shows the bicyclol-induced changes in hepatic index in normal as well as cholesterol/bile salt-treated or high-fat diet/cholesterol-fed mice. Bicyclol (100 and 300 mg for 7 days) increased the hepatic index (by 8–32%) in normal mice, when compared with that of mice receiving the vehicle. Bicyclol treatment at the same dosage further increased the hepatic index significantly in hypercholesterolaemic mice, when compared with the drug-untreated control. Fenofibrate treatment also caused an increase in hepatic index, but to a much larger extent than that of bicyclol treatment, in hypercholesterolaemic mice.

Discussion

Lipid disorders, encompassing hypercholesterolaemia, hypertriglyceridaemia (or their combination) and fatty liver, are prevalent in modern society (Park et al 2003). Hypercholesterolaemia is commonly associated with atherosclerotic vascular disease and is present as a risk factor for coronary heart disease (Aronow 2006). While fatty liver is commonly found in obese individuals aged over 30, it also occurs in children (Mager & Roberts 2006). The incidence of fatty liver is in the

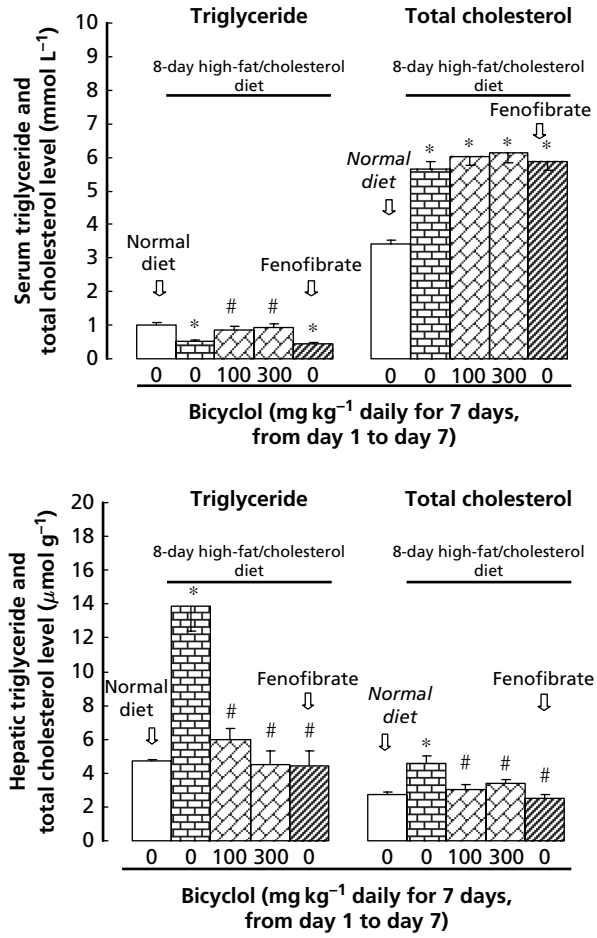

Figure 3 Effect of bicyclol treatment on serum and hepatic triglyceride and total cholesterol levels in mice fed with high-fat diet/cholesterol. Mice were fed with the diet supplemented with lard/cholesterol (10/1%, w/w) for 8 days. Control mice were fed with normal diet. Bicyclol (100 or 300 mg kg^{-1} daily, p.o.) or fenofibrate (100 mg kg^{-1} daily, p.o.) co-treatment was performed for 7 days (from day 1 to day 7), as described in Materials and Methods. Drug-untreated mice received the vehicle (0.5% CMC 10 mL kg^{-1} daily, p.o.) only. Twenty-four hours after the last dosing with bicyclol or fenofibrate, serum/hepatic triglyceride and total cholesterol levels were determined. Values given are the mean ± s.e.m., n = 10. $*P < 0.05$, compared with the normal diet group; $\#P < 0.05$, compared with the drug-untreated high-fat/cholesterol diet group.

range 15–30% in the USA, Europe and Asia and 50–90% among obese people (Benedetti 2005; Fan et al 2005; Lin et al 2005; Tilg & Kaser 2005). Although non-alcoholic fatty liver rarely leads to more serious liver problems, it may interfere with normal liver function or cause inflammation and fibrosis secondary to hepatocyte injury, with resultant liver failure, hepatocirrhosis or hepatocellular carcinoma (Sanval 2005; Farrell & Larter 2006). In this study, to explore whether bicyclol, a clinically used drug for viral hepatitis (Liu 2001; Yao et al 2002), could reduce hepatic lipid levels, the effects of bicyclol treatment were investigated in two mouse models of hypercholesterolaemia. The results indicated that bicyclol treatment, while decreasing the hepatic triglyceride and total cholesterol levels under both normal and hypercholesterolaemic conditions, also caused an increase in serum triglyceride level in mice.

High fat and excessive carbohydrate intake can cause hypertriglyceridaemia, wherein the inhibition of β-oxidation leads to the production of excessive triglycerides from the esterification of fatty acids (Eaton et al 1997). While the mechanism involved in the elevation of serum triglyceride level by bicyclol treatment remains to be elucidated, it is possible that bicyclol may stimulate the esterification of fatty acids or inhibit the β-oxidation. Despite the fact that the triglyceride is just one type of lipid molecule for energy storage in the body, hypertriglyceridaemia has been regarded as an independent risk factor for cardiovascular disease (Hokanson & Austin 1996). Given the hypertriglyceridaemic action induced by bicyclol, the clinical use of this drug should be exercised with caution, particularly in those patients with hyperlipidaemia. Nevertheless, the dose regimen of bicyclol adopted in this experimental investigation was up to 300 mg kg^{-1} daily, which was much higher (100–200 fold) than the recommended human adult daily dose of 75–150 mg (i.e., 1.5–3.0 mg kg^{-1} for 50 kg body weight). Likewise, the dosage of fenofibrate adopted in this study was 17–25 fold higher than the human dose.

Fenofibrate, a broad spectrum lipid-lowering drug, is clinically used in patients suffering from primary combined dyslipidaemias or secondary dyslipidaemias (McKenney et al 2006). Pharmacological investigations have suggested that the ability of fenofibrate to reduce hepatic and serum lipid levels may be related to several biochemical actions. In essence, fenofibrate was found to stimulate lipolysis and the elimination of triglyceride-rich particles from plasma through activating lipoprotein lipase and reducing the production of apolipoprotein C-III, an inhibitor of lipoprotein lipase activity, as well as inducing enzymes for catalysing β-oxidation of fatty acids in mitochondria (Packard 1998; Andersson et al 1999; Miura et al 2005). A recent study has shown that an extract of *Salacia oblonga*, an Ayurvedic medicine that possesses PPARα (peroxisome proliferator-activated receptor-alpha) activating activity, can improve the hepatic steatosis in rat models of diabetes and obesity (Huang et al 2006a, b). Whether or not bicyclol, a synthetic compound based on an active ingredient from *Schisandra chinensis*, can lower hepatic lipid content via action mechanisms similar to those of fenofibrate and *Salacia oblonga* extract awaits further investigation.

The mechanism underlying the hepatoprotective action of bicyclol in patients suffering from hepatitis has been shown to be mediated by its anti-inflammatory, anti-peroxidative and detoxifying actions, as well as the inhibition on Fas/FasL mRNA expression and TNF-α secretion (Lu & Li 2002; Li & Liu 2004; Liu et al 2005a). The finding of hepatic lipid-modulating effect of bicyclol further suggests the involvement of reduction in hepatic triglyceride and total cholesterol levels in the hepatoprotection afforded by bicyclol treatment. While, in this study, bicyclol treatment increased the liver weight in mice, a recent study indicated that bicyclol treatment at doses up to 600 mg kg^{-1} daily for 6 months did not produce any detectable changes in liver weight in rats (Liu et al 2005b).

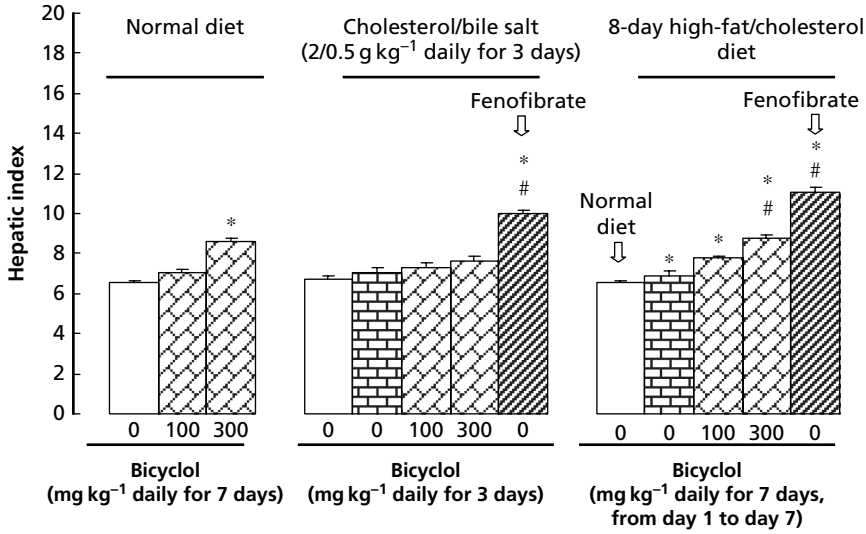

Figure 4 Effect of bicyclol treatment on hepatic index in normal and hypercholesterolaemic mice. Experimental details are described in Figures 1, 2 and 3, respectively. Hepatic index was estimated by the ratio of the whole liver weight to body weight. Values given are the mean ± s.e.m., n = 10. *$P < 0.05$, compared with the control or normal diet group; #$P < 0.05$, compared with the drug-untreated hypercholesterolaemic group.

The hepatotrophic effect of bicyclol may therefore be species specific. The lesser degree of liver hypertrophy caused by bicyclol than fenofibrate might be more desirable for the treatment of fatty liver. In addition, while schisandrin B or bifendate treatment increased hepatic triglyceride level in normal animals (Pan et al 2006a, b, c), bicyclol treatment did not produce any effect on hepatic triglyceride level in normal mice.

Conclusion

The results indicate that bicyclol treatment can invariably decrease hepatic total cholesterol levels in normal and hypercholesterolaemic mice. Both serum triglyceride level and liver weight were also increased by bicyclol treatment under normal and hypercholesterolaemic conditions. Similar lipid-modulating effects were also observed in fenofibrate-treated mice, except that fenofibrate did not cause hypertriglyceridaemia. Bicyclol might be a candidate drug, as is the case for fenofibrate (Tsutsumi & Takase 2001), for the treatment of fatty liver. Considering the plasma triglycerides, a risk factor for cardiovascular disease, care must be taken in patients with coronary heart disease if using bicyclol clinically.

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