JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 399–403

Received September 25, 2007
Accepted November 22, 2007
DOI 10.1211/jpp.60.3.0017
ISSN 0022-3573

Communication

Schisandrin B from *Schisandra chinensis* reduces hepatic lipid contents in hypercholesterolaemic mice

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Abstract

The effects of schisandrin B (Sch B) on liver and serum lipid contents were investigated in mice with experimentally-induced hypercholesterolaemia. Hypercholesterolaemia was induced either by oral administration of a cholesterol/bile salts mixture (2/0.5 g kg^{-1}) for four days or by feeding a high fat/cholesterol/bile salts (10/1/0.3%, w/w) diet for seven days. Daily co-administration of Sch B (50–200 mg kg^{-1}, i.g.) for four or six days, respectively, decreased hepatic total cholesterol (TC) and triglyceride (TG) levels (by up to 50% and 52%, respectively) in hypercholesterolaemic mice. Sch B treatment also increased hepatic indices (14–84%) in hypercholesterolaemic mice. The results indicated that Sch B treatment could decrease hepatic TC and TG levels, and increase liver weight, in mouse models of hypercholesterolaemia. Fenofibrate treatment (100 mg kg^{-1}) produced effects similar to those of Sch B on the hepatic index and lipid levels of hypercholesterolaemic mice.

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Funding: This work was supported by grants from the Natural Science Foundation of Beijing City (grant no.7022017) and Hong Kong Baptist University (grant no. FRG/06-07/II-67).

Introduction

Non-alcoholic fatty liver disease represents a spectrum of liver diseases characterized mainly by macrovesicular steatosis in the absence of significant alcohol ingestion. Although a non-alcoholic fatty liver rarely leads to more serious liver problems, this condition may interfere with normal liver function or cause inflammation and/or fibrosis secondary to hepatocyte injury, with resultant liver failure, hepatocirrhosis, or hepatocellular carcinoma (Festi et al 2004; Sanval 2005; Farrell & Larter 2006). The incidence of fatty liver is 15–30% in the general population, but rises to 50–90% in obese people (Fan et al 2005; Tigl & Kaser 2005; Mager & Roberts 2006). At present, orthodox medicine offers no specific drug to reverse the condition of fatty liver, although antioxidants, insulin sensitizers, hepatoprotectants, and general lipid-lowering agents are used to this end (Comar & Sterling 2006). Some hypocholesterolaemic drugs have been shown to cause disorders in liver function resulting from non-specific actions (Comar & Sterling 2006).

Schisandrin B (Sch B) is an abundant, active, dibenzocyclooctadiene derivative isolated from the fruit of *Schisandra chinensis*, a traditional Chinese herb prescribed clinically for the treatment of hepatitis (Ko & Mak 2004). Recent studies have shown that although treatment with Sch B and other synthetic dibenzocyclooctadiene derivatives, such as bifendate, elevate serum triglyceride (TG) levels, the drugs significantly decrease hepatic total cholesterol (TC) levels in mice and rabbits with normal cholesterol levels (Pan et al 2006a, b). In addition, bifendate treatment was found to attenuate hepatic steatosis in hypercholesterolaemic mice (Pan et al 2006c). The aim of this study was to investigate whether Sch B treatment might suppress hepatic lipid levels in mice with experimentally-induced hypercholesterolaemia.

Materials and Methods

Chemicals and reagents

Sch B was purified from the petroleum ether extract of dried fruits of *Schisandra chinensis*, as described by Ip et al (1995). The purity of Sch B, as determined by high performance liquid chromatography, was > 95%. Cholesterol and bile salts were from the Beijing Chemical Reagent Co. (Beijing, China). Fenofibrate was purchased from Beijing Yongkang Medical Ltd (Beijing, China). Assay kits for total cholesterol and triglyceride were purchased from Zhongsheng Beikong Bio-Technology and Science Inc. (Beijing, China).

Animal treatment

Male ICR mice (18–20 g; grade II, certificate No. SCXK(jing) 2002-0003) were supplied by the Vital River Laboratory Animal Co. Ltd (Beijing, China). All animals 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%. Mice were allowed free access to water and food. Experiments were performed when the animals had attained body weights of 24–26 g and each group in this study contained 10 mice. Hypercholesterolaemia was induced either by oral administration of a cholesterol/bile salts mixture (2/0.5 g kg^{-1} daily for 4 days, suspended in olive oil) or by feeding a high fat/cholesterol/bile salts (10/1/0.3%, w/w) diet. In drug treatment groups, Sch B or fenofibrate (suspended in olive oil) was intragastrically administered at doses of 50–200 mg kg^{-1} or 100 mg kg^{-1}, respectively. Normal animals received the vehicle (olive oil) at 10 mL kg^{-1} and were fed a normal diet. Blood and liver tissue samples were obtained from ether-anaesthetized animals which had been fasted for 6 h, and these samples were subjected to biochemical analysis. The 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 of the Beijing University of Chinese Medicine.

Determination of total cholesterol and triglyceride levels

Serum samples were prepared by centrifuging whole blood obtained from the orbital vein for 8 min at 2000 *g* and lipid levels were then measured. Liver tissue samples were homogenized in 9 vol 0.9% (w/v) NaCl solution (Pan et al 2006a). Hepatic supernatants, 10 and 40 μL, were used to determine total cholesterol and triglyceride levels, respectively. Serum and hepatic total cholesterol and triglyceride concentrations were measured by assay kits using enzyme-coupled colour reactions.

Statistical analysis

The data were expressed as means ± s.e.m. and were analysed by one-way analysis of variance. Significant differences amongst groups were detected by Dunnett's test using SPSS version 12.0 software. A difference was considered significant when $P < 0.05$.

Results

Effects of Sch B treatment on serum lipid levels in hypercholesterolaemic mice

Table 1 shows the effects of Sch B treatment on serum lipid levels in hypercholesterolaemic mice. Serum TC levels was increased (by 70–160%), but TG levels were reduced (by 37–55%), in mice treated with cholesterol/bile salts for four days or fed a high fat/cholesterol/bile salts diet for seven days, when compared with the levels seen in mice fed a normal diet. Although Sch B treatment did not produce any detectable effect on serum TC levels, Sch B treatment significantly elevated serum TG contents (by 19–170%) when compared with the level seen in hypercholesterolaemic mice receiving no drug treatment. Fenofibrate treatment decreased the mean serum TC level (by 37–43%) in hypercholesterolaemic mice. In contrast, fenofibrate increased the average serum TG level (by 31%) in mice with hypercholesterolaemia induced by the high fat/cholesterol/bile salts diet, but did not affect the serum TG level in animals with hypercholesterolaemia induced by the cholesterol/bile salts treatment.

Effects of Sch B treatment on hepatic total cholesterol levels in hypercholesterolaemic mice

Treatment with the cholesterol/bile salts regimen for four days or feeding a high fat/cholesterol/bile salts diet for seven days caused a significant increase in mean hepatic TC levels (173% or 96%, respectively) in mice. Co-treatment with Sch B (50–200 mg kg^{-1}) resulted in a dose-dependent decrease in hepatic TC levels (3–50%), compared with those of hypercholesterolaemic mice receiving no drug treatment. Fenofibrate co-treatment significantly suppressed the average hepatic TC level (by 56% or 67% in the two models) in hypercholesterolaemic mice (Figure 1A and B).

Effects of Sch B treatment on hepatic triglyceride levels in hypercholesterolaemic mice

The cholesterol/bile salts treatment increased the average hepatic TG level only slightly. Sch B treatment did not show any detectable effect on mean hepatic TG level in cholesterol/bile salts-treated mice (Figure 2A). Feeding of a high fat/cholesterol/bile salts diet to mice significantly increased the mean hepatic TG level (by 68%). Sch B treatment decreased the hepatic TG level in hypercholesterolaemic mice, with an apparent biphasic dose response that showed a maximum extent of suppression (52%) at a Sch B dose of 100 mg kg^{-1} when compared with the mean hepatic TG level in hypercholesterolaemic mice receiving no drug treatment (Figure 2B). Fenofibrate co-treatment significantly lowered hepatic TG levels (by 33 or 44% in the two models) in hypercholesterolaemic mice.

Effect of Sch B treatment on the hepatic indices of hypercholesterolaemic mice

The hepatic index was increased (5–24%) in hypercholesterolaemic mice, compared with that of mice fed a normal diet. Sch B or fenofibrate treatment further increased the hepatic

Table 1 Effects of Sch B treatment on serum total cholesterol levels and triglyceride levels in hypercholesterolaemic mice

Group	Dose (mg kg^{-1}/day)	Serum total cholesterol levels (mmol L^{-1})	Serum triglyceride levels (mmol L^{-1})
Normal diet Cholesterol/bile salts (2.0 g/0.5 g kg^{-1} daily for 4 days)		3.55 ± 0.20	0.62 ± 0.06
Vehicle		6.04 ± 0.66*	0.28 ± 0.03*
Sch B	100 × 4	5.00 ± 0.26*	0.73 ± 0.05#
	200 × 4	6.41 ± 0.48*	0.76 ± 0.08#
Fenofibrate	100 × 4	3.45 ± 0.23#	0.27 ± 0.04*
Normal diet Seven day high fat/cholesterol/bile salts (10/1/0.3%, w/w) diet		3.62 ± 0.18	0.92 ± 0.08
Vehicle		9.42 ± 0.44*	0.58 ± 0.03*
Sch B	50 × 6	9.13 ± 0.66*	0.73 ± 0.07
	100 × 6	9.14 ± 0.45*	0.76 ± 0.06
	200 × 6	8.36 ± 0.45*	0.79 ± 0.09#
Fenofibrate	100 × 6	5.98 ± 0.46#,*	0.76 ± 0.05#

Experimental details are described in the legend to Figure 1. Twenty-four hours after the last dosing with Sch B or fenofibrate, serum total cholesterol and triglyceride levels were measured. Values given are means ± s.e.m., with n = 10. *$P < 0.05$ vs mice fed a normal diet; #$P < 0.05$ vs hypercholesterolaemic mice receiving no drug treatment.

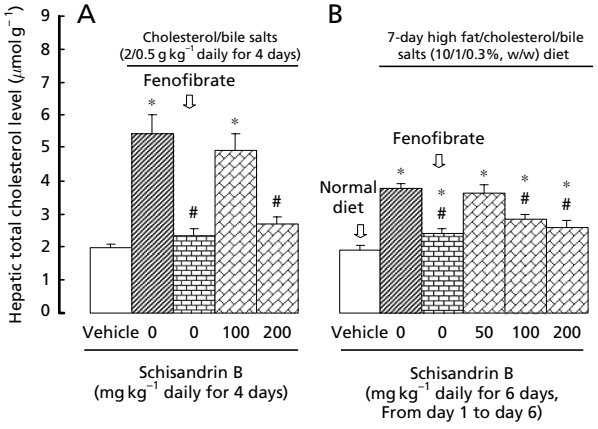

Figure 1 Effects of Sch B treatment on hepatic total cholesterol levels in hypercholesterolaemic mice. Mice were given cholesterol/bile salts (2/0.5 g kg^{-1}, i.g.) for four days, or fed a high fat/cholesterol/bile salts diet (10/1/0.3%, w/w) for seven days. Sch B (50–200 mg kg^{-1}, i.g.) or fenofibrate (100 mg kg^{-1}, i.g.) co-administration was performed for four or six days, respectively. Control (normal diet) animals and hypercholesterolaemic mice receiving no drug treatment were given vehicle only. Hepatic total cholesterol levels were measured 24 h after the last dosing with Sch B or fenofibrate. Values given are means ± s.e.m., with n = 10. *$P < 0.05$ vs mice fed with vehicle/normal diet; #$P < 0.05$ vs hypercholesterolaemic mice receiving no drug treatment.

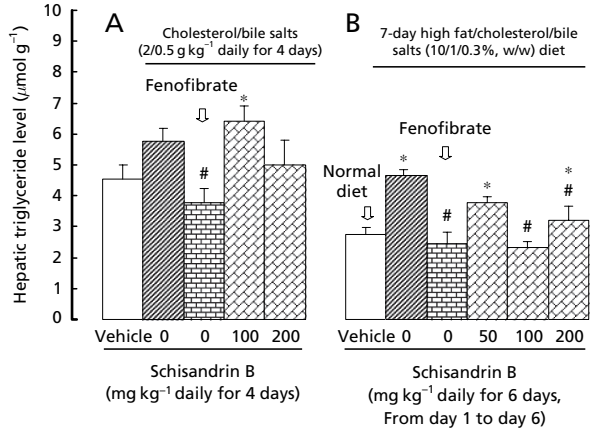

Figure 2 Effects of Sch B treatment on hepatic triglyceride levels in hypercholesterolaemic mice. Experimental details are described in the legend to Figure 1. Twenty-four hours after the last dosing with Sch B or fenofibrate, hepatic triglyceride levels were measured. Values are means ± s.e.m., with n = 10. *$P < 0.05$ vs mice fed with vehicle/normal diet; #$P < 0.05$ vs hypercholesterolaemic mice receiving no drug treatment.

index (by 14–84% or 46–84% in the two models), when compared with that of hypercholesterolaemic mice receiving no drug treatment (Figure 3A and B).

Discussion

The condition of fatty liver, which is recognized as a common cause of a number of liver disorders, has become an important area of experimental and clinical research. Although it is

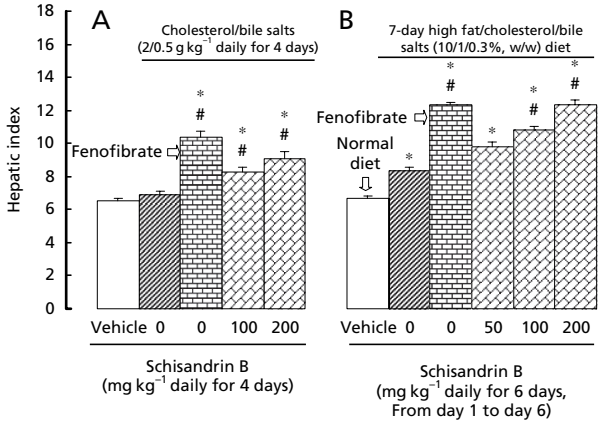

Figure 3 Effects of Sch B treatment on the hepatic indices in hypercholesterolaemic mice. Experimental details are described in the legend to Figure 1. Twenty-four hours after the last dosing with Sch B or fenofibrate, hepatic indices were estimated (the hepatic index is the ratio of whole liver weight to body weight). Values are means ± s.e.m., with n = 10. $^{*}P < 0.05$ vs mice fed with vehicle/normal diet; $^{\#}P < 0.05$ vs hypercholesterolaemic mice receiving no drug treatment.

known that fat accumulates in the liver under a number of conditions, the elevation of hepatic lipid levels is related to the excessive intake of fatty foods and/or cholesterol, as shown in this study. In addition, fatty liver is associated with a number of pathological conditions, such as chronic hepatitis, diabetes mellitus, and tuberculosis, as well as the chronic use of drugs, such as valproic acid, tamoxifen, and corticosteroids (Akyol et al 2005; Grieco et al 2005; Jian et al 2006).

In this study, to investigate the effect of Sch B treatment on fatty liver, two acute mouse models of fatty liver were established by use of a cholesterol/bile salts regimen or by feeding a diet high in fat, cholesterol and bile salts. The acute models of fatty liver adopted in this study are more suitable than a long-term fatty liver model for screening hepatic lipid-lowering drugs, as the long-term models involve the feeding of a high fat diet or fat milk for six weeks or three months (Deng et al 2007; Zhang et al 2007). The use of two acute models of hypercholesterolaemia, which showed differential changes in serum and hepatic lipid levels, allowed the investigation of Sch B treatment effects under varying experimental conditions. As only a small amount of ether was inhaled during brief anaesthesia, there should have been no ether effect on hepatic lipid metabolism in mice. In the experimental models of acute fatty liver used in this paper, the elevations of serum TC and hepatic lipid levels were associated with reductions in serum TG levels. The increases in serum TC and hepatic TG levels, as well as the hepatic weight increases, in mice fed the high fat/cholesterol/bile salts diet were found to be larger than in mice given cholesterol/bile salts orally. The extent of the decrease in the serum TG level, and the increase in the hepatic TC level in the cholesterol/bile salts model were found, however, to be larger than seen in the high fat/cholesterol/bile salts model. Reduction in serum TG levels in animals with experimentally-induced hypercholesterolaemia may have been related to alterations in hepatic TG metabolism upon high loading of dietary lipids.

Interestingly, the hypertriglyceridaemic effect produced by Sch B treatment, as reported by Pan et al (2006a), tended to restore the normal level of serum TG in hypercholesterolaemic mice. Unlike fenofibrate, which could decrease both hepatic cholesterol and serum cholesterol in hypercholesterolaemic mice, Sch B treatment did not affect the mean serum TC level. This finding suggested that the hepatic lipid-lowering action of Sch B may have been mediated by an as yet undefined biochemical mechanism distinct from that of fenofibrate. In this regard, Sch B has been shown to influence various aspects of liver function and to protect against toxin-induced hepatic injury (Ip et al 2001; Chiu et al 2003). Whether Sch B could produce any effect on hepatic lipid metabolism remains to be investigated. On the other hand, fenofibrate, a broad spectrum lipid-lowering drug, is clinically used in patients suffering from dyslipidaemias and alcoholic fatty livers (Tsimihodimos et al 2005; McKenney et al 2006). The ability of fenofibrate to reduce hepatic and serum lipid levels may be related to several biochemical actions including activation of lipoprotein lipase, reduction of apolipoprotein C-III production, and induction of enzymes catalysing β-oxidation of fatty acids in mitochondria (Packard 1998; Andersson et al 1999; Miura 2005).

The average liver weight was increased in mice fed the high fat/cholesterol/bile salts diet. Both Sch B and fenofibrate further enhanced the increase in liver weight of hypercholesterolaemic mice, with the effect of fenofibrate being more prominent. Although the mechanisms involved in the hepatotrophic actions of Sch B and fenofibrate remain to be determined, these may be related to the induction of xenobiotic-metabolizing and/or lipid-metabolizing enzymes (Mounie et al 1988; Frederiksen et al 2003). Sch B, which was found to cause a lesser degree of liver hypertrophy than did fenofibrate, might be more desirable than fenofibrate as a drug for fatty liver, even though fenofibrate was more potent than Sch B in lowering liver lipids, regardless of whether lipid levels or total lipid contents were considered. Acute and chronic toxicological studies have indicated that Sch B treatment did not cause any detectable systemic toxicity in mice (Ko & Mak 2004).

In conclusion, the results indicated that Sch B invariably decreased hepatic (but not serum) lipid levels in two acute mouse models of hypercholesterolaemia. In addition to beneficial effects on liver function and tissue protection against oxidative damage, Sch B may also be used as a specific drug for the treatment of fatty liver disorders.

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