The hepatic protective mechanism of Ginkgo biloba extract in rats with obstructive jaundice

Ming-Zhe Weng, Xian-Ping Zhou, Jian-Guang Jia, Jing Ding, Cui-Fu Fang, Yi-Yu Qin, Shao-Fu Tao, Long-Hua Rao, Ji-Yu Li, Zhi-Wei Quan*

Department of General Surgery, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road, 200092, Shanghai, China

ABSTRACT

The objective of our study was to examine the hepatic protective mechanism of Ginkgo biloba extract (GBE) in rats with obstructive jaundice (OJ). Twenty rats underwent bile duct ligation and received daily intraperitoneal injections of either control saline or Ginkgo biloba extract for 14 days. Ten sham-operated rats had their bile duct exposed but not ligated or sectioned. Serum alanine transaminase (ALT) was analyzed for liver function tests and liver damage was further assessed by histologic examination. The levels of endothelin 1 (ET-1) and nitric oxide (NO) in blood and liver homogenate were measured. The serum alanine transaminase was elevated in the bile duct ligation rats (BDL rats); GBE could significantly lower serum transaminase level and ameliorate liver histological damage. ET-1 and NO levels in both plasma and liver tissue were also elevated in common bile duct (CBD)-ligated rats, but this increase was significantly decreased by GBE treatment. Moreover, the degree of liver damage severity positively correlates with high levels of ET-1 and NO. GBE mediated the liver protective effect at least in part by suppressing overproduction of ET-1 and NO and restoring a proper balance between ET-1 and NO to some extent.

INTRODUCTION

Obstructive jaundice (OJ) is characterized by an interruption to the drainage of bile in the biliary system, resulting in intracellular accumulation of bilirubin, bile acids and cholesterol. The biliary obstruction causes cholestatic liver injury, and the absence of bile in the intestine facilitates bacterial translocation across the intestinal mucosa, which, in turn, may cause sepsis and further liver injury [1]. The main treatment for obstructive jaundice includes surgical removal of obstruction and endoprosthesis introduced either percutaneously or endoscopically. This mechanism of liver injury during obstructive jaundice is involved in chemical mediators such as superoxide, nitric oxide (NO), endothelin 1 (ET-1) and cytokines [2, 3, 4, 5]. Among these cytokines, ET-1 and NO have been considered to be the main effectors in liver injury [3, 4, 5]. Obviously, it is important to reduce the release of ET-1 and NO in the perioperative period in order to prevent or minimize the related complications.

Standardized Ginkgo biloba extract (GBE) derived from leaves and nuts of Ginkgo tree is a valuable therapeutic drug for a variety of disorders. A recent study showed that treatment significantly reduced pathological changes in liver and terminal ileum in obstructive jaundice [6]. However, currently the mechanism of action of GBE in obstructive jaundice is not clear. A number of studies provided evidence for a role of GBE in the regulation of production of NO and ET-1 [7, 8, 9, 10]. Since the ET-1 and NO appears to be more important in obstructive jaundice, we postulate that GBE can exert a protective effect on liver injury induced by obstructive jaundice through the regulation of production of NO and ET-1. Thus, the present study was undertaken to verify the above hypothesis and elucidate GBE protective mechanisms related to liver injury induced by obstructive jaundice. To our knowledge, this is the first study to report GBE liver protective mechanism in obstructive jaundice.

MATERIAL AND METHODS

Drugs and animal models

GBE injection was obtained from Taiwan Chi Sheng Chemical Corporation with dosage of 17.5 mg/ 5 ml. All animal experiments were conducted following guidelines set forth in the National Institutes of Health Guide for the Care
and Use of Laboratory Animals. Adult Wistar rats of either sex, weighing 330 to 370g were kept in a temperature-controlled environment, and were fed standard chow and water. Thirty Wistar rats were randomly divided into three groups, each involving 10 rats, and sex ratio in each group was the same (Male/Female 1:1). Sham-Operated group (SO group): The bile duct was mobilized but not ligated; Obstructive Jaundice group (OJ group): Ligation of the bile duct + injection of saline solution; Ginkgo biloba extract group (GBE group): Ligation of the bile duct + injection of Ginkgo biloba extract (50 mg/kg /day from the 1st to the 14th postoperative days). The rats were sacrificed by cervical dislocation 14 days later, and venous blood was collected and livers were harvested for hematoxylin/eosin staining.

Measurement of biochemical parameters
Plasma and tissue ET-1 concentrations were measured via radioimmunoassay (RIA; Phoenix Pharmaceuticals, Mountain View, CA). Plasma and tissue NO concentrations were measured via nitrate reductase assay (RIA; Phoenix Pharmaceuticals, Mountain View, CA). Serum ALT levels were assessed by L-glutamic acid colorimetric method using commercial kits (Nanjing Jiancheng Bio Company, Co09, Nanjing, China). Serum total bilirubin level was assayed caffeine/sodium assay. All experimental procedures were carried out according to the manufacturer’s instructions.

Liver histological examination
Serially harvested liver specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections were made at 5-mm and stained with hematoxylin and eosin (H&E).

Statistical analysis
All data were expressed as the mean ± SD. The differences among the groups were evaluated by using one-way analysis of variance followed by post-hoc test (Tukey’s HSD test). p values of less than 0.05 were considered as significant. The data were analyzed by statistical software (SPSS for Windows 10.0; SPSS, Chicago, Illinois, USA).

RESULTS
Sham group rats were agile, responsive to painful stimuli. There was no change in urine color in Sham group. All CBD-ligated rats were slightly jaundiced third days postoperative and profoundly jaundiced on the seventh day, and they had poor appetite, lost weight and listlessness. The rats in GBE group also demonstrated these symptoms above, such as poor appetite, lost weight and listlessness, etc. However, compared with the OJ group, they had less severe symptoms above. Although the Child-Pugh classification can be used clinically to assess patients’ general condition liver injury related, it has not been applied to animal studies. Therefore, the preliminary qualitative assessments of general condition of experimental animals were performed in our experiment. The levels of ALT and total bilirubin in the OJ groups and GBE group were significantly higher than that of the sham-operated group (Figure 1). But plasma ALT level in GBE group was found to be significantly reduced compared to OJ group (Figure 1 B), and there was no difference statistically significant in plasma total bilirubin level between OJ groups and GBE group (Figure 1 A). No histological damage was seen in the sham-operated group (Figure 2 A). Whereas marked destruction of the

FIGURE 1. The serum total bilirubin concentrations in 3 groups (A). The serum ALT concentrations in 3 groups (B). * p<0.01 vs SO group, Δ p<0.05 vs OJ group.
liver tissue with broad areas of necrosis was observed in OJ group (Figure 2 B). While liver injury was moderate in GBE group as compared to OJ group. Treatment with GBE clearly reduced this cholestatic liver damage (Figure 2 C). The plasma and tissue levels of ET-1 in OJ and GBE group increased compared with the Sham-Operated group, but its levels in the GBE group were significantly lower than in the OJ group (Figure 3 A). The plasma and tissue levels of NO were also measured. The levels of NO in blood and liver tissue in OJ group and GBE group increased significantly, compared with SO group, but the GBE group had significantly lower plasma and tissue NO levels than the OJ group (Figure 3 B). Together, as was shown in these data, ET-1 and NO concentrations in plasma and tissue were correlated to liver injury indicators (ALT, pathological index). The higher the concentration of ET-1 and NO, the more severe liver damage, which suggested that liver damage severity correlated positively with high level of ET-1 and NO in plasma and tissue. In addition, a significant change was observed in ET-1/NO in Plasma and liver tissue. In sham group, ET-1/NO in Plasma and liver tissue was 0.78±0.02 and 0.49±0.09 respectively, and the counterpart in OJ group was 1.13±0.11 and 0.73±0.04 respectively, while the counterpart in GBE group was 0.80±0.06 and 0.56±0.03 respectively. It suggested that a proper balance between ET-1 and NO was restored to some extent in the GBE group.

**DISCUSSION**

Hepatic injury is one of the main consequences of biliary obstruction, which markedly increases patient morbidity and mortality. Thus, novel treatment options are needed to reduce
The equilibrium between NO and ET-1 is essential in maintaining liver's blood supply. Biliary obstruction leads to excessive production of ET and NO [2, 3, 4, 5], which causes the destruction of this dynamic equilibrium and the disorders of hepatic hemodynamics, which further aggravates liver injury. Under physiological conditions, ET-1 and NO are mutually antagonistic in their effects on vascular tension. The equilibrium between NO and ET-1 is essential in maintaining liver's blood supply. Biliary obstruction leads to excessive production of ET and NO [2, 3, 4, 5], which causes the destruction of this dynamic equilibrium and the disorders of hepatic hemodynamics, which further aggravates liver injury.

Consistent with previous research, our data demonstrated that GBE treatment led to an increase of coronary artery blood flow through restoration of the equilibrium between NO and ET-1 [7]. Although the experimental model employed in the study is different from the present study, there is strong support for its role as restoration of the balance between ET-1 and NO in obstructive jaundice. In addition to antioxidation and antiinflammatory, GBE has other pharmacological effects including anti-apoptotic effects [18], modulation of immune response [19] and inhibition of platelet-activating factor [20]. Therefore, it was probable that additional hepatic protective mechanisms were also involved besides the abovementioned mechanism. This needs to be investigated in future studies.

GBE could act as free radical scavengers to capture oxygen-derived free radicals, such as NO, superoxide anion, hydroxyl and peroxyl radicals, and act as a donor of hydrogen atom to terminate pathological aggravation of free radical chain reaction and lipid peroxidation [9]. We have tested the scavenging ability of GBE toward NO in the experimental model of obstructive jaundice. Our data indicated that the levels of NO remarkably were reduced both in liver tissue and plasma, which provided evidence that the GBE may act as a good antioxidant in obstructive jaundice. Some data in the literature have indicated the extract and its ingredients could exhibit potent inhibitory effects on the expression of inducible nitric oxide synthase in hepatic fibrosis rats. Therefore the decrease in levels of NO in our experiment may in part due to the direct suppression of inducible nitric oxide synthase. In our experiment, GBE not only suppressed overproduction of ET-1 and NO induced by obstructive jaundice, but also restored a proper balance between ET-1 and NO to some extent, which sequently improved hepatic perfusion and attenuated the liver injury. A recent study showed that GBE treatment led to an increase of coronary artery blood flow through restoration of the equilibrium between NO and ET-1 [7]. Although the experimental model employed in the study is different from the present study, there is strong support for its role as restoration of the balance between ET-1 and NO in obstructive jaundice.
CONCLUSION

This study found that treatment with GBE reduced liver injury in the obstructive jaundice model, and GBE mediated the liver protective effect at least in part by suppressing overproduction of ET-1 and NO and restoring a proper balance between ET-1 and NO to some extent. Although surgical and endoscopic decompression is the principal treatment of biliary obstruction in clinical practice, treatment with GBE may be helpful to reduce cholestasis-induced liver damage and its related complications in perioperative period.

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DECLARATION OF INTEREST

None to declare

REFERENCES