Preventive effetc of Dai-saiko-tô (Da-Chai-Hu-Tang) extract on gallstone formation in mice fed a lithogenic diet

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Abstract

The preventive effect of an extract of Dai-saiko-tô (a traditional Chinese medicine) on gallstone formation was studied in mice fed a lithogenic diet containing 0.5% cholesterol and 0.25% sodium cholate (control diet). The Dai-saiko-tô extract was added to the control diet at a level of 0.5%. The incidences of gallstones were significantly lower in animals given the Dai-saiko-tô extract (15%) than in control mice (57%). The result demonstrates that the Dai-saiko-tô extract may prevent gallstone formation induced by a lithogenic diet.

Key words Dai-saiko-tô, gallstone formation
Abbreviation Dai-saiko-tô (Da-Chai-Hu-Tang), 大柴胡湯

Introduction

Kampohozai (a traditional Chinese medicine) is a drug therapy which has been developed from clinical experience accumulated over thousands of years in China. An extract of Dai-saiko-tô (Da-Chai-Hu-Tang) used in the experiment is one of the Kampohozai containing Bupleuri Radix as a main crude component. The Dai-saiko-tô extract is used clinically for therapy of arteriosclerosis, chronic hepatic diseases and cholelithiasis.[1] However, effects of Dai-saiko-tô extract on these diseases, particularly cholelithiasis, have not always been proven experimentally.

In the present study, we examined the preventive effect of a Dai-saiko-tô extract on gallstone formation induced by a lithogenic diet in mice.

Materials and Methods

Dai-saiko-tô extract : The Dai-saiko-tô extract used in this study was kindly supplied by Kanebo Yakuhin Co., Ltd., Tokyo, Japan. It was a mixture of eight crude drugs (Bupleuri Radix 6 g, Pinelliae Tuber 4 g, Scutellariae Radix 3 g, Zingiberis Rhizoma 1 g, Zizyphi Fructus 3 g, Aurantii Fructus Immaturus 2 g, Paeoniae Radix 3 g and Rhei Rhizoma 1 g). These weights indicate the dry weights of the crude drugs given per day to humans. This mixture of crude drugs was boiled for 60 min with water and the aqueous extract was evaporated and spray-dried.

Diet : The basal diet used was of the following composition : casein ; 20%, soy bean oil ; 5%, salt mixture[2] ; 4%, vitamin mixture[2] ; 1% and sucrose to make 100%. Lithogenic diet (control diet) was prepared by the additions of 0.5% (W/W) cholesterol and 0.25% (W/W) sodium cholate to the basal diet as described by Ebihara *et al*.[3] The test diet was prepared by addition of Dai-saiko-tô extract to the control diet at a level of 0.5% (W/W).

Animals : Female ICR mice aged 5 weeks from Charles River Breeding Laboratory (Kanagawa, Japan) were used. Animals were maintained on basal diet for one week and then 20 animals were randomly divided into two groups. They were kept 10 mice to a cage with free access to food and water in a room maintained at

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23±1℃ with a 12 hr light−12 hr dark cycle for 5 weeks. The experiments were repeated six times in the same way as above.

Incidences of gallstones : At the end of each experiment, animals were anesthetized with pentobarbital and then their abdominal cavity was opened. Gallstone formation was observed by naked eye. Data of gallstone incidence were pooled for six experiments. The extent of gallstone formations were determined as the following : + ; presence of crystal-like gallstones, ++ ; presence of particle-like gallstones, +++ ; presence of particle-like gallstones and turbid bile and ++++ ; presence of particle-like gallstones and strongly turbid bile.

Statistical analysis : Data were analyzed by G-test.[4]

Results and Discussion

The Dai-saiko-tô extract is a Kampohozai (traditional Chinese medicine) that is used clinically in treatment of cholelithiasis.[5] In this study, we examined the effect of oral administration of Dai-saiko-tô extract on gallstone formation in mice fed a lithogenic diet containing 0.5% cholesterol and 0.25% sodium cholate.

The growth and the food intake of mice given the control diet with or without Dai-saiko-tô extract were virtually similar in each trial. In both groups, however, some of the mice with gallstones decreased in body weight. Data on the incidence of gallstones are shown in Table I. The cholesterol gallstones were observed at high incidence, 34 of 60 mice (57%), in mice given control diet. The extent of gallstone formation was the grade ++ and grade +++ in most of control mice with gallstones. Furthermore, one of two mice with grade ++++ died during the experiment. In contrast, gallstone formation was observed at significantly lower incidence ($p<0.01$), 9 of 60 mice (15%), in mice given diet supplemented with Dai-saiko-tô extract as compared to the control group. Dai-saiko-tô extract also reduced the extent of gallstone formation in mice with gallstones. These findings are consistent with the results that Dai-saiko-tô tended to inhibit formation of gallstones in a few hamsters fed a lithogenic diet reported by Saitoh.[6] Dai-saiko-tô extract with Bupleuri Radix as a main crude component has been reported to contain saponins such as saikosaponins which increase the excretions of cholesterol and bile acid in the feces.[7,8] This kampohozai may prevent the formation of cholesterol gallstones by inhibiting cholesterol absorption. In bile, cholesterol is solubilized as mixed micelles of bile acids, phospholipids, *etc.* and the solubility of cholesterol in the bile depends on the relative proportions of these constituents. According to Redinger and Small,[9] gallstone formation is initiated when biliary cholesterol concentration increased in relation to the other biliary lipids. Improvement of the lithogenicity of bile with respect to the oral Dai-saiko-tô extract may be responsible for the preventive effect on the gall-

Table I Effect of Dai-saiko-tô extract on gallstone formation in mice fed a lithogenic diet.

Diet	Gallstone[a] incidence	Extent of gallstone formation[b]			
		+	++	+++	++++
Control diet (C)	34/60 (57%)	3	18	11	2
C+0.5% Dai-saiko-tô extract	9/60 (15%)[c]	1	6	2	0

a : Number of mice with gallstones/total mice in the group. The data was pooled for six experiments.
b : The extent of gallstone formation is determined as the following : + ; presence of crystal-like gallstones, ++ ; presence of particle-like gallstones, +++ ; presence of particle-like gallstones and turbid bile and ++++ ; presence of particle-like gallstones and strongly turbid bile.
c : Result is significantly different at $p<0.01$ when compared with the control group.

stone formation.

The present study demonstrated that the oral administration of Dai-saiko-tô extract prevented the cholesterol gallstone formation induced by a lithogenic diet in mice. The mechanisms responsible for the preventive effect of Dai-saiko-tô extract are investigated in progress.

和文抄録

胆石形成における大柴胡湯エキスの予防効果を0.5% コレステロール及び0.25% コール酸ナトリウムを含む胆石形成飼料を与えたマウスで検討した。大柴胡湯エキスを0.5% 濃度で飼料中に添加した。胆石発生率は対照群（57%）よりも大柴胡湯を与えた群（15%）で低かった。この結果は，大柴胡湯エキスが胆石形成飼料によって誘導される胆石形成を予防することを実証している。

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