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研究課題名（和文）原発性胆汁性胆管炎および原発性硬化性胆管炎に対するハスカップ果実抽出物の改善効果

研究課題名（英文）Improving effects of Haskap fruit extracts on primary biliary cholangitis and primary sclerosing cholangitis

研究代表者
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研究成果の概要（和文）：ハスカップ果実抽出物の原発性胆汁性胆管炎モデルマウスに対する効果を調べる研究では、中用量および高用量のハスカップ果実抽出物の投与により、肝細胞障害や胆汁うっ滞の血清学的マーカー、および胆管炎や線維化の組織病理学所見が改善する傾向が見られた。肝組織におけるT細胞浸潤は、中用量ハスカップ投与により有意に抑制された。
ハスカップ果実抽出物の原発性硬化性胆管炎モデルマウスに対する効果を調べる研究では、血清ALT値は低用量のハスカップ果実抽出物の投与により、ALP値は高用量ハスカップ果実抽出物の投与により有意に改善した。総ビリルビン値は、高用量投与群が低用量投与群よりも有意に低値であった。

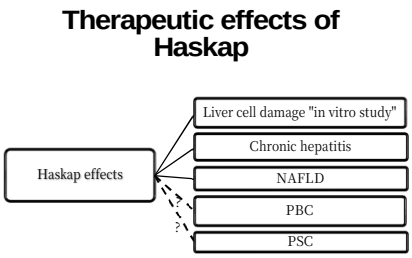
研究成果の学術的意義や社会的意義
The Haskap fruit extract improves PBC and PSC in model mice through reducing inflammation, bile duct damage, or fibrosis. Thus, Based on above findings we believe that this our preliminary achievement will lead to the development of new therapeutic agents for PBC and PSC.

研究成果の概要（英文）：In the first study, investigating the effects of haskap fruit extract on a mouse model of primary biliary cholangitis, showed improved serological markers of hepatocellular injury, cholestasis, cholangitis. and histopathological findings of fibrosis in the medium- and high-dose haskap fruit extracts administrating groups. Total T-lymphocyte infiltration in liver tissue was significantly suppressed in the medium-dose haskap administration group.
In the second study, investigating the effects of haskap fruit extract on primary sclerosing cholangitis model mice, the serum levels of ALT were significantly improved by administration of low-dose haskap fruit extract, and ALP levels were significantly improved by administration of high-dose haskap fruit extract. Total bilirubin levels were significantly lower in the high-dose group than in the low-dose haskap group.

研究分野：59040

キーワード：PSC　PBC　Haskap　Fruit extract　animal model　Low dose　Medium dose　High dose

１．研究開始当初の背景



PBC and PSC are the most common chronic cholestatic diseases in adults. To date a variety of therapeutic agents with different mechanisms of action have been evaluated in the treatment of those diseases, none of which shown convincing benefit yet. Haskap fruit contains anti-inflammatory and antioxidant properties and has been shown a potential effect against some liver diseases, however its effects on PBC and PSC are remains unknown.　. Haskap fruit extract suppresses hepatocellular injury in rats in vitro (J Agric Food Chem 2009; 57: 6584-9), and chronic hepatitis induced by lipopolysaccharide in rats (Food Funct 2016; 7: 4267-77).) and nonalcoholic fatty liver disease (NAFLD) in mice (Int J Mol Med, in press). In this study, we hypothesized the Haskap fruit extract improves PBC and PSC in model mice through improving inflammation, bile duct damage, and portal fibrosis.

２．研究の目的

The purpose of this research is to investigate the ameliorating effects of haskap fruit extract on PBC and PSC model animals, and to explore the possibility of developing new therapeutic agents. PBC is a common chronic liver disease that slowly progresses to cirrhosis and has a poor prognosis in advanced cases. UDCA, the current first-line drug for PBC, has limitations, and there is an urgent need to expand drug treatment options for refractory cases. PSC is also a chronic cholestatic disease leading to liver cirrhosis, and drug treatment has not been established, and there are many cases of death or liver transplantation 5 to 17 years after diagnosis. Therefore, establishing a drug therapy for PSC is an urgent task.
Although the effects of haskap fruit on chronic liver disease have been historically well known however, there is no direct evidence yet. As mentioned above, the effects of Haskap fruit on several liver diseases and liver disorders have recently been scientifically proven, however its ameliorating effects on PBC and PSC are remains unknown.

３．研究の方法

Studies investigating the effect of Haskap fruit extract on PBC model mice

Twenty-four 8-week-old female Cyp2c70/Cyp2a12 double knockout mice injected 2-octynoic acid coupled to bovine serum albumin (2-OA-BSA) intraperitoneally and divided them into4 groups: -Control group: Normal diet -Low-dose group: Normal diet supplemented with 0.15% Haskap fruit extract -Medium-dose group: Normal diet supplemented with 0.75% Haskap fruit extract -High-dose group: Normal diet supplemented with 1.5% Haskap fruit extract. After 8 weeks treatment, mice were sacrificed and serum biochemical and histopathological analysis were performed.

Serum ALP, γ-GTP, total bilirubin, AST, ALT and anti-mitochondrial antibody titers are measured by Oriental Yeast Co., Ltd. In addition, the liver tissue is taken are fixed in formalin to prepare HE specimens. HE specimens are observed microscopically to semi-quantitatively evaluate the degree of inflammatory cell infiltration in the portal region, the degree of interlobular bile duct damage/destruction, the number of granulomas, and the degree of fibrosis. The degree of fibrosis is objectively evaluated by image analysis using a Sirius red-stained specimen. In addition, immunostaining for CD3, CD4, CD8, CD20, CD56, and CD68 is performed to identify the types of infiltrating inflammatory cells.

2. Studies investigating the effect of Haskap fruit extract on PSC model mice

Thirty 6-week-old male PSC model mice (Abcb4-/- mice) were divided into the following 5 groups (6 mice in each group);
1) Control group: Control diet (CRF-1) ad libitum
2) UDCA group: 0.5% UDCA-added CRF-1 to be fed ad libitum
3) Low dose group: 0.15% Haskap fruit extract-added CRF-1 was given ad libitum.
4) Mid-dose group: 0.75% CRF-1 with honeysuckle fruit extract added ad libitum
5) High dose group: 1.5% Haskap fruit extract-added CRF-1 was given ad libitum.
UDCA is purchased from Wako Pure Chemical Industries. Haskap fruit extract is provided by a Mongolian research team member. We asked Oriental Yeast Industry for the preparation of special feeds added the fruit extract. Abcb4−/− mice were purchased from the Jackson Laboratory and bred at the International University of Health and Welfare Medical School Animal Facility. All mice were sacrificed when they reach 15 weeks of age. Blood is collected at the time of slaughter, and serum ALP, γ-GTP, total bilirubin, AST, and ALT levels are measured by Oriental Yeast Co., Ltd.

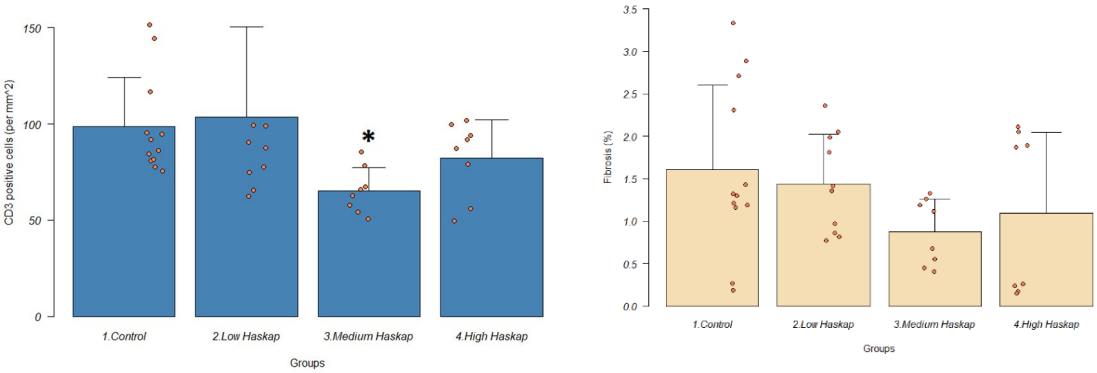
RNA will be extracted from the remaining liver tissue, and inflammatory cytokines tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-12p40, IL-12p70, and IL are willing to be detected by real-time RT-PCR. We will also measure the damage marker (4-hydroxynonenal: 4-HNE) caused by active oxygen in the liver.

4．研究成果

PBC experiment: Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) tended to be lower in the medium and high haskap groups than in the control group. Serum bilirubin levels were abnormally low in the present animal model, and they approached to the normal values in the low haskap group.

On histopathological analysis using by Nakanuma's classification, cholangitis activity, fibrosis score, and PBC stage tended to be lower in the medium haskap group than in the control group. Cholangitis activity of the high haskap group tended to be lower than that of the control group (Table 1, Fig. 1).

On quantitative image analysis, the number of CD3-positive cells per mm^2 (reflecting the extent of T cell infiltration) was smaller in the medium and high haskap groups than in the control group, and significant difference was observed between the medium haskap groupand the control group (Fig. 2A). The frequency of Sirius red-positive fibrotic area tended to be lower in the medium and high haskap groups than in the control group (Fig. 2B).



With regard to PSC-study, we found the serum AST levels were tended to be lower whereas, the serum ALT levels were significantly lower in the low haskap group than in the control group. ALP and T-Bil levels were significantly lower in the high haskap group than in the control and low haskap groups respectively. In the future, we planned to perform the second main experiment with histopathological and molecular analysis extensively.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　1件）

１．著者名 Dungubat E, Watabe S, Togashi-Kumagai A, Watanabe M, Kobayashi Y, Harada N, Yamaji R, Fukusato T, Lodon G, Sevjid B, Takahashi Y	４．巻 12
２．論文標題 Effects of caffeine and chlorogenic acid on nonalcoholic steatohepatitis in mice induced by choline-deficient, L-amino acid-defined, high-fat diet	５．発行年 2020年
３．雑誌名 Nutrients	６．最初と最後の頁 3886
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/nu12123886	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Takahashi Y, Watabe S, Togashi-Kumagai A, Watanabe M, Dungubat E, Kusano H, Kobayashi Y, Harada N, Yamaji R, Sugimoto K, Fukusato T	４．巻 87
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オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

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５．総ページ数
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１．著者名
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〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関