様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

# 科学研究費助成事業　　研究成果報告書

令和　５ 年　 ６ 月 ２６ 日現在

機関番号：１１３０１
研究種目：若手研究
研究期間：2021～2022
課題番号：２１Ｋ１４８１３

研究課題名（和文）核内受容体を介したビタミンKの胆汁酸代謝への影響

研究課題名（英文）Effect of vitamin K on bile acid metabolism via nuclear receptors

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,500,000円

研究成果の概要（和文）：今回の研究の目的は、ビタミンKがヒトPXRを介して胆汁酸（BA）代謝に及ぼすメカニズムを明らかにすることであった。その結果、コール酸（CA）を1週間または2週間投与すると、hPXR雌マウスの肝臓に障害が起こることを発見した。ALP値は有意に増加しなかったが、ALT、LAP、AST、総ビリルビン、BA値はCA処理により血清中で有意に増加した。さらに、MK-4処理は、これらのマウスのALT、AST、LAP、および総BAの血清レベルを低下させる傾向がある。これらのCA処理マウスにおいて、BA関連遺伝子の発現に対するMK-4処理の有意な効果は見いだせなかった。

研究成果の学術的意義や社会的意義
It was found that VK defficiency is commonly observed in cholestatic liver disease. However,the mechanism of the effect of VK on cholestasis-related liver disease is not revealed yet. This research could reveal the validation of VK treatment on cholestatic liver disease.

研究成果の概要（英文）：The purpose of the current study was to reveal the mechanism of Vitamin K on bile acids (BA) metabolism via human PXR. I found that cholic acid (CA) administration for 1 or 2 weeks causes damage to the liver of hPXR female mice. Though ALP level was increased insignificantly, ALT, LAP, AST, total bilirubin, and BA levels were increased significantly in the serum due to CA treatment. Moreover, MK-4 treatment tends to reduce the serum level of ALT, AST, LAP, and total BA in these mice. I found no significant effect of MK-4 treatment on the expression of BA-related genes in these CA-treated mice.

研究分野：Vitamin K, PXR

キーワード：Vitamin K　Bile acid metabolism　PXR

１．研究開始当初の背景

One of the fat-soluble vitamins, vitamin K (VK), acts as a cofactor of gamma-glutamyl carboxylase and plays an important role in blood clotting and bone metabolism. There are 2 types of VK (VK1 and VK2) in nature, VK1 (phyloquinone) is synthesized in green plants, and VK2 (menaquinone) is produced mainly by microorganisms.VK2 has isoprene side chains of varying lengths (from 1 to 15), while menaquinone-4 (MK-4), which has a geranyl group (4 isoprene units), is the most detected VK2 in animal tissues, including humans [Shirakawa, 2014]. MK-4 in this tissue is known to be formed by the side chain dislocations of VK in the diet after absorption and the introduction of geranyl-geranyl groups derived from the mevalonic acid pathway. MK-4, like other VKS, has a unique role as a cofactor of carboxylase, and has unique activities such as inhibition of osteoclast differentiation and apoptosis induction, and inhibition of osteoblast apoptosis. It is used as a preventive and therapeutic agent for osteoporosis in Japan and other Asian countries. Furthermore, it was shown that MK-4 acts as a ligand for the nuclear receptor PXR (pregnane X receptor) and regulates the expression of several genes at the transcriptional level [Tabb, 2003].

PXR is one of 48 nuclear receptor superfamily in humans and is activated by various fat-soluble substances such as pharmaceuticals, food ingredients, and foreign bodies, and is involved in the expression control of many metabolism-related proteins including drug metabolic enzymes [Watkins, 2003; Zhou, 2009]. PXR has different ligand specificity depending on the species. For example, rifampicin, an anti-tuberculous drug, strongly activates human PXR, but does not activate mouse or rat PXR. On the other hand, the synthetic steroid pregnane 16α-carbonitrile functions as a ligand for rodent PXR, but does not activate human PXR [Jones, 2000]. These differences in ligand specificity have led to the inability to accurately assess PXR-mediated responses in rodent-based drug efficacy and toxicity tests. To overcome this problem, genetically modified animals have been created in which human PXR is introduced [Igarashi, 2012]. The authors report that MK-4 is administered to human PXR mice and changes are observed in the expression of PXR-dependent genes, but not in wild-type mice [Sultana, 2018]. This suggests that extensive preclinical studies on the effects of VK in rodents have shown that PXR-mediated pathways have not been accurately evaluated, and that the effects of VK should be re-evaluated using humanized PXR animals. In addition, I found that MK-4 controls the expression level of bile acid (BA) synthase Cyp7a1 and Cyp8b1 mRNA in human PXR mice [Sultana, 2018].

BA are physiological surfactants necessary for the absorption of lipids in the diet, such as fat-soluble vitamins [Chiang, 2009]. BA, secreted into the intestinal tract, are metabolized by intestinal bacteria into secondary BA such as deoxycholic acid and litocholic acid. The mRNA expression of BA synthesis and related transporters are regulated by a variety of transcription factors, including PXR [Jung, 2006]. Excessive synthesis of BA and hyposecretion into the intestinal tract cause liver damage due to

their toxicity. Cholestasis is a disorder of the secretion of bile, which eventually causes liver fibrosis, cirrhosis, and even liver cancer. In cholestasis model mice with bile duct ligation, PXR deficiency cause more severe liver damage than wild-type mice [Stedman, 2005]. In addition, the administration of PXR ligand reduces bilirubin and BA in the blood by increasing the expression of BA metabolic enzymes, and reduces liver damage [Wagner, 2005]. In addition, PXR has been reported to have anti-fibrillation and anti-inflammatory effects in addition to anti-cholestasis.

As mentioned earlier, MK-4 may act as a ligand for human PXR, negatively regulating BA synthesis and protecting against cholestasis. Furthermore, when human PXR mice were fed a VK-deficient diet for 4 weeks, BA content in the liver increased, and it was estimated that the effect was due to changes in the expression of BA transporters by PXR. These results suggest that VK converts into MK-4 in the tissues, activates PXR, and is involved in the homeostasis of BA metabolism, and that high doses of MK-4 may lead to the development of new drugs for cholestasis. By clarifying the role of MK-4 mediated PXR activation in cholestasis, it is possible to show a new role of VK.

２．研究の目的

As mentioned earlier, PXR has a significant difference in ligand specificity by species, and previous studies on the action of VK in rats and mice have not accurately evaluated the pathway through PXR. By using human PXR mice, this study is an unprecedented study that clarifies the action of VK and its mechanism of action, which was not revealed in previous studies. This study had the following specific goals.

**(1)Investigation of the effect of menaquinone-4 administration on cholestasis liver injury model**

**(2)Investigation of the effect of VK deficiency on cholestasis liver injury model**

**(3)Investigation of the effects of intestinal bacteria on cholestasis during VK deficiency (*experiment was not done*)**

We planned to create human type PXR mice without enterobacteria to reveal the involvement of enterobacteria in bile accumulation in the liver due to a VK-deficient diet.

**(4) Investigation of the effect of menaquinone-4 on bile acid metabolism using human liver-derived and gastrointestinal-derived cells (*experiment was not done*)**

Using human PXR-positive cells, we planned to analyze the effect of menaquinone-4 on the expression of bile acid synthesis and transport-related proteins.

３．研究の方法

**(1)Effect of VK administration on cholestasis hepatic disorder model**

Human PXR mice and wild-type mice were fed a 1% cholic acid diet with different VK content and kept for 4 weeks. After feeding, the amount of VK in the liver is measured by fluorescent HPLC method, and the amount of bile acid in the liver is measured by LC-MS/MS method. In addition, liver damage markers (plasma AST, ALT activity,

bilirubin), and expression levels of inflammatory cytokines in the liver were performed. Furthermore, the mechanism of bile acid accumulation is analyzed by quantitative RT-PCR analysis of genes related to bile acid synthesis and transporters in the liver and ilium.

**(2)Effect of VK deficiency on humanized PXR mice and wild type mice**

Human PXR mice and wild-type mice were fed a VK-deficient diet for 4 weeks. The effect of VK deficiency was analyzed in humanized PXR mice and wild type mice by analyzing the expression of genes related to bile acids synthesis and transporters in liver and intestine using RT-PCR.

４．研究成果

In the first year of the project, I have found that cholic acid treatment for both 1 and 2 weeks causes damage to the liver of hPXR female mice. Though ALP level was increased insignificantly, ALT, LAP, AST, total bilirubin, and BA level were increased significantly in the serum due to cholic acid treatment. Furthermore, I have found that administration of menaquinone-4 (type 2 VK) tends to reduce the serum level of ALT, AST, LAP, and total BA in these mice. I analyzed the expression of genes related to bile acid synthesis and transporters in the liver and ilium. Unfortunately, I have found no significant effect of menaquinone -4 treatment on the expression of genes related to BA in these CA treated mice.

In this second year of the project, I had a plan to focus on finding the effect of VK deficiency on hPXR mice compared to the WT mice by analyzing BA metabolism-related proteins and transporters. Furthermore, I had a plan to analyze the effects of menaquinone-4 on mRNA and proteins related to BA synthesis and transport in human liver-derived cells (HepaRG) and colon-derived cells (Caco-2).

However, though I found that VK deficiency changed several genes related to the BA metabolism, I could not continue this project research since I moved to another laboratory in Tohoku University.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　0件／うちオープンアクセス　1件）

| １．著者名<br>Halima Sultana, Michio Komai, and Hitoshi Shirakawa | ４．巻<br>13(8) |
|---|---|
| ２．論文標題<br>The Role of Vitamin K in Cholestatic Liver Disease | ５．発行年<br>2021年 |
| ３．雑誌名<br>Nutrients | ６．最初と最後の頁<br>2515 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.3390/nu13082515 | 査読の有無<br>有 |
| オープンアクセス<br>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<br>－ |

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

| | 氏名<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（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 |
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