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α-Tocopherol and lipid profiles in plasma and the expression of α-tocopherol-related molecules in the liver of *Opisthorchis viverrini*-infected hamsters

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ABSTRACT

Opisthorchis viverrini infection induces inflammation-mediated oxidative stress and liver injury, which may alter α-tocopherol and lipid metabolism. We investigated plasma α-tocopherol and lipid profiles in hamsters infected with *O. viverrini*. Levels of α-tocopherol, cholesterol, and low-density lipoprotein increased in the acute phase of infection. In the chronic phase, α-tocopherol decreased, while triglyceride and very low-density lipoprotein increased. Notably, high-density lipoprotein decreased both in the acute and chronic phases. In the liver, cholesteryl oleate, triolein, and oleic acid decreased in the acute phase, and increased in the chronic phase. Such chronological changes were negatively correlated with the plasma α-tocopherol level. The expression of α-tocopherol-related molecules, ATP-binding cassette transporter A1 (ABCA1) and α-tocopherol transfer protein, increased throughout the experiment. These results suggest that *O. viverrini* infection profoundly affects on lipid and α-tocopherol metabolism in due course of infection.
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1. Introduction

Opisthorchis viverrini is a trematode parasite causing opisthorchiasis in humans and other mammalian hosts. *O. viverrini* infection is endemic in the Southeast Asia including Thailand, Lao PDR, Cambodia, and Vietnam [1]. In northeastern Thailand, the prevalence of *O. viverrini* infection is the highest in the world and closely associated with the high incidence of cholangiocarcinoma (CCA) [1]. The severity of the disease is determined primarily by worm burden, re-infection, and the duration of infection [1]. Infection induces inflammation, resulting in free radical-mediated oxidative and nitrative stress to cause liver/bile duct injury during the acute phase [2]. In the chronic phase, progressive periductal fibrosis is the most prominent histopathological finding [1,3,4].

Under normal circumstances, the liver, fat tissues and intestinal epithelial cells play a crucial role in lipid, lipoprotein, and bile acid metabolism. In general, infection and inflammation are known to alter lipid and lipoprotein patterns in plasma [5]. Conversely, alterations in lipid metabolism are shown to mediate inflammation, fibrosis, and proliferation in a mouse model of chronic cholestatic liver injury [6]. *Schistosoma mansoni* infection affects the lipid metabolism of the host, which contributes to the progression of the disease [7]. Moreover, decrease of vitamin E, a chain-breaking antioxidant, causes a variety of oxidative stress-related diseases in humans [8]. Vitamin E metabolism is regulated by the α-tocopherol transfer protein (α-TTP) [9] and ATP-binding cassette transporter A1 (ABCA1) [10] in the liver. We have previously reported that *O. viverrini* infection induces inflammation-mediated oxidative/nitrative stress in hamsters [2]. However, it remains unclear whether *O. viverini* infection induces inflammation-mediated alteration in lipid and lipoprotein metabolism. In this study, alteration of plasma α-tocopherol and lipid profiles in relation to disease progression was investigated in *O. viverrini*-infected hamster model. Alteration of α-tocopherol and the systemic changes of the lipid metabolism may provide a new clue for better understanding of clinical features and prognosis of opisthorchiasis patients.

2. Materials and methods

2.1. Parasites and animals

Methods for collection of *O. viverrini* metacercariae were described previously [2]. Cyprinid fish obtained from the endemic area of Khon Kaen province, Thailand, were digested by artificial gastric juice (0.25% pepsin–HCl, Wako, Japan). *O. viverrini* metacercariae

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were collected under a dissecting microscope and viable cysts were used for infection in hamsters (50 metacercariae per animal). Sixty male Syrian golden hamsters (*Mesocricetus auratus*) of 4–6 week-old were obtained from the Animal Unit, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. They were housed under conventional conditions and fed a stock diet and given water *ad libitum*. They were divided into 30 normal and 30 *O. viverrini*-infected groups. Infected animals (5 animals per designated time) were sacrificed on 21 days, 1, 3, 4, 5 and 6 month(s) post-infection. All hamsters were received well care in accordance with the guideline of the Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand, and this experiment was approved by the Committee (AEKKU14/2553).

2.2. Determination of α-tocopherol

α-Tocopherol in plasma was measured by a high-performance liquid chromatography (HPLC) system. Sixty μL of plasma was placed into a tube and vortexed together with 240 μL of absolute ethanol containing 0.1% butylated hydroxytoluene and 10 μg/mL tocopherol acetate (an internal standard). The sample solution was then mixed with 3 mL of *n*-heptane and vigorously shaken for 2 min. After centrifugation at 3000 rpm for 15 min, the supernatant was taken up and evaporated under nitrogen gas. The residue was dissolved in 100 μL of mobile phase (methanol: acetonitrile: methylene chloride = 200:200:50 v/v/v), and was injected into an HPLC system. α-Tocopherol was quantified by UV detection at the wavelength of 292 nm.

2.3. Plasma lipid profiles

Total cholesterol, triglyceride, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) in plasma were determined using an automated analyzer (Hitachi 912, Japan).

2.4. Thin layer chromatography

Neutral lipids were extracted as described previously [7], with some modifications. Briefly, 50 mg of liver tissues was homogenized in 0.5 mL of chloroform:methanol (2:1). The supernatant was collected after centrifugation at 13,000 rpm for 6 min and the chloroform–methanol extraction was repeated once. One mL of the supernatant was added to 0.23 mL of 0.88% (w/v) KCl in water. After mixing by vortex, the lipid-containing layer was removed and stored at −20 °C. Twenty μL aliquots of each liver extract and the neutral lipid standards (Sigma, USA) were run in parallel in the same gel on a 10 cm × 20 cm silica TLC plate. The plate was developed vertically with a petroleum ether:diethyl ether:acetic acid (80:20:1) mobile phase in a chromatography tank and was then developed in an iodine vapor atmosphere. The relative intensity of each spot was analyzed with the computer-assisted imaging 150 densitometer system (Scion image, Scion Corporation, USA).

2.5. Quantitative real-time reverse transcription–polymerase chain reaction (qRT-PCR)

Total RNA was extracted from each liver sample (150 mg) with TRIzol® reagent (Invitrogen, USA). An aliquot of total RNA was reverse transcribed into cDNA using reverse transcriptase (Fermentas, Germany) following the manufacturer's protocol. PCR was carried out in duplicate using Faststart Universal SYBR Green Master (ROX, Roche Applied Science, Germany) and performed in the Applied Biosystems 7500 thermal cycler (Applied Biosystems, USA). The primers for ABCA1 were: (5′TACTTGCAGGATGTGGTGGA3′ and 5′AGTCATGAAGGGCATTG3′), and for α-TTP were: (5′CCAAGAAGATTGCTGCTG3′ and 5′ATGTCTGGGAAG TGCTGA3′). All data were analyzed using the Rotor Gene 5 software (Corbett Research, Australia) with a cycle threshold (Ct) in the linear range of amplification and then processed by the $2^{-\Delta\Delta Ct}$ method. Relative mRNA expression was calibrated using the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene as the standard and the results were expressed as the fold change over the normal control.

2.6. Western blotting

Twenty μg of liver protein was separated by SDS-PAGE and the resolved proteins were transferred to a polyvinylidene difluoride membrane. The membranes were incubated with the primary antibody [rabbit polyclonal anti-ABCA1 (1:1000; Santa Cruz Biotechnology, USA), rabbit polyclonal anti-α-TTP (1:1000; GeneTex, USA), or mouse monoclonal anti-actin antibody (1:2000; Abcam, USA)]. After washing, a 1:2000 dilution of the secondary antibody [horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG antibody (GE Healthcare, Bio-Sciences Corp., USA)] was applied to the membrane. The immunoreactive signal was visualized using the enhanced chemiluminescence detection kit (GE Healthcare, Bio-Sciences Corp.) and the relative band intensity was analyzed with the computer-assisted imaging 150 densitometer system (Scion image).

2.7. Statistical analysis

Data were presented as the mean ± SD. Student's *t*-test was used to compare between infected group and normal control. Pearson's correlation coefficient was used for parametric data. Statistical analyses were performed using SPSS version 11.5. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. α-Tocopherol, lipid and lipoprotein profiles in plasma of O. viverrini-infected hamsters

Fig. 1 shows the chronological changes of α-tocopherol and lipid in the plasma of *O. viverrini*-infected and control hamsters. α-Tocopherol and cholesterol levels of *O. viverrini*-infected hamsters were significantly higher than those in normal hamsters at the acute phase of infection (21 days–1 month) ($P<0.05$). In the chronic phase at 3 to 6 months, α-tocopherol level was significantly decreased ($P<0.05$). The triglyceride and the ratio of triglyceride/cholesterol levels were increased at 5 months post-infection.

Fig. 2 shows the changes of lipoprotein (VLDL, LDL and HDL) in the plasma of *O. viverrini*-infected hamsters. *O. viverrini* infection caused significant increase in the LDL level compared to normal hamsters in the acute phase (1 month). In the chronic phase, a significant increase in the VLDL levels was observed at 5 months post-infection. Notably, the level of HDL and the ratios of HDL/LDL and HDL/cholesterol of infected animals were significantly lower than that of the control group in all time points.

3.2. Alteration of neutral lipids in the liver of O. viverrini-infected hamsters

To examine whether *O. viverrini* infection affects on liver lipids, we examined neutral lipids, cholesteryl oleate, triolein, oleic acid, and cholesterol, in the livers of *O. viverrini*-infected and normal hamsters. Thin layer chromatograph revealed significant reduction of cholesteryl oleate, triolein, and oleic acid in the liver of infected hamsters in the acute phase (21 days–1 month) but these levels increased compared to the normal group ($P<0.05$) in the chronic phase (3–6 months) (Fig. 3a–d).

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