Andrographolide Ameliorates Beta-Naphthoflavone-Induced CYP1A Enzyme Activity and Lipid Peroxidation in Hamsters with Acute Opisthorchiasis

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Background: *Opisthorchis viverrini (OV) infection generates oxidative stress/free radicals and is considered as a primary cause of cholangiocarcinoma since it primarily triggers sclerosing cholangitis.*
Objective: *In this study, the impacts of andrographolide on acute opisthorchaisis in β-naphthoflavone (BNF)-exposed hamsters were investigated.*
Material and Method: *Ethoxyresorufin O-deethylase (EROD) and methoxyresorufin O-demethylase (MROD) activities and Thiobarbituric acid reaction substances (TBARS) assay of andrographolide in acute opisthorchiasis in the BNF-exposed hamsters were assessed.*
Results: *The results showed that andrographolide ameliorated the hepatic CYP1A1 and CYP1A2 activities by decreases of the specific enzymatic reactions of EROD and MROD, respectively, in the BNF-exposed hamsters. Moreover, andrographolide lowered the formation of malondialdehyde in the livers and brains of the hamsters.*
Conclusion: *These observations revealed the promising chemo-protective and antioxidant activities of andrographolide via suppression of the specific EROD and MROD reactions and lipid peroxidation against acute opisthorchiasis in the BNF-exposed hamsters.*

Keywords: *Opisthorchiasis, Andrographolide, β-naphthoflavone, CYP1A, Lipid peroxidation, Hamster*

J Med Assoc Thai 2016; 99 (Suppl. 1): S22-S27
Full text. e-Journal: *http://www.jmatonline.com*

Infection of liver fluke or *Opisthorchis viverrini* (OV) is a major health problem in tropical countries of Southeast Asia[1]. The excystedmetacercariae then develop into juvenile flukes within the biliary tree of the liver, leading to acute cholangitis and develop into cholangiocarcinoma[2]. Besides the OV infection, cytochrome P450 1A (CYP1A) inducers such as the synthetic flavone, β-naphthoflavone (BNF), are known to induce oxidative stress and have a liver tumor-promoting effect in rats[3]. The oxidative stress is normally generated by microsomal electron systems including CYPs and nicotinamide adenine dinucleotide phosphate (NADPH)-CYP reductase, leading to the production of reactive oxygen species (ROS) through metabolism processes, followed by a subsequent formation of oxygenated substrates and water[4]. CYP1A is the major enzyme involved in metabolic activation of a large number of procarcinogens to form reactive intermediates that can interact with cellular nucleophiles and ultimately trigger carcinogenesis[5]. The activities of CYP1A1 and CYP1A2 are associated with progression of various cancers[6].

Andrographolide is the major diterpenoid constituent of *Andrographispaniculata*, a traditional medicine claimed for various pharmacological benefits[7], i.e., hepatoprotective, anti-oxidative, anti-inflammatory, and anti-cancer activities, etc[8,9]. The diverse effects of *A. paniculata* extract or andrographolide on the expression of a limited number of CYPs in vivo in rat livers and in vitro in human and rat hepatocyte cultures have been noted[10]. Therefore,

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it is worth determining how andrographolide exhibits pharmacological actions against acute opisthorchiasis in the BNF-exposed hamsters. The present study aims to investigate chemo-protective and antioxidative effects of andrographolide in acute opisthorchiasis in the BNF-exposed hamsters via the assessment of EROD and MROD activities and thiobarbituric acid reactive substance (TBARS) assay. The results assured andrographolide as a promising chemo-protective candidate based on the significant suppression of the specific CYP1A1 and CYP1A2 enzymatic reactions and lipid peroxidation in the BNF-exposed hamsters with acute opisthorchiasis.

Material and Method

Materials

Andrographolide was a product of Wako Pure Chemical Industries (Osaka, Japan). BNF, ethoxyresorufin (ER), methoxyresorufin (MR), were products of Sigma Aldrich (St. Louis, MO, USA). All other laboratory chemicals were of the highest purity available from commercial suppliers.

Animals

Female Syrian Golden hamsters at 3 weeks of age were supplied by the Animal Unit of Faculty of Medicines (Khon Kaen University, Khon Kaen, Thailand). Animal handling and the treatment protocols were approved by the Animal Ethic Committee for use and care of Khon Kaen University, Thailand (Approval No. AEKKU01/2555). The hamsters were infected by a single intragastric gavage given 50 metacercaria of OV. Three weeks after the infection to OV, the hamsters were daily intra-peritoneally administered with BNF (30 mg/kg/day) for 3 days, and/or andrographolide (5 mg/kg/day) for 5 days. The livers and brains were collected by liver perfusion 24 h after the last treatment and immediately kept at -80°C for further analysis.

Preparation of hepatic microsome

The liver was weighed, minced, and homogenized on an ice-bath in three volumes of cold 1.15% (w/v) potassium chloride. The microsomal fraction was prepared by centrifugationas described[11].

Assessment of alkoxyresorufin O-dealkylase activity

Hepatic AROD activities were determined by the method of Jarukamjorn[11], with modifications.

Determination of lipid peroxidation

Lipid peroxidation was determined by thiobarbituric acid fluorometric method[12].

Statistical analysis

The results were expressed as means of triplicate independent duplicate experiments ±SD and analyzed by using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test (version 19; SPSS Inc., Chicago, IL, USA).

Results

Effect of andrographolide on hepatic EROD and MROD activities

The induction of EROD activity was noted in the acute opisthorchiatic hamsters (Fig. 1A). BNF1 significantly elevated the level of hepatic EROD activity in the acute OV-infected hamsters while andrographolide itself did not affect. Interestingly, andrographolide significantly restored the BNF-induced EROD activity to a similar level as that of the OV-infected hamsters, though it could not reach to the

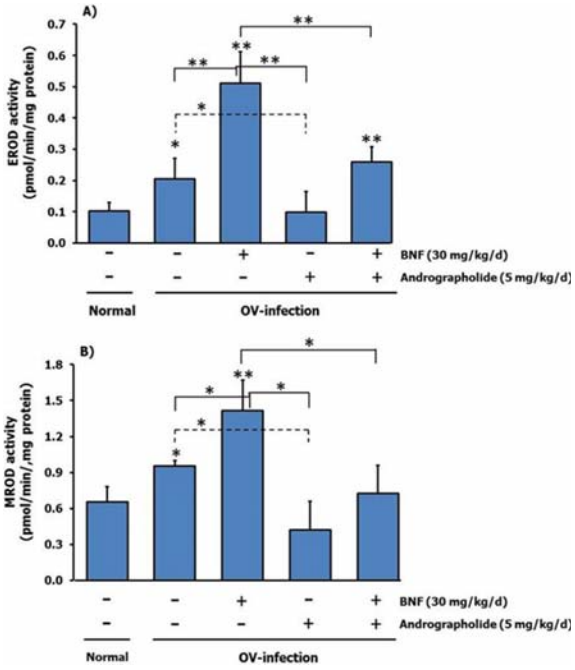

Fig. 1 EROD (A) and MROD (B) activities in the livers of female Syrian Golden hamsters. The hamsters were infected by a single intragastric administration of 50 OV metacercaria. Three weeks after the infection, the hamsters were daily administered with BNF (30 mg/kg/day) for 3 days, and/or andrographolide (5 mg/kg/day) for 5 days. The data is presented as the mean ± SD (n = 3) from two independent experiments. * $p<0.05$, ** $p<0.01$.

baseline level of the non-treated hamsters. Besides the benefit of andrographolide on suppression of CYP1A1 in the BNF-exposed hamsters with acute opisthorchiasis, the BNF-induced MROD activity was returned to the baseline by andrographolide (Fig. 1B). Consistent with the EROD activity, BNF excessively increased the level of hepatic MROD activity in the acute OV-infected hamsters while andrographolide itself did not. These observations implied that andrographolide assures the chemo-protective effect by restoring the BNF-induction of CYP1A1 and CYP1A2 activity in the acute opisthorchiatic hamsters.

Effect of andrographolide on lipid peroxidation

The results from both liver and brain showed that the formation of MDA was significantly higher in either the OV-infected alone or the OV-infected with BNF-exposed hamsters (Fig. 2A&B). Likewise, andrographolide did not change the level of lipid peroxidation in the livers and brains of the OV-infected hamsters. Expressively, andrographolide significantly restored the level of lipid peroxidation in the BNF-exposed hamsters with acute opisthorchiasis to the same level as those of the controls.

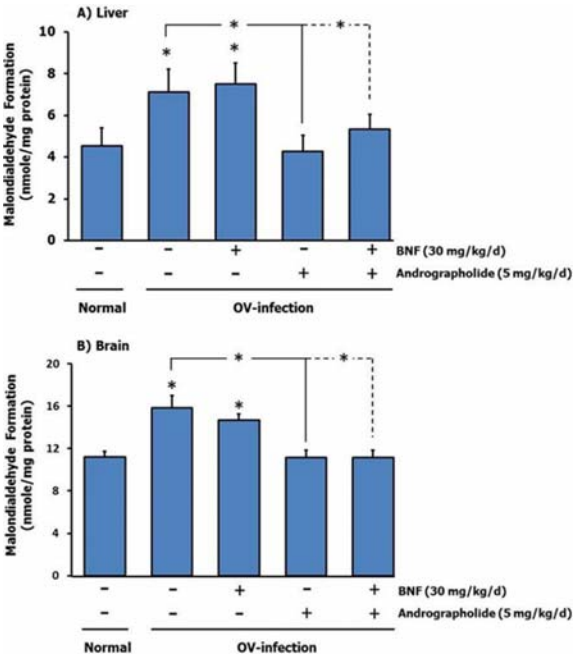

Fig. 2 Lipid peroxidation in the livers (A) and the brains (B) of female Syrian Golden hamsters. The hamsters were infected by a single intragastric administration of 50 OV metacercaria. Three weeks after the infection, the hamsters were daily administered with BNF (30 mg/kg/day) for 3 days, and/or andrographolide (5 mg/kg/day) for 5 days. The data is presented as the mean ± SD (n = 3) from two independent experiments. * $p<0.05$.

Discussion

Infection with OV due to eating certain traditional freshwater-fish dishes is a principal risk factor for cholangiocarcinoma in the Northeast of Thailand where the infection is endemic and the incidence of this form of primary liver cancer has been the highest in the world[13]. During chronic inflammation, ROS and reactive nitrogen species are generated from inflammatory and epithelial cell and play a key role in pathologic conditions and carcinogenesis[14]. OV infection triggers inflammation surrounding the bile duct lumen in hamsters, and during acute inflammation, the oxidative/nitrative stress also induces inflammation liver injury[15]. The study of Pinlaor et al, 2004[15] revealed that MDA level in the blood of hamsters fed with 50 metacercariae of OV by intragastric intubation for single infections was significantly increased from 3 to 90 days, agreed with our result. The MDA level was increased in the liver and brain of hamsters after 21 days of the OV infection. Andrographolide potentially returned the lipid peroxidation in both of the liver and brain of the acute opisthorchiasis hamsters. These observations corresponded to the previous study proposed that the antioxidant property of andrographolide could be due to activation of antioxidant enzymes[16]. Furthermore, the present findings supported the hepatoprotective property of andrographolide from previous studies in mice, rats, and guinea pigs[16-18]. The nuclear factor erythroid 2-related factor (Nrf2) is a transcriptional factor that functions as a key controller of the redox homeostatic gene-regulatory network[19]. Enhancement of oxidative stress and nitrative stress in the OV-infected hamsters may be mediated through the Nrf2 pathway by increasing expression of superoxide dismutase and Glutathione peroxidase enzymes (GPx), which suggested host defensive mechanisms against oxidative stress[20]. Andrographolide was also found to be the most potent Nrfs activator among 200 biologically active compounds[21].

Until now, praziquantel has been widely used for the treatment of OV infection because of its apparent effectiveness[22]. Praziquantel might produce general side effects, i.e., nausea, vomiting, headache, and abdominal discomfort[23]. In addition, praziquantel treatment for short term induced inflammation, and

resulted in oxidative and nitrative stress through OV antigen release[20]. Therefore, andrographolide might convey a benefit as an adjuvant of an anthelmintic agent because of its antioxidative property to reduce lipid peroxidation.

Besides the effect of ROS on cholangitis or cholangiocarcinoma, some hepatic metabolizing enzymes related cancer should be concerned. CYP1A1 and CYP1A2 play the role in metabolic activation of carcinogenic polycyclic aromatic hydrocarbons (PAHs) through aryl hydrocarbon receptor (AhR)[24]. The implication of CYP1A induction in human risk evaluation has been concerned and remained a central focus of interest in cancer research, toxicology, and food safety[24]. EROD and MROD activities were reduced in *Schistosomamansoni* infected DBA/2 mice[25]. Furthermore, the activity of CYP1A1 and expression of CYP1A1/2 protein were significantly increased in the liver of *Taeniataeniformis* metacestodes infected rats[26]. However, the effect of OV infection on expression of CYP1A was still unknown. This is the first time to report the induction of EROD and MROD in the OV-infected hamsters. BNF also additional increased the EROD and MROD activities. When people were infected with OV, they possibly exposed to procarcinogens in daily life via char-food and/or environmental pollutants, resulted in increasing cancer risk. Hence, it is of interest to have a dietary compound or natural produce to delay or lower the risk.

The very recent publication presented the anticancer potential of andrographolide in cholangiocarcinoma cells[27]. These findings support that andrographolide possesses chemo-protective property with the ability to down-regulate CYP1A in the OV-infected hamsters exposed to a CYP1A inducer. Taken data together, andrographolide had chemo-protective property and anti-lipid peroxidation due to its ability to down-regulate both EROD and MROD activities, and MDA formation in the BNF exposed hamsters to acute opisthorchiasis.

This is the first time to report a powerful chemo-protective activity and antioxidant property of andrographolide via the specific responsible CYP1A1 and CYP1A2 enzymatic reactions, EROD and MROD, and formation of MDA, against the acute opisthorchiasis in BNF-exposed hamsters. Therefore, andrographolide might be a promising adjuvant against cholangiocarcinoma development in an OV-infected patient, at least via amelioration of CYP1A1/2-related carcinogenesis and oxidant-antioxidant balance pathways.

What is already known on this topic?

Opisthorchis viverrini (OV) infection is considered as a primary cause of cholangiocarcinoma since it primarily triggers sclerosing cholangitis. A chronic infection of OV generates oxidative stress/free radicals and inflammation. Andrographolide is a Thai herbal medicine and has antioxidant property via activation of antioxidant enzymes. Taken data together, andrographolide might be a benefit to those who have OV infection.

What is this study adds?

The study implied that andrographolide has a chemo-protective effect by restoring the BNF-induction of CYP1A1 and CYP1A2 activity in the acute opisthorchiatic hamsters. Moreover, formation of MDA shows that andrographolide significantly restored the level of lipid peroxidation in the BNF-exposed hamsters with acute opisthorchiasis.

Acknowledgement

This work was supported by the Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. Special thanks for the technical assistance of OV metacercaria-intragastric administration is brought to Vet. Dr. Tidarat Boonmars, Khon Kaen University.

Potential conflicts of interest

None.

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ผลของสารแอนโดรกราโฟไลด์ต่อสมรรถนะของเอนไซม์ CAP1A ในภาวะที่ถูกกระตุ้นด้วยสารบีตาแนพโธฟลาโวน และภาวะลิปิดเปอร์ออกซิเดชั่นในหนูแฮมเสตอร์ที่เกิดภาวะ opisthorchiasis แบบเฉียบพลัน

ลติพร อุดมสุข, วรัญญา จตุพรประเสริฐ, กนกวรรณ จารุกำจร, ไพบูลย์ สิทธิถาวร

ภูมิหลัง: *การติดเชื้อพยาธิใบไม้ตับ (Opisthorchis viverrini) ทำให้เกิดภาวะเครียดออกซิเดชั่นและอนุมูลอิสระ และถือเป็นสาเหตุสำคัญที่ทำให้เกิดภาวะตับอ่อนอักเสบและนำไปสู่โรคมะเร็งถุงน้ำดีได้*

วัตถุประสงค์: *การศึกษาในครั้งนี้จึงมุ่งเน้นศึกษาผลของสารแอนโดรกราโฟไลด์ต่อการเกิดภาวะ opisthorchaisis แบบเฉียบพลันในหนูแฮมสเตอร์ที่ได้รับสารบีตาแนพโธฟลาโวน*

วัสดุและวิธีการ: *หาสมรรถนะของเอนไซม์ ethoxyresorufin O-deethylase (EROD) และ methoxyresorufin O-demethylase (MROD) และใช้วิธี TBARS ของสารแอนโดรกราโฟไลด์ในแฮมสเตอร์ที่ได้รับสารบีตาแนพโธฟลาโวนและเกิดภาวะ opisthorchiasis แบบเฉียบพลัน*

ผลการศึกษา: *ผลการทดลองพบว่าสารแอนโดรกราโฟไลด์สามารถลดสมรรถนะของเอนไซม์ CYP1A1 และ CYP1A2 ผ่านปฏิกิริยา EROD และ MROD ในแฮมสเตอร์ที่ได้รับสารบีตาแนพโธฟลาโวน นอกจากนี้สารแอนโดรกราโฟไลด์ยังสามารถลดการสร้างสารมาลอนไดอัลดีไฮด์ในตับและสมองของแฮมสเตอร์อีกด้วย*

สรุป: *จากผลการทดลองดังกล่าวแสดงให้เห็นถึงศักยภาพของสารแอนโดรกราโฟไลด์ในการเป็นสารต้านมะเร็ง และต้านอนุมูลอิสระผ่านการลดการทำงานของปฏิกิริยา EROD และ MROD และลดการเกิดลิปิดเปอร์ออกซิเดชั่น ในหนูแฮมเสตอร์ที่ได้รับสารบีตาแนพโธฟลาโวนที่มีภาวะ opisthorchiasis แบบเฉียบพลัน*