ผลของเจตมูลเพลิงแดงและพลัมบาจินต่อการแสดงออกของไซโตโครม พี 450 2 อี 1 ในตับ และ ไซโตโครม พี 450 2 เอฟ 2 ในปอดหนูถีบจักร

Effect of *Plumbago indica* Linn. and plumbagin on the expression of hepatic cytochrome P450 2e1 and lung cytochrome P450 2f2 in mice

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บทคัดย่อ

เจตมูลเพลิงแดง (*Plumbago indica* Linn.) ถูกใช้ในการแพทย์แผนไทยเพื่อฆ่าพยาธิ กระตุ้นความอยากอาหาร กระตุ้นระบบไหลเวียนโลหิตและทำแท้ง เจตมูลเพลิงแดงมีพลัมบาจินเป็นสารสำคัญที่ถูกรายงานฤทธิ์ทางเภสัชวิทยาในการต้านการอักเสบ กดภูมิคุ้มกัน เพิ่มระบบไหลเวียนโลหิต และต้านมะเร็ง การศึกษานี้มีวัตถุประสงค์เพื่อประเมินผลของสารสกัดหยาบส่วนเมธานอลของเจตมูลเพลิงแดงและพลัมบาจินต่อการแสดงออกของไซโตโครม พี 450 2 อี 1 (Cyp2e1) ในตับและไซโตโครม พี 450 2 เอฟ 2 ในปอดของหนูถีบจักร เพศผู้ สายพันธุ์ ICR อายุ 7 สัปดาห์ที่ได้รับพลัมบาจิน ขนาด 1, 5 และ 15 มิลลิกรัมต่อกิโลกรัมต่อวัน และสารสกัดหยาบเจตมูลเพลิงแดง ขนาด 20, 200 และ 1,000 มิลลิกรัมต่อกิโลกรัมต่อวัน โดยการป้อนทางปากทุกวันติดต่อกันเป็นระยะเวลา 14 วัน การแสดงออกที่ระดับเอ็มอาร์เอ็นเอของ Cyp2e1 ในตับและ Cyp2f2 ในปอดถูกวิเคราะห์ด้วยเทคนิคปฏิกิริยารีเวิร์สทรานสคริปชั่นร่วมกับปฏิกิริยาลูกโซ่โพลีเมอเรสแบบเวลาจริง (RT real-time PCR) ผลการศึกษาพบว่าพลัมบาจินเพิ่มระดับการแสดงออกของ Cyp2e1 เอ็มอาร์เอ็นเอในรูปแบบที่สัมพันธ์กับขนาดของพลัมบาจิน ส่วนสารสกัดหยาบมีผลเพิ่มระดับการแสดงออกของ Cyp2e1 เพียงเล็กน้อย ในทำนองเดียวกันพลัมบาจินและสารสกัดหยาบมีผลต่อระดับการแสดงออกของ Cyp2f2 เอ็มอาร์เอ็นเอในปอดในรูปแบบที่คล้ายคลึงกับการแสดงออกของ Cyp2e1 ในตับแม้ว่าการแสดงออกตรวจพบในระดับที่ต่ำกว่า โดยพลัมบาจินเหนี่ยวนำการแสดงออกของ Cyp2f2 ในปอดให้เพิ่มขึ้นอย่างมีนัยสำคัญในขณะที่สารสกัดหยาบแสดงผลการเหนี่ยวนำเพียงเล็กน้อยเท่านั้น จากการศึกษานี้พบว่าพลัมบาจินและสารสกัดหยาบเจตมูลเพลิงแดงทำให้อนุมูลอิสระเพิ่มขึ้นและเกิดภาวะเครียดออกซิเดชั่นในตับและปอด ดังนั้นการใช้พลัมบาจินหรือสารสกัดหยาบเจตมูลเพลิงแดง รวมถึงผลิตภัณฑ์ที่มีส่วนประกอบของสารดังกล่าวในการแพทย์ทางเลือกควรระมัดระวังเนื่องจากอาจเกิดความเป็นพิษต่อตับและปอดผ่านการเหนี่ยวนำภาวะเครียดออกซิเดชั่นที่มีความสัมพันธ์กับเอนไซม์ Cyp2e1 และ Cyp2f2

คำสำคัญ: เจตมูลเพลิงแดง พลัมบาจิน ไซโตโครม พี 450, Cyp2e1, Cyp2f2

Abstract

Plumbago indica Linn. (Rose-colored Leadwort) has been used in Thai traditional medicine as anthelminth, appetite stimulant, rubefacient, and abortifacient. Plumbagin, an active constituent of *P. indica*, has been reported to be anti-inflammatory, immunosuppressive, abortifacient, and anti-cancer agents. In this study, methanolic crude extract of *P. indica* and plumbagin were examined for their effects on hepatic cytochrome P450 2e1 (Cyp2e1) and lung

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cytochrome P450 2f2 (Cyp2f2) in mice. The 7-week-old male ICR mice were daily intragastrically adminsitered plumbagin (1, 5, and 15 mg/kg/day) or *P. indica* extract (20, 200, and 1,000 mg/kg/day) consecutively for 14 days. At 24 hours after the last treatment, total RNA was prepared form liver and lung. The expression of Cyp2e1 and Cyp2f2 mRNA was determined using RT real-time PCR. The expression of hepatic Cyp2e1 mRNA was significantly increased by plumbagin in a dose-dependent pattern whereas it was slightly increased by *P. indica* extract. The influence of plumbagin and *P. indica* extract on the expression of lung Cyp2f2 mRNA was nearly similar to those of hepatic Cyp2e1, though it was at the less extent. Plumbagin significantly induced the lung Cyp2f2 expression whereas *P. indica* extract slightly did. These observations suggested that plumbagin and the *P. indica* extract caused excessive ROS formation and mediated oxidative stress in the mouse livers and lungs. Therefore, the use of plumbagin and/or *P. indica* contained product as alternative medication is of caveat due to its hepatotoxicity and lung toxicity via the induction of oxidative stress-related Cyp2e1 and Cyp2f2.

Keywords: *Plumbago indica*, plumbagin, cytochrome P450, Cyp2e1, Cyp2f2

Introduction

Plumbago indica Linn. (Rose-colored Leadwort) is one of important ingredients in many for mulations of Thai traditional medicine for the ailments of heart, blood, lung, stomach, and reproductive organs [1-2]. A yellowish quinonoid compound, plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone, Fig. 1A), is the major constituent of *P. indica* which contributes to their various medicinal properties [1-2], including anthelmintic [2], antimalarial [3], antimicrobial [4], anti-inflammatory [5], immunosuppressive [6], abortifacient [7], anticancer [8-9], and possibly antidiabetic [10]. Despite its medicinal properties, plumbagin was claimed to have several side effects [11] due to its ability as a strong inducer of reactive oxygen species (ROS) and a depleting agent of cellular glutathione [12-13] which contributes to its hepatotoxicity and cytotoxicity [1,11-13]. According to similarity of molecular structure between plumbagin and menadione, a synthetic naphthoquinone (Fig. 1B), which was found to exert toxic effects on several organs including lung and liver in murine via oxidative stress [14], it is likely that plumbagin has similar effect [12]. In addition, styrene-induced lung and liver tumors in mice via metabolism of styrene to 7,8-styrene oxide is critically dependent on metabolism by Cyp2e1 and Cyp2f2, respectively. Therefore, the aim of this study was to examine the impact of *P. indica* crude extract and plumbagin on the profiles of hepatic Cyp2e1 and lung Cyp2f2, two main cytochrome P450 enzymes responsible for oxidative stress, in mice.

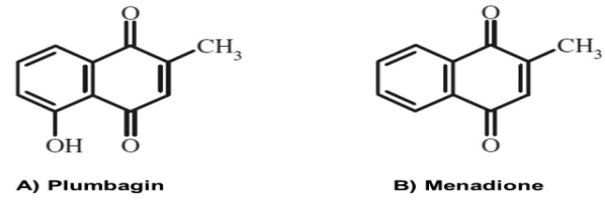

Fig. 1 Structure of plumbagin and menadione

Materials and Methods

Chemicals

Plumbagin was supplied by LKT Laboratories (St. Paul, MN, USA). ReverTraAce® reverse transcriptase was a product of Toyobo Co., Ltd. (Osaka, Japan). Taq DNA polymerase and RNase inhibitor were products of Invitrogen Life Technologies (Carlsbad, CA, USA). SYBR® Green I was from Cambrex Bio Science. (Rockland, ME, USA). The TaqMan® Gene Expression Assays (Inventoried) for Cyp2e1 (Mm00491127_m1) and CYP2f2 (Mm00484087_m1) were suppliedby Applied Biosystems (Branchburg, NJ, USA). All other laboratory chemicals were of the highest available purity from commercial suppliers.

Preparation of the *P. indica* crude extract

P. indica was bought from the Mor Tong-In Thai Traditional Medicine (Mahasarakam, Thailand) in June, 2014. It was dried at 50°C in a hot air oven then shredded and extracted with methanol using a soxhlet apparatus for 3 hours. The extract was then evaporated and freeze-dried into powder with the yield of 33.40%.

Animal Design and Treatments

Seven-week-old male ICR mice from the National Laboratory Animal Center, Mahidol University (Nakorn Pathom, Thailand) were housed in the Animal Unit, Faculty of Pharmaceutical Sciences, Khon Kaen University (Khon Kaen, Thailand). The animal handling and treatment protocol were approved by the Animal Ethics Committee for Use and Care of Khon Kaen University (AEKKU01/2558) under the supervision of a certified veterinary medical doctor. At all times, the mice were housed on wood shaved bedding in polysulfone cages, with *ad libitum* access to water and commercial animal diet under the controlled temperature (23±2°C) and humidity (45±2%). The mice were administered 0.5% carboxymethylcellulose (CMC) as vehicle-control, plumbagin in 0.5% CMC (1, 5, and 15 mg/kg/day), or the *P. indica* extract in 0.5% CMC (20, 200, and 1,000 mg/kg/day) consecutively for 14 days. At 24 hours after the last treatment, the mice were sacrificed, and the livers and lungs were collected for further analysis.

Quantitative determination of hepatic Cyp2e1 and lung Cyp2f2 mRNA expression

Total RNA was prepared using guanidine thiocyanate-phenol-chloroform method [15] and the cDNAs was subsequently synthesized using ReverTraAce® reverse transcriptase under the conditions recommended by the supplier: 25°C for 10 min, 42°C for 60 min, and 95°C for 5 min. The expression of the target mRNA was quantified by real-time PCR [15] using the specific TaqMan® Gene Expression Assay for Cyp2e1 and CYP2f2, or SYBR® Green I with the specific forward (5'-CCTCGTCCCGTAGACAAAATG-3') and reverse (5'-TGAAGGGGTCGTTGATGGC-3') primers for Gapdh using the real-time PCR system and the software of Bio-Rad® (Hercules, CA, USA). The level of each CYP mRNA was normalized to a reference housekeeping gene, Gapdh.

Statistical Analysis

The data were presented as the mean±SD and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test (SPSS 11.5). Values of $p<0.05$ were considered to be statistically significant.

Results

The expression of hepatic Cyp2e1 mRNA was significantly increased by plumbagin in a dose-dependent pattern whereas the *P. indica* extract slightly increased those expressions (Fig. 2). The influence of plumbagin and the *P. indica* extract on the expression of lung Cyp2f2 mRNA was nearly similar to those of hepatic Cyp2e1 though it was at the less extent. Plumbagin significantly induced the lung Cyp2f2 expression whereas the *P. indica* extract slightly did (Fig. 3).

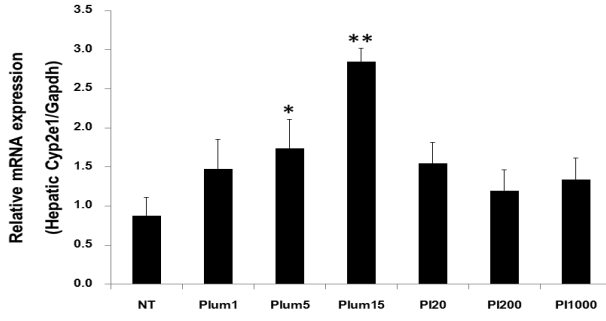

Fig. 2. Modified expression of hepatic Cyp2e1 mRNA by plumbagin and *P. indica* extractPlum 1, 5, 15, plumbagin 1, 5, 15 mg/kg/day; PI20, 200, 1000, *P. Indica* extract 20, 200, 1000 mg/kg/day; *,** $p<0.05$, 0.01 VS control (Non-treatment, NT).

Discussion and Conclusion

Hepatic Cyp2e1 metabolizes a variety of small molecules, hydrophobic substrates, and some drugs [16]. From a toxicological study, Cyp2e1 plays an important role in metabolism and activation of many toxicologically important compounds such as ethanol, carbon tetrachloride, acetaminophen, benzene, halothane, and many other halogenated substrates [16]. Cyp2e1 can be induced under a variety of metabolic or nutritional conditions such as chronically obese, overfed rats, and in rats fed a high-fat diet [17]. In addition, Cyp2e1 is the major enzyme in ethanol-induced oxidative stress and is a minor pathway of ethanol oxidation leading to an increase of ROS in hepatocytes [18]. In this study, we found the induction of Cyp2e1 mRNA by plumbagin and *P. indica*. Hence, the hepatotoxicity via Cyp2e1 induction pathway is of concern.

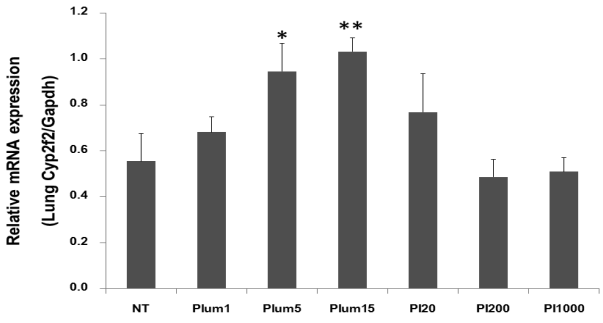

Fig. 3. Modified expression of lungCyp2f2 by plumbagin and *P. indica* extract Plum1, 5, 15, plumbagin 1, 5, 15 mg/kg/day; PI20, 200, 1000, *P. Indica*extract 20, 200, 1000 mg/kg/day; *,** p<0.05, 0.01 VS control (Non-treatment, NT).

Cyp2f2 is a member of cytochromes P450 family results in a cytotoxicity-driven mode of action in nasal and lung tissue in mice [19]. Coumarin is metabolized by Cyp2f2 to coumarin-3,4-epoxide in mouse lung which can induce mouse lung cytotoxicity and is believed to cause lung tumors [20]. Moreover, Cyp2f2 can activate naphthalene in lung and nasal tissues, leading to bronchiolar cytotoxicity [21]. The induction of Cyp2f2 was presently found. Hence, the Cyp2f2 activation might cause lung toxicity.

These observations suggested that plumbagin and the *P. indica* extract induced the excessive ROS formation and mediated oxidative stress in the mouse livers and lungs. Therefore, the use of plumbagin and/or the *P. indica* contained product as alternative medication is of caveat due to its hepatotoxicity and lung toxicity via the induction of oxidative stress and toxicity related to Cyp2e1 and Cyp2f2 activation.

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