อิทธิพลของเคอร์คูมินต่อพัฒนาการแสดงออกของยีนที่เกี่ยวข้องกับภาวะเครียดในตับหนูไมซ์ที่ถูกแยกเลี้ยงเดี่ยว

Effect of curcumin on the improvement of stress-related gene expression in the liver of social isolation mice

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บทคัดย่อ

เคอร์คูมินมีฤทธิ์ทางเภสัชวิทยาที่หลากหลาย อาทิ ฤทธิ์ต้านอักเสบ ต้านมะเร็ง และต้านซึมเศร้า ในการศึกษานี้ทำการประเมินผลของเคอร์คูมินต่อยีนที่เกี่ยวข้องกับกระบวนการต้านออกซิเดชั่นในตับของหนูไมซ์ที่ถูกแยกเลี้ยงเดี่ยว หนูไมซ์ถูกกระตุ้นให้เกิดภาวะเครียดโดยการแยกเลี้ยงเดี่ยว เป็นระยะเวลานาน 6 สัปดาห์ หลังจากถูกแยกเลี้ยงเดี่ยว หนูไมซ์ได้รับสารเคอร์คูมิน ขนาด 10 และ 20 มิลลิกรัมต่อกิโลกรัมต่อวัน หรือได้รับยาอิมิพรามีน (ยาต้านซึมเศร้า) ขนาด 20 มิลลิกรัมต่อกิโลกรัมต่อวัน เป็นระยะเวลา 2 สัปดาห์ จากนั้นการแสดงออกของยีนในตับที่เกี่ยวข้องกับภาวะเครียด ได้แก่ อะโปไลโปโปรตีน อี (ApoE) ไซโตโครมซีออกซิเดส (cytochrome c oxidase หรือ Cox11) ไฮดรอกซีแอซิด ออกซิเดส 1 (Hao1) ออกซิเดทีฟสเตรสเรสปอนซีฟ 1 (Oxsr1) สเตรสอินดิวซ์ฟอสฟอโปรตีน 1 (Stip1) และยูบิควิตินบี (Ubb) ที่ระดับเอ็มอาร์เอ็นเอ (mRNA) ถูกประเมินโดยใช้เทคนิคปฏิกิริยาลูกโซ่ Reverse transcription polymerase chain reaction (RT-PCR) ผลการศึกษาพบว่าการแสดงออกของ ApoE เอ็มอาร์เอ็นเอ ลดลงอย่างมีนัยสำคัญทางสถิติ และการได้รับเคอร์คูมินหรืออิมิพรามีน สามารถทำให้ระดับการแสดงออกของ ApoE กลับสู่ภาวะปกติ การแสดงออกของยีน Stip1 ลดลงในหนูที่ได้รับยาอิมิพรามีน และยีน Ubb เพิ่มขึ้นในหนูที่ได้รับเคอร์คูมินหรืออิมิพรามีน การแสดงออกของยีน Oxsr1 เพิ่มขึ้นเมื่อได้รับเคอร์คูมินขนาดสูง ในขณะที่ยีนอื่นๆ ได้แก่ Cox11 และ Hao1 ไม่พบการเปลี่ยนแปลงอย่างมีนัยสำคัญในตับหนูไมซ์ที่ถูกแยกเลี้ยงเดี่ยว โดยสรุปเคอร์คูมินสามารถรักษาระดับการแสดงออกของยีนต้านออกซิเดชั่น ApoE ให้อยู่ในระดับปกติ และเพิ่มการแสดงออกของ Oxsr1 และ Ubb ในหนูไมซ์ที่ถูกแยกเลี้ยงเดี่ยว ดังนั้นเคอร์คูมินอาจมีประโยชน์ในการต้านออกซิเดชั่นในตับเนื่องจากภาวะซึมเศร้า

คำสำคัญ: เคอร์คูมิน ยีนที่เกี่ยวข้องกับภาวะเครียด แยกเลี้ยงเดี่ยว อะโปไลโปโปรตีน อี

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Abstract

Curcumin possesses several pharmacological effects including anti-inflammation, anti-tumor, and antidepressant. In this study, the effects of curcumin on the antioxidant related genes in liver of the social isolation (SI) mice were determined. Mice were caused stress using chronic social isolation (SI) for six weeks. After SI induction, mice were treated with curcumin at the dose of 10 and 20 mg/kg/day, or imipramine (tricyclic antidepressant) at the dose of 20 mg/kg/day for two weeks. The hepatic mRNA expression of stress related genes namely apolipoprotein E (ApoE), stress-induced phosphoprotein 1 (Stip1), oxidative-stress responsive 1 (Oxsr1), COX11 homolog (cytochrome c oxidase, Cox11), hydroxyacid oxidase 1 (Hao1), and ubiquitin B (Ubb) were determined using semi-quantitative RT-PCR. The expression of ApoE mRNA was significantly reduced in SI mice. Interestingly, curcumin or imipramine restored the ApoE mRNA expression to the normal level. The expression of Stip1 was reduced in imipramine treated mice. The expression of Ubb was increased by curcumin or imipramine treatments. Hepatic Oxsr1 was induced by the high dose of curcumin while the expression of Cox11 and Hao1 were not affected in SI model. In conclusion, curcumin restored the expression of ApoE to the normal level and increased the expression of Oxsr1 and Ubb in SI mice. Therefore, curcumin might provide extra-benefit, especially hepatic antioxidative property due to depression.

Keywords: Curcumin, stress-related gene, social isolation, apolipoprotein E

Introduction

Curcumin, a major constituent of the traditional medicinal plant *Curcuma longa* L., has been showed to be a potent antioxidant, antitumor promoter, and anti-inflammatory agent, both *in vitro* and *in vivo* [1-3]. Extensive researches have demonstrated the antidepressant activity of curcumin [4-7]. Curcumin reduced the neuroinflammation, plaque deposition, and protein oxidation in Alzheimer's disease [6] and produced a marked increase of serotonin and noradrenaline levels in the frontal cortex and the hippocampus of depressive behavior mice [4]. Moreover, curcumin showed an antidepressant-like action by modulating synapse associated proteins within the lateral amygdala in an unpredictable chronic mild stress (UCMS) rat model [8].

However, there are several genes in many organs involve in the stress or depression status. Therefore, in this study the effect of curcumin on the stress related-genes including apolipoprotein E (ApoE), stress-induced phosphoprotein 1 (Stip1), oxidative-stress responsive 1 (Oxsr1), COX11 homolog (cytochrome c oxidase, Cox11), hydroxyacid oxidase 1 (Hao1), and ubiquitin B (Ubb) were determined in the liver of social isolation mice. The success of this study might provide the extra-benefit of curcumin in the stress condition.

Materials and Methods

Animal Design and Treatments

Three-week-old male ICR mice were provided by the National Laboratory Animal Center (Mahidol University, Nakhon Pathom, Thailand) and were housed in the Laboratory Animal Unit of the Faculty of Pharmaceutical Sciences (Khon Kaen University, Khon Kaen, Thailand). The research protocols were approved by the Animal Ethics Committee for Use and Care, Khon Kaen University, Khon Kaen, Thailand (Approval No. AEKKU05/2551 and AEKKU54/2553).

For the normal condition, five mice were housed in a polysulfone cage on wood chip bedding with water and commercial animal diet supplied ad libitum and 12 h dark/light cycle under controlled temperature (23±2°C) and humidity (45±2%).

For the social isolation (SI) model, mice were housed one mouse per cage for six weeks under normal conditions. After the stress induction, the mice were randomly divided into four groups (n=5-6) for treatment initiation. Curcumin was dissolved in 0.5% carboxymethylcellulose (CMC) in 0.9% normal saline solution (NSS). Mice received daily intraperitoneal injections of either curcumin (10 or 20 mg/kg/day) or imipramine (20 mg/kg/day) for two weeks. The control groups received daily intraperitoneal injections of 0.5% CMC in NSS (0.1 ml/mouse/day) for the same period.

Expression of hepatic antioxidation related genes mRNAs

The hepatic mRNA of ApoE, Stip1, Oxsr1, Cox11, Hao1, and Ubb were semi-quantified by RT-PCR. Hepatic total RNA was reverse-transcribed using ReverTraAce® reverse transcriptase, and then the cDNA was amplified under the conditions recommended by Invitrogen™ (Life Technologies Corporation, Carlsbad, CA). The PCR programs were described in table 1. After separation of the PCR products by gel electrophoresis in 1.5-2.0% agarose, the target cDNA was detected under ultraviolet light in the presence of Novel Juice of GeneDirex® (Bio-Helix Co., Ltd., Taiwan) and was semi-quantified by Syngene® gel documentation (Ingenius L, Cambridge, UK) and the GeneTools match program (Syngene®). The mRNA level of the target was normalized to that of GAPDH.

Statistical Analysis

The data are presented as the mean±SD and were 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

Social isolation (SI) for six weeks significantly suppressed the expression of ApoE mRNA (Fig 1A), and slightly decreased the expression of Ubb mRNA (Fig 1F). Curcumin (Cur) at both the dose of 10 and 20 mg/kg/day and imipramine (Imi, 20 mg/kg/day) significantly restored the expression of ApoE to the normal level (Fig 1A). Both Cur and Imi increased the expression of Ubb in SI mice (Fig 1F). Interestingly, Cur at the high doses increase the expression of Oxsr1 (Fig 1D) while Imi

decreased the expression of Stip1 (Fig 1E). The expression of Cox11 and Hao1 were not significantly modified by any treatments (Fig 1B&C).

Discussion and conclusion

Apolipoprotein E (ApoE) is a multifunctional protein synthesized by the liver and tissue macrophages.The level of apoE may influence the balance between oxidants and antioxidants in hippocampus [9]. ApoE is involved in uptake and degradation of lipoprotein by liver and ApoE-deficient animals develop spontaneous hyper cholesterol and atherosclerotic lesion [10].

Table 1 Primers for RT-PCR

Gene	Accession number	Forward primer (5'→3')	Reverse primer (5'→3')	Product size(bp)
Apolipoprotein E (ApoE)	NM_009696.3	CCTGAACCGCTTCTGGGATT	TGCCTTGTACACAGCTAGGC	406
Cytochrome c oxidase assembly protein 11 (Cox11)	NM_199008.2	ACCGTTTGAAGCTGGACAGT	AACTGGCAACTTGTGCCCTT	205
Hydroxyacid oxidase 1 (Hao1)	NM_010403.2	TCCTGGATGGGGGAGTAAGG	TCACATTCTGGCACCCACTC	192
Oxidative-stress responsive 1 (Oxsr1)	NM_133985.2	CGAGCTGCAGGAGGTGATAG	TATCCGCTTGATCGCCACTC	94
Stress-induced phosphoprotein 1 (Stip1)	NM_016737.2	ACCTGGGCACGAAACTACAG	ATAAGCCAAGCGCTCCTGTT	569
Ubiquitin B (Ubb)	NM_011664.3	TCTGAGGGGTGGCTATTAA	TGCTTACCATGCAACAAAAC	167

Our result showed that ApoE mRNA expression was suppressed in the social isolation mice. Curcumin and imipramine restored the expression of ApoE. This might be a benefit of these compounds to prevent the diseases in social isolation status. The oxidative stress responsive gene 1 (Oxsr1) controls cell proliferation and cell apoptosis. Oxsr1 is known to be involved in cytoskeleton rearrangements, and reacts on osmotic arrange [11]. Stress-induced phosphoprotein 1 (Stip1) protein and Oxsr1 were also

reported to be enhanced in response to oxidative stress [12]. Curcumin slightly increased the expression of Oxsr1 that might stimulate the response of Oxsr1 to oxidative stress. However, the effect of Oxsr1 up-regulation by curcumin should be further investigated. Imipramine suppressed the expression of Stip1. Hence, imipramine might reduce the response of Stip1 to oxidative stress.

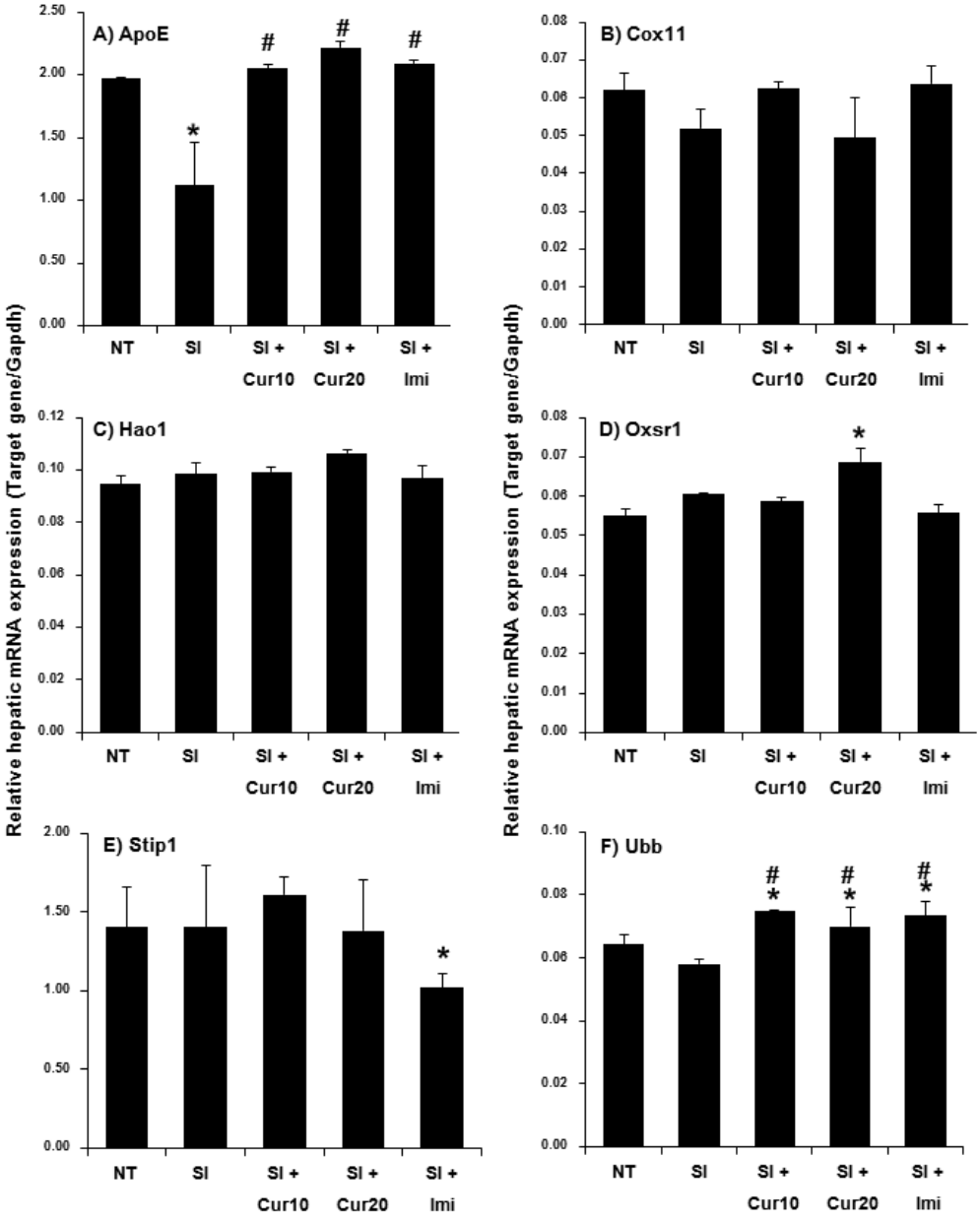

Figure 1 The mRNA expression of stress-related genes in the liver of social isolation (SI) mice (n=5-6). NT, non-treatment (0.5% CMC in NSS i.p.); SI, social isolation; Cur, curcumin (10 or 20 mg/kg/day); Imi, imipramine (20 mg/kg/day). * p<0.05 VS NT; # p<0.05 VS SI.

Under the stressful conditions, polyubiquitin genes are up-regulated, which results in an increase of the monomeric ubiquitin within the cytoplasm. The enhancement presumably facilitates the removal of damaged or denaturated proteins. The ubiquitin mRNA levels, therefore, can be used as a general indicator of oxidative stress [13]. Both of curcumin and imipramine increased the expression of ubiquitin B (Ubb) might indicate oxidative stress occurred or stimulation of the ubiquitin system to prevent oxidative stress. Cytochrome c oxidase (Cox11) and hydroxyacid oxidase 1 (Hao1) also get involved in stress pathway, but in this study we did not observe any alteration of these genes.

In conclusion, curcumin restored the expression of ApoE to the normal level and increased the expression of Oxsr1 and Ubb in SI mice. Therefore, curcumin might provide extra-benefit, especially hepatic antioxidative property due to depression.

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