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EXPERIMENTAL STUDY

Lichong decoction reduces Matrix Metalloproteinases-2 expression but increases Tissue Inhibitors of Matrix Metalloproteinases-2 expression in a rat model of uterine leiomyoma

Wang Yasong, Li Donghua, Xu Xin, Qian Ruiya, Zhang Yalan, Huang Yuhua, Geng Jianguo, Zou Xiaoli, Han Hongjuan, Zhang Wufang

Wang Yasong, School of Traditional Chinese Medicine, Capital Medical University, and School of General Practice and continuing Education, Capital Medical University, Beijing Xicheng District Desheng Community Health Service Center, Beijing 100120, China

Li Donghua, Geng Jianguo, Zou Xiaoli, Han Hongjuan, Zhang Wufang, School of Traditional Chinese Medicine, Capital Medical University, Beijing Key Lab of TCM Collateral Disease Theory Research, Beijing 100069, China

Xu Xin, Huang Yuhua, Department of Gynecology of Beijing Hospital of Traditional Chinese Medicine, Beijing 100010, China

Qian Ruiya, Zhang Yalan, The Fourth Ward of Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing Maternal and Child Health Care Hospital, Beijing 100026, China

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Correspondence to: Li Donghua, School of Traditional Chinese Medicine, Capital Medical University, Beijing 100069, China. dududoctor@163.com

Telephone: +86-10-83911670

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Abstract

OBJECTIVE: To study the effect of Lichong decoction (LD) on expression of matrix metalloproteinase-2 (MMP-2) and metalloproteinase-2 (TIMP-2) in a rat model of uterine leiomyoma (UL).

METHODS: UL was induced in rats using exogenous estrogen and progesterone. LD was administered (p.o.) for 4 weeks, and mifepristone (RU-486) used as a control. To observe the effect of LD on the uterine coefficient and uterine transverse diameter, a radioimmunoassay method was used to detect serum levels of sex hormones. Light microscopic analyses of pathologic changes in the tissues of UL rats were evaluated. Expression of the proteins of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in uterine tissues was assessed by immunohistochemical staining and western blotting.

RESULTS: A UL model in rats was established successfully. LD reduced uterine weight, uterine coefficient, and uterine transverse diameter compared with untreated controls. LD reduced levels of estradiol, progesterone, follicle-stimulating hormone, and luteinizing hormone in our UL models. LD improved the pathologic condition of uterine muscle. Expression of MMP-2 protein decreased to varying extents in LD-treated groups, but TIMP-2 levels were enhanced. LD appears to reduce MMP-2 expression and increase TIMP-2 expression in UL tissue.

CONCLUSION: These data suggest that the mechanism of action of LD on ULs may involve reduction of MMP-2 expression and increase in TIMP-2 expression in rats.

Key words: Leiomyoma; Matrix metalloproteinase 2; Tissue inhibitor of metalloproteinase-2; Lichong decoction

INTRODUCTION

Uterine leiomyomas (ULs; also known as "uterine fibroids") grow *via* hyperplasia of uterine smooth muscle. ULs are a common benign tumor in the female reproductive system. Main manifestations of ULs are abdominal masses, depressive symptoms, pain, infertility, spontaneous abortion, menstrual disorders, and dysmenorrhea.[1]

Traditional Chinese Medicine infers that the pathogenesis of UL is "blood stasis" caused by a disorder of viscera, hindrance in the functional activities of *Qi*, and internal static blood obstruction. ULs have a long course, which damages healthy *Qi* and leads to other diseases and deficiency syndromes.[2] Deficiency of vital *Qi* is the basis of ULs, whereas blood stasis is the external manifestation. Recently, this pathogenesis has been treated by strengthening body resistance and eliminating blood stagnation. This is a suitable and viable method to cure ULs that is now used widely.

Lichong decoction (LD) is documented in *Yi Xue Zhong Zhong Can Xi Lu*,[3] written by a famous physician in the *Qi*ng Dynasty: Zhang Xichun. The formula used to prepare the decoction is Huangqi (*Radix Astragali Mongolici*), Dangshen (*Radix Codonopsis*), Baizhu (*Rhizoma Atractylodis Macrocephalae*), Shanyao (*Rhizoma Dioscoreae Oppositae*), Tianhuafen (*Radix Trichosanthis*), Zhimu (*Rhizoma Anemarrhenae*), Sanleng (*Rhizoma Sparganii*), Ezhu (*Rhizoma Curcumae Phaeocaulis*), and Jineijin (*Endothelium Coreneum Gigeriae Galli*). This formula was designed to treat ULs by strengthening healthy *Qi*, enhancing blood flow, and eliminating pathogens.

Our research team has revealed that LD can significantly suppress UL growth, and trigger apoptosis of UL cells.[4] Research has also shown that the prevalence and development of ULs are related to an imbalance in the metabolism of the extracellular matrix (ECM). Also, ECM degradation appears to be a signal for tumor cells to attack and migrate into normal tissue. ECM degradation is dependent upon the ratio of matrix metalloproteinases (MMPs) to their endogenous inhibitors: tissue inhibitors of metalloproteinases (TIMPs). An imbalance in expression of MMP-2 and TIMP-2 has a key role in this process.[6]

We established a rat model of ULs to study further the effect of LD on expression of MMP-2 and TIMP-2, and to investigate its possible mechanism of action.

MATERIALS AND METHODS

Experimental animals

Homologous, healthy, adult, female, specific pathogen-free Sprague-Dawley rats [n = 50; 3 months; (200 ± 20) g] were provided by Beijing Weitong Lihua Experimental Animal Tech (Beijing, China). Rats were kept in the Center of Laboratory Animals at our university. After adaptive feeding for 1 week, rats were divided into five groups of ten using the random numbers method: normal control (N); model (M); mifepristone (Ru-486, R); low-dose LD (LLD); high-dose LD (HLD). The study protocol was approved by the Ethics Committee of Capital Medical University (Beijing, China).

Drugs and reagents

LD was prepared according to standard procedures. All Chinese herb ingredients were purchased from Beijing Tongrentang Drug Store (Beijing, China). Mifepristone (Ru-486) was obtained from Zhejiang Fairy Ju Pharmaceuticals (Zhejiang, China). Estradiol benzoate was purchased from Tianjin Jin Yao Amino Acids (Tianjin, China). Progesterone was obtained from Shanghai General Pharmaceuticals (Shanghai, China). Rabbit polyclonal anti-MMP-2 kits and rabbit polyclonal anti-TIMP-2 kits were from Wuhan Boster Biological Engineering (Wuhan, China). Rabbit streptavidin-horseradish peroxidase (HRP) kits were purchased from Kangwei Biological Technology (Beijing, China). HRP-labeled goat anti-rabbit IgG was obtained from Jackson (Jackson, MI, USA), as was HRP-labeled goat anti-rat IgG. HRP-labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was purchased from Shanghai Kang Cheng Biological (Shanghai, China). Mouse TIMP-2 was obtained from Abcam (Cambridge, UK). Rabbit MMP-2 was from CST (Boston, MA, USA).

Induction of a rat model of UL and treatments

A rat model of UL was established based on previous reports.[7,8] Rats received estrogen (0.5 mg/kg body weight, i.p.) once daily for the first 4 weeks, followed by estrogen and progesterone at an identical dose and via the same route for 5 days. The LLD dose was equivalent to an adult dose (10.15 $g \cdot kg^{-1} \cdot d^{-1}$), whereas the HLD dose was three-times the adult dose (30.45 $g \cdot kg^{-1} \cdot d^{-1}$). The dose administered to rats in the R group was equivalent to the dose for adults (2.92 $mg \cdot kg^{-1} \cdot d^{-1}$). Rats in N and M groups were gavaged with an equivalent volume of water once daily for the final 4 weeks. Drugs used in this process were stored at 4 ℃.

Radioimmunoassay (RIA) used to detect serum levels of sex hormones

Serum levels of sex hormones in groups were assessed using a RIA. Blood samples were taken *via* cardiac puncture for RIA analyses one day after treatment had been completed. Serum was separated using commercial kits according to manufacturer instructions.

Calculation of the uterine coefficient (UC) and uterine transverse diameter (UTD)

The general status of rats was recorded. Then, rats were killed and blood samples taken. The uterus was weighed and uterine wet weight (mg) recorded. Angles at several points above the cervix and at the uterus root were measured (mm) using vernier calipers to assess its