

Original Research Article

Effect of *Kang Fu Yan* capsule on phenol mucilage-induced intrauterine adhesion injury in female rats

Qinghong Fan¹, Wei Xiao¹, Xiaomeng Chai¹, Zhe Zhang¹, Tao Zhu¹, Dan Tang¹, Kaihe Ye¹, Tianjun Luo², Qing Liu², Gang Huang², Yulan Yang², Hong Nie^{1*}

¹Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, Guangdong, ²Long-Range Pharmaceutical Co., Ltd, Guiyang 550001, Guizhou, China

*For correspondence: **Email:** hongnie1970@163.com; **Tel:** +86-20-85222810; **Fax:** +86-20-85224476

Sent for review: 12 December 2018

Revised accepted: 26 April 2019

Abstract

Purpose: To investigate the effect of Kang fu yan capsule (KFYC) on phenol mucilage-induced intrauterine adhesion (IUA) in a rat model, and the underlying mechanisms.

Methods: An IUA model was established by injecting 0.06 mL of 25 % phenol mucilage into the uterus of female Sprague-Dawley rats. The IUA model rats (n=59) were randomly divided into 5 groups: IUA group, fuke qianjin tablet group (FKQJT, 0.22 mg/kg), and 3 KFYC groups given different doses of the drug i.e. 0.13, 0.39 and 1.17 mg/kg. A group of 10 healthy female rats served as control. After 19 days treatment, blood samples were collected for determination of IL-2 and IL-10 by ELISA, while uterine tissues were subjected to histological examination using hematoxylin and eosin staining (H&E) and Masson staining. Expressions of Notch1, recombination signal binding protein-JK (RBP-JK), a disintegrin and metalloprotease (ADAM)-12, ADAM-15, matrix metalloprotein-9 (MMP-9), and inhibitor of NF-κB (IκB) in uterine tissues were determined using RT-qPCR and western blot analysis.

Results: Compared to IUA group, histological results showed reduced inflammatory cell infiltration in rat uterine tissue of KFYC group. Moreover, KFYC significantly reversed uterine fibrosis ($p < 0.05$). Serum concentrations of IL-2 significantly decreased in KFYC groups ($p < 0.05$ or $p < 0.01$), and there was significant increases the serum concentrations of IL-10 in KFYC groups ($p < 0.05$ or $p < 0.01$), when compared to IUA group. The mRNA and protein expressions of Notch1, RBP-JK, ADAM-12, ADAM-15, MMP-9 were also significantly down-regulated ($p < 0.05$), while protein expression of IκB was up-regulated in KFYC group, when compared to IUA group.

Conclusion: KFYC exerts an anti-IUA effect via amelioration of uterine inflammation and fibrosis, probably via a mechanism involving regulation of Notch1/ADAM pathway.

Keywords: Intrauterine adhesion, Kang Fu Yan capsule, Notch1, ADAM-12

This is an Open Access article that uses a fund-ing model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Since 1900, intrauterine adhesions (IUAs) have been recognized as the cause of secondary

amenorrhea. In the mid-20th century, Asherman described an eponymous condition that occurs after pregnancy as intrauterine adhesion or Asherman syndrome [1,2]. IUAs are usually

caused by trauma or infection, especially after an abortion [3]. Endometrial fibrosis is currently recognized as a pathological basis for IUA [3,4].

The inflammation caused by endometrial trauma and infection produces a variety of inflammatory cytokines such as IL-2, IL-10, and IL-1 β . Subsequent secretions of large amounts of extracellular matrix components ultimately lead to IUA and tissue fibrosis [5-7]. Over the past 70 years, several studies have focused on IUA, but their prevalence and their effects on reproductive outcomes have not been elucidated. Currently, hysteroscopy is the main diagnostic strategy for IUA, and drugs used for treatment are mostly antibiotics and estrogens [8,9]. However, after hysteroscopy and medical treatment, IUA easily relapses. Thus, there is need to evolve novel drugs with minimal side effects for treating IUA.

Kang fu yan capsule (KFYC) is a patent Chinese medicine which is used in the treatment of pelvic inflammation. Widely used in Asia [10-12], it contains several kinds of Chinese herbal medicines such as angelica and sophora [11], which promote blood circulation. It is used mainly to treat pelvic inflammatory disease, vaginitis and chronic cervicitis. However, the anti-IUA effect of KFYC, and its potential mechanisms of action have not been investigated. The *fuke qianjin* tablet (FKQJT) is also an effective patent Chinese medicine which has been used in the treatment of pelvic inflammation as a positive control. In the present study, the anti-inflammatory effect of KYFC against IUA, and the underlying mechanisms were studied in a 25% phenol mucilage-induced IUA rat model [13].

EXPERIMENTAL

Reagents

Phenol, gum tragacanth and glycerine were purchased from Guangzhou Dongzheng Chemical Co., Ltd. (Guangzhou, China). *Kang fu yan* capsule was provided by the Long-Range Pharmaceutical Co., Ltd (Guizhou, China). *Fuke qianjin* tablet (FKQJT) was purchased from Qianjin Pharmaceutical Co. Ltd (Zhuzhou, China).

Animals

Female Sprague-Dawley rats (180 - 220 g, 8 weeks old, certificate no. SCXK Guangdong, 2013-0002) were purchased from the Animal Center of Guangdong Province. All rats were housed in an environment with a 12 h light/12 h dark cycle. The experiments were approved by

the Laboratory Animal Ethics Committee of Jinan University (approval no. 20160729131210), and performed according to the guidelines of National Institutes of Health (OLAW/NIH Revised 2015) [14].

Animal studies

Anesthesia was induced in the rats by intraperitoneal injection of 3 % pentobarbital sodium at a dose of 1ml/kg. Then, the abdomen was shaved. The operation procedure was carried out under aseptic conditions. The pelvic region was examined to exclude any adhesions or macroscopic abnormalities. The uterine horns were exposed by an abdominal midline vertical incision. Then, 0.06 ml of phenol mucilage was injected into right uterine tissue at the upper 1/3 of the horn [13]. The left horn was used as control, and was injected with an equivalent volume of normal saline. The uteri were gently returned to their pelvic location without disruption. Next, 59 IUA model rats were randomly divided into 5 groups: IUA group (n=12), FKQJT group (0.22 mg/kg, n=11), and 3 KFYC groups (12 rats each) given different doses of the drug i.e. 0.13, 0.39 and 1.17mg/kg. A group of 10 healthy female rats was used as control. The rats were treated with appropriate drugs or normal saline for 18 days post-operation. At the end of 19 days, rats were sacrificed after an overnight fast and all blood samples were collected through the abdominal aorta. Bilateral uterine tissue was removed and several small transverse sections were fixed overnight in 4% paraformaldehyde at 4°C prior to histological evaluations. Some of the uterine tissue sections were cryopreserved in liquid nitrogen for molecular studies.

Histopathological examination

The small transverse sections of uterine tissues fixed in 4 % paraformaldehyde were dehydrated in alcohol, paraffin- embedded, sectioned and subjected to H&E or Masson staining. The stained sections were observed under the light microscope.

Measurement of serum concentrations of IL-2 and IL-10

The serum concentrations of IL-2 and IL-10 were measured with ELISA kit (Cusabio, China). In line with the manufacturer's instructions, standard or sample (100 μ L) was added to each well and incubated for 2 h at 37 °C. Then, the medium in each well was discarded, and biotin antibody (100 μ L) was added to each well, followed by incubation for 1 h at 37 °C. The

medium in each well was aspirated and the wells were washed 3 times. Thereafter, HRP-avidin (100 μ L) was added to each well and incubated for 1 h at 37 °C. The medium in each well was aspirated and the wells were washed 5 times, followed by addition of tetramethylbenzidine substrate (90 μ L) and incubation for 20 min at 37 °C in the dark. Finally, the stop solution (50 μ L) was added to each well, and the absorbance of the wells were read at 450 nm within 5 min.

RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR)

The mRNA levels of Notch1, RBP-JK, ADAM-12 and ADAM-15 in each uterus were measured using RT-qPCR. Total RNA was extracted with TRIZOL reagent (Takara, Japan) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from 1000 ng of the total RNA using a PrimeScriptTMRT reagent Kit (Takara, Japan). Each cDNA sample was amplified for the target gene and β -actin in a 15 μ l reaction volume. The primers used are listed in Table 1. Real-time PCR conditions were 95 °C for 30 sec followed by 40 cycles of 95 °C for 5 sec, 55 °C for 30 sec and 72 °C for 60 sec. The mRNA levels of all genes were normalized against that of β -actin.

Western blot analysis

Frozen uterine tissue (100 mg) was rapidly thawed and homogenized at 4 °C in 1 ml of RIPA lysis buffer. The homogenates were centrifuged at 13700 g for 15 min at 4 °C to obtain supernatants. The protein concentrations in the supernatants were determined with a BCA protein assay kit (Beyotime, China) Each sample containing 80 μ g protein was run in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 10 % acrylamide resolving gel. The separated proteins were transferred to a PVDF membrane, blocked in TBST containing 5 % non-fat milk for 2 h at room temperature.

Thereafter, the membrane was incubated with primary antibodies for Notch1, I κ B, MMP-9, ADAM-12, ADAM-15 antibody (1:1000 dilution each) , or GAPDH antibody (1:1000 dilution) at 4 °C overnight, washed, and further incubated with the horseradish peroxidase-conjugated secondary antibody (1:1000) for 2 h at room temperature. The blots were visualized using enhanced chemiluminescence reagent (Beyotime, China), and UVP BioSpectrum Imaging System was used to expose immune-positive bands. The bands were semi-quantitatively analyzed with Image J , and the results are expressed as ratios of Notch1, I κ B, MMP-9, ADAM-12, ADAM-15 to GAPDH densitometry readings.

Statistical analysis

Quantitative data are expressed as mean \pm standard error of the mean. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test using SPSS 17.0 software. The count data were statistically analyzed with *t* test and χ^2 test. Values of *p* < 0.05 were considered statistically significant.

RESULTS

Anti-inflammatory effect of KFYC on IUA

As shown in Figure 1 A, compared with the control group, serum IL-2 in IUA group was higher (*p* < 0.05). In the 3 KFYC groups, the serum concentrations of IL-2 were significantly decreased, when compared with the IUA group (*p* < 0.05). Figure 1 B shows that the IUA group had a significant decrease in the serum IL-10 concentrations (*p* < 0.01). There were significant increases the serum concentrations of IL-10 in the 3 KFYC groups, when compared with the IUA group (*p* < 0.05 or *p* < 0.01).

Table 1: Primers used for RT-qPCR

Gene	Primer	Sequence 5'-3'
Notch1	Forward primer	5'-CTGCCTTCGTGCTCCTGTTCTTT-3'
	Reverse primer	5'-GAGGGGTTCTCTCCGTTCTTCT-3'
RBP-JK	Forward primer	5'-TTCCACCAGCCTTACCTTTACC-3'
	Reverse primer	5'-GTGCTGGCGTTTGTGTAACCTC-3'
ADAM12	Forward primer	5'-GGAAAGGCCCACTGATGAG-3'
	Reverse primer	5'-ATTGAGGGGCTGCTGATG-3'
ADAM15	Forward primer	5'-GAAGCTTAGACAATATGGCATCCA-3'
	Reverse primer	5'-AATTCAGTGGGTCCCAGGTACTAC-3'
β -actin	Forward primer	5'-CAGGCTGTGTTGCCCTGTA-3'
	Reverse primer	5'-CTCTCAGCTGTGGTGGTCAA-3'

Interestingly, the 1.17 mg/kg KFYC group had significantly increased serum concentrations of IL-10. This indicates that KFYC had better anti-inflammatory effect than FKQJT.

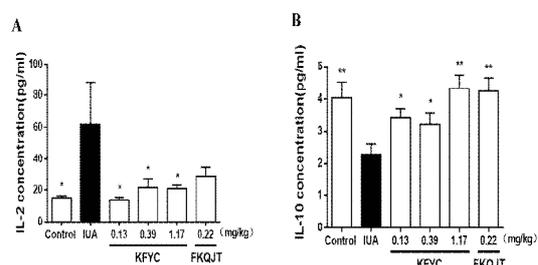


Figure 1: The effect of KFYC on serum concentrations of IL-2 and IL-10 in IUA rats. A: IL-2; B: IL-10. $p < 0.05$, $^{**} p < 0.01$, compared with IUA group

Histological results

Uterine tissue from the control group showed that architecture with intact endothelium and patent wide uterine cavity with different layers (Figures 2A and 2a). In the IUA group, the uterine cavity was widely opened. Under the epithelium, there was polymorphonuclear cell infiltration. Part of endometrium was barred from absence of mucosal folds, and there was a moderate increase in collagen fibers deposition under the endometrium and in the stroma surrounding uterine glands (Figures 2B, 2b, 3B and 3b). In the KFYC groups, the uterine cavity was narrowed down, epithelium polymorphonuclear cell infiltration was decreased, part of endometrium recovered mucosal folds, and the collagen fibers deposition under the endometrium was comparatively alleviated (Figure 2 C to E, Figure 3 C to E). Results of semi-quantitative analysis of fibrosis (Figure 4) revealed that the area of collagen fibers in the groups treated with 0.13 or 1.17 mg/kg KFYC were significantly lower than in the untreated IUA group ($p < 0.01$ or $p < 0.05$).

Effect of KFYC on expressions of Notch1, RBP-JK, ADAM-12 and ADAM-15 mRNA

As shown in Figure 5, when compared with the control group, the relative mRNA expression levels of Notch1, RBP-JK, ADAM-12 and ADAM-15 were significantly increased in the IUA groups ($p < 0.01$). However, KFYC decreased the relative mRNA expressions of Notch1, ADAM-12 and ADAM-15, when compared with the IUA group ($p < 0.05$ or $p < 0.01$). In addition, the relative mRNA expression of RBP-JK was decreased in the 0.39mg/kg and 1.17mg/kg KFYC groups ($p < 0.01$ or $p < 0.05$).

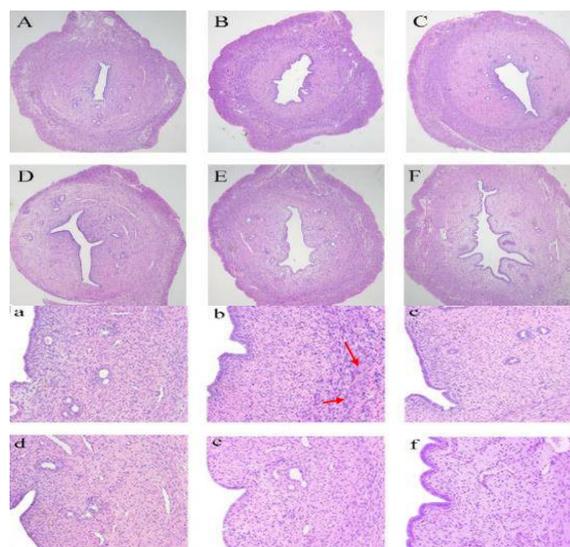


Figure 2: Photomicrographs showing variations in the uterus after KFYC treatment in the IUA rats (The red arrows represent the positive areas of H & E staining). A and a: Control group ; B and b: IUA group; C and c: Low-dose KFYC group (0.13 mg/kg); D and d: Medium-dose KFYC group (0.39 mg/kg); E and e: High-dose KFYC group (1.17 mg/kg) F and f: FKQJT group (0.22 mg/kg). Upper panels scale bar = 500 μ m, lower panel scale bar = 100 μ m

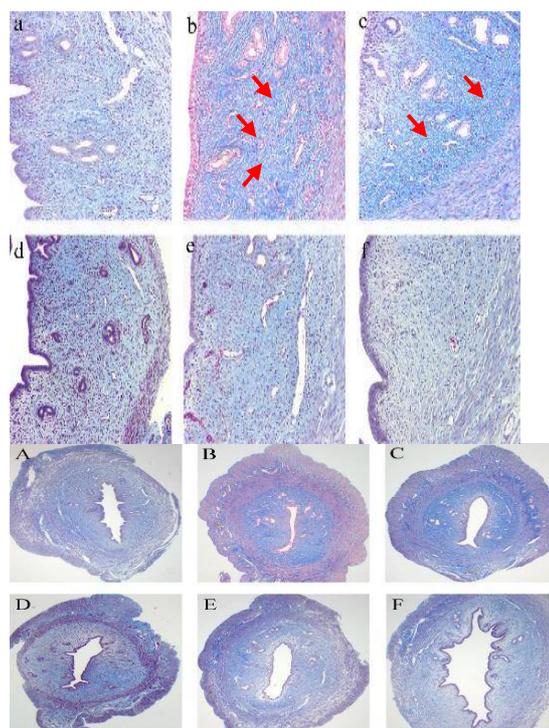


Figure 3: Photomicrographs of rat' uterus of all groups after Masson staining (The red arrows represent positive areas of Masson staining). A and a: Control group; B and b: IUA group; C and c: Low-dose KFYC group (0.13 mg/kg); D and d: Medium-dose KFYC group (0.39 mg/kg); E and e: High-dose KFYC group (1.17 mg/kg); F and f: FKQJT group (0.22 mg/kg). Upper panels scale bar = 500 μ m, lower panel scale bar = 100 μ m

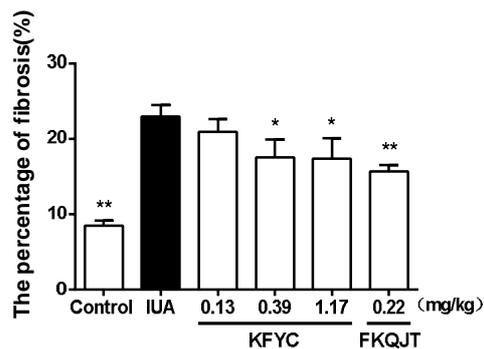


Figure 4: Semi-quantitative analysis of fibrosis; * $p < 0.05$, ** $p < 0.01$, compared with IUA group (n = 4)

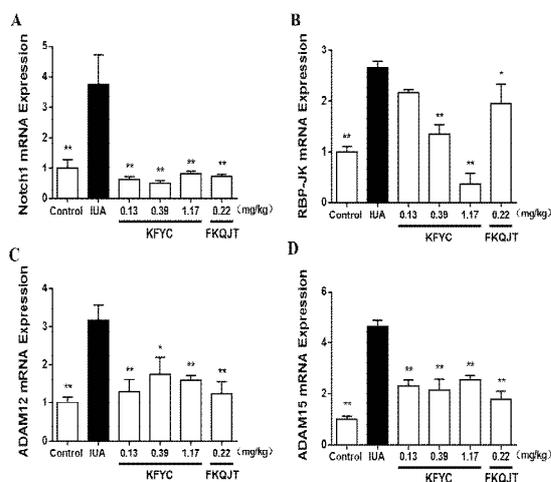


Figure 5: Effect of KFYC on the relative mRNA expressions of Notch1, RBP-JK, ADAM-12 and ADAM-15 in the IUA rats. A: Notch1; B: RBP-JK; C: ADAM-12; D: ADAM-15; * $p < 0.05$, ** $p < 0.01$, compared with IUA group (n = 4)

Effect of KFYC on Notch1, MMP-9, IκB, ADAM-12 and ADAM-15 protein expression

Results from RT-qPCR showed that the Notch1/ADAM pathway was associated with IUA. In order to further investigate the exact role of KFYC in the IUA model, the protein expressions of Notch1, MMP-9, IκB, ADAM-12, and ADAM-15 were determined by Western blot assay. As shown in Figure 6, the protein expressions of Notch1, MMP-9, ADAM-12, and ADAM-15 in the IUA group were increased, when compared with control group, but, the protein expression of IκB was decreased, when compared with control group. However, after treatment with KFYC, the protein expressions of Notch1, MMP-9, ADAM-12, and ADAM-15 were decreased, while the protein expression of IκB was increased, relative to the IUA group.

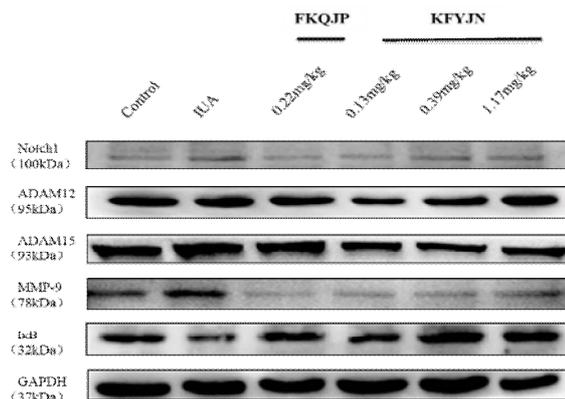


Figure 6: Effect of KFYC on protein densities of Notch1, MMP-9, IκB, ADAM-12 and ADAM-15 in IUA rats

DISCUSSION

Although clinical treatment of IUA restores the anatomical morphology of the uterine cavity, the recovery of endometrial function and prevention of adhesion relapse remain as serious challenges [8]. It is necessary to have an animal model that is similar to the human model with which to study the pathogenesis and treatment strategies of IUA.

Twenty-five percent phenol mucilage, which is widely used for female sterilization was used to induce IUA in a rat model in the present study [13]. The IUA model induced with phenol mucilage has a clear advantage over other methods e.g. mechanical injury, because it is easy to control the degree of damage when using liquid phenol mucilage in IUA model [16]. As shown in the results of H & E staining, the endometrial glands disappeared completely, and infiltration of inflammatory cells and fibroblasts increased in the uterus of the IUA group. The result of Masson staining showed that the positive areas which identified the area of fibrosis had increased in the IUA group. Thus, a successful establishment of IUA by phenol mucilage in a rat model was achieved. This study showed that KFYC reduced the number of endometrial glands, decreased collagen fibers deposition under the endometrium, and lessened infiltration of inflammatory cells, suggesting that KFYC has good anti-inflammatory effects.

Currently, endometrial fibrosis is recognized as a pathological basis for IUA, and Masson staining is the best way to detect the degree of fibrosis [3,17]. In this study, Masson staining was used to check the degree of fibrosis, and Image Pro Plus was used to analyze the Masson-stained sections. The fibrotic areas are usually stained blue. From the results, the fibrotic area was

significantly increased in IUA group, and significantly decreased after KFYC treatment, relative to control group. This suggests that KFYC ameliorated the degree of fibrosis in the IUA model.

The IUA is commonly caused by trauma or infection [3,18]. An understanding of the causes and related molecular mechanisms of IUA is essential for the prevention and treatment of IUA. The early stage of IUA is an inflammatory process, and it has been reported that inflammatory factors play important roles in the pathogenesis of IUA [6,7]. The nine-member family of IL-10 cytokines appears before the adaptive immune response and plays important roles in many inflammatory diseases [19,20]. Interleukin-10 (IL-10) is the most important anti-inflammatory cytokine. It represses pro-inflammatory responses and controls tissue disruptions caused by inflammation [21]. Interleukin-2 (IL-2) is a multiple-effect cytokine first considered to be a T-cell growth factor [22]. It is a pro-inflammatory cytokine which is produced by B-cells. It has since been reported to drive regulatory T cell (Treg) differentiation as well as natural killer cell-mediated cytotoxicity [23], and activation-induced cell death (AICD) [24,25]. Rat serum concentrations of IL-2 and IL-10 in the different groups were determined in the present study. The concentrations of IL-2 were significantly decreased in KFYC groups, while the concentrations of IL-10 were significantly increased in KFYC groups, when compared with IUA group. Thus, KFYC alleviated the inflammation in the IUA rats.

The Notch signaling plays a key role in cell differentiation, survival, and proliferation through diverse mechanisms [26]. Recently, increasing evidence has shown that Notch signaling is associated with inflammation [15]. To date, active Notch signaling has been observed under a variety of inflammatory conditions including rheumatoid arthritis [27,28], systemic lupus erythematosus [29,30], and bacterial and viral infections [23,31]. The RBP-JK is a key nuclear mediator of the canonical Notch pathway. In this study, the translational level of Notch1 and the transcriptional level of Notch1 and RBP-JK were significantly increased in the IUA model group, but after the treatment with KFYC, the protein expression level of Notch1 and the relative mRNA expressions of Notch1 and RBP-JK were decreased, when compared with the IUA model group. This finding revealed that the KFYC might exert anti-IUA effects via the Notch pathway.

The ADAM proteins are a family of transmembrane proteins which contain a disintegrin and metalloprotease domains and perform important functions in adhesion and proteolytic processing [29,32]. Currently, the ADAM protein family has 29 members. Studies have revealed that ADAM-12 is a proteolytic member of the ADAM family, involved in the pathogenesis of various cancers, as well as liver fibrogenesis [33]. Some researchers have demonstrated that the expression of ADAM-12 is also activated by Notch [34]. Indeed Notch activates RBP-JK and then stimulates the activity of inhibitor of nuclear factor kappa-B kinase, thereby increasing the degradation of NF- κ B inhibitors, which results in NF- κ B activation [34].

This activation results in reduced transcription of miR-29, and thus increases the transcription of ADAM-12. It has been shown that ADAM-15 is the only member of the ADAM family with the integrin binding motif Arg-Gly-Asp (RGD) in its disintegrin-like domain [35]. It plays an important role in cell-cell and cell-matrix interactions and in the proteolysis of molecules on the cell surface or the extracellular matrix. It also participates in the body inflammatory process [35-37]. In the early stages of endometrial trauma, ADAM-12 and ADAM-15, through cell-cell and cell-extracellular matrix interactions adhere more hemamoeba thus enhancing inflammation. In this study, it was found that the translational and transcriptional levels of ADAM-12 and ADAM-15 decreased after the treatment with KFYC. This study has revealed that ADAM-12 and ADAM-15 might play key roles in the treatment of IUA. Further investigations will be necessary to determine if other family members also play anti-IUA roles.

CONCLUSION

These results indicate that KFYC reduces the number and morphology of endometrial glands, ameliorates inflammatory cell infiltration and reduces the degree of fibrosis and inflammation in a rat model of IUA. Thus KFYC exerts an anti-IUA effect by amelioration the degree of uterine inflammation and fibrosis in the uterus through the Notch1/ADAM signaling pathway.

DECLARATIONS

Acknowledgement

This work was supported by Long-Range Pharmaceutical Co. Ltd (no. 40116075). Thanks to Dr. Bill Milne for modifying the manuscript.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hong Nie designed the experiment and revised the paper. Qinghong Fan, Wei Xiao, Xiaomeng Chai, Zhe Zhang and Tao Zhu performed the experiments while Qinghong Fan, Wei Xiao, Dan Tang, Kaihe Ye, Tianjun Luo, Qing Liu, Gang Huang and Yulan Yang collected and analyzed the data. In addition, Qinghong Fan and Wei Xiao prepared the manuscript.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

- Asherman JG. Amenorrhoea traumatica (atretica). *J Obstet Gynaecol Br Emp* 1948; 55: 23-30.
- Asherman JG. Traumatic intra-uterine adhesions. *Bjog An Int J Obstet Gynaecol* 1950; 57: 892-896.
- Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman syndrome-one century later. *Int J Fertil Steril* 2008; 89: 759-779.
- March CM. Update: Intrauterine adhesions. *Obstet Gynecol Clin North Am* 1995; 22: 491-505.
- Liu F, Zhu ZJ, Li P, He YL. Creation of a female rabbit model for intrauterine adhesions using mechanical and infectious injury. *J Am Acad Orthop Surg Glob Res Rev* 2013; 183: 296-303.
- Sillem M, Prifti S, Monga B, Arslan T, Runnebaum B. Integrin-mediated adhesion of uterine endometrial cells from endometriosis patients to extracellular matrix proteins is enhanced by tumor necrosis factor alpha (tnf alpha) and interleukin-1 (Il-1). *Eur J Obstet Gynecol Reprod Biol* 1999; 87: 123-127.
- Tao Z, Duan H. expression of adhesion-related cytokines in the uterine fluid after transcervical resection of adhesion. *Chinese Journal of Obstetrics and Gynecology* 2012; 47: 734.
- Bosteels J, Weyers S, Kasius J, Broekmans FJ, Mol BW, D'hooghe TM. Anti-adhesion therapy following operative hysteroscopy for treatment of female subfertility. *Cochrane Database Syst Rev* 2015; 11: CD011110.
- Thomson AJ, Abbott JA, Deans R, Kingston A, Vancaillie TG. The management of intrauterine synechiae. *Curr Opin Obstet Gynecol* 2009; 21: 335.
- Sun HM, Li JJ, Wang ZX, Wang FF. The effect of Kang Fu Yan capsule on the treatment of chronic pelvic inflammatory disease was treated with the treatment of large blood cane soup enema and microwave. *Hebei Journal of Traditional Chinese Medicine* 2014; 1504-1505.
- Zhang SY, Jiang YL, Shen YA. Determination of matrine and matrine in the capsule of Kang Fu Yan Capsule. *Lishizhen Medicine and Materia Medica Research* 2006; 17: 728-729.
- Zhang WL, Lei WF. The treatment of 54 cases of chronic pelvic inflammation with antiphlogistic capsule and traditional Chinese medicine enema. *Shanxi Journal of Traditional Chinese Medicine* 2010; 31: 784-785.
- Richart RM. Female sterilization using chemical agents. *Res Front Fertil Regul* 1981; 1: 1.
- Office of Laboratory Animal Welfare/National Institutes of Health. 2015. Public Health Service policy on human care and use of laboratory animals. National Institutes of Health, Bethesda, Maryland. Available from: <https://olaw.nih.gov/policies-laws/phs-policy.htm>.
- Shang Y, Smith S, Hu X. Role of notch signaling in regulating innate immunity and inflammation in health and disease. *Protein Cell* 2016; 7: 159-174.
- Wang X, Ma N, Sun Q, Huang C, Liu Y, Xin L. Elevated nf-kb signaling in asherman syndrome patients and animal models. *Oncotarget* 2017; 8: 15399-15406.
- Semedo P, Cenedeze CCM. Mesenchymal stem cells attenuate renal fibrosis through immune modulation and remodeling properties in a rat remnant kidney model. *Stem Cells* 2009; 27: 3063-3073.
- Taylor PJ, Cumming DC, Hill PJ. Significance of intrauterine adhesions detected hysteroscopically in eumenorrhoeic infertile women and role of antecedent curettage in their formation. *Am J Obstet Gynecol* 1981; 139: 239-242.
- Howard M, O'garra A, Ishida H, Malefyt RDW, Vries JD. Biological properties of interleukin 10. *Immunol Today* 1992; 12: 239-247.
- Opal SM, Wherry JC, Grnt P. Interleukin-10: Potential benefits and possible risks in clinical infectious diseases. *Clin Infect Dis* 1998; 27: 1497-1507.
- Boshtam M, Asgary S, Kouhpayeh S, Shariati L, Khanahmad H. Aptamers against pro- and anti-inflammatory cytokines: A review. *Inflammation* 2016; 40: 1-10.
- Cote-Sierra J, Foucras G, Guo L, Chiodetti L, Young HA, Hu-Li J. Interleukin 2 plays a central role in Th2 differentiation. *Proc Natl Acad Sci U S A* 2004; 101: 3880.

23. Ito T, Schaller M, Hogaboam CM, Standiford TJ, Sandor M, Lukacs NW, Kunkel SL. Tlr9 regulates the mycobacteria-elicited pulmonary granulomatous immune response in mice through dc-derived notch ligand delta-like 4. *J Clin Invest* 2009; 119: 33.
24. Bao Y, Cao X. The immune potential and immunopathology of cytokine-producing b cell subsets: A comprehensive review. *J Autoimmun* 2014; 55: 10-23.
25. Liao W, Lin JX, Leonard WJ. Il-2 family cytokines: New insights into the complex roles of Il-2 as a broad regulator of t helper cell differentiation. *Curr Opin Immunol* 2011; 23: 598-604.
26. Hoyne GF. Notch signaling in the immune system. *Immunity* 2003; 74: 971-981.
27. Ando K, Kanazawa S, Tetsuka T, Ohta S, Jiang X, Tada T, Okamoto T. Induction of notch signaling by tumor necrosis factor in rheumatoid synovial fibroblasts. *Oncogene* 2003; 22: 7796-7803.
28. Nakazawa M, Ishii H, Aono H, Takai M, Honda T, Aratani S, Kobata T. Role of notch-1 intracellular domain in activation of rheumatoid synoviocytes. *Arthritis Rheum* 2001; 44: 1545-1554.
29. Edwards DR, Handsley MM, Pennington CJ. The adam metalloproteinases. *Mol Aspects Med* 2008; 29: 258.
30. Murea M, Park JK, Sharma S, Kato H, Gruenwald A, Niranjana T, Melamed ML. Expression of notch pathway proteins correlates with albuminuria, glomerulosclerosis, and renal function. *Kidney Int* 2010; 78: 514-522.
31. Narayana Y, Balaji KN. Notch1 up-regulation and signaling involved in mycobacterium bovis bcg-induced socs3 expression in macrophages. *J Biol Chem* 2008; 283: 12501-12511.
32. Seals DF, Courtneidge SA. The adams family of metalloproteases: Multidomain proteins with multiple functions. *Genes Dev* 2003; 17: 7-30.
33. Nyrenerickson EK, Jones JM, Srivastava DK, Mallik S. A disintegrin and metalloproteinase-12 (adam12): Function, roles in disease progression, and clinical implications. *Biochim Biophys Acta* 2013; 1830: 4445-4455.
34. Li H, Solomon E, Muggy SD, Sun D, Zolkiewska A. Metalloprotease-disintegrin adam12 expression is regulated by notch signaling via microma-29. *J Biol Chem* 2011; 286: 21500.
35. Langer H, May AE, Bültmann A, Gawaz M. Adam 15 is an adhesion receptor for platelet gpiib-iiiA and induces platelet activation. *Thromb Haemost* 2005; 94: 555.
36. Lu D, Scully M, Kakkar V, Lu X. Adam-15 disintegrin-like domain structure and function. *Toxins* 2010; 2: 2411.
37. Martin J, Eynstone LV, Davies M, Williams JD, Steadman R. The role of adam 15 in glomerular mesangial cell migration. *J Biol Chem* 2002; 277: 33683.