Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Effects of Kangfuxinye on NF-κB and inflammatory cytokines in gingival crevicular fluid of patients with orthodontic gingivitis caused by orthodontic treatment

Hui Li*, Yan Chen, Weiguo Sun

Department of Stomatology, The First Affiliated Hospital of Anhui University of Science and Technology (The First People's Hospital of Huainan), Huainan, 232007, Anhui Province, China

ARTICLE INFO

Original paper

Article history:
Received: December 09, 2022
Accepted: February 11, 2023
Published: February 28, 2023

Keywords:

Kangfuxinye, orthodontic gingivitis, NF-κB, inflammatory cytokine

ABSTRACT

Explore the Kangfuxinye effection on the expressions of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and inflammatory cytokines (IC) in the gingival crevicular fluid of patients with orthodontic gingivitis caused by orthodontic treatment. 98 patients with orthodontic gingivitis in Qingdao Stomatological Hospital caused by orthodontic treatment were divided into two groups, namely, the control treatment group and the Kangfuxinye treatment group. In this study, the expressions of those proteins and IC in gingival crevicular fluid before and after treatment were analyzed at first, and the correlations of the NF-κB p65 expression with IC were explored. Then the differences in the expressions of those proteins and IC and the efficacy between the control treatment group and the Kangfuxinye treatment group were analyzed. Compared with those before treatment, the expressions of NF-κB-related proteins and IC interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α) and vascular endothelial growth factor (VEGF)] were significantly decreased after treatment (p<0.05). After treatment, the expression of NF-κB p65 was positively correlated with IL-1β, TNF-α and VEGF, but negatively related to IL-4 and IL-10. In addition, compared with the control treatment, Kangfuxinye significantly reduced the expressions of those proteins and their messenger ribonucleic acids (mRNAs) (p<0.05), decreased the expressions of IL-1β, TNF-α and VEGF (p<0.05) but improved the total effective rate of treatment. Kangfuxinye can reduce the NF-κB expressions and IC in the gingival crevicular fluid of patients with orthodontic gingivitis caused by orthodontic treatment and enhance the efficacy.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.2.15

Introduction

Orthodontic treatment is an adaptive biological response that interferes with maxillofacial structures by exerting forces on the outside (1). The mechanical force is applied to promote periodontal tissue remodeling, and tooth dynamics during bone reconstruction is a biological mechanism involved in acute inflammatory responses (2). Tissue changes induced by an external force, combined with the recombination of cells and extracellular matrix, lead to the synthesis and release of neurotransmitters, cytokines, growth factors, and arachidonic acid metabolites, and so on (3).

As tiny protein molecules, inflammatory cytokines (IC) have a great role in regulating cell communication and function, which could induce cell proliferation and differentiation (4, 5). Interleukin-1 (IL-1) mainly exists in two forms: IL-1α and IL-1β, among which IL-1β is related to bone metabolism. In the initial stage of orthodontic tooth movement (OTM), IL-1β is one of the most effective cytokines in the periodontal environment. IL-1β is mainly secreted by fibroblasts, osteoblasts and osteoclasts (6). Studies have shown that cytokine release is involved in the tissue response to mechanical stimulation at the early stage of OTM. This mechanical stress can lead to acute inflammation in periodontal tissues, promote tooth movement and biological processes, and induce bone resorption (7, 8). The value of gingival crevicular fluid in evaluating the biological states of the periodontal tissues, bone, and the innate tissues of related tooth structures extends to the source of biomarkers for specific clinical conditions (9). Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) has long been considered as the target of new anti-inflammatory drugs. But data from mouse genetic studies indicate that NF-κB may be a biological target for intractable inflammatory diseases. The NF-κB pathway regulates the production of pro-IC, leukocyte recruitment, which is an important contributor to the inflammatory response. Nevertheless, with the survival of epithelial cells and the integrity of mucosal barriers, NF-κB's anti-apoptosis function can prevent and maintain the inflammatory response through continuous leukocyte activation.

The main pharmacological effects of Kangfuxinye include anti-inflammation, anti-tumor, anti-oxidation and wound healing promotion. This study aims to explore the Kangfuxinye affection on the expressions of NF-κB and IC in the gingival crevicular fluid of patients with orthodontic gingivitis caused by orthodontic treatment.

Materials and Methods

Kangfuxinye was purchased from Hunan Kelun Pharmaceutical Co., Ltd., NF-κB and ELISA kit from Shanghai Guduo Biotechnology Co., Ltd., and cytokine ELISA

* Corresponding author. Email: julianyi962039@163.com

kit from Shanghai Lengton Biotechnology Co., Ltd.

Source of cases

A total of 98 patients with fixed orthodontic gingivitis admitted to the Department of stomatology, The First Affiliated Hospital of Anhui University of Science and Technology from 2018 to 2022 were selected. They were randomly divided into two groups: the control treatment group (treated with conventional gingival cleaning); the Kangfuxinye treatment group (treated with Kangfuxinye based on conventional gingival cleaning). The expressions of p50, p65, an inhibitor of NF-κB (IκBα) and IC in the gingival crevicular fluid of patients in the Kangfuxinye treatment group were measured before treatment, which was used to compare the differences before and after treatment.

Detection of the expressions of p50, p65 and IκBα in gingival crevicular fluid via ELISA

The operation was performed according to the instructions of commodities, and the ELISA detection kit was used for detection. The corresponding sample concentration was calculated according to the standard curve regression equation.

Detection of the expression of IC in gingival crevicular fluid via ELISA

The operation was performed according to the instructions of commodities, and the ELISA detection kit was used for detection. The corresponding sample concentration was calculated according to the standard curve regression equation.

Polymerase chain reaction (PCR) detection

The total ribonucleic acid (RNA) in the gingival crevicular fluid was extracted with TRIzol reagent, and the extracted nucleic acid was rapidly transcribed according to the instruction manual of the reverse transcription (RT) kit, and the messenger RNA (mRNA) expression was evaluated by PCR. Primers: NF-κB p50: ATTTCTACGCTATGTG (forward) and TCCGCCTGCTTGTAGTA (reverse), NF-κB p65: GCTCCTTCTCAAGCTGAGT (forward) and CAGAAGTTGAGTTTCGGGTAGG (reverse), IκBα: CTAGAATTCGCCATGTTCCAG (forward) and CATGAATTCTTGCACTCATACGTCAG (reverse), and β-actin: CCCTGTATGCTCTGGTC (forward) and TTTACGGATGTCAACG (reverse).

Western blotting

The protein in the gingival crevicular fluid was extracted in lysis buffer, added with a complete protease inhibitor mixture and 0.1 mM β-glycerophosphate) and determined by Bio-Rad protein assay. The nucleoprotein fraction for Western blotting analysis was obtained according to the method described by Pilon et al (10). The protein immobilized on the nitrocellulose membrane was imprinted overnight with the primary antibody at 4°C and bound with the horseradish peroxidase-bound secondary antibody, followed by color development.

Evaluation method of clinical efficacy

The evaluation indexes of efficacy mainly include: Gingival color and shape returning to normal and the depth of probing being less than 1 mm represented recovery, gingival color and shape returning to normal and the probing depth being 1.0-2.0 mm significant effectiveness, the detection depth value being 2.0-3.0 mm improvement, and the detection depth value being above 3.0 mm for ineffectiveness. Total effective rate = 1 - ineffective rate.

Statistical analysis

GraphPad 6.0 was adopted for data analysis. Two-way ANOVA was used to analyze the difference between the two groups. $p<0.05$ indicated that the difference was statistically significant, and $p<0.01$ suggested that the difference was statistically extremely significant. The correlations of the NF-κB p65 expression with IC were analyzed by linear regression and correlation analyses.

Results

NF-κB protein expressions in gingival crevicular fluid before and after treatment

In this study, the expression of the NF-κB-related protein in the Kangfuxinye treatment group and control group was analyzed using ELISA. The results manifested that NF-κB p50, NF-κB p65 and IκBα in the Kangfuxinye treatment group were remarkably reduced ($p<0.01$) (Figure 1).

Expressions of IC before and after treatment

The effects of Kangfuxinye on the expression of IC were detected by the ELISA kit. The results revealed that the expressions of IL-1β, TNF-α and VEGF in gingival

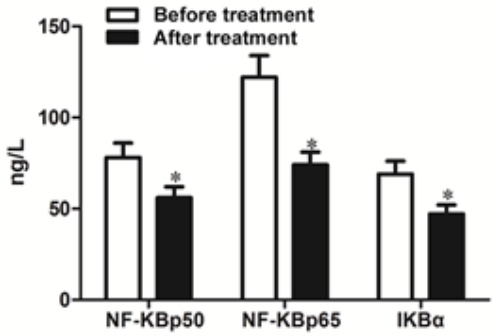

Figure 1. Detection of the expressions of NF-κB p50, NFκB p65 and IκBα in gingival crevicular fluid via ELISA. *p<0.05 represents an extremely significant difference.

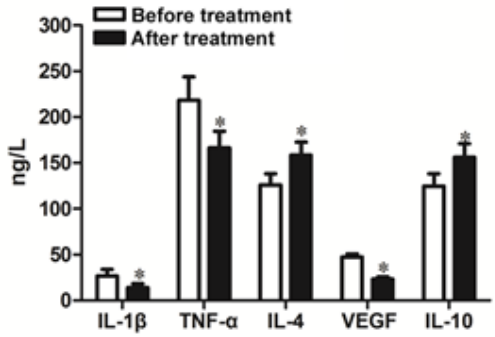

Figure 2. Effects of Kangfuxinye on the expression of inflammatory cytokines in the gingival crevicular fluid of orthodontic gingivitis patients caused by orthodontic treatment. *p<0.05 represents a significant difference.

crevicular fluid in the Kangfuxinye treatment group were notably decreased compared with those in the control treatment group (p<0.05), while the expressions of IL-4 and IL-10 were markedly increased (p<0.05) (Figure 2).

Correlation analysis of the NF-κB p65 expression with IC after treatment

The relationship between the NF-κB p65 expression level and the cytokine expression in the gingival crevicular fluid of 49 patients with fixed orthodontic tooth caries after treatment was analyzed. The results of linear regression and correlation analyses showed that the NF-κB p65 expression in cytokines had different correlations with different cytokines, among which it was significantly positively related to IL-1β, TNF-α and VEGF (p<0.001, r^2=0.7491, 0.4164 and 0.3269, respectively) (Figure 3A-3C), but significantly negatively associated with IL-4 and IL-10 (p<0.001, r^2=0.517 and 0.5895, respectively) (Figure 3D-3E).

Effects of Kangfuxinye on the NF-κB-related protein expression after treatment

NF-κB-related proteins in the blood of patients treated with Kangfuxinye were further examined using RT-PCR and Western blotting. According to the results (Figure 4), the expressions of NF-κB-related proteins and their mRNAs were significantly reduced in the Kangfuxinye treatment group compared with those in the control treatment group (p<0.05).

Effects of Kangfuxinye and control treatment on the expression of IC

Furthermore, inflammatory cytokine-related proteins in the blood of patients treated with Kangfuxinye were examined via ELISA, and the effects of Kangfuxinye treatment on them were analyzed. The results indicated that Kangfuxinye treatment reduced the expressions of pro-

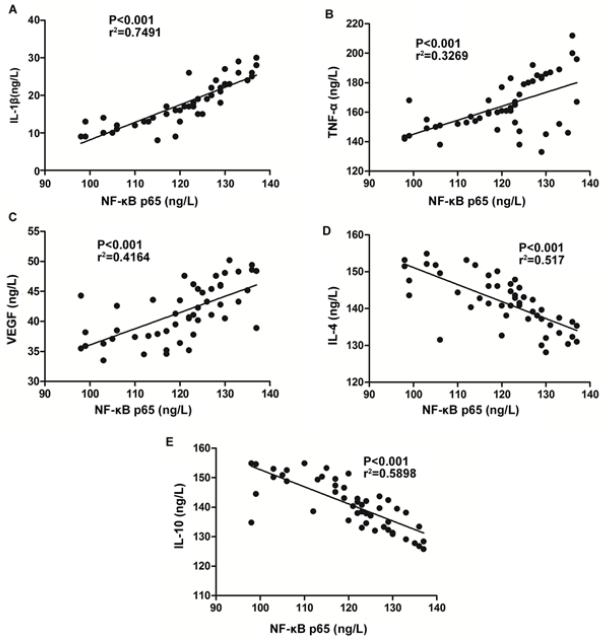

Figure 3. . Correlation analysis of the NF-κB p65 expression with inflammatory cytokines after treatment A-E represents the expressions of IL-1β, TNF-α, VEGF, IL-4, IL-10 and NF-κB p65, respectively. p<0.001 indicates a significant correlation.

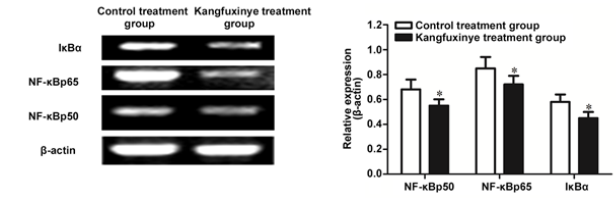

Figure 4. Detection of the expressions of NF-κB p50, NF-κB p65 and IκBα and their mRNAs via Western blotting and RT-PCR. *p<0.05 represents a significant difference.

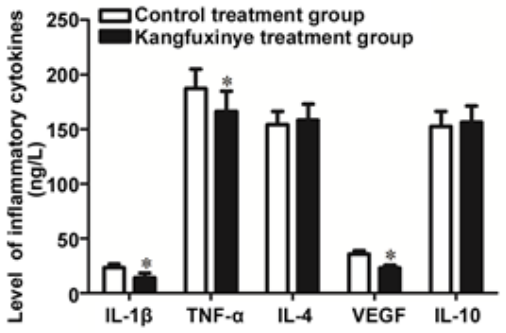

Figure 5. Changes in inflammatory cytokines after treatment with Kangfuxinye detected by ELISA. *p<0.05 represents a significant difference.

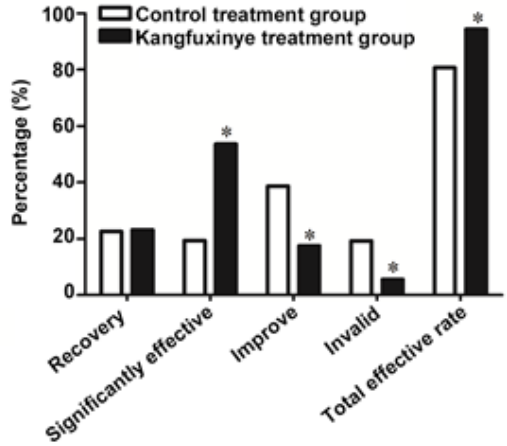

Figure 6. Efficacy analysis of Kangfuxinye in the treatment of fixed orthodontic tooth caries. *p<0.05 represents a significant difference.

IC (IL-1β, TNF-α and VEGF) (p<0.05). It promoted the increases of anti-IC (IL-4 and IL-10), but the differences were not significant (p<0.05) (Figure 5).

Clinical efficacy of Kangfuxinye on patients

Finally, the efficacy of Kangfuxinye in the treatment of fixed orthodontic tooth caries was analyzed. It was found that compared with those in the control group, the total effective rate of treatment was improved (control treatment group: 80.67% vs. Kangfuxinye treatment group: 94.38%) and the ineffective rate was reduced (control treatment group: 19.33% vs. Kangfuxinye treatment group: 5.62%) in Kangfuxinye treatment group (Figure 6).

Discussion

In fixed orthodontics, the ideal state is to produce the highest rate of OTM without irreversible damage to the

root or periodontal ligament (PDL) (11). This study aims to investigate the Kangfuxinye effects on NF-κB-related proteins expressions and IC in the gingival crevicular fluid of patients with orthodontic gingivitis caused by orthodontic treatment. Compared with oral cleaning treatment alone, Kangfuxinye treatment significantly reduces the levels of NF-κB-related proteins expression and IC and brings higher efficacy.

Cytokine content and tooth movement rate depend on the efficiency of alveolar bone reconstruction because osteoclast fusion, survival and activation correspond to it (12). In addition, IL-1β is related to bone resorption, induces the receptor activator of NF-κB (RANKL) expression in PDL and osteoblasts cells, and stimulates osteoclast precursors differentiation (13). In our study, NF-κB level in gingival crevicular fluid during OTM was evaluated, which might be a valuable biological marker and also play a major role in the inflammatory process related to OTM. NF-κB has been considered a typical pro-inflammatory pathway (14, 15). However, as a complex physiological process, the role of NF-κB in the inflammatory response cannot be inferred from in-vitro studies (16). At present, most of the studies on inflammatory signal transduction focus on the IL-1 and TNF receptor families (17). IL-1 and TNF-α are prototyped pro-IC released rapidly when the tissue is damaged or infected (18). NF-κB also plays a great role in cartilage degradation. The expressions of TNF-α, IL-6 and IL-1 mediated by NF-κB in chondrocytes can enhance matrix metalloproteinase (MMP) production, regulate proteoglycan synthesis, and enhance NF-κB activation via positive feedback loops (19). Moreover, the inhibition of NF-κB in chondrocytes can lead to the decreased expression of MMP-3/13 induced by IL-1 (20). Furthermore, TNF-α has the strongest anti-tumor effector among IC (21, 22). It can enhance the differentiation and proliferation of T lymphocytes and stimulate monocytes phagocytosis of and neutrophils (23-25). The comparative observation before and after treatment found that fixed orthodontics could increase the content of pro-IC, and some studies have speculated that it may be mainly due to the distortion of the anterior PDL reconstructed by the bone. However, after treatment, the pro-IC was significantly decreased, but the anti-IC expression was raised. It might cause a reduced biological response in the periodontal tissue on the experimental side. Studies have revealed that bone remodeling induces the releases of IL-1β, TNF-α and VEGF in PDL, which up-regulates RANKL and MMPs through osteoblasts during OTM (26). RANKL could stimulate mononuclear precursor cells to enter the bone surface and degrade the function and formation of osteoclasts with a mineralized matrix (27). Besides, the correlation analysis showed that NF-κB-related proteins were positively correlated with the expression of pro-IC, but negatively related to the expression of anti-IC. NF-κB-related proteins are correlated with IC, inflammatory responses and bone movement after fixed orthodontic treatment.

In the contrast treatment experiment, the levels of IL-1β, TNF-α and VEGF in the Kangfuxinye treatment group were further reduced compared with those in the control treatment group. The main ingredient of Kangfuxinye is the extract from Periplaneta. Studies have confirmed that it has a significant role in eliminating infection and inflammation. It can improve tissue necrosis and promote blood circulation. In addition, it can enhance immunity and promote the metabolism of necrotic cells (28). This study manifested that Kangfuxinye not only reduced the expressions of IC, IL-1β and TNF-α but also significantly enhanced the efficacy and improved periodontal conditions.

In a word, NF-κB and IC are closely related to tooth root resorption. Although its internal mechanism is not clear, the contents described in this study have strong practical significance. The detailed analysis to identify the degree and severity of this phenomenon needs further study.

References

1. Sandler J. Handbook of Orthodontics 2nd Edition. J Orthod 2016; 43: 157.
2. Czerniuk MR, Gorska R, Filipiak KJ, Opolski G. Inflammatory response to acute coronary syndrome in patients with coexistent periodontal disease. J Periodontol 2004; 75: 1020-1026.
3. Luppanapornlarp S, Kajii TS, Surarit R, Iida J. Interleukin-1beta levels, pain intensity, and tooth movement using two different magnitudes of continuous orthodontic force. Eur J Orthod 2010; 32: 596-601.
4. Sun AY, Hinck B, Cohen BR, Keslar K, Fairchild RL, Monga M. Inflammatory Cytokines in the Papillary Tips and Urine of Nephrolithiasis Patients. J Endourol 2018; 32: 236-244.
5. Cardoso EM, Reis C, Manzanares-Cespedes MC. Chronic periodontitis, inflammatory cytokines, and interrelationship with other chronic diseases. Postgrad Med 2018; 130: 98-104.
6. Han Y, You X, Xing W, Zhang Z, Zou W. Paracrine and endocrine actions of bone-the functions of secretory proteins from osteoblasts, osteocytes, and osteoclasts. Bone Res 2018; 6: 16.
7. Kirschneck C, Fanghanel J, Wahlmann U, Wolf M, Roldan JC, Proff P. Interactive effects of periodontitis and orthodontic tooth movement on dental root resorption, tooth movement velocity and alveolar bone loss in a rat model. Ann Anat 2017; 210: 32-43.
8. Hamamci N, Acun Kaya F, Uysal E, Yokus B. Identification of interleukin 2, 6, and 8 levels around miniscrews during orthodontic tooth movement. Eur J Orthod 2012; 34: 357-361.
9. Diomede F, Gugliandolo A, Scionti D, Merciaro I, Cavalcanti MF, Mazzon E, Trubiani O: Biotherapeutic Effect of Gingival Stem Cells Conditioned Medium in Bone Tissue Restoration. Int J Mol Sci 2018; 19: 329.
10. Cui JJ, Wang XX, Wang Y, Chen PP, Ma D, Zhang J. Effect of akebiasaponin D with different concentrations on orthodontic tooth movement in rats. Shanghai Kou Qiang Yi Xue 2018; 27: 129-134.
11. Singh A, Gill G, Kaur H, Amhmed M, Jakhu H: Role of osteopontin in bone remodeling and orthodontic tooth movement: a review. Prog Orthod 2018; 19: 18.
12. Kobayashi Y, Udagawa N. Mechanisms of alveolar bone remodeling. Clin Calcium 2007; 17: 209-216.
13. Francica BJ, Ghasemzadeh A, Desbien AL, Theodros D, Sivick KE, Reiner GL, Hix Glickman L, Marciscano AE, Sharabi AB, Leong ML. TNFalpha and Radioresistant Stromal Cells Are Essential for Therapeutic Efficacy of Cyclic Dinucleotide STING Agonists in Nonimmunogenic Tumors. Cancer Immunol Res 2018; 6: 422-433.
14. Wang W, Wu H, Yu H, Zhang X, Cui G, Wang K, Mao S, Pan Y. Typhonium giganteum Lectin Exerts A Pro-Inflammatory Effect on RAW 264.7 via ROS and The NF-kappaB Signaling Pathway. Toxins (Basel) 2017; 9.
15. Wu H, He M, Yang R, Zuo Y, Bian Z. Astrocyte elevated gene-1 participates in the production of pro-inflammatory cytokines in dental pulp cells via NF-kappaB signalling pathway. Int Endod J 2018; 51: 1130-1138.

16. Kouba DJ, Nakano H, Nishiyama T, Kang J, Uitto J, Mauviel A. Tumor necrosis factor-alpha induces distinctive NF-kappa B signaling within human dermal fibroblasts. J Biol Chem 2001; 276: 6214-6224.
17. Lobito AA, Ramani SR, Tom I, Bazan JF, Luis E, Fairbrother WJ, Ouyang W, Gonzalez LC. Murine insulin growth factor-like (IGFL) and human IGFL1 proteins are induced in inflammatory skin conditions and bind to a novel tumor necrosis factor receptor family member, IGFLR1. J Biol Chem 2011; 286: 18969-18981.
18. Orlando S, Matteucci C, Fadlon EJ, Buurman WA, Bardella MT, Colotta F, Introna M, Mantovani A. TNF-alpha, unlike other pro- and anti-inflammatory cytokines, induces rapid release of the IL-1 type II decoy receptor in human myelomonocytic cells. J Immunol 1997; 158: 3861-3868.
19. Liang Y, Chen S, Yang Y, Lan C, Zhang G, Ji Z, Lin H. Vasoactive intestinal peptide alleviates osteoarthritis effectively via inhibiting NF-kappaB signaling pathway. J Biomed Sci 2018; 25: 25.
20. Wang M, Shen G, Xu L, Liu X, Brown JM, Feng D, Ross RA, Gao B, Liangpunsakul S, Ju C. IL-1 receptor like 1 protects against alcoholic liver injury by limiting NF-kappaB activation in hepatic macrophages. J Hepatol 2017.
21. Luna T, Santos SB, Nascimento M, Porto MA, Muniz AL, Carvalho EM, Jesus AR. Effect of TNF-alpha production inhibitors on the production of pro-inflammatory cytokines by peripheral blood mononuclear cells from HTLV-1-infected individuals. Braz J Med Biol Res 2011; 44: 1134-1140.
22. Akanda MR, Kim IS, Ahn D, Tae HJ, Nam HH, Choo BK, Kim K, Park BY. Anti-Inflammatory and Gastroprotective Roles of Rabdosia inflexa through Downregulation of Pro-Inflammatory Cytokines and MAPK/NF-kappaB Signaling Pathways. Int J Mol Sci 2018; 19.
23. Derouich-Guergour D, Aldebert D, Vigan I, Jouvin-Marche E, Marche PN, Aubert D, Ambroise-Thomas P, Pelloux H. Toxoplasma gondii infection can regulate the expression of tumour necrosis factor-alpha receptors on human cells in vitro. Parasite Immunol 2002; 24: 271-279.
24. Pietruczuk K, Lisowska KA, Grabowski K, Landowski J, Witkowski JM. Proliferation and apoptosis of T lymphocytes in patients with bipolar disorder. Sci Rep 2018; 8: 3327.
25. Deuel TF, Senior RM, Huang JS, Griffin GL. Chemotaxis of monocytes and neutrophils to platelet-derived growth factor. J Clin Invest 1982; 69: 1046-1049.
26. Kambayashi Y, Fujimura T, Furudate S, Lyu C, Hidaka T, Kakizaki A, Sato Y, Tanita K, Aiba S. The Expression of Matrix Metalloproteinases in Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL)-expressing Cancer of Apocrine Origin. Anticancer Res 2018; 38: 113-120.
27. Ruest LB, Ranjbaran H, Tong EJ, Svoboda KK, Feng JQ. Activation of Receptor Activator of Nuclear Factor-kappaB Ligand and Matrix Metalloproteinase Production in Periodontal Fibroblasts by Endothelin Signaling. J Periodontol 2016; 87: e1-8.
28. Ren PW, Yang WJ, Wang DD, Shan JY, Kang DY, Hong Q, Wen S, Zhang RW. Kangfuxinye Enema Combined with Mesalamine for Ulcerative Colitis: A Systematic Review and GRADE Approach. Evid Based Complement Alternat Med 2017: 6019530.