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Original Article

Synovial fractalkine plays important role in cytokines' related knee edema variation in rat arthritis model

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Abstract

Introduction: The systemic and local content of inflammatory cytokines and chemokines play substantial roles in pathophysiology of arthritis. This study was purposed to verify the roles of synovial TNF-α, IL-6 and fractalkine (Fkn) in edema changes during different stages of Complete Freund's Adjuvant (CFA)-induced knee arthritis in rats.

Methods: 168 male Wistar rats were divided in 7 groups and each group was divided to 4 subgroups. Each subgroup contains 6 male rats. Arthritis was evoked into the right knee joint. Changes in knee edema were evaluated by caliper and synovial TNF-α and IL-6 levels were assayed by rat standard ELISA kit in homogenized synovial tissues on days 0, 7, 14 and 21 of study. Synovial Fkn content was assessed during different stages of study using western blot. For analysis of within-groups differences, ANOVA followed by post hoc Tukeys was used. Unpaired student t-test was used for analysis of differences between groups.

Results: CFA injection caused intense knee edema which was reduced by anti-TNF-α and anti-Fkn administration. In anti-IL-6 treated rats, knee edema was reduced in the first two weeks but increased on day 21 of study. Remarkable increase in synovial TNF-α, IL-6 and Fkn levels were observed after CFA treatment. Anti-TNF-α treatment reduced synovial levels of IL-6 and Fkn. Anti-IL-6 administration caused a reduction in synovial IL-6 level and an increase in TNF-α synovial level. Anti-Fkn administration caused a reduction in Fkn and TNF-α level.

Conclusion: It seems that Fkn plays an important role in modulating the TNF-α and IL-6 effects on edema changes in CFA-induced inflammation.

Keywords:
Inflammation;
TNF-α;
IL-6;
Fractalkine;
Complete Freund's Adjuvant (CFA)

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Introduction

Cytokines are low-molecular weight signaling proteins that play important roles in many physiological, pathophysiological responses and have therapeutic potential (Tedgui and Mallat, 2006; Tekieh et al., 2014). They are especially important for regulating immune and inflammatory responses and have crucial functions in regulating the immune system (Tedgui and Mallat, 2006). Inflammatory reaction occurs through the production of specific soluble

inflammatory mediators like cytokines (Foyet et al., 2014). Cytokines are significant component of inflammatory disorders such as Rheumatoid Arthritis (RA) (Tekieh et al., 2011). It was revealed that there is high concentration of cytokines within the serum and synovial fluids of RA patients (Rose-John et al., 2006). Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6) are cytokines and are considered to be important participants in pathophysiology of inflammatory diseases (Katsikis et al., 1994). Inflammation is a key component of biological response to harmful stimuli (Liao et al., 2002; Foyet et al., 2014). Prolonged inflammation can lead to numerous diseases including RA (Foyet et al., 2014). RA is a chronic, autoimmune, inflammatory disease characterized by inflammation of the synovium with symptoms such as edema, joint pain, stiffness and sustained production of inflammatory cytokines such as TNF-α and IL-6 (Yoshida et al., 2011; Zaringhalam et al., 2014). TNF-α, IL-6 are produced by synovial membrane in RA (Katsikis et al., 1994; Boehme et al., 2000). It was shown that the blocking of Soluble IL Receptor (SIL-R) - mediated events can lead to improved experimental model of RA (Rose-John et al., 2006). Also, it was demonstrated that targeting TNF-α by related antagonists can reduce RA symptoms (Boehme et al., 2000; Jones et al., 2012).

Moreover, it has been shown that cytokines can exert some of their intracellular effects via chemokines (Gilory, 2004). Studies demonstrated that chemokines have immune regulatory properties consistent with that of cytokines (Ruth et al., 2001). Among them, it was revealed that TNF-α induces chemokine production and delivery in rat aortic endothelial cells (Gilory, 2004). Chemokines are composed of a super family of chemo attractant, low molecular weight proteins responsible for coordinating cell trafficking in both homeostatic and inflammatory contexts (Boehme et al., 2000; Kastenbauer et al., 2003). Some of chemokines have been identified as crucial mediators of the inflammation. They are involved in the pathogenesis of inflammatory diseases, including RA (Kastenbauer et al., 2003) and act as important mediators of inflammation in the joint affected with RA (Kastenbauer et al., 2003). Fractalkine (Fkn) is the solitary member of a unique CX3C class of chemokines (Kastenbauer et al., 2003). It is biologically active as either a membrane- anchored protein, or a secreted chemokine upon protease cleavage from the mucin stalk (Boehme et al., 2000). Fkn expression is enhanced by inflammatory stimuli, including TNF-α (Boehme et al., 2000; Hyc et al., 2007). It was shown that Fkn has diverse pro- and anti-inflammatory properties (Santora et al., 2007) and similar to other chemokines, seems to play a role in recruitment of inflammatory immune cells in pathological conditions (Multag, 2012). It has been shown that soluble Fkn (sFkn) is up-regulated in RA Synovial Fluids (SF) (Kastenbauer et al., 2003). It is involved in the development of numerous inflammatory pathological processes including RA (Hyc et al., 2007). It has been also revealed that Fkn is an endothelial cell-derived CX3C chemokine that is chemotactic mainly to mono-nuclear cells (Garcia et al., 2000). Fkn, as a chemokine, participates in recruitment of cells to sites of active inflammation. Therefore, it is intricately involved in immunopathogenesis of inflammatory diseases including RA (Ruth et al., 2001). Fkn is chemotactic for monocytes and lymphocytes. It has been shown that Soluble Fkn (SFkn) is up-regulated in RA synovial fluid and described to have a novel role in monocyte chemotaxis. These findings together underscore the importance of monocytes in Fkn-mediated inflammation in RA (Ruth, 2001).

Therefore, given the key roles of TNF-α, IL- 6 and Fkn in arthritis; our hypothesis was that increased levels of IL-6, TNF-α and Fkn increases the synovial membrane edema during different phases of CFA-induced arthritis and the purpose of this study was to investigate the role of synovial Fkn in cytokines related knee edema variations during different stages of adjuvant-induced arthritis in male rats.

Materials and methods

Laboratory animals

Adult male Wistar rats, weighing 180-200 g in the beginning of experiments, were used in our study. The animals were housed at a room temperature of 22.0±1°C and a 12h light-dark cycle. Except during experiments, food and water were available. The study protocol was approved by the local ethics committee for the use of animals in research and we followed the guidelines of ethical standards for investigation of experimental pain in animals

(Zimmerman, 1983). The experimental groups were arranged as: CFA; CFA control; CFA + Anti IL-6; CFA + Anti TNF-α; CFA + Anti Fkn; Control + Anti Fkn; CFA + PBS. Each group was divided to 4 subgroups as: 0, 7th, 14th and 21st days' subgroups to assess the variations during different days of study (0, 7th, 14th and 21st days) and each subgroup contained 6 male rats.

CFA-induced arthritis

CFA-induced arthritis was evoked on day 0 by a single intraarticular injection (100 µL) of heat-killed *Mycobacterium tuberculosis* suspended in sterile mineral oil (10 mg/ml; CFA; Sigma, St Louis, MO, USA), into the rat right knee joint. Control rats, only received sterile mineral oil (100 µL) injection. This animal model was chosen because it exhibits a rapid primary inflammation response to the adjuvant (Cicala et al., 2000).

Evaluation of CFA-induced arthritis

Arthritis development was assessed by measuring the rat knee diameter during different times of the study (on days 0, 7, 14, and 21). The changes made in the knee size on the different days of the study, were evaluated using a caliper (Taniguchi et al., 2004).

Chemicals and reagents

To evaluate the role of TNF-α, IL-6, Fkn in CFA-induced arthritis and edema variation, the rats were treated with TNF-α, IL-6 and Fkn antibodies to deplete serum levels of these factors. In each group, rats received the drug from day 1 to day 7, 14 or 21 according to the protocol specified for each drug. The anti-rat IL-6 antibody was obtained from Abcam/CA (Anti-IL-6 antibody, ab9770, Abcam/ CA, UK). The anti-rat TNF-α antibody was obtained from Abcam/CA (Anti-TNF-α antibody, ab9755, Abcam/ CA, UK). The anti-rat Fkn antibody was obtained from R & D (CX3CL1/ Fractalkine chemokine domain antibody R & D, AF537). According to manufacturer, the neutralization dose 50 (ND50) for anti-rat IL-6 antibody was approximately 0.145- 0.165 µg/ml in the presence of 20 ηg/ml rat IL-6. The ND50 for anti-rat TNF-α antibody was approximately 0.145- 0.165 µg/ml in the presence of 20 ηg/ml rat TNF-α and the ND50 for anti-rat CX3CL1 was approximately 0.3- 1.2 µg/ ml in the presence of 40 ηg/ml recombinant rat CX3CL1/ Fractalkine. The anti-IL-6, anti-TNF-α and anti-Fkn neutralizing antibodies dissolved in sterile phosphate-buffered saline (PBS) and the control animals received only PBS as vehicle. Other reagents were all of analytical grade. All solutions were prepared freshly within 30 min prior to injection and were equilibrated at room temperature. The volume of injected solution was equal for all groups (1 ml/ rat) and done in specified and same times for all groups.

Synovial tissue extraction

For molecular measurements, the knee joint synovial tissue of rats was removed. To achieve this, right knee joint of the animals was opened and synovial tissue was removed carefully from the surrounding enclosures (Rodriguez-Vita and Lawrence, 2010). Then, the tissue weight was calculated and noted. The obtained sample was first placed in liquid nitrogen for 30 minutes and then transferred to -80 $^{\circ}$C freezer for later uses.

Synovial IL-6 and TNF-α measurement

After removal of the synovial membrane, it was homogenized by the lysis buffer. Synovial IL-6 and TNF-α levels were evaluated by rat standard ELISA kits on day 0 (before CFA injection) and at different phases of study according to the manufacturer's protocol. The collected solution showed 100% cross reactivity with the ELISA kits.

Western blot analysis

To evaluate the synovial level of Fkn; western blot method (Zaringhalam et al., 2013) was used. The synovial tissue was quickly removed after the rat was killed and it homogenized in lysis buffer. Then, it was centrifuged at 13000 rpm (4ºC) for 25 min and the supernatant was isolated. Protein concentration was determined (Bradford, 1976). Equal amounts of proteins were diluted with sample buffer. After boiling for 2 min, an aliquot of the diluted sample (24µL) was loaded on 12.5% sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE) gels and run at 100V for approximately 2 hr. A sample from an individual synovium tissue was loaded on each lane. Proteins were transferred to Immobilon-P PVDF membrane (Millipore, Bed ford, MA) using mini-

PROTEAN II (Bio-Rad) at 100V, 0.35 A, for 1h. Non-specific binding sites on the membrane were blocked by incubation (75 min at room temperature) in blocking buffer (2% aurora blocking agent) followed by incubation (3h at room temperature) with primary antibody in blocking buffer (Goat polyclonal IgG for CX3CL1/ Fractalkine (1/1000), R&D systems). Membrane was washed three times with TBST buffer and then it was incubated (75 min at room temperature) with secondary antibody in blocking buffer (Anti Goat IgG (1/2000), AbCam/ CA for Fractalkine). Membrane was then washed thrice with TBST buffer. The immunoreactivity of the proteins on the membrane was visualized using the chemiluminescence detection system (ECL, Amersham). The membrane was then incubated in stripping buffer at 37oc for 15 min and incubated with β-actin primary antibody (Rabbit polyclonal IGg for β-actin (1/1000), Cell Signaling) as a loading control. Band density was measured densitometrically using NIH Image (1. 60) and expressed as the ratio of the Fkn bands to β-actin to account for any difference in Fkn proteins. Each experiment was replicated three times with new groups of rats.

Experimental procedures

Animals were randomly divided into 7 different experimental groups (n=24/ group) as: CFA; CFA control; CFA + Anti IL-6; CFA + Anti TNF-α; CFA + Anti Fkn; Control + Anti Fkn; CFA + PBS. Each group was divided to 4 subgroups (0, 7^{th}, 14^{th} and 21^{st} days subgroups), each contained 6 male rats.

Arthritis was induced by single intra-articular injection of CFA in the rat right knee joint on day 0 (under light anesthesia) in experimental groups. To determine the effects of serum TNF-α, IL-6 and Fkn levels on knee diameter, and whether a time -dependent relationship exists, experiments were performed at different time points. A neutralizing dose of anti-TNF-α, anti-IL-6 and anti-Fkn antibodies diluted in PBS were injected (intra peritoneal) from day 1 continuing until the day 21 of the study (Daily injection for anti-TNF-α antibody, weekly injection for anti-IL-6 antibody and three times per week injection for anti-Fkn antibody). Considering the different phases described for CFA-induced arthritis, knee diameter, synovial TNF-α and IL-6 levels measurements were made on day 0 (immediately before CFA injection) and on days 7 (inflammatory phase); 14 and 21 (arthritic phase). Rats were killed according to animal study ethics guidelines at the end of each experiment and synovial membranes were dissected, snap-frozen immediately in liquid nitrogen and kept at -80ºC. Synovial TNF-α, IL-6 levels were assessed by rat ELISA kit on days 0, 7, 14 and 21 in experimental groups. This procedure was also applied in control groups.

Statistical analysis

The results are presented as the Mean ± standard error of mean (SEM). For analysis of within-groups differences, repeated measurements and one way analysis of variance (ANOVA) followed by *post hoc Tukeys* multiple comparison tests (spss, 16) were used. Unpaired student *t*-test was used to determine significant differences in knee diameter and synovial level TNF-α and IL-6 between groups. Statistical significance was accepted at $p \leq 0.05$.

Results

Knee diameter variations during different stages of study

Intra-articular CFA injection significantly increased ipsilateral knee diameter, which continued until day 21 of study. Knee size significantly increased on days 7, 14 and 21 after CFA injection when compared to day 0 ($p \leq 0.001$ for all days).

Our results indicate that CFA-injected knee size was significantly reduced by anti-TNF-α administration on days 7, 14 and 21 compared with the same days in the CFA control group ($P \leq 0.001$ for day 7 and $P \leq 0.01$ for day 14 and day 21).

In the CFA + anti-IL-6 group, our results demonstrated that CFA-injected knee diameter was significantly reduced by anti-IL-6 administration on days 7, 14 compared to the same days in the CFA control group ($P \leq 0.001$ for day 7 and $P \leq 0.05$ for day 14). Anti-IL-6 administration caused significant increase in knee diameter on day 21 compared to the same day in the CFA control group ($P \leq 0.05$). There were no significant differences in knee diameter variations between CFA injected rats and CFA + PBS (vehicle) group.

In the CFA + Anti-Fkn group, our results showed that Anti-Fkn antibody administration caused significant

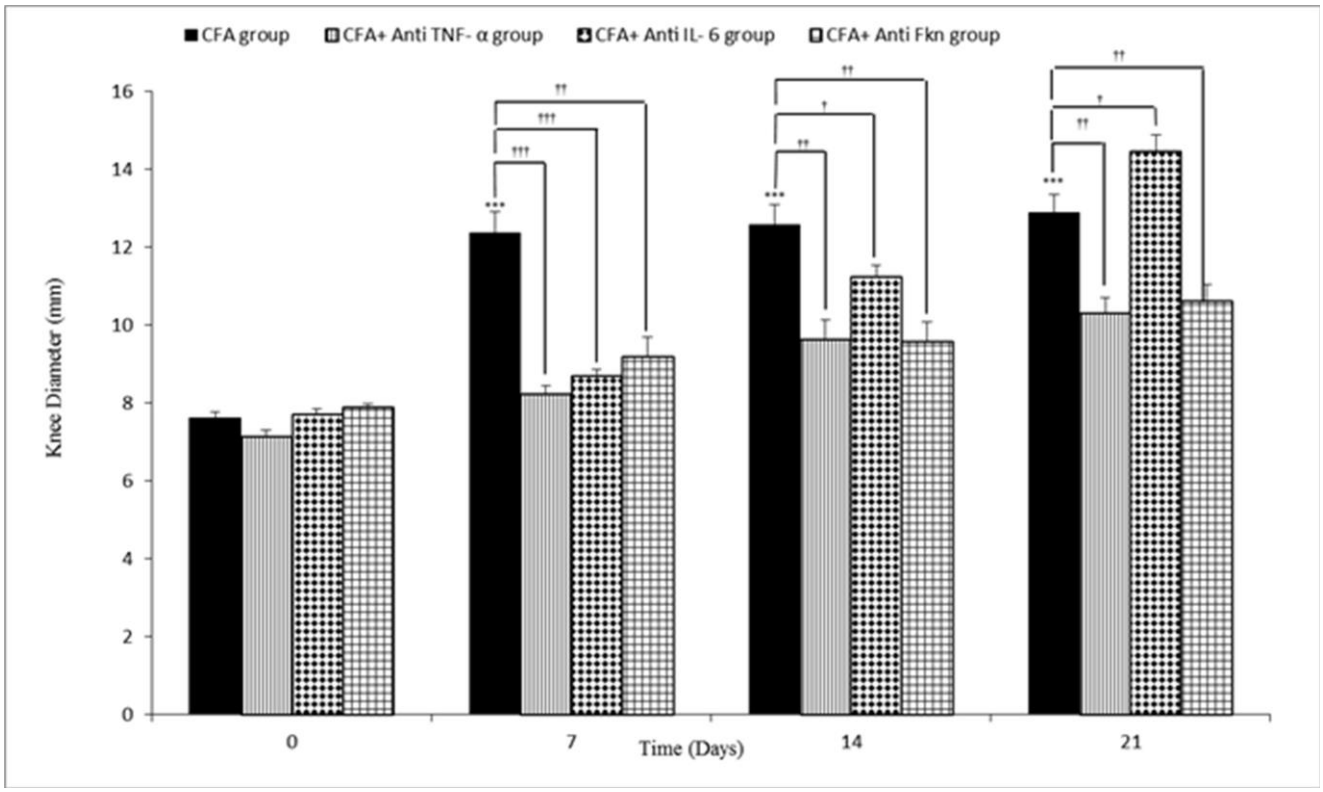

Fig.1. knee diameter significantly increased in CFA injected rats. Anti-TNF-α, anti-IL-6 and anti-Fkn antibodies administration significantly changed inflamed knee diameter. Data are presented as mean± SEM (n=6/ group)
***p≤0.001: significant difference between 7th, 14th and 21st days compared with day 0.
††p ≤0.01, †††p≤0.001: comparison of knee diameter variation between CFA and CFA+ anti-TNF-α groups.
†p≤0.05, †††P≤0.001: comparison of knee diameter variation between CFA and CFA+ anti-IL-6 antibody treated rats.
††p ≤0.01: comparison of knee diameter variation between CFA and CFA+ anti-Fkn antibody treated rats.

reduction in knee size on days 7, 14 and 21 of the study compared to the same days in the CFA control group (p≤0.01 for all days). There were no significant differences in knee size between CFA injected rats and CFA + PBS (vehicle) group (Figure 1).

Variation of synovial TNF-α level during different stages of study

Significant increase in synovial TNF-α concentration after CFA treatment was seen. Synovial TNF-α level significantly increased on days 7, 14, and 21 compared to day 0 in the CFA-injected rats (p≤0.001 for all days).

Anti-TNF-α antibody administration in the CFA injected rats returned serum TNF-α levels to those of day 0 (before CFA injection), and the CFA + anti-TNF-α rats indicated significant reduction in synovial TNF-α level in whole study period compared to the same days in the CFA group (p≤0.001 for all (7, 14 and 21) days).

Anti-IL-6 antibody administration in the CFA- injected rats caused a significant elevation in serum TNF-α level. Anti-IL-6 antibody treated rats indicated significant increase in synovial TNF-α level on day 21 compared to the same day in the CFA group (p≤0.01).

Although synovial TNF-α level in CFA + anti-Fkn group varied during different stages of study, but it was only significant on day 21 of study in comparison to CFA rats (p≤0.05) (Figure 2).

Synovial IL-6 level variation during different stages of study

Significant increase in IL-6 concentration after CFA treatment was observed. Synovial IL-6 level significantly increased on 7th, 14th, and 21st days, compared to day 0, in the CFA injected rats (p≤0.001 for all).

administration of anti-IL-6 antibody in the CFA injected rats returned synovial IL-6 levels to those of day 0 (before CFA injection), and the anti-IL-6 antibody treated rats indicated a significant reduction

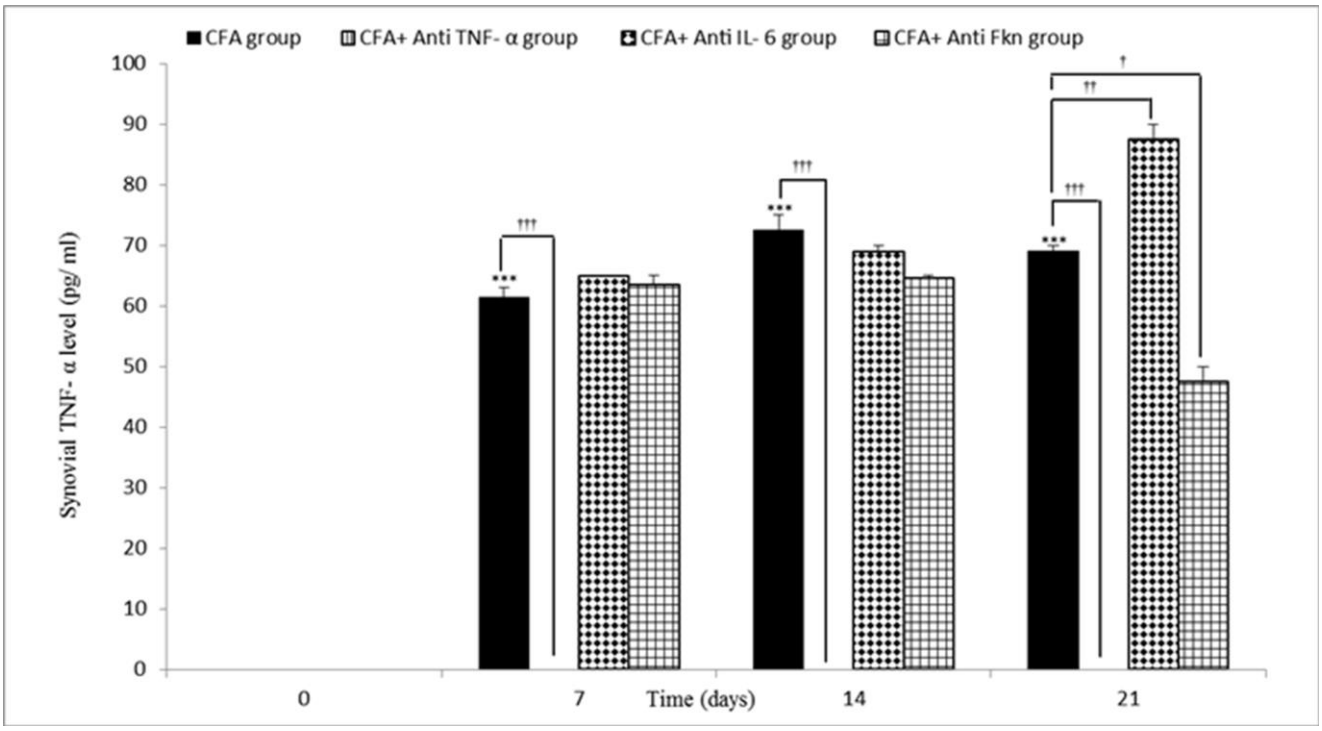

Fig.2. ELISA analysis of synovial TNF-α level during different stages of CFA-induced arthritis. Anti-TNF-α, anti-IL-6 and anti-Fkn antibodies administration significantly changed synovial TNF-α level. Data are presented as mean± S.E.M (n=3- 4/ group).

***p≤ 0.001: significant increase in synovial TNF-α level in CFA injected rats during different stages of study.

†††p≤0.001: significant decrease in synovial TNF-α level in CFA+ anti-TNF-α group when compared to the CFA group.

††p≤0.01: significant increase in synovial TNF-α level in CFA+ anti-IL-6 group when compared to the CFA group.

†p≤0.05: significant decrease in synovial TNF-α level in CFA+ anti-Fkn group when compared to the CFA group.

in synovial IL-6 level on days 7, 14 and 21compared to the same days in the CFA group (p≤0.001 for all).

Anti-TNF-α antibody administration caused significant reduction in synovial IL-6 level. The anti-TNF-α antibody treated rats indicated a significant reduction in synovial IL-6 level throughout the study compared to the same days in the CFA group (p≤0. 05 for days 14, p≤0.01 for days 7 and days 21).

Anti-Fkn administration caused a non-significant decrease in synovial IL-6 level on 7th, 14th, and 21st days in the CFA injected rats, compared with the CFA control group (Figure 3).

Synovial Fkn/ β-actin ratio variation during different stages of study

Based on our data, intra-articular CFA injection caused significant changes in synovial Fkn/ β-actin ratio when compared with the control group. Our results indicated that, on the synovial membranes derived from the CFA injected rats, synovial Fkn/ β-actin ratio significantly increased on days 7, 14 and 21 when compared to day 0 of the study (p≤0.05 for day 7 and day 14, p≤0.01 for day 21).

Based on our foundings, daily administration of anti-TNF-α antibody in CFA-injected rats caused significant reduction in synovial Fkn/ β-actin ratio on days 7, 14 and 21 of this study compared to the CFA control group (p≤0.05 for day 7 and day 14, p≤0.01 for day 21).

Western blot analysis demonstrated that administration of anti-IL-6 antibody in the CFA treated rats caused a significant reduction in synovial Fkn/ β-actin ratio on 7th day of study and increased this ratio on 14th and 21st days of study compared with the CFA control group (p≤0.05 for day 14, p≤0.01 for day 21).

Treatment with anti Fkn antibody significantly reduced synovial Fkn/ β-actin ratio on days 7, 14, and 21 of study compared with the CFA control group (p≤0.01 for day 14 and day 21, p≤0.001 for day 7) (Figure 4a, b).

Discussion

Our results indicated that intra-articular CFA injection

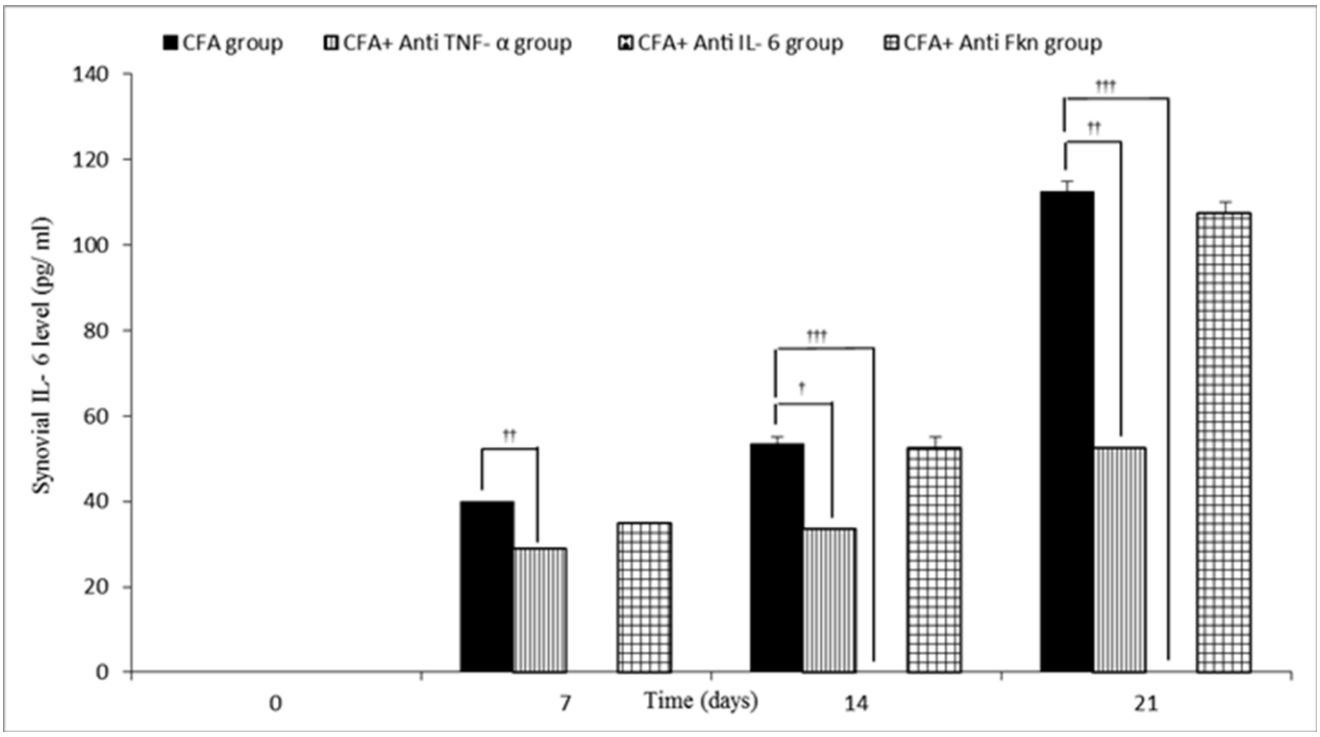

Fig.3. ELISA analysis of synovial IL-6 level during different stages of CFA-induced inflammation. Anti-TNF-α, anti-IL-6 and anti-Fkn antibodies administration significantly changed synovial IL-6 level. Data are presented as mean± S.E.M (n=3- 4/ group).

$^{***}p \leq 0.001$: significant increase in synovial IL-6 level in CFA injected rats during different stages of study.

$^{\dagger}p \leq 0.05$, $^{\dagger\dagger}p \leq 0.01$: significant decrease in synovial IL-6 level in CFA+ anti-TNF-α group when compared to the CFA group.

$^{\dagger\dagger\dagger}p \leq 0.001$: significant decrease in synovial IL-6 level in CFA+ anti-IL-6 group when compared to the CFA group.

caused constant synovial edema. CFA-induced arthritis increased synovial TNF-α, IL-6 and Fkn levels. These findings also demonstrated that synovial Fkn plays important role in variations of knee edema that caused by synovial cytokines during different stages of CFA-induced arthritis.

Intra-articular CFA injection caused permanent synovial inflammation and edema during 21 days of study. Previous studies confirmed that local CFA injection is one of the usual scientific models of inflammatory diseases like RA induction and is proper for evaluation of various behavioral and molecular changes made during acute and chronic inflammatory condition (Cahill et al., 2003). Our previous studies also demonstrated that plantar injection of CFA induces constant inflammation and edema during a 21 days study (Zaringhalam et al., 2013). It is revealed that cytokines and chemokines play main roles in pathophysiology of inflammation and its related symptoms like edema and hyperalgesia (Lin et al., 2001; Zaringhalam et al., 2013). It was also demonstrated that arthritis results in elevation of inflammatory mediators such as TNF-α, IL-6 and IL-1β (Eskandari et al., 2003; Gilory, 2004). Our previous research showed that plantar injection of adjuvant, increases serum TNF-α and IL-6 levels during the entire 21 days of study (Tekieh et al., 2011). In this study, again our findings indicated that CFA injection in knee synovial space increases synovial TNF-α and IL-6 levels along with knee edema. Based on our results, weekly anti-IL-6 antibody administration lessens knee edema during the first two weeks of study. But edema increased during the chronic phase of inflammation due to anti-IL-6 antibody treatment. These results showed that IL-6 plays a dual role (pro- and anti-inflammatory) in our model. Align with our results, important roles of serum IL-6 in pain and edema modulation during plantar arthritis caused by CFA injection was revealed previously and it certified that neutralization of IL-6 can reduce some inflammatory symptoms (Tekieh et al., 2011). Our data showed that long term anti-TNF-α antibody treatment reduced knee edema during all stages of study. In this regard, previous studies revealed important role of serum TNF-α in pathophysiology of pain and edema during CFA-

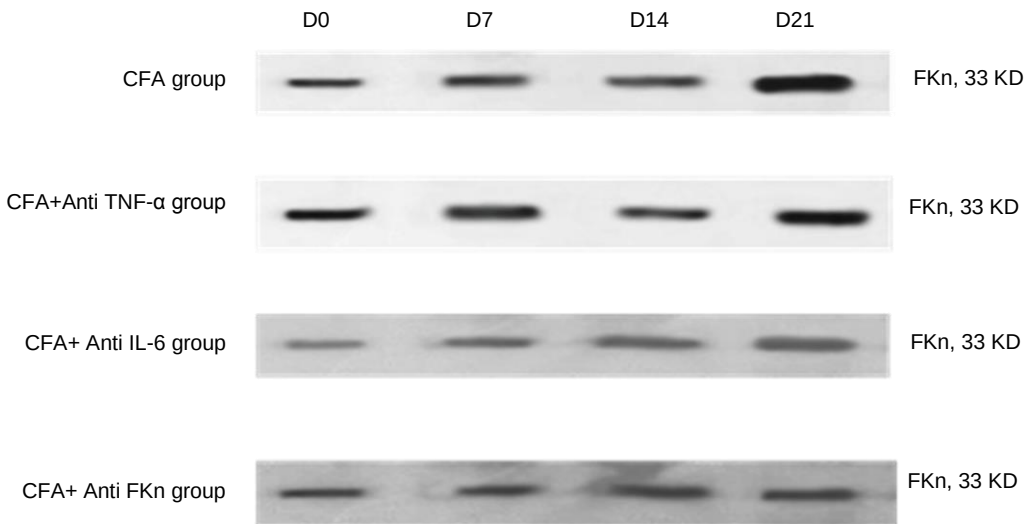

Fig.4a. Immunoblots of Fractalkine extracted from the synovial membranes, which normalized to beta actin during different stages of study (days 0, 7, 14, 21).
Variation of synovial Fractalkine/ β-actin ratio during different days in CFA, CFA+ Anti-TNF-α, CFA+ Anti-IL-6 and CFA+ Anti-Fkn, groups.

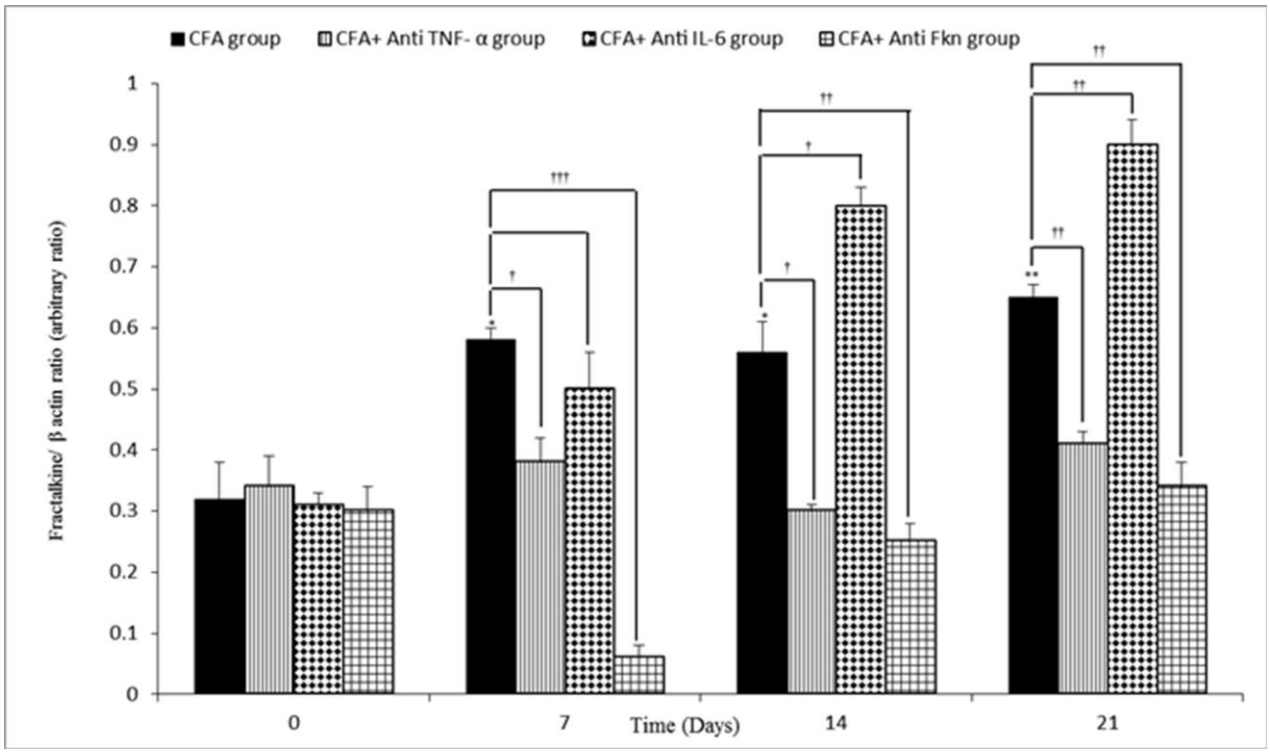

Fig.4b. Synovial Fractalkine/ β-actin ratio changes in different days of study compare to day 0 in CFA group. Anti-TNF-α, anti-IL-6 and anti-Fkn antibodies administration significantly changed synovial Fractalkine/ β-actin ratio. Data are presented as mean ± SEM (n= 3- 4/ group).
$^{*}p\leq0.05$ and $^{**}p\leq0.01$: significant difference between days 7, 14 and 21with day 0 in CFA group.
†p≤0.05 and ††p≤0.01: comparison of synovial Fractalkine/ β-actin ratio band intensity between different days in CFA and CFA+ anti- TNF-α groups.
$^{\dagger}p\leq0.05$ and $^{\dagger\dagger}p\leq0.01$: comparison of synovial Fractalkine/ β-actin ratio band intensity between different days in CFA and CFA+ anti-IL-6 groups.

induced inflammation (Ruth et al., 2001; Eskandari et al., 2003). Our results .in line with those studies, revealed the importance of synovial TNF-α in knee edema changes during a 21-day study period.

On the other hand, we found that synovial Fkn increased during both phases of our adjuvant arthritis model consistent with synovial TNF-α and IL-6 levels. Our data showed that long term anti-IL-6 antibody administration caused an elevation in synovial level of Fkn during the last two week of study. But, anti TNF-α antibody treatment reduced the chemokine level in synovial tissue of arthritic rats during whole period of study. Based on studies, Fkn is as an inflammatory chemokine and secreted by synovial fibroblast cells during inflammatory arthritis. It was shown that Fkn and its receptor are important in immune system responses associated with inflammatory conditions (Garcia et al., 2000; Lin et al., 2001). In addition, inflammatory cytokines was revealed to induce the release of chemokines. In this regard, studies revealed that increase in serum IL-6 level can regulate chemokine expression (McLoughlin et al., 2004). Also, it was revealed that soluble interleukin-6 receptor-α inhibits the cytokine-induced Fkn expression in human vascular endothelial cells in culture (Matsumiya et al., 2001). Studies also demonstrated that increase in serum TNF-α level can stimulate Fkn secretion in many cell types such as vascular endothelial cells; intestinal, bronchial and renal tubular epithelial cells (Sukkar et al., 2004). Align with some previous studies, we showed that chemokine Fkn is an important chemokine in the pathophysiology of inflammatory responses during CFA-induced arthritis. Also we demonstrated that Fkn has an essential role in cytokines (TNF-α and IL-6) mediated inflammatory symptoms like edema, leading to the conclusion that at least a part of cytokines effects on arthritis pathophysiology can be mediated by Fkn.

Conclusion

Results demonstrated that intra-articular CFA injection caused arthritis in rat knee joint. This inflammation was accompanied d with increased levels of synovial TNF-α, IL-6 and Fkn. Also, inhibition of these factors could be effective in alleviation of inflammatory responses like edema. In addition, we demonstrated that at least a part of TNF-α and IL-6 effects on arthritic inflammation, is mediated by Fkn.

Acknowledgments

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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