Long-Term Treatment by Vitamin B$_1$ and Reduction of Serum Proinflammatory Cytokines, Hyperalgesia, and Paw Edema in Adjuvant-Induced Arthritis

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ABSTRACT

Introduction: Immune system is involved in the etiology and pathophysiology of inflammation and vitamins are important sources of substances inducing nonspecific immunomodulatory effects. Given the proinflammatory role of cytokines in the inflammation and pain induction, this study aimed to assess the effects of long-term administration of vitamin B$_1$ on the proinflammatory cytokines, edema, and hyperalgesia during the acute and chronic phases of adjuvant-induced arthritis.

Methods: On the first day of study, inflammation was induced by intraplantar injection of complete Freund's adjuvant (CFA) in the hindpaws of rats. Vitamin B$_1$ at doses of 100, 150, and 200 mg/kg was administrated intraperitoneally during 21 days of the study. Antinociceptive and anti-inflammatory effects of vitamin B$_1$ were also compared to indomethacin (5 mg/kg). Inflammatory symptoms such as thermal hyperalgesia and paw edema were measured by radiant heat and plethysmometer, respectively. Serum TNF-α and IL-1ß levels were checked by rat standard enzyme-linked immune sorbent assay (ELISA) specific kits.

Results: The results indicated that vitamin B$_1$(150 and 200 mg/kg) attenuated the paw edema, thermal hyperalgesia, and serum levels of TNF-α and IL-1ß during both phases of CFA-induced inflammation in a dose-dependent manner. Effective dose of vitamin B$_1$(150 mg/kg) reduced inflammatory symptoms and serum levels of TNF-α and IL-1ß compare to indomethacin during the chronic phase of inflammation.

Conclusion: Anti-inflammatory and antihyperalgesic effects of vitamin B$_1$ during CFA-induced arthritis, more specifically after chronic vitamin B$_1$ administration, suggest its therapeutic property for inflammation.

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1. Introduction

Inflammation can result in tissue damage and loss of function in the long term (Rodriguez –Vita, & Lawrence, 2010). In the inflammatory diseases, rapid release of inflammatory mediators, including chemokines such as interleukins-1 and -6(IL-1 and IL-6) and tumor necrosis factor α (TNF-α) is reported (Lipsky, 2006; Möller & Villiger, 2006). Inflammatory factors lower the membrane thresholds and sensitize the specialized sensory neurons that comprise the nociception pathway leading to pain and disability, the principal clinical features of inflammation (Inglis et al., 2005). Rheumatoid arthritis, characterized by chronic synovitis, progressive joint damage, and significant pain disability, is the most common form of inflammatory arthritis.

Complete Freund's adjuvant (CFA) is used for induction of an arthritic immune-pathological condition that mimics many pathological features of human RA (Billiau & Matthys, 2011). This model has been used extensively, not only for analyses of the cellular and molecular aspects of RA, but also for evaluation of the anti-inflammatory and antinociceptive effects of newly developed drugs on chronic arthritis (Lee et al., 2009; Martin & Eisenach, 2001). In CFA-induced inflammation, there is a distinctive biphasic inflammatory response termed early (acute) and late (chronic) phases (Philippe et al., 1997; Taniguchi et al., 2004).

Unbalanced production of acute phase proteins and proinflammatory cell-mediated cytokines such as IL-1, IL-6, and TNF-α are considered as the main reasons for hyperalgesia and edema induction in acute and chronic inflammatory situations (Fonseca, Santos, Canhao, & Choy, 2009). IL-1ß is not only involved in autoimmune diseases and malignancy but also plays an important role in inflammation-induced edema and hyperalgesia (Billiau & Matthys, 2011; Choy & Panay, 2001). Altogether, TNF-α and IL-1β are known as cytokines that carry out pleiotropic functions in immunity, inflammation, cell proliferation control, differentiation, and apoptosis (Caminero, Comabella, & Montalban, 2011; Woolf, Allchorne, Safieh-Garabedian, & Poole, 1997). Although the etiology of RA remains unknown, it has been revealed that some proinflammatory mediators such as IL-1β, TNF-α, IL-6, and IL-8 play a pivotal role in the pathogenesis of many chronic inflammatory disorders such as RA (Bingham, 2002; Cuenda & Rousseau, 2007; Garfield, Krahl, Appel, Cooper, & Rincon, 2005). TNF-α has been reported to sensitize nociceptive neurons indirectly via the induction of a proinflammatory

cytokine cascade involving IL-1β, IL-6, and IL-8, resulting in the release of prostaglandins and other mediators from immune cells (Hackett, Holloway, Holgate, & Warner, 2008; Woolf et al., 1997).

Therapies directed toward TNF-α and IL-1β are effective in the treatment of RA and reduction of pain scores associated with this condition (Inglis et al., 2005; Möller & Villiger, 2006). Using biological therapies targeting key proinflammatory molecules and their receptors has emerged as a powerful tool for the control of many systemic inflammatory disorders in the past few years (Garfield et al., 2005). The high cost of acquiring synthetic drugs, their inadequate supplies, side effects associated with drug administration (Carpenter, 1997), and believing in therapeutic effect of vitamins in many disease conditions (such as pain and inflammatory conditions) have led researchers and health care providers to use vitamins and vitamin products in recent years (Basu & Dickerson, 1996; Edmonds et al., 1997).

Vitamins are rich sources of substances, which induce nonspecific immunomodulatory responses (Rossato, Hoffimeister, Tonello, de Oliveira Ferreira, & Ferreira, 2015). Vitamin B_1, more commonly known as thiamin, is a member of the B vitamin family. Several studies have demonstrated the antinociceptive and anti-inflammatory effects of B vitamins in different animal pain models (Bartoszyk & Wild, 1990; Jurna, 1998; Moallem, Hosseinzadeh, & Farahi, 2008; Reyes-García, Medina-Santillán, Terán Rosales, Mateos-Garcia, & Castillo-Henkel, 1999; Tadano et al., 1995). In this regard, given the critical roles of TNF-α and IL-1β on induction of hyperalgesia and edema during arthritis and suggested anti-inflammatory effect for vitamin B family, we aimed to assess the effect of vitamin B_1 administration on the proinflammatory cytokines, edema, and hyperalgesia during the acute and chronic phases of adjuvant-induced arthritis in male Wistar rats.

2. Methods

2.1. Laboratory animals

Forty-two adult male Wistar rats, weighing 200-220 g were used in the current study. Rats were housed in polypropylene cages under hygienic and standard environmental conditions (humidity 60%-70%; temperature 22±2°C; 12 h light/dark cycle). The animals were allowed to access standard food and water. Each animal was tested only once. The protocol was approved by the local Ethics Committee for the use of animals in research. We followed the guidelines of ethical

standards for investigations of experimental pain in animals (Zimmermann, 1983).

2.2. Preparation of the materials

Thiamine (Hakim Co., Tehran, Iran) is water soluble, thus appropriate solutions (100, 150, and 200 mg/kg) were prepared by dissolving thiamine in normal saline. Thiamin in known to cause low acute toxicity but a single intraperitoneal (ip) injection of vitamin B_1(300 mg/kg) is lethal in rats and mice. However, injection of various doses of vitamin B_1 revealed that 200 mg/kg was the maximum tolerated dose. Accordingly, 100, 150, and 200 mg/kg of B_1 solutions were prepared (Jurna, 1998; Franca et al., 2001; Reyes-García et al., 1999). Indomethacin (Daru Pakhsh, Tehran, Iran) was used as a positive control (5 mg/kg). Complete Freund's adjuvant (Sigma, St Louis, MO, USA) at the concentration of 10 mg/mL was provided by suspending heat-killed Mycobacterium tuberculosis in sterile mineral oil and injected subcutaneously (100 μL) to induce inflammation.

2.3. Experimental design

Animals were divided into 5 experimental groups: 1) Control group: sterile mineral oil (sc), 2) Adjuvant arthritis (AA) group: CFA (sc), 3) AA+B_1 groups: CFA (sc)+different doses of vitamin B_1 (ip), 4) AA+indomethacin group: CFA (sc)+indomethacin (ip), 5) AA+vehicle group: CFA (sc)+saline (ip). Rats were randomly divided into different groups (6 male Wistar rats in each group). On the first day of the study, inflammation was induced by subcutaneous injection of 100 μL CFA into the rats' right hindpaws. The effects of treatment with vitamin B_1 were assessed during the acute (first week) and chronic (next 2 weeks) phases of CFA-induced inflammation. Experimental CFA-injected rats received different doses of vitamin B_1 from the first day after CFA injection till day 21. Thermal hyperalgesia, paw edema and molecular parameters i.e. serum levels of TNF-α and IL-1ß were assessed on days 0 (immediately before CFA injection), 3, 7, 14, and 21, thirty minutes after the treatment (saline, vitamin B_1 or indomethacin administration).

2.4. CFA-induced inflammation

Inflammation was induced on the day 0 by a single subcutaneous injection of 100 μL CFA into the right hindpaw (Zaringhalam et al., 2014). This animal model indicates a rapid primary inflammation response to the adjuvant (Santora, Rasa, Visco, Steinetz, & Bagnell, 2007). First hour after CFA injection in hindpaw, unilateral edema was established (acute phase) and continued during the 3 weeks later (chronic phase) (Philippe et al., 1997; Taniguchi, Kanai, Kawarnoto, Endo, & Higashino, 2004).

2.5. Paw edema measurement

To confirm proper injection, each paw volume was measured before and after CFA injection during different time points of the study. Paw volume measurements were conducted by displacement of an electrolyte solution in a plethysmometer (Model 7141; Ugo Basile; Comerio VA, Italy), as it was mentioned previously (Rezazadeh, Zaringhalam, Manaheji, & Kebryaeezadeh, 2009). Synoptically, the rats' hindpaws were immersed up to the tibiotarsal joint into an electrolyte-filled Perspex cell of the plethysmometer after their taking out from cages. The volume of electrolyte solution displacement, which is equivalent to the paw volume, was displayed on a digital screen. Volume measurement was carried out. The edema was quantified by measuring the paw volume differences between the day zero and other time points of the study. The paw volumes were shown as the percentage of the day zero (Zaringhalam et al., 2014).

2.6. Thermal hyperalgesia assessment

Paw withdrawal latency (PWL) from noxious heat was assessed using the plantar test (Ugo Basile, Verse, Italy) in both experimental and control groups (Fraser, Gaudreau, Clarke, Ménard, & Perkins, 2000). Rats were placed in Plexiglas boxes for 10 to 15 minutes before testing to habituate to test environment. Paw withdrawal latency (PWL) from noxious heat was assessed using the plantar test (Ugo Basile, Verse, Italy) in both experimental and control groups (Fraser et al., 2000) on days 0, 7, 14, and 21. Rats were placed in a Plexiglas chambers for 15 minutes before examination in order to habituate to the test environment.

The infrared light was positioned under the plantar surface of the rats' hindpaws and projected focally. PWL was automatically recorded by digital timer to the nearest 0.1 s which was connected to the heat source (infrared light). Heating was stopped at 20 s cut off to prevent tissue damage. PWLs were measured 3 times for each paw at an interval of 5 minutes and then the mean latency was computed. Afterward, the mean value for the inflamed paw was subtracted from the control paw and the result was considered as the hyperalgesia sign in the affected paw.

2.7. Blood sampling, serum TNF-α and IL-1ß measurements

The serum levels of TNF-α and IL-1ß were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kits (Koma Biotech, Seoul, Korea) before CFA injection and at different phases of study according to the manufacturer's protocol. The rats were lightly anaesthetized and retroorbitally bled into heparinized tubes. The
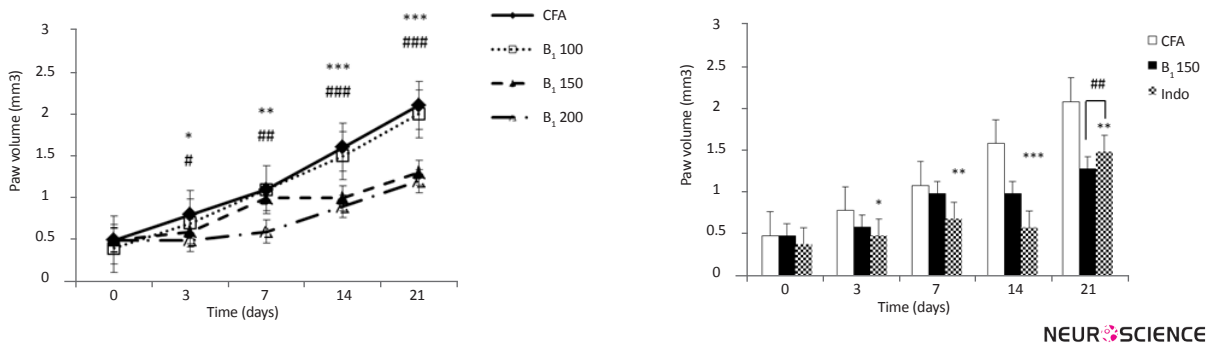
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Figure 1. (a) Effect of vitamin B_1 administration on paw edema during different stages of CFA-induced inflammation. Daily B_1 administration (100 mg/kg) did not significantly change paw volume compared to the CFA group. Long-term administration of higher doses of vitamin B_1(150 and 200 mg/kg) reduced paw edema in a dose-dependent manner. *P<0.05, **P<0.01 and ***P<0.001 for comparison of paw volume changes between day 0 and different days of the study in the CFA group. #P<0.05, ##P<0.01 and ###P<0.001 for comparison of paw volume changes between CFA group and CFA+B_1(200 mg/kg) group.

(b) Effect of administration of indomethacin on paw edema during different stages of CFA-induced inflammation compared to vitamin B_1 administration (150 mg/kg). *P<0.05, **P<0.01 and ***P<0.001 for comparison of paw volume changes between indomethacin and CFA groups. ##P<0.01 for comparison of paw volume changes between indomethacin group and B_1(150 mg/kg) group.

samples were centrifuged and stored at -80°C. The collected serum showed 100% cross-reactivity with the ELISA kit (Zaringhalam, Akbari, Tekieh, Manaheji, & Shamsali, 2010).

2.8. Statistical analyses

Data analysis was performed by SPSS (version 18.0) software (Chicago, Illinois, USA). Unpaired Student t-test, repeated measure 1-way ANOVA followed by Tukey post-hoc test were also used. P values less than 0.05 were considered

statistically significant. Continuous data are expressed as mean±standard error of mean (SEM).

3. Results

3.1. Effect of vitamin B_1 on paw edema during different stages of CFA-induced inflammation

Subcutaneous CFA injection into the right hindpaws of rats in CFA group on day 0 induced inflammation and edema in the same paw and paw volume increased continuously until
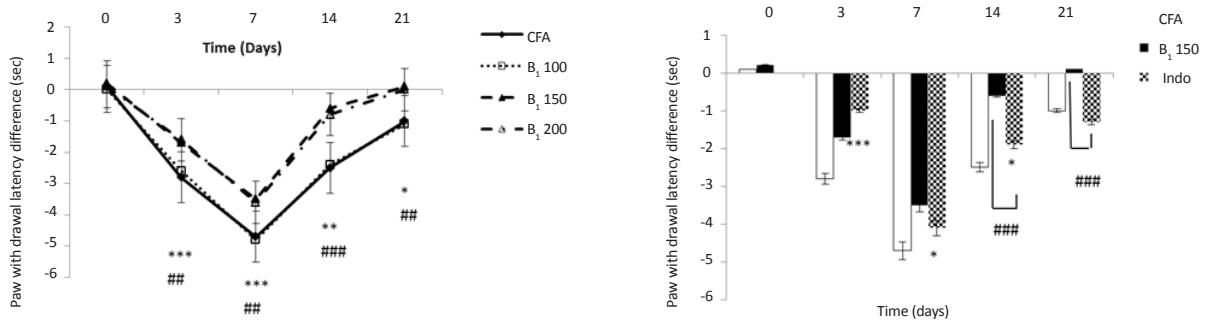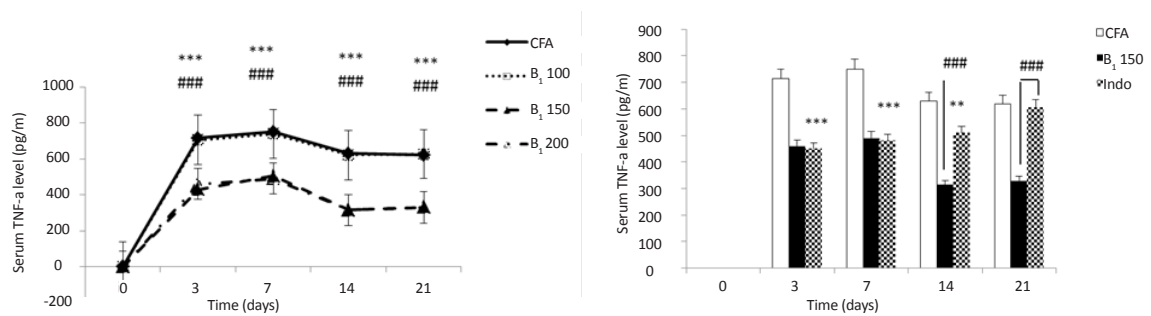

Figure 2. (a) Effect of vitamin B_1 administration on paw withdrawal latency during different stages of CFA-induced inflammation. Paw withdrawal latency increased until day 7 after CFA injection and decreased during following days. Higher doses of vitamin B_1(150 and 200 mg/kg) increased paw withdrawal latency compared to the CFA group. There were no significant differences in paw withdrawal latency between the vitamin B_1(100 mg/kg) group and CFA group. *P<0.05, **P<0.01 and ***P<0.001 for comparison of paw withdrawal latency between day 0 and different days of the study in the CFA group. ##P<0.01 and ### P<0.001 for comparison of paw withdrawal latency changes between CFA group and AA+vitamin B_1(150 mg/kg) group.

(b) Effect of administration of indomethacin on paw withdrawal latency during different stages of CFA-induced inflammation compared to vitamin B_1(150 mg/kg) group. *P<0.05 and ***P<0.001 for comparison of paw volume changes between indomethacin and CFA groups. ###P<0.001 for comparison of paw withdrawal latency changes between indomethacin group and vitamin B_1(150 mg/kg) group.

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Figure 3. (a) Effect of vitamin B_1 administration on serum level of TNF-α during different stages of CFA-induced inflammation. Serum level of TNF-α increased until day 21 after injection. Higher doses of vitamin B_1(150 and 200 mg/kg) decreased serum level of TNF-α compared to the CFA group. There were no significant differences regarding serum levels of TNF-α between the vitamin B_1(100 mg/kg) group and CFA group. ***$P<0.001$ for comparison of serum level of TNF-α between day 0 and different days of the study in the CFA group. ###$P<0.001$ for comparison of serum level of TNF-α changes between the CFA group and AA+vitamin B_1(150 mg/kg) group.

(b) Effect of administration of indomethacin on serum level of TNF-α during different stages of CFA-induced inflammation compared to vitamin B_1 administration (150 mg/kg). Indomethacin reduced serum level of TNF-α only on days 7 and 14. Effect of vitamin B_1 on reduction of serum level of TNF-α on day 7 was more than that of indomethacin. **$P<0.01$ and ***$P<0.01$ for comparison of serum level of TNF-α changes between indomethacin and CFA groups. ###$P<0.001$ for comparison of serum level of TNF-α changes between indomethacin group and vitamin B_1 group (150 mg/kg).

21 days after injection ($F_{3,21}$=136.317, $P<0.001$). Repeated measure 1-way ANOVA followed by Tukey post-hoc test showed that 3 and 7 days after CFA injection paw volume increased significantly in comparison to day 0($P<0.05$ and $P<0.01$ respectively). The maximum changes were observed 14 and 21 days after paw CFA injection ($P<0.001$) (Figure 1a). Administration of vitamin B_1(150 mg/kg) in CFA group reduced paw edema during different stages of our study ($F_{3,20}$=43.176, $P<0.001$).

Vitamin B_1 treatment in CFA rats at the dose of 150 mg/kg caused significant decrease in paw volume on day 21 compared to day 7 of the study ($P<0.01$). Moreover, administration of vitamin B_1 in CFA group caused reduction in paw edema only on days 14 and 21 compared to just CFA group ($P<0.001$). There were no significant differences between 150 and 200 mg/kg doses of vitamin B_1 treatment regarding paw edema reduction in CFA rats during time points of the study. Unpaired Student t-test showed that vitamin B_1 administration at the dose of 100 mg/kg during different stages of the study did not induce significant changes in paw edema compared to the CFA group (Figure 1a).
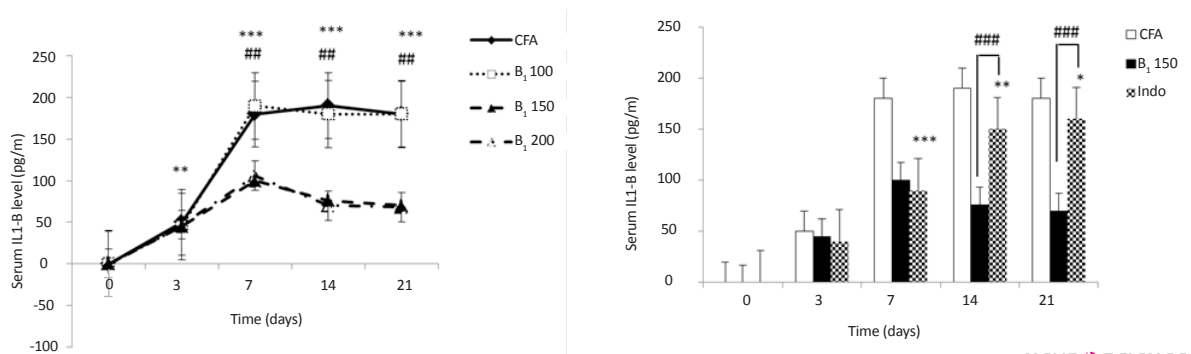
In another set of experiments, repeated measure 1-way ANOVA showed that treatment by indomethacin caused significant reduction in CFA-induced paw edema on different days of the study compared to CFA group ($F_{3,20}$=51.421, $P<0.01$). Unpaired Student t-test revealed that anti-inflammatory effect of indomethacin was more than effective dose of vitamin B_1(150 mg/kg) on the days 3, 7, and 14. However-

er, anti-inflammatory effect of vitamin B_1(150 mg/kg) was more than indomethacin on day 21($P<0.01$) (Figure 1b).

3.2. Effect of vitamin B_1 on thermal hyperalgesia during different stages of CFA-induced inflammation

According to Figure 2a, rats in CFA group showed different thermal hyperalgesia in right hindpaws during different stages of the study. CFA administration caused attenuation in paw withdrawal latency corresponding to an increase in responses to the heat stimulation ($F_{3,21}$=126.421, $P<0.001$). Hyperalgesia continuously increased until day 7 and decreased in the following weeks. One-way ANOVA followed by Tukey post-hoc test indicated that compared to day 0 in CFA group, hyperalgesia was significantly increased on days 3 and 7 after CFA-injection ($P<0.001$).

Our findings revealed that although hyperalgesia significantly decreased on days 14 and 21 of the study compared to days 3 and 7, it was still significantly higher in comparison to day 0($P<0.01$ and $P<0.05$ for days 14 and 21, respectively). Treatment by 150 mg/kg dose of vitamin B_1 caused a significant reduction in thermal hyperalgesia during all time points of the study ($F_{3,19}$=109.141, $P<0.001$). Administration of 150 mg/kg vitamin B_1 in CFA treated rats reduced affected paw hyperalgesia on days 3, 7, 14, and 21 in a dose-dependent manner compared to CFA group ($P<0.01$ for days 3, 7 and 21; $P<0.001$ for day 14). Furthermore in CFA+B_1(150 mg/kg) group, paw hyperalgesia significantly reduced on days 14 and 21 in comparison to day 7($P<0.001$). There were no significant differences be-

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Figure 4. (a) Effect of vitamin B₁ administration on serum level of IL-1β during different stages of CFA-induced inflammation. Serum level of IL-1β increased until day 21 after injection. Unlike the CFA group, higher doses of vitamin B₁(150 and 200 mg/kg) decreased serum level of IL-1β. There were no significant differences regarding serum level of IL-1β between the vitamin B₁(100 mg/kg) group and CFA group. $^{**}P<0.05$ and $^{***}P<0.001$ for comparison of serum level of IL-1β between day 0 and different days of the study in the CFA group. $^{##}P<0.01$ for comparison of serum level of IL-1β changes between CFA group and AA+vitamin B₁(150 mg/kg) group.

(b) Effect of indomethacin administration on serum level of IL-1β during different stages of CFA-induced inflammation compared to vitamin B₁ administration (150 mg/kg). Indomethacin reduced serum level of IL-1β on days 3, 7 and 14. Effect of vitamin B₁ on reduction of serum level of IL-1β on days 14 and 21 was more than that of indomethacin. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ for comparison of serum level of IL-1β changes between indomethacin and CFA groups. $^{###}P<0.001$ for comparison of serum level of IL-1β changes between indomethacin group and vitamin B₁(150 mg/kg) group.

tween the antihyperalgesic effects of 150 and 200 mg/kg doses of vitamin B₁. Unpaired Student t-test showed that administration of 100 mg/kg dose of vitamin B₁ had no significant effects on hyperalgesia variations compared to the CFA group during different days of the study.

One-way ANOVA showed that indomethacin administration caused a significant reduction in hyperalgesia on different days of the study in comparison to CFA group ($F_{3,21}=95.216$, $P<0.01$). Antihyperalgesic effect of indomethacin during the acute phase of CFA-induced hyperalgesia (on day 3) was more than that of effective dose of vitamin B₁(150 mg/kg). In comparison to indomethacin, antinociceptive effect of vitamin B₁(150 mg/kg) was significantly more on days 14 and 21($P<0.001$) (Figure 2b).

3.3. Effect of vitamin B₁ on Serum TNF-α level during different stages of CFA-induced inflammation

Figure 3a shows that CFA injection in AA group caused a significant increase in serum levels of TNF-α and this rise continued until 21 days after injection ($F_{3,19}=577.311$, $P<0.001$). One-way repeated measure ANOVA followed by Tukey test showed that serum TNF-α level significantly increased on days 3, 7, 14, and 21 after plantar injection of CFA compared to that level on day 0($P<0.001$). Administration of 150 mg/kg vitamin B₁ in CFA rats significantly reduced serum TNF-α level in different time points of study ($F_{3,20}=267.756$, $P<0.001$). Our results revealed that serum TNF-α level in AA+B₁ group (150 mg/kg) significantly reduced on days 14 and 21 in comparison to that level on day

7($P<0.001$). Compared to CFA group, long-term administration of vitamin B₁(150 mg/kg) significantly decreased the serum TNF-α level on days 3, 7, 14, and 21($P<0.001$ for all groups) in a dose-response manner. Moreover, during different stages of the study, there were no significant differences in serum TNF-α level between groups which had received vitamin B₁ at the doses of 150 and 200 mg/kg. Unpaired Student T-test showed that administration of 100 mg/kg dose of vitamin B₁ had no significant effects on serum TNF-α level variation compared to that level in CFA group during different days of the study.

One-way ANOVA showed that administration of indomethacin (5 mg/kg) significantly reduced serum TNF-α level on different days of the study in comparison to that level in CFA group ($F_{3,21}=310.342$, $P<0.001$). Unpaired Student t-test revealed that serum levels of TNF-α on days 3 and 7 were similar in indomethacin and vitamin B₁(150 mg/kg) groups. However, serum level of TNF-α on days 14 and 21 reduced more in vitamin B₁(150 mg/kg) group in comparison to indomethacin treated group ($P<0.001$) (Figure 3b).

3.4. Effect of vitamin B₁ on serum IL-1ß level variation during different stages of CFA-induced inflammation

Subcutaneous CFA injection in CFA group induced a significant increase in serum levels of IL-1ß and this increase continued until day 21 of injection ($F_{3,21}=130.642$, $P<0.001$) (Figure 4a). One-way repeated measure ANOVA followed by Tukey post-hoc test showed that plantar injection of CFA

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significantly increased serum levels of IL-1ß on days 3, 7, 14, and 21 after injection compared to day 1(P<0.01 for day 3 and P<0.001 for other days). Furthermore, the results showed that long-term administration of vitamin B_1 at doses of 150 and 200 mg/kg reduced serum levels of IL-1ß in CFA rats ($F_{3,20}$=78.352, P<0.001). Moreover, serum levels of IL-1ß in CFA+B_1(150 mg/kg) group on days 14 and 21 were significantly lower than that level on day 7(P<0.01). In CFA+B_1(150 mg/kg) group serum level of IL-1ß significantly reduced on days 7, 14, and 21 in comparison to CFA group (P<0.01).

There were not any remarkable differences between serum levels of IL-1ß in AA+B_1(150 and 200 mg/kg) groups during different study days. Unpaired Student t-test showed that animals which in addition to subcutaneous injection of CFA had received 100 mg/kg dose of vitamin B_1 during different stages of study did not show any significant differences regarding their serum levels of IL-1ß compared to those levels in CFA group.

Unpaired Student T-test showed that indomethacin treated rats showed significant decrease in serum level of IL-1ß in comparison to CFA control group (P<0.001). Serum levels of IL-1ß were decreased on days 14 and 21 but with less significant changes compared to CFA control group (P<0.01 and P<0.05, respectively). In other words, maximum effect of indomethacin was observed on day 7. While, serum level of IL-1ß in vitamin B_1 group (150 mg/kg) showed significant decrease on days 14 and 21 compared to indomethacin group (P<0.001). There were no significant differences between CFA group and vitamin B_1 group on day 7 (Figure 4b).

Moreover, our results showed that subcutaneous administration of sterile mineral oil in control group did not present any inflammatory or hyperalgesic profile. Also, edema and hyperalgesia variations in AA+vehicle group were not significantly different from CFA (AA) group.

4. Discussion

Modulation of immune responses to alleviate pain and inflammation has been of interest for many years (Woolf et al., 1997). In the current study, anti-inflammatory and antinociceptive effects of vitamin B_1 were tested against two phases of inflammation induced by subcutaneous injection of CFA. Here are the most important findings of this study:

1) Administration of vitamin B_1 reduced paw edema, hyperalgesia, and serum levels of TNF-α and IL-1ß in a dose-dependent manner;

2) The effect of vitamin B_1 administration during the chronic phase of CFA-induced inflammation was more than that during the acute phase;

3) Short-term administration of indomethacin was more effective in the reduction of paw edema, hyperalgesia, and serum levels of TNF-α and IL-1ß compared to long-term administration;

4) Effect of vitamin B_1 on reduction of inflammatory and nociceptive parameters during the chronic phase of CFA-induced inflammation was more prominent than that of indomethacin.

A number of studies have documented 2 inflammatory phases during the CFA-induced monoarthritis driven by an increase in the secretion of various proinflammatory chemokines like IL-1β and TNF-α, which cause hyperalgesia and edema (Choy, & Panay, 2001; Rossato et al., 2015). In the present study, for the first time, we assessed the effects of vitamin B_1 on the acute phase of CFA-induced inflammation. Our finding was in line with the previous studies showing that CFA-induced inflammation was accompanied by increase in the activities of cytokines (Akhtari, Zaringhalam, Eidi, Manaheji, & Tekieh, 2012; Rossato et al., 2015).

The results showed that subcutaneous injection of CFA not only increased serum levels of TNF-α and IL-1β during both phases of CFA-induced inflammation but also indicated that these levels remain elevated up to 21 days after CFA intervention. In this respect, vitamin B_1 administration reduced paw edema during both phases of CFA-induced inflammation.

Anti-inflammatory effect of vitamin B_1 has also been reported not only in carrageenin-induced edema (Bartoszyk & Wild, 1990) but also in formaldehyde-induced paw edema (Franca et al., 2001). As it has already been claimed by Hackett, Holloway, Holgate, and Warner (2008) and Woolf et al. (1997), our findings showed that TNF-α secretion precede the increase in serum level of IL-1β. In accordance with previous studies in various inflammatory models in animals and humans (Bartoszyk & Wild, 1990; Franca et al., 2001; Jurna, 1998; Moallem et al., 2008; Reyes-García et al., 1999; Tadano et al., 1995), reduction in the serum levels of TNF-α and IL-1β after vitamin B_1 administration proposes that anti-inflammatory effect of vitamin B_1 may be due to inhibition of action and or synthesis of inflammatory mediators.

Several line of studies have demonstrated that vitamins B_1, B_6, and B_{12} potentiate analgesic effect of anti-inflam-

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matory painkillers (Jurna, 1998; Reyes-García et al., 1999) or reduce nociception against formaldehyde-induced nociception and heat-induced pain or some disorders such as neuropathic disorders (Bartoszyk & Wild, 1990; Franca et al., 2001; Moallem et al., 2008; Reyes-García, Castillo-Henkel, Medina-Santillán, Terán-Rosales, & Granados-Soto, 2002). Antinociceptive effect of vitamin B_1 was observed from the third day along with the beginning of paw edema attenuation. Furthermore, the highest anti-inflammatory effect of vitamin B_1 was correlated with its highest analgesic effect, i.e. during the chronic phase of CFA-produced inflammation. Then, given the fact that vitamin B_1 diminishes serum levels of TNF-α and IL-1β, antinociceptive effect of vitamin B_1 can be attributed to anti-inflammatory modulatory role of vitamin B_1.

This effect is, at least in part, indirectly mediated through reduction of cytokine production. Cytokines which release at the site of inflammation contribute in nociception through sensitization and attenuation of membrane thresholds of nociceptors at the inflammation site (Woolf et al., 1997). As antinociceptive effect of B vitamins have been observed only during the inflammatory phase of formalin-induced pain, it can be suggested that pain modulatory role of B vitamins is strictly related to their anti-inflammatory function (Franca et al., 2001).

On the other hand, some studies have revealed that antinociceptive effect of B vitamins is partially mediated by inhibition of nitric oxide synthesis (Jurna, 1998; Moallem et al., 2008). Some studies have suggested that vitamin B_1 could produce antinociception by either releasing endogenous opioids or activating opioid receptors. In these studies, authors have noted that antinociceptive effect of B vitamins was blocked by the opioid receptor antagonist naloxone (Moallem et al., 2008; Tadano et al., 1995). In this respect, we have already demonstrated that spinal mu-opioid receptor expression increased and pain perception attenuated during the chronic phase of CFA-induced inflammation, an effect that is blocked by naloxone (Akhtari et al., 2012; Zaringhalam, Manaheji, Maghsoodi, Farokhi, & Mirzaiee, 2008; Zaringhalam, Tekieh, Manaheji, & Akhtari, 2013). Complementary molecular studies will provide more information about the mechanisms underlying the anti-inflammatory and antinociceptive activities of vitamin B_1.

Indomethacin as a nonselective cyclooxygenase inhibitor and nonsteroidal anti-inflammatory drug, inhibit production of prostaglandins. In this study, indomethacin reduced serum levels of TNF-α and IL-1β as well. The anti-inflammatory and antinociceptive effects of vitamin B_1 were more potent than those of indomethacin. There-fore, the anti-inflammatory and antinociceptive properties of vitamin B_1, compared to indomethacin, are probably mediated through different pathways. Furthermore, in comparison to indomethacin, long-term administration of vitamin B_1 may be more effective in reduction of inflammatory symptoms. It could be due to vitamin B_1 accumulative antihyperalgesic long-term effects against indomethacin, and need more investigation.

In conclusion, our study showed that vitamin B_1 significantly decreases the serum levels of IL-1β and TNF-α accompanied with a significant reduction in hyperalgesia and paw edema during both acute and chronic phases of CFA-induced inflammation in rats. Altogether, this study proposes clinical uses of vitamin B_1 because of its remarkable antihyperalgesic and anti-inflammatory effects, especially for treatment of arthritis which has some remarkable similarities with adjuvant-induced arthritis.

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Conflict of Interest

All authors declared no conflict of interest.

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