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The Antinociceptive Effect of Adalimumab, A TNF-Alpha Inhibitor, in a Formalin Induced Inflammatory Pain Model in Mice

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Abstract: The aim of this study, evaluate the nociceptive effect of adalimumab, a TNF-alpha inhibitor, on formalin-induced inflammatory pain in mice. The antinociceptive activity of adalimumab was determined on adult male BALB-C mice (6 mice in each group) by a formalin-induced inflammatory pain model and hot plate test applied. Diclofenac, which we used as a positive control, showed anti-nociceptive activity in both phases of the formalin test. The effect of the hot plate test at the thirtieth minute was statistically significantly different. Adalimumab was not effective in the neuronal phase of the formalin test but showed an antinociceptive effect in the inflammatory phase. Changes due to adalimumab in the hot plate test did not reach statistical significance. The findings of this study showed that adalimumab, which we applied as a single dose, had antinociceptive activity in the inflammatory phase of the formalin test. This result indicated that the peripheral analgesic effect of adalimumab is stronger.

Farelerde Formalin Kaynaklı İnflamatuar Ağrı Modelinde Bir Tnf-Alfa İnhibitörü Olan Adalimumabın Antinosiseptif Etkisi

Anahtar Kelimeler
Adalimumab, Tnf-alfa, Formalin testi, İnflamatuar ağrı, Sıcak plaka testi

Öz: Bu çalışma farelerde formalin testiyle uyarılmış inflamatuar ağrı üzerine bir tnf-alfa inhibitörü olan adalimumabın nosiseptif etkisinin değerlendirilmesi amacıyla gerçekleştirildi. Adalimumab'ın anti-nosiseptif aktivitesi formalin ile uyarılmış inflamatuar ağrı modeli ve sıcak tabaka testleri ile erişkin erkek BALB-C ırkı fareler (her grupta 6 fare) kullanılarak gerçekleştirildi. Adalimumab tek doz (10 mg/kg) olarak formalin enjeksiyonundan 120 saat önce uygulandı. Pozitif kontrol olarak uyguladığımız diklofenak sodyum formalin enjeksiyonundan 15 dakika önce 10 mg/kg dozunda uygulandı. Diklofenak sodyum, formalin testinin her iki fazında da anti-nosiseptif aktivite gösterdi. Sıcak plaka testinde otuzuncu dakikadaki etkisi anlamlı olarak farklıydı. Adalimumab, formalin testinin nöronal fazında etkili değildi, inflamatuar fazda antinosiseptif etki gösterdi. Sıcak plaka testinde adalimumaba bağlı değişiklikler anlamlılığa ulaşamadı. Sonuç olarak, tek doz uyguladığımız adalimumabın formalin testinin inflamatuar fazında anti-nosiseptif aktiviteye sahip olduğunu gösterdi. Bu sonuç adalimumabın periferik analjezik etkisinin daha güçlü olduğunu göstermektedir.

1. INTRODUCTION

Pain is one of the global health problems that negatively affect the quality of life and can be defined as excessive neuronal activity caused by the activation of many ion channels and related receptors, which causes depolarization of nociceptive nerve endings and accordingly creates action potentials [1]. Pain is actually a symptom and often does not occur spontaneously, but is accompanied by a chronic metabolic disease such as diabetes and rheumatoid arthritis [2]. It has also been reported to be associated with anxiety and depression in some societies [3]. It is possible to classify pain into different categories as acute pain and chronic pain according to duration, inflammatory pain and neuropathic pain in terms of pathogenesis, somatic pain and visceral pain according to the region, mild-moderate and severe pain according to intensity [1]. Inflammatory pain occurs in response to tissue damage and inflammation. The sensory nervous system responds to stimuli by undergoing certain changes in its response capacity to repair the affected part of the body. This is sometimes in the form of pain response to normally

harmless stimuli (allodynia) and sometimes in the form of a widening of the response to the stimulus that produces a response (hyperalgesia), and the pain associated with the perception of harmful stimuli is called nociceptive pain [4]. Although non-steroidal anti-inflammatory drugs such as opioids and glucocorticoids are widely used to treat inflammation and related diseases, chronic applications of these drugs bring the use of new drugs to the agenda due to side effects such as stomach irritation and ulcers, hepatotoxicity and kidney failure [5].

While the evidence for the role of proinflammatory cytokines in the formation of inflammation is increasing, it is known that tumor necrosis factor-alpha (TNF-alpha), interleukin 6 (IL-6), and IL-1β take an active role in this process [6]. Adalimumab, an anti-TNF-alpha monoclonal antibody, binds directly to TNF-alpha and blocks its receptor binding or after dissolving to inhibit TNF-alpha binding, it binds to membrane TNF receptors and exerts a bidirectional effect [7]. Although it is used to modify inflammatory responses in rheumatic diseases such as rheumatoid arthritis and ankylosing spondylitis [8], and inflammatory bowel diseases such as Crohn's disease [9], increasing evidence now shows the efficacy of adalimumab in more pathological conditions. Adalimumab, ameliorates cognitive impairment by attenuating neuroinflammation in the mouse model of Alzheimer's disease [10], reduces methotrexate-induced organ toxicity with toxic side effects on tissues and organs [11], limits the production of proinflammatory cytokines in the experimental animal model of acute lung injury, and improves tissue inflammation [12], prevents cognitive impairment and neuroinflammation caused by chronic cerebral hypoperfusion [13], reduces pulmonary edema and histological damage due to mechanical ventilation [14], alleviates endotoxin-induced cardiac damage by modulating cytokine secretion [15], protective effect by reducing blood sugar and cytokine secretion in obese diabetic rats [16], prevents lipid peroxidation caused by spinal cord trauma in rats [17]. It contributes to the regulation of cognitive and emotional processes in response to anti-inflammatory therapy with adalimumab in patients suffering from inflammatory bowel disease [18].
Findings in current studies that adalimumab controls inflammation processes by regulating proinflammatory cytokine release and is therapeutically effective suggest that it may be effective in the inflammatory pain model. For this purpose, this study was planned to evaluate the efficacy of adalimumab in mice with formalin induced inflammatory pain and to compare it with another therapeutic agent with proven efficacy.

2. MATERIAL AND METHOD

2.1. Animals

The mice used in our study were obtained from Selcuk University Experimental Medicine Research and Application Center and the study was carried out in this center. BALB/C male mice weighing 27-30 grams, 8-10 weeks old, were kept in a climate-controlled room with a temperature of 22±2 ºC and relative humidity of 50±5% in a 12-hour light and 12-hour dark period. Mice received standard mouse chow and water ad libitum. This study was started after obtaining the necessary permission from the Experimental Medicine Research and Application Center Experimental Animals Local Ethics Committee of Selcuk University (Ethical approval date: 22.05.2022, number: 2022/15).

2.2. Groups and Procedures

Our study consisted of four groups with 6 mice in each group. No drug was administered to the control group. In our second group, 20 microliters (µl) of 1% formalin were injected subcutaneously into the dorsal part of the right hind legs of the mice. The third group received formalin injection as in the second group. Diclofenac sodium (Sigma Aldrich, St Louis, MO, USA), at a dose of 10 mg/kg was injected intraperitoneally (i.p.) 15 minutes before the formalin injection [19]. The fourth group was injected adalimumab (Humira, 40 mg/0.8 ml AbbVie Laboratories, Istanbul, Turkey) with i.p., a TNF-alpha inhibitor at a dose of 10 mg/kg, 5 days before the formalin injection [15]. In order to avoid the placebo effect, the carriers of the drug-administered groups were injected in the same way as the drug-administered groups.

2.3. Formalin Test

All mice were placed in the observation cage 30 minutes before the test and acclimatized to the environment. For the formalin test, nociception was induced by subcutaneous administration of 20 µl of 1% formalin to the dorsal part of the right hind legs of the mice [20]. As soon as the formalin was administered, each mouse was placed in a clear plexiglass box to observe the spontaneous activity of the treated foot. The observable biphasic pain response after subcutaneous formalin injection was evaluated in two time periods, 0-10 (neurogenic phase) and 10-30 (inflammatory phase) minutes. The time spent (in seconds) for the mice to lick or bite the injected hind paw during the respective periods was measured in each experimental group, and this value was considered an indicator of nociceptive behavior.

2.4. Hot Plate Test

Mice were individually placed on a metal hot plate (MAY 9619, Ankara, Turkey) set at 55 ± 1 °C; The time it took either as soon as he started licking his front and back paws or when he tried to jump off the hot plate was recorded and taken from the hot plate. Mice were kept on the hot plate for a maximum of 30 seconds to avoid tissue damage [20]. Mice were allowed to spend some time on the plate one hour before testing to acclimate to the experimental setup.

2.5. Statistical Analysis

The data obtained from our study are presented as mean ± standard error of the mean. One-way analysis of

variance (ANOVA, SPSS v.20.0) and TUKEY multiple comparison tests were used for statistical analysis of the results. A value of $p<0.05$ was accepted as significant.

3. RESULTS

3.1. Formalin Test Results

Injection of 1% formalin, which we administered 20 µl subcutaneously in mice, produced nociceptive behaviors in both phases of the test ($p<0.05$). The effect of adalimumab, which we administered as a single dose of 10 mg/kg 5 days before the formalin test, was biphasic. The effect on the neuronal phase (between 0-10 minutes after formalin injection), which is considered the early phase of the formalin test, was not statistically significant ($p>0.05$). However, in the late phase of the test (in the inflammatory phase-between 10-30 minutes after the formalin injection), adalimumab improved the nociceptive activity due to formalin injection ($p<0.05$). Mice in the group receiving diclofenac sodium, which we used as a positive control, exhibited ($p<0.05$) antinociceptive behavior both in the neuronal phase and in the inflammatory phase (Fig. 1A-B)

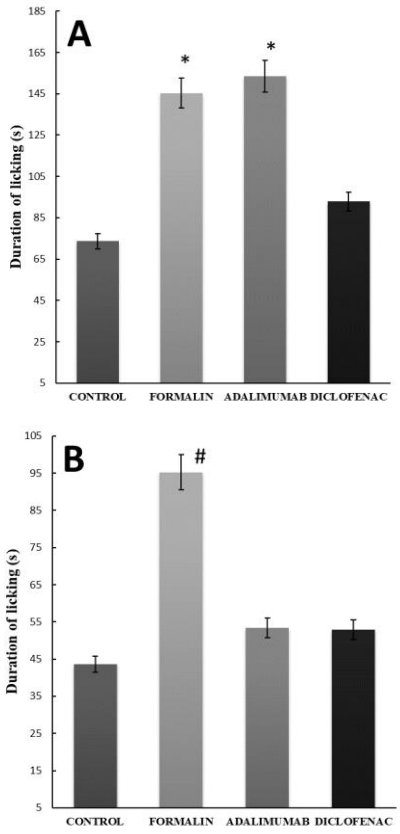

Figure 1. Results of paw-licking test (formalin test). (A) Early phase (0-10 minutes after injection) (B) Late phase (10-30 minutes after injection). The data are expressed as the means ± SD. Asterisk (*) indicates significance compared with Control and Diclofenac groups. Hash (#) indicates significance compared with other groups. $p<0.05$; one-way ANOVA.

3.2. Hot Plate Test Results

In the hot plate test, measurements were taken before the start of the formalin test (0. minutes) and at the 15th and 30th minutes after it started. There was no significant difference between all groups in the measurements made at the 0th and 15th minutes ($p>0.05$). The delay response detected in the diclofenac sodium group at the 30th minute was found to be significantly different ($p<0.05$). Adalimumab-induced changes were not significant ($p>0.05$) in all measurements of the test in mice with formalin-induced inflammatory pain (Fig. 2).

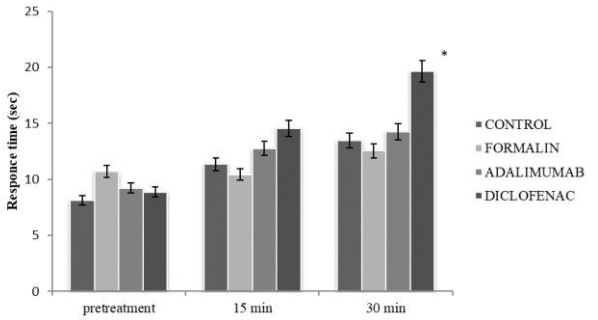

Figure 2. Hot-plate test of the antinociceptive activity of intraperitoneally administered adalimumab in mice. The data are expressed as the means ± SD. Asterisk (*) indicates significance compared with other groups. $p<0.05$; one-way ANOVA.

4. DISCUSSION AND CONCLUSION

Formalin test in experimental animals is widely used to evaluate the effects of pain relief agents. This study was carried out to evaluate the effect of adalimumab, a TNF-alpha inhibitor, on nociceptive behaviors in inflammatory pain processes in mice with a formalin-induced inflammatory pain model. In this model, diclofenac sodium was used as a positive control. In the second phase of the formalin test, adalimumab reduced paw licking behavior compared to the plain formalin group but was not effective in the neuronal phase. The effects were less pronounced in the hot plate test, and no statistically significant difference was observed between the groups. Our study revealed that adalimumab is effective in the second stage of the formalin test, rather than in the first stage, in which the inflammatory process is evaluated.

The most commonly used experimental animals for nociception and inflammation studies are mice and rats. Some tests used for this purpose include various chemical agents such as formalin, acetic acid and carrageenan, as well as physical stimuli such as hot and cold [21]. The formalin test is used as a model for finding antinociceptive and anti-inflammatory substances [20]. Subcutaneous injection of dilute formalin is given to the dorsal or plantar part of the paw of the experimental animal, causing an inflammatory response characterized by swelling in the rodent's paw [22]. Subsequently, the release of mediator cytokines such as TNF-alpha, IL-1β, and IL-6, which cause inflammation from glial cells in the spinal cord, leads to tissue damage over time [23]. Formalin-induced nociception is an in vivo model of acute pain and leads

to characteristic biphasic licking, biting, and pulling behaviors. The initial phase also called the early phase or neurogenic phase, is characterized by central neurogenic pain caused by direct chemical stimulation of C-fiber nociceptors and occurs within the first (0-10 min) minutes of formalin administration. Nociceptive behaviors in this process can be prevented with opioid drugs due to the central effect [20]. The late phase also called the inflammatory phase, is the result of the inflammatory pain response from peripheral mechanisms. It is characterized by an inflammatory process involving mediators such as prostaglandins, neuropeptides and nitric oxide and lasts 10-30 minutes [20], [24]. This information indicates that the two phases observed in the formalin test-induced inflammatory pain model may have different nociceptive mechanisms. In general, centrally acting drugs inhibit both phases, while peripherally acting drugs inhibit only the second phase. In our study, we showed that the nociceptive behaviors observed in the adalimumab group due to the formalin test were not different from the formalin group in the early neuronal phase of the test, but were lower in the inflammatory phase compared to the formalin group. It is not surprising that adalimumab is active in the inflammatory process rather than the neuronal phase. Due to the role of proinflammatory cytokines such as TNF-alpha, IL-1β and IL-6 released from spinal cord glial cells [23], especially in the second phase of the test, inhibition of these cytokines may be associated with the antinociceptive response in the second phase. In addition, TNF-alpha plays a key role in neuronal inflammation [25]. While there are clinical and experimental studies of adalimumab in many pathological conditions associated with inflammation in the literature, to the best of our knowledge, our study is the first report evaluating the nociceptive effect of adalimumab in an inflammatory pain model.

Adalimumab; has curative effects in mood disorders observed in patients suffering from chronic bowel disease [18], contributes to recovery in lipopolysaccharide-induced lung injury by reducing lung tissue TNF-alpha immunoreactivity more effectively than the steroid group [12], cognitive impairment observed in rats in the chronic cerebral hypoperfusion model, TNF-alpha ameliorates by inhibiting IL-6 and NF-kB signaling [13]. We cannot say whether the antinociceptive effect of adalimumab we observed in our study is related to proinflammatory cytokine release, this is the first limitation of our study. Since there has not been such a study before, we cannot make a comparison about the effect of different adalimumab doses and administration regimens. However, a recent review reported that adalimumab reduces pain symptoms associated with the disease in people suffering from hidradenitis suppurativa, without mentioning any mechanism of action [26]. Although the investigation of the underlying mechanism of the antinociceptive effect of adalimumab is the subject of future studies, our study is important as it is the first report showing the antinociceptive effect of adalimumab. Diclofenac sodium showed antinociceptive activity in both the early and late phases, and the findings were consistent with the literature [27], [28]. The hot plate test is used to evaluate centrally mediated anti-nociceptive and analgesic effects after pain induced by an appropriate method. This test is based on recording the delay in the experimental animal pulling its paw off the hot plate or licking the paw. In our study, adalimumab did not change this effect in all measurements made throughout the hot plate test. The peripheral analgesic effect is mediated by the inhibition of inflammatory mediators, while the inhibition of central pain receptors is related to the central analgesic effect. In our study, the insufficient statistical power of the effect of adalimumab in the hot plate test indicates that it does not have a strong analgesic activity that can be mediated centrally. Our study is the first to evaluate the antinociceptive effect of adalimumab, therefore, it is necessary to better explain the subject and to conduct new studies on how adalimumab has an effect in different doses and different administration regimens, and through which pathways it works in these evaluations.

In conclusion, our study showed that adalimumab has an antinociceptive effect in the formalin test. Because the effect is more pronounced in the inflammatory phase of this test and the effect is weaker in the hot plate test, this indicates that its peripheral analgesic effect is stronger rather than the central effect.

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