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Investigation of The Effect of Adalimumab on Experimental Brain Injury in Mice

Adalimumab'ın Farelerde Deneysel Beyin Hasarına Etkisinin Araştırılması

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ABSTRACT

Aim: We aimed to investigate the neuroprotective role of adalimumab based on the hypothesis that "TNF-alpha inhibitor adalimumab may affect inflammation-related neuronal injury due to its anti-inflammatory effect".
Methods: To investigate the effects of adalimumab, we induced brain injury in mice using a cold trauma model and evaluated the underlying cell survival/ death mechanisms via cresyl violet and calculated infarct/edema volume with image analyze system.
Results: Although our data indicated a tendency to decreased infarct and edema volume, these findings are not significant statistically.
Conclusion: To the best of our knowledge, this is the first study evaluating the neuroprotective effect of adalimumab on injured neurons.

Key Words: brain injury, adalimumab, neural protection

ÖZ

Amaç: "TNF-alfa inhibitörü Adalimumab'ın, anti-inflamatuvar etkisi nedeniyle inflamasyonla ilişkili nöronal hasarı etkileyebileceği" hipotezine dayanarak adalimumab'ın nöroprotektif rolünü araştırmayı amaçladık.
Metotlar: Adalimumab'ın etkilerini araştırmak için soğuk travma modelini kullanarak farelerde beyin hasarı oluşturduk ve krezil viyole boyasıyla hücre sağ-kalım/ölüm oranları ile görüntü analiz sistemi ile hesaplanan infarktüs/ödem hacmini değerlendirdik.
Bulgular: Verilerimiz infarkt ve ödem hacminde azalma eğilimi göstermesine rağmen istatistiksel olarak anlamlı değildir.
Sonuç: Bildiğimiz kadarıyla çalışmamız adalimumab'ın hasarlı nöronlar üzerindeki nöroprotektif etkisini değerlendiren ilk çalışmadır.

Anahtar Kelimeler: beyin hasarı, adalimumab, nöroprotektif etki

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INTRODUCTION

Brain injury (BI) is a significant cause of morbidity and mortality around the world. Besides, the financial costs are rapidly increasing due to hospitalization duration, physical rehabilitation, and medical treatments after BI. Unfortunately, although preclinical studies have suggested many promising pharmacologic agents for BI, more than 30 Phase III prospective clinical trials have failed to show the significance of their primary endpoint [1, 2].

The neural structures has been damaged by two different mechanisms after BI. The primary insult is characterized by a mechanical injury, while the second phase is caused by mainly the inflammatory response of the organism's to the primary insult which is including neutrophils, monocytes, macrophages, T lymphocytes and proinflammatory mediators such as TNF-α play a critical role during this process [3]. Studies are showing that TNF-α is one of the cytokines that play a role in the pathophysiology of inflammatory diseases such as rheumatoid arthritis, psoriatic arthritis, Crohn's disease, psoriasis and ankylosing spondylitis, and neuroinflammatory-induced neuronal tissue damage [4, 5]. Therefore, TNF-α blockers are currently used in the treatment of some inflammatory diseases [3, 6].

In this context, adalimumab is a well known human recombinant monoclonal IgG1 antibody specific for cytokine TNF-α [7]. Adalimumab commonly possess the Fc portion of IgG1, whose CH2 domain activates the first component of complement (C1) activation [8]. Adalimumab has a long serum half-life (10-20 days) [9].

Considering these anti-inflammatory effects of adalimumab, we aimed to evaluate its neuroprotective effect on injured neurons after BI. Here, we have hypothesized that its TNF-α receptor blocking effect might exert a decreasing effect on neuroinflammation.

MATERIALS AND METHODS

Ethics statement

This study has been conducted in accordance with the ethical standards and according to the Declaration of Helsinki and has been approved by the Ethics Committee of Istanbul Medipol University (approval number: 09.10.2019/74).

Animals

The study was performed at Meditam Research Laboratories of Istanbul Medipol University. A total of 24 male C57BL/6 mice at 8-10 weeks of age and weighing between 24 and 32 grams were included in this study. The animals were maintained under a constant 12:12-h light-darkness regimen (with the lights on daily at 7.00 a. m.) and with ad libitum access to food and water. The mice were housed separately in cages after the operation.

Experimental groups and adalimumab treatment

In the literature, there are showing that adalimumab is used at different doses ranging from 0.5 mg/kg to 70 mg/kg in many studies in mice. Based on this information, an average adalimumab dose (8 mg/kg) and a high adalimumab dose (80 mg/kg) were preferred in our study [10-13].

In our study mice were randomly divided into three groups. Group 1 (control group): 5% ethanol in normal saline (n = 8); group 2: adalimumab 8 mg (n = 8); group 3: adalimumab 80 mg (n = 8).

All injections were administered intraperitoneally immediately after the experimental procedure.

Cold injury

The brain injury was performed as previously described for a cryogenic trauma model [14, 15]. All the mice were anesthetized with intraperitoneal (i.p) ketamine (60 mg/ kg) and xylazine (6 mg/ kg) and fixed in the stereotaxic device. A parietal craniotomy (3 mm diameter, 2.5 mm lateral, 2.5 mm posterior to the bregma) was done using a dental drill. The cold injury was performed using a liquid nitrogen-cooled copper probe (tip diameter 2.5 mm), which was placed on the dura for 60s and then removed. After that, the scalp was sutured. The rectal temperature was continuously monitored and kept between 36.5 and 37°C with a homeothermic blanket during the procedure. The animals were then taken to the feeding room, and the experimenters waited for the animals to recover during the following 24 h post-trauma. At the end of this 24 h, the animals were anesthetized again with high doses of i.p xylazine (20 mg/ kg)

and ketamine (100 mg/ kg). The mice were sacrificed, and their brains were dissected and put on dry ice. Coronal 18 µm-thick brain sections were taken from four equidistant levels for histopathologic and protein analyses using cryostat (Leica model).

Cresyl violet staining

The sections were dried at room temperature for 30 min in order to remove the moisture, followed by fixation in a 4% paraformaldehyde solution for 7 min. After washing with distilled water, the sections were placed in a glass chamber containing phosphate-buffered saline (PBS) with subsequent shaking of the samples for 5 min at 140 rpm. Then, cresyl violet dye was applied to the sections for 15 min on a shaker with 80 rpm. After staining, the sections were rinsed three times with distilled water, and they were dipped into four chambers containing sequentially increasing concentrations of ethanol (70%, 90%, 95%, and 100%) for 20 s in each chamber. Finally, xylene was applied to the sections for 3 min at room temperature and the mounting medium was placed onto each slide [14].

Analysis of brain injury

Coronal brain sections from equidistant brain levels, 0.5 mm apart, were stained with Cresyl violet staining according to a standard protocol [16]. On the sections, the border between the injured and non-injured areas was outlined using an image analysis system (Image J; NIH, Bethesda, MD, USA), and the area of the injury was assessed by subtracting the area of the non-lesioned ipsilateral hemisphere from that on the contralateral side. The volume of the injury was calculated by the integration of these lesion areas. Edema was calculated as the volume difference between the ischaemic and the non-ischaemic hemisphere and expressed as a percentage of the intact hemisphere [15].

Statistical analysis

After the using Shapiro-Wilk test and the found that P values> 0.05, the data were evaluated by a one-way ANOVA followed by LSD test. All the values are given as mean ± SEM with n values indicating the number of animals analyzed. P <0.05 is considered significant.

RESULTS

Infarct Volume and Brain Swelling

To analyze the effects of adalimumab on BI, the damage volume was measured. In vehicle-treated animals, reproducible brain infarcts were observed after 24 h. In the control group (group 1), the infarct volume was measured as 24.670 ± 4.517 mm3 (mean ± SEM). Besides, infarct volumes in the adalimumab-8 mg group and the adalimumab-80 mg group were measured as 19.234 ± 5.413 (mean ± SEM) and 24.672 ± 4.090 (mean ± SEM), respectively (Figure 1A). And there was no statistical significance between the groups.

When brain swelling was measured, control, adalimumab-8 mg and adalimumab-80 mg groups were found to be 5.241 ± 0.736 mm3 (mean ± SEM), 7.059 ± 2.330 mm3 (mean ± SEM), 5.619 ± 1.899 mm3 (mean ± SEM), respectively. There was no statistically significant difference between the groups (Figure 1B).

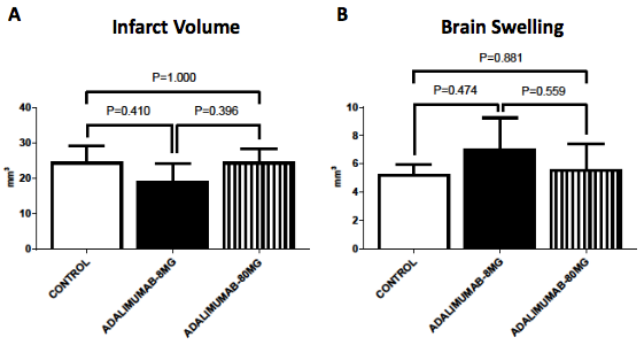

Figure 1. (A) Infarct volume and (B) brain swelling. 24 h after brain injury, adalimumab treatments did not change infarct volume and brain swelling development significantly. The values are given as mean ± SEM.

DISCUSSION

It has been already revealed that proinflammatory cytokines such as TNF, interleukin-1-ß, and interleukin-6 are upregulated within hours from injury. Accordingly, clinical studies have shown that TNF levels were significantly increased in CSF and serum of patients with TBI. Also, rat models of TBI, including the modulation of TNF, have revealed that increased expression of TNF is detrimental for TBI [17-20]. Despite this, there have been studies [21-23] showing that TNF and TNF-alpha receptor knockout mice exerted increased mortality

rates and long term recovery after TBI suggesting that TNF might have a dual role including pro- and anti-inflammatory effect during the recovery period of TBI [19, 24]. Under the light of these TNF findings, it may be assumed that our study may not have reached an effective level of TNF-alpha for decreasing the volume of edema and ischemia. We have revealed that 8 and 80 mg/kg adalimumab administration led to decreased ischemia and edema volume in the injured area even though this did not reach a statistically significant level.

It is difficult to estimate what caused this result; however, it can be assumed that an effect limited only with TNF-alpha receptor blockage may not sufficiently block the neuroinflammatory process. This can be due to that also other well-known cytokines might be involved in this process. Hence, a comprehensive anti-inflammatory therapy approach could be more useful to augment the therapeutic response. However, it should be also noted that small sample size in our study could be responsible for the inconsistency in our results. Thereby, more accurate number of samples could be included for the experimental design in the future .

From another point of view, despite its long half-li-fe [25], considering the low affinity of adalimumab in mice species, our dosages could be too low to exert a neuroprotective effect. In this respect, future studies with higher dosages and different routes of application (i.e., intraventricular or i.v.) could be logical further steps to be undertaken.

Besides the above-mentioned weaknesses, our major strength is that we have applied Adalimumab in the critical period of TBI, where TNF-alpha and cytokines peak. Here, it is logical to assume that adalimumab might have blocked the complicated process of neuroinflammation via altering additional cytokines and proinflammatory enzymes, which play a critical role during this phase. Despite our above-mentioned recommendation that higher doses of adalimumab might be more effective in TBI, it should also not be forgotten that TNF exerts a dual role, and further studies should consider this issue. Also, our small sample size could be responsible for unsignificant statistical results, and similar studies with a larger number of animals could provide more consistent results. In conclusion, our results indicate that adalimumab might lead to improved outcomes in TBI. However, further studies with optimal sample size and dosages of adalimumab are needed.

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