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Original Article

Protective Effects of Infliximab on Lung Injury Induced by Methotrexate☆

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ABSTRACT

Introduction: Methotrexate (MTX) is used to treat cancers, several forms of arthritis and other rheumatic conditions, although MTX may cause pulmonary toxicity related to the production of free oxygen radicals, various cytokines. Infliximab (IB) with its potent effect on tumor necrosis factor-alpha (TNF-α) inhibition also inhibits the release of endothelin-1 (ET-1). We aimed to investigate whether IB reduces pulmonary damage induced by an overdose of MTX.
Method: The rats were divided into 3 groups of 8 animals. The control group was given only saline. One dose of 20 mg/kg MTX intraperitoneal was administered in the MTX group. IB 7 mg/kg was given to the MTX + IB (MI) group. Three days after IB was administered, 20 mg/kg MTX was given. Five days after MTX was administered, all rats were sacrificed.
Results: The TNF-α, ET-1, malondialdehyde (MDA), myeloperoxidase (MPO) and caspase-3 levels in MTX group were significantly higher than in control groups of TNF-α (*P*=.001), ET-1 (*P*=.001), MDA (*P*=.001), MPO (*P*=.001) and caspase-3 levels (*P*=.001) and MI groups of TNF-α (*P*=.009), ET-1 (*P*=.001), MDA (*P*=.047), MPO (*P*=.007) and caspase-3 levels (*P*=.003). The MI group had less histopathological damage in lung tissue than the MTX group.
Conclusion: Overdose of MTX leads to cytokine release and the formation of reactive oxygen species in addition to increased ET-1 secretion release that causes lung damage. IB, as a potent proinflammatory agent, TNF-α blocker, can decrease ET-1 release and oxidative stress, it may show significant protective effects in lung tissue against damage caused by MTX overdose.

Efectos protectores de infliximab sobre el daño pulmonar inducido por metotrexato

Palabras clave:
Toxicidad pulmonar
Metotrexato
Infliximab
Factor de necrosis tumoral-alfa
Endotelina-1

RESUMEN

Introducción: El metotrexato (MTX) se emplea para tratar el cáncer, varias formas de artritis y otras patologías reumáticas, pero puede causar toxicidad pulmonar debido a la producción de radicales libres del oxígeno y varias citocinas. Infliximab (IB) es un potente inhibidor del factor de necrosis tumoral-alfa (TNF-α) e inhibe también la liberación de endotelina-1 (ET-1). Nos propusimos investigar si IB reduce el daño pulmonar inducido por una sobredosis de MTX.
Método: Las ratas se dividieron en 3 grupos de 8 animales. Al grupo control solamente se le administró solución salina. Al grupo MTX se le administró una dosis intraperitoneal de 20 mg/kg de MTX. Al grupo de MTX + IB (MI) se le administraron 7 mg/kg de IB. Tres días después de la administración de IB se

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administraron 20 mg/kg de MTX. Todas las ratas se sacrificaron 5 días después de la administración de MTX.

Resultados: Las concentraciones de TNF-α, ET-1, malondialdehído (MDA), mieloperoxidasa (MPO) y caspasa-3 fueron significativamente más altas en el grupo MTX que en el grupo control: TNF-α (p < 0,001), ET-1 (p < 0,001), MDA (p < 0,001), MPO (p < 0,001) y caspasa-3 (p < 0,001) y en el grupo MI: TNF-α (p < 0,009), ET-1 (p < 0,001), MDA (p < 0,047), MPO (p < 0,007) y caspasa-3 (p < 0,003). El grupo MI mostró menos daño histopatológico en el tejido pulmonar que en el grupo MTX.

Conclusión: La sobredosis de MTX induce la liberación de citocinas y la formación de especies reactivas de oxígeno, además de una mayor secreción de ET-1 que provoca daño pulmonar. IB es un agente proinflamatorio potente, bloquea el TNF-α, puede reducir la liberación de ET-1 y el estrés oxidativo y mostrar importantes efectos protectores del tejido pulmonar frente al daño causado por una sobredosis de MTX.

Introduction

Methotrexate (MTX) is a folic acid analog widely used to treat systemic inflammatory diseases such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis, as well as many malignancies, including lung and breast cancer.[1,2] Pharmacological doses of MTX suppress proinflammatory cytokines, and have a weak tumor necrosis factor-alpha (TNF-α) suppressive effect. Long-term use at therapeutic doses or overdose of MTX can cause significant dosage-dependent pulmonary side effects, such as acute and subacute respiratory failure, nonproductive cough, dyspnea, fever, pneumonitis, interstitial lung disease, and pulmonary fibrosis.[2,3]

Although the reasons for MTX toxicity in the lungs are unclear, some explanations have been proposed. MTX-induced immune suppression causes recurrent viral or bacterial infections and hypersensitivity reactions. MTX may also have a direct toxic effect on the alveolar epithelial walls.[4] Furthermore, MTX causes toxicity by increasing apoptosis and fibrosis of lung tissue.[5] Although no human studies have been conducted, experimental studies have shown that MTX may cause acute pulmonary toxicity by increasing secretion of cytokines such as TNF-α, interleukin-1 (IL-1), interleukin-8 (IL-8), and monocyte chemotactic protein-1.[6,7] In addition, overdose of MTX can lead to proinflammatory cytokine release due to the increase in oxidative stress and reactive oxygen species (ROS) formation.[8] Overdose also leads to pulmonary tissue damage by increasing the caspase enzyme system and activating ROS formation.[5,9]

Infliximab (Ib), a chimeric monoclonal antibody, is used as an anti-TNF-α agent in rheumatic, gastrointestinal, and dermatological disorders, as well as in chronic eye diseases and sarcoidosis.[10–12] Ib targets TNF-α activity in a selection of in vitro bioassays by human fibroblasts, endothelial cells, neutrophils, lymphocytes, and epithelial cells.[13] Ib diminishes the secretion of proinflammatory cytokines and reduces the formation of ROS by inhibiting TNF-α. It prevents tissue damage by inhibiting excessive cytokine release and reducing the formation of ROS, and thus reduces tissue damage by decreasing the stimulation of apoptosis.[14] In addition to blocking TNF-α and inhibiting endothelin-1 (ET-1), Ib has been reported to have a protective effect in lung tissue. ET-1, a potent vasoconstrictor and bronchoconstrictor, is released from the bronchial epithelium and has been implicated in fibrosis.[15] ET-1 increases the release of proinflammatory cytokines, while release of ET-1 increases with increased cytokine levels in lung tissue.[16]

In this study, we aimed to measure major proinflammatory cytokines ET-1 and TNF-α, malondialdehyde (MDA) levels, and myeloperoxidase (MPO) enzyme activity in lung tissue injury induced by high doses of MTX, in order to evaluate the role of ROS formation and apoptosis. Furthermore, we aimed to investigate whether Ib affects these parameters and has a protective role in lung toxicity caused by MTX overdose.

Materials and Methods

Animals

This study was performed on 24 Wistar albino rats. Rats were on average 12–15 weeks old and weighed 250–300 g. The experimental animals were randomly divided into 3 groups: a control group (n=8), MTX (n=8) group, and MTX + Ib (MI) group (n=8). The research was conducted according to the Guide for the Care and Use of Laboratory Animals (NIH, 1985) and was approved by local ethics committee (approval number: 2014/12).

Experimental Design

The control group received isotonic saline solution only (equal to the volume of intraperitoneal MTX). Intraperitoneal injections were performed with 20 mg/kg single-dose MTX (Emthexat-s, 50 mg ampoule) in the MTX group. In the MI group, the rats were administered a single 7 mg/kg Ib dose intraperitoneal injection, and after 3 days, a single 20 mg/kg dose of MTX was administered. All rats in all groups were sacrificed on the same day, 5 days after administration of MTX. All groups were anesthetized with ketamine hydrochloride (ketamine and 50 mg/kg, intramuscularly, Parke-Davis Eczacibasi, Istanbul, Turkey) before they were sacrificed. Lung tissue from the rats was stored at −80 °C until the analysis was conducted.

Tissue Homogenates

Lung tissue samples were homogenized with phosphate-buffered saline (PBS; pH 7.4). The samples were centrifuged at 10,000 × *g* for 20 min. The supernatant was removed, aliquoted to tubes and frozen at −80 °C. The parameters were studied within 1 month.

Measurement of Protein

The Lowry protocol was used to measure tissue homogenate protein levels. The method is based on the Biuret reaction, in which the peptide bonds of proteins react with copper under alkaline conditions to produce Cu^{+}, which reacts with the Folin reagent (Folin–Ciocalteu reaction).[17]

Tissue TNF-α

TNF-α concentrations were measured using the enzyme-linked immunosorbent assay (ELISA) method. We used the commercially available rat TNF-α ELISA kit (eBioscience, Vienna, Austria).