Original Article

Effects of methotrexate in a toluene diisocyanate-induced allergic rhinitis rat model

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ABSTRACT

Objectives: This study aims to investigate the therapeutic effect of low-dose topical administration of methotrexate (MTX) in a toluene diisocyanate (TDI)-induced allergic rhinitis rat model.

Materials and Methods: The experiments were performed on 18 healthy male Wistar albino rats weighing between 250-270 g. Rats were divided into four study groups: control group (n=5), sham group (n=3), steroid group (n=5), and MTX group (n=5). After the drugs were administered, multiple biopsies were taken bilaterally from the nasal mucosal areas and evaluated histologically for fibrosis, loss of cilia and goblet cells, edema, squamous cell metaplasia, and vascular proliferation.

Results: Fibrosis, loss of cilia cells, edema, and vascular proliferation were significantly lower in both MTX and steroid groups than in the control group ($p<0.05$). There were no statistically significant differences with respect to histopathological parameters between the steroid group and the MTX group ($p>0.05$). There were no statistically significant differences with respect to loss of goblet cells and squamous cell metaplasia among the studied groups ($p>0.05$).

Conclusion: Methotrexate may be an alternative or adjuvant therapeutic agent in allergic rhinitis.

Keywords: Allergic rhinitis; methotrexate; toluene diisocyanate.

The prevalence of allergic rhinitis (AR) in the general population has been reported to be increasing year by year, and recent research suggested that approximately 20% of the world population is affected by AR.[1] Allergic rhinitis has been described as an inflammatory disorder of the upper airway mucosa, characterized by a local influx of eosinophils.[2] Mast cells express the immunoglobulin Fc epsilon receptor I that triggers specific antigens to immunoglobulin E (IgE). After IgE-dependent stimulation, mast cells release allergic mediators such as histamine, α-hexosaminidase, cytokines, chemokines and arachidonic acid derivatives, mediating acute and chronic inflammation. Therefore, treatment options for AR consist of allergen avoidance, symptomatic treatment and allergen immunotherapy to further improve the control of allergic responses.[1] A better therapeutic agent is required, as the current treatment of AR is limited to antihistamines, nasal corticosteroids, antileukotrienes, and antiallergen immunotherapy. Since these therapies are still not

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perfect, it is important to continue to study the pharmacology of this disease as part of the search for better drugs.[3]

Methotrexate (MTX) is a competitive inhibitor of dihydrofolate reductase, and is a cytotoxic agent widely used for the treatment of malignancy and inflammatory conditions. The inhibition of dihydrofolate reductase, with subsequent impaired thymidylate and DNA synthesis, is the mechanism by which MTX (N-10-methyl-aminopterin) effects the treatment of cancer.[4] The mechanism of action of MTX as an anti-inflammatory and anti-proliferative drug is not fully understood. These effects of MTX are attributed to inhibition of several key enzymes involved in folate, methionine, adenosine and de novo nucleotide synthesis pathways.[5] Methotrexate, used at low dosages to treat a variety of chronic inflammatory diseases, has been proposed to have steroidsparing effects in steroid-dependent asthmatic patients.[6]

To our knowledge, there is no study in the literature on the topical application of MTX in the treatment of AR. The aim of the present study was to investigate the therapeutic effect of low-dose topical administration of MTX in a toluene diisocyanate (TDI)-induced AR rat model.

MATERIALS AND METHODS

Subjects and setting: This study was approved by the "Committee for Ethics in Animal Experiments" of the university (2011-109-407). The experiments were performed on 18 healthy male Wistar albino rats weighing 250-270 g. Animals were maintained on a 12 h light/dark cycle at a constant temperature (22±2°C) with ad libitum access to food and water. The experiments were performed in accordance with the requirements of the "Helsinki Declaration of Research Ethics". Every attempt was made to minimize both the number and suffering of animals used in these experiments. Animals with signs of active or recent nasal infection were discarded. Cephazoline (17 mg/kg, IM) was administered intramuscularly to all rats to reduce the possibility of a bacterial infection confounding experimental results.

Procedure: Parenteral MTX solution (50 mg/mL) was diluted to 0.025 mg/mL concentration, which was placed into a special spray bottle that applied 0.1 mL solution (2.5 µg MTX) with each puff. Sensitization to TDI was performed using the method described by Tanaka et al.[7] Briefly, 10 µL of a 10% solution of TDI (Tıp Kim San, Istanbul, Turkey) in ethyl acetate was painted bilaterally on the nasal vestibules once a day for five consecutive days (sensitization). This sensitization procedure was then repeated after a two-day interval. Allergic rhinitis induced rats were randomly divided into four study groups. All rats were anesthetized with intraperitoneal ketamine (Ketalar®, Eczacibasi, Turkey; 60 mg/kg) and intraperitoneal xylazine (Alfazyne®, Alfasan International B.V. Woerden, Holland; 10 mg/kg). All procedures were performed under sterile conditions. The physicians were blinded to the group assignment of the rats. Only the Chief Investigator had access to the randomized code.

Group I (Control group) (n=5): No procedure was applied.

Group II (Sham group) (n=3): Only anesthesia was administered, no drug was applied on the nasal mucosa.

Group III (Steroid group) (n=5): Mometasone furoate aqueous nasal spray (Nasonex, Schering-Plough Corporation, Istanbul, Turkey) 50 µg was administered topically to both nasal cavities once daily for seven days.

Group IV (MTX group) (n=5): MTX solution (Methotrexate-Ebewe, Ebewe Pharma, Austria) 0.1 mL was administered topically to both nasal cavities once daily for seven days.

Outcome parameters: After the drugs were administered, the rats were decapitated. The nasal cavities, nasal septa, paranasal sinuses and turbinates were dissected, fixed in 10% buffered formaldehyde and kept in this solution at 4ºC for 24 h. All specimens were dehydrated, embedded in paraffin and serially cut into 5-µm slices. Sections were stained with hematoxylin and eosin for light microscopic examination by a single pathologist.

Biopsies were evaluated histologically for fibrosis, loss of cilia and goblet cells, edema, squamous cell metaplasia, and vascular proliferation. Each parameter was scored between 0 and 3, according to the method of Ercan et al.[8]

Table 1. Histological parameters evaluated in the steroid group and control group

Histological parameters	Group (n=10)	Mean±SD	*p*
Fibrosis	Steroid	0.5000±0.52705	<0.001
	Control	2.6000±0.51640	
Loss of goblet cells	Steroid	0.7000±0.48305	0.660
	Control	0.6000±0.51640	
Loss of cilia	Steroid	1.3000±0.48305	<0.001
	Control	2.7000±0.48305	
Edema	Steroid	0.9000±0.56765	0.025
	Control	2.4000±0.51640	
Squamous cell metaplasia	Steroid	0.5000±0.52705	1
	Control	0.5000±0.52705	
Vascular proliferation	Steroid	0.4000±0.51640	<0.001
	Control	2.5000±0.52705	

SD: Standard deviation.

using numerical scores (0= no change, 1= mild, 2= moderate, 3= severe).

Statistical analysis

Data was analyzed using the Statistical Package for Social Sciences (SPSS) for Windows version 10.0 software (SPSS Inc., Chicago, IL, USA). All differences associated with a chance probability of 0.05 or less were considered statistically significant. Continuous variables were presented as mean ± standard deviation. Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov test. Histological parameters among groups were compared with independent-sample t test and one-way ANOVA test.

Table 2. Histological parameters evaluated in the methotrexate group and control group

Histological parameters	Group (n=10)	Mean±SD	*p*
Fibrosis	Methotrexate	0.5000±0.52705	<0.001
	Control	2.6000±0.51640	
Loss of goblet cells	Methotrexate	0.6000±0.51640	0.08
	Control	0.4500±0.51640	
Loss of cilia	Methotrexate	1.3000±0.48305	<0.001
	Control	2.7000±0.48305	
Edema	Methotrexate	0.8000±0.63246	<0.001
	Control	2.4000±0.51640	
Squamous cell metaplasia	Methotrexate	0.5000±0.52705	0.102
	Control	0.7500±0.52705	
Vascular proliferation	Methotrexate	0.4000±0.51640	<0.001
	Control	2.5000±0.52705	

SD: Standard deviation.

Table 3. Histological parameters evaluated in the steroid group and methotrexate group

Histological parameters	Group (n=10)	Mean±SD	*p*
Fibrosis	Steroid	0.5000±0.52705	1
	Methotrexate	0.5000±0.52705	
Loss of goblet cells	Steroid	0.7000±0.48305	0.660
	Methotrexate	0.6000±0.51640	
Loss of cilia	Steroid	1.3000±0.48305	1
	Methotrexate	1.3000±0.48305	
Edema	Steroid	0.9000±0.56765	0.714
	Methotrexate	0.8000±0.63246	
Squamous cell metaplasia	Steroid	0.5000±0.52705	1
	Methotrexate	0.5000±0.52705	
Vascular proliferation	Steroid	0.4000±0.51640	1
	Methotrexate	0.4000±0.51640	

SD: Standard deviation.

RESULTS

Fibrosis, loss of cilia cells, edema, and vascular proliferation were significantly lower in the steroid group than in the control group ($p<0.05$) (Table 1). There were no statistically significant differences with respect to loss of goblet cells and squamous cell metaplasia between the steroid and control groups ($p>0.05$).

Fibrosis, loss of cilia cells, edema, and vascular proliferation were significantly lower in the MTX group than in the control group ($p<0.05$) (Table 2). There were no statistically significant differences with respect to loss of goblet cells and squamous cell metaplasia between the MTX and control groups ($p>0.05$).

There were no statistically significant differences with respect to histopathological parameters between the steroid group and the MTX group ($p>0.05$) (Table 3).

As the basement membrane thickness did not differ among the study groups and histologically showed "no change" in all rats, this parameter was excluded from statistical evaluation.

DISCUSSION

Although MTX has been a therapy of choice for patients with chronic inflammatory conditions for a long time and several studies have been published since then, no firm conclusion has been reached on whether MTX can be considered an alternative or adjuvant drug in AR. In the present study, we attempted to demonstrate whether MTX might be considered as a therapeutic agent in AR.

Although animal models are not always closely reflective of human responses, they can certainly improve our understanding of the cellular and molecular mechanisms associated with TDI-induced allergic disorders.[9] Toluene diisocyanate is capable of inducing different types of immune reactions, depending on T-cell polarization toward the type 1 T helper (Th1) or type 2 T helper (Th2) cells. Th1 cells promote cell-mediated immunity and are defined by their secretion of cytokines, mainly interferon gama (IFN-γ). Th 2 cells are recognized by the secretion of interleukins, such as interleukin (IL)-4, IL-5 and IL-13, which support the humoral immune response. In experimental studies, the divergence might be because of different animal experimentation conditions, e.g. sensitization way and dose applied.[10] In the treatment of AR, antihistamines, leukotriene inhibitors, systemic and local steroids, cromolyn sodium, and decongestants can become inadequate except those used as a prophylaxis against allergens.[11] Shimizu et al.[12] used a Th2 cytokine inhibitor suplast tosylate in

sensitized rats. Intranasal cyclosporine was used in eosinophilic rhinosinusitis with nasal polyps in a mouse model.[13]

Long-term low-dose MTX has been an established treatment for rheumatoid arthritis and Crohn disease since many decades. Other inflammatory disorders including Wegener granulomatosis, sarcoidosis, psoriasis, asthma and atopic dermatitis have also been reported to respond to such treatment.[14] Its mode of action in these conditions has not been fully elucidated, although several mechanisms have been postulated. Methotrexate is a potent inhibitor of the enzyme dihydrofolate reductase, leading to the inhibition of purine and pyrimidine synthesis and reduced T-cell proliferation. Additionally, it is believed that the anti-inflammatory action of MTX stems from the extracellular accumulation of adenosine, which acts as an endogenous anti-inflammatory agent. Adenosine binds to specific adenosine receptors and inhibits lymphocyte proliferation as well as the production of the proinflammatory cytokines TNF-α, IL-6, and IL-8, while stimulating transcription of the gene encoding IL-1 receptor antagonist and the production of IL-10.[15] Symptoms of AR are sneezing, nasal rubbing, nasal congestion and rhinorrhea, caused by the interaction between chemical mediators and sensory nerves through activation of specific receptors. Nasal vasodilatation and increased vascular permeability are important features of AR. Angiogenic factor vascular endothelial growth factor (VEGF) has also been shown to increase in the nasal mucosa of patients with AR, as a result of the increase in nasal vascular permeability and congestion.[16] Vascular endothelial growth factor participates in nasal mucosa swelling from increased blood vessel permeability in an immediate reaction of AR.[17] Vascular endothelial growth factor may therefore play an important role in nasal mucosal inflammation in AR. Park et al.[18] showed that MTX decreased levels of VEGF in patients with nasal polyposis. Recently, Buyukozturk et al.[19] described two patients with steroid-dependent asthma whose nasal polyps dramatically decreased in size after a course of MTX therapy, administered as an auxiliary treatment. Corrigan et al.[20] reported that MTX therapy in asthma increases T cell susceptibility to corticosteroid inhibition. Goecke et al.[21] demonstrated that MTX regulates the expression of glucocorticoid receptor alpha and beta isoforms in lymphocytes. Another study supports the use of biological agents in preference to using oral MTX as a steroid-sparing agent at the first instance.[22] A theoretically interesting approach is topical administration of chemotherapy. In a study by Ercan et al.,[8] MTX was sprayed topically on the nasal mucosa of rats in various doses for a month. The drug was well tolerated both locally and systemically.

Structural alterations such as ciliary cell loss, epithelial metaplasia, mucous gland hypertrophy, goblet cell hyperplasia, and vascular changes have been reported to occur in patients with AR. These pathologic changes are called tissue remodeling and occur via mediators released from inflammatory cells.[23] In this study using animal AR models, hematoxylin and eosin staining demonstrated ciliary loss in respiratory epithelial cells, and marked vascular proliferation of connective tissue vessels. In the group treated with MTX, and steroids, a decrease in ciliary loss in respiratory epithelial cells, vascular proliferation, edema and fibrosis was observed, and the severity of these changes came close to those seen in the control group. In another study, a higher percentage of goblet cells were determined in patients with nonallergic noninfectious rhinitis.[24] In still another study, no change in the density of goblet cells in biopsy materials retrieved from the inferior nasal turbinate of the patients with perennial rhinitis could be demonstrated.[25] In our study changes in the number of goblet cells did not display a statistically significant difference. In a study performed by Ercan et al.[8] no toxic effect of topical MTX application on nasal mucosa was detected.

Methotrexate can be associated with some side effects, such as oral ulcerations, nausea, diarrhea, post dose reactions like arthralgia, fatigue, and less commonly abnormal liver function tests and myelosuppression. Most of these side effects, including myelosuppression, are dose dependent and occur infrequently at the doses typically used in non-malignant diseases.[26]

Conclusion

We conclude that MTX can be an alternative or adjuvant therapeutic agent in AR. However, additional experimental studies are required to support these findings.

Declaration of conflicting interests

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REFERENCES

1. Bousquet J, Schünemann HJ, Zuberbier T, Bachert C, Baena-Cagnani CE, Bousquet PJ, et al. Development and implementation of guidelines in allergic rhinitis-an ARIA-GA2LEN paper. Allergy 2010;65:1212-21.
2. Jang TY, Jung AY, Kyung TS, Kim DY, Hwang JH, Kim YH. Anti-allergic effect of luteolin in mice with allergic asthma and rhinitis. Cent Eur J Immunol 2017;42:24-29.
3. Baroody FM, Brown D, Gavanescu L, DeTineo M, Naclerio RM. Oxymetazoline adds to the effectiveness of fluticasone furoate in the treatment of perennial allergic rhinitis. J Allergy Clin Immunol 2011;127:927-34.
4. Marder W, McCune WJ. Advances in immunosuppressive therapy. Semin Respir Crit Care Med 2007;28:398-417.
5. Chaabane S, Marzouk S, Akrout R, Ben Hamad M, Achour Y, Rebai A, et al. Genetic Determinants of Methotrexate Toxicity in Tunisian Patients with Rheumatoid Arthritis: A Study of Polymorphisms Involved in the MTX Metabolic Pathway. Eur J Drug Metab Pharmacokinet 2016;41:385-93.
6. Domingo C, Moreno A, Amengual MJ, Comet R, Luján M. Twelve years' experience with methotrexate for GINA treatment step 5 asthma patients. Curr Med Res Opin 2009;25:367-74.
7. Tanaka K, Okamoto Y, Nagaya Y, Nishimura F, Takeoka A, Hanada S, et al. A nasal allergy model developed in the guinea pig by intranasal application of 2,4-toluene diisocyanate. Int Arch Allergy Appl Immunol 1988;85:392-7.
8. Ercan I, Cakir BO, Başak T, Ozbal EA, Sahin A, Balci G, et al. Effects of topical application of methotrexate on nasal mucosa in rats: a preclinical assessment study. Otolaryngol Head Neck Surg 2006;134:751-5.
9. Muti AD, Pârvu AE, Muti LA, Moldovan R, Mureşan A. Vitamin E effect in a rat model of toluene diisocyanate-induced asthma. Clujul Med 2016;89:499-505.
10. Ban M, Morel G, Langonné I, Huguet N, Pépin E, Binet S. TDI can induce respiratory allergy with Th2-dominated response in mice. Toxicology 2006;218:39-47.
11. May JR, Dolen WK. Management of Allergic Rhinitis: A Review for the Community Pharmacist. Clin Ther 2017;39:2410-2419.
12. Shimizu S, Hattori R, Majima Y, Shimizu T. Th2 cytokine inhibitor suplatast tosilate inhibits antigen-induced mucus hypersecretion in the nasal epithelium of sensitized rats. Ann Otol Rhinol Laryngol 2009;118:67-72.
13. Chang DY, Joo YH, Kim SJ, Kim JH, Jung MH, Kim DW, et al. Therapeutic effects of intranasal cyclosporine for eosinophilic rhinosinusitis with nasal polyps in a mouse model. Am J Rhinol Allergy 2015;29:e29-32.
14. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. Pharmacol Rev 2005;57:163-72.
15. Morabito L, Montesinos MC, Schreibman DM, Balter L, Thompson LF, Resta R, et al. Methotrexate and sulfasalazine promote adenosine release by a mechanism that requires ecto-5'-nucleotidase-mediated conversion of adenine nucleotides. J Clin Invest 1998;101:295-300.
16. Matsune S, Ohori J, Yoshifuku K, Kurono Y. Effect of vascular endothelial growth factor on nasal vascular permeability. Laryngoscope 2010;120:844-8.
17. Yamashita T, Terada N, Hamano N, Kishi H, Kobayashi N, Kotani Y, et al. Involvement of vascular endothelial growth factor in nasal obstruction in patients with nasal allergy. Allergol Int 2000;49:183-8.
18. Park SK, Kim HI, Yang YI, Hur DY. Effects of methotrexate on vascular endothelial growth factor, angiopoietin 1, and angiopoietin 2 in nasal polyps. Am J Rhinol Allergy 2011;25:e129-32.
19. Buyukozturk S, Gelincik A, Aslan I, Aydin S, Colakoglu B, Dal M. Methotrexate: can it be a choice for nasal polyposis in aspirin exacerbated respiratory disease? J Asthma 2009;46:1037-41.
20. Corrigan CJ, Shiner R, Shakur BH, Ind PW. Methotrexate therapy in asthma increases T cell susceptibility to corticosteroid inhibition. Clin Exp Allergy 2003;33:1090-6.
21. Goecke IA, Alvarez C, Henríquez J, Salas K, Molina ML, Ferreira A, et al. Methotrexate regulates the expression of glucocorticoid receptor alpha and beta isoforms in normal human peripheral mononuclear cells and human lymphocyte cell lines in vitro. Mol Immunol 2007;44:2115-23.
22. Bilocca D, Hargadon B, Pavord ID, Green RH, Brightling CE, Bradding P, et al. The role of oral methotrexate as a steroid sparing agent in refractory eosinophilic asthma. Chron Respir Dis 2018;15:85-87.
23. Nakaya M, Dohi M, Okunishi K, Nakagome K, Tanaka R, Imamura M, et al. Prolonged allergen challenge in murine nasal allergic rhinitis: nasal airway remodeling and adaptation of nasal airway responsiveness. Laryngoscope 2007;117:881-5.
24. Canakcioglu S, Tahamiler R, Saritzali G, Alimoglu Y, Isildak H, Guvenc MG, et al. Evaluation of nasal cytology in subjects with chronic rhinitis: a 7-year study. Am J Otolaryngol 2009;30:312-7.
25. Berger G, Marom Z, Ophir D. Goblet cell density of the inferior turbinates in patients with perennial allergic and nonallergic rhinitis. Am J Rhinol 1997;11:233-6.
26. Kavanaugh A, Broide DH. Immunomodulators. In: Adkinson NF, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's Allergy Principles & Practice. 7th ed. China: Elsevier; 2009. p. 1643-56.

