INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of severe visual loss in developed countries (1-3). Angiogenesis has a role in many diseases, including AMD and choroidal neovascularization (4). Identification of the most important regulating factors, such as vascular endothelial growth factor (VEGF) (5), angiopoietin (6), and pigment epithelium growth factor (7), has facilitated the development of new therapeutic agents that target angiogenic
Intravitreal bevacizumab and MTX for neovascular AMD

pathologic pathways. Nowadays, many medications are designed for intravitreal injection in AMD. Pegaptanib (8), ranibizumab (9), and bevacizumab (10) are used to treat choroidal neovascularization (CNV) in AMD. In clinical comparison of other therapeutic modalities such as photodynamic therapy, which has less effect on visual acuity outcomes (11), anti-VEGF agents can improve visual acuity outcomes from 28% to 43% (8-10). Nevertheless, more than half of patients treated with bevacizumab are considered nonresponders (12). Methotrexate (MTX) has been used as an anti-inflammatory agent since 1965 (13, 14). Methotrexate can inhibit many enzymes involved in metabolic pathways of folic acid, which can inhibit production of thymidylates, purines, and methionine (15). Since CNV in AMD can be caused by inflammatory (16,17), angiogenic (4), and proliferative (5) processes, and MTX may act to inhibit these processes (18-30), it may be an alternative therapy for CNV due to AMD. Since the safe dose for intravitreal injection of MTX has been investigated (31-33), we designed a pilot study to determine the safety and effect of the combination of intravitreal MTX and bevacizumab (IVB+IVMTX) on regression of CNV in ARMD.

PATIENTS AND METHODS

This pilot study was conducted according to the guidelines of the declaration of Helsinki. Patients were recruited from April 2008 to April 2009. The Review Board/Ethics Committee of the Ophthalmic Research Center, Labbafinejad Medical Center, Shahid Beheshti University, approved the study. All patients gave informed consent to participate. Inclusion criteria included age more than 50 years, subfoveal CNV due to AMD, occult and classic types of CNV according to fluorescein angiography, and size of lesion no more than 4 DD (including scars, hemorrhage, atrophy, and neovascularization). Exclusion criteria included subfoveal scar, history of ocular surgery, photodynamic therapy, transpupillary thermotherapy, diabetic retinopathy, and aphakia. All patients underwent a complete history and comprehensive ocular examination including best-corrected visual acuity (BCVA; Snellen chart expressed as logMAR), intraocular pressure (IOP), slit-lamp, and fundus examinations. Baseline central macular thickness (CMT) assessment by optical coherence tomography (OCT; spectral domain 3D, Topcon Corporation, Tokyo, Japan) using standard software and fluorescein angiography (Heidelberg Retina Angiograph II, Heidelberg, Germany) were performed and repeated at 1.5-month intervals, if indicated. In all patients, electroretinography (ERG, Metrovision, Monopack 2, Pernes-chies, France) was performed before and 1 week after intravitreal injection. Changes in a- and b-wave amplitude more than 15% were considered meaningful. Patients received combined IVB (1.25 mg) with IVMTX (400 μg) in 2 separate sites. If patients required retreatment, only IVB injection was repeated. It was performed at 1.5-month intervals which was sufficient to achieve a level of >500 ng/mL of drug in the vitreous to completely block VEGF-A (34). Criteria for reinjection were based on no improvement in vision, no changes or increase in subretinal fluid, presence and enlargement of hemorrhage and exudation, increase or no changes in CMT, increase or no changes in size of active lesion, no scar formation, and increase or no changes in pigment epithelial detachment height, either together or alone.

Intravitreal injection

Under local anesthesia with tetracaine 0.5% and sterile conditions, bevacizumab (made for F. Hoffmann-La Roche Ltd., Basel, Switzerland, by Genentech Inc.; South San Francisco, CA) 0.05 cc (1.25 mg) was injected through a 27-gauge needle at 3.5 mm of the supratemporal limbus. Methotrexate (Ebetrex, Ebewe Pharma Ges.m.b.H. Nfg., Unterach, Austria) 400 μg was also injected through a 27-gauge needle 3.5 mm from the infratemporal limbus. All eyes underwent examination at 1, 3, and 7 days after injection, mainly for anterior chamber and vitreous reaction and increase in IOP. A complete eye examination was repeated at 1.5-month intervals.

Statistical analysis

Statistical analysis was performed using SPSS software version 17.0. To describe data, we used mean ± SD and frequency (%). Changes were evaluated by paired *t* test. A *p* value less than 0.05% was considered significant.

RESULTS

Table I presents patient information and follow-up for visual acuity (VA), BCVA, CMT, and number of intravitreal injections for 7 eyes of 7 patients. Mean age was 65.43±5.96
Results of fluorescein angiography, OCT, and electroretinography in patient 7 are shown in Figures 1-3. Electroretinography showed no significant changes in a- and b-wave amplitude after injection. No adverse reactions after injection (e.g., endophthalmitis, anterior chamber reaction, retinal detachment, corneal epitheliopathy, optic atrophy, increase in IOP) were seen.

DISCUSSION

This pilot study did not disclose any adverse effect of intravitreal MTX injection (400 μg dose) when combined with 1.25 mg bevacizumab. In most patients, BCVA was improved compared to baseline, and in all eyes CMT decreased, except in one patient (no. 1) despite improvement of VA. No scar formation or increase of fibrous component was noted in any eye.

Methotrexate is widely used in many intraocular disorders, such as intraocular non-Hodgkin lymphoma and uveitis (31, 33). It also has been used in proliferative diabetic retinopa-
Intravitreal bevacizumab and MTX for neovascular AMD

Fig. 1 - Patient 7. (A) Before intravitreal injection of bevacizumab and methotrexate, fluorescein angiography shows classic choroidal neovascularization with fibrovascular retinal pigment epithelium detachments nasal and supranasal to the classic choroidal neovascularization; (B) 6 weeks after combined intravitreal injection of bevacizumab and methotrexate, fluorescein angiography shows leakage reduction particularly from fibrovascular retinal pigment epithelium detachments.

Fig. 2 - Patient 7. (A) Before intravitreal injection of bevacizumab and methotrexate, optical coherence tomography shows retinal edema and retinal pigment epithelium detachment; (B) 3 months after combined intravitreal injection of bevacizumab and methotrexate, decrease of macular thickness and flattening of retinal pigment epithelium detachment occurred.

Fig. 3 - Patient 7. (A) Electroretinography before intravitreal injection of bevacizumab and methotrexate, and (B) 1 week after shows no changes after injection.
thy, cystoid macular edema, epithelial downgrowth, retinitis, and proliferative vitreoretinopathy due to chronic infection or trauma (32). Methotrexate has many mechanisms of action that include antiproliferative and anti-inflammatory properties (18-30). Indirect evidence of MTX mechanisms of action on cell proliferation and apoptosis through increasing reactive oxygen species (ROS) production is given by studying the role of ornithine decarboxylase overexpression (18, 19). Ornithine decarboxylase overexpression leads to increased polyamine levels—spermine and spermidine—which are ROS scavengers. Methotrexate indirectly inhibits polyamine-producing enzymes that ultimately induce ROS. As a result, methotrexate can inhibit the production of ROS found in active lymphocytes, and may induce an apoptotic response (18, 19). A novel mechanism of antiproliferative effects of MTX has also been reported; it can inhibit isoprenylcysteine carboxyl methyltransferase (ICMT), which is a critical component of the antiproliferative effect of methotrexate. Inhibition of ICMT may lead to reduced RAS protein methylation. RAS protein is a central component in signal transduction pathways, regulating cell growth and differentiation (20). Choroidal neovascularization is the result of proliferation, migration, invasion of endothelial cells, and organization of endothelial cells into functional tubular structures, and maturation of vessels (4). Thus, MTX may affect CNV through its antiproliferative effect. Methotrexate can inhibit production of cytokines induced by T-cell activation (26-29) and also can suppress intracellular adhesion molecules and lymphocyte-associated antigen in activated lymphocytes (30). Many reports show that MTX directly or indirectly releases endogenous anti-inflammatory adenosine (23, 34, 35). Regarding the presence of immune response proteins in drusen of postmortem AMD retinas, Hageman and colleagues (16) were the first to suggest a link between inflammation and AMD. Thus, due to anti-inflammatory effects of MTX, it may act on CNV through this mechanism.

Many mechanisms of angiogenesis are involved in the pathogenesis of CNV. Changes in vascular permeability due to VEGF inhibition could have contributed to improving BCVA or decreasing subretinal fluid in CNV. Although current data do not provide evidence that MTX directly affects angiogenesis, it may affect angiogenesis through indirect mechanisms such as disruption of macrophages and fibroblast-like cell interaction or reduced cellular adhesion molecule expression (22-24). It is not clear whether improvement in BCVA or CMT is the result of bevacizumab action, the result of MTX action, or both. In our study, disciform scar formation—the end stage and natural course of AMD (36)—was not observed during follow-up, although the follow-up period was short. The small number of patients, short-term follow-up, and concurrent use of MTX with bevacizumab are shortcomings of this pilot study. However, no adverse effect of intravitreal MTX injection when combined with bevacizumab was noted.

Future studies should be done to support these observations. A randomized clinical trial is planned at our institution to further evaluate the safety and efficacy of intravitreal MTX injection alone or in combination with bevacizumab.

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REFERENCES

6. Otani A, Takagi H, Oh H, Koyama S, Matsamura M, Honda Y. Ex-