IS A DECREASED NITRIC OXIDE CONCENTRATION AFTER TRIAMCINOLONE ACETONIDE INTRAVITREAL INJECTION ONE OF THE REASONS FOR INTRAOCULAR PRESSURE RISE?

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SUMMARY – Diabetic macular edema is the most common cause of vision loss in patients affected by diabetes mellitus. For eyes with persistent retinal thickening despite anti-VEGF therapy, treatment with intravitreal triamcinolone may be considered, especially in pseudophakic eyes. The aim of this study was to examine aqueous humor nitric oxide concentration changes in pseudophakic eyes with persistent diffuse diabetic macular edema after intravitreal injection of triamcinolone acetonide, as well as the potential impact of these changes on the intraocular pressure values. In 10 pseudophakic eyes with persistent diffuse diabetic macular edema, paracentesis of anterior chamber with aspiration of aqueous humor and nitric oxide concentration measurements were done on the day of the intravitreal application of 20 mg triamcinolone acetonide, and after 1, 3, 6 and 9 months. Also, we were recording intraocular pressure values before the intravitreal triamcinolone acetonide injection and during the next 9 months. One month after the intravitreal triamcinolone acetonide injection, we noticed a decrease of nitric oxide concentration (45.37±5.55 μmol/L) by 31.79% compared to the initial values (66.52±7.66 μmol/L). After that, nitric oxide concentrations began to rise slightly, and at the end of the ninth month the mean nitric oxide concentration was similar to that recorded at the beginning of the study. Intraocular pressure values had increasing trend one month after the intravitreal triamcinolone acetonide injection (23.70±4.08 mm Hg) compared to the initial values (16.21±1.55 mm Hg), but after nine months these values returned to normal levels. Decreased concentration of nitric oxide could be one of the reasons for increased intraocular pressure after intravitreal application of triamcinolone acetonide in the treatment of diffuse diabetic macular edema.

Key words: *Diabetic macular edema; Intravitreal triamcinolone acetonide injection; Intraocular pressure; Nitric oxide*

Introduction

Diabetic macular edema (DME) is the most common cause of vision loss in patients affected by diabe-

tes mellitus, especially in type 2 diabetes[1]. The pathogenesis of DME is multifactorial, but inflammation plays a very important role[2,3]. Prior to and following cataract surgery, monitoring of diabetic maculopathy is required because it has been shown that the severity of diabetic retinopathy and DME can progress more rapidly after cataract surgery[4,5]. Ideally, maculopathy should be completely resolved before cataract surgery, but in some cases macular edema persists at the time

of cataract surgery despite the treatment performed. Currently, the anti-vascular endothelial growth factor (anti-VEGF) injections are considered the new gold standard of therapy for eyes with center-involving macular edema and reduced vision.

Corticosteroids are important drugs for the treatment of DME patients, but mostly as a second-level option. They block the expression of VEGF and other inflammatory mediators, inhibit leukostasis, improve the barrier function of endothelial cell tight junctions, and decrease vascular leakage[6-8]. Triamcinolone acetonide (TA) is a synthetic glucocorticoid agonist. The exact mechanism of its action remains unknown but data suggest that TA applied intravitreally (IVTA) could reduce retinal vascular leakage and retinal thickening with consequently improved visual acuity. Its therapeutic effect lasts for approximately 2 to 4 months with 4 mg IVTA and up to 9 months with 20 mg IVTA[9].

For eyes with persistent retinal thickening despite anti-VEGF therapy, treatment with IVTA may be considered, especially in pseudophakic eyes. The Diabetic Retinopathy Clinical Research Network has reported on a randomized trial where TA and ranibizumab were similarly effective when combined with laser treatment in pseudophakic eyes[10].

The most frequent side effects of TA treatment are increase in intraocular pressure (IOP) and cataract. Steroid-induced ocular hypertension was observed in one-third to more than one-half of patients who received IVTA injections[11-17]. It has been suggested that trabecular outflow obstruction may occur due to crystalline steroid particles after receiving IVTA[18].

Nitric oxide (NO) acts as a signaling mediator in various physiological and pathological processes throughout the body. There are three isoforms of nitric oxidase (NOS) synthetase that produce NO and they are encoded by different genes, i.e., neuronal NOS1, inducible NOS2, and endothelial NOS3[19]. All three isoforms of NOS enzyme are expressed in ocular tissues. Under physiological conditions, NO mediates a variety of ocular effects, including maintenance of IOP. NO relaxes trabecular meshwork by altering contractility in conventional outflow tissues and thus allows for an increased outflow of aqueous humor[20-23]. Patients with open-angle glaucoma have been shown to have reduced levels of NO markers[24]. Currently, the use of NO donors is already a relevant therapeutic approach to treat elevated IOP[25].

In this study, we examined a possible correlation between NO concentrations in aqueous humor of pseudophakic patients with diffuse diabetic macular edema after intravitreal application of TA and IOP values during a specific period.

Patients and Methods

The study included 10 diabetic pseudophakic patients (10 pseudophakic eyes) with persistent diffuse macular edema despite receiving two intravitreal injections of anti-VEGF therapy and recruited for IVTA. The study was conducted at the Department of Ophthalmology, Kragujevac Clinical Center, Kragujevac, Serbia, from January 2018 until January 2019. The study design was approved by the local Ethics Committee, and all enrolled patients gave their written consent at the beginning of the study.

All patients had passed complete ophthalmologic examination including visual acuity, IOP measurement, slit lamp and fundus examination. All patients had diffuse diabetic macular edema documented by fundus photography and fluorescein angiography (Carl Zeiss, Meditec, Inc., Dublin, CA, USA) and optical coherence tomography image (Stratus Optical Coherence Tomography, OCT3, Carl Zeiss Meditec, Inc., Dublin, CA, USA). As a pathologic status of increased macular thickness, OCT 3 definition ≥305 μm for males and ≥290 μm for females was used[26].

Complete eye examination was performed at follow up visits at 1 and 3 months, and then every 3 months until 9 months. Paracentesis of anterior chamber was done on the day of the IVTA application and at 1, 3, 6 and 9 months. Aqueous humor was aspirated and samples were immediately frozen in liquid nitrogen and stored at -80 °C.

Intravitreal injections of TA were administered to all patients as an official procedure by a technique described by Jonas *et al.*[27]. Patients were informed that it was an off-label use of TA and signed an informed consent.

All eyes received an intravitreal injection of 20 mg of crystalline TA (Kenalog, Bristol Myers Squibb, Athens, Greece). The eye was prepared for the intervention with 5% povidone-iodine solution and with lidocaine 1% applied subconjunctivally in the infero-temporal quadrant. The IVTA injection was applied in that quadrant, 3.5 mm posterior to the limbus using a 27 gauge needle.

Crystalline cortisone was prepared by aspirating the whole volume of a 1-mL bottle containing 40 mg triamcinolone acetonide (Kenalog, Bristol Myers Squibb, Athens, Greece) into a tuberculin syringe of 1-mL volume. The syringe was vertically positioned on the operating table for at least 15 minutes for sedimentation. Then, the sedimented part of the vehicle was left in the syringe, and upper 0.8 mL was slowly eliminated out of the syringe. Ringer`s solution was used to fill it up and position it vertically for 5 minutes. The procedure of removing 0.8 mL of the drug was repeated. The procedure was repeated twice. Then the remaining 0.2 mL, containing about 20 mg TA, was injected into the vitreous cavity. Ciprofloxacin drops (Floxal, Dr. Gerhard Mann, Chem.-Pharm. Fabrik GmbH, Berlin, Germany) were administered as prophylactic treatment for five days after intravitreal injection.

Total NO concentrations in the samples were measured using the technique described by Green *et al.*[28]. Analyzed solutions of 0.2 mL, PCA 3M 0.1 mL, and EDTA 20 mm 0.4 were pipetted into Eppendorf tube (aqueous humor-diluted 1/10). The samples were incubated at -4 °C for 10 minutes, centrifuged for 4 minutes at 1500 rpm. Precipitated material was then mixed in Green's reagent (prepared before use), after the supernatant was decanted.

Distilled water was used for control measurements. In the test tube, we put 0.1 mL of extracted sample, 250 μL of Green's reagent and 125 μL of ammonia puffer (pH 9). After stabilization of the mixture, NO concentration was measured by spectrophotometric method at 550 nm.

The IOP was measured before the injection, on the post injection day 1 and day 7, then at 1, 3, 6 and 9 months after IVTA application. When the IOP was more than 25 mm Hg, IOP reducing medications were prescribed. The initial reducing IOP medication was the fixed combination of dorzolamide hydrochloride-timolol maleate (Cosopt, MSD, Haarlem, The Netherlands). If the achieved IOP was suboptimal, we added brimonidine tartarate (Alphagan, Allergan, Westport, Co., Mayo, Ireland) and acetazolamide (Diamox, Lederle, Maidenhead Berks, UK). If normal IOP was not reached, filtering surgery would have been planned (trabeculectomy). Excluding criterion for patients was pre-existing glaucoma due to the possibility that NO concentration in aqueous humor could have

already been changed in that ocular condition.

On statistical data analysis, SPSS 20.0 statistical software package (SPSS Inc., Chicago, IL, USA) was used. The normality of distribution was tested by Shapiro-Wilk test. For comparative statistical procedures, we used nonparametric Friedman test or parametric one-way ANOVA and Student's t-test. The results were expressed as the mean. The levels of statistical significance were set at $p<0.05$ and $p<0.001$ (as indicated in Figure 1).

Results

The mean age of our participants was 59.47±9.53 (range 45-84) years. The male to female ratio was equal (male and female, 5 each). All patients had diabetes mellitus type 2. The mean duration of diabetes mellitus was 15.12±6.8 years.

All examined eyes were pseudophakic and cataract surgery was performed by phacoemulsification technique one year before current examination. Also, all examined eyes had persistent diffuse diabetic macular thickening despite 2 intravitreal injections of anti-VEGF agent (bevacizumab) received approximately 3 months before and 6 months after cataract surgery.

The mean NO concentration in diabetic participants before IVTA was 66.52±7.66 μmol/L (range 48.80-82.11 μmol/L). Paracentesis was performed at each next patient follow up visit. At one month, we recorded a statistically significant decrease of NO concentration by 31.79% (mean 45.37±5.55 μmol/L, range 35.50-56.22 μmol/L; $p<0.001$). In the next period, NO concentration showed a slight increase, with a statistically significant difference compared to the mean value before IVTA; it was 51.79±6.37 μmol/L (range 40.11-60.62 μmol/L; $p<0.001$) at 3 months and 61.63±8.01 μmol/L (range 41.10-74.45 μmol/L; $p<0.001$) at 6 months. At the end of the study, nine months after IVTA injection, the mean NO concentration was similar to that before IVTA (mean 68.77±7.64 μmol/L, range 49.97-80.26 μmol/L), without any statistically significant difference ($p=0.1$).

Before IVTA application, the mean IOP in our patients was 16.21±1.55 mm Hg (range 13-19 mm Hg). We noticed a slight rise of the IOP on post injection day 1 (17.77±2.43 mm Hg; range 14-21 mm Hg) and day 7 (18.1±1.89 mm Hg; range 15-21 mm Hg), without a statistically significant difference.

The highest increase of IOP was recorded at the end of the post injection month 1, when the mean IOP value in all examined eyes was 23.70±4.08 mm Hg (range 18-30 mm Hg) and statistically significantly higher than the pre-injection values (p<0.001). In 5 (50%) eyes, IOP exceeding 22 mm Hg was recorded.

Until the end of the post injection month 6, IOP value differed statistically significantly as compared to the pre-injection level: month 3: 22.0±2.66 mm Hg (range 18-26 mm Hg) statistically significant (p<0.001); and month 6: 21.20±2.53 mm Hg (range 18-25 mm Hg) statistically significant (p<0.001). Although at the end of the post injection month 9, normal IOP values were achieved (mean 19.2±1.68 mm Hg, range 17-22 mm Hg), a statistically significant difference was still present compared to the pre-injection value (p=0.001).

We noticed that maximum IOP elevation by 46.3% was recorded at one month after IVTA. Elevated IOP values persisted throughout the measurements until the end of the study; at month 3, the mean IOP was by 35.8% higher, at month 6 by 30.8%, and at month 9 by 18.5% than before IVTA application. Figure 1 shows the mean values of NO concentration and IOP during the study.

According to our results, the first and biggest elevation of IOP over 22 mm Hg was noticed in 5 (50%) eyes at one month after IVTA (27.20±1.92 mm Hg, range 25-30 mm Hg). The mean age of patients with elevated IOP during the study was 55.22±6.72 years, and three of them were males. Antiglaucomatous drug was prescribed to them. On the next visit, despite prescribed therapy, these 5 patients still had elevated IOP, but with a lower mean IOP (24.20±1.48 mm Hg, range 22-26 mm Hg). For that reason. we added the maximum planned antiglaucomatous therapy. Six months after IVTA injection, with prescribed therapy, we achieved significant decrease of IOP in these 5 patients (23.40±1.14 mm Hg, range 22-25 mm Hg). At the end of the study, normal IOP values were reached in these patients (20.6±0.89 mm Hg, range 20-22 mm Hg), so there was no need for filtering surgery. In these eyes, from month 1 to the end of the post injection month 9, the mean IOP values were statistically significantly higher compared to the pre-injection values (p<0.001).

In the other 5 eyes, there was no IOP increase over 22 mm Hg. The mean IOP values were as follows: month 1: 20.20±1.79 mm Hg (range 18-22 mm Hg); month 3: 19.80±1.30 mm Hg (range 18-21 mm Hg); month 6: 19.00±1.10 mm Hg (range 18-20 mm Hg); and month 9: 17.8±0.83 mm Hg (range 17-19 mm Hg). Comparing them to those eyes who had increased IOP over 22 mm Hg, high statistical significance was noticed in all measurements (p<0.001).

Comparing NO concentrations between patients with increased IOP over 22 mm Hg and those without it, no statistically significant difference was observed before IVTA (69.60±7.57 *vs.* 64.21±5.88; p=0.186). Statis-
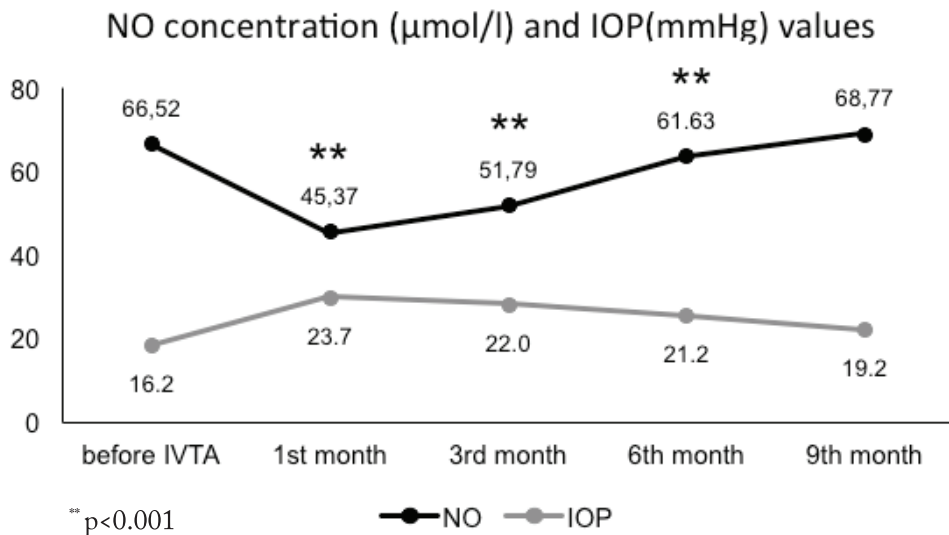

Fig. 1. Values of nitric oxide (NO) concentration and intraocular pressure (IOP) during the study.

tical significance was only recorded at one month after IVTA (48.95 ± 5.36 *vs.* 42.65 ± 4.82; p=0.005). Measurements at three (53.57 ± 4.25 *vs.* 49.91 ± 8.30; p=0.243), six (65.28 ± 3.14 *vs.* 61.70 ± 7.83; p=0.324), and nine months (72.20 ± 5.58 *vs.* 67.85 ± 5.87; p=0.384) yielded no statistical significance. We calculated a strong negative correlation between IOP values and NO concentration in those 5 eyes with IOP increase (-0.77), and mild correlation in the other 5 eyes (-0.31) during the study.

Discussion

Diabetic macular edema is the most common cause of vision loss in patients affected by diabetes mellitus, especially type 2 diabetes. The pathogenesis of DME is multifactorial, but inflammation plays a very important role. For this reason, corticosteroids are a reasonable choice for DME treatment. They produce an anti-inflammatory effect through various mechanisms, including decrease in the synthesis of inflammatory mediators, block the expression of VEGF, inhibit leukostasis, improve barrier function of endothelial cell tight junctions, and decrease vascular leakage[29-32]. TA is a synthetic glucocorticoid agonist. Intravitreal TA injections, used off label to treat DME, improve vision in many patients[16]. Therapeutic effect of IVTA is reported to last for approximately 2 to 4 months with 4 mg IVTA and up to 9 months with 20 mg IVTA[9].

Prior to and following cataract surgery, monitoring of diabetic maculopathy is required because it has been shown that the severity of diabetic retinopathy and DME can progress more rapidly after cataract surgery[4,5]. Ideally, maculopathy should be completely resolved before cataract surgery, but in some cases macular edema persists at the time of cataract surgery despite treatment performed. Currently, anti-VEGF injections are considered the new gold standard of therapy for eyes with center-involving macular edema and reduced vision[33-35]. Corticosteroids are important drugs for the treatment of DME patients, but mostly as a second-level option. For eyes with persistent retinal thickening despite anti-VEGF therapy, treatment with intravitreal triamcinolone may be considered, especially in pseudophakic eyes[10]. As first-line therapy, the use of steroids may be considered in patients with a history of severe cardiovascular disease[36]. The Diabetic Retinopathy Clinical Research Network has reported on a randomized trial where TA and ranibizumab were similarly effective when combined with laser treatment in pseudophakic eyes[10,37].

The most frequent side effects of TA treatment are IOP increase and cataract. IOP elevation after intravitreal depot steroid injection was observed in 28%-77% of patients[11-17,38]. Rhee *et al.* report IOP elevation in 53.2% of eyes following a single IVTA injection, 50.6% of which were at least 30% above baseline[39]. In their study using 25 mg doses, Jonas *et al.* report that IOP elevation occurred about 2 months after the injection in about 50% of eyes[40].

Steroid-induced glaucoma is a form of open angle glaucoma. The precise mechanism of IOP increase after steroid use is not completely clear, but it occurs primarily due to the increased outflow resistance. Steroids cause stabilization of lysosomal membranes and accumulation of polymerized glycosaminoglycans in the trabecular meshwork. Corticosteroids cause inhibition of phagocytotic properties of endothelial cells in the trabecular meshwork, which leads to the accumulation of aqueous debris. Steroids reduce the synthesis of prostaglandins, which regulate water outflow[41]. Also, genetic influence in steroid responsiveness has been proposed. Besides the myocilin gene alpha1 antichymotrypsin, for which the role is well established[42], many other genes have been postulated to play a role in steroid responsiveness[43].

After intravitreal injection of TA, trabecular outflow obstruction can occur due to the accumulation of crystalline steroid particles in the trabecular meshwork[18]. Following intravitreal injection of triamcinolone, dissolution of insoluble triamcinolone crystals into soluble triamcinolone within the vitreous occurs. One part of the drug targets the retina, but a larger portion diffuses anteriorly to the crystalline lens and outflow pathways. In our study, in one eye, in the early post injection period, greater accumulation of crystalline particles occurred in the anterior chamber of the eye in the form of a transient 'triamcinolone pseudohypopyon', but surprisingly without a significant IOP increase (Fig. 2).
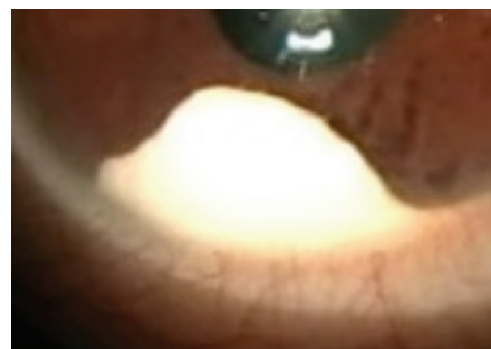

Fig. 2. Triamcinolone pseudohypopyon.

Exposure of glucocorticoid to the outflow tissues alters the extracellular matrix and increases outflow resistance and may increase IOP[44]. Also, data suggest that IOP was elevated until TA crystals could be viewed in the vitreous[45]. Younger males were more common among the participants with elevated IOP[46].

Earlier studies by Wingate and Beaumont indicated that increased IOP after intravitreal triamcinolone injection could occur in the first 3 months[47], but it could also be raised until 6 months without other known local risk factors[48]. Our IOP measurements indicated that IOP was elevated in 5 patients one month after the IVTA application. Also, males were dominant (3 patients) and the mean age of patients with elevated IOP was lower than in the whole study group (55.22±6.72 years). All these patients had mean IOP values statistically significantly higher than other patients until the end of the study. After month 9, IOP returned to normal values in these patients, which probably was the result of complete pharmacokinetic elimination of TA from the eye[49,50]. With prescribed antiglaucomatous therapy, we managed to achieve partial lowering of IOP in the period from the post injection month 1 to month 6. Antiglaucomatous surgery was not performed in any of these 5 eyes because there is still no clear recommendation when this procedure should be performed in secondary ocular hypertension caused by IVTA. Data also indicate that in most patients, normalization of IOP can be expected after 9 to 12 months[40,51]. In the mentioned period, these patients had detailed ophthalmologic examination, and none had glaucomatous optic nerve damage or visual field defect.

Nitric oxide acts as a signaling mediator in various physiological and pathological processes throughout the body[52]. There are three isoforms of NO synthetase (NOS) that produce NO and they are encoded by different genes, i.e., neuronal NOS1, inducible NOS2, and endothelial NOS3[19]. All three isoforms of NOS enzyme are expressed in ocular tissues. In addition to vasodilatory function, eNOS plays an important role in mediating VEGF-induced vascular permeability and angiogenesis, which is of great importance in retinal vascular disorders[53].

Under physiological conditions, NO mediates a variety of ocular effects, including maintenance of IOP. NO plays an important role in regulating trabecular meshwork contractility. NO relaxes trabecular meshwork by altering contractility in conventional outflow tissues and thus allows for an increased outflow of aqueous humor[54-56]. Since NO is a local mediator for improving and decreasing contractility in the conventional outflow tract, deficiency of NO and/or dysfunctional NO signaling may be a root cause of increased trabecular meshwork rigidity[22-25]. NO decreases IOP by increasing aqueous outflow facility in the trabecular meshwork and/or Schlemm's canal. However, cellular mechanisms are still unknown. It is believed that contractility of endothelial cells of trabecular meshwork regulates outflow facility. The predominant isoform in the trabecular meshwork is iNOS, likely because of the presence of macrophages, while eNOS is the predominant isoform expressed by endothelial cells within Schlemm's canal cells and macrophages found in the trabecular meshwork[57,58].

It has been shown that patients with open angle glaucoma present with decreased levels of NO markers. Interestingly, NO concentration in the vitreous humor correlates with the type and severity of glaucoma[24]. There are certain proofs which suggest that dysregulation of NO in trabecular meshwork, Schlemm's canal, and ciliary muscle in patients with primary open angle glaucoma could be connected to IOP elevation[59].

Several studies have shown that intravitreal injection of TA leads to a significant decrease in the concentration of VEGF and many other inflammatory cytokines in the vitreous and aqueous humor[60,61]. Since NO is implicated in VEGF mediated vascular permeability and angiogenesis, it can be assumed that changes in the VEGF concentration also lead to changes in NO concentration after intravitreal injection of TA.

Our measurements undoubtedly showed that NO concentration in aqueous humor was lower than before IVTA throughout the following period, indicating that intravitreal corticosteroid application leads to decreasing NO concentration in the eye.

Those measurements showed reduced NO concentration by about 31.79% at month 1, by 22.12% at month 3, and by 7.36% at month 6 as compared to the pre-injection level. At month 9, the mean NO concentrations were similar as at the beginning of the study. Comparing NO concentration between patients with and without elevated IOP, statistical significance was only seen at one month after IVTA. These results suggest that decreased NO concentration could be one, but not the only cause of IOP elevation. It is well known that corticosteroid potential for IOP rise after

local or systemic use of corticosteroids is genetically determined[42,43].

Corticosteroids increase IOP by inhibition of the extracellular matrix degradation, as well as by decreasing local (ocular) NO concentrations. When TA was metabolized, its local effects vanished, and NO concentrations returned to the starting level. Consequently, IOP values were also normalized.

Strong negative correlation was recorded between NO concentration and IOP in 5 eyes with elevated IOP. In other eyes, the correlation was mild because these eyes also had the mean IOP values statistically significantly higher than the pre-injection values, but without the need for antiglaucomatous therapy.

Conclusion

Although the primary reason for IVTA application was treatment of persistent diffuse DME, in this study we paid attention to its effect in changing NO concentration and IOP values. We observed transient reduction of NO concentrations in aqueous humor, which coincided with IOP elevation after the application of IVTA. Although our study had a small number of eyes analyzed, due to the delicacy of the design and examination procedures, its results led us to a conclusion that the decreased concentration of NO could be one of the reasons for the transient IOP increase after IVTA injection. Temporarily decreased NO concentration can affect aqueous humor outflow pathways with a possible consequence of transitional elevation of IOP. Currently, the use of NO donors is already a relevant therapeutic approach to treat elevated IOP, and the results of our work could confirm the importance of changes in NO concentration in the pathogenesis of IOP increase.

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Sažetak

JE LI SMANJENA KONCENTRACIJA DUŠIKOVA MONOKSIDA NAKON INTRAVITREALNE INJEKCIJE TRIAMCINOLON ACETONIDA JEDAN OD RAZLOGA PORASTA OČNOG TLAKA?

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Dijabetični edem makule najčešći je uzrok gubitka vida kod bolesnika s dijabetesom melitusom. Za oči s trajnim zadebljanjem mrežnice unatoč anti-VEGF terapiji liječenje intravitrealnim triamcinolonom može se razmotriti, osobito kod pseudofakičnih očiju. Cilj ovog istraživanja bio je ispitati promjene koncentracije dušikova monoksida u očnoj vodici u pseudofakičnim očima s trajnim difuznim dijabetičnim edemom makule nakon intravitrealnog ubrizgavanja triamcinolon acetonida, kao i potencijalni utjecaj tih promjena na vrijednosti očnog tlaka. U 10 pseudofakičnih očiju s ustrajnim difuznim dijabetičnim edemom makule paracenteza prednje očne sobice s aspiracijom očne vodice i mjerenjem koncentracije dušikova monoksida obavljena je na dan intravitrealne primjene 20 mg triamcinolon acetonida te nakon 1, 3, 6 i 9 mjeseci. Također smo bilježili vrijednosti očnog tlaka prije intravitrealnog ubrizgavanja triamcinolon acetonida i tijekom sljedećih 9 mjeseci. Mjesec dana nakon intravitrealnog ubrizgavanja triamcinolon acetonida primijetili smo smanjenje koncentracije dušikova monoksida (45,37±5,55 μmol/L) za 31,79% u odnosu na početne vrijednosti (66,52±7,66 μmol/L). Nakon toga koncentracije dušikova monoksida počele su lagano rasti, a na kraju devetog mjeseca srednja koncentracija dušikova monoksida bila je slična kao i na početku studije. Vrijednosti očnog tlaka imale su trend porasta mjesec dana nakon ubrizgavanja intravitrealnog triamcinolon acetonida (23,70±4,08 mm Hg) u usporedbi s početnim vrijednostima (16,21±1,55 mm Hg), ali nakon devet mjeseci te su se vrijednosti vratile na normalnu razinu. Smanjena koncentracija dušikova monoksida mogla bi biti jedan od razloga povišenog očnog tlaka nakon intravitrealne primjene triamcinolon acetonida u liječenju difuznog dijabetičnog edema makule.

Ključne riječi: *Dijabetični edem makule; Intravitrealna injekcija triamcinolon acetonida; Intraokularni tlak; Dušikov monoksid*