ABSTRACT

Subclinical inflammation occurring in diabetic retinopathy is supposed to be, at least partly, the cause of breakage of blood-retinal barrier. It may be expected that anti-inflammatory drugs have favorable effect on the course of diabetic macular oedema.

The aim was to investigate the effect of intravitreally applied triamcinolone acetonide (TA) on resolution of diffuse diabetic macular oedema and visual acuity improvement.

A single intravitreal application of 20 mg/0.2 mL triamcinolone was made in 88 eyes with developed diffuse macular oedema, where no laser treatment had been done before. Biomicroscopic assessment of foveal thickness and test of visual acuity were made in three-month intervals.

In the eyes with the longest period of follow-up (12 months), before application of triamcinolone, the average visual acuity was 0.14±0.16, and the average value of foveal thickness above the normal foveal thickness about 350 µm. During the first 3 months, visual acuity had shown tendency of improvement, it was stable during 4 to 6 months, and then started to fall gradually (in the 1st month: 0.29±0.24; 3rd month: 0.26±0.23; 9th month: 0.25±0.24; 12th month: 0.22±0.20). Average foveal thickness in the first 3 months after application of the drug showed tendency of decrease, while after that period thickness of the oedema started to grow slowly (in the 1st month: about 130µm; 3rd month: about 80µm; 6th month: about 100µm; 9th month: about 150µm; 12th month: about 190µm). Regardless of gradual decrease visual acuity and return of the oedema as noticed in the 6th month, the values of the visual acuity and foveal thickness even 6, 9 and 12 months following the application of the drug were statistically much better than those before the treatment.

Triamcinolone given intravitreally stabilizes the blood-retinal barrier, helps in resolution of diffuse macular oedema and slows further decrease of visual acuity.

Key words: diabetic retinopathy, macula lutea, edema, triamcinolone acetonide (TA), visual acuity.

INTRODUCTION

Diabetic macular oedema is a thickening of macular surface caused by abnormal accumulation of liquid in macula. It progressively disturbs macular anatomy and leads to slow irreversible degradation of photoreceptors. It is also the most frequent complication of diabetic retinopathy and the main reason for decreased visual acuity, particularly in the patients suffering from type 2 diabetes mellitus. Wisconsin epidemiological study of diabetic retinopathy estimated that after 15 years of diabetes, macular oedema exists in 20% of the type 1 diabetic patients, in 25% of the type 2 diabetic patients treated with insulin, and in 14% of the type 2 diabetic patients who did not take insulin (1). Macular oedema, although rarely leads to total blindness, often reduces central visual acuity to the level which makes the patients unable to perform their professional activities and, consequently, impairs their quality of life.

Almost a half of the patients with macular oedema loosens 2 and more lines of visual acuity in the subsequent two years of spontaneous evolution of maculopathy. Till now, the only approved therapeutic procedure in the treatment of diabetic maculopathy is laser photocoagulation. A special therapeutic problem are the eyes with diffuse macular oedema resulting from generalized hyperpermeability of the overall vascular net of macular region. In such cases, there is no possibility to identify the particular leakage spots that could be treated by focal laser photocoagulation. In these eyes the failure rate of laser treatment and impairment of visual acuity ranges between 25% and
VEGF is increased very early in diabetic retinopathy, long before extensive proliferation, suggesting important role in development of diabetic macular oedema (5,6).

If the subclinical inflammatory disease contributes to development of diabetic retinopathy, then anti-inflammatory drugs could have a favourable effect on the course of the disease. Corticosteroids, with their anti-inflammatory properties, could significantly inhibit expression of VEGF gene and thereby mitigate a VEGF-induced hyperpermeability of retinal blood vessels and slow or diminish development of diabetic macular oedema.

In addition, it has been found that intravitreal injection of cortisone does not exhibit any toxic reaction on intraocular tissues, and is eliminated from the eye within 24 hours. Based on these facts, in 1999 the intravitreal application of triamcinolone was reported, a syntetic corticosteroid drug, in the form of depot preparation. Its intravitreal absorption time is very prolonged, from 2 to 5 months, thus enabling much longer contact of the drug with retina (7,8). In the beginning, this technique has been used only in patients with previously unsuccessful laser photocoagulation but now it is often used as primary therapy in some patients with diffuse diabetic macular oedema (9).

However, many treatment issues are still unresolved like selection of patients who might expect the greatest benefit, optimal dosing regimen, proper technique, duration of treatment and need for additional treatment and follow-up. Therefore, the aim of our study was to investigate the effect of single intravitreal injection of triamcinolone acetonide (TA) in the dosage of 20 mg/0.2 mL, as the only treatment, on macular oedema and visual acuity in the patients with diffuse diabetic maculopathy.

PATIENTS AND METHODS
The study had prospective, open, interventional design without control group. The study included 68 diabetic patients (68 eyes) with nonproliferative diabetic retinopathy and diffuse diabetic macular oedema. There were 25 (36.7%) men and 43 (63.3%) women with average age of 60.4±7.2 years. All these patients had type 2 diabetes mellitus and the average time they suffered from it was 14.8±5.4 years. Before entering the study all of the patients were fully informed about the experimental character of this treatment and they were given explanations concerning the procedure of treatment of diabetic maculopathy by intravitreal application of corticosteroid drugs. All of the patients signed an informed consent. The study has been approved by the Ethical Committee of Clinical Centre Kragujevac, and was performed during 2005–2006 year.

The eyes included in this study were chosen by the following inclusion criteria:

a) current persistent, clinically significant, diffuse macular oedema untreated previously by laser photocoagulation,

b) progressive decrease of visual acuity, as found in previous ophthalmologic check-ups,

c) the absence of other ophthalmologic disease, such as glaucoma or cataract.

At baseline and also at every subsequent check-up, each eye was subjected to a detailed ophthalmologic examination which included: determination of visual acuity by Snellen chart, aplanation tonometry, biomicroscopic examination of anterior eye segment and particularly assessment of transparency of eye lens, biomicroscopic examination of the fundus and biomicroscopic estimation of macular thickness by means of Goldman lens with 3 mirrors and Volk lens +90 Dp. The assessment of retinal thickness has been made by means of indirect binocular biomicroscopy with 50 µm graduation. Based on previous reports concerning indirect binocular biomicroscopy, it has been considered that initial macular oedema correspond to increased foveal thickness exceeding normal foveal thickness by 60% (i.e. about 100 µm) (10). All ophthalmologic examinations as well as biomicroscopic assessment of the size of foveal thickness, and intravitreal application of the drug were carried out by the same investigator.

For intravitreal application of triamcinolone acetonide (TA), the commercially available Kenalog 40® (Bristol Myers-Squib, Princeton, NJ) was used. The intravitreal injection of TA was performed under topical anesthesia, using 3 mirrors and Volk lens +90 Dp. The study drug was prepared from the original preparation of Kenalog® ampoule containing 40 mg of triamcinolone in the volume of 1 mL, using the technique of triple sedimentation. Application of the drug was made through pars plana of the ciliar body at 3.5–4 mm from the limbus of cornea in inferotemporal quadrant, using a small diameter needle (27 or 30 gauge).

In order to reduce intraocular volume intravenous infusion of 250 mL of 20% manitol had been applied 2 hours before the drug was injected. After application, the patients were told to keep their heads in upright position for 2 hours, in order to prevent settling of triamcinolone crystals upon the macular region itself.
All the patients were examined on the first day after intravitreal injection, and then after 7 days, 1 month, 3 months, 6 months and further on in three-month intervals. When appropriate the color pictures of the fundus and fluorescein angiography were taken before application of triamcinolone as well as after 3–6 months.

The statistical data processing was done by using commercially available statistical software package (SPSS for Windows, version 11.5, SPSS, Chicago, IL, USA)). Non-parameter Friedman test was used for comparison of changes in visual acuity and macular thickness. The level of significance was 0.05 (two sided) in all statistical testing.

RESULTS

The patients were followed-up from 6 to 12 months: for 15 eyes the study period was 12 months, for 35 eyes 9 months, while all 68 eyes treated had minimum monitoring period of 6 months. The average duration of follow-up was $8.2\pm2.4$ months (The mean± Stand.dev.).

Table 1 shows the values of visual acuity during the monitoring period. In 15 eyes with the longest monitoring time of 12 months, the initial visual acuity before application of TA was $0.14\pm0.16$. During the first 3 months after application of the drug the visual acuity tended to improve and grow very quickly (at the end of the 1st month $0.29\pm0.25$; at the end of the 3rd month $0.29\pm0.24$). Between the 3rd and 6th month the visual acuity was maintained rather stable but with a slow tendency of decline (at the end of the 6th month $0.26\pm0.23$). This slow-falling trend continued even after nine months ($0.25\pm0.24$), and 12 months of monitoring ($0.23\pm0.23$). The similar results were obtained also with the group of eyes with shorter monitoring time of 6 and 9 months. Figure 1 shows the changes of visual acuity during the monitoring period. Regardless the tendency of slow decline of visual acuity observed in all eyes tested at the end of the 6th month, the values of visual acuity even after 6, 9 and 12 months following the application of the drug were statistically much better than those before the beginning of treatment ($p<0.001$, $p<0.001$, $p<0.001$).

Table 2 shows the values of macular (foveal) thickness during the study period.

In 15 eyes with the longest monitoring time of 12 months, the average thickness of foveal zone before application of the drug was $353\pm130.2$ µm. During the first 3 months after application of the drug the macular thickness decreased significantly (at the end of the 1st month $130.67\pm85.5$ µm; at the end of the 3rd month $83.33\pm74.8$ µm). At the end of the 6th month, a slow increase of foveal thickness ($100.00\pm100.00$ µm) was observed again, and this trend of increase of foveal thickness continued even at the end of the 9th month of monitoring ($146.67\pm124.6$ µm) and at the end of the 12th month of monitoring ($186.67\pm140.75$ µm). The similar results were obtained also in the group of eyes with shorter monitoring time of 6 and 9 months. Figure 2 shows the changes in macular (foveal) thickness during the monitoring period. Regardless of the tendency of slow increase of foveal thickness observed in all eyes tested at the end of the 6th month, the values of foveal thickness even after 6, 9 and 12 months following the application of the drug were statistically much lower than those before the beginning of the treatment ($p<0.001$, $p<0.001$, $p<0.001$).
**Table 2.** Macular (foveal) thickness during 6, 9 and 12 months of follow-up.

<table>
<thead>
<tr>
<th>Tested parameter</th>
<th>Macular thickness (µm) (X±SD)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macular thickness during 12 months of monitoring (n=15)</td>
<td></td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>before</td>
<td>353.33±130.2</td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>136.67±65.5</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>83.33±74.8</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>100.00±100.0</td>
<td></td>
</tr>
<tr>
<td>9 months</td>
<td>146.67±124.6</td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>186.67±140.75</td>
<td></td>
</tr>
<tr>
<td>Macular thickness during 9 months of monitoring (n=35)</td>
<td></td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>before</td>
<td>371.43±142.6</td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>148.71±97.3</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>81.43±62.42</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>108.57±122.17</td>
<td></td>
</tr>
<tr>
<td>9 months</td>
<td>171.43±146.67</td>
<td></td>
</tr>
<tr>
<td>Macular thickness during 6 months of monitoring (n=68)</td>
<td></td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>before</td>
<td>372.06±143.36</td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>144.12±96.01</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>79.41±88.62</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>107.35±120.09</td>
<td></td>
</tr>
</tbody>
</table>

*Friedman test; (X±SD): The mean ± Stand. dev; n: number of eyes

Figure 3 show the view of macula: a) before and b) 3 months after triamcinolone was given. A significant reduction of number of hard deposits in the macula can be observed; usually it is also noted that those with decreased lipoprotein deposits demonstrated also the best improvement of visual acuity.

Figure 4 show fluorescein angiography: a) before and b) 3 months after application of the drug; significant decrease of capillary leakage was noted due to an improvement in functional status of blood-retinal barrier.

**DISCUSSION**

Increased vascular permeability in the course of diabetic retinopathy occurs as a result of damage to the blood-retinal barrier, that is, impaired architecture of intercellular junctions between endothelial cells. The key event that causes conformation changes and destabilization of tight junctions is phosphorylation of proteins of tight junctions. Antonetti and others have demonstrated that Vascular endothelial growth factor (VEGF) can increase permeability of blood vessels by increasing phosphorylation of protein of tight junctions, such as occludin and zonula occludens 1. In experimental conditions VEGF may increase vascular leakage of retinal vasculature even 50.000 times more than histamin and result in opening almost 30% of retinal tight junctions (11). VEGF also increases expression of Intercellular adhesion molecule 1 (ICAM-1) which is critical component that takes part in leukocyte adhesion to endothelium (12,13). In diabetic patients leukocytes are considered to be less deformable and more strongly adhering to vascular endothelium. This leukocytic stasis in macula
can deepen macular hypoxia and contribute to development of macular oedema (6). Increased permeability of capillary net were in macula causes progressive accumulation of liquid in retinal tissue which results in increase of its thickness and slow necrosis of photoreceptor cells in the macula. The macular thickness is considered to be the most sensitive parameter related to macular oedema associated with loss of visual acuity (14).

All this suggests that numerous pathological phenomena inherent in diabetic retinopathy occur following the pattern of „chronic subclinical inflammation“ (4). If diabetic retinopathy is really a subclinical inflammatory disease then anti-inflammatory drugs could have a favorable effect on its course. It is known that circulating glucocorticoids in their physiological concentrations regulate permeability of endothelial and epithelial barriers. Although the precise mechanism of action of triamcinolone in diabetic retinopathy is not completely understood, there are two main actions that could be helpful: restoration of blood-retinal barrier and anti-inflammatory action.

It was found that hydrocortisone greatly reduces phosphorylation of occludin and increases its concentration; it also increases the quantity of ZO-1 which results in conformational changes and binding of tight junctions (15). This could lead to improvement of barrier-like features of endothelium as manifested by reduced intensity of vascular leakage by 5 times and increased transendothelial electric resistance by 3 times (16). It was also reported that after 2–7 weeks of treatment by glucocorticoids resulted in doubled number of tight junctions between endothelial cells, tripled concentration of ZO-1 and tenfold reduction of interendothelial gaps (17).

The other, well-known actions of corticosteroids such as inhibition of the pathway of arachidonic acid, reduction of cytokine synthesis, reduction of expression of adhesion molecules and production of VEGF (18,19) could be very helpful in stabilization of blood-retinal barrier and reduction of intensity of vascular leakage. Additional explanations for reduction of macular oedema may lay in the fact that triamcinolone crystals, due to their sedimentation, can lead to posterior detachment of vitreous body (20). The mechanism of improvement of visual acuity after application of TA is unknown, but it can be assumed that activation of glucocorticoid receptors inhibits apoptosis of photoreceptors; also, in the experiments on rabbit's retinas, an improvement in electroretinographic amplitudes of a and b waves by 10–25% was observed after intravitreal injection of TA (21, 22).

So far, all clinical studies like ours have indicated a significant improvement of macular function and structure after injection of TA, at least within a short time period. In the largest series in which triamcinolone was the only treatment for diabetic macular oedema, a dose of 20 mg resulted in an improvement by at least two Snellen lines in regards to initial values after 1, 3 and 6 months (25). In our study, the greatest increase of visual acuity after the injection of TA in the eyes treated was observed in the first month after application of the drug, and a slow rise continued until the end of the third month. In that period visual acuity was approximately doubled. However, our results confirmed previous findings that the phase of rapid improvement was followed by plateau phase, and consequently regression of visual acuity and slow return of macular oedema. Between the third and sixth month the visual acuity of our patients was maintained stable, but with a slow downward tendency that continued even at 9 and 12 months, although these values were significantly better than those before the beginning of treatment. Also worth emphasizing is the fact that most eyes with initial visual acuity of 0.05 and lower, rarely showed an improvement of visual acuity by more than 0.1 after application of TA. This means that the function of central macula in these patients had been already deeply damaged by the oedema preventing improvement.

Improvement of visual acuity is associated with reduction of thickness of macular oedema after application of TA. This again underlines the fact that macular thickness is the most sensitive parameter concerning macular oedema associated with loss of vision (26). In clinical studies it has been found that corticosteroids can greatly reduce macular thickness by almost 50% as confirmed by Optical Coherent Tomography (OCT). Using OCT measurement Ciardella and associates have shown reduction of average thickness of central macula from 540 µm to 240 µm after injection of TA (27). In our study biomicroscopic assessment of foveal thickness has shown the greatest reduction of oedema in the first 3 months after application. Although there was the tendency of slow increase of foveal thickness, beyond the 6th month the values of foveal thickness even after 6, 9 and 12 months following application of the drug were significantly lower than those before the beginning of treatment.

One final unresolved issue concerning the use of intravitreal triamcinolone is the optimal dose to maximize efficacy and minimize side effects. The three most frequently used doses of TA for treating diffuse maculopatthy are 4 mg, 8 mg, and 20 mg. In our study we have chosen the maximum dose of triamcinolone used so far (20 mg). Our decision was based on the results of the study published by Spandau and others who compared the effects of 3 different doses of TA of 4 mg, 8 mg and 25 mg (28). They reported that improvement of visual acuity was greater and duration of the effect of TA was significantly longer when larger doses of the drug were used. On the other hand, the increase of intraocular pressure, as the most frequent complication of this type of treatment, was not dose-dependent.

Another unresolved issue with the use of this technique is duration of the action of intravitreally applied TA. The mean maximum duration of effect of triamcinolone coincides with the presence of triamcinolone crystals in vitreous body. In our study the triamcinolone crystals
were visible in vitreous body 115±36 days after injection time. It is close to the results obtained by Audren and others who used pharmacokinetic-pharmacodynamic modelling of OCT readings and estimated the maximum duration of effect intravitreally applied TA to be 140 days (29).

The most frequent complication of intravitreal application of TA in our study, as in most other studies as well, was the increase of intraocular pressure, observed in about 35% of the eyes. In all cases it was possible to regulate this increase of pressure by using one or two topical antiglaucomatous drugs, and in none of the eyes there was the need for surgical antiglaucomatous operation. This corresponds to other published studies in which the increase of intraocular pressure occurred in approximately 30–40% of the patients and it returned to the pre-injection values about 8–9 months following the injection (30).

REFERENCES

The fall of visual acuity which occurred 6 months after application of the drug was mostly caused by recurrence of foveal oedema but also by progression of cataract, which was observed in 17% of the eyes at the end of the 9th and in 20% of the eyes at the end of the 12th month of monitoring. The results of our study indicate that single intravitreal injection of 20 mg triamcinolone acetonide stabilizes blood-retinal barrier, helps resolution of diffuse macular oedema, prevents further decrease of visual acuity and has an acceptable profile of side effects. However, short-time of action, as well as the risks associated with repeated intravitreal injections of triamcinolone limit the possibility of choosing this type of treatment as the long-term treatment in diabetic macular oedema. Nevertheless, it is important to emphasize that intravitreally applied triamcinolone has the ability to improve macular structure and function in diabetic maculopathy, at least within a short time period.