The impact of octreotide in experimental proliferative vitreoretinopathy

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Aims: This study aims to investigate the effects of intravitreal octreotide on the growth factors, which have significant roles in the pathogenesis of proliferative vitreoretinopathy (PVR). **Settings and Design:** An experimental trial. **Materials and Methods:** 21 guinea pigs were randomly assigned to form 3 groups each including 7 animals. In group 1 (the control group), 0.2 ml saline solution was applied intravitreally in a location of 1.5 mm behind the limbus. In group 2 (the sham group), 0.07 IU dispase in 0.1 ml and 0.1 ml saline solution were applied via the same route. The guinea pigs in group 3 (the treatment group) were applied 0.07 IU dispase in 0.1 ml and 1 mg octreotide in 0.1 ml via the same route. Octreotide injection was applied twice during the period of 10 weeks of the experiment. At the end of the 10 weeks, eyes were enucleated and retinal homogenates were prepared. The platelet derived growth factor (PDGF), insulin-like growth factor (IGF 1) and transforming growth factor (TGF ß) levels in homogenized retina tissue were measured by Enzyme Linked-Immuno-Sorbent Assay (ELISA) method. **Statistical Analysis Used:** Kruskal-Wallis variance analysis and Mann-Whitney U test. **Results:** In the treatment group, a significant decrease was observed in retinal PDGF levels ($P < 0.01$) while decreases in TGF ß and IGF 1 levels were not found to be significant ($P > 0.05$). **Conclusions:** Intravitreally applied octreotide at a dose of 1 mg has a highly strong effect on PDGF. This study suggests that intravitreal octreotide may suppress PVR development and that octreotide may merit investigation for PVR prophylaxis.

Key words: Insulin-like growth factor 1, octreotide, platelet derivated growth factor, proliferative vitreoretinopathy, transforming growth factor ß

Proliferative vitreoretinopathy (PVR) is an abnormal tissue response, which is characterized by the proliferation of non-neoplastic cells on both surfaces of the retina, resulting in membrane formation and traction on the retina. This abnormal tissue response occurs in the substance of vitreous and retina.[1-6] Currently, PVR is one of the most important complications experienced in vitreoretinal surgery as well as one of the most important reasons for serious vision loss worldwide.[7]

Current therapy for the treatment of PVR is vitreoretinal surgery including the removal of scar tissue, peeling of the membranes on the retina and flattening the retina with various intraocular tamponades. As a result of the advances in the surgical techniques, anatomic success rate of 60% - 80% has been reported while the functional success rate remains around 30% - 40%.[8] The difficulties in surgical treatment of PVR, the necessity for more than one surgery in many cases and the fact that the resulting blindness rate is considerably high, have led the researchers to seek for an ideal PVR treatment. Although the preventive effects of various pharmacological agents in adjuvant treatment for PVR have been studied, up to now, none of these drugs have been put to routine use due to their relative ineffectiveness and toxicity.

Octreotide is a long-term effective analogue of somatostatin formed by neuro-endocrine tissues and the gastrointestinal system. Somatostatin is a natural inhibitor of the growth hormone and insulin-like growth factor 1 (IGF-1), and it has great inhibitory effects on some hormones including thyroid stimulant hormone (TSH), prolactin (PRL), glucagon and insulin. It causes decrease in tumor growth fraction via its direct anti-proliferative effect.[9-14] To our knowledge, although octreotide has not been used widely in the ophthalmology practice, it might be a promising drug, in particular for its anti-proliferative and anti-angiogenic effects. Octreotide has been reported to inhibit retinal pigment epithelium (RPE) cells, which have important roles in PVR development and endothelial cells of choriocapillary induced by growth factors, thus causing a decrease in retinal neovascularisation.[11,14-23] However, there has been no study carried out with octreotide in animal PVR models in the literature.

Up to date, although there have been some unexplained points in the pathogenesis of PVR; the role of growth factors is clear and precise. It has been demonstrated from the samples taken from vitreous and sub-retinal liquid that there are 5 significant growth factors - the platelet derivated growth factor (PDGF), IGF 1, transforming growth factor (TGF ß), fibroblast growth factor (FGF) and epidermal growth factor (EGF), which have active roles in the proliferation of RPE cells.[24-38]

Taking into account the above-mentioned features of octreotide and the fact that it has inhibitory effect on the growth factors, which play important roles in the PVR pathogenesis, it might be thought that octreotide will inhibit RPE cell proliferation, which is considered to be the key stage for PVR pathogenesis. Under the light of this information, we aimed to investigate the effects of intravitreally-applied octreotide on the growth factors, which have significant roles in the pathogenesis of PVR.

Materials and Methods

The study was carried out under the permission of the institutional ethics Committee. A total of 21 pigmented guinea

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pigs (mean weight: 500 grams) were used in the experiment. 1 eye each of 21 pigmented guinea pigs were used in the study. The animals were kept in Institutional Medicine Research Center under appropriate diet conditions and in special cages throughout the study.

The guinea pigs were randomly divided into 3 groups, each including 7 animals:

Group 1: Control group

Group 2: Sham group (in which PVR was developed)

Group 3: Treatment group (in which PVR was developed and octreotide was applied)

Anesthesia technique

A combination of intramuscular 50 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Turkey) and 6 mg/kg xylazine hydrochloride (Rompun, Bayer, Turkey) was used for the anesthesia and analgesia. Before the operation, 1 drop of 0.5% proparacaine hydrochloride (Alcaine, Alcon, Turkey) was instilled on the animals' eyes.

Application of the experiment

After 10 mg of octreotide in the form of powder (Sandostatin LAR 10 mg flacon, Novartis Pharma AG, Basel, Switzerland) was solved by means of a special solvent containing 12.5 mg sodium carboxymethyl cellulose and 15 mg mannitole, 1 mg octreotide in 0.1 mL was prepared. 0.07 IU dispase in 0.1 mL was prepared by means of combining 25 IU of dispase in powder form (Boehringer Mannheim, Indianapolis, IN; Collaborative Biomedical Products, Bedfort, MA) and saline. In order to provide the prophylaxis of endophthalmitis, prior to all intravitreal applications, the surgical area was cleaned with 10% povidone iodine, and 5% povidone iodine was applied to the conjunctival sac; after waiting for 3 minutes, the conjunctival surface was washed with saline solution in all animals.

In the treatment group, a 27 G needle was inserted into the right eyes of the 7 guinea pigs in a location of 1.5 mm behind the limbus and 0.1 ml vitreous was aspired (vitreous tap). 0.07 U dispase and 1 mg octreotide were consequently injected without removing this needle from the globe. The vitreous tap was performed in order to minimize the 0.2 ml of volume effect.

A period of 10 weeks (70 days) was waited to provide an adequate time for dispase-induced PVR development.[39,40] Taking into account the fact that octreotide remains for 35 days in the vitreous; intravitreal octreotide injection was applied twice within 70 days. The first injection of octreotide was performed simultaneously with dispase while the second injection was applied 35 days after the first injection.[41,42]

In the sham group, a 27 G needle was inserted into the right eyes of the 7 guinea pigs via the same and 0.1 mL of vitreous tap was performed with the same method. Without removing the needle from the globe, 0.07 U dispase and 0.1 mL saline solution were injected intravitreally. Similarly, 10 weeks (70 days) were waited. On the 35th day, a second injection of 0.1 mL saline solution was repeated similar to the treatment group.

In order to maintain the similar mechanic effect formed in the eyes of the treatment and in the sham groups, 0.2 saline solution was intravitreally injected into the right eyes of the 7 subjects of the control group via the same method. On the 35th day, the same injection was repeated with 0.1 ml of saline solution.

At the end of the 10th week, the guinea pigs were applied a combination of intramuscular 50 mg/kg ketamine hydrochloride and 6 mg/kg xylazine hydrochloride, and enucleation was performed. The eyes, which were enucleated, were dissected into 2 in the direction of the sagittal surface passing through the optic nerve. The hematoxylen-eosine stained sections obtained from one half of the globe were analyzed.

After observing epiretinal membrane formation and retinal folds in the eyes of the animals of the sham and treatment groups by slit lamb examination, the eyes were enucleated on the 70th day.

Homogenization and biochemical assessment

The PDGF, IGF-1 and TGF-β levels in homogenized retina tissue were measured with Enzyme Linked- Immuno-Sorbent Assay (ELISA) method using Quantikine PDGF (R and D Systems, Inc., Minneapolis, USA) kit, Quantikine IGF-1 (R and D Systems, Inc., Minneapolis, USA) kit and Biosource TGF-β (Invitrogen Corporation, Carlsbad, USA) kit, respectively.

Statistical analysis

The means and standard deviations of the obtained data were calculated. Statistical analysis was performed with Statistical Package for the Social Sciences 13 (SPSS 13.0, Chicago, IL, USA). Kruskal-Wallis variance analysis was performed for multiple comparisons. Mann-Whitney U test was applied for between groups comparison. A P value <0.05 was accepted as significant.

Results

The maximum, minimum and mean values and the standard deviations of retinal PDGF, TGF-ß and IGF-1 levels in the groups are presented in Table 1. The comparisons of retinal PDGF, TGF-ß and IGF-1 levels in the groups are given in Figs. 1-3, respectively.

When compared with the control group, it was observed that mean PDGF level of the sham group was significantly higher than that of the control group ($P < 0.05$). A comparison of the mean PDGF levels of the sham and treatment groups revealed a significant decrease in PDGF level in the treatment group ($P < 0.05$). It was also found out that there is no significant difference between the PDGF levels of control and treatment groups ($P = 0.937$), and the mean PDGF level of the treatment group were close to that of control group.

It was observed that there was an increase in the mean TGF-ß level of the sham group in comparison with the control group, yet this increase was not significant ($P = 0.056$). It was also seen that there was a decrease in the mean TGF-ß level in the treatment group in comparison with the sham group. However, this difference was also not significant ($P = 0.273$). As for the comparison of mean TGF-ß levels of control and treatment groups, no significant difference was observed between them, and it was noted that the mean level of the treatment group were close to that of control group ($P = 0.329$).

A comparison between the IGF-1 levels of the sham group and control group demonstrated that there was a significant increase in the sham group ($P < 0.05$). As for the sham group and treatment group, no significant difference was found between them ($P = 0.731$). Finally, a significant level of

Table 1: The maximum, minimum, mean values and standard deviations of retinal platelet derived growth factor, transforming growth factor-ß and insulin-like growth factor-1 levels in study groups

Group	PDGF levels (pg/mL) Range (Mean ± SD)	TGF-β levels (pg/mL) Range (Mean ± SD)	IGF–1 levels (pg/mL) Range (Mean ± SD)
Control	2.69 - 3.82 (3.35 ± 0.36)	14.84 - 78.94 (49.24 ± 30.74)	11.21 - 29.65 (19.05 ± 5.06)
Sham	4.00 - 7.02 (4.87 ± 1.18)	34.78 - 135.64 (121.21 ± 61.69)	19.75 - 76.93 (39.15 ± 20.49)
Treatment	2.49 - 5.44 (3.50 ± 1.10)	47.66 - 120.56 (89.90 ± 30.47)	21.74 - 47.59 (35.93 ± 8.86)

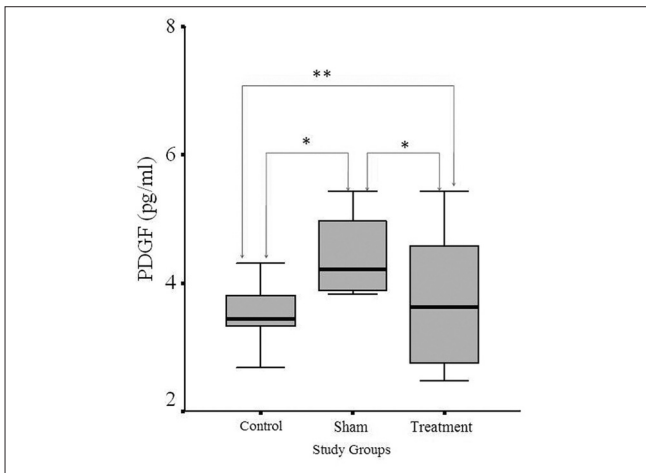PDGF: Platelet derived growth factor, TGF-β: Transforming growth factor, IGF–1: Insulin-like growth factor-1

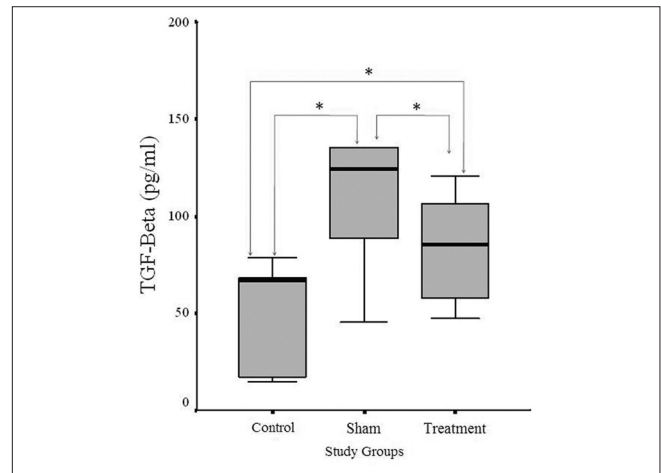**Figure 1:** A comparison of the levels of retinal PDGF among the groups.* $P < 0.05$ **$P > 0.05$

Figure 2: A comparison of the levels of retinal TGF-β among the groups. *$P > 0.05$
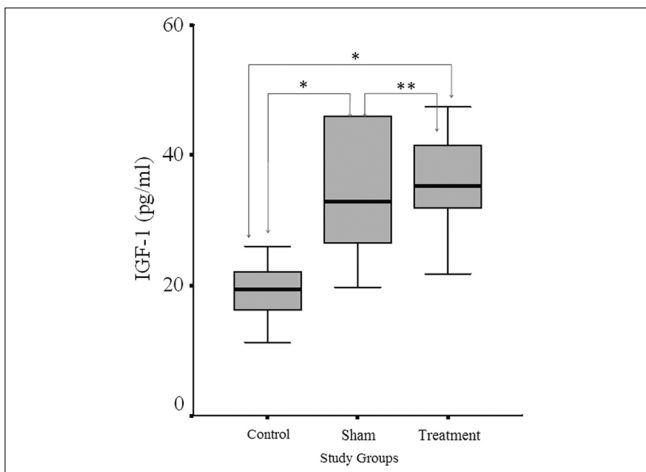

Figure 3: A comparison of the levels of retinal IGF-1 among the groups. *$P < 0.05$ **$P > 0.05$

increase was found in the treatment group in comparison with the control group ($P < 0.05$).

Discussion

The most important stage in PVR development is the proliferative stage where the RPE cells and the myofibroblasts assumed to be formed with the transformation of the proliferation of these cells. Thus, for PVR prophylaxis, the emphasis has been widely put on anti-proliferative agents. As a matter of fact, many anti-proliferative drugs have been used experimentally or clinically in order to prevent PVR. Although they are available as potent drugs for the prevention of PVR, they have not been put in common use due to the critical retinal toxicity they have.[43-45]

In miscellaneous experimental studies, autologous and homologous fibroblasts and RPE cells, endothelial cells, chondrocytes, embrional cells, and photoreceptor cells stimulated by transgenic PDGF have been used in intravitreal injection for PVR induction. The PVR model performing by use of intravitreal dispase injection, described by Frenzel *et al*, is an easy and inexpensive method.[39] Dispase is a proteolytic enzyme derived from Bacillus polymyxa. It selectively breaks down type 4 collagen and fibronectin in the structure of the basement membrane. Basement membrane plays an important role in continuity of RPE cell layer. When RPE cells from disrupted RPE layer were dispersed into vitreal cavity, they can cause the formation of intravitreal contractile membranes, retinal detachment and consequently PVR development. In recent studies, it was shown that an effective dose of dispase is 0.05 - 0.07 U, and that average duration is 8 - 10 weeks for PVR development. In our study, dispase model was used for PVR induction because this method is easy and inexpensive method, and it does not need laboratory conditions compared to the others.[39,40]

Octreotide, whose efficacy was not previously studied *in vivo* PVR models, was used in this study. The fact that octreotide, which has been used in various fields recently, has lower side effects when compared to the strong anti-proliferative agents like 5-fluorouracil, mitomycin-C and daunomycin. Thus, this increases the importance of the drug.[43-46] Recent studies have shown that

when octreotide was applied intravitreally in doses of 1 mg or lower, it does not lead to retinal toxicity.[41,42] On the basis of this knowledge, in order to minimize present systemic side effects, intravitreal application was preferred in the present study.

As it is known, during PVR development, inflammatory blood cells and pro-inflammatory serum elements besides RPE cells also pass into vitreal cavity. The platelets which pass from the serum induce to release growth factors including PDGF, TGF-β and EGF. TGF-ß stimulates the synthesis of collagen and fibronectin in fibroblasts; in addition, they are chemo attractants for the monocytes. PDGF is a chemoattractant and mitogen for both RPE and glial cells.[8,47,48]

Octreotide inhibits DNA synthesis stimulated by IGF-1 and cell proliferation beside hormone excretion centrally in some tissues and at the same time IGF-1 excretion peripherally, and it regulates cell proliferation and secretion directly by affecting at least 3 different signal transmissions.[49,50] In a study on rats, it was observed that IGF-1 is effective in especially the proliferative phase of premature retinopathy, which is one of the vitreoretinal disorders characterized with proliferation.[37] Also, it was demonstrated that in the studies performed *in vitro*, IGF-1 increases the contraction stimulating effect of Muller cells together with PDGF.[36,38] In another *in vitro* study by Mukherjee *et al.*,[51] it was shown that IGF-1 have the stimulating effect for traction on RPE cells, and it was considered that IGF-1 may have a significant physiopathological role in fibro-contractile disorders like PVR. In the light of this issue, the IGF-1 levels and octreotide efficacy in retina were analyzed in animals, in which PVR was induced in this study. As expected, a significant increase was observed in IGF-1 levels in the sham group in comparison with the control group. This finding supports the potential role of IGF-1 in PVR. However, when the treatment and sham groups were compared, the expected decrease in IGF-1 level was not observed. The fact that the values of the treatment group were not close to those of the control group, we considered that intravitreally applied octreotide had no effect on IGF-1. The fact that octreotide could not maintain the expected inhibition on IGF-1 levels, the non-toxic 1 mg intravitreal dose is thought not to be sufficient for the inhibitory effect on IGF-1. Thus, further studies should be carried out in order to examine the effect of octreotide in higher doses on IGF-1 inhibition.

In various studies, it was demonstrated that TGF-ß levels were high in the vitreous of the eyes with PVR and that these elevations were in accordance with the degree of intraocular fibrosis in various diseases such as PVR. Therefore, TGF-ß has a critical role in disorders, which appear with fibrosis.[26-30,46,52,53] In some studies, it was reported that octreotide decreases wound healing response similar to corticosteroids and mitomycin.[54,55] In this the present study, it was observed that there was an increase in the TGF-ß levels of the animals in which PVR was formed; however, this increase was not found to be significant in comparison with the control group. The fact that TGF-ß levels increase in accordance with fibrosis degree, has been shown in previous studies, leads one to think that the experimental PVR model applied in this study may have led to a lower level of fibrosis when compared to previous ones. Although no significant differences were observed in the comparisons between the treatment, control and sham groups, numerically the treatment group values were found to be highly close to control group, and the sham group values were found to be higher than these 2 groups. This shows that the intravitreally applied octreotide in treatment group has inhibiting effect over TGF-ß levels, yet this effectiveness is limited.

The fact that somatostatin inhibits growth factor effects by maintaining activation of tyrosine phosphatase enzyme, which establishes inactivation of growth factors, makes one think that it will also have an inhibiting effect over PDGF, which is a growth factor.[12,13] PDGF is a significant mitogen, chemo attractant and mediator, playing a role in cellular contraction, therefore, it has a key role in PVR pathogenesis.[30-35] In this study, when compared to the control group, a significant increase was observed in the PDGF levels of animals in which PVR was formed. This is a supportive finding on the potential role of PDGF in PVR pathogenesis. In an *in vitro* study by Amann *et al.*, it was demonstrated that octreotide significantly decreases RPE proliferation stimulated by PDGF.[56] However, there has been no *in vivo* study on the inhibiting effect of octreotide on PDGF. In this study, a significant decrease was observed in PDGF values of the treatment group in comparison with sham group. The fact that there was no significant difference between the treatment group levels and control group levels, leads one to consider that intravitreally applied octreotide at this dose has an inhibitory effect on PDGF levels.

In conclusion, it was found that intravitreally applied octreotide at a dose of 1 mg has no effect over IGF 1, yet has a partial effect on TGF-ß and a highly strong effect on PDGF. In this study, it was concluded that octreotide may inhibit PVR effectively. Further studies are needed for the usage of intravitreal octreotide in the PVR prophylaxis.

Acknowledgements

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- The style as well as bibliographic elements should be 100% accurate to get the references verified from the system. A single spelling error or addition of issue number / month of publication will lead to error to verifying the reference.
- Example of a correct style
  Sheahan P, O'leary G, Lee G, Fitzgibbon J. Cystic cervical metastases: Incidence and diagnosis using fine needle aspiration biopsy. Otolaryngol Head Neck Surg 2002;127:294-8.
- Only the references from journals indexed in PubMed would be checked.
- Enter each reference in new line, without a serial number.
- Add up to a maximum 15 reference at time.
- If the reference is correct for its bibliographic elements and punctuations, it will be shown as CORRECT and a link to the correct article in PubMed will be given.
- If any of the bibliographic elements are missing, incorrect or extra (such as issue number), it will be shown as INCORRECT and link to possible articles in PubMed will be given.