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Aqueous Humor Adrenomedullin Levels Differ in Patients With Different Types of Glaucoma

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Purpose: Adrenomedullin is a multifunctional 52 amino acid large peptide. Recent studies have reported that it is expressed in the iris-ciliary body in the eye and that it decreases intraocular pressure (IOP) by increasing outflow facility via specific adrenomedullin receptors, suggesting a role for this peptide in controlling IOP. In the present study, we aimed to explore clinically the possible involvement of adrenomedullin in the pathophysiology of glaucoma.

Methods: Reverse-phase high-performance liquid chromatography was used to determine the aqueous humor adrenomedullin levels in 41 patients (41 eyes) undergoing elective surgery for a variety of ocular diseases in the Research Hospital of Inönü University Medical Faculty between 1999 and 2000. The ocular diseases of the patients included primary open-angle glaucoma (POAG, n = 16), neovascular glaucoma (NG, n = 11), and cataract (n = 14). The study was an open trial with purposive sampling. Aqueous humor samples were taken by paracentesis. Mann-Whitney *U*-test was used in the statistical analysis and $P < .05$ was considered as significant. Results were expressed as mean ± SE.

Results: The mean age and sex distribution between groups were comparable. Mean adrenomedullin levels in patients with POAG (22.3 ± 0.6 pmol/L) were significantly higher than those in patients with NG (5.6 ± 0.2, pmol/L; $P < .001$) and cataract (11.9 ± 0.5, pmol/L; $P < .001$). On the other hand, the mean aqueous humor adrenomedullin levels in patients with NG were significantly ($P < .001$) lower than those in cataract patients.

Conclusions: This first clinical in vivo study on aqueous humor adrenomedullin levels showed that this peptide may be involved in the pathophysiology of glaucoma. Increased aqueous humor adrenomedullin levels in patients with POAG may indicate a compensatory defense response against increased IOP to slow the formation and progression of a vicious cycle. On the other hand, there may be deficient production of the peptide in patients with NG, or adrenomedullin-producing cells may be lost because of very high IOP during the course of the disease. The control of adrenomedullin levels in the eye might be a target that could be considered in the therapeutic strategies for glaucoma. Further studies in this respect are needed. **Jpn J Ophthalmol 2002;46:203–208**

Key Words: Adrenomedullin, aqueous humor, glaucoma, high performance liquid chromatography, pathophysiology.

Introduction

High intraocular pressure (IOP) is the major factor in the development and progression of glaucoma. While the outflow of aqueous is controlled by bulk flow, the ciliary processes produce aqueous humor by an energy-dependent process. Although the trabecular meshwork is the major site in outflow control, the real nature of this resistance and the mechanism for the equilibrium between aqueous production and outflow to maintain IOP in a normal range remain to be clarified.[1]

Received: July 27, 2001

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Published by Elsevier Science Inc.

0021-5155/02/$–see front matter
PII S0021-5155(01)00501-9

Jpn J Ophthalmol
Vol 46: 203–208, 2002

Adrenomedullin is a multifunctional bioactive 52 amino acid large vasorelaxant peptide formed by an intramolecular disulphide bridge and a carboxy-terminal amidated residue originally discovered in acid extracts of human phaeochromocytoma.[2] It has been reported that this recently identified peptide is produced not only in normal adrenal medulla but in vascular smooth muscle cells and endothelial cells as well.[3] In addition, functional adrenomedullin receptors in these cells have also been reported.[4]

More recent in vitro studies have demonstrated that adrenomedullin is expressed in the iris-ciliary body in the eye and that it decreases IOP mainly via specific adrenomedullin receptors, suggesting a role for adrenomedullin in controlling IOP.[5,6] Some authors have suggested that adrenomedullin may increase outflow facility to reduce IOP.[5] Therefore, aqueous humor adrenomedullin levels during the course of glaucoma remain to be specified. In the present clinical study, we measured the aqueous humor adrenomedullin levels in patients with primary open-angle glaucoma (POAG) and neovascular glaucoma (NG), and in age- and sex-matched cataract patients as controls. To our knowledge, this is the first report on aqueous humor adrenomedullin levels in patients with glaucoma.

Materials and Methods

In total, 41 consecutive patients (41 eyes) were enrolled in this study after meeting entry criteria; 16 patients with POAG (9 men and 7 women), 11 patients with NG (6 men and 5 women) and, as controls, 14 age- and sex-matched cataract patients without glaucoma (7 men and 7 women). We selected cataract patients as controls for practical purposes, because many previous studies have also used such patients as controls in their comparative studies.[7–9] In addition, to our knowledge, no association between adrenomedullin and cataract has been reported to date.

POAG was defined as glaucomatous optic nerve damage as documented by repeatable visual field loss on the 30-2 Humphrey visual field analyzer (Humphrey Systems, Dublin, CA, USA) full-threshold (white-on-white) standard visual field program. Visual field reliability criteria included less than 25% fixation losses and false-positive and false-negative rates. The glaucomatous optic nerve head damage was defined by the presence of excavation or undermining of the cup, documented cupping, retinal nerve fiber layer defects characteristic of glaucoma, notching, or cup-disk asymmetry greater than 0.2. The glaucomatous optic nerve damage was associated with achromatic visual field loss in the corresponding hemifield location.

The patients with POAG were between 64 and 77 years of age (mean ± SE, 69.8 ± 0.9 years) and had a visual acuity of 20/40 or better, refractive error not exceeding 2 diopters sphere and/or 1 diopter cylinder, and no prior incisional surgery. Patients with coexisting retinal disease, intraocular inflammation, uveitis, or nonglaucomatous optic neuropathy were excluded from this investigation. All subjects underwent a complete ophthalmic examination including slit-lamp microscopy. These patients had been diagnosed as having POAG a mean of 76.8 (SE = 8.2) months before surgery (range, 16–136 months). The mean (± SE) preoperative IOP was 30.8 ± 0.4 mm Hg (range, 28–34 mm Hg).

The control subjects with cataract but without glaucoma had no history of any other ocular or systemic disease. All had IOP less than 21 mm Hg (mean ± SE, 15.8 ± 0.3 mm Hg) by Goldmann applanation tonometry, normal optic disk appearance and normal perimetry. Absence of glaucomatous optic neuropathy was defined as vertical cup:disk asymmetry less than 0.2, cup:disk ratio less than 0.4, and intact neuroretinal rim without peripapillary hemorrhages, notches, localized pallor, or retinal nerve fiber layer defect.

The patients with NG were between 61 and 75 years of age (mean ± SE, 68.4 ± 1.3 years) and all had visual acuity of 20/400 or worse with the mean (± SE) preoperative IOP of 46.9 ± 0.8 mm Hg (range, 40–49 mm Hg). NG patients had been diagnosed as having the disease a mean of 21.7 (SE = 1.1) months before surgery (range, 11–32 months). All patients had anterior segment neovascularization and the etiology was diabetes (n = 7), central retinal vein occlusion (n = 3), and central retinal artery occlusion (n = 1).

Informed written consent was obtained from all subjects in the three groups. The Ethics Review Board of Inönü University approved the study protocol. When both eyes met the enrollment criteria, only the right eye was included in the present study. The major indications for trabeculectomy in patients with POAG were as follows: unsatisfactory controlled IOP (>22 mm Hg) despite maximum tolerable medical treatment (n = 10), progression of visual field defects despite good IOP control (n = 2), progression of optic disc cupping (n = 1), side effects of medical treatment (n = 1), and unsatisfactory compliance with treatment (n = 2). The glaucoma patients receiving maximum tolerable medical treat-

ment to control IOP were using topical β-blockers (betaxolol 0.5%) twice daily, parasympathomimetic agent (pilocarpine 4%) three times a day and topical carbonic anhydrase inhibitors (dorzolamide 2%) three times a day.

Aqueous Humor Samples

The samples of undiluted aqueous humor (100–200 μL) were manually aspirated into disposable tuberculin syringes by clear corneal paracentesis made at the 10 o'clock position at the start of eye surgery in all groups, avoiding vascular contact or damage to the intraocular tissues. Each sample was then transferred immediately to a sterile tube and frozen at −20°C until analysis. The surgery for patients with POAG was trabeculectomy without antimetabolite application. On the other hand, the surgery for patients with NG was trabeculectomy with antimetabolite application during the surgery (n = 8) and Molteno tube implantation (n = 3).

Adrenomedullin Analysis

Aqueous humor adrenomedullin concentration was measured by using reverse-phase high-performance liquid chromatography (HPLC). Aqueous humor samples were subjected to reverse-phase HPLC (C-18 column, 4.6 × 250 mm, Cecil 1100, Supelco, Cambridge, UK), with a linear-gradient dilution of CH_3CN from 10% to 60% in a solution of 0.1% trifluoroacetic acid. Rat adrenomedullin (1–50 pmol/mL) was used as the standard (Phoenix Pharmaceutical, Mountain View, CA, USA), the absorbance being read at 210 nm as described before.[10,11]

Statistics

The results are expressed as mean ± SE and analyzed statistically by using the Mann–Whitney *U*-test. A *P*-value less than .05 was considered as significant. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 8.0; Chicago, IL, USA).

Results

The difference in mean age among the patients with POAG (69.8 ± 0.9 years; range, 64–77), NG (68.4 ± 1.3 years; range, 61–75) and cataract (67.5 ± 1.0 years; range, 61–73) was not significant ($P > .05$). There were also no differences between the groups with regard to sex distribution. All patients with POAG included in the present study had an open chamber angle, increased IOP (measurements above 22 mm Hg), an abnormal appearance of the optic nerve head, and abnormal glaucomatous visual field defects in perimetric examination. An abnormal appearance of the optic nerve head was the presence of an abnormal shape of the neuroretinal rim including rim notches, localized or diffuse loss of the retinal nerve fiber layer, and disc hemorrhages. The mean cup-to-disk ratio was 0.69 ± 0.04, 0.80 ± 0.04, and 0.21 ± 0.03 for patients with POAG, NG and cataract, respectively.

The mean aqueous humor adrenomedullin levels in patients with POAG (22.3 ± 0.6 pmol/L; range, 16.8–28.1) were significantly (for each, $P < .001$) higher than in patients with cataract (11.9 ± 0.5 pmol/L; range, 9.8–16.7) and in NG patients (5.6 ± 0.2 pmol/L; range, 4.1–7.1). In addition, the patients with NG had significantly ($P < .001$) lower aqueous humor adrenomedullin levels than cataract patients (Table 1).

Discussion

After the discovery of adrenomedullin by Kitamura et al[2] in 1993, many studies evaluated the significance of adrenomedullin in systemic and ocular diseases. This large peptide was found to be widely distributed in various tissues and organs, including heart, spleen, kidney and lung.[5,12] Furthermore, adrenomedullin was found to circulate in blood.[13] Several peripheral actions of adrenomedullin have been reported including hypotension, increased coronary blood flow, vasodilation, inhibition of K^+-stimulated aldosterone secretion, increased renal blood flow, inhibition of ACTH secretion and increased cerebral blood flow.[6,14]

Adrenomedullin has recently gained major attention in ocular experimental studies in vivo and in vitro. It has been demonstrated that adrenomedullin levels in the vitreous of patients with proliferative vitreoretinopathy were significantly higher than those of patients with proliferative diabetic retinopathy, age-related macular degeneration, and macular hole. Authors suggested that adrenomedullin may be involved in the pathophysiology of proliferative vitreoretinopathy.[15] Taniguchi et al[5] recently demonstrated for the first time the expression and effects of adrenomedullin in the eye. They found in their in vitro study that adrenomedullin was expressed in the rat iris-ciliary body and had an hypotensive effect, suggesting its possible role in controlling IOP. Then, in the same year, Yousufzai et al[6] have demonstrated the functional adrenomedullin receptors in the iris-ciliary body isolated from several mammalian spe-

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Table 1. Patient Characteristics and Aqueous Humor Adrenomedullin Levels at Time of Surgery: Statistical Analysis*

	POAG†			Neovascular Glaucoma‡			Cataract§		
	Mean ± SE	SD	Range	Mean ± SE	SD	Range	Mean ± SE	SD	Range
Age‖ (years)	69.8 ± 0.9	3.7	64–77	68.4 ± 1.3	4.3	61–75	67.5 ± 1.0	3.7	61–73
Preoperative IOP (mm Hg)	30.8 ± 0.4	1.7	28–34	46.9 ± 0.8	3.5	40–49	15.8 ± 0.3	1.1	14–18
Glaucoma duration (months)	76.8 ± 8.2	33.0	16–136	21.7 ± 1.1	5.5	11–32		—	
Aqueous humor AM (pmol/L)	22.3 ± 0.6¶	2.6	16.8–28.1	5.6 ± 0.2#	0.9	4.1–7.1	11.9 ± 0.5	1.9	9.8–16.7

*SE: standard error of mean, SD: standard deviation, IOP: intraocular pressure, AM: adrenomedullin.
†POAG: primary open-angle glaucoma. N = 16 (9 men, 7 women).
‡N = 11 (6 men, 5 women).
§N = 14 (7 men, 7 women).
‖The age between the three groups was comparable ($P > .05$).
¶Significantly higher than in cataract group by Mann–Whitney *U*-test ($P < .001$).
#Significantly lower than in cataract group by Mann–Whitney *U*-test ($P < .001$).

cies including man. Likewise, it has been demonstrated that human retinal pigment epithelial (RPE) cells produce and secrete adrenomedullin, and that adrenomedullin itself stimulates RPE cell proliferation.[16] Furthermore, Clementi et al[17] have reported that adrenomedullin causes dose-dependent conjunctival hyperemia accompanied by an increase in inflammatory cell number and prostaglandin E_2 concentration in aqueous humor when administered peripherally in rabbits. The present clinical investigation is the first clinical study to demonstrate aqueous humor adrenomedullin concentration in patients with POAG and NG and to compare it with that found in age- and sex-matched control subjects.

Elevated IOP is the major risk factor in the development of the commonest form of glaucoma, POAG. Aqueous production by the ciliary processes is mainly an energy-dependent process. The balance of IOP levels depends on the rate of aqueous humor production and drainage, which is controlled by bulk flow. Important in this respect are the vasoactive agents such as endothelin and nitric oxide, which are detected in plasma and are widely present in many organs and tissues, including the eye. Nitric oxide is a relaxing agent while endothelin is a contracting agent; both are thought to participate in the regulation of IOP.[18] Moreover, it has been demonstrated that aqueous humor endothelin levels were significantly higher in glaucoma subjects than in matched control subjects.[18] In addition, endothelin is known to be related to optic nerve ischemia in mammalians.[19] On the other hand, our previous study showed that mean aqueous humor nitric oxide levels were found to be significantly decreased in patients with POAG compared to those in matched cataract patients serving as controls.[20] Recently, it has been reported that adrenomedullin mRNA is expressed in the iris-ciliary body, mediating the IOP response, and that exogenous adrenomedullin has a potent effect on IOP primarily via specific adrenomedullin receptors, suggesting that adrenomedullin could be an endogenous IOP modulator. However, the target sites for the action of adrenomedullin remain to be elucidated.[5] Furthermore, the authors have suggested that adrenomedullin may also increase the outflow facility to reduce IOP.

Although the exact physiological role of adrenomedullin in the eye has not been fully understood, the potent hypotensive action of adrenomedullin when injected intravitreally suggests its pathophysiological implication in the aqueous humor production-drainage procedure.[5] In the present study, we found that the aqueous humor adrenomedullin level was significantly higher in patients with POAG and lower in patients with NG when compared with age- and sex-matched cataract subjects. Too little is known to infer the mechanism and the significance of increased or decreased aqueous humor adrenomedullin levels in these two types of glaucoma. At the present time, the major source of aqueous humor adrenomedullin is unknown. However, it may be possible that the iris-ciliary body produces a large amount of adrenomedullin in patients with POAG as a defense response to increased IOP, ie, to compensate by increasing outflow facility.

An alternative explanation for the aqueous humor adrenomedullin concentrations in different types of glaucoma may be the adrenomedullin-binding protein, complement factor H, which was very recently identified.[21] The authors reported that a specific binding protein for adrenomedullin exists in mammalian and avian blood that might influence the bioactivity and function of adrenomedullin in health and disease. We do not know at present whether this adreno-

medullin-binding protein is present in aqueous humor, as it is in blood. If it is, the differential release of adrenomedullin from this adrenomedullin-binding protein could be the cause for the differences in the adrenomedullin levels in the POAG, NG, and cataract subjects.

Assuming the implication of adrenomedullin in the integrity of the iris-ciliary body system, adrenomedullin may be involved in the protective mechanism against increased IOP. It may also be speculated that an increase in adrenomedullin may contribute to preserving intraocular regional blood flow in the various stages of POAG, and thereby may slow formation and progression of a vicious cycle. On the other hand, decreased adrenomedullin levels in patients with NG could be explained by the damage to adrenomedullin-producing cells by very high IOP during the course of the disease.

When discussing our findings, some limitations concerning the study methodology have to be mentioned. On the one hand, the group of patients with NG was heterogeneous. Whether the relatively low level of aqueous adrenomedullin in this group is due to the elevation of IOP or the neovascularization, or due to the underlying diseases like diabetes, remains unclear. However, the aqueous humor adrenomedullin levels of these subgroups of NG were all lower than the mean level of the control subjects. Therefore, we think that the increased IOP pressure, which is the common feature of the three groups of subjects, is responsible for this finding. On the other hand, patients with glaucoma were under treatment with antiglaucoma drugs. It is therefore possible that their medications could have influenced the level of adrenomedullin in the aqueous humor samples analyzed. However, the IOPs found in the present study were different for the glaucoma groups (higher in POAG patients and lower in NG patients) when compared with those of cataract subjects, although all operated subjects were using the same maximum tolerable medical treatment. This discrepancy should be further investigated. The other limitation is that cataract subjects were enrolled as the control group. One should consider that cataract is also a disease. We know that cataract patients may not be ideal controls; however, we selected such patients for practical reasons because most previous studies used cataract subjects as controls in their intraocular comparative studies.[7–9,20] Furthermore, to our knowledge, there is no report regarding any association between cataract and adrenomedullin. This limitation has also been discussed previously in the Materials and Methods section.

Finally, we have not measured plasma adrenomedullin levels in this study to assess whether there is a correlation between serum and aqueous adrenomedullin concentrations. It is likely that some, if not all, of the adrenomedullin detected in the aqueous humor was derived from the systemic circulation. However, it has been recently demonstrated that adrenomedullin has no effect on the blood—aqueous barrier[5] and blood—brain barrier[22] when injected peripherally. Furthermore, the profound IOP reduction by adrenomedullin was not accompanied by a significant increase in aqueous protein. Thus, the ocular hypotensive effect of adrenomedullin is dissociated from its effect on the blood—aqueous barrier. As a result, we think that intraocular adrenomedullin overproduction or deficient production is responsible for our findings in the present study. This hypothesis requires further investigation.

In conclusion, adrenomedullin has been found to act as a multifunctional regulatory peptide in various tissues including the eye, as do nitric oxide and endothelins. The present preliminary clinical investigation demonstrated that aqueous humor adrenomedullin levels are significantly higher in patients with POAG and lower in those with NG when compared with the levels in patients with cataract without glaucoma. Considering the intraocular production by the iris-ciliary body and the increasing effect on outflow facility, increased aqueous humor adrenomedullin levels may be involved in the defense mechanism against further IOP elevation in POAG. On the other hand, deficient adrenomedullin production or deficient adrenomedullin release from the adrenomedullin-binding protein may be responsible for the higher IOP in patients with neovascular glaucoma. Therefore, further in vivo and in vitro studies are needed to exactly clarify the ocular effects and the role of adrenomedullin in glaucoma patients.

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