이학석사 학위논문

안질환 환자의 유리체액과 혈청에 존재하는 아데노부속바이러스 특이적 중화항체 분석

Determination of pre-existing neutralizing antibody levels specific to adeno-associated viruses in the vitreous humor and serum of ocular disease patients

울 산 대 학 교 대 학 원

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이 논문을 이학석사 학위 논문으로 제출함

2019 년　8 월

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2019 년 8 월

Abstract

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Purpose

We determine the prevalence of neutralizing antibodies (NAbs) to adeno-associated virus (AAV) in the vitreous humor and serum of patients with vitreoretinal diseases and investigate the relationship between NAb titers in the vitreous humor and serum.

Methods

We analyzed NAbs to AAV serotypes 2, 5, 8, and 9 via in vitro neutralization in the vitreous humor and serum from 32 patients requiring vitrectomy for vitreoretinal diseases. The blood–retinal barrier (BRB) was evaluated for integrity based on preoperative examinations, with vitreous hemorrhage (VH) on funduscopy or dye leakage on fluorescein angiography observed indicating disruption.

Results

NAb levels were much lower in the vitreous humor than in the serum regardless of serotype. Patients with VH had higher levels of NAbs in the vitreous humor than those without VH. The NAb ratio (ratio between NAb titers in the serum and vitreous humor) was much lower in patients with epiretinal membrane with than in those without leakage. A significantly lower NAb ratio was noticed in patients with than in those without BRB disruptions.

Conclusions

The NAb ratio between levels in serum and vitreous humor varies according to the condition of the BRB. Therefore, BRB integrity should be examined when planning retinal gene therapy. This study provides substantial basis for retinal gene therapy using AAVs and how maintenance of BRB integrity in target diseases should be considered.

Keywords

Neutralizing Antibody(NAb); Adeno-Associated Virus(AAV); Vitreous humor; serum; Gene Therapy; Blood Retinal Barrier(BRB)

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Introduction

Adeno-associated virus (AAV) vectors are common gene delivery tools in gene therapy due to their major safety and efficiency advantages.[1–4] As AAV vector-based retinal gene therapy proved to be successful in early clinical trials of inherited disorders, the clinical application of gene therapy recently has expanded to acquired retinal diseases, such as age-related macular degeneration (AMD).[5,6]

Humoral anti-AAV antibody-mediated immunity, especially pre-existing immunity resulting from childhood exposure to AAVs, is a well-known, significant limiting factor to the efficacy of gene therapy using AAV as a gene delivery vehicle.[7,8] Thus, the possibility of such baseline serum antibodies directed against AAV having negative effects on transgene expression is a crucial subject that must be investigated. Several previous studies conducted in humans and large animal models suggest that high levels of pre-existing neutralizing antibodies (Nabs) in the serum could interfere with transgene expression in retinal gene therapy, especially after intravitreal delivery.[6,9]

However, whether the presence of serum antibodies against AAVs would have a direct effect on transgene expression of retinal gene therapy remains controversial. This is because retinal gene therapy uses a subretinal or intravitreal space, which is considered immunologically different from other tissues.[10,11] Nonetheless, little information regarding pre-existing NAbs is present in the human vitreous humor, which would be the basis for answering these questions. Accordingly, the correlation between NAb titers in the serum and vitreous humor also is not established to our knowledge. Moreover, many components of the immune-privileged status of the eye, such as the presence of blood–retinal barriers (BRBs), could be compromised under pathologic conditions.[12] Therefore, an investigation of the immunologic status of the intraocular space of a diseased eye in relation to the healthy eye is necessary for future developments.

We report, to our knowledge, the first analytical examination of NAbs against four different serotypes of AAV (2, 5, 8, and 9) in the vitreous humor and serum of patients with various vitreoretinal diseases. Each serotype represents a phylogenetically divergent clade of the AAV family; the origin of serotypes 2, 5, and 9 is human, whereas serotype 8 is thought to have originated in the rhesus macaque.[13,14] The usefulness of these AAVs with distinct tissue tropisms has been investigated intensively in preclinical and clinical trial studies.[14,15] The relationship between NAb titers in the serum and vitreous humor was scrutinized further to determine whether differences existed depending on the pathologic disease status of the retina.

Materials and Methods

Patients and Grouping

Patients requiring pars plana vitrectomy for treatment of vitreoretinal diseases at the Department of Ophthalmology of Asan Medical Center, Seoul, Korea, from September 21, 2017, to January 18, 2018, were included consecutively in this study. The study was approved by the institutional review board and ethics committee of Asan Medical Center (2017-0968) and adhered to the tenets of the Declaration of Helsinki. All patients gave their written informed consent before enrollment. Patients younger than 18 or older than 80 years and those who had previously undergone pars plana vitrectomy were excluded from the study. Each patient underwent complete ophthalmologic examination preoperatively according to disease status. Based on preoperative evaluation, especially with regard to the presence of vitreous hemorrhage (VH) on fundus examination and leakage at the peak phase on fluorescein angiography (FA), we assessed the status of BRB maintenance (Fig. 1). The integrity of the BRB was considered disrupted when there was evidence of VH or leakage on FA.

Samples

Pars plana vitrectomy was performed by a vitreoretinal surgeon (JYL) using the standard 25gauge vitrectomy system. The vitreous samples were collected in the operating room through undiluted lines using a vitreous cutter. Undiluted vitreous samples were obtained using a 3-mL syringe connected directly to the handpiece until the eye was visibly noted to soften. At least 2 mL of undiluted vitreous was collected in each case; 3 mL serum samples were collected on the day before or day of surgery. The samples were maintained in the collection syringe and were delivered to the clinical laboratory immediately after they were obtained. Blood was clotted overnight at 4°C, and the serum was collected after centrifugation. Both samples were aliquoted and stored at–80°C until use.

Cell Culture and Preparation of AAV Serotypes

HeLa cells were cultured in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS; Invitrogen), 15 mM HEPES (Sigma-Aldrich Corp., St. Louis, MO), GlutaMAX-1 (2 mM), and penicillin (100 IU/mL)/ streptomycin (50 lg/mL) and then maintained at 37°C under humidified 5% CO_2. The AAV serotypes 2, 5, 8, 9 expressing green fluorescent protein (GFP) were produced using a triple cotransfection method as described previously[16] and were supplied by CdmoGen Co., Ltd. (Cheongju, Korea).

Neutralizing Antibody Determination

HeLa cells in 96-wells were infected with AAV2, 5, 8, and 9 to achieve approximately 50% of GFP positive cells at multiplicity of infection (MOI) values of 200, 200, 5000, and 10,000, respectively. The cells were coinfected with adenovirus 5 for efficient transduction. After 2 days, the percentage of cells showing GFP signal was determined under fluorescence microscopy. Neutralizing titers of the sample were calculated as the highest dilution that inhibited 50% of transduction. Samples were considered to have neutralizing activity if 1:20 dilution inhibited vector transduction by at least 50%.

Statistical Analysis

The Wilcoxon signed rank test was used to compare the NAb titers in serum and vitreous humor for each patient. The Mann–Whitney U test was used to determine the significant differences between the two groups. For correlation between the NAb titers in serum and vitreous humor, we used Spearman's correlation test. All statistical analyses were performed using SPSS 22.0 for Windows (SPSS, Chicago, IL), and $P < 0.05$ was considered statistically significant.

Results

Patient Characteristics

A total of 32 consecutive patients (16 men, 16 women; median age, 65.5 years; interquartile range, 54.3–70.0) were enrolled in this study (Supplementary Table S1). The presence of an epiretinal membrane (ERM) was the most common reason for surgery (n = 15, 46.9%), followed by diabetic VH (n = 10, 31.3%), macular hole (n = 3, 9.3%), intraocular lens dislocation (n = 2, 6.3%), and VH associated with retinal vein occlusion (n = 1, 3.1%) and with AMD (n = 1, 3.1%). Among patients with an ERM, nine did not and six did show dye leakage on preoperative FA (Fig. 1).

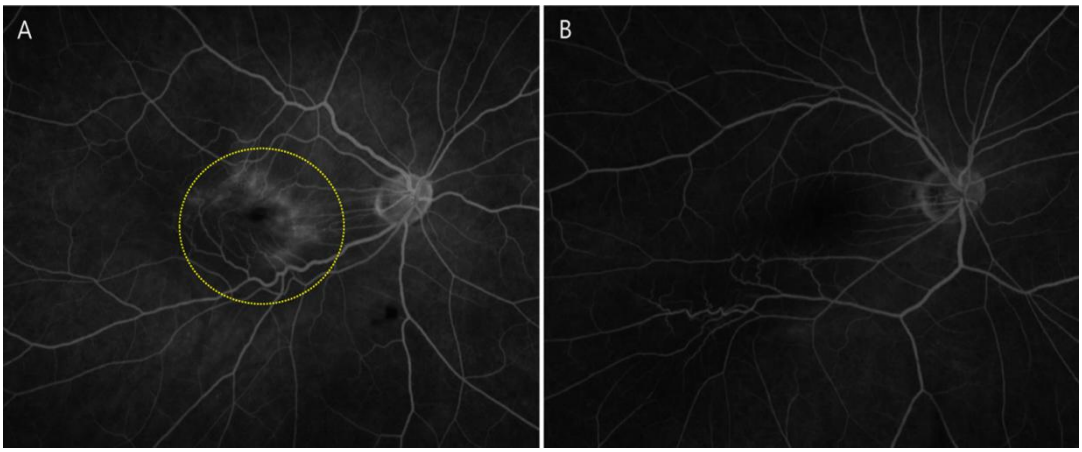

Figure 1. Classification of patients with ERM according to dye leakage in FA images.

(A) FA image of patient with leakage observed in the macula area at peak phase (dotted yellow line). (B) FA image of patient without leakage.

ERM = epiretinal membrane; FA = fluorescein angiography

Supplementary Table S1. Patient demographics, clinical characteristics, and peak NAb titers listed by patient number

Patient	Age (years)	Gender	Disease	Presence of VH or leakage in FA	NAb titers in sera				NAb titers in vitreous humor			
					AAV2	AAV5	AAV8	AAV9	AAV2	AAV5	AAV8	AAV9
1	75	Male	ERM	Leakage	3413	53	18	28	40	5	2	2
2	67	Male	VH RVO	VH	11947	46	18	299	95	2	2	2
3	73	Male	ERM	None	4096	64	36	299	20	2	2	2
4	60	Female	ERM	Leakage	5120	427	41	224	20	2	2	2
5	54	Female	ERM	None	1707	320	18	224	16	2	2	2
6	55	Female	MH	None	13653	171	10	128	7	2	2	2
7	44	Male	Diabetic VH	VH	2786	171	10	85	57	2	2	2
8	70	Male	ERM	None	13653	171	10	91	60	2	2	2

Supplementary Table S1. 계속

9	68	Female	ERM	None	2560	171	10	21	2	2	2	2
10	65	Female	ERM	None	2560	43	10	64	2	2	2	2
11	57	Male	Diabetic VH	VH	13653	5291	100	512	593	134	2	40
12	55	Male	IOL dislocation	none	768	160	11	171	2	2	2	2
13	67	Male	Diabetic VH	VH	192	38	3	2	8	6	2	2
14	70	Male	ERM	Leakage	2194	187	40	313	35	4	2	2
15	71	Female	ERM	Leakage	213	48	6	27	33	4	2	2
16	68	Male	ERM	Leakage	160	10	2	2	2	2	2	2
17	64	Female	ERM	None	3150	201	5	11	9	2	2	2
18	75	Male	ERM	None	596	84	4	10	2	2	2	2

Supplementary Table S1. 계속

19	53	Female	Diabetic VH	VH	10240	640	34	160	213	10	2	7
20	67	Female	ERM	Leakage	160	13	2	2	2	2	2	2
21	47	Female	ERM	None	10240	640	160	320	10	2	2	2
22	72	Female	Diabetic VH	VH	160	10	2	2	3	2	2	2
23	57	Male	Diabetic VH	VH	640	551	7	80	40	12	2	2
24	75	Female	ERM	Leakage	160	10	50	2	2	2	2	2
25	53	Male	IOL dislocation	None	13653	853	80	213	2	3	2	2
26	60	Female	MH	None	640	160	10	93	40	2	2	2
27	49	Female	Diabetic VH	VH	20480	187	40	160	853	3	2	4
28	29	Male	Diabetic VH	VH	5	6	2	2	2	2	2	2

Supplementary Table S1. 계속

29	66	Female	Diabetic VH	VH	20480	2560	320	640	640	40	2	53
30	70	Female	MH	None	20480	5120	213	1143	187	34	2	6
31	76	Male	VH wAMD	VH	640	53	10	40	131	2	2	2
32	54	Male	Diabetic VH	VH	13653	640	53	640	211	13	2	13

VH = vitreous hemorrhage ; FA = fluorescein angiography ; NAb = neutralizing antibody ; ERM = epiretinal membrane ;

RVO = retinal vein occlusion ; MH = macular hole ; IOL = intraocular lens ; wAMD = wet age-related macular degeneration

Pre-existing NAbs in Serum

The results of NAb titers against each serotype in serum are shown in Table 1 and Figure 2. Among all serotypes, serotype 2 showed overwhelmingly high NAb titers, followed by AAV5, AAV9, and AAV8. When the seropositive criteria were set at 1:100 and 1:400 dilution, NAbs against serotype 2 were observed most frequently. Although no patient had an NAb titer > 2000 against AAV8 or AAV9, 19 (59.4%) and three (9.4%) patients had NAb titers > 2000 against AAV2 and AAV5, respectively. No patient had an NAb titer > 400 against AAV8. Most patients with NAbs to serotype 5, 8, or 9 had coprevalent NAbs to serotype 2. Only one patient was negative for NAbs against AAV2, but positive for another serotype at the seropositive criterion of 1:100 (Supplementary Figure S1).

Table 1. Neutralizing Titers to AAV in Serum and Vitreous Humor from 32 Patients

Serotype	Neutralizing titer in serum Median (IQR)	Neutralizing titer in vitreous humor Median (IQR)	*p* value
AAV2	2673.0 (607.0–13226.5)	20.0 (2.0–86.3)	<0.001
AAV5	171.0 (46.5–520.0)	2.0 (2.0–4.8)	<0.001
AAV8	10.5 (6.3–40.8)	2.0 (2.0–2.0)	<0.001
AAV9	92.0 (13.5–280.3)	2.0 (2.0–2.0)	<0.001

AAV = adeno-associated virus

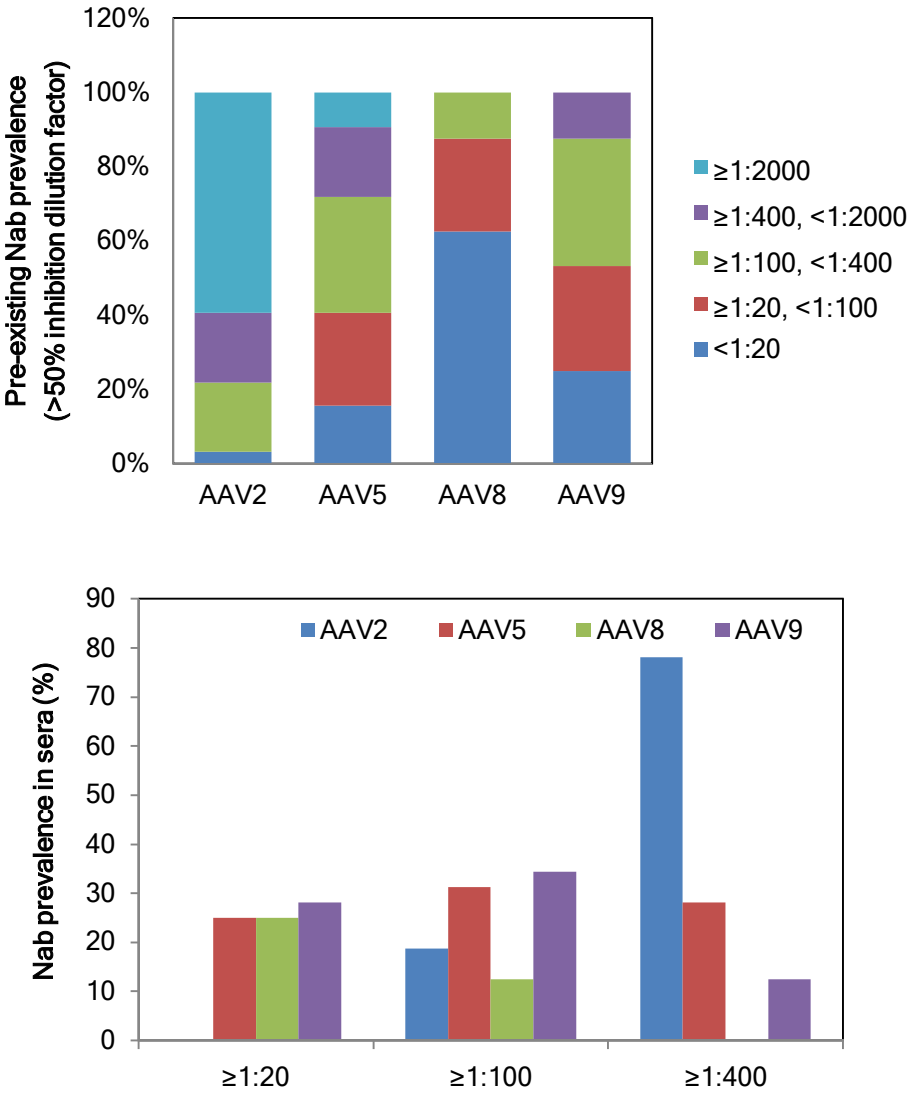

Figure 2. Prevalence of pre-existing NAbs in the serum. Samples from 32 subjects were assayed within 1 month after pars plana vitrectomy. The percentages of serum titer ranges are shown for samples positive for NAbs against each serotype, as well as the distribution by range of neutralizing titers against AAV2, AAV5, AAV8, and AAV9.

AAV = adeno-associated virus; NAbs = neutralizing antibodies

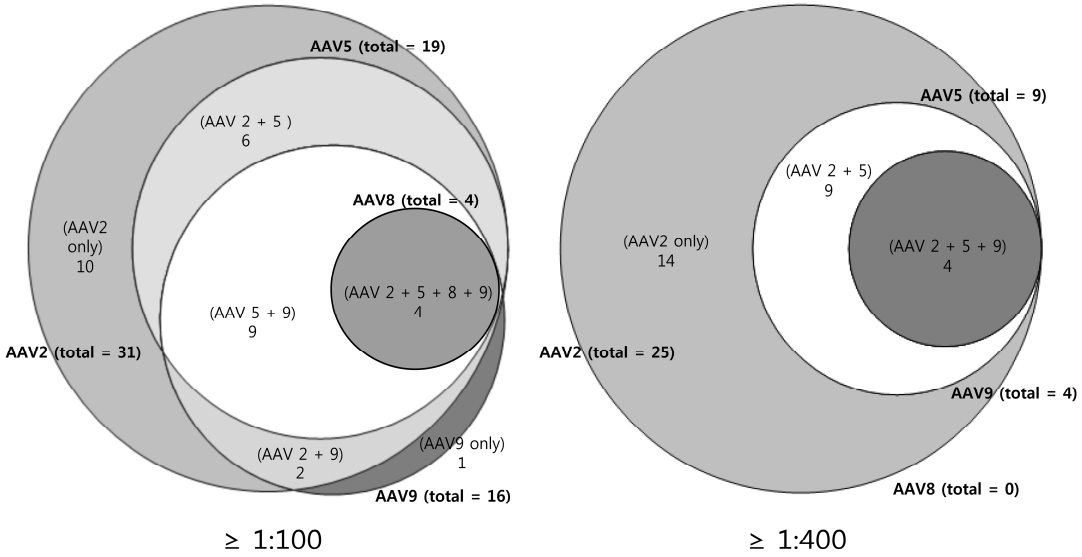

Figure S1. Co-prevalence of Nabs against AAV2, AAV5, AAV8 and AAV9 in sera for (≥ 1:100) and (≥ 1:400) dilutions. 32 total samples demonstrated AAV neutralization for at least one of the four serotypes. With the exception of one sample, all sera samples at (≥ 1:100) and (≥ 1:400) neutralized AAV2. Venn diagram was generated using Venn Diagram Plotter software version 1.5.5228.29250 (originally written by Kyle Littlefield for the Department of Energy (PNNL, Richland, WA, USA)).

Pre-existing NAb Titer in the Vitreous Humor

The neutralizing activities for vitreous humor are shown in Table 1 and Figure 3. Similar to the results for serum, NAb titer against serotype 2 in the vitreous humor was confirmed to be the highest and NAb titer against serotype 8 was the lowest. However, the absolute value of NAb titer in the vitreous humor was much lower than that in the serum. NAb titers > 100 were observed against serotype 2 in seven patients (21.9%) and against serotype 5 in one (3.1%); titers > 400 were observed only against serotype 2 in three patients (9.4%). Among the underlying vitreoretinal disease conditions in the eight patients with NAb titers > 100, five patients had diabetic VH, one had VH associated with AMD, and one had a macular hole. All patients with NAb titers > 400 had diabetic VH. All patients with NAbs to serotypes 5 or 9 had coprevalent NAbs to serotype 2 (Supplementary Figure S2).

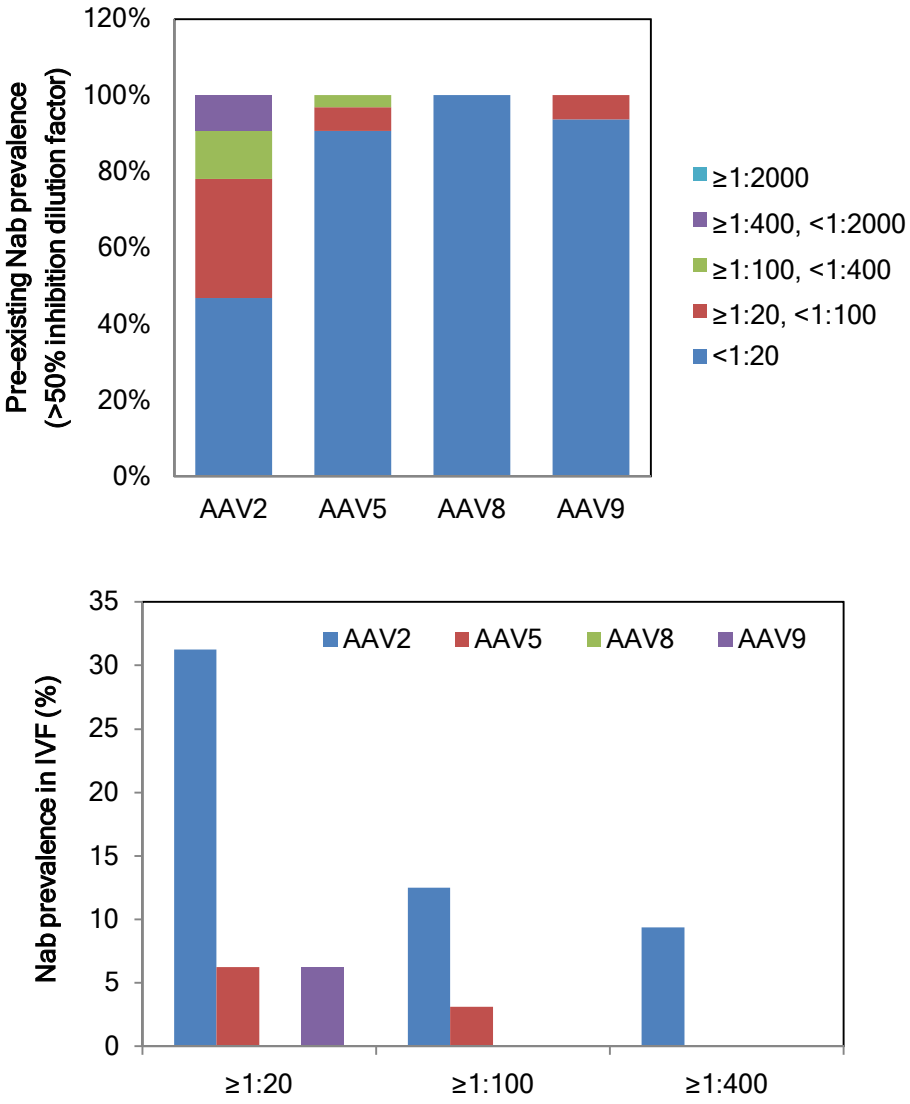

Figure 3. Prevalence of pre-existing NAbs in the vitreous humor. The percentages of vitreous humor titer ranges are shown for samples positive for NAbs against each serotype, as well as the distribution by range of neutralizing titers against AAV2, AAV5, AAV8, and AAV9. AAV = adeno-associated virus; NAbs = neutralizing antibodies

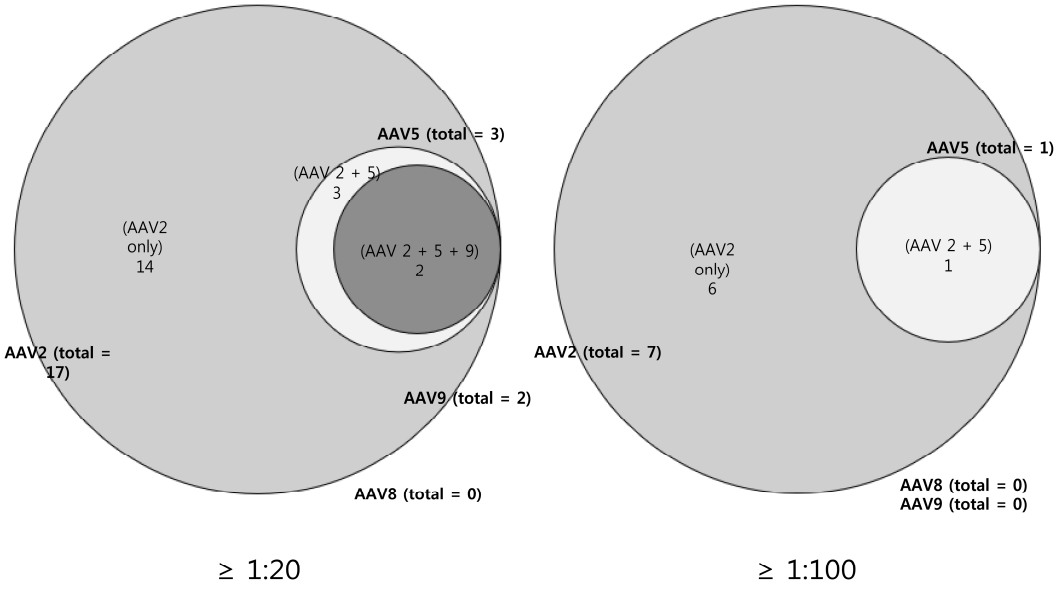

Figure S2. Co-prevalence of Nabs against AAV2, AAV5, AAV8 and AAV9 in vitreous humor for (≥ 1:20) and (≥ 1:100) dilutions. Venn diagram was generated by using Venn Diagram Plotter software version 1.5.5228.29250 (originally written by Kyle Littlefield for the Department of Energy (PNNL, Richland, WA, USA)).

Comparison According to Disease Characteristics

Presence or Absence of VH

Of the 32 patients, 12 had VH and 20 did not. Table 2 shows the NAb titers in the serum and vitreous humor, and the ratio between NAb titers in the serum and vitreous humor (NAb ratio) against serotypes 2 and 5 according to the presence or absence of VH. There was no difference between the two groups in NAb titers in the serum, but patients with VH had significantly higher NAb titers to serotypes 2 and 5 in the vitreous humor (113.0 vs. 9.5, $P = 0.004$, and 4.5 vs. 2.0, $P = 0.028$) and lower NAb ratios to serotype 2 (28.0 vs. 216.2, $P < 0.001$) compared with patients who did not have VH.

Table 2. Comparison of NAb Titer and NAb Ratio to AAV2 and AAV5 According to Presence or Absence of VH

Serotype	Variable	Patients with VH (n = 12) Median (IQR)	Patients without VH (n = 20) Median (IQR)	*p* value
AAV2	NAb titer in serum	6513.0 (304.0-13653.0)	2560.0 (607.0-8960.0)	0.639
	NAb titer in vitreous humor	113.0 (16.0-498.0)	9.5 (2.0-34.5)	0.004
	NAb ratio (serum / vitreous humor)	28.0 (17.8-52.2)	216.2 (80.0-864.0)	<0.001
AAV5	NAb titer in serum	179.0 (40.0-640.0)	165.5 (49.3-290.3)	0.716
	NAb titer in vitreous humor	4.5 (2.0-12.8)	2.0 (2.0-2.75)	0.028
	NAb ratio (serum / vitreous humor)	42.7 (10.5-63.6)	80.0 (14.4-138.1)	0.106

AAV = adeno-associated virus; NAb = neutralizing antibody; VH = vitreous hemorrhage

Presence or Absence of Leakage in Patients with ERM

Table 3 shows the NAb titers in the serum and vitreous humor, and the ratio between NAb titers in the serum and the vitreous humor (NAb ratio) against serotypes 2 and 5 according to the presence or absence of leakage in patients with ERM. Patients with leakage that was observed on a preoperative FA scan had significantly lower NAb ratios to serotypes 2 and 5 than those without leakage (80.0 vs. 324.0, P = 0.004 and 10.6 vs. 85.5, P = 0.037).

Table 3. Comparison of NAb Titer and NAb Ratio to AAV2 and AAV5 According to Presence or Absence of Leakage in Patients with ERM

Serotype	Variable	Patients with ERM accompanying leakage in FA (n = 7) Median (IQR)	Patients with ERM not accompanying leakage in FA (n = 8) Median (IQR)	*p* value
AAV2	NAb titer in serum	213.0 (160.0-3413.0)	2855.0 (1920.3-8704.0)	0.081
	NAb titer in vitreous humor	20.0 (2.0-35.0)	9.5 (2.0-19.0)	0.632
	NAb ratio (serum / vitreous humor)	80.0 (62.7-85.3)	324.0 (210.5-1216.0)	0.004
AAV5	NAb titer in serum	48.0 (10.0-187.0)	171.0 (69.0-290.3)	0.105
	NAb titer in vitreous humor	2.0 (2.0-4.0)	2.0 (2.0-2.0)	0.047
	NAb ratio (serum / vitreous humor)	10.6 (5.0-46.8)	85.5 (34.5-145.1)	0.037

AAV = adeno-associated virus ; ERM = epiretinal membrane ; FA = fluorescein angiography ; NAb = neutralizing antibody

Presence or Absence of a Disrupted BRB

Nineteen patients with preoperative VH or ERM with preoperative fluorescein leakage were classified as having a disease in which the BRB had been disrupted (group A), and 13 were classified as having a disease in which the BRB was intact (group B; Table 4). Among the 19 group A patients, 12 had VH and 7 had ERM along with leakage. Group B had eight patients with ERM without leakage, three with a macular hole, and two with intraocular lens dislocation. There were no differences between the two groups in NAb titers in the serum and NAb titers to serotype 2 in the vitreous humor, but group A patients had significantly higher NAb titers to serotype 5 in the vitreous humor (3.0 vs. 2.0, $P = 0.036$) and lower NAb ratios to serotypes 2 and 5 (48.9 vs. 350.0, $P < 0.001$, and 26.5 vs. 85.5, $P = 0.001$) than group B patients.

Table 4. Comparison of NAb Titer and NAb Ratio to AAV2 and AAV5 According to Maintenance of the BRB

Serotype	Variable	GroupA: Patients with disrupted BRB (n=19) Median(IQR)	GroupB: Patients with intact BRB (n=13) Median(IQR)	*p* value
AAV2	NAb titer in serum	2194.0 (160.0-11947.0)	3150.0 (1237.5-13643.0)	0.218
	NAb titer in vitreous humor	40.0 (3.0-211.0)	9.0 (2.0-30.0)	0.062
	NAb ratio (serum / vitreous humor)	48.9 (23.0-80.0)	350.0 (157.2-1280.0)	<0.001
AAV5	NAb titer in serum	53.0 (13.0-551.0)	171.0 (122.0-480.0)	0.233
	NAb titer in vitreous humor	3.0 (2.0-10.0)	2.0 (2.0-2.0)	0.036
	NAb ratio (serum / vitreous humor)	26.5 (6.3-62.3)	85.5 (61.0-155.3)	0.001

Relationship Between Neutralizing Activity Against Serotype 2 in the Serum and Vitreous Humor

Overall, there were no significant correlations between the titers of NAbs in the serum and vitreous humor (Fig. 4). However, when the data were analyzed according to BRB maintenance, a strong positive correlation was found between the NAbs titers in the serum and vitreous humor in patients with disrupted BRBs ($r = 0.917$, $P < 0.001$), whereas no significant correlation was found in patients with intact BRBs ($r = 0.381$, $P = 0.199$). In most cases of intact BRB, NAb titers in the vitreous humor remained low, regardless of NAb titers in the serum.

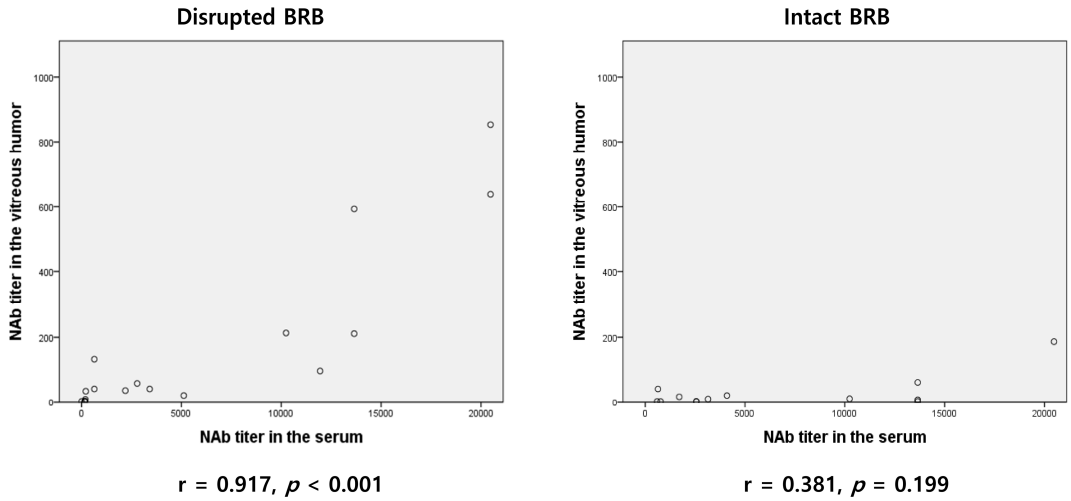

Figure 4. Difference in the relationship between neutralizing activity against AAV serotype 2 in serum and vitreous humor according to BRB maintenance.

AAV = adeno-associated virus; BRB = blood-retina barrier; NAb = neutralizing antibody

Discussion

We surveyed the prevalence of pre-existing NAbs against various AAV serotypes in the human vitreous humor. The relationship between NAb titers in the serum and vitreous humor also was examined by determining differences in these values according to BRB robustness. Of significance, we found that the level of pre-existing NAbs in the vitreous humor was much lower than that in the serum, regardless of AAV serotype. This novel finding supplements previous studies of the NAbs level in the aqueous humor.[17,18] For instance, Amado et al.[17] reported that NAb titers to AAV2 in the anterior chamber in control human subjects were at or near background levels, even in cases of very high serum NAb titers. In patients with Leber hereditary optic neuropathy (LHON), baseline NAb levels in the anterior chamber also were much lower than serum levels in all participants.[18] Although the NAb levels in the aqueous humor cannot represent those in the vitreous humor, which directly affect retinal gene therapy, these findings are concordant with our results. The differences in NAb levels between the systemic circulation and the intraocular space could be a result of the immune-privileged nature of the healthy eye.

The origin of immune privilege in the ocular system is a complex and dynamic process involving multiple physiologic, anatomic, and immunologic properties.[10,11] The BRB, which is composed of nonfenestrated capillaries of retinal vessels forming the inner part, as well as tight junctions of the retinal pigment epithelium forming the outer layers, is an important anatomic structure that maintains the immune privilege of the posterior segment of the eye. This restricts the movement of inflammatory cells and inflammatory macromolecules from the circulatory system into the eye.[12,19] Several retinal diseases are associated with alterations in the inner or outer BRB, leading to disruption of the immune-privileged state of the retina. Our study showed that the preexisting immune status of the vitreous humor may be different under such conditions.

Patients with VH (i.e., severely disrupted inner BRB) had higher titers of NAbs in the vitreous humor than patients with an intact inner BRB, regardless of their serum titers. Significantly lower NAb ratios were observed in patients with ERM whose FA scan revealed dye leakage (i.e., mildly disrupted inner BRB) compared to those of patients without leakage. Taken as a group, patients with VH and ERM with leakage had significantly lower NAb ratios than patients with intact BRB.

The difference that BRB-dependent, pre-existing immune status makes is clearer when the relationship between the NAb titers in the serum and vitreous humor is considered. Our study showed that when the BRB is intact, the NAbs level in the vitreous humor remains low even if the level in the serum is high. However, the levels in serum and vitreous humors are positively correlated when BRB is disrupted.

These results indicated that the level of NAbs in the vitreous humor could be higher in cases of BRB disruption, and that the absolute value of NAbs in the vitreous humor could be influenced by the extent of BRB disruption and the level of NAbs in the serum.

Recently, two major clinical trials of human retinal gene therapy using AAV2 vectors delivered by intravitreal injection reported conflicting results regarding the serum NAbs levels and the efficacy of gene therapy.[6,18] One trial examined the safety and tolerability of AAV2-sFLT01 in patients with advanced neovascular AMD, and the other trial investigated the effects of AAV2(Y444,500,730F)P1ND4v2 in LHON patients. The study with AMD patients reported an inverse correlation between the presence of NAbs to AAV2 and the ability of AAV2sFLT01 to produce the sFLT01 protein. On the other hand, the study with LHON patients suggested that high serum NAbs levels may not be a barrier to successful ocular gene therapy, because the four patients who showed the most improvement in visual acuity with the treatment had the highest serum NAbs levels. Based on our findings, these conflicting results could be explained by the differences in BRB maintenance status in the target disease. Neovascular AMD is a frequent retinal disease that involve the breakdown of

the BRB, especially the outer BRB, whereas LHON is an inherited mitochondrial disorder that is not associated with BRB disruption, as shown by the absence of dye leakage in a typical FA image. However, the results of a nonhuman primate study were different from those of human studies, because the primate study reported that the presence of pre-existing NAb titers in the serum of healthy monkeys was correlated strongly with weak transgene expression after intravitreal delivery of AAV.[9] It is presumed that immunologic heterogeneity and differences in BRB structure between nonhuman primates and humans may account for the differences between the results of these studies.[20]

Another factor to consider regarding the immunologic aspect of retinal gene therapy is the route of administration; that is, the subretinal or intravitreal space, as they are immunologically slightly different.[17,21,22] A mouse study demonstrated that intravitreal administration of AAV generated a humoral immune response against AAV capsid equivalent to that of systemic administration, whereas subretinal administration did not trigger a humoral immune response and did not affect subsequent intravitreal or subretinal administration of AAV.[21] Other studies demonstrated that initial subretinal injection of AAV2 does not prevent readministration of AAV2 to the contralateral eye.[17,22] Human studies targeting neovascular AMD with subretinal injections of AAV2 have reported that the presence of anti-AAV antibodies in the serum at baseline does not affect the outcome, in contrast to the findings of Heier et al. for intravitreal injections in patients with the same disease.[5,6,23] Because of difficulties in measuring the level of pre-existing NAbs in the subretinal space, the relationship between the serum and subretinal space pertaining to pre-existing immunity is difficult to evaluate. However, this relationship may be different from the relationship between the serum and intravitreal space because of the differential effect of serum NAbs on transgene expression based on the injection route for the same disease; that is, neovascular AMD, which is associated with breakdown of the BRB.

The significance of our study is that, to our knowledge, it is the first to measure pre-existing NAb titers in the human vitreous humor and to examine pre-existing NAb titers in Korean populations to provide insights on AAV vector-based gene therapy. By surveying patients with various vitreoretinal diseases, we demonstrated the possibility of differences in the relationship between NAb titers in the serum and vitreous humor according to BRB status, and we determined the factors to be considered when performing retinal gene therapy in relation to the inhibitory effect of NAbs.

Nonetheless, our study has some limitations. The small sample size limited the statistical strength of our analysis, and there is potential ethnic bias, as only Korean patients were represented. Further large-scale studies that span a wider demographic will be crucial to confirm our findings. Our results are important for the development of gene therapy for neovascular AMD in the future. However, because VHs caused by wet AMD are uncommon,[24] only one patient with neovascular AMD has been included in this study. We investigated the integrity of the BRB qualitatively with FA. However, future studies are required to determine whether quantification of vitreous albumin is a comparatively reliable method for evaluating the BRB integrity.

In conclusion, the presence of baseline NAbs in serum may not be a limiting factor for successful retinal gene therapy using AAV vectors. This is particularly true if their BRB is intact. However, baseline NAb levels in the serum and the extent of BRB breakdown must be considered when planning gene therapy for diseases associated with breakdown of the BRB, because NAb levels in the vitreous humor are affected by these two factors and may be high enough to impact successful transduction. Therefore, the characteristics of diseases associated with maintenance of the BRB should be considered in retinal gene therapy using AAV vectors.

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국문 요약

연구 목적

안질환 환자의 혈청과 유리체액을 동시에 확보하여 유리체강 내 아데노부속바이러스에 대한 중화항체 수준과 혈청과 유리체액 사이 중화항체의 상관관계를 알아보고자 한다.

연구 대상 및 방법

본 연구는 안질환 환자 중 유리체 절제술이 요구되는 환자 32 명을 대상으로 환자의 혈청과 유리체액을 확보하여 Neutralizing antibody assay 프로토콜에 따라 AAV2, 5, 8, 9 에 대한 중화항체 수준을 확인하였다. 또한 안저검사와 혈관 조영술을 통해 유리체 출혈과 조영제의 누출을 분석하여 종합적으로 혈액망막장벽의 손상여부를 평가하였다.

연구 결과

전반적인 중화항체 수준은 바이러스 종류에 상관없이 혈청보다 유리체액에서 현저히 낮은 수준을 나타내었다. 다만 유리체 출혈이 있는 환자에서는 출혈이 없는 환자보다 유리체액에서 높은 중화항체 수준을 보였고 혈청과 유리체액 사이 중화항체 비율(혈청에서 중화항체 수준/유리체액에서 중화항체 수준)은 망막 전막에 누출이 있는 환자가 그렇지 않은 환자보다 훨씬 낮았다. 유리체 출혈과 망막 전막의 누출은 혈액망막장벽에 손상이 있는 질환으로 구분될 수 있었고 혈액망막장벽에 손상이 있는 환자들은 유리체액에서 중화항체 수준이 올라감에 따라 중화항체 비율이 현저히 낮아짐을 확인 할 수 있었다.

고찰

혈청과 유리체액 사이의 중화항체 비율은 혈액망막장벽의 상태에 따라 달라진다. 그러므로 아데노부속바이러스를 안질환 관련 유전자 치료에 적용할 때 혈액망막장벽의 손상여부를 고려해야 한다.

중심 단어

중화항체; 아데노부속바이러스; 유리체액; 혈청; 유전자 치료; 혈액망막장벽