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Influence of ranibizumab treatment on the extracellular matrix in patients with neovascular age-related macular degeneration

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Background: We know the influence of the intravitreal anti-vascular endothelial growth factor (VEGF) injections on the choroidal neovascularization in the course of exudative age-related macular degeneration (AMD). However, the influence of the ranibizumab therapy in question on the extracellular matrix (ECM) remains unknown. We aimed to estimate the influence of Lucentis intravitreal injections on the gene expression of structural components of the extracellular matrix in patients with neovascular AMD.

Material/Methods: Patients with subfoveal localization of neovascularization in AMD, which was clinically active and observed using optical coherence tomography, were treated with ranibizumab (0.5 mg/0.05 mL) in accordance with the PrONTO scheme. Total RNA was extracted from peripheral blood mononuclear cells, and an oligonucleotide microarray technique enabled comparison of the expression level of genes encoding collagens, elastin, and laminins in AMD patients compared to control subjects.

Results: After 3 intravitreal injections of ranibizumab (Lucentis), *COL1A1* and *COL6A1* genes showed increased expression, whereas decreased expression mainly occurred for the following genes: *COL4A5*, *COL11A1*, *COL4A6*, *LAMB4*, and *LAMC2*.

Conclusions: Anti-VEGF local therapy influences the gene expression of structural components of the ECM as measured from blood samples. The loading dose of ranibizumab for the retina changes the expression of collagen and laminin genes, but does not influence the expression of the elastin gene.

MeSH Keywords: **Neovascular (Exudative) Age-Related Macular Degeneration • Ranibizumab • Extracellular Matrix • Bruch's Membrane • Collagen • Elastin • Laminin**

Abbreviations: **AMD** – age-related macular degeneration; **CNV** – choroidal neovascularization; **ECM** – extracellular matrix; **MMP** – matrix metalloproteinase; **RPE** – retinal pigment epithelium; **TIMP** – tissue inhibitor of metalloproteinase; **VEGF** – vascular endothelial growth factor

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3667 3 — 66

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Background

Age-related macular degeneration (AMD) is the main cause of practical and irreversible central blindness (scotoma in the central visual field makes impossible the following: reading and writing, stereoscopic vision, and recognition of colors and details) in patients after the age of 50 years in industrialized countries in Europe and North America [1,2]. In 10% to 15% of these patients, advanced (late) AMD occurs in the exudative form, characterized by the presence of choroidal neovascularization (CNV) resulting from angiogenesis and the pathological proliferation of choroid vessels in the extracellular matrix (ECM) and under the retina [1,2]. The pathology of AMD starts the dysregulation of metabolic processes at the level of retinal pigment epithelium (RPE) cells; however, the area of these disorders is the extracellular matrix, which is Bruch's membrane [1–3]. Bruch's membrane, the main components of which are collagens, elastin, and laminins, constitutes a sort of scaffold for RPE cells, a physical barrier for the passage of RPE cells and the endothelial tissue of blood vessels, and the interface regulating the diffusion of nutritional molecules between the retina and the choroid [3,4]. In the pathogenesis of CNV/AMD, a key role is played by vascular endothelial growth factor-A (VEGF-A) [5,6]; however, changes in the ECM are also necessary in order to create the conditions for adhesion of the proliferating and migrating endothelial tissue and for the expansion of the newly created vessels [7]. Intravitreal injections of ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA), an antigen-binding fragment of a recombinant, humanized monoclonal antibody, inhibit all of the known biologically active forms of VEGF, which in turn inhibits the proliferation of endothelial tissue, vessel leakage, and angiogenesis [8–11]. Ranibizumab reduces edema in the central retina [9]: in 90% to 95% of patients, it stabilizes visual acuity, and in one-third of patients, it improves it [8,9]. It is an effective cure for CNV/AMD [8,10,12]; however, it is not devoid of adverse effects [10,13,14].

To date, neither the general nor the regional effects of Lucentis injections on the ECM in patients with CNV/AMD have been described, and to the best of our knowledge, this is the first study to examine these effects. Our aim was to estimate the influence of the loading dose of ranibizumab (Lucentis) on the expression of collagen, elastin, and laminin genes in patients with the exudative form of AMD.

Material and Methods

Material

Patients with the active, exudative form of AMD qualified for participation in the study if they met the criteria presented in Table 1.

Methods

Ophthalmological tests

The following ophthalmological tests were given to each participant: (1) best corrected visual acuity on the ETDRS charts; (2) indirect biomicroscopy in mydriasis (+78 D lens; Volk Optical,

Table 1. Inclusion and exclusion criteria for patients with active CNV qualified for intravitreal injections of ranibizumab.

Inclusion criteria
– age ≥60 years
– interview (decrease in BCVA, metamorphopsia) no longer than 3 months
– BCVA 20/25 to 20/200 Snellen equivalent
– CNV activity in biomicroscopy (central edema of retina, hard exudates, hemorrhages), OCT (hyperreflectivity characteristic for CNV, presence of SRF and/or IRF), FAG/ICG (vascular leakage)
– each of the angiographic CNV subtypes of subfoveal localization (predominantly classic, minimally classic, fibrovascular RPE detachment, leakage of unknown source)
– no previous CNV therapies: anti-VEGF injections (Lucentis, Avastin, Macugen), photodynamic therapy with verteporfin, laser therapy, steroid therapy (triamcinolone)
– written consent for both the Lucentis injections and participation in the study
Exclusion criteria
– polypoidal choroidal vasculopathy, retinal angiomatous proliferations
– idiopathic CNV as well as CNV in cases of high myopia, angioid streaks, inflammation, or infection (histoplasmosis, sarcoidosis, multifocal choroiditis, punctuate inner choroidopathy), choroid tumors (melanoma, hemangioma, osteoma), injuries (choroidal rupture, laser photocoagulation), retinal vein occlusion
– coexisting diabetic maculopathy, epiretinal membrane

BCVA – best corrected visual acuity; CNV – choroidal neovascularization; FAG – fluorescein angiography; ICG – indocyanine green angiography; IRF – intraretinal fluid; OCT – optical coherence tomography; RPE – retinal pigment epithelium; SRF – subretinal fluid; VEGF – vascular endothelial growth factor.

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Mentor, OH); (3) Goldmann applanation tonometry; (4) 6 diagonal high-density 6-mm scans in optical coherence tomography (OCT) at 30-degree intervals (Stratus III OCT; Carl Zeiss, Dublin, CA); and (5) fluorescein angiography with or without indocyanine green angiography (5 mL 10% fluorescein/25 mg indocyanine green administered by IV; Fundus Camera FF 450 plus IR; Carl Zeiss).

Intravitreal injections

Intravitreal injections were performed by 1 ophthalmologist, who applied an aseptic technique of the procedure, using infiltration anesthesia in the injection site (most often the upper temporal quadrant, 4 mm from the limbus). The vial contents were withdrawn 30 min before the injection procedure, at which time 0.5 mg/0.05 mL ranibizumab was injected (Lucentis, Novartis Pharma Ayringe G: Basel, Switzerland, and Genentech, South San Francisco, CA) with a 30-gauge needle [10]. The scheme PrONTO was applied [15,16], in which the loading dose regimen was applied in the form of 3 injections every month, the reinjections dependent on CNV activity: relapse or maintenance of subretinal fluid and/or intraretinal fluid in OCT, loss of 5 lines on the ETDRS charts in association with fluid in OCT, new hemorrhages, and/or angiographically confirmed enlargement of the CNV [15,16]. Three days before and 3 days after the injections, the patients were given ofloxacin regionally 4 times a day. The patients treated with anticoagulants before the injections and after the consultations with the general practitioner were advised to apply Clexane. All patients were treated in the Department of Ophthalmology in Katowice, Poland. The profile of ECM expression of the chosen genes was analyzed after the application of 3 injections of Lucentis.

Tissue samples

Venous blood samples of patients with AMD before and after intravitreal injections were collected in tubes containing ethylenediaminetetraacetic acid. The samples were centrifuged on a Ficoll-Conray gradient (specific gravity 1.077; Immuno-Biological Laboratories Co., Gumma, Japan) immediately after blood collection and stored at –20°C for 24 h until RNA extraction.

Ribonucleic acid extraction from tissue specimens

Total RNA was extracted from peripheral blood mononuclear cells using TRIzol reagent (Invitrogen, Carlsbad, CA). RNA extracts were treated with DNase I (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. The quality of extracts was checked electrophoretically using 0.8% agarose gel stained with ethidium bromide. The results were analyzed and recorded using the 1D Bas-Sys gel documentation system (Biotech-Fisher, Perth, Australia). Total RNA concentration was determined by spectrophotometric measurement in 5-µL capillary tubes using the GeneQuant II RNA/DNA Calculator (Pharmacia Biotech, Cambridge, UK).

Oligonucleotide microarray analysis

All samples were selected for microarray analysis. Analysis of the expression profile of genes related to the selected ECM components: collagens, laminin, and elastin in samples collected before (3 samples) and after (5 samples) ranibizumab intravitreal injection, was performed by using commercially available oligonucleotide microarrays of HG-U133A (Affymetrix, Santa Clara, CA) according to the manufacturer's recommendations. Each gene chip contained 22 238 probe sets corresponding to more than 18 400 transcripts and 14 500 well-characterized human genes. Approximately 8 µg of total RNA was used for complementary cDNA synthesis using the SuperScript Choice System (GIBCO BRL Life Technologies, Carlsbad, CA). During the next step, complementary DNA was used as a template to produce biotin-labeled complementary RNA (cRNA) with the BioArray HighYield RNA transcript labeling kit (Enzo Life Sciences, Farmingdale, NY). Complementary RNA was purified on Rneasy Mini Kit columns (Qiagen Gmbh, Hilden, Germany). Next, the biotin-labeled cRNA was fragmented by using the GeneChip Sample Cleanup Module (Qiagen) and hybridized with the HG-U133A microarray (Affymetrix). The hybridized cRNA were stained with streptavidin phycoerythrin conjugate and scanned using a GeneArray Scanner G2500A (Agilent Technologies, Santa Clara, CA). The scanned data were processed for signal values using Microarray Suite 5.0 software (Affymetrix). The results obtained were normalized with RMAExpress software (Robust Multichip Average).

Statistical analysis

Microarray data analysis was performed with the GeneSpring 12.0 platform (Agilent Technologies UK Ltd., South Queensferry, UK) to identify transcriptome differences between the control and the study groups. The oligonucleotide microarrays of Affymetrix HG-U133A enabled analysis of 22 283 mRNA transcripts. For further study, we selected only 98 transcripts from the Affymetrix NetAffx Analysis Center database (*http://www.affymetrix.com/analysis/index.affx*). Normalized data were used to compile a list of 7 genes, the expression of which appeared to be up- or down-regulated by an arbitrary cutoff of at least 2-fold. Significant differential gene expression was identified by a 2.0-fold change at $P<0.05$. The results obtained through the oligonucleotide microarray technique were grouped with the hierarchical clustering method using the Euclidean distance measure.

The study was approved by the Bioethics Committee of the Medical University in Katowice, in accordance with the

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Table 2. Characteristics of patients with CNV/AMD treated with intravitreal injections of ranibizumab and control group patients with CNV/AMD.

	Treated group N=5 eyes	Control group N=3 eyes
Sex		
Female	3 (60%)	2 (67%)
Male	2 (40%)	1 (33%)
Age (y) Mean (SD)	76.80 (6.10)	75.33 (2.31)
Range	72.0–86.0	74.0–78.0
Eyes		
Right	3 (60%)	2 (67%)
Left	2 (40%)	1 (33%)
BCVA (log MAR)		
before therapy Mean (SD)	0.78 (0.35)	0.73 (0.25)
Range	1.0–0.2	1.0–0.5
after therapy Mean (SD)	0.44 (0.19)	
Range	0.7–0.2	
CNV subfoveal localization	5 (100%)	3 (100%)
CNV component:		
Minimally classic	1 (20%)	3 (100%)
Occult with no classic	4 (80%)	1 (33%)
fibrovascular RPE detachment	1 (20%)	2 (67%)
leakage of unknown source	3 (60%)	
Central retinal thickness before/ after therapy µm Mean (SD)	319.1 (103.9)/233.4 (77.6)	337.5 (118.3)
Lens status		
Phakic	5 (100%)	2 (67%)
Pseudophakic		1 (33%)
IOP mmHg Mean (SD)	15 (4.30)	16.33 (2.31)
General disorders		
AH	2 patients	2 patients
IHD	2 patients	1 patient
Osteoporosis	1 patient	
Hypothyreosis		1 patient
Diet supplementation	All patients	All patients
Post-injection complications	None local and general	

AH – arterial hypertension; AMD – age-related macular degeneration; BCVA – best corrected visual acuity; CNV – choroidal neovascularization; IHD – ischemic heart disease; IOP – intraocular pressure; logMAR – logarithm of the minimum angle of resolution; RPE – retinal pigment epithelium; SD – standard deviation.

Declaration of Helsinki regarding medical research involving human subjects. The study and its purpose were explained to each participant or his or her legal guardian, who gave written informed consent.

Results

The characteristics of the patients participating in the test are shown in Table 2.

The effect of the Lucentis injection on the change in expression of the collagen and laminin genes is presented in Table 3. Of note, therapy with ranibizumab did not have any influence on the expression of elastin genes.

Discussion

Ranibizumab (Lucentis) is a biological medication, with regional and general activity, as can be demonstrated by its

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Table 3. Change in expression of collagen and laminin genes after three injections of ranibizumab.

Name of probe	Gene	Name of gene	FC	*P
202312_s_at	*COL1A1*	Collagen, type I, alpha 1	2.19↑	0.0068
212938_at	*COL6A1*	Collagen, type VI, alpha 1	2.41↑	0.0153
213110_s_at	*COL4A5*	Collagen, type IV, alpha 5	2.09↓	0.0001
216622_at	*LAMB4*	Laminin, beta 4	2.14↓	0.0005
202267_at	*LAMC2*	Laminin, gamma 2	2.24↓	0.0038
204320_at	*COL11A1*	Collagen, type XI, alpha 1	2.74↓	0.0011
213992_at	*COL4A6*	Collagen, type IV, alpha 6	3.32↓	<0.0001

FC – multiple of difference in fluorescence signals, statistical importance: * $P<0.05$, *t* test; ↑ overexpression of gene, ↓ underexpression of gene.

pharmacokinetics [6,10,11,17]. Injected into the vitreous body, it penetrates the whole retina to the level of neovascularization. Next, it undergoes slow absorption into the circulatory system: its concentration in the serum is approximately 90 000–140 000 times less and it decreases simultaneously with the decrease in its level in the vitreous body [6]. In patients with CNV/AMD, after multiple injections, there was less than 11 ng/mL of ranibizumab in the serum, a level that blocks the biological activity of VEGF-A [6]. After being catabolized in the kidneys, the medication is eliminated in the urine [17]. Ranibizumab is a protein, which in approximately 1% to 8% of treated patients stimulates antibody production; antibodies may reduce its therapeutic effectiveness, enter into reactions with endogenic proteins, and cause anaphylactic reactions [18]. Apart from its local effects, it can also cause general adverse effects: hypertension, nasopharyngitis, headache, arthralgia, stroke, cardiac infarction, hemorrhage, thrombosis, and emboli, and even death that is related or unrelated to the blood vessels [10,19]. Ranibizumab also influences the ECM, as shown in our study. Under the influence of the loading dose of Lucentis, the genes coding collagen type I (gene COL1A1) and collagen type VI (gene COL6A1) were overexpressed.

An increase in the amount of interstitial **collagen type I,** being the most abundant component of the ECM, may favor cell adhesion, as well as maintenance of tension and mechanical endurance of the collagen fibers of various tissues, such as bones, joints, and skin [20,21]. Theoretically, this increase may also favor the rebuilding process of Bruch's membrane after being degraded by neovascularization, since the structural and functional rebuilding of the collagen layers can improve the mechanical stability of Bruch's membrane, facilitate RPE cell adhesion, transport nutrients and products of metabolism, and transmit signals.

Increased interstitial collagen type I may also have a negative impact on the condition of the retina and destroy the effects of regional anti-VEGF therapy, because collagen type I induces genes involved in angiogenesis, has strong activity in the process of angiogenesis, and facilitates the development of CNV [22]. Increased collagen I occurring after injections of Lucentis may stimulate and sustain angiogenesis, and, as has been shown by Nguyen [23], in the case of illness, activate 2 endopeptidases crucial to the process: gelatinase A (matrix metalloproteinasis-2; MMP-2) and membrane-type metalloproteinase-1 (MMP-14) [23]. MMP-2, the most abundant and significant metalloproteinase synthesized by RPE and endothelial cells, digests the main structural element of basement membranes (ie, collagen IV); moreover, similar to interstitial collagenases, it digests stromal collagen type I and V, as well as digesting gelatin, elastin, laminin, and fibronectin [23–25]; plays a key role in Bruch's membrane remodeling [26–28]; and is crucial in angiogenesis [23] and CNV/AMD [23,29]. It also promotes the activation of latent interstitial collagenases and their ability to degrade stromal collagen [30]; interstitial collagen digestion produces gelatin (denatured collagen), which acts as a specific substrate for MMP-2 [23]. MMP-14 activates MMP-2 directly and by mediation of tissue inhibitor of metalloproteinase-2 (TIMP-2) in a 3-molecule complex: pro-MMP-2/TIMP-2/active MMP-14 [23]. MMP-14 influences the ECM by activating MMP-2 in the range of the 3-molecule complex, but it can also direct the degradation of interstitial collagen type I, II, and III [25,31]. ECM digestion is necessary to the deposition and breakdown of choriocapillaris basement membrane, to opening space to endothelial cell invasion, to capillary tube formation and sprouting of new vessels, and to the mobilization of ECM receptor shedding and activation of pro-/anti-angiogenic growth factors to enable cell migration and proliferation [32]. By activating both of the metalloproteinases having such a broad spectrum of activity, collagen I triggers the so-called vicious cycle of auto-degradation, as well as sustaining pathological angiogenesis for a lengthy period until the endothelial tissues do not shape their own basement membranes, which in turn becomes a barrier for contact with collagen and further stimulation by both enzymes [23].

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Moreover, neovascularization sustains pathological angiogenesis, while leakage of its various components to the basal laminar deposits, located under the RPE layer (ie, the basal laminar deposits and soft drusen, as well as in the area of 2 collagen layers and elastic layer of Bruch's membrane), favors untypical polymerization of molecules into aggregates similar to ribbons and long fibers of collagen [33]. Accumulation of the new structures thickens the basal laminar deposits much more, which in turn worsens the supply of the RPE/photoreceptors complex with nutrients and oxygen, favoring hypoxia and sustaining neovascularization [33].

An increased expression of genes of collagen type VI, a component of the basement membranes [34], occurring after the injections, may be profitable because it increases the amount of a factor that favors the adhesion of cells to the basement membranes, as well as the interactions in the range of the ECM throughout the organism and in the retina [34]. On the other hand, it may theoretically worsen the retina's condition in the treated and in the adjacent eye, since it causes enlargement of basal laminar deposits (BLamD) and soft drusen (SD) and, through them, may favor pathological angiogenesis [35–37]. BLamD that are larger than 25 μm become clinically visible SD, which intensify the process of lipofuscinogenesis in the RPE, worsen RPE cell metabolism and their ability to supplement the photoreceptors, and separate RPE cells from the internal collagen layer of Bruch's membrane [35,38,39]. On the other hand, SD bigger than 125 µm and confluent is a risk factor for the development of advanced forms of AMD, which means geographical atrophy or neovascularization within the next 5–10 years [40,41].

Because of the loading dose of ranibizumab, the genes coding collagen type IV (*COL4A5*, *COL4A6*) and type XI (*COL11A1*), as well as the genes coding beta–lamin (*LAMB4*) and gamma-lamin (*LAMC2*), undergo a silencing process. The genes *COL4A5* and *COL4A6* are linked in the genome [42]. Moreover, both underwent a silencing process in our analysis; however, the *COL4A6* gene showed the highest loss of expression. A decrease in collagen type IV after injection of Lucentis may be considered disadvantageous for the following reasons.

From a structural point of view, the decrease in the amount of the main and structural components of the basement membranes may disintegrate, as well as destabilize, the basement membranes in the entire body (including the basement membranes of the RPE cells and the choroid endothelial tissues) and it may distort the cells' adhesive properties to the basement membranes [43–45].

In the eyes, in the case of the neovascular form of AMD, the loss of collagen type IV may also be theoretically undesirable because of its anti-angiogenic properties [46–48]. Collagen type IV acts anti-angiogenically on different levels (mechanisms, manners). It can inhibit both proliferation and migration of the endothelial tissues in the eye tissues [47]; in the process of adhesion, it binds with the non-integrin and integrin receptors of the cell (mainly β1 integrins), as some of them work in an anti-angiogenic manner [49]; and it participates in homeostasis [46,48].

In patients undergoing local anti-VEGF therapy, apart from the changes in the area of the vitreous body (over the age of 40 years, it appears as gradual liquefaction of the vitreous body's gel-synchysis senilis) [50,51], overlap also occurs in the changes resulting from the numerous injections, because the loading dose of the medication, with time, is combined with reinjections, which are dependent on relapse/lasting of subretinal or intraretinal fluid in OCT, loss of 5 lines on the ETDRS chart, and/or angiographically proven enlargement of CNV [15,16].

Development of persistent vitreomacular attachment may cause a macular hole or cystoid macular edema, together with the relapse of metamorphopsia; such cases are not a result of CNV relapse, but a consequence of the changes within the vitreous body [52–54].

The ECM of the eye contains numerous components typical for hyaline cartilage, namely, collagen type II, VI, IX, and XI [34,43,55–57]. After the application of 3 anti-VEGF injections in our material, a decrease appeared in the expression of the genes of collagen type XI (*COL11A1*), which is closely related both structurally and actively to collagen type V, regardless of the fact that they have different localizations in the genome [55–57]. Collagen type V and XI constitute approximately 10% of the collagen fibers of the vitreous body [57,58]. Mutations of the gene *COL11A1* distort fibrillogenesis, causing the formation of rare, thickened, and irregular collagen fibers of the vitreous body, which are typical for the Stickler and Marshall syndrome [59,60].

Loss of collagen XI, after the loading dose of Lucentis, may theoretically distort renewal of the vitreous body after it is exposed to numerous iatrogenic injuries. On the other hand, after the injections occurred, the expression of the collagen VI gene (*COL6A1*) increased, which, next to collagens being coded by the genes *COL6A2* and *COL6A3*, also belongs to the so-called eye-cartilage-collagens [34,43,55–57]. Collagen VI stabilizes the gel-like structure of the vitreous body by binding its various fibers with hyaluronic acid [50,55]. Increased expression of collagen VI may, theoretically, favor both stabilization and renewal of the vitreous body that is changed by the injections, as well as limiting the decreased expression of the collagen XI gene.

Theoretically, the change of expression of collagen type I, IV, and VI genes after loading dose of ranibizumab, influences in the eye not only by the condition of Bruch's membrane, but also the ECM in trabecular meshwork, sclera, and lamina cribrosa.

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Collagen is the main structural component and therefore, contributes to the mechanical properties, organization, and the shape of these tissues, and it plays an important role in glaucoma pathogenesis [61]. Therefore, the change of expression of collagen genes may hypothetically favor glaucoma development in patients being treated with Lucentis, which can be demonstrated by the following study results: 1) collagen type I, the main component of ECM in the trabecular meshwork and uveoscleral aqueous humor outflow pathways, may be involved in increased aqueous humor outflow resistance in trabecular meshwork and elevation of intraocular pressure; 2) changes of synthesis of type IV collagen have been found in glaucomatous trabecular meshwork; and 3) collagen types I, IV, and VI (as well as types III and V) constitute the main composition in the lamina cribrosa, and the content and/or the composition of the collagen molecules in the lamina cribrosa is significantly changed in glaucoma [61]. However, the above hypothesis needs further and detailed study.

The loading dose of Lucentis caused decreased expression of beta-laminin (*LAMB4*) and gamma-laminin (*LAMBC2*). Laminin, next to collagen type IV, is the key structural component of basal membranes [4,62], and is classified as a so-called big-sized component of the basement membrane [62]. Laminin fills up the eyelets in the 3-dimensional network of collagen IV, to which it joins directly or via bridges (entactin/nidogen) [48,63] and joins the cell surface via surface receptors [4,48,64]. Laminin initiates the process of membrane formation and is therefore indispensable [4,44,64], in contrast to collagen IV, which despite its ubiquity and abundance, does not initiate the formation of basement membranes, but does play a role in supporting their structure [44].

Decreased laminin gene expression after the injections may not be beneficial, both generally and regionally, and may favor neurological defects or disorders of muscle, skin, or kidneys [63,64].

A decrease in the factor necessary for building the basement membranes may theoretically limit their formation, including the restoration of basement membranes in RPE cells, as well as in the endothelial cells of the choroid, which are damaged in the process of angiogenesis. It may disturb the process of cell adhesion and the passing of signals in the range of the ECM in the entire body and in Bruch's membrane.

On the other hand, the process of neovascularization requires a certain change in the ECM in such a way that the migrating endothelial cells can adhere, and moreover, not undergo the process of apoptosis. Such a change in the ECM is regulated at various levels by many factors, such as laminin (mainly LAM-1) [7,65]. A decrease in the amount of laminin after the injections (in this context) can be beneficial, since it may not facilitate the pathological rebuilding of Bruch's membrane; moreover, it can hamper the adhesion of the migrating endothelial cells and therefore limit the development of CNV [66].

Conclusions

Apart from its regional influence, ranibizumab also works generally since it changes the gene expression of structural components of the ECM, as measured in blood levels (i.e., collagens and laminins); however, it does not influence the expression of elastin genes. Changes in the structural collagens in Bruch's membrane under the influence of ranibizumab (increased collagen I and decreased collagen IV) may theoretically favor relapse of neovascularization and reinjections in the treated eye; moreover, this may theoretically accelerate the development of the exudative phase of AMD in the adjacent eye, which has so far represented only the early symptoms of AMD. The beneficial effects of the therapy in question may be the decrease in laminin, which has pro-adhesive activities. Ranibizumab also changes the gene expression of the structural collagens of the vitreous body (i.e., it increases the gene expression of collagen VI and decreases the gene expression of collagen XI). The necessity of repeating the anti-VEGF injections may not only result from the natural tendency of the vessels to recanalize, including pathological vessels, but may also be connected to changes in ECM structure, which favors the development of CNV, mainly in terms of the changed amount of collagen type I (which favors the development of CNV and stimulates the activity of 2 strong pro-angiogenic metalloproteinases characterized by a wide spectrum of activity) and collagen type IV (because of its anti-angiogenic properties).

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Competing interests

No competing interests. All authors declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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