Chondroitinase as a Vitreous Interfactant: Vitreous Disinsertion in the Human

VI.H.

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Chondroitinase Study Group (Addendum 1)

Outline

Keywords

Vitreous • Pharmacologic vitreolysis • Chondroitinase • Posterior vitreous detachment • Vitreous disinsertion • Monkey • Electrophysiology • Ultrastructure • Human vitrectomy

Key Concepts

1. Studies in monkeys have demonstrated that chondroitinase disinserts the posterior vitreous cortex from the ILM, the vitreous base from the peripheral fundus, and the anterior vitreous cortex from the lens. It has thus been found to be an effective interfactant agent for pharmacologic vitreolysis, but does not induce liquefaction.
2. Proof-of-principle studies in human brain-dead patients demonstrated efficacy as an interfactant without any untoward effects upon ocular morphology, in particular retinal ultrastructure.
3. Phase I/II FDA trials demonstrated safety and efficacy as a surgical adjunct in treating macular holes and proliferative diabetic retinopathy.

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I. Introduction

Despite the initial discovery and testing of chondroitinase as a pharmacologic vitreolysis agent that began nearly three decades ago, contractual restrictions in the initial years and intellectual drift in more recent years have limited the peer-reviewed publication of most of the animal (monkey) and human studies that evaluated this promising agent. Detailed investigator, institutional, corporate, and registered FDA regulatory documents that are available to us provided the basis for the majority of this chapter. Two devastating floods of Dr. Gregory Hageman's data room at the University of Iowa, however, reduced the number and variety of images available from which to provide supplementary visual illustration of the human results that will be discussed.

II. Biochemistry

A full appreciation of the rationale and initial results of chondroitinase pharmacologic vitreolysis requires an understanding of the localization of the proteoglycans substrate within the vitreous body and at the vitreoretinal interface as well as the biochemical properties and mechanism of action of chondroitinase.

A. Proteoglycans and the Vitreoretinal Interface

Proteoglycans are complex macromolecules found in all extracellular matrices including the vitreoretinal interface (Figure VI.H-1). Proteoglycans' function in basement membranes is to hydrate and resist compression, constrain diffusion by anionic charge, and provide mechanical adhesion between tissues [1]. They are identified by their core protein sequence and many, but not all, have been sequenced, characterized, and named, as for example decoran, versican, and perlecan [2]. Chondroitin sulfate proteoglycans have been previously shown to provide adhesion between basement membranes and adjacent extracellular matrices within the eye and other tissues. For instance, inhibiting the secretion of chondroitin sulfate proteoglycans within the insoluble interphotoreceptor matrix has been shown to result in reduction of adhesion between the retina and the retinal pigment epithelium [3–6].

As target substrates, proteoglycans consist of proteins that are heavily glycosylated by the attachment of linear glycosaminoglycan (GAG) polymer side chains attached via a serine (Ser) residue through which the GAG is attached by a tetrasaccharide bridge (i.e., <chondroitin sulfate>–glucose-galactose-galactose-xylose-serine) (Figure VI.H-1) [1]. In addition to their sequence, proteoglycans are characterized by the predominant saccharide dimer-pair that makes up the GAG side chains. For instance, a proteoglycan is called a chondroitin sulfate proteoglycan if the majority of the GAGs consist of alternating N-acetylgalactosamine and glucuronic acid.

In earlier sections of this book, the localization and significance of vitreous attachment(s) in development and disease have been documented [see chapters II.A. Vitreous Embryology; II.E. Vitreo-retinal interface & ILM; III.B. Anomalous PVD & vitreoschisis; III.C. Pathology of vitreomaculopathies]. The vitreous fibrillar structure can be clinically appreciated in aging [see chapter II.C. Vitreous aging & posterior vitreous detachment] and may be histologically visualized with a variety of particulates, stains, and probes. An illustration of the relative orientation and density of this fibrillar architecture is given in Figure VI.H-2. Regional differences in the adherence of the vitreous to the neural retina correspond to the density of the fibrillar vitreous structure, particularly to the density of fibrils orthogonal to the vitreoretinal surface. Central portions of the vitreous fibrillar material co-localize with collagen II [7]. However, our understanding of the terminations of the fibrillar structure and its insertions remains incomplete. Other fibrillar proteins and/or proteoglycans contribute to the vitreous and to retinal linkage as has been confirmed by Paul Bishop and colleagues [8–10] [see chapter I.A. Vitreous proteins].

Several mechanisms of adhesion of the vitreous fibrils are clinically known. These include the constitutive attachments found in younger humans and other primates. In addition, however, attachments of a pathologic nature may also add to or replace the constitutive adhesive mechanisms. For instance the development of retinal neovascularization onto or into the posterior vitreous cortex in proliferative diabetic vitreoretinopathy or the formation of organized tissue may complicate the attachment paradigm. Differing models of the constitutive attachments incorporate data derived from transmission electron microscopy, immunohistochemistry, and enzymatic response of the vitreoretinal interface. Based upon our observations of the behavior of the primate and human vitreoretinal interface to chondroitinase, we conclude that a structurally significant, omnipresent layer exists between the normal fibrillar/lamellar vitreous cortex and the neural retina (as suggested in the schematic representation of the vitreoretinal interface shown in Figure VI.H-3) [see "ECM" in chapter II.E. Vitreo-retinal interface & ILM]. The orientation and attachment site(s) of the chondroitin sulfate proteoglycan core protein has not been established.

We have previously shown that an incompletely identified, chondroitin sulfate-containing proteoglycan (core size approx. 240KDa) is associated with regions of strong adhesion between the vitreous and the retina, with the proteoglycan being localized to the vitreoretinal interface (a human example illustrating these adhesions in retinopathy of prematurity is

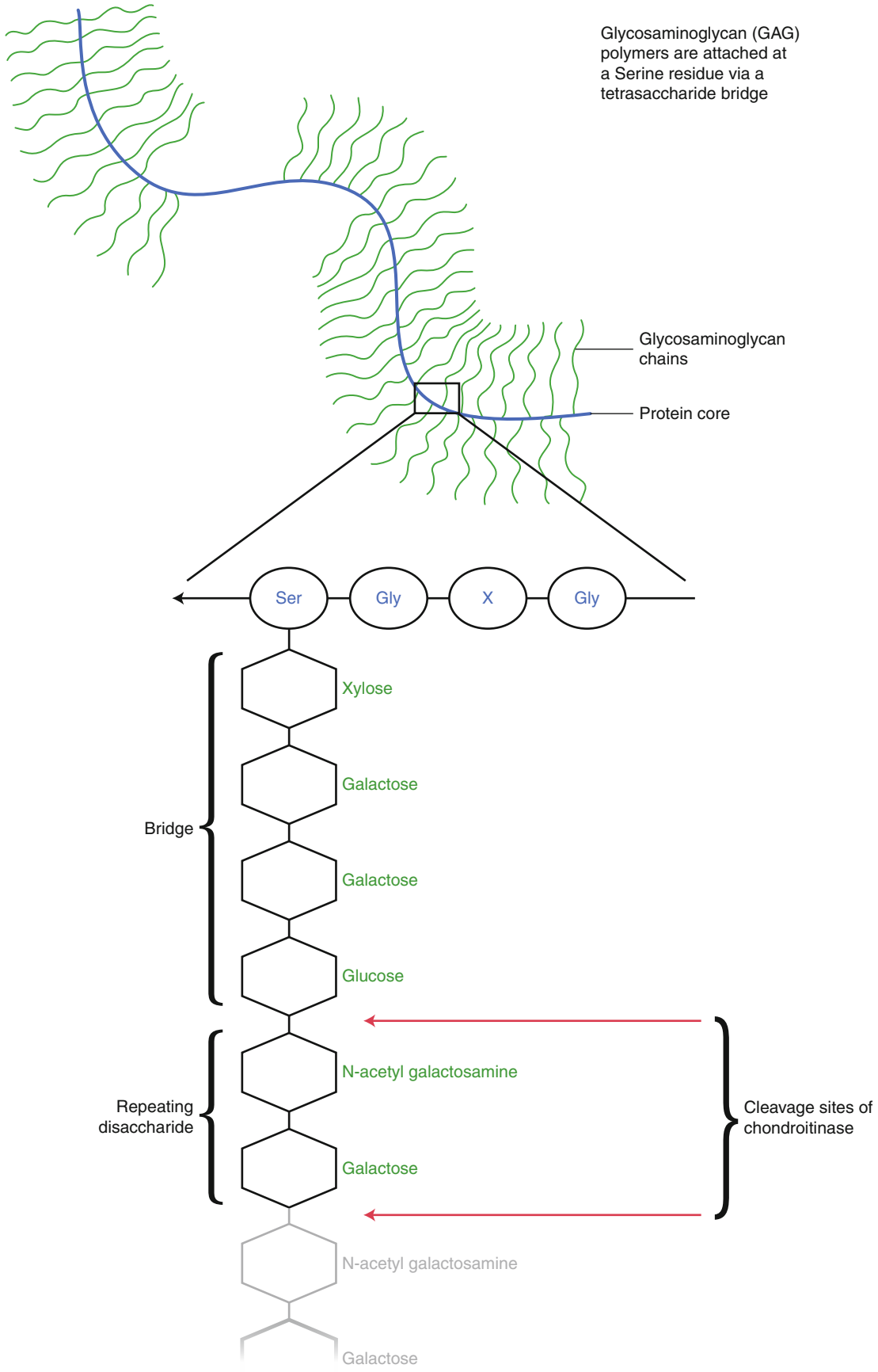

Figure VI.H-1 Proteoglycan structure and relationship of glycosaminoglycan (GAG) attachments. Glycosaminoglycan polymers are attached to a core protein via a serine residue ("O-linked") and a tetrasaccharide bridge. Exposure of the disaccharide repeats within the GAGs to chondroitinase facilitates isolation of the protein core, which may be adherent or covalently attached to other structural proteins at one or more sites

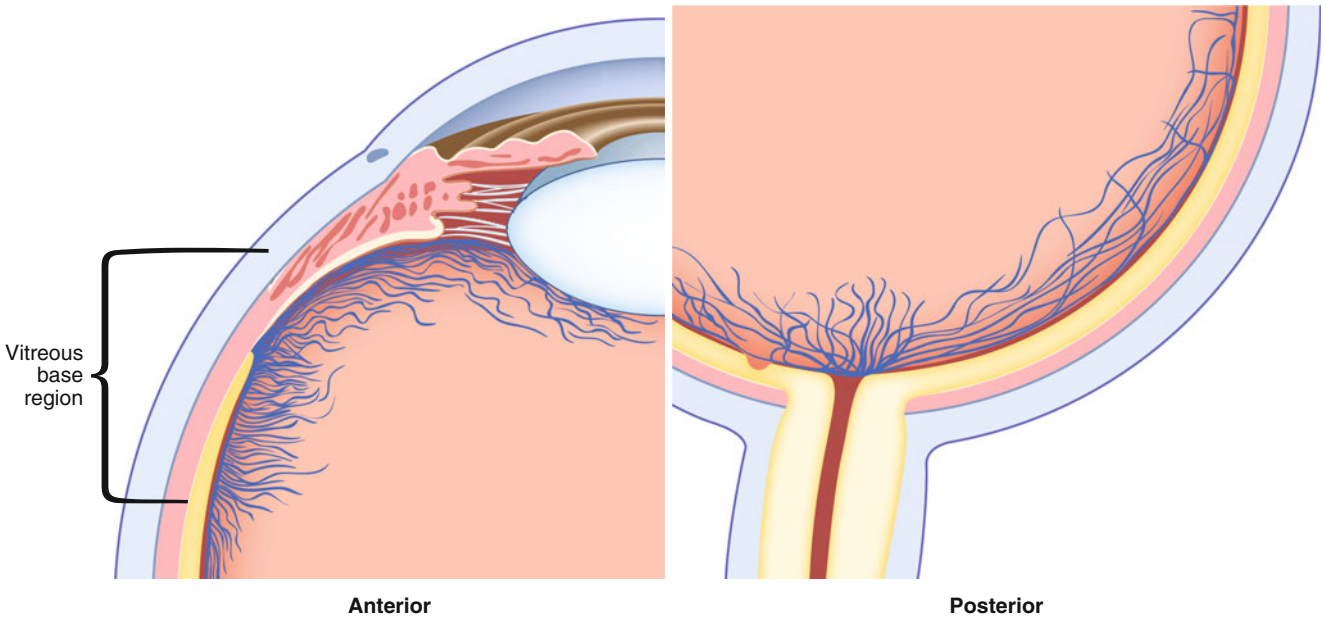

Figure VI.H-2 Schematic illustration of the fibrillar anatomy of the human vitreous. Vitreous adhesion and fibrillar density co-localize and are highest where the fibrils are dense and orthogonal to the vitreous base (region anterior and posterior to the ora serrata), intermediate in adhesion where there is intermediate orthogonal fibrillar density at the inner optic disk and lowest in adhesion and density where the fibers are sparsely distributed over the posterior inner retinal surface

shown in Figure VI.H-4) [11]. In the case of the incompletely identified vitreoretinal interface proteoglycan, the lectin peanut agglutinin or PNA binds with highest affinity to its glycosaminoglycans or GAGs which contain chondroitin-4-sulfate and/or chondroitin-6-sulfate. Subsequently we developed an antibody to the proteoglycan that facilitated more specific identification of this molecule associated with vitreous/retinal adhesion [12]. In a series of pilot experiments using commercially available chondroitinase, cynomolgus monkeys were treated with various doses of chondroitinase for varying time (incubation) periods prior to vitrectomy in order to determine if the enzyme could facilitate disinsertion of the vitreous from the neural retina [11, 13].

B. Biochemistry of Chondroitinase Enzymes and Substrates

Chondroitinase is an enzyme complex that acts to cleave and fragment chondroitin-sulfate-containing glycosaminoglycan (GAG) side chains of proteoglycan "core" molecules [12]. Chondroitinase demonstrates a specificity to cleave and degrade only bonds between and within the disaccharide N-acetylgalactosamine-glucuronic acid. As formulated for human clinical trials, chondroitinase is a biologic agent that consists of a mixture of two enzymes, chondroitinase I and chondroitinase II [12]. Chondroitinase I (110 KDa) is a lyase that attacks chondroitin sulfate in an endolytic fashion, breaking the polymeric structure into oligosaccharides and disaccharides [12]. The second enzyme, chondroitinase II (112 KDa), has no activity against the intact polymer but can digest tetrasaccharides produced by the chondroitinase I-catalyzed reaction [12]. The combined activity of these two enzymes results in a more rapid degradation than use of chondroitinase I alone. This type of depolymerization activity utilizing more than one enzyme to attack a large polymeric substance is analogous to many other enzymatic systems among microorganisms capable of degrading such polymers. The amino acid sequences of the chondroitinase enzymes are known [12].

Chondroitinase is produced by *Proteus vulgaris* bacteria, which can be induced to produce high concentrations of chondroitinase when incubated on a carbon diet consisting exclusively of chondroitin sulfate, such as shark cartilage. When other bacterially derived enzymes, secreted products, and waste are removed, the purified chondroitinase is a white, lyophilized powder that can be reconstituted with balanced salt solution. Purification methods often inactivate the enzymatic active sites or otherwise modify their protein structure, conformation, or glycosylation. Collaboration with Storz/American Cyanamid Company resulted in developing batch-produced, incubation product that was physically and chemically purified to acceptably low concentrations of endotoxins and pyrogens and achieved physiologic stoichiometry of chond roitinase I and II that permitted human FDA IND approval of a pharmacologically purified and biologically active chondroitinase in 1994 [12]. Multiple attempts to develop recombinant forms of chondroitinase I and chondroitinase II were unsuccessful, which may have led to elevated production cost estimates and ultimate discontinuance of human testing after completion of the FDA phase I/II study. Difficulty or failure to purify enzymes

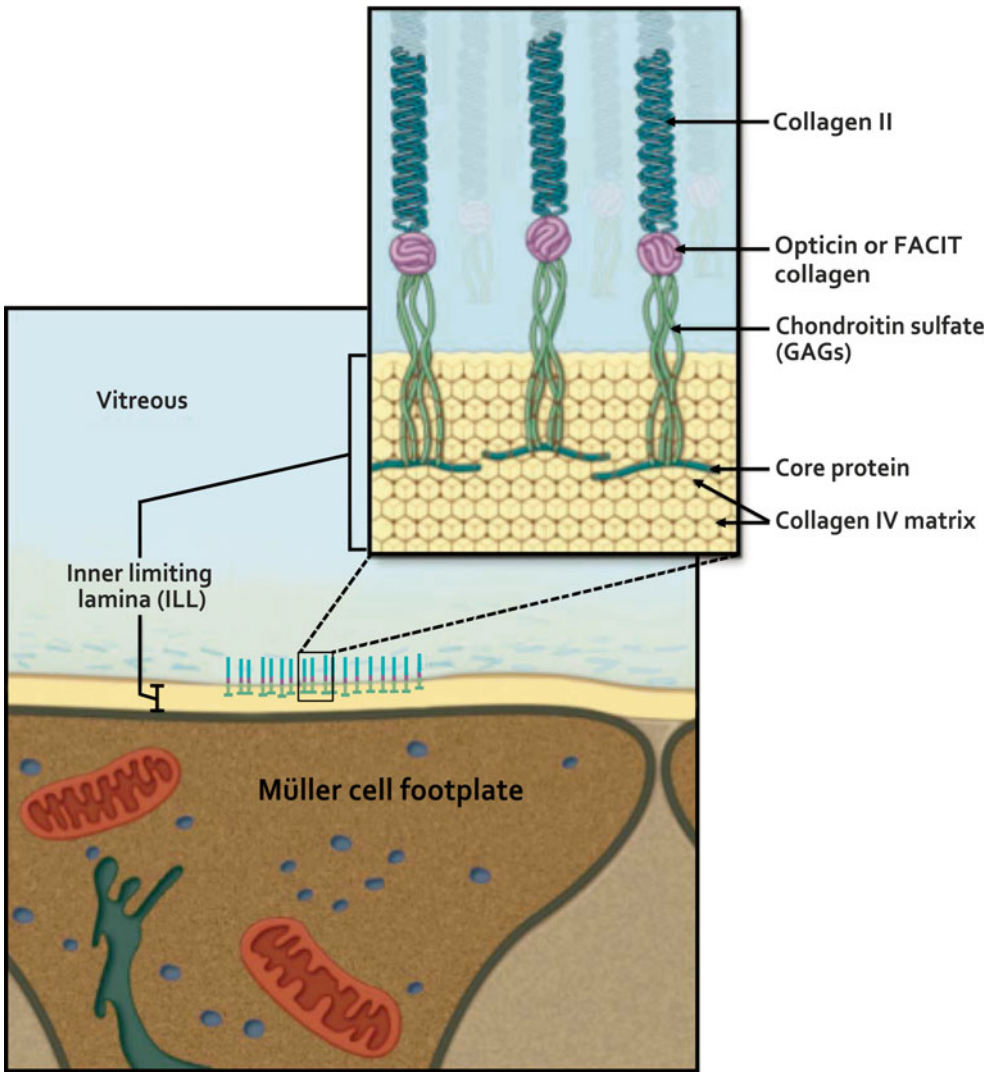

Figure VI.H-3 Schematic illustration of the human vitreoretinal interface. The inner limiting membrane (lamina) is represented by the yellow structure. Note the layer of chondroitin sulfate GAGs within the vitreoretinal interface. Cleavage or disruption of the saccharide layer (chondroitinase) or degradation of the fibrillar proteins or basement membrane (proteases) may result in weakening or separation of the vitreous from the inner limiting membrane of the retina (Reprinted from the journal *Retina*, reference [11], courtesy of Wolters Kluwer Health)

in an experimental setting commonly results in residual contamination by other enzymes that may have different substrates and/or activities. Chondroitinase contamination found in off-the-shelf enzyme formulations, which may be of adequate purity for biochemical purposes but insufficient for pharmacologic testing, has resulted in unexpected inflammation due to breakdown of unintended structures and/or reaction to degradation products [14].

III. Chondroitin Sulfate at the Vitreoretinal Interface

As previously mentioned, Dr. Greg Hageman and I began our collaboration in 1988 to evaluate the potential usefulness of chondroitinase for human application, a project which preceded the development of plasmin by years [11]. Preliminary survey studies of lectin binding on transverse primate globe sections revealed that fluorescein-conjugated peanut agglutinin or PNA, a marker for chondroitin sulfate linkages, localized to the vitreoretinal interface, posterior lens capsule, and surface of the optic disk (Figure VI.H-4) [11, 12, 15]. Further, the intensity of PNA binding and the width of binding by immunofluorescence microscopy corresponded to the strength or degree of vitreoretinal adhesion. For instance, the PNA binding was expressed as most intensely overlying the vitreous base region, less intensely adjacent to the optic disk and posterior lens capsule, and finely in a linear binding pattern to the majority of the remaining vitreoretinal interface (Figure VI.H-4). In human, primate, and pig eye sections exposed to chondroitinase, PNA binding was extinguished, and adhesion between the vitreous collagen network and the retina/optic nerve head/lens could be appreciated by separation with minimal effort or force [11, 12]. Comparison to other enzymatic degradation agents such as trypsin, hyaluronidase, dispase, heparinase, and others confirmed that the most complete loss of PNA binding signal and reduction in adhesion could be obtained with chondroitinase.

Follow-on studies demonstrated that exposure of the human vitreoretinal interface to chondroitinase resulted in complete disruption of the adhesion, a process we termed

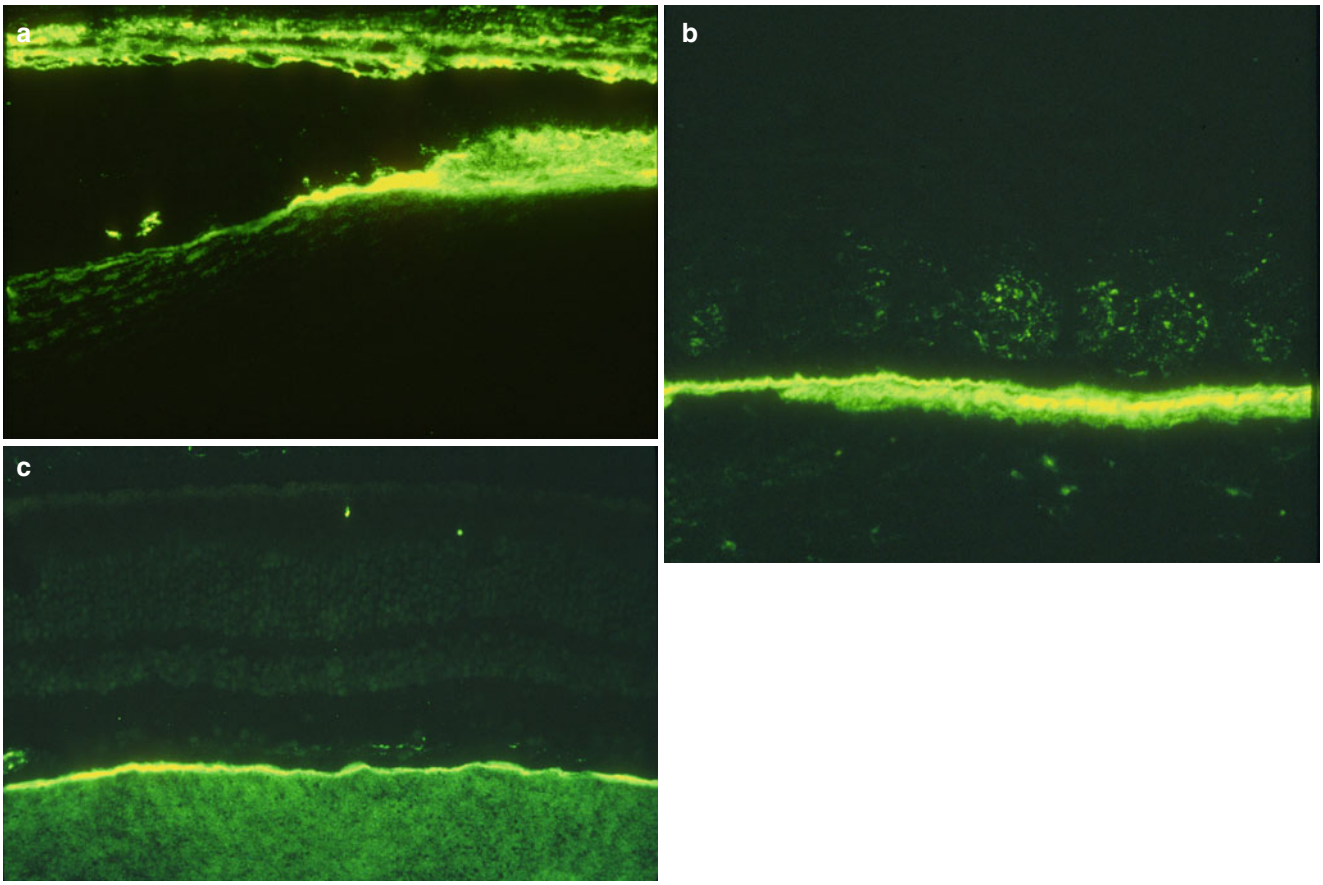

Figure VI.H-4 Epifluorescence microscopy of fluorescein-labeled anti-CS proteoglycan antibody binding at human vitreoretinal interface in retinopathy of prematurity. In this 17-week-old premature human donor, the localization of the proteoglycan core is demonstrated by the intensity and distribution of the antibody binding. Note the intense binding in the region of the vitreous base (**a**), intermediate density in the region of the inner optic disk (**b**), and lesser density in the other regions of the vitreo-retinal interface (**c**)

disinsertion. We argued for several weeks over what was the best term to use when describing this unique and characteristic effect of exposing chondroitinase to the human vitreoretinal, vitreopapillary, and vitreolenticular interfaces. Unlike known proteolytic enzymes, chondroitinase exposure resulted in complete, rather than incomplete, separation of the vitreous from the primate retina and did not liquefy the vitreous as might be suggested by the term "pharmacologic vitreolysis." The absence of vitreous liquefaction by chondroitinase has since been confirmed [16]. As illustrated in cynomolgus eyes during preclinical testing (Figure VI.H-5), "complete" disinsertion refers to the mechanical separation of the vitreous from the inner limiting membrane, optic disk, vitreous base, and posterior lens attachments. We refer to "incomplete" separation as effecting separation only in regions of relatively weaker adhesion that corresponds to regions of less PNA binding (i.e., posterior pole and optic disk). Phylogenetic analysis showed that complete vitreous cleavage or "disinsertion" was only observed in higher primates and pigs. Extensive testing of chondroitinase on mice, rats, and rabbits showed no effect on vitreous attachment strength [12].

IV. Chondroitinase Pharmacologic Vitreolysis

As mentioned above, Dr. Hageman and I published very few peer-reviewed publications on this topic from 1988 to 2012. However we did publish on related issues such as the generation of preretinal membranes that resulted from chondroitin sulfate injection into rabbit vitreous and the outcome of the control arm of one of our primate studies [15, 17, 18]. A few representative observations were reported by Sebag based upon interim discussions [13]. Since that first publication, Sebag proposed a classification system for pharmacologic vitreolysis agents based on biological activity [19]. Whereas he described chondroitinase as both a *liquefactant* (agent that liquefies the gel vitreous) and an *interfactant* (agent that induces vitreoretinal dehiscence), it is now clear that the former is not an effect of chondroitinase [16].

A. Preliminary Monkey Studies

After determining that lower primates and non-primates failed to show the same degree and type of response to chon-

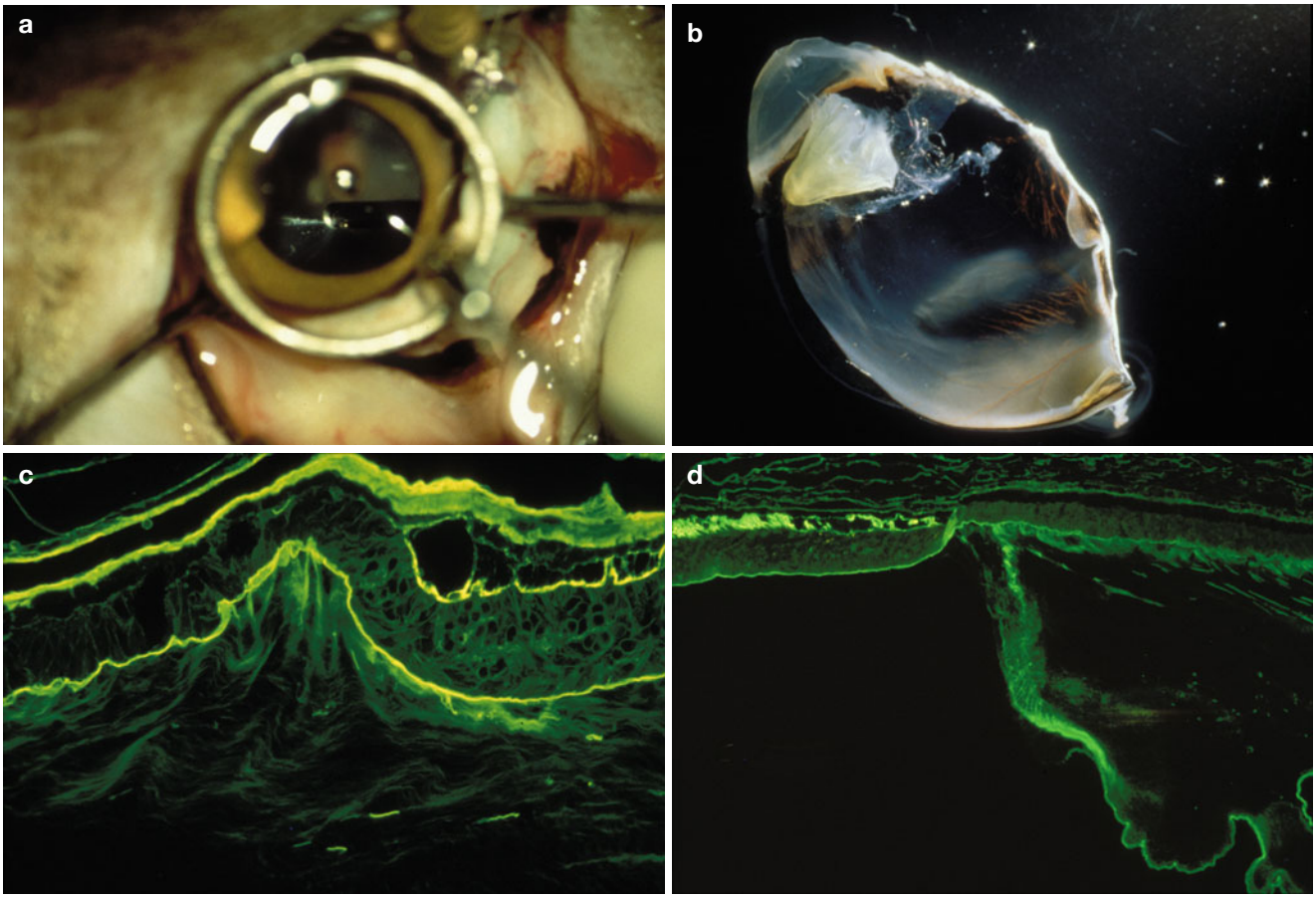

Figure VI.H-5 Disinsertion of vitreoretinal adhesion by chondroitinase in the cynomolgus monkey (**a**) Intraoperative photograph illustrates that traction applied to the illuminated vitreous following chondroitinase treatment reveals that the residual vitreous attachment is anterior to the crystalline lens. (**b**) The companion gross anatomical section depicts fixed visible vitreous (translucent white) unattached to the retina or ciliary body but maintaining connection to the zonules. (**c**) Epifluorescence microscopy of cynomolgus globe sections treated with fluorescein-linked PNA that bisects the ora serrata. In the left panel is a control untreated eye. In the right panel is a chondroitinase-treated eye. Under normal conditions the adhesion of the vitreous to the retina in this region is stronger than either the vitreous or retinal tissue. The separation of the vitreous from the retina in the region of the vitreous base supports the contention that all regions of constitutive vitreoretinal adhesion are mediated by the same, chondroitinase-sensitive mechanism (Reprinted from reference [11], courtesy of Wolters Kluwer Health)

droitinase as in higher primates, we completed a series of studies which evaluated the efficacy, safety, and toxicology of exposing cynomolgus and rhesus monkey vitreous to chondroitinase. Although these studies go beyond the scope of this chapter, an enumeration of a subset of these may be illustrative of the extent of preclinical data that were collected. We initially conducted a non-survival study on 6 animals to determine approximate concentrations and exposure times for effectiveness. We then performed a short- and long-term treatment trial (one eye treated, one eye control) to evaluate for efficacy and longer-term side effects. In a third study we evaluated whether chondroitinase was effective in facilitating the removal of preretinal membranes. These were generated in primates by vitreous injection of chondroitin sulfate GAGs, a technique we had demonstrated to successfully generate preretinal membranes in rabbits [17]. We also examined a number of human postmortem specimens to determine whether the chondroitinase-sensitive (constitutive) vitreoretinal mechanism was recapitulated between human preretinal membranes and the inner limiting membrane. We subsequently performed non-survival studies on a number of batches of recombinant chondroitinase. We evaluated suprathreshold doses in 12 animals for evidence of toxicity or inflammation. Once we determined that the Storz-produced incubation product would be used for clinical trial, we performed an extended short-term survival study utilizing radioactive enzyme to trace its metabolism and excretion.

However, because this chapter focuses on the effects of chondroitinase on the human vitreoretinal interface and because our primate testing of chondroitinase went well beyond the scope of this chapter, these data will be summarized and published elsewhere (Russell SR and Hageman GS, American Ophthalmological Society, thesis in preparation).

B. Human Proof-of-Concept Study

In 1992, Dr. Hageman and I undertook a key proof-of-concept human study. We decided that to accelerate our understanding of the value of chondroitinase, we needed to determine whether this agent could "disinsert" the vitreous *in vivo* in the human. Until then all of our testing *in vivo* had been on rhesus and cynomolgus monkeys, as described above. Since testing in living humans was impossible, as the agent had not received an FDA Investigational New Drug approval (IND), Dr. Hageman approached the Missouri Eye Bank and affiliated organ transplant collection agencies for access to brain-dead organ donors. Following delicate negotiations with donor groups and our institutional human subjects and ethics committees, we obtained permission to perform vitrectomy and place chondroitinase into two eyes of subjects who had been declared brain-dead and were undergoing multiple organ donation (liver, kidney, and heart harvest). Both subjects were young males (22 and 30 years old), unlikely to have spontaneous vitreous separation. One died from a motor vehicle accident and the other from a non-ocular gunshot wound. Fundus evaluations revealed normal fundi, retinal vessels, optic nerves, and peripheral retinal vessels in the gunshot victim. In the subject who succumbed to motor vehicle accident, the fundus examination revealed scattered intraretinal hemorrhages, judged to be a result of the trauma. The retinal vessels in this subject were otherwise normal as were the optic nerves.

Vitrectomy surgery was performed on one eye of each subject using a method similar to that used for the monkey and subsequent human studies. In the first study, a 500-unit dose of chondroitinase (pharmaceutical grade chondroitinase ABC, SeikagakuCorporation, Tokyo, Japan) was placed for a treatment period of 35–40 min. In the second study 600 units were used for a treatment period of 15–18 min. In each case, both the treated and untreated eyes were enucleated following the vitrectomy procedure and portions of the eyes were fixed for histology, immunohistochemistry, and transmission electron microscopy. In addition, the anterior segment was fixed for routine histologic evaluation of the lens, posterior lens capsule, cornea, iris, and ciliary body. Based on the impression of the surgeon and the gross observation of the enucleated eye, the vitreous appeared to be disinserted in the chondroitinase-treated eye. Histology, immunohistochemistry, and TEM confirmed that the vitreous was disinserted from the inner limiting membrane of the neural retina and the posterior lens in the chondroitinase-treated eye but not in the control eye (Figure VI.H-6). All other ocular structures (i.e., retina, RPE, optic nerve, ciliary body, lens, iris, and cornea) appeared normal in both eyes. These two experiments indicated that the human vitreous could be disinserted using chondroitinase and revealed no acute adverse effects resulting from chondroitinase-mediated surgery.

Analysis of the surgical procedures showed spontaneous separation of the posterior vitreous from the optic disk and retina. No posterior vitreous detachment was present in the untreated eyes of either brain-dead donor. Sectioning the treated eyes showed disinsertion of the vitreous from the anterior retina and posterior lens surface, with residual vitreous attached where zonules passed though the formed vitreous gel [see chapter IV.D. Physiology of accommodation & role of vitreous].

Light-level immunofluorescence microscopy demonstrated loss of PNA binding along the inner limiting membrane and within the vitreous gel. PNA binding was reduced but not extinguished over the pars plana and pars ciliaris. Transmission electron microscopy demonstrated that the ILM was intact and smooth, suggesting that no proteoglycan remnants remained attached to the ILM surface (Figure VI.H-6). The eyes from both brain-dead donors demonstrated similar features (although only those of the 22-year-old donor are shown in Figure VI.H-6). No inflammation was noted on light-level microscopy, immunofluorescence, or electron microscopy.

C. FDA Phase I/II Study and its Implications

Subsequent to FDA IND approval, twenty-five subjects enrolled in a registered FDA phase I/II dose-ranging trial conducted by the Chondroitinase Study Group (see Addendum 1) between August and December 1995. The study was sponsored by Storz (later American Cyanamid and subsequently American Home Products) and utilized the batch-produced chondroitinase derived and purified from a 1,000 l incubation of *P. vulgaris* on shark cartilage (see above) [12]. Subjects were assigned into one of three escalating-dose treatment groups, 500 units, 1,000 units, or 1,500 units (reconstituted in 300 μl of balanced salt solution, respectively). The starting dose of 500 units of chondroitinase was selected based upon the effectiveness of vitreous disinsertion in our human brain-dead study. For each of the three study sites, the indication for surgery and entry criteria for surgery differed.

Drs. Peter Campochiaro and Julia Haller (co-principal investigators) at Wilmer Eye Institute of Johns Hopkins University enrolled 10 subjects with stage II to IV full-thickness macular hole (Protocol DP-123-1). Dr. Jerry Sebag at the VMR Institute in Huntington Beach enrolled 6 subjects with proliferative diabetic retinopathy, macular edema, and vitreomacular traction (Protocol DP-123-2). Dr. Paul Sternberg at Emory University enrolled 9 subjects with proliferative diabetic retinopathy and macular tractional retinal detachment with or without vitreous hemorrhage (Protocol DP-123-2). Subjects were followed up to 1 year. Summary data are shown in Table VI.H-1.

Subjects ranged in age from 36 to 81 years old and included 16 women and 9 men. Within the trial, one subject died of a stroke, unrelated causally or temporally to chon-

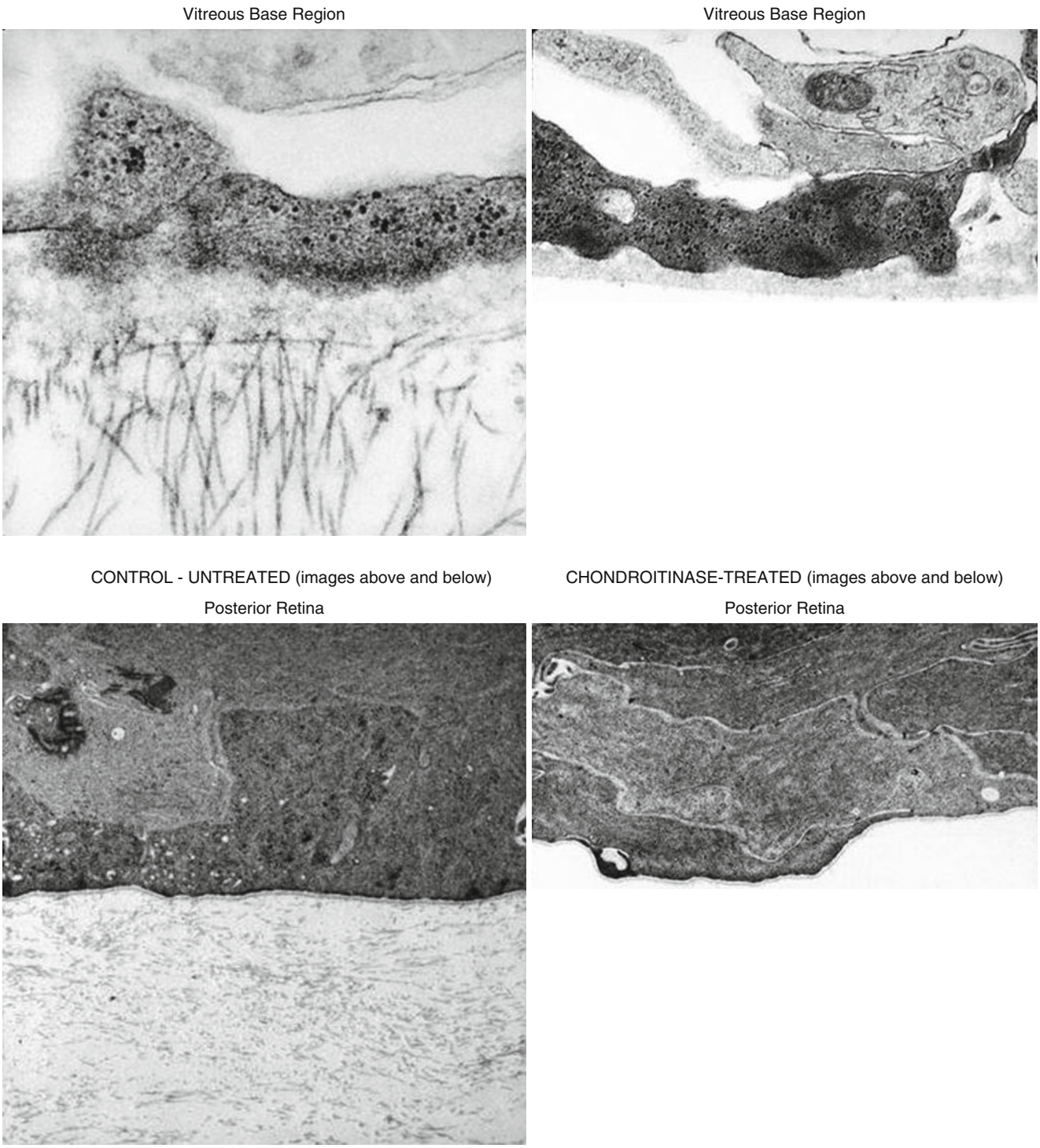

Figure VI.H-6 Transmission electron micrographs (TEMs) of chondroitinase-treated and control eyes from brain-dead human study. Eyes were fixed in half-strength Karnovsky fixative, pH 7.4 for 2–4 h followed by secondary fixation with 2.0 % osmium tetroxide for 1–2 h. Sections were imaged on a JEOL 1200EX TEM. In this subject, 600 units of chondroitinase were applied for 15–18 min. In left panels, vitreous fibrils can be seen to insert or attach to the inner limiting membrane (ILM) or basement membrane of the retina (Muller cells). In the right panels, the vitreous fibrils have been entirely disinserted without evidence of residual fibrillar attachments. Ultrastructurally, the ILM is undisrupted. Note the complete separation of the vitreous fibrils from the ILM in the region of the vitreous base, a region in which vitreoretinal adhesive strength exceeds the strength of either the ILM or vitreous fibrils [20]

Table VI.H-1 Summary of FDA phase I/II chondroitinase-treated subjects

Subject #	Indication	(Stage)	Dose	Age/ gender	Pre op VA	Op time	Resid vit att	PVD ease	MP ease	MP ease NV	MP ease NoV	Comment	Post op VA	Late compl
1	PDR OS	TRD	500 u	50 F	1/200	3:30	Ant to equ	Easy	Difficult	Difficult	Easy	Drug assisted vitreous removal but not membrane peel	20/100	Stroke unrelated to drug
2	PDR OS	TRD	500 u	46 F	2/200	2:00	Ant to equ	Easy	Difficult	Difficult	Easy	No drug effect noted	20/63	Pneumonia unrelated to drug
3	PDR OD	VH	500 u	62 F	20/HM	2:30	Ant to equ	Easy	Easy	Easy	Easy	No drug effect noted	20/32	None
4	PDR OS	VH	1,000 u	55 M	CF	2:36	NA	Easy	Difficult	Difficult	Easy	Drug facilitated complex procedure	20/63	None
5	PDR OS	VH/ TRD	1,000 u	36 M	20/100	1:35	Equ	Easy	Difficult	Easy	Difficult	Equivocal effect of drug	20/16	None
6	PDR OD	TRD	1,000 u	44 F	20/HM	1:05	NA	Easy	Easy	Easy	Easy	Felt vitreous largely separated preop	20/100	None
7	PDR OS	VH/ TRD	1,500 u	36 F	20/200	1:38	NA	Easy	Difficult	Difficult	Difficult	Drug did not facilitate surgery	20/25	None
8	PDR OS	VH	1,500 u	73 F	5/200	1:08	Ant to equ	Easy	Easy	Easy	Easy	Minimal premacular membrane and vitreous attachments	20/100	None
9	PDR OD	T/RRD	1,500 u	61 M	6/200	2:02	Ant to equ	Easy	Easy	Easy	Easy	Drug may have effect, membranes peeled easily	20/100	None
10	FTMH OD	Stage III	500 u	78 F	20/70	2:05	Ant to equ	Easy				Posterior vitreous cortex came up within 30 secs of aspiration	20/40	None
11	FTMH OS	Stage III	500 u	62 F	20/100	1:35	Ant to equ	Easy				Easy as any with normal aspiration	20/30	None
12	FTMH OS	Stage III	500 u	75 M	20/70	1:13	Ant to equ	Easy				Spontaneous vitreous separation to equator	20/25	None
13	FTMH OD	Stage III	500 u	69 F	20/80	1:27	Ant to equ	Easy				Lens opacity, cleared post op	20/40	None, mild lens feathering post op
14	FTMH OS	Stage IV	1,000 u	63 F	20/400	1:30	Ant to equ	Easy				Posterior vitreous cortex separated within 5 sec of aspiration	20/40	None
15	FTMH OS	Stage II	1,000 u	68 M	20/50	1:20	Equator	Difficult				No effect noted by surgeon	20/25	None
16	FTMH OS	Stage III	1,000 u	66 F	20/160	2:25	Ant to equ	Unable				Temporal limited choroidal hemorrhage	20/63	RD repaired with SBP
17	FTMH OS	Stage III	500 u	81 F	20/80	2:16	Ant to equ	Easy				Posterior vitreous cortex separated within 5 sec of aspiration	20/20	None

Subject #	Indication	(Stage)	Dose	Age/ gender	Pre op VA	Op time	Resid vit att	PVD ease	MP ease	MP ease NV	MP ease NoV	Comment	Post op VA	Late compl
18	FTMH OD	Stage III	1,500 u	65 F	20/400	1:20	Ant to equ	Easy				No perceived effect by surgeon	20/400	hole remained open
19	FTMH OS	Stage III	1,500 u	37 F	20/100	1:38	Ant to equ	Easy				Bubble came around lens into anterior chamber	20/40	None
20	PDR OS		1,000 u	64 M	20/100	1:05	NA	Easy	NA	NA	NA		20/80	None
21	PDR OD		1,000 u	74 M	CF	1:30	NA	Easy	Easy	Difficult	Easy	Tears near sclerotomy in pars plana	20/200	MI 4 months post op
22	PDR OD		1,000 u	34 M	20/100	1:15	PP	Difficult	Difficult	Difficult	Difficult	Agent did not alter surgery as usual	20/30	Periph ring RD
23	PDR OS		1,500 u	54 F	CF 5'	1:45	NA	Easy	NA	Easy	Easy	Vitreous gel remained formed	HM	VH last visit
24	PDR OS		1,500 u	75 F	CF	1:15	NA	Difficult	NA	NA	NA	Agent did not alter surgery as usual	20/70	None
25	PDR OS		1,500 u	52 M	CF	1:15	Ant to equ	Easy	Difficult	Difficult	Easy	Agent did not alter surgery as usual	CF	None

droitinase treatment. One subject withdrew with nonsurgically related pneumonia. Two of 25 subjects developed retinal detachment (one a peripheral "ring" detachment outside of the peripheral scatter photocoagulation and one successfully repaired with a scleral buckle). One macular hole failed to close in an eye in which the surgeon did not appreciate any chondroitinase effects. Other complications included limited choroidal hemorrhage (subject 16), transitory crystalline lens opacification (subject 13), intraretinal hemorrhage (subjects 2, 4, 7, 22, and 25), and retinal tears (subjects 1, 3, 4, 7, and 21), although most hemorrhages and tears developed in subjects that underwent membrane peeling for proliferative diabetic retinopathy.

1. Efficacy

Vision improved in 23 of 25 subjects but remained stable at counting fingers in one subject and was reduced from counting fingers to hand motions in one subject (due to non-clearing vitreous hemorrhage, patient lost to follow-up). Because this was an unmasked trial, the effectiveness of chondroitinase was assessed by the ease of vitreous separation from the retina (iatrogenic or surgical posterior vitreous detachment) and ease of delaminating nonvascularized preretinal membranes. In 21 subjects vitreous separation was assessed by the operating surgeon as easy and in three as difficult. In one subject who developed a temporal choroidal hemorrhage, the macular hole was later found to be closed and vision improved to 20/25 (Subject 16). In seven subjects the operating surgeon could not determine the location of the anterior insertion of the vitreous. In two subjects the residual vitreous was inserted at the equator and in 16 subjects vitreous was inserted anterior to the equator or pars plana (complete disinsertion including vitreous base, similar to Figure VI.H-4, top left panel).

a. Proliferative Diabetic Retinopathy

Among the 15 subjects with proliferative diabetic retinopathy, in whom retinal neovascularization could confound recognition or effectiveness of chondroitinase application, the operating surgeon reported that surgical separation of the vitreous from the retina was easy. In only 2 cases was this process described as difficult. However, when peeling preretinal membranes, only 5 were described as easy and 7 were difficult (as might be anticipated).

b. Macular Hole

The effects of chondroitinase were most easily recognized in the macular hole group. Of the 9 subjects in whom vitreous separation could be assessed, 8 were recognized as easy, and in 4 of these, the vitreous separated within 5 to 30 s of gentle aspiration. The vitreous was found to be inserted anterior to the equator (i.e., residual attachment to zonules) in 9 of 10 subjects. In one phakic patient intraocular gas migrated around the crystalline lens, which did not affect long-term visual outcome (20/40).

2. Safety

Electroretinograms (ERGs) were obtained from 23 subjects. Two subjects with normal pre-chondroitinase treatment ERGs refused posttreatment ERGs. In one subject, the posttreatment ERG improved but remained abnormal. In the remaining subjects (12 with abnormal and 8 with normal pretreatment ERGs), the posttreatment ERGs remained unchanged.

Conclusions

Chondroitinase remains an intriguing agent as an adjunct in the vitreoretinal surgeons' armamentarium. Even after extensive testing, the limits of use and potential benefits remain incompletely determined. Development of recombinant or altered enzyme may prove more potent and appealing than our current offerings for "vitreolysis" given the demonstrated potential of chondroitinase to disinsert the vitreous from the retina especially in the region of the vitreous base and posterior lens capsule.

Recent regulatory and marketing developments continue to highlight the attraction of pharmacological separation of the loose connective tissue within the eye, the vitreous, from its adjacent basement membranous attachments of the optic disk, retina, uvea, and lens capsule. Controlling or restricting enzymatic activity in topography, degree of cleavage, and effect on surrounding tissues will likely require further assessment of the expected and potential unanticipated effects of pharmacologically breaking these and similar physiological attachments.

Abbreviations

AC	Anterior chamber
Asp	Aspiration
CF	Count fingers
CS	Chondroitin sulfate
Complic	Complication
ECM	Extracellular matrix
equ	Equator
ERG	Electroretinography
F	Female
FDA	US Food and Drug Administration
FTMH	Full-thickness macular hole
GAG	Glycosaminoglycans
hemor	Hemorrhage
HM	Hand motions
ILM	Inner limiting membrane
IND	Investigational new drug
kDa	KiloDalton
M	Male
MI	Myocardial infarction
OD	Right eye

Op Time	Operative time
OS	Left eye
PDR	Proliferative diabetic retinopathy
periph	Peripheral
PNA	Peanut agglutinin
post op	Postoperative
PP	Pars plana
Pre Op	Preoperative
PVD	Posterior vitreous detachment
RD	Retinal detachment
ResidVitAtt	Residual vitreous attachment location
RRD	Rhegmatogenous retinal detachment
SBP	Scleral buckle procedure
sec	Seconds
TEM	Transmission electron microscopy
TRD	Traction retinal detachment
u	Units
VA	Visual acuity
VH	Vitreous hemorrhage
vit	Vitreous

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