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Enzymatic-assisted vitrectomy

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Abstract

Purpose **This paper discusses the approach of enzymatic vitrectomy and potential applications.**
Methods **A description of available agents for enzymatic vitreous surgery will be given and the techniques that have been suggested.**
Results **Both animal and human results will be presented in this article regarding trials of enzymatic vitreous surgery.**
Conclusion **Enzymatic vitreous surgery may be a useful adjunct or additional agent to treat several vitreoretinal diseases.**

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Introduction

There are many reasons to pursue enzymatic-assisted vitreous surgery. The common goal of such surgery is to manipulate the vitreous collagen, both centrally achieving liquefaction, as well as along the vitreoretinal surface to be able to achieve a cleavage plane cleaner than can be mechanically achieved currently. Hopefully, this would allow us the ability to manage some retinal diseases that are currently managed in the operating room with mechanical manipulation by pharmacologic technique or even in an office setting. Those eyes that have a more difficult clinical course currently may be able to achieve better anatomic results by the use of pharmacologic manipulation of the vitreous. Some of these more difficult eyes are thought of conceptually as having incomplete removal of vitreous collagen from the anterior retinal surface, or vitreous schisis, which has been described particularly in diabetic eyes.

Materials and methods

Potential enzymatic candidates

Although many enzymes have been used over the years in ocular surgery, alpha-chymotrypsin is perhaps the most common example having been used to digest zonules in the past to make intracapsular cataract surgery safer. Wydase also has been used in retrobulbar injections as a spreading agent for anesthetic throughout the orbit. Enzymes have been suggested as adjunctive therapy to vitreous surgery and have included chondroitinase, hyaluronidase, dispase, and plasmin enzyme. In addition, other agents changing the osmolarity of the vitreous in the past have also been suggested to be able to manipulate the vitreous and create either more liquefied vitreous or perhaps even posterior-vitreous separation. The issues of enzymatic assembly must be considered a recombinant *vs* autologous enzymatic agents. In addition, agents that activate endogenous enzymes have been considered such as tissue plasminogen activator. All of these biochemical pathways have as their common goal to potentially manipulate either central vitreous collagen or the vitreoretinal interface.[1–6] The enzymes that seem to be most likely to achieve the clinical goals of either vitreous liquefaction or manipulation of the vitreoretinal juncture seem to be either hyaluronidase (the trade name Vitrase) to cause liquefaction of the central vitreous, or plasmin enzyme to manipulate vitreous collagen, which seems to be able, in a dose-dependent fashion, to liquefy the vitreous, but, in addition to act on the laminin and fibronective glue of the vitreoretinal juncture. The role of other agents such as tissue plasminogen activator (tPA) has worked through a similar pathway as plasmin enzyme by activating endogenous plasminogen. The endogenous plasminogen then converts to plasmin enzyme and works on the vitreoretinal juncture. Vitrase is currently in clinical trials and plasmin enzyme will soon

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begin clinical trials having completed the pre-clinical data and human pilot studies in macular holes and diabetic retinopathy.

Background of enzymatic manipulation of the vitreous

Both experimental studies and clinical observations confirm the central role of the vitreous in the pathobiology of ocular trauma as well as many other ocular diseases.[1–6] This suggests a potentially beneficial effect of vitrectomy in many of these diseases. The current management includes mechanical vitrectomy for many of these diseases as well as dissection along the vitreoretinal interface. This dissection can be quite arduous and can result in retinal damage. For this reason, people have sought agents to reduce the risk of mechanical injury to the anterior retinal surface. These techniques as well as techniques to liquefy the vitreous have been discussed for many years including the use of previous enzymatic agents such as alpha-chymotrypsin and its effect on zonular protein. The search for an appropriate or multiple enzymatic agents to perform manipulation of vitreous collagen continues today and several candidates currently are available.

Chondroitinase A 240-kDa chrondroitin sulfate proteoglycan is associated with the vitreoretinal interface.[7] The greatest immunoreactivity of this proteoglycan is at the vitreous base and the optic nerve, suggesting a role in vitreoretinal adhesion. Chondroitinase lyses this proteoglycan and has been studied as an adjunct in vitrectomy. In cynomolgus monkeys and human organ donors, intravitreal injections of chondroitinase separated the vitreous from the retina without damage to the ILM. Chondroitinase has been studied in phase I human trials, but no results have been yet reported. Chondroitinase appears to be a promising agent for pharmacological manipulation of the vitreous.

Hyaluronidase Hyaluronidase (Vitrase) has been suggested as an agent to liquefy the central vitreous acting on the hyaluronan, which is a very large component of the vitreous body. This liquefaction may lend itself to more rapid clearing of the vitreous cavity. The liquefaction may also contribute to the formation of a posterior vitreous separation over a period of time. It appears that the effect of liquefaction and vitreous hemorrhage by hyaluronidase, trade name Vitrase, may take weeks to months to see its final effect.

Plasmin enzyme The use of plasmin enzyme for the induction of vitreoretinal separation derives from the observation that plasmin, a non-specific protease, acts on laminin and fibronectin, which to a large extent comprises the biologic 'glue' that holds the vitreous to the retina. This was originally tested in a rabbit model. In the rabbit model, it was shown that a reliable posterior vitreous separation could be achieved with a very clear anterior retinal surface demonstrated by scanning electron microscopy following injection of enzyme and irrigation of the vitreous cavity without any manipulation along the anterior retinal surface. In addition, no evidence by electroretinogram of retinal toxicity was seen.[8] Recent animal studies have shown that doses as high as 3 IU of autologous plasmin enzyme do not result in electroretinographic evidence of toxicity.[9] The activity curves show plasmin enzyme to reach its peak activity in 15–30 min and to remain at this peak for an hour and a half. This activity then falls off over the next several hours to an immeasurable level.[8] The animal studies have been confirmed at multiple sites and stimulated the desire to pursue human trials.[9,10]

Results

Human trials

Human trials have been conducted on chondroitinase, Vitrase, and plasmin enzyme, as well as the use of intraocular injection of t-PA to stimulate endogenous plasminogen and create the effects of plasmin enzyme based on the endogenous plasminogen load.[11]

Chondroitinase Human trials with chondroitinase were curtailed without data being released. Studies were presented that showed an effective liquefaction and posterior vitreous separation in cadaver eyes, which had chondroitinase injected into the mid vitreous cavity. Human trials of diabetic retinopathy eyes and macular holes have never been released to allow complete analysis, but theoretically, chondroitinase could act on the chondroitin sulfate of the vitreoretinal juncture and lead to a spontaneous or more easy to peel vitreoretinal juncture.

Hyaluronidase Hyaluronidase has been tested in a clinical trial and currently is in Phase 3 testing seeking FDA approval. The hyaluronidase is a sheep product which injected into the mid vitreous cavity is felt to liquefy the vitreous gel. The study population is what is described as dense vitreous hemorrhages that, due to liquefaction of the vitreous, the manufacturers feel may settle sooner and allow photocoagulation to treat

the underlying proliferative diabetic retinopathy. These trials have not come to conclusion and have proceeded though to Phase 3 clinical trials, so soon results of these trials may be available. Some reports of hypopyon have been concerned with the use of Vitrase; however, these hypopyons appear to spontaneously resolve. Initially, these were reported to be present in a third of patients treated with hyaluronidase. In addition, other reports have focused attention on the possibility of using hyaluronidase to induce a posterior vitreous separation. These reports are still pilot type studies and have not yet undergone the rigors of a controlled clinical trial.

Plasmin enzyme Plasmin enzyme has been reported in cadaver eyes by Kampik and co-workers who showed a posterior vitreous separation testing both 1 and 2 IU of plasmin enzyme. The cadaver eyes did show liquefaction and posterior vitreous separation to be present.[10] In addition, human pilot trials in traumatic macular holes, macular holes, and in diabetic eyes have been reported. In these eyes, the enzyme has been shown to allow the vitreous gel to be easier to peel than expected or spontaneously separated in the majority of eyes. The use of plasmin enzyme in diabetics is also based on its ability to degrade fibrin and allow more easy access to the space between the epiretinal membrane and neurosensory retina, which sometimes can be very atrophic, and biochemically remove the last layer of vitreous due to vitreoschisis.[12,13] The dose of plasmin enzyme has ranged in these studies from 0.4 to 0.8 IU. Dosing studies, that tested up to 3 IU, have revealed that very large amounts of plasmin enzyme do not seem to show clinical or histologic toxicity.[14] The liquefaction of vitreous gel by plasmin enzyme is based on its activity on collagenases. Fortunately, plasmin enzyme spares type 4 collagenase, leaving the internal limiting lamina intact, but causing liquefaction in a dose-dependent fashion in the central vitreous cavity. The dose of 0.8 IU plasmin enzyme appears to be very helpful in liquefying vitreous in the human eye. This combination of loosening or leading to spontaneous posterior vitreous separation in eyes, as well as liquefaction of the vitreous gel, suggests many possible applications for this enzymatic intervention.[15–17]

Discussion

The applications of enzymatic vitreous surgery are numerous. The immediate application was felt to be an adjunctive effect of enzymes on vitreous surgery leading to an easier and perhaps safer peeling of the posterior hyaloid along the anterior retinal surface. This safer and perhaps more complete peeling might lend itself to less residual collagen on the anterior retinal surface and therefore less tractional effect on the underlying neurosensory retina.[11] This seemed to be true in the diabetic eyes with macular edema treated with autologous plasmin enzyme that appeared to achieve their best visual acuities more rapidly than eyes with similar clinical findings receiving conventional vitreous surgery.

In addition, the use of enzymatic adjuncts to manipulate vitreous collagen may allow vitrectomy to be performed with smaller instrumentation. This small instrumentation may not require conjunctival incision and with this absence of conjunctival manipulation and suturing, it may be that some of these vitreous surgery techniques may be able to be performed in an office setting. Currently, 25-gauge instrumentation is gaining in popularity. The 25-gauge instruments however, even with high levels of suction and high cutting rates, still have limitations due to the resistance of more viscous vitreous collagen. The liquefaction action of many of the enzymatic candidates may be an aid in making 25-gauge instrumentation usable both in the operating room and in the office setting. This may allow us to perform some vitreous surgery techniques in a fashion similar to a fluid–gas exchange in a vitrectomized eye. The other applications of enzymatic manipulation of the vitreous cavity include the effects on matrix metalloproteinases. Plasmin enzyme plays a role in the manipulation of MMPs 2 and 9. These particular MMPs have been implicated in the process of diabetic retinopathy. In addition, any agent that causes a posterior vitreous separation may contribute to a mechanical factor involved in reducing progression of diabetic retinopathy. This mechanical feature of posterior vitreous separation accompanied by biochemical manipulation of VEGF or addition of VEGF inhibitors may lead to a combined biochemical and mechanical control of diabetic retinopathy. In addition, other vitreoretinal tractional processes, such as vitreomacular traction syndrome and macular holes of stage 1, may become enzymatic vitreous surgery candidates with liquefaction of the vitreous and cleavage of the vitreoretinal juncture, allowing these to be treated by injection alone.

In conclusion, the future of enzymatic manipulation of the vitreous collagen and indeed vitreous cavity biochemistry may allow us a new mechanical and biochemical way of managing vitreoretinal diseases that today consistently require surgical intervention. Even diseases such as rhegmatogenous retinal detachment with obvious vitreoretinal traction might possibly be able to be cleaved enzymatically and treated effectively by a pneumatic type technique

without the fear of additional areas of vitreoretinal traction and formation of other retinal breaks. Certainly, there is a great deal to be learned about the manipulation of vitreoretinal collagen and the biochemistry of the vitreous cavity.

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