Asteroid hyalosis: pathogenesis and prospects for prevention

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Abstract

Asteroid hyalosis (AH) is a common degenerative process in which fatty calcium globules collect within the vitreous humour. The condition rarely causes visual disturbances, and surgical removal is only rarely required. The presence of AH has been associated with systemic diseases such as diabetes; however, research in this area has been hampered by the lack of an animal model of AH. Recently, we have reported that AH occurs in galactose-fed beagles that develop the advanced stages of diabetes-like retinopathy. Comparisons of vitreous humour containing asteroid bodies (ABs) collected from these galactose-fed beagles and vitreous samples from age-matched normal beagles without ABs indicate that ABs contain calcium and phosphorous. Subtraction analysis of chloroform extracts of the vitreous samples by electrospray mass spectroscopy resulted in the identification of the quasimolecular ion of 1,2-dipalmitoyl-glycero-3-phosphoethanolamine (DPPE) as the main component of ABs. Since several reports indicate that ABs are composed of lipid–calcium complexes, we have proposed that the main component of ABs in the galactose-fed dogs with AH is a quasimolecular ion of DPPE in which two molecules of DPPE are complexed through their phosphates groups with calcium. We suggest that these lipid components diffuse into the vitreous from the degenerating retinas of these dogs.

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Introduction

The vitreous is a highly hydrated gel-like substance that provides structural support to

the eyeball while providing a clear unobstructed path for light to reach the retina. In addition, it hinders the forward diffusion of oxygen from the retinal blood supply to the anterior segment of the eye where it can cause oxidation damage to the lens. The vitreous is an extracellular matrix that is composed of collagen fibrils with the carbohydrate polymer hyaluronan (hyaluronic acid) serving as the major component between the fibrils.¹ With age, it may partially liquefy as a result of hyaluronan breakdown associated with oxidative stress.^{2,3} Once liquefied or surgically removed, the vitreous does not reform significantly.

Opacities may form in the clear vitreous as a result of developmental abnormalities, injury, or disease. Developmental opacities present from birth are the remnants of the hyaloid system that are located in Cloquet's canal, whereas degenerative opacities, associated with inflammatory debris, often appear after ocular inflammation, ocular haemorrhage, posterior vitreous detachment, or vitreous haemorrhage. Ocular trauma or inflammation may also result in the accumulation of glittering cholesterol crystals in the vitreous humour along with the anterior chamber and subretinal space (synchysis scintillans). However, the most common clinically observed degenerative opacity of the vitreous is asteroid hyalosis (AH). Accurately differentiated from synchysis scintillans in the late 1800s, it was first called asteroid hyalitis; however, the name was changed in the 1960s to AH because of the absence of inflammatory changes. AH is characterized by spherical white bodies that move along with the vitreous humour when the eye moves. They are present unilaterally in approximately 75% of patients and are more predominant in men than women unilaterally.^{4–6} Although their presence is obvious upon ocular examination and in severe cases make retinal examination difficult, patients are usually unaware of AH because the asteroid bodies

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(ABs) rarely cause visual disturbances. Surgical removal of these opacities is usually not required unless AH compromises the patient's vision.

Pathogenesis of AH

Although AH has been associated with several systemic diseases, the cause(s) and mechanism(s) of AH formation remain unknown. Three major studies conclude that AH is primarily associated with ageing. In the Beaver Dam Study of Wisconsin where 4952 patients were examined, AH was present in 1.2% of the population with prevalence increasing from 0.2% in subjects 43–54 years of age to 2.9% in those aged 75–86 years.⁶ AH was primarily unilateral and was more prevalent in men. The Australian Blue Mountains Eye Study of 3654 patients aged 49–97 years confirmed a similar 1% prevalence of AH.⁵ In this group, the prevalence of AH also increased with age from 0% of persons 49–55 years of age to 2.1% of persons aged 75–97 years, and AH was again more prevalent in men. A higher, 1.96%, prevalence of AH was observed in the third study from the University of California at Los Angeles (UCLA) autopsy eye database from 1965 to 2000 where 10 801 patients were examined.⁴ This retrospective study, which demographically matched the characteristics of the US population on the 2000 census, also showed an inverse relationship between AH and posterior vitreous detachment.

Although these three major studies found AH to primarily correlate with ageing, others suggest that AH is associated with diabetes mellitus (DM),^{7–16} hypertension,^{14,17} hypercholesterolaemia or lipidemia,^{7,15,16} or increased serum calcium levels.^{18,19} Of these, a link to DM is most often cited; however, this link is also the most controversial. Although AH predominantly occurs unilaterally,^{4–6} AH should be bilateral if linked to systemic diseases such as DM because each eye is equally exposed to hyperglycaemia. Bilateral 'trends' of AH have been suggested among diabetic populations¹¹ and the UCLA autopsy study,⁴ which observed a statistical association between diabetes and bilateral AH before adjusting the data for age and sex. However, in the UCLA study, AH was present only in 20 of 780 diabetics. AH formation in diabetics has been suggested to be secondary to retinal vascular changes.¹⁰ The overall relationship between DM and AH is further complicated by data suggesting that AH may arrest the process of vitreous collapse or contraction in eyes with proliferative diabetic retinopathy.^{8,20}

Structure and composition of ABs

The structure and composition of ABs is currently unknown. Studies to date have utilized clinically

obtained human AH. However, the small size and amounts of ABs available for study, along with their strong adherence to vitreous humour, have limited applications to histological and microscopic techniques. Histological studies indicate that unlike the vitreous humour, ABs readily stain for the presence of lipids (Sudan black B and oil red O) and calcium (von Kossa). However, ABs do not dissolve in common fat solvents. This led Verhoeff²¹ in the early 1920s to postulate that ABs are calcium soaps with calcium primarily bound to the fatty acid carboxylate groups. Forty years later, Rodman *et al*²² confirmed that calcium is bound to the main acidic lipid components of ABs. However, its behaviour is not consistent with fatty acid calcium salts because the ABs did not dissolve in glacial acetic acid or 0.8% HCl but did dissolve in methanolic HCl. Moreover, two types of AH were observed. In the first, Type I AH, the ABs were suspended in and surrounded by vitreous fibrils. The ABs appeared composed of two zones, a large inner zone filled with birefringent particles and a thin cortical rim composed of condensed nonbirefringent vitreous particles. In the second, Type II AH, the ABs were surrounded by cellular elements and this whole complex was enmeshed with vitreous fibrils or inflammatory tissue. The appearance of these two types of ABs suggest potential differences in their pathogenic mechanism(s); however, both types have been documented to be present in the same patient.¹² Moreover, despite the apparent link with inflammatory tissues, uveitis is not associated with AH.

In the 1970s, analyses of ABs by X-ray microanalyser revealed that phosphate, in addition to calcium, is present as a major element.²³ This suggests that calcium is present in ABs in the form of a calcium phosphate complex (apatite) rather than as fatty acid calcium salts. Electron microscopy revealed the presence of 'satellite' ABs composed of a number of round particles of equal size and in symmetrical arrangement. These particles appeared embedded in a matrix, which merged with the surrounding vitreous. These observations led March and Shoch²³ to minimize the lipid character of ABs and to suggest that ABs may simply be a physicochemical phenomenon or shift of a part of the normal vitreous from the liquid to a solid phase. In this process, 'satellite' asteroids are initially crystallized from 'seeds' formed in the supersaturated vitreous. These 'satellites' then conglomerate in the solid phase to form the larger, experimentally observed ABs. The basic processes leading to this hypothetical supersaturated solution were not identified.

In contrast to the suggestion that ABs are derived from the vitreous, which is naturally low in lipids, Streeten²⁴ reported in 1982 that ABs must be derived from sources exogenous to the vitreous. This was based on

observations that large quantities of complex lipids and calcium are present in ABs. He reported that larger ABs lying in a homogeneous background matrix are composed of intertwined ribbons of multilaminar membranes with a 6-nm periodicity characteristic of complex lipids, especially phospholipids. Interspersed throughout the ABs are clumps of dense material with swirls of thin membranous strands often surrounding them. The structures of the smaller ABs suggest that the lipid ribbons result from the deposition of successive lipid layers on thin membranes so that various degrees of lipid were contained. In ABs of all sizes, energy-dispersive X-ray analysis indicated that calcium and phosphorus were the main elements, and electron-diffraction structural analysis indicated that calcium hydroxyapatite and possibly other forms of calcium phosphate crystals are present in the focal densities of the ABs. Others using electron microscopy have subsequently reported that ABs contain intertwined ribbons of multilaminar membranes with a periodicity (10–60 Å) that is characteristic of complex lipids, especially phospholipids, along with calcium and phosphate.^{12,25} Miller *et al*²⁶ suggested that ABs are not true crystals but rather liquid crystals of phospholipids in the vitreous humour. This was based on their ability under appropriate conditions of fixation and high-resolution transmission electron microscopy to observe that ABs had a lamellar arrangement with a periodicity of 4.6 nm, which is typical of liquid crystalline phases of lipids in water.

After another nearly 20-year lapse, two recent publications provide additional evidence for the apatite-like nature of ABs.^{27,28} Using element mapping with electron spectroscopic imaging, Winkler and Lunsdorf²⁷ in 2001 demonstrated that calcium, phosphorus, and oxygen are homogeneously distributed in ABs and that the electron energy loss spectra of these elements shows details similar to those found for hydroxyapatite. Moreover, they found that ABs were time dependent and degraded in the presence of the calcium-specific chelator Na-EGTA. Winkler and Lunsdorf²⁷ also observed by immunofluorescence that chondroitin-6-sulphate is present at the periphery of ABs and that part of the inner matrix of ABs is composed of carbohydrates specific for hyaluronic acid.

Komatsu *et al*²⁸ in 2003 confirmed the apatite-like structure of ABs by comparing the surface patterns of artificial hydroxyapatite produced *in vitro* with simulated body fluid containing Na⁺, K⁺, Ca²⁺, Mg²⁺, HCO₃⁻, Cl⁻, HPO₄²⁻, and SO₄²⁻ to those of AH particles on collagen-coated styrene plates. They also reported that the ABs by scanning electron microscopy appeared as snowballs with a pumice stone-like structure whose surfaces were covered with a clotted matrix of vitreous

substances. From these observations, they suggested that AH is produced not only by changes of ionic tension in the vitreous fluid but also by changes of vitreous matrix associated with the ageing process or disease. Since the observed structural characters of the ABs resemble stones in the living body (lithiasis), Komatsu *et al*²⁸ suggested that AH is a condition of lithiasis in the body.

Although studies have focused on the structure and elemental composition of ABs, little progress has been made on identifying the actual biochemical components present in ABs. Such analyses are hampered by contamination since ABs are virtually impossible to separate from the surrounding vitreous. Using the techniques of thin layer and gas chromatography available in the 1960s, Feldman²⁹ reported that the lipid composition of ABs from humans contains 21% cholesterol, 5% cholesteryl esters, 38% sphingomyelin, 23% ceramide dihexoside, 10% cerebroside, and trace amounts of triglycerides and lecithin (3% of lipids were unidentified). Until now, no other investigators have addressed this problem.

Currently, Lin *et al*³⁰ have reported the presence of β -carotene in ABs in one of two patients with AH whose ABs were obtained by vitrectomy and examined by confocal Raman microspectroscopy and high pressure liquid chromatography (HPLC) analysis. β -Carotene was identified by Raman spectroscopy in the presence of two unique peaks at 1525 and 1158 cm⁻¹. Subsequently, the presence of β -carotene was confirmed by comparison of HPLC retention times with standard β -carotene.

The galactose-fed dog

In addition to humans, AH has been observed in dogs,^{31,32} chinchillas,³³ cats,³² and rabbits.³⁴ While none of these specific animals consistently develop bilateral AH, we have observed that young (9-month old or less) male beagles fed a diet containing 30% galactose for 42 months all developed AH.³⁵ Galactose feeding activates the enzyme aldose reductase, which is the first step of the Sorbitol Pathway in which excess glucose is converted to fructose through the intermediate sugar alcohol sorbitol. Under hyperglycaemic conditions, the activation of aldose reductase results in the intracellular accumulation of sorbitol and this accumulation has been linked to the development of diabetic complications, which in the eye include cataract, retinopathy, and keratopathy. In galactose-fed animals, intracellular galactose is more rapidly converted to galactitol by aldose reductase than glucose is to sorbitol. Unlike sorbitol, galactitol is not further metabolized. The net result is a more rapid and higher intracellular accumulation of sugar alcohol in the galactose-fed compared to diabetic animal, and this results in a more rapid and more severe development of

Table 1 Development of diabetes-like ocular lesions in 9-month old beagles fed 30% galactose diet

Months diet	Ocular lesions
2–6	Cataract
19–24	Pericyte ghost formation
27–31	Microaneurysms
33–36	Dot and blot haemorrhages
36–48	Confluent haemorrhages, areas of nonperfusion
42	Asteroid hyalosis
56	IRMA
60	Occluded arterioles; A-V shunts; exudates; gliosis of nerve fibre layer
66	Intravitreal haemorrhage; partial posterior vitreous detachment
72	Proliferative retinopathy, NVD

Figure 1 Appearance of AH in the dog vitreous.

cataract, retinopathy, and keratopathy. The advantage of galactose feeding is that it induces aldose-reductase associated lesions in a non-diabetic animal within an earlier time frame than in the diabetic animal.³⁶ Galactose-fed dogs that develop retinal vascular changes identical to diabetic dogs are more healthy, vigorous, and capable of a longer lifespan than their diabetic counterparts whose fragile lives must be maintained by insulin injections. This has the advantage of producing diabetes-like complications in a shorter time frame while maintaining the health of the animal. The galactose-fed dog is unique because it is the only animal model that demonstrates both the histological and clinical lesions associated with all stages of diabetic retinopathy, ranging from background to the proliferative stages.^{37–41} It is also the only animal model that develops bilateral AH.

The time frame for diabetes-like ocular changes in initially 9-month-old galactose-fed beagles are summarized in Table 1. These begin with the development of cataract within weeks after the initiation of the galactose diet.^{42–44} The development of retinal lesions follows with the degeneration of retinal capillary pericytes between 18 and 24 months, the histological development of microaneurysms (27–33 months), and the clinical appearance of dot and blot haemorrhages at 33–36 months.^{37,38,45} At this time, morphological changes in corneal endothelial cells also occur.⁴⁶ Between 36 and 48 months, significant changes in retinal perfusion develop, which result in retinal hypoxia and the subsequent development of lesion association with the preproliferative and subsequently proliferative stage.^{38–41} It is during this time frame that AH appears.

Figure 2 ABs from dog demonstrating birefringence (a) and lipid staining with oil red O (b).

Figure 1 illustrates the appearance of AH in the vitreous of an enucleated eye from a galactose-fed beagle. Attempts at physically extracting the white ABs suspended in the viscous vitreous humour without contamination from the adhering vitreous were unsuccessful. Therefore, vitreous 'smears' containing ABs were prepared and examined by light microscopy. The ABs demonstrated birefringence (Figure 2a) and stained positive for lipids (Oil Red O) (Figure 2b). Subsequently, samples of the vitreous from dogs with and without AH were lyophilized and subjected to elemental analysis that indicated that the percent of calcium and phosphorus were significantly higher in the vitreous containing ABs (Figure 3), thus verifying that dog ABs also contain calcium and phosphorus. A key feature of the eyes of galactose-fed dogs is the prolonged

Figure 3 Results of elemental analysis of calcium and phosphorus of vitreous with/without AH from dogs. $n = 3-4$, mean $\pm$ SD, (* $P < 0.05$).

presence of mature cortical cataracts. Since mature sugar cataracts are more permeable and lens resorption is known to occur in dogs,⁴⁷⁻⁴⁹ the possibility that ABs contain lens crystallins was investigated. Vitreous with/without AH was subjected to polyacrylamide gel electrophoresis and subjected to western blot analysis using antibodies against bovine α -, β -, and γ -crystallins. This technique failed to show the presence of crystallins in the vitreous containing AH.

To identify potential components present in the ABs, electrospray mass spectroscopy (ESI-MS) studies were conducted using a Finnigan LCQTM mass spectrometer (ThermoQuest; San Jose, CA, USA). Since the ABs are impossible to separate from the vitreous humour, identical studies were conducted with the normal and the vitreous-containing AH. The vitreous samples were sonicated in HPLC grade water using a Sonicator[®] ultrasonic processor (Misonix Inc., Farmingdale, NY, USA) for 1-2 min, and the homogenate was then extracted three times with HPLC grade chloroform. Following removal of chloroform *in vacuo*, the residue was dissolved in HPLC grade methanol for mass spectroscopic analysis. Peaks in the extract associated with ABs were identified by subtraction analysis of vitreous with/without AH. These analyses indicated the presence of three distinct peaks at m/z peak 690.5, 547.1, and 1430.6, respectively (Figure 4). The major m/z 690.5 peak was identified by computer analysis as the quasimolecular ion of 1,2-dipalmitoyl-glycero-3-phosphoethanolamine (DPPE) and confirmed by MS/MS using a relative collision energy of 34 eV and comparing these results to a similar MS/MS analysis of standard

Figure 4 Subtraction spectrum of electrospray (ESI-MS) conducted in the negative mode on samples of vitreous with/without AH. Samples were directly infused into the heated capillary of the detector at 1.5 μ l/min. Spray voltage was 5 kV, temperature of the capillary was 275°C; capillary voltage and tube lens offset voltage were both set up at -30 V. The mass range was 150-2000. Results show the presence of three peaks that are absent in vitreous without AH.

Figure 5 Structure of hydroxyapatite complex of 1,2-DPPE identified in ABs.

DPPE (Sigma-Aldrich). The identity of the m/z 547.1 and 1430.6 peaks remain unknown. On the basis of the identification of DPPE, the presence of Ca and P in ABs, and the suggested structural and elemental similarity of the AB with hydroxyapatite, the major component of the AH body is proposed to be a complex between two molecules of DPPE and calcium as outlined in Figure 5.³⁵

Since there are no reports on the presence of DPPE in the eye, where can the components of this phospholipid come from? Palmitic acid is the most abundant free fatty acid present in bovine and rabbit aqueous humour, and in the lens its metabolism to CO_2 and glutamic acid generates energy.⁵⁰ In the retina, palmitate along with other free fatty acids are known to increase with diabetes, and increased levels of retinal palmitate can initiate apoptosis of retinal capillary pericytes.⁵¹ Palmitic acid is also a major saturated free fatty acid present in the vitreous of humans and dogs,⁵² and its concentration has been shown to increase with proliferative retinopathy.⁵³ Phosphatidylethanolamine has been reported to be the only phospholipid that is present at higher concentrations in the vitreous than in serum.⁵⁴ This increase has been suggested to be due to direct diffusion from the retina.⁵⁴ The retina contains relatively high amounts of glycerophosphorylethanolamine,⁵⁵ and both diabetes and galactosemia are associated with both increased palmitic acid incorporation into diacylglycerol and increased levels of diacylglycerol.⁵⁶ The elevated levels of diacylglycerol, in turn, can increase PKC activity.⁵⁷

Since AH in the galactose-fed dog appears during a period in which significant retinal perfusion changes occur, we propose that the DPPE observed in the ABs of the galactosemic dogs may be derived from the retina

and linked to the formation of retinal changes in these dogs. Diffusion of substances from the degenerating retina into the vitreous have been established. For example, evidence suggests that in the Royal College of Surgeons (RCS), posterior subcapsular cataracts (PSC) are initiated by toxic products generated by the peroxidation of polyunsaturated lipid components from the degenerating photoreceptor outer segments in the retina that diffuse anterior through the vitreous to the lens.⁵⁸ Active lipid metabolism has also been reported to occur in the vitreous from Irish setters with hereditary rod-cone dysplasia (RCD).⁵² AH has also been documented to be present in patients with retinitis pigmentosa,^{59–61} or pigmentary retinal degeneration.⁶²

In addition to retinal changes, other factors appear to influence the development of AH in dogs. In studies in which dogs were made aphakic by phacoemulsification surgery before galactose feeding,⁶³ AH was clinically observed to occur after 25 months of galactose feeding. Moreover, it appeared similarly in dogs treated with aldose-reductase inhibitor, which was observed to significantly delay the development of retinal lesions and reduce the areas of retinal nonperfusion. Since surgery results in an inflammatory response, the ABs in these animals may be different. Unfortunately, the vitreous from these animals was not available for analysis.

These results clearly indicate that AH is a complex lesion associated with a number of biochemical changes in the eye. Although currently there are no prospects for its prevention, investigations on the development of AH in the galactose-fed dog animal model should provide an insight into the pathogenesis, future management and treatment of this ocular lesion.

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