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Cathepsin A activity of normal bovine ocular tissues and pathological human intraocular fluids

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Cathepsin A activity assayed with N-Cbz-Phe-Ala, N-Cbz-Glu-Tyr and N-Cbz--Glu-Phe as substrates, was measured in fresh corneas, lenses, aqueous humor, vitreous humor and choroid plus retinal pigment epithelium taken from normal bovine eye balls and in human intraocular fluids from the eye balls in various ocular diseases (cataract, glaucoma, diabetes, intraocular tumors).

Cathepsin A exhibited a pH optimum at 5.0 and showed the highest specificity towards N-Cbz-Phe-Ala as a substrate.

In bovine ocular tissues high cathepsin A activity was found in the choroid plus retinal pigment epithelium and in cornea. The lens and the vitreous humor showed low enzyme activity and the aqueous humor none at all.

In the human aqueous humor of the eye with cataract cathepsin A activity was more than three times higher then in the eye with choroid tumor. In human vitreous humor in absolute glaucoma the activity was twice as high as in melanoma and almost three times higher than in the case of lung metastatic tumor. Diabetes in glaucoma increased seven fold cathepsin A activity in the vitreous humor.

Proteolytic enzymes, including cathepsin A, play a very important role in protein turnover and maintenance of dynamic equilibrium between proteins and their degradation products in normal and morbid tissues.

The tissues of eye ball contain several proteinases. The presence of cathepsin B, D and collagenolytic cathepsin ([1–3] respectively) has been demonstrated in all ocular tissues of humans and in many animals species. Aminopeptidase and dipeptidase activities were found in cornea [4] and lens [5, 6] and γ-glutamyl transpeptidase in lens, retina and uvea [7]. Collagenase and gelatinase are the major enzymes responsible for collagenolysis in cornea [8]. Metalloproteinases were found in the interphotoreceptor matrix of retina [9]. The results of investigations on proteinases of the various ocular tissues point to their importance in pathogenesis of many ocular diseases such as: cataract [10, 11], intraocular inflammation, glaucoma [12], retinal dystrophy [13] and detachment [14], keratoconus [15], corneal ulcus [16], etc.

Although there are numerous data concerning proteolytic enzymes, the occurrence of cathepsin A in ocular tissues has not been previously reported, except for a single report on the presence of cathepsin A in the vitreous humor samples from cadavers on autopsy [17]. The authors of that report have studied enzymatic activity in relation to the diseases leading

Abbreviations: N-Cbz, *N*-carbobenzoxy residue; PBFI, potassium-binding benzofuran isophtalate.

to death and they found higher values of cathepsin A activity in patients with severe brain and vertebral column damage (craniocerebral trauma, multiple trauma) and significantly lower values in subjects who died of natural death. Thus cathepsin A activity in vitreous humor proved to be a useful criterion in the postmortem diagnosis of brain damage.

We have attempted to evaluate cathepsin A activity in bovine normal ocular tissues and in human ocular fluids (aqueous humor and vitreous humor) in various eye diseases: absolute glaucoma, diabetes in the course of glaucoma, cataract and intraocular tumors. Cataract, glaucoma and diabetes are the main causes of amaurosis. Whereas opacification of the lens (cataract) can be effectively treated by surgery, glaucoma and diabetes result in progressive and irreversible loss of vision [18, 19]. Glaucoma is characterized by increased intraocular pressure which destroys retinal and optic nerve structures responsible for perception and transmission of visual stimuli. Microangiopathies in blood vessels of the eye in diabetes result in degenerative and ischemical changes of retina and nerve fibers and in haemorrhage into the vitreous humor. The eye ball, similarly as other organs of human body, is the site of primary and secondary neoplasms. These diseases, detected as a rule very late due to absence of perceptible symptoms, very often end in enucleation or orbital exenteration.

The present work is a preliminary step aiming at elucidation of a role of cathepsin A in normal and malignant eye tissues.

MATERIALS AND METHODS

The studies were performed on fresh bovine eye balls and human intraocular fluids. The aqueous humor was aspirated from 40 bovine eye balls with a sterile syringe from the anterior chamber. Then each eye ball was cut equatorially to remove the vitreous humor and lens. Next, the retinal pigment epithelium plus choroid was separated from the sclera with a spatula. The cornea was cut off within limits of its limbus.

The human aqueous humor was obtained during surgery from the anterior chamber of 30 patients operated for cataract. The aqueous humor of 7 patients with choroid tumor was aspirated from the anterior chamber after enucleation. The human vitreous humor was taken from eye balls which were enucleated because of absolute glaucoma (5 eyes) and intraocular tumors: melanoma (7 eyes) and carcinoma planoepitheliale metastatic (2 cases). Besides, cathepsin A activity was measured in vitreous humor in 4 diabetic patients with glaucoma after enucleation. All intraocular fluids were from patients operated in the years 1994–1996.

Homogenates of the tissues (10% in 0.15 M NaCl containing 0.2% Triton X-100) were prepared by homogenizing the samples at 4°C for 3 × 15 s in a Potter homogenizer. The homogenates were centrifuged at 15000 × *g* for 30 min at 4°C and the supernatant was used for the enzyme assay. Cathepsin A activity was determined by the ninhydrin colorimetric method with *N*-carbobenzoxy-L-phenylalanyl-L-alanine (N-Cbz-Phe-Ala), *N*-carbobenzoxy-L-glutamyl-L-tyrosine (N-Cbz-Glu-Tyr) or *N*-carbobenzoxy-L-glutamyl-L-phenylalanine ((N-Cbz-Glu-Phe) as substrates [20]. Studies were performed over a pH range from 3.0 to 6.0 at intervals of 0.5 pH unit. A mixture containing 0.25 ml of the tissue homogenate and 0.25 ml of 0.125 M substrate was incubated at 37°C for 4, 8, 16, 20 and 24 h. The reaction was stopped by adding 1.25 ml of 10% trichloroacetic acid. The samples precipitated with the acid at zero time served as a control. After centrifugation 0.5 ml of the ninhydrin reagent was added to 0.5 ml of the supernatant and the mixture was heated for 20 min in a water bath. After cooling and addition of 2 ml of n-propanol/water mixture (1:1, v/v) α-amino nitrogen released during incubation was measured spectrophotometrically at 570 nm. The resulting absorbance was then converted to leucine equivalents basing on a calibration curve. Correction was made for degradation of endogeneous proteins of the investigated tissues in 0.1 M acetate buffer. Specific cathepsin A activity was expressed in μmol/ml.

To prevent a possible effect of bacterial contamination during long incubation period, sterile saline with an antimicrobial agent was added prior to homogenization.

The protein content was determined by the biuret method.

RESULTS

The enzyme exibited a pH optimum at 5.0. A significant increase of cathepsin A activity appeared after 24 h of incubation. The highest activity was found with N-Cbz-Phe-Ala as a substrate in all investigated ocular tissues.

Cathepsin A activity was demonstrated in all investigated bovine eye tissues except for aqueous humor (Table 1). The highest activity of the enzyme was found in the choroid plus retinal pigment epithelium and in cornea. The lens and the vitreous humor showed low activity of the enzyme.

In humans with ocular disease the localization to various ocular tissues is different than in normal bovine eye. In patients with cataract cathepsin A activity in the aqueous humor was more than three times higher than in patients with choroid tumor (melanoma) (Table 2).

Glaucoma was found to be associated with a very pronounced increase in cathepsin A activity which in the human vitreous humor was twice as high in absolute glaucoma as in melanoma, and almost three times as high as in metastatic tumor from the lung — carcinoma planoepitheliale (Table 3). Cathepsin A activity of the vitreous humor in primary tumor of choroid was only slightly higher than the activity in metastatic tumor of choroid. It is noteworthy that in diabetic patients with absolute glaucoma cathepsin A activity in the vitreous humor was seven times higher than in the eye of patients without diabetes.

DISCUSSION

To our knowledge, this is the first report evidencing the existence of the lysosomal protease, cathepsin A, in ocular tissues. This information extends our knowledge on the range and specificity of protein substrates in ocular tissues. Cathepsin A is the only known enzyme in eye which exhibits a carboxypeptidase type of activity. The enzyme catalyses the release of C-terminal hydrophobic residues having an unsubstituted α-carboxyl group [21]. It is generally known that cathepsin A occurs in multiple forms in various tissues. In liver the enzyme can form free homodimers or can associate with lysosomal β-galactosidase and neur-

Table 1

Activity of cathepsin A in bovine ocular tissues at pH 5 with synthetic substrates.

Means of 40 ocular tissues.

Tissue	Cathepsin A (μmol/ml per 24 h) ±S.D.		
	N-Cbz-Phe-Ala	N-Cbz-Glu-Tyr	N-Cbz-Glu-Phe
Choroid + retinal pigment epithelium	39.23 ± 1.89	28.41 ± 1.18	25.62 ± 0.90
Cornea	15.25 ± 0.70	11.28 ± 0.63	8.28 ± 0.61
Lens	2.00 ± 0.30	1.10 ± 0.17	0.13 ± 0.02
Vitreous humor	0.60 ± 0.08	0.10 ± 0.02	0.05 ± 0.01
Aqueous humor	0.00	0.00	0.00

Table 2

Activity of cathepsin A in human aqueous humor ocular diseases measured by the increase of α-amino nitrogen at pH 5.0 with N-Cbz-Phe-Ala as a substrate

Aqueous humor	Number of patients	Cathepsin A (μmol/ml per 24 h) ±S.D.
In cataract	30	0.572 ± 0.060*
In malignant melanoma of choroid	7	0.169 ± 0.045*

* Statistically different at $P \leq 0.05$

Table 3
Activity of cathepsin A in human vitreous humor ocular diseases measured by increase of α-amino nitrogen at pH 5.0 with N-Cbz-Phe-Ala as a substrate

Vitreous humor	Number of patients	Cathepsin A (μmol/ml per 24 h) ±S.D.
In absolute glaucoma	5	1.690 ± 0.110*
In absolute glaucoma with diabetes	4	12.232 ± 0.329*
In malignant melanoma of choroid	7	0.845 ± 0.061*
In metastatic tumor of choroid	2	0.585 ± 0.034*

* Statistically different at $P \leq 0.05$

aminidase, towards which it exerts a protective function necessary for their stability and activity [22]. In other tissues cathepsin A can form aggregates and complexes with other lysosomal peptides.

Differences in cathepsin A activity towards N-Cbz-Phe-Ala, N-Cbz-Glu-Tyr and N-Cbz-Glu-Phe and relative different rates of these activities in various bovine eye tissues might suggest heterogeneity of bovine ocular cathepsin A. According to Matsuda [20] cathepsin A preferentially cleaves N-Cbz-Phe-X dipeptide bonds and consistently the enzyme activity in bovine eye towards N-Cbz-Phe-Ala was the highest. N-Cbz-Glu-Tyr and N-Cbz-Glu-Phe were hydrolyzed to a lower extent.

It was previously demonstrated that among bovine ocular tissues the retinal pigment epithelium showed the highest specific activities of cathepsin B, D and other lysosomal enzymes [23]. From the presented results it appears that distribution of cathepsin A in the bovine eye is similar to that of cathepsin D and B [1, 2], and that cathepsin A in the retinal pigment epithelium plus choroid also exhibits the highest specific activity. High cathepsin A activity in these tissues suggests its functional importance, especially since the retinal pigment epithelium exhibits a highly developed phagolysosomal system and plays a very important role in protein turnover of retinal photoreceptors. The outer segments of mature rods and cones continually undergo renewal, and the pigment epithelium is responsible for removing the discs of the terminal photoreceptor outer segments and digesting rhodopsin. There is thus a strong possibility that cathepsin B and D play a role in hydrolysis of rhodopsin and other rod outer segment proteins and even in hydrolysis of protein of neighboring tissues under some pathological conditions [13, 24]. It seems also likely that cathepsin A cooperates with other lysosomal enzymes in the metabolism of choroid and retina since the rate of protein hydrolysis in lysosomes could be enhanced by exopeptidase acting in concert with endopeptidases.

Our results on cathepsin A in pathological human intraocular fluids provide the next evidence for the role of this enzyme in ocular proteolysis. The results obtained on enzyme activity in human vitreous humor are similar to those of other authors concerning proteolytic activity of human vitreous humor in the course of some ocular diseases [12]. As we have demonstrated, this activity was two or three times as high in glaucoma as in intraocular tumors and was the highest in intraocular inflammation. Cathepsin A activity in human glaucomatous vitreous humor was also higher than in normal bovine vitreous humor. These data suggest that the liberation of proteolytic enzymes from different ocular tissues including the vitreous humor, may participate in the pathogenesis of absolute glaucoma and that proteolysis may play a significant role in local destruction of the retina and the optic nerve. Retinal destructive changes in diabetes (microangiopathies, degenerative changes and neovascularization) may be an additional risk factor in glaucoma, as it increases the release of the proteolytic enzymes from damaged tissues [25].

Our results on cathepsin A activity in aqueous humor from the anterior chamber in cataract is of special interest because this activity was absent in the normal bovine aqueous humor while it occurs in the bovine lens. This suggests its importance in cataract pathogenesis. It is

well known that occurrence of proteolytic enzymes is associated with formation of vacuoles and liquification of lens contents and dissolution of lens cortex in hypermature cataract [26]. Leucine aminopeptidase and alanine aminopeptidase [10] participate in development of these changes, and their activities increase with ageing. Aminopeptidase activities are three times as high in human cataractous lens as in human normal lens. Dipeptidase activity e.g. DPP II, DPP III [11] behave in a very similar way. The activities of these exopeptidases are increased in the aqueous humor cataract but, as in the case of cathepsin A, it was not found in normal aqueous humor. This implies that cathepsin A, is involved in development of lens opacity and is found in the aqueous humor due to diffusion from cataractous lens in which the proteolytic process prevails.

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