

Human Corneal Aldehyde Dehydrogenase : Localization and Its Clinical Properties

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Abstrak

Sindrom perlunakan kornea tepi dikenal sebagai suatu proses imunologik. Protein kornea dengan berat molekul 54 kD dan yang baru saja dipastikan sebagai enzim aldehyd dehidrogenase (ALDH) diduga sebagai antigen penyebab sindrom perlunakan tersebut. Dari berbagai teori yang diajukan mengenai peranan ALDH di kornea, belum satupun yang menjelaskan kaitan antigenitasnya dengan sindrom perlunakan ini. Pada kornea manusia, dengan kombinasi dua teknik pewarnaan histologik berdasarkan sifat enzim yang spesifik, ternyata enzim ALDH terdapat di dalam dan di luar sel. Hal itu juga menunjukkan kemungkinan adanya perbedaan pada struktur alami kedua jenis ALDH tersebut. Pada binatang percobaan, telah diketahui bahwa baik antibodi maupun reaksi selular terhadap protein kornea ini ternyata tidak dapat mencapai sel epitel yang intak. Sebaliknya, antibodi tersebut dapat dengan mudah memasuki stroma kornea. Oleh karena itu, adanya ALDH yang berada di luar sel ini tentunya dapat menjadi antigen sasaran pada penyakit perlunakan kornea tepi.

Abstract

It is known that immune processes are involved in the so-called the peripheral corneal melting syndromes. Moreover, the wellknown 54 kD corneal protein, which was recently identified as corneal aldehyde dehydrogenase (ALDH) is said to be the triggering antigen. Despite several proposed hypothesis concerning the properties of this corneal ALDH, none have addressed the antigenic issue of ALDH in this melting syndromes. In this histochemical study, we used two modified ALDH enzyme-specific staining methods. The results indicate that human corneal ALDH was found to be located either intra and extracellularly in the cornea. Moreover, based on ALDH visualization by different staining methods, we speculated that there might be slight differences in the globular, native structure of the two corneal ALDH. In animal experiments, it has been shown that a circulating antibody and cellular reaction against this corneal protein could not reach intact epithelial cells, and that a serum-derived antibody could easily diffuse in the corneal stroma. In this respect, the extracellular location of ALDH may become a target antigen in the corneal melting diseases.

Keywords : Corneal aldehyde dehydrogenase (ALDH), Corneal Protein 54 kD, Histochemistry, Peripheral corneal melting diseases.

INTRODUCTION

Circulating antibodies and cellular immune reaction against the 54 kD corneal protein have been found in patients suffering from peripheral corneal melting diseases, anterior uveitis, Behçet's disease, Fuch's heterochromic cyclitis, and following corneal transplantation.¹⁻⁴ Experimentally, due to the localization and tissue specificity of the protein, the production of antibody against this 54 kD corneal antigen is easily triggered. However, the circulating auto-antibodies do

not seem to be able to reach intact corneal epithelium.^{1,5}

Based on DNA sequence homology this soluble 54 kD corneal protein has recently been identified as class 3 aldehyde dehydrogenase (ALDH). It was then strengthened by an enzymatic assay in bovine and human corneal extracts.⁶⁻¹¹ There are at least four proposed functions of this intriguing 54 kD corneal ALDH. First, the enzymatic property of ALDH, which enables it to detoxify cytotoxic aldehydes. Cytotoxic aldehydes are generated within the cornea following

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lipid membrane peroxidation triggered by free radicals species.^{7,8,9} Abedinia⁷ proposed the second possibility suggesting that corneal ALDH may act as an absorbing target of the UV light. The third proposed function is a structural role of corneal ALDH. This hypothesis is based on the so-called "gene sharing" phenomenon which involves the use of one gene encoding a protein that has two entirely different functions at the same time,¹⁰ and the studies on the embryonal development of the bovine cornea which indicate that the level of this principal corneal soluble protein correlates with the increasing transparency of the tissue.¹¹ The last hypothetical function of this protein is that ALDH is involved in the extracellular processing of corneal collagen by regulating cross-linking of fibrils, thereby contributing to the unique arrangement of collagen and to corneal transparency.¹²

Up to now, it has not been clear whether these hypothetical functions can explain some pathologic corneal conditions which may be related to this 54 kD, corneal ALDH. In this study we investigated the human cornea using histochemical techniques and concluded that human corneal ALDH is present in the cornea both intracellularly and extracellularly. In the latter case this protein might become the target antigen in corneal melting diseases.

MATERIALS AND METHODS

ALDH Histochemistry

Normal human corneal tissue stored in liquid nitrogen was embedded in Tissue-Tek, O.C.T. compound (1988 Miles Inc., Elkhart, NY, USA), and 10 μ m-thick frozen sections were cut at 20^o C. About two to three serial sections were usually prepared per slide. The sections were allowed to air dry at room temperature for approximately 30 min.¹³

Sections were stained for ALDH using two different methods. The first method was a slight modification of the techniques describes by Gabler.¹⁴ The staining solution contained 10 mg MgCl₂.6H₂O (E.Merck, Darmstadt, Germany), 2 mg M.T.T.(3-[4,5 dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) from Sigma Co, St. Louis, USA; 10 mg NADP (Boehringer Mannheim GmbH, Germany), and 1 ml 1,2-Propylene Glycol (BDH, Poole, UK) and 10 μ l Benzaldehyde (Sigma, St. Louis, USA) in 9 ml Phosphate buffer pH 7.5. Sections were incubated for 30-60 min at 37^o C in the dark.

In the second staining method, slides were pre-stained with 2 mg Nitro Blue Tetrazolium (NBT, Sigma) in 5 ml of Acetone (E.Merck) for 30-60 seconds.¹⁵ The staining solution ingredient were 2 mg

NBT, 20 mg NADP and 1 mg Phenazyl Methosulphate (PMS, Sigma) and 22% w/v Polyvinyl Alcohol (PVA, Sigma) in 10 ml Phosphate buffer pH 7.5. Sections were incubated for 10-15 min at room temperature in the dark.¹³ After staining, the slide was rinsed with the same buffer and fixated with 3.8% buffered formalin for 10 min, and rinsed again with 0.9% NaCl solution for 1 min. A drop of glycerol was placed on the sections and covered with a cover-slide.

The specificity of the reaction was tested with the staining medium in the absence of either substrate or coenzyme.

RESULTS

A positive histochemical reaction for ALDH is indicated by the deposition of fine, purple, formazan precipitates at the site of enzyme activity. In the human cornea, formazan staining was found in the epithelium, especially in the basal cells layer. Staining was also present in the keratocytes of the stromal layer (Figure 1a). This result was in concordance to the former finding in rat and bovine corneas^{3,5,14}.

Histochemical staining using the NBT dye resulted in a better morphologic visualization of the ALDH as compared to the MTT dye. The MTT techniques, demonstrated unexpected staining in the periphery of Bowman's membrane which was stained diffusely, whereas down to the central part of the cornea no enzymatic staining could be seen (Figure 1b).

DISCUSSION

More than a decade ago, it was proposed that corneal ALDH, the most abundant intrinsic soluble protein in the human and vertebrate corneas have to play an important role in the corneal metabolism.¹⁶⁻¹⁸ Our previous studies¹⁹⁻²³ in human corneal ALDH indicates that the function of this intriguing 54 kD corneal protein tend to be a structural one rather than as a functional enzyme.

This conclusion is based on the fact that corneal ALDH has a high k_m value even for the preferential substrate of the human and bovine corneal ALDH¹⁹ as compared to the other studies on other class 3 ALDH members from recombinant source.²⁴ Moreover, 4-hydroxynonenal and malonaldehyde, as the most cytotoxic products of lipid membrane peroxidation are not substrates for class 3 ALDH.²⁴ Although, the human cornea has a lower ALDH relative content as compared to the bovine eye (Gondhowiardjo, unpublished observation), however, its levels still greatly

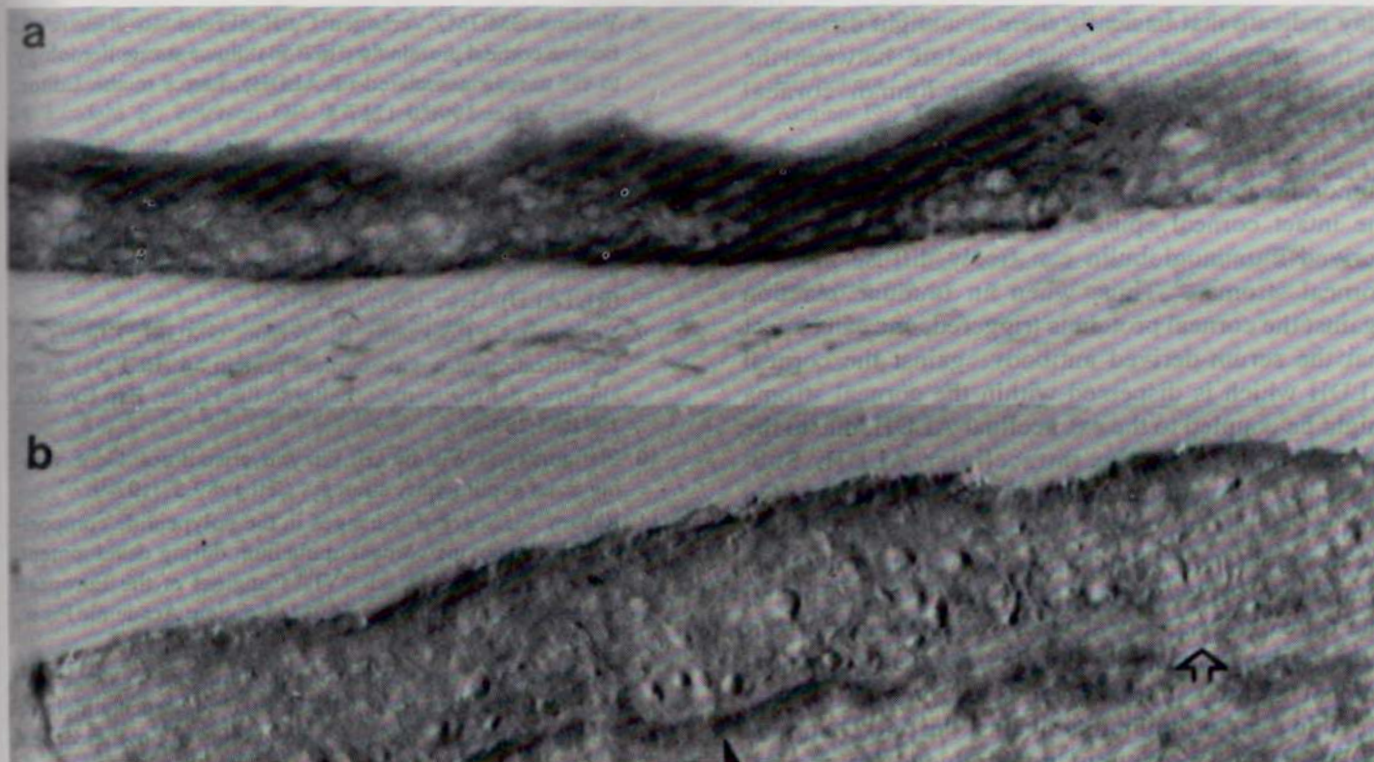


Figure 1. Histochemical staining for ALDH

exceed any likely catalytic requirement. The most striking finding was that whatever the underlying pathological process i.e inflammation, dystrophy or degeneration of the cornea, it had a single effect on the native, globular structure of the human corneal ALDH. Furthermore, this phenomenon seems to be related to the alteration of the glutathione redox system.²² Further studies confirmed this issue, with the finding that pathologic corneas have also a deviation in the activity of the glutathione related enzymes, namely the glutathione S-transferase and the glutathione reductase.²³

It is likely that this histochemistry study may help to clarify the antigenic properties of this 54 kD corneal antigen. In the rat cornea by using immuno-histochemistry techniques, the 54 kD corneal epithelium antigen was found to be accumulated intra-cytoplasmically in the epithelium, stromal keratocytes and the corneal endothelium.^{3,5} Earlier, histochemistry presentations of the aldehyde dehydrogenase in the bovine cornea was demonstrated by the work of Gabler.¹⁴ Since then, to our knowledge, up to now there were no reports concerning the locations of human corneal ALDH.

Experimentally, Hoekzema¹² showed that the 54 kD corneal protein had a strong relation with the col-

lagen type IV and type I. In this study, we found that by using the MTT dye, the anterior basement membrane, the peripheral portion of Bowman's membrane were stained diffusely for ALDH. This was in agreement with the study of Cleutjens²⁵ using immunohistochemistry techniques on the human corneal epithelial basement membrane. She demonstrated that the type IV collagen is only present in the peripheral portion of corneal anterior basement membrane. The anterior basement membrane is known to be the product of basal cell epithelium, which is the major source of corneal ALDH.

In histological practice, MTT dye is more readily reduced than the NBT and both dyes are entirely independent of the oxygen tension in the medium. However, NBT dye is the most widely used tetrazolium salt, due to its rapid and strong reaction and very precise localisation.^{26,27} Because in this experiment NBT was used in combination with PMS, a potent catalysator, it is presumable that the two staining techniques had a comparable potency in detecting the corneal ALDH. Nevertheless, it is known that the MTT had a lower substantivity staining than NBT dyes,^{26,27} thus, the extracellular ALDH staining is not just a novel of substantivity. In this respect, the extracellular corneal ALDH which only can be detected by MTT staining,

may indicate that basically there is a slight difference in the native conformational structure between the intracellular and the extracellular human corneal ALDH.

It is known, that experimentally, the cellular and humoral reactions against corneal ALDH cannot reach the intact corneal epithelial cells,¹ although serum-derived immunoglobulin can freely diffuse into the corneal stroma.²⁸ Thus, when an immune reaction against the corneal protein is triggered, one can expect that the serum-derived antibody against the corneal ALDH which is dispersed within the corneal stroma might be coupled to the extracellular ALDH, due to the identical epitope as the intracellular ALDH. Subsequently, this antigen-antibody complex, scattered in the peripheral portion of Bowman's and anterior basement membrane of the cornea, as well as in the surrounding of the stromal keratocytes in the peripheral one-third of the anterior stroma, will subsequently start the inflammatory process of the so-called peripheral corneal melting diseases. However, it may also be possible that autoimmunity against the 54 kD corneal ALDH is an epiphenomenon induced by corneal trauma or diseases. Nevertheless, this proposed hypothesis is in concordance to the recent editorial in *Lancet* which stated that the enzyme's specific target organ might become the target or even the triggers of auto-immune diseases.²⁹

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Table 1. Sensitivity testing performed in The Health Laboratory of

The Department of Health in Indonesia. These islands are inhabited by about 180 million people.

No	Name of Island	Tested	Antibiotic
1	Sumatra	KB	nm
2	Jawa	KB	10
3	Bali	KB	12
4	Banten	KB	10
5	DKI	KB	10
6	Sumatra	KB	nm
7	Jawa	KB	10
8	Bali	KB	12
9	Banten	KB	10
10	DKI	KB	10
11	Sumatra	KB	nm
12	Jawa	KB	10
13	Bali	KB	12
14	Banten	KB	10
15	DKI	KB	10

Table 2. Sensitivity testing performed in The Health Laboratory of

The Department of Health in Indonesia. These islands are inhabited by about 180 million people.

No	Name of Island	Tested	Antibiotic
1	Sumatra	KB	nm
2	Jawa	KB	10
3	Bali	KB	12
4	Banten	KB	10
5	DKI	KB	10
6	Sumatra	KB	nm
7	Jawa	KB	10
8	Bali	KB	12
9	Banten	KB	10
10	DKI	KB	10
11	Sumatra	KB	nm
12	Jawa	KB	10
13	Bali	KB	12
14	Banten	KB	10
15	DKI	KB	10

microbiology of the medical schools had been looking into these problems earlier. Unfortunately, antibiotic monitoring and reporting has not been done regularly, but with the recent awareness of nosocomial infection problems, its role are becoming more prominent. On the other hand more attention have to be paid for the standardization of antibiotic sensitivity testing since the same language is needed to interpret whether an isolate is sensitive or resistant to a certain antibiotic.

PRESENT CONDITION

Indonesia with about 180 million people has twenty seven provinces. Not all of health laboratories in each province. The Technical Institute of Health in Jakarta is the only institution that has a good laboratory. Most of health laboratories are still in the

introduction of antibiotic sensitivity testing. Many infectious diseases are still a major problem in Indonesia. The frequent and irrational use of antibiotics might cause the emergence of resistant strains. Many groups of antibiotics are available in this country, i.e. the sulfonamides, tetracyclines, β -lactam antibiotics, sider, macrolides, quinolones, etc. Generators of antibiotics are free to choose one of those drugs for the treatment of their patients. Today many laboratories try to anticipate the problem drug resistance by preparing themselves to perform the sensitivity testing. For academic reasons the university laboratories especially the department of