Abstract: New cysteine protease inhibitors in human tears and milk and their medical significance are reviewed in this paper. As protective components against bacterial infection in the eyes, we detected four kinds of anti-bacterial proteins in normal human tears including lysozyme and three kinds of cysteine protease inhibitors. Using our reverse zymography of normal tears, three kinds of cysteine protease inhibitors were found to be 78kDa, 20kDa and 15kDa and were determined to be lactoferrin, Von Ebner's Gland (VEG) protein and cystatin S, respectively. All of them belong to the cystatin super family and VEG protein and cystatin S are well known cysteine protease inhibitors. The C-terminus area 17mer peptide, Y679-K695, of lactoferrin showed strong homology with a common active domain of the cystatin family and the synthesized peptide showed inhibition of cysteine proteases. Not only were disease-specific changes found in these inhibitor profiles, but also disease-specific new inhibitors in patients tears with certain autoimmune diseases. A 35kDa inhibitor, which was detected specifically in tears with Behcet's disease, an typical autoimmune disease, was determined to be a lacrimal acidic proline-rich protein based on the N-terminus sequence analysis. A65kDa inhibitor of tears with Harada's autoimmune disease was determined to be an Ig heavy chain V-III region. In addition, lactoferrin content in Harada's disease was very low. We found two cathepsin inhibitors in bovine milk using reverse zymography, namely lactoferrin and —casein. The L133-Q151, in the human —casein molecule is the active inhibitory domain. They may play an important role in antiseptic and anti-infectious functions. J. Med. Invest. 50: 154-161, 2003

Keywords: cysteine protease inhibitor, human tears, reverse zymography, Behcet's disease, lactoferrin, milk, autoimmune disease
Inhibition analysis of the transferrin family against cysteine proteases

Synthesized peptide of near C-terminus 17 mer peptide of lactoferrin

Preparation of intramolecular peptides of β-casein

Analysis of the N-terminus amino acid sequence

Negative staining method of the SDS-PAGE gel

[II] Physiological functions of endogenous cysteine protease inhibitors.

In situ
Reverse zymography for the detection of cysteine protease inhibitors in natural materials

[1] Reverse zymography for the detection of cysteine protease inhibitors in natural materials

[2] Basic demonstrations for detection of authentic protease inhibitors using the reverse zymography method
Disease-specific expression of new inhibitors in human tears

Identification and properties of normal tears inhibitors

Identification and properties of normal tears inhibitors

Coomassie Brilliant Blue staining of all proteins in tears

Neatline staining of all proteins in tears

Reverse Zymography of papain inhibition in tears

Reverse Zymography of papain inhibition in pure lactoterm in

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Characteristic changes in the inhibitor profiles in pathological tears and detection of new disease-specific inhibitors in tears with specific autoimmune diseases

Lactoferrin

<table>
<thead>
<tr>
<th>89% Homology</th>
<th>61% Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>t Y E K Y L G p Q Y V A G I T N L K k</td>
<td></td>
</tr>
<tr>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
</tbody>
</table>

Cystatin Family

| Y E K F k V V Q V V A G I T N I K v |
| 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |

Transferrin

<table>
<thead>
<tr>
<th>78% Homology</th>
<th>28% Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y E K Y L G e E Y V k A V g N L R k</td>
<td></td>
</tr>
</tbody>
</table>

N. Katunuma et al. New cathepsin inhibitors in tears and milk
[4] Lactoferrin and β-casein in mammalian milk as cysteine protease inhibitors

(1) Detection of lactoferrin and β-casein as cysteine protease inhibitors in human and cow milk

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(2) Inhibition characteristics of human β-casein to cysteine proteases

β-casein, a protein found in milk, contains a cysteine protease inhibitor domain that is important in protecting the milk from enzymatic degradation. This inhibitor domain interacts with cysteine proteases, which are enzymes that catalyze the cleavage of peptide bonds. The interaction between β-casein and cysteine proteases is essential for maintaining the functionality and stability of milk products.

In a study by N. Katunuma et al., new cathepsin inhibitors in tears and milk are presented. These inhibitors play a crucial role in the protection of the eye and the maintenance of milk quality. The inhibitors are effective in preventing the degradation of β-casein and other milk proteins, ensuring the overall health of the products.

Advances in Enzyme Regulation 43 (2005)
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lococcus aureus

α

in vitro

β

in vivo