PB-09

ANTI-ALLERGIC EFFECT OF IMIDAZOLE PEPTIDES *IN VITRO* and *IN VIVO*

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Introduction

It has been reported that imidazole dipeptides such as anserine or carnosine produce effects of pollinosis depression, fatigue restorative and anti-aging. We have discovered that HAQ(His-Ala-Gln) imidazole tripeptide inhibited the degranulation of RBL-2H3 cells.

Materials & Methods

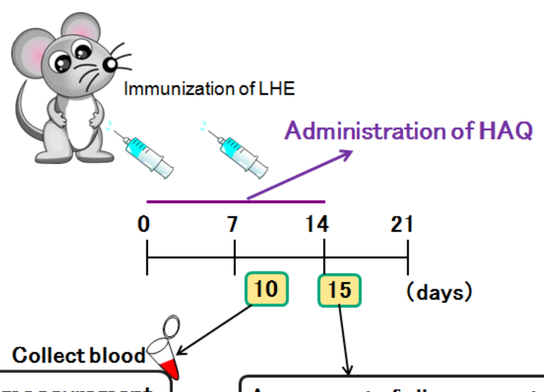
In vitro assay

- **The peptides used were low molecular weight HAQ (His-Ala-Gln), HHH (His-His-His), QHA (Gln-His-Ala), AQH (Ala-Gln-His), Carnosine (β-Ala-His).**
  Peptide purification levels were 95%.
- **Measurement of Degranulation**

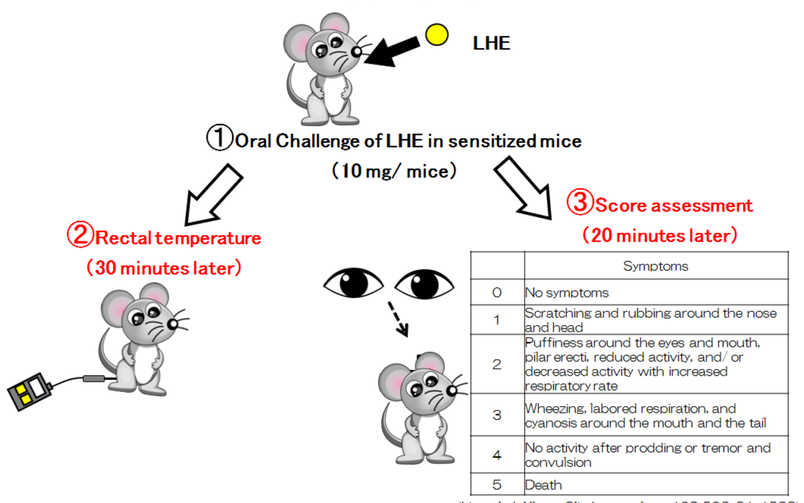
Degranulation of RBL-2H3 cells was monitored by measuring activity of released β-hexosaminidase. For antigen stimulation, DNP-specific IgE-primed RBL-2H3 cells were preincubated for 10 min with various concentrations of peptides, then stimulated with antigen (mouse anti-DNP IgE). After 30 min, the medium was collected and 0.2% Triton X-100 was added to the cells.
Levels of β-hexosaminidase released into the medium and within cells were determined by colorimetric assay using *p*-nitrophenyl-2-acetamide-2-deoxy-β-glucopyranoside and expressed as the percentage of activity release compared to total activity.

In vivo assay

- **Animals:** Female C3H/HeJ mice weighting 10~20g
- **Oral administration of HAQ peptide:**
  1~14 days, 1 mg/ mice/ day.
- **Schedule of Lysozyme from hen egg white (LHE) sensitization:** Intraperitoneal injection
  1st: 100μg of LHE/ 1mg $Al(OH)_3$
  2nd: (7days) : 50μg of LHE/ 1mg $Al(OH)_3$
  An interval of 7 days between 2 injections



- **Assesment of allergy symptoms**

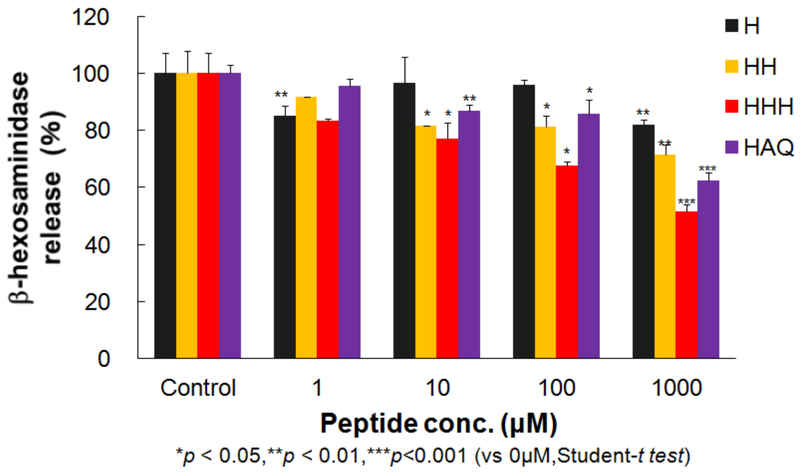


	Symptoms
0	No symptoms
1	Scratching and rubbing around the nose and head
2	Puffiness around the eyes and mouth, pilar erecti, reduced activity, and/ or decreased activity with increased respiratory rate
3	Wheezing, labored respiration, and cyanosis around the mouth and the tail
4	No activity after prodding or tremor and convulsion
5	Death

(Li et al. J Allergy Clin Immunology. 103 206-21, 1999)

Results

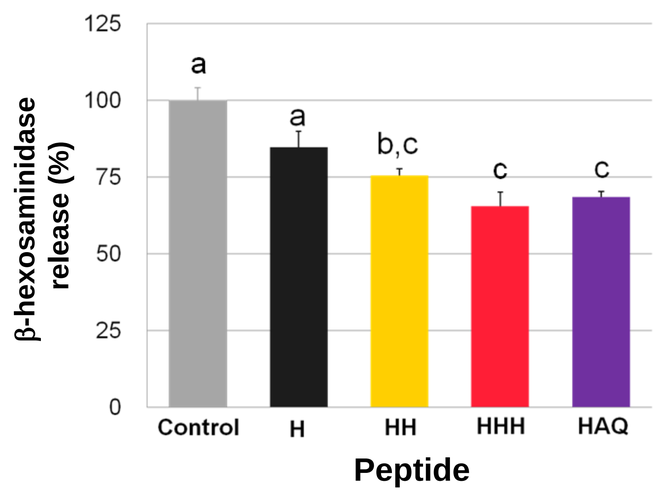
Fig.1 Effects of H, HH, HHH and HAQ peptides on β-hexosaminidase release from RBL-2H3 cells.



A significant inhibitory effect on the release of β-hexosaminidase was found with all imidazole peptides, depending on the dose.

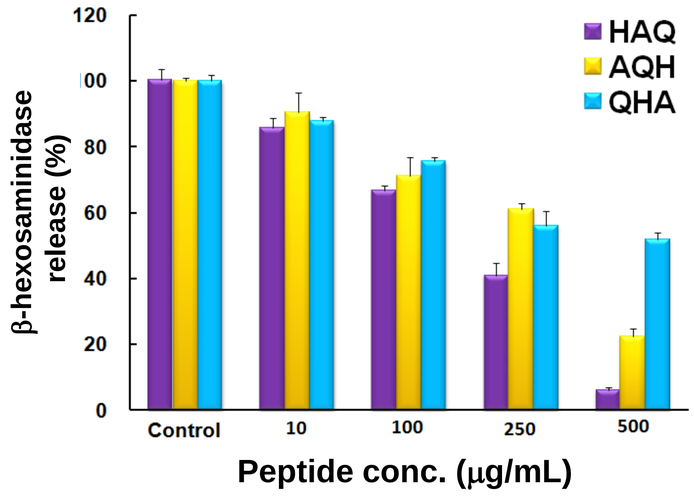
Fig.2 Effects of four imidazole peptides on β-hexosaminidase release from RBL-2H3 cells. (peptide conc. 100μM)

Post hoc test for One-Way ANOVA, p<0.05



The strength of the anti-allergic effect of the peptides was, in decreasing order, **HAQ=HHH>HH**.

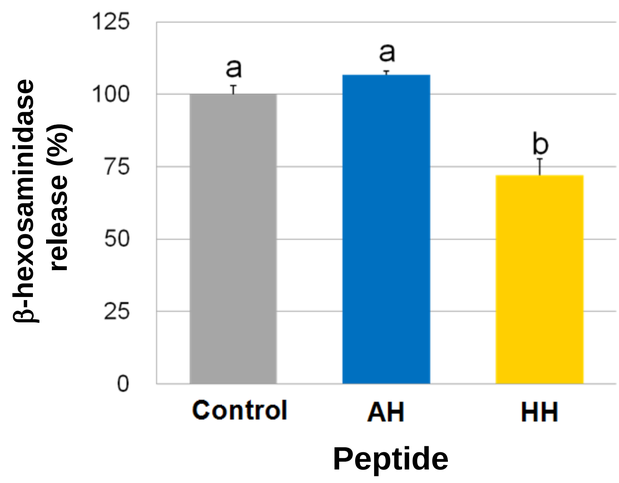
Fig.3 Effects of HAQ, AQH and QHA peptides on β-hexosaminidase release from RBL-2H3 cells.



Strong inhibition of degranulation was found under HAQ peptide stimulation. The strength of the anti-allergic effect of the peptides was, in decreasing order, **HAQ>AQH>QHA**.

Fig.4 Effects of Carnosine (AH) and HH peptides on β-hexosaminidase release from RBL-2H3 cells.

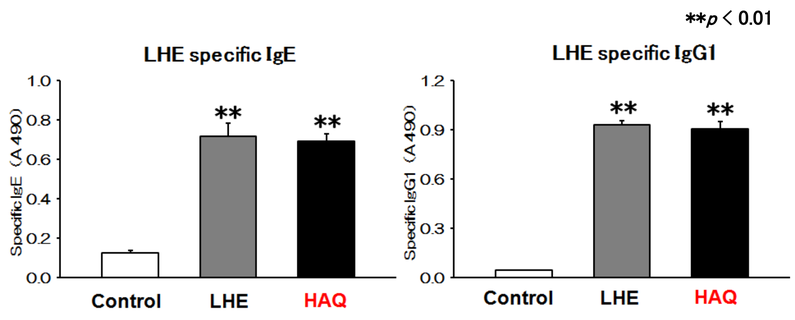
Post hoc test for One-Way ANOVA, p<0.05



Inhibitory effect on the release of β-hexosaminidase wasn't found with carnosin (AH) peptide.

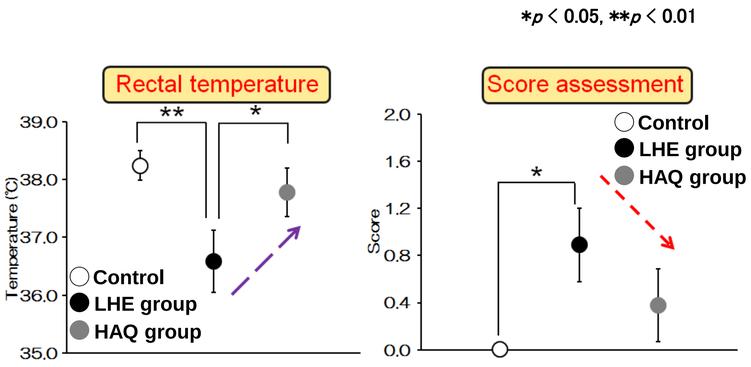
Peptide sequence specificity is important for the manifestation of degranulation inhibitory activity.

Fig.5 Effects of HAQ peptide on the specific anti-LHE IgE and IgG1



The specific anti-LHE IgE and IgG1 of HAQ group didn't decrease significant more than LHE group.

Fig.6 Effects of HAQ peptide on allergic symptoms



LHE group evoked significant decreases in body temperature than control group, but HAQ group did not. In score assessment , LHE group was significantly higher than control group and HAQ group.

Conclusion

The level of degranulation inhibitory activity depended on the number of histidine residues in peptide, and peptide sequence specificity is important for the manifestation of degranulation inhibitory activity. It was suggested that HAQ peptide has anti-allergic effect *in vitro* and *in vivo*.