

Synergistic Effect of Lysozyme on Bactericidal Activity of Magnolol and Honokiol against a Cariogenic Bacterium, *Streptococcus mutans* OMZ 176

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Abstract □ A combination of magnolol or honokiol with lysozyme isolated from the egg white of the Korean Ogol fowl (Korean natural monument No. 265) exhibited synergistic effect of bactericidal activity against a typical cariogenic bacterium, *Streptococcus mutans* OMZ 176. The synergistic ratio increased with time dependence.

Keywords □ Magnolol, honokiol, lysozyme, Ogol fowl, *Streptococcus mutans* OMZ 176.

The role of *Streptococcus mutans* in the initiation of dental caries in humans has been extensively studied¹⁾. Lysozyme inhibits growth and lyses gram-positive oral bacteria, including *S. mutans*²⁻⁵⁾. Twetman also reported that human salivary lysozyme reduced incorporation of the nonutilizable glucose analogue 2-deoxyglucose (2DG) and L-(+)-lactic acid production from glucose in *S. mutans* BHT, a serotype b strain⁶⁾ and the effect of human lysozyme on 2-deoxyglucose uptake by *S. mutans* including other oral microorganisms⁷⁾. On the other hand, magnolol and honokiol isolated from the stem bark of *Magnolia obovata* had been elucidated to show potent antibacterial activity against *S. mutans* by us⁸⁾. The minimal concentration (MIC) of both components was 6.3 μ g/ml, their antibacterial activity was more potent than that of berberine known as a typical naturally occurring antibacterial component. In view of these findings, it was of interest to study of synergistic effect of lysozyme on the bactericidal activity of magnolol and honokiol against a cariogenic bacterium, *S. mutans* OMZ 176, a serotype d.

Lysozyme used in the experiment was isolated and purified partially from the egg white of Korean native Ogol fowl by the method of crystallization⁹⁾. Ogol fowl's egg white lysozyme was compared its bacteriolytic activity against *Micrococcus lysodeikticus* with that of hen's egg white lysozyme.

EXPERIMENTAL METHODS

*Lysozyme preparatin*⁹⁾

The egg white 1 kg of Ogol fowl (Korean natural monument No. 265) was purchased from Nosan, Chungnam (Aug. 1987), and homogenated. The homogenate was stirred after adding 50g NaCl and adjusted to pH 9.5 with 1 N-NaOH. The seed lysozyme was added to the homogenate and stored at 4° for 3 days. The precipitated lysozyme was obtained from centrifuging at 5,000 rpm for 10 minutes. The produced lysozyme was suspended with distilled water and adjusted to pH 4.5 with acetic acid, then insoluble residue was discarded by centrifuging. The purified lysozyme was recovered from repeated procedures described as above. The bacteriolytic activity of purified lysozyme was determined spectrophotometrically at 450 nm using *M. lysodeikticus* (Sigma) as the substrate.

Isolation of magnolol and honokiol

The compounds were isolated from the stem bark of *Magnolia obovata* by the method of Fujita *et al.*,¹⁰⁾ and identified with the spectra of NMR and IR.

Bactericidal activity

Prior to testing, *S. mutans* was activated in brain heart infusion (BHI, Difco) at 37° overnight. The turbidity of bacterial suspension was adjusted with the same broth to the optical density of about 0.07 absorbance at 550 nm. The samples, 1% lysozyme, 0.0001% magnolol and 0.0001% honokiol, a mixture of 1% lysozyme and 0.0001% magnolol or the one of 1% lysozyme and 0.0001% honokiol were added to Bausch and Lomb Spectronic-20 col-

orimeter tubes. They were incubated at 37° and, at intervals, 1, 24, 48 and 72 hours, the optical density was measured at 550 nm.

RESULTS AND DISCUSSION

Under the condition of our assay, 1% lysozyme, 0.0001% magnolol and 0.0001% honokiol did not show any significant drop in absorbancy (Fig. 1, 2 and Table I). The inhibitory effects (IE, %) of 1% lysozyme were 5.7, 4.9, 9.8 and 7.4% during the time intervals, 1, 24, 48 and 72 hours, respectively. The IEs of 0.0001% magnolol and 0.0001% honokiol were in the range of 7.3 to 13.2%. The concentration of magnolol and honokiol used in this experiment was diluted less than the MIC value ($6.3 \mu\text{g}/\text{m}l$)⁸) to see the synergistic effects with 1% lysozyme. Therefore, it was considered that 0.0001% (1 $\mu\text{g}/\text{m}l$) magnolol and 0.0001% honokiol showed about 10% IEs only (Table I).

In contrast, the combination of 1% lysozyme and 0.0001% magnolol showed a significant decrease in optical density indicative of evident synergistic effect (Fig. 1). The same bacteriolytic phenomenon was also found with 1% lysozyme and 0.0001% honokiol (Fig. 2). The IEs of combination of 1% lysozyme and 0.0001% magnolol were 14.3, 19.5, 41.0 and 41.2% during the time intervals, 1, 24, 48 and 72 hours, respectively. The synergistic ratios (SR, %) of them were 0, 7.3, 18.1 and 21.0% for 1, 24, 48 and 72 hours, that is, the SR increased with time dependence (Table I). The same synergistic

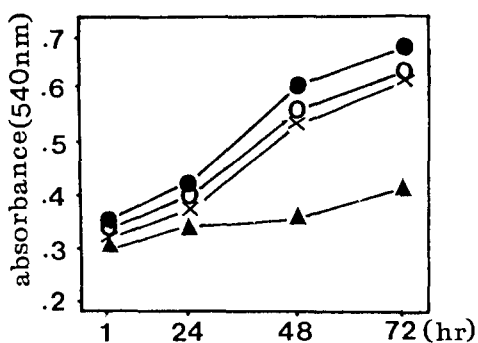


Fig. 1. Bactericidal activity of magnolol and synergistic effect with lysozyme against *Streptococcus mutans* OMZ 176.

Changes in optical density of a suspension of *S. mutans* incubated at 37° were investigated in the presence of nutrient broth control.

● : Control, ○ : 1% Lysozyme, × : 0.0001% Magnolol, ▲ : 0.0001% Magnolol + 1% Lysozyme.

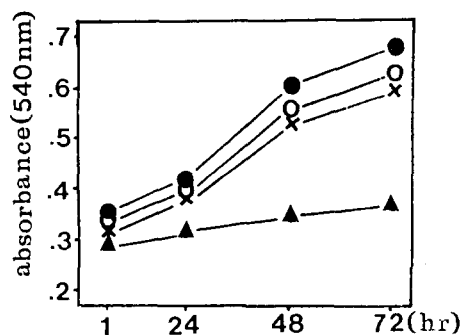


Fig. 2. Bactericidal activity of honokiol and synergistic effect with lysozyme against *Streptococcus mutans* OMZ 176.

● : Control, ○ : 1% Lysozyme, × : 0.0001% Honokiol, ▲ : 0.0001% Honokiol + 1% Lysozyme.

Table I. The inhibitory effects of Lysozyme, Magnolol and Honokiol, and their synergistic ratio against *Streptococcus mutans* OMZ 176.

Compounds	Inhibitory effect (%) ¹⁾				Synergistic ratio (%) ²⁾			
	1 hr	24 hr	48 hr	72 hr	1 hr	24 hr	48 hr	72 hr
1% L	5.7	4.9	9.8	7.4				
0.0001% M	8.6	7.3	13.1	11.8				
0.0001% H	8.6	10.0	14.8	13.2				
1% L + 0.0001% M	14.3	19.5	41.0	41.2	0	7.3	18.1	21.0
1% L + 0.0001% H	17.1	22.0	44.3	45.6	2.8	7.1	21.0	25.0

The L, M and H denote Lysozyme, Magnolol and Honokiol, respectively.

¹⁾ Inhibitory effect (IE, %)

$$= 100 - \frac{\text{Absorbance at 540 nm of Compounds}}{\text{Absorbance at 540 nm of Control}} \times 100$$

²⁾ Synergistic ratio (%) = IE of Lysozyme with Synergistic compounds - IE of (Lysozyme + Synergistic compounds)

phenomenon was also occurred in the combination of 1% lysozyme and 0.0001% honokiol. The present experiments suggest a common basis for the bacteriolytic and bactericidal effects produced by lysozyme and magnolol (or honokiol). The results allowed to conclude that the bactericidal activity of magnolol and honokiol had been rendered to increase when occurred bacteriolysis by lysozyme.

The bacteriolytic activity of Ogo fowl's egg white lysozyme against *M. lysodeikticus* was 45,000 units, and almost same one of hen's egg white lysozyme.

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