Arch. Pharm. Res. Vol.19, No.6, pp. 447-449, 1996

Anti-*Helicobacter pylori* Activity of Mushrooms

Dong-Hyun Kim[1]*, Eun-Ah Bae[2], Il-Sung Jang[1] and Myung Joo Han[2]

[1]College of Pharmacy and [2]Dept of Foods and Nutrition, Kyung-Hee University, Dongdaemun-ku, Seoul 130-701, Korea

(Received July 2, 1996)

Inhibitory effects of the mushrooms on the growth and urease of *Helicobacter pylori* (HP), which is associated with human gastroduodenal diseases such as gastritis, peptic ulcer and gastric carcinoma, were investigated. Most of the mushroom extracts did not show inhibitory effect on HP urease except *Coriolus versicolar, Auricularia auricula, Sarcodon aspratus* and *Flammulina velutipes*. The extract of *Ganoderma lucidum, Coriolus versicolar, Gyropora esculenta* and *Agaricus bisporus var. albidus* inhibited the growth of HP. When their extracts were fractionated, the ether fraction of *Ganoderma lucidum* and *Agaricus bisporus var. albidus* were the most effective. Among seven components seperated from the ether fraction of *G. lucidum* extract by silica gel column chromatography, P3 was the most potent: MIC was 200 μg/ml. However, P3 did not inhibit the urease.

Key words : *Helicobacter pylori*, MIC, mushrooms, *Ganoderma lucidum*

INTRODUCTION

Helicobacter pyolri (HP) was isolated from the gastric antrum of chronic gastritis patients by Warren and Marshall in 1983. Pathogenic HP produces urease strongly, which hydrolyzes urea to CO_2 and ammonia. This ammonia generated by HP protects itself from the environment of gastric acid in stomach, and damages directly the gastric mucosal cell. HP also produces a vacuolating toxin and its toxicity may be potentiated by urease-mediated ammonia production (Marshall *et al.*, 1990; Smoot *et al.*, 1990; Tsujii *et al.*, 1992). HP urease is considered to play critical roles in the pathogenesis of gastritis and peptic ulcer. HP is also believed to promote gastritis to gastric cancer. Therefore, eradication of the bacteria and inhibition of the urease are important for the treatment of patients with gastroduodenal diseases (Eaton *et al.*, 1991).

Several trials in USA and Western Europe have shown that HP could be eradicated by mixed therapeutic agents such as antibiotics, bismuth subsalicylate, proton pump inhibitors and H_2-blockers (Hentschel *et al.*, 1993). However, all these drugs were administered for a long period for eradication so that adverse side-effects often occurred in patients. On the other hand, mushrooms extracts have been used as the oriental traditional medicine or the health food for thousands of years in Korea, China, Japan and other Asian countries. Also, mushrooms as well as herbs have been used for the treatment of gastritis in folk cure. Anti-*Helicobacter pylori* activity of traditional medicines has not been studied except the report of Kobashi *et al.* (1995) that water extract of some traditional herbs inhibited the growth of HP. Therefore, it is valuable to screen what kinds of the mushrooms could inhibit the growth and urease of HP. In the present study, water extracts of nine mushrooms were investigated for their inhibitory effects against urease activity and growth of HP *in vitro*.

Correspondence to: Dong-Hyun Kim, College of Pharmacy, Kyung-Hee University, Dongdaemun-ku, Seoul 130-701, Korea

METHODS

Bacterial strain

HP ATCC43504 was purchased from ATCC. It was inoculated into brucella agar plates supplemented with 7% horse serum and cultured for three days at 37°C in an anaerobic jar with AnaeroPak Campylo.

Preparation of water extract of each mushroom

Nine species of mushrooms were purchased from Kyung-Dong Market, Seoul, Korea. These materials were extracted with D.W. at 80°C for 6h and then the extracts were evaporated to concentrate. These extracts were fractionated with ether, ethylacetate, butanol and residue (water).

Preparation of HP urease

HP was inoculated from an agar plate into 30 ml of

brucella broth supplemented with 10% fetal calf serum in a 100 ml flask, which was placed in an anaerobic jar. The harvested cells were washed with 10 ml of 20 mM phosphate buffer, pH 7.0, sonicated and centrifuged at 5000×g for 30 min. The resulting supernatant was used as the crude enzyme.

Assay of urease activity

Urease activity was determined according to the method of Gutmann and Bergmyer (1994).

Reaction mixture was composed of 50 µl enzyme solution, 50 µl distilled water (or each extract of the mushroom) and 50 µl urea. It was incubated for 30 min at 37°C and then added 100 µl of 1 N H_2SO_4. 1 ml each of phenol reagent (1% phenol and 0.005% sodium nitroprusside) and alkali reagent (5.5% Na_2HPO $4.12H_2O$, 0.5% NaOH and 0.1%NaOCl) were added to each mixture. After incubation at 60°C for 20 min, the absorbance at 660 nm was measured. All experiment were done in triplicate.

Growth inhibition assay of HP

Each extract was added into petri dish containing unsolidified brucella agar supplemented with 7% horse serum: final concentration of each extract was 10 mg/ml. And then HP was inoculated into the agar plates and cultured microaerobically for three days at 37°C in an anaerobic jar. All experiments were performed in duplicate. The inhibitory activities of the extracts on the growth of HP was determined by judging visually with four grades as very strong (+++, 100% inhibition), strong (++, 100-75% inhibition), weak (+, 75-30% inhibition) and no(-). Those of the compounds from ether extract of G. lucidum were determined by the standard two fold-dilution agar method. The MIC of the compound was determined by judging visually the microbial growth in the series of test agar plates. Ampicillin was used as a positive control.

RESULTS AND DISCUSSION

Effect of each mushroom extract on HP urease

Table I. Inhibitory effects of mushrooms on the urease of *Helicobacter pylori*

Mushroom[1)]	Inhibition (%)
Lentinus edodes	0
Ganoderma lucidum	1
Sarcodon aspratus	10
Coriolus versicolar	8
Auricularia auricula	9
Gyropora esculenta	3
Flammulina velutipes	12
Pleurotus ostreatus	1
Agaricus bisporus var. albidus 0	0

[1)]Final concentration was 0.3 mg/ml.

The inhibitory effect of the water extract of each mushroom on urease activity was shown in Table 1. Most of the mushroom did not show inhibitory effect. Among them, the extracts of *S. aspratus, C. versicolar, A. auricular* and *F. velutipes* weakly inhibited the urease activity at 0.3 mg/ml. Therefore, the water extracts of these mushrooms were not fractionated.

Effect of each mushroom extract on HP growth

The effect of each water extract of the mushrooms on the growth of HP was shown in Table II. The extracts of *G. lucidum, C. versicolar, G. esculenta* and *A. bisporus var. albidus* inhibited the growth of HP. As a standard, ampicillin, which is used for eradication of HP, was tested under the same procedure and condition. MIC of ampicillin was found to be 0.5 µg/ml media. In order to isolate the active component of the mushrooms, their extracts were frac-

Table II. Inhibitory effects of mushrooms on the growth of *Helicobacter pylori*

Mushroom[1)]	Growth Inhibition
Lentinus edodes	-
Ganoderma lucidum	+++
Sarcodon aspratus	-
Coriolus versicolar	++
Auricularia auricula	-
Gyropora esculenta	++
Flammulina velutipes	-
Pleurotus ostreatus	-
Agaricus bisporus var. albidus	+++
Ampicillin	+++

[1)]Final concentration of the mushroom was 10mg/ml except ampicillin (1 µg/ml).

Table III. Inhibitory effect of each fraction of mushrooms on the growth of *Helicobacter pylori*

Mushroom[1)]	Fraction	Growth Inhibition
G. lucidum	Ether	+++
	EtOAc	-
	BuOH	-
	Residue	-
C. versicolar	Ether	+++
	EtOAc	-
	BuOH	-
	Residue	-
A. bisporus var. albidus	Ether	+++
	EtOAc	+++
	BuOH	-
	Residue	-
G. esculenta	Ether	+++
	EtOAc	+
	BuOH	-
	Residue	-
Ampicillin		+++

[1)]Final concentration of the mushroom was 10 mg/ml except ampicillin (1 µg/ml).

Table IV. Inhibitory effect of compounds seperated from *G. lucidum* on the growth of *Helicobacter pylori*

Compound	Rf value[1)]	MIC (μg/ml)
P1	0.76	>200
P2	0.74	>200
P3	0.68	100
P4	0.57	>200
P5	0.47	>200
P6	0.39	>200
P7	0.29	>200
Ampicillin	0.5	

[1)]TLC Developing conditions: plate, silica gel $60F_{254}$ (Merk); solvents, $CHCl_3$/MeOH (4:1)

tionated and their inhibitory potency on the growth of HP was measured. As shown in Table III, the most effective fraction was ether fraction of the tested mushrooms. Among them, the ether fraction of *G. lucidum* and *A. bisporus var. albidus* were the most effective. And then we seperated seven components from the ether fraction of *G. lucidum* extract by silica gel column chromatography and the inhibitory potency was compared. Among them, P3 was the most potent: MIC was 100 μg/ml. However, P3 did not inhibit urease. Hydroxamic acid known as a potent urease inhibitor inhibited strongly urease of HP and in vivo the growth of HP (Kobashi *et al.*, 1974). Thus, the HP growth inhibition of P3 is different to that of hydroxamic acid.

The eradication of HP has been known to cure gastritis and prevents the relapse of duodenal ulcer. Ampicillin showed inhibitory effects on growth of HP at two-order lower concentration. Therefore, further isolation of active components in mushrooms or the other source are necessary for clinical use.

Finally, mushrooms are consumed frequently in our diet. Therefore, they are believed to contribute to the prevention of the gastritis in some degree, even if they are not the potent growth inhibitor.

REFERENCES CITED

Eaton, K. A., Brooks, C. L. Morgan, D. R. and Krakowka, S., Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. *Infec. Immun.*, 59, 2470-2475, (1991)

Goldie, J., Veldhuyzen van Zanten, S. J. O., Jalali, S., Richardson, H. and Hunt, R. H., Inhibition of urease activity but not growth of *Helicobacter pylori* by acetylhydroxamic acid. *J. Clin. Pathol.*, 44, 695-697, (1991).

Gutmann, I. and Bergmeyer, H. U., Urea, In Bergmeyer, H. U. (Eds.), *Methods of enzymatic analysis.* vol.4, Academic Press, Inc., New York, 1974, pp. 1791-1794.

Hentschel, E., Brandstatter, G., Dragsics, B., Hirschil, A. M., Nemec., H., Schutze, K., Taufer, M. and Wurzer, H., Effect of ranitidine and amoxacillin plus metronidazole on the eradication of *Helicobacter pylori* and the recurrence of duodenal ulcer. *N. Engl. J. Med.*, 328, 308-312, (1993).

Immamura, L., Tsuchiya, M., Inada, A., Nakanishi, T. and Kobashi, K., Inhibition of urease and growth of *Helicobacter pylori* by herb extracts. *J. Traditional Medicines,* 12, 129-136, (1995).

Kobashi, K., Hase, J. and Uehara, K., Specific inhibition of urease by hydroxamic acid. *Biochim. Biophys. Acta*, 65, 380-383, (1962).

Marshall, B. J., Barrett, L. J., Prakash, C., McCallum, R. W. and Guerrant, R. L., Urea protects *Helicobacter pylori* from the bactericidal effect of acid. *Gastroenterology*, 99, 697-702, (1990).

Smoot, D. T., Mobley, H. L. T., Chippendale, G. R., Lewison, J. F. and Resau, J. H., *Helicobacter pylori* urease activity is toxic to human gastric epithelial cells. *Infect. Immun.*, 58, 1992-1994, (1990).

Tsujii, M., Kawano, N., Tsujii, S., Fusamoto, H., Kamada, T. and Sato, N., Mechanism of gastric mucosal damage induced by ammonia. *Gastroenterology* 102, 1881-1888, (1992).

Warren, J. R. and Marshall, B. J., Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet*, 1, 1273-1275, (1983).