韓 國 菌 學 會 誌 第15卷 第2號
Kor. J. Mycol. Vol.15, No.2, 87-91, 1987

Effects of Ginseng Saponin on the Antimicrobial Activities of Some Antibiotics

Hack-Seang Kim, Seong-Sun Han, Ki-Wan Oh, Tae-Seup Jeong and Ki-Yeul Nam*

College of Pharmacy, Chungbuk National University, Cheongju 310, *Korea*
*Korea Ginseng & Tobacco Research Institute**, *Daejeon* 300-01, *Korea*

數種 抗生物質의 抗菌作用에 對한 人蔘사포닌 分劃의 效果

金學成・韓星淳・吳基完・鄭太燮・南基烈*
忠北大学校 薬学大学 韓国人蔘煙草研究所*

ABSTRACT: The in vitro interactions between antibiotics(ampicillin, kanamycin, cephalexin, oxytetracycline and chloramphenicol) and ginseng saponin were studied by the chessboard method against bacteria(*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Mycobacterium smegmatis*).

Ampicillin, kanamycin, oxytetracycline and chloramphenicol against *Bacillus subtilis,* and ampicillin and cephalexin against *Staphylococcus* aureus were synergistic in the presence of ginseng saponin. Whereas kanamycin, oxytetracycline and chloramphenicol against *Staphylococcus aureus,* and ampicillin, Kanamycin, cephalexin, oxytetracycline and chloramphenicol against *Escherichia coli,* and ampicillin, kanamycin, and chloramphenicol against *Pseudomonas aeruginosa,* and ampicillin and chloramphenicol against *Mycobacterium smegmatis* were indifferent in the presence of ginseng saponin. Antagonisms between antibiotics and ginseng saponin were not observed in this study.

KEYWORDS: Ginseng Saponin. Interactions. Antibiotics. Chessbord method.

Panax ginseng has been used as a tonic and drug of longivity for thousands years in many countries such China, Korea, Japan and Russia.(Lee, 1980). Studies of the effects of the ginseng on microorganisms have shown that both the volatile fraction and the root juice are stimulants for the growth of bacteria(*Bacillus megatherium, Serratia marcescens* and organisms of garlic root), and the water-alcohol extract from leaves, blossoms and rootlets has bactericidal activities, while the same water-alcohol extract from main root, dried sprouts and stems acts as mild stimulants. (Gramenitskaya *et al.,* 1956).

It has been reported that the growth of *Bacillus subtilis* in a medium containing 0.3% ginseng saponin was faster than that in a medium without it.(Cho *et al.,* 1981). When the *Escherichia coli* were grown on a medium containing 0.1% ginseng saponin, the growth was faster than that of the control(Cho *et al.,* 1981). And in combination with 6 MFA(antiviral substance of fungal origin), ginseng saponin enhanced significantly the protection of mice against Semliki Forest Virus(SFV) compared to 6 MFA alone when administered orally(Singh *et al.,* 1983), and the combined administration of INAH and ginseng saponin did not show any inhibition of *Mycobacterium*(Pyun *et al.,* 1969).

Nowadays, Panax ginseng or Panax ginseng products with other drugs are widely used all over the world without any research on their interactions. In a preliminary study of the administration of ginseng combined with other drugs, we investigated the in vitro interaction of antibiotics and

Table I. Test strains

Gram positive Bacteria	*Bacillus subtilis* ATCC 6633
	Staphylococcus aureus ATCC 25923
Gram negative Bacteria	*Escherichia coli* ATCC 2592 25923
	Pseudomonas aeruginosa ATCC 25619
Acid-fast Bacteria	*Mycobacterium Smegmatis* R 31

the types produced, namely synergistic, indifferent (or additive) and antagonistic (Victor, S. 1980).

Materials and Method

Materials

The test strains appeared in Table I were supplied from the Department of Microbiology, Chungbuk National University. The antibiotics were provided from the pharmaceutical companies appeared in Table II, and ginseng saponin (obtained and purified from ginseng butanol fraction by the usual method) was kindly supplied from the Korea Ginseng and Tobacco Research Institute.

Table II. Antibiotics and ginseng saponin

Test drugs	Activities (μg/mg)	Donors
Ampicillin sodium	910	Chong Kun Dang Co.
Kanamycin sulfate	610	Dong A PHarm. Co.
Cephalexin	970	Dong Wha Pharm. Ind. Co.
Oxytetracycline HCI	980	Chong Kun Dang Co.
Chloramphenicol	980	Chong Kun Dang Co.
Ginseng saponin		Kor. Gin. & Toba. Res. Ins.

Test strains, antibiotics and ginseng saponin used in this study were as follows.

Method

Interaction studies between antibiotics and ginseng saponin were carried out by the chessboard method(Findly *et al.*, 1983 ; Stenesh, 1980 ; Neuman, 1981). The basic procedure was to determine the minimum inhibitory concentration(MIC) of antibiotics by preparing in both serial two dilutions of each at one half, one fourth and one eighth, etc. Test tubes containing approximately 10^{5-6} orgainsms were examined for growth after 18-20 hours of incubation at 37℃. MICs were determined visually for lack of turbidity(Han *et al.*, 1971). Certain antibiotics were only to test some strains depending on their sensitvities.

Table III. Effects of ginseng saponin on the antimicrobial activities of ampicillin, kanamycin, oxytetracycline and chloramphenicol against *Bacillus subtilis*

Antibiotiics (μg/ml)		Ginseng Saponin (μg/ml)			
		0	150	300	600
Ampicillin	1.8	+	+	+	+
	3.3	+	+	+	−
	7.2	+	+	−	−
	14.4	−	−	−	−
	28.8	−	−	−	−
Kanamycin	0.04	+	+	+	+
	0.08	+	+	+	+
	0.16	+	+	−	−
	0.32	−	−	−	−
	0.64	−	−	−	−
Oxytetracycline	0.03	+	+	+	+
	0.06	+	+	+	+
	0.12	+	+	−	−
	0.25	−	−	−	−
	0.50	−	−	−	−
Chloramphenicol	1.0	+	+	+	+
	2.0	+	+	+	+
	4.0	+	+	−	−
	8.0	−	−	−	−
	16.0	−	−	−	−

+ growth − not growth

Results and Discussion

Their own MICs of ampicillin, kanamycin, oxytetracycline and chloramphenicol were 14.4 μg/m*l*, 0.32 μg/m*l*, 0.25 μg/m*l* and 8.0 μg/m*l* against *Bacillus subtilis* respectively in this study. These values were in agreement with available literature(Victor, 1980). In 300 μg/m*l* of ginseng saponin, MICs of ampicillin, kanamycin, oxytetracycline and chloramphenicol were 7.2 μg/m*l*, 0.16 μg/m*l*, 0.12 μg/m*l* and 4.0 μg/m*l* respectively, and in 600 μg/m*l* of ginseng saponin MIC of only ampicillin appeared lower than that in 300 μg/m*l* of ginseng saponin. Their own MICs of ampicillin and cephalexin were 7.2 μg/m*l* and 8.0 μg/m*l* respectively. In 300 μg/m*l* of ginseng saponin, MICs of ampicillin and cephalexin were 3.6 μg/m*l* and 4.0 μg/m*l*, showing synergistic effects. Kanamycin, oxytetracycline and chloramphenicol against *Sta*

Table IV. Effects of ginseng saponin on the antimicrobial activities of ampicillin, kanamycin, oxytetracycline, cephalexin and chloramphenicol against *Staphylococcus aureus*

Antibiotics (μg/ml)		Ginseng Saponin (μg/ml)			
		0	150	300	600
Ampicillin	1.8	+	+	+	+
	3.6	+	+	−	−
	7.2	−	−	−	−
	14.4	−	−	−	−
	28.8	−	−	−	−
Kanamycin	0.02	+	+	+	+
	0.04	+	+	+	+
	0.08	−	−	−	−
	0.16	−	−	−	−
	0.32	−	−	−	−
Oxytetracycline	0.03	+	+	+	+
	0.06	+	+	+	+
	0.12	−	−	−	−
	0.25	−	−	−	−
	0.50	−	−	−	−
Cephalexin	1.0	+	+	+	+
	2.0	+	+	+	+
	4.0	+	+	−	−
	8.0	−	−	−	−
	16.0	−	−	−	−
Chloramphenicol	1.0	+	+	+	+
	2.0	+	+	+	+
	4.0	−	−	−	−
	8.0	−	−	−	−
	16.0	−	−	−	−

Table V. Effects of ginseng saponin on the antimicrobial activities of ampicillin, kanamycin, oxytetracycline, cephalexin and chloramphenicol against *Escherichia coli*

Antibiotics (μg/ml)		Ginseng Saponin (μg/ml)			
		0	150	300	600
Ampicillin	0.9	+	+	+	+
	1.8	+	+	+	+
	3.6	+	+	+	+
	7.2	−	−	−	−
	14.4	−	−	−	−
Kanamycin	0.04	+	+	+	+
	0.08	+	+	+	+
	0.16	+	+	+	+
	0.32	−	−	−	−
	0.64	−	−	−	−
Oxytetracycline	0.03	+	+	+	+
	0.06	+	+	+	+
	0.12	+	+	+	+
	0.25	−	−	−	−
	0.50	−	−	−	−
Cephalexin	1.0	+	+	+	+
	2.0	+	+	+	+
	4.0	+	+	+	+
	8.0	−	−	−	−
	16.0	−	−	−	−
Chloramphenicol	1.0	+	+	+	+
	2.0	+	+	+	+
	4.0	+	+	+	+
	8.0	−	−	−	−
	16.0	−	−	−	−

Table VI. Effects of ginseng saponin on the antimicrobial activities of ampicillin, kanamycin and chloramphenicol against *Pseudomonas aeruginosa*

Antibiotics (μg/ml)	Ginseng Saponin (μg/ml)			
	0	150	300	600
Ampicillin 1.8	+	+	+	+
3.6	+	+	+	+
7.2	+	+	+	+
14.4	−	−	−	−
28.8	−	−	−	−
Kanamycin 5.1	+	+	+	+
10.2	+	+	+	+
20.4	−	−	−	−
40.8	−	−	−	−
81.6	−	−	−	−
Chloramphenicol 1.0	+	+	+	+
2.0	+	+	+	+
4.0	+	+	+	+
8.0	−	−	−	−
16.0	−	−	−	−

Table VII. Effects of ginseng saponin on the antimicrobial activities of ampicillin and chloramphenicol against *Mycobacterium smegmatis*

Antibiotics (μg/ml)	Ginseng Saponin (μg/ml)			
	0	150	300	600
Ampicillin 1.8	+	+	+	+
3.6	+	+	+	+
7.2	+	+	+	+
14.4	−	−	−	−
28.8	−	−	−	−
Chloramphenicol 1.0	+	+	+	+
2.0	+	+	+	+
4.0	−	−	−	−
8.0	−	−	−	−
16.0	−	−	−	−

phylococcus aureus, ampicillin, cephalexin and oxytetracycline against *Pseudomonas aseruginosa* and ampicillin and chlorampenicol against *Mycobacterium smegmatis* in the presence of ginseng saponin were indifferent. Anatgonisms between antibiotics and ginseng saponin were not observed in this study.

Cho *et al.*, reported that the lysis of Gram-positive and negative bacteria grown in a medium containing ginseng saponin by lysozyme was faster than that of control(Cho *et al.*, 1981 : Cho *et al.*, 1981). The lysozyme caused lysis of bacteria by hydrolyzing the(1-4) glycosidic bonds of the polysaccharide backbone of peptidoglycan(Findly *et al.*, 1983). The cell wall acted as a corseting structure, protecting the cell against osmotic lysis. Thus those agents that destroyed the cell wall such as lysozyme and prevented its normal synthesis, such as penicillins, may cause lysis of the cell(Stenesh, 1980). The combined interactions between antibiotics and ginseng saponin were synergistic against Gram-positive bacteria but not Gram-negative bacteria. And these synergistic effects may be related to the fact that the peptidoglycan layer is much thicker in the cell of Gram-positive bacteria than in the cell wall of Gram-negative bacteria.

Ampicillin and cephalexin showing antimicrobial actions through the inhibition of cell wall synthesis were more effective against Gram-positive bacteria than against Gram-negative bacteria.

One of the most intriguing questions is whether or not in vitro synergism is associated with improved clinical outcome. The opposite situations may hide a clinically useful combination. The conditions in which antibacterial agents and ginseng saponin are expected to exert their effect in treatment in clinical situations may be different from the conditions of in vitro situations in which antibiotics and ginseng saponin were tested(Neuman, 1981).

摘 要

수종 항생물질의 항균력에 대한 인삼사포닌의 영향에 대하여 시험관 내에서 항균력 실험을 실시한 바 다음과 같은 결과를 얻었다.

Ampicillin. kanamycin, oxytetracycline 및 chloramphenicol은 *Bacillus subtilis*에 대하여 chloramphenicol은 *Staphylococcus aureus*에 대하여 상승효과를 나타냈으며 kanamycin, oxytetracy-

cline 및 chloramphenicol은 *Staphylococcus aureus*에 대하여, ampicillin, kanamycin, cephalexin 및 oxytetracycline은 *Escherichia coli*에 대하여, ampicillin, kanamycin 및 chloramphenicol은 *Pseudomonas aeruginosa*에 대하여, ampicillin 및 chloramphenicol은 *Mycobacterium smegmatis*에 대하여 각각 상가효과를 나타내었으나 길항작용은 관찰되지 않았다.

Acknowledgement

Ginseng saponin was kindly supplied by the Korea Ginseng and Tobacco Research Institute. We express our gratitude for this generous assistance.

References

Cho, Y.D., Kim, N.D. and Kim, C.W.(1981) : A study on the effect of ginseng saponin fraction on cell wall(II), *Korean Biochem. J.* **14** : 295-302.

Cho, Y.D., Kim, U.T. and Cho, H.G.(1981) : A study on the effect of ginseng saponin fraction on cell wall, *Korean, J. ginseng Sci.* **5** : 65-72.

Findly, J.E. and Akag J.M.(1983) : *Assessment of antimicrobial activity and resistance.* 299-303.

Gramenitskaya, V.G. and Grashvitskii I.V. (1956) : Effect of ginseng on microorganimsm. Mikrobiologiya. **25** : 221-6 CA 50 : 12186.

Han, S.S., Kim, B.K., Jeong, D.S. and Lee, J.K. (1971) : *Experimental Microbiology,* Chung Ang Univ. Press, p.83-85.

Lee, C.H.(1985) : *An introduction to Korean ginseng,* Korea Ginseng and Tobacco Research Institute. 3-13.

Neuman, M.(1981) : *Useful and Harmful interactions of antibiotics.* p.21-35.

Pyun, H.W. and Lee, C.S.(1969) : Influence of ginseng on experimental tuberculosis. *Katorik Taehak Uihakbu Normunjip,* **17** : 109-16.

Stenesh, J.(1980) : *Experimental Biochemistry.* 149-150.

Singh, V.K., George, C.S., Singh, N., Agarwal, S.S. and Gupa, B.M.(1983) : Amplification of host resistance to Semliki Forest virus, *Planta Medica* **47** : 234.

Victor, L.(1980) : *Antibiotics in Laboratory Medicine,* p.395-400.

Accepted for Publication 22 June 1987