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Effect of Hexane Extract of Acori graminei Rhizoma on the Growth of Chloramphenicol Resistant Bacteria

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Abstract – The combination of hexane extract (E4) of rhizome of *Acorus gramineus* with chloramphenicol (Cm) was applied to Gram negative Cm resistant microbials to find the possibility of clinical use and to clarify the relationship of the activity of chloramphenicol acetyltransferase (CAT). The combination of 1,000 μg/ml of E4 and 8 μg/ml of Cm entirely ceased the growth of S. aureus SA2, a gram positive resistant strain to 10 antibiotics. But in Gram negative strains which possess CAT activity, some showed considerably strong resistances to Cm and some did weakly.

Keywords – *Acorus gramineus*, antibiotics resistance, chloramphenicol, Gram negative bacteria, Gram positive bacteria, resistance inhibition, *Staphyloccoccus aureus*

Introduction

In spite of continuous investigation of new antibiotics, the newly investigated potent antimicrobial agent soon loose its activity due to the development of resistance (Shanson, 1981 and Jacoby and Archer, 1991) and many patients are sacrificed by several infectious diseases every year (Possner and Farr, 2002). So the reduction of resistance is thought to be valuable as well as developing newer and stronger antibiotics. In previous study, acorenone, an essential oil component from *Acorus gramineus* Soland (Araceae) was shown to be the active principle against multi-drug resistant microorganisms such as *Staphylococcus aureus* SA2 (Kim, *et al.*, 1998), which has resistances to 10 usual antibiotics including chloramphenicol (Cm). In this study the combination of hexane extract with Cm was applied to Gram negative Cm resistant microbials to clarify the possibility for clinical trials and relationship of the activity to chloramphenicol acetyltransferase.

Experiemental

Reagents – Tryptic soy broth (TSB) and agar were purchased from Difco Co., Cm, buffers and chloramphenicol acetyltransferase (CAT) were purchased from Sigma Co. Supplies for TLC were purchased from E. Merck.

Fractionation of acorus – The essential oil was collected by steam distillation and applied to column chromatography to obtain fractions ACH 1~10 with different TLC patterns. The fractions ACH 5~10, which showed same TLC pattern with hexane fraction of previous study (Kim, *et al.*, 1998) were pooled (E4) and used for the study.

Microbial strain (Table 1) – The strain *S. aureus* SA2 used in the studies was cultivated in the medium containing 10 antibiotics at the laboratory of our university (Kang and Moon, 1990; Kim, *et al.*, 1992; Lee and Moon, 1993), *E. tarda* JH10, *E. tarda* RE1, *V. damsella* JE1, *A. hydrophila* HA were kindly donated from Prof. Jung(?) of Pukyung Univ. and *S. enterotidis* #4 from Busan Metropolitan City Institute of Health and Environment.

MIC (Minimal inhibitory concentration) – The MIC of Cm was measured by serial dilution method. The tryptic soy agar containing 1×10^4 microorganisms and each diluted Cm was incubated for 24 hrs at 37°C and the growth of microorganisms was checked.

Table 1. Various Strains and Resistance Patterns used in this Study

Strains	Resistant to*
Staphylococcus aureus SA2	Am, Cm, Clm, Em, Gm, Km, Mc, Sm, Tc, Tm
Edwardsiella tarda JH10	Am, Cm, Cl, Km, Tc
Edwardsiella tarda RE1	Cm, Cl, Km, Na, Nf, Tc
Vibrio damsella JE1	Am, Cm, Km, Tc
Aeromonas hydrophila HA	Am, Cm, Cl, Km, Na, Oa, Tc
Salmonella enterotidis #4	Cm

*Abbreviation: Am, ampicillin; Cm, chloramphenicol; Cl, colistin; Clm, clindamycin; Em, erythromycin; Gm, gentamicin; Km, kanamycin; Na, nalidixic acid; Nf, norfloxacin; Oa, oxolinic acid, Tc, tetracyclin.

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CAT activity – CAT activity was determined by the method of Seed and Sheen (1988) based on the amount of acetyl-CoA measured by 5,5'-dithio-*bis*-2-nitrobenzoic acid, DTNB, Ellman, 1959).

Results and Discussion

Effect of the combination of E4 and Cm in *S. aureus* – The possibility of resistance inhibition will come from the lowering of MIC which was elevated by development of resistance. The reduction of MIC of Cm in *S. aureus* SA2 due to the combination with E4 is shown in Table 2. The MIC of Cm itself to *S. aureus* SA2 was shown 128 μg/ml. In previous study the MIC of Cm to *S. aureus* RN4220, a non-resistant strain, was 4 μg/ml (Kim, *et al.*, 2000). Differences of MIC value to microbial strain imply the possibility for clinical trials. The MIC of Cm was reduced dose-dependently when combined with E4. When 1,000 μg/ml of E4 was combined with Cm, the MIC was reduced to 1/16 of Cm itself (128 μg/ml). Single treatment of E4 in this level showed no effect on the growth of *S. aureus* SA2.

Effect of the combination in Gram negative strains – As Cm is generally used for Gram negative microbials, the effects of the combination in such strains were checked (Table 3). Some of the Gram negative microorganism generally showed less reduction of resistance contrary to S. aureus SA2, a Gram positive strain, by treatment of the combination. The MIC's of Cm itself to the strains *V. damsella* JE1 and *S. enteritidis* #4 were determined as high as 512 μg/ml and the MIC's of the combination were larger than 128 μg/ml. Such results mean that the strain possess resistance causing other mechanism than CAT.

Table 2. Effect of E4 on MIC of Cm in *S. aureus* SA2

E4 (μg/ml)	MIC (μg/ml)
0	128
200	32
400	32
600	16
800	16
1,000	8

Table 3. Effect of E4 on MIC to Cm in Gram negative bacteria

Strains	MIC (μg/ml)	
	Cm without E4	with E4 (500 μg/ml)
E. tarda JH10	256	16
E. tarda RE1	128	32
V. damsesela JE1	512	>128
A. hydrophila HA	128	32
S. enteritidis #4	512	>128

Table 4. CAT Activities of Cm Resistant Microbials

Strains	CAT activity (μM/min/10^{10}cells)
S. aureus SA10	63.6
E. tarda JH10	38.1
E. tarda RE1	28.6
V. damsela JE1	40.0
A. hydrophila H	23.0
S. enteritidis #4	22.0

Effect of single treatment of Cm on CAT activity – As acorenone, the active principle of *A. graminei*, showed resistance inhibition by the inactivation of enzyme CAT in *S. aureus* SA2 (Kim, *et al.*, 1998), the inherent CAT activities in above strains was checked (Table 4). All of the strains showed considerable maintenance of CAT activity through cultivation with Cm. The result means that E4 has another mechanism to reduce the resistance to antibiotics in Gram positive and negative microbials.

Conclusion

The combination of Cm and essential oil fraction E4 of the rhizome of *A. graminei* showed resistance inhibitory effect in Gram negative strains by lowering MIC (128 to 8 μg/ml). Generally the Cm resistant strains possess the CAT activity but the results were varied. Such result implied the intervention of other mechanism for reduction of resistance. Nevertheless of uncertain results there remains still some possibility that the combination can be introduced for clinical use and the possibility will grow according to isolation of active principle or purification of the fraction.

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