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*Platycodi radix*로부터 신경독성방어 트라이테프펜 사포닌의 활성

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Neuroprotective Activity of Triterpenoid Saponins from *Platycodi radix* Against Glutamate-induced Toxicity in Primary Cultured Rat Cortical Cells

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Objectives

The roots of *Platycodon grandiflorum*(Jacq.) A.DC. (Campanulaceae) have been used in the traditional Chinese folk medicine "*Platycodi radix*". Modern pharmacological research suggests that extracts from *P. radix* possess wide-ranging health benefits and this plantis presently a hot research topic in South Korea and Japan because of its potential health care uses. Many novel and remarkable activities have been discovered in succession. However, the components responsible for the neuroprotective effects of *P. radix* still remain unidentified. In the context of our natural product chemistry program dealing with the development of new potent neuroprotection agents, we have examined the isolation of triterpenes saponin compounds as leads for novel glutamate-induced toxicity inhibitors

Materials and Methods

Materials: The *P. radix* was purchased in a local Oriental medicine market in Seoul, South Korea and voucher specimens (WKU-0705) have been deposited in the Herbarium of the Inam Neuroscience Research Center, Sanbon, Wonkwang University.

Isolation of platycodins from total saponins: The total saponin lyophilized powder (80g) was chromatographed on a silica gel column and eluted using a $CHCl_3$-MeOH-water elution system (65:35:10, v/v/v, lower layer) to yield 10 fractions; these fractions were further separated by repetitive silica gel, Sephadex LH-20, ODS column chromatography. Four known triterpenoid saponincompounds were isolated. The structures of these compounds were identified by comparing their physicochemical and spectroscopic data with previous reported results

Cortical cell culture and Cell viability assessment: Primary cultures of mixed cortical cells containing both neurons and glia were prepared from 17~19-day-old fetal rats (Sprague-Dawley) as described previously [8]. Cultures were allowed to mature for at least 2 weeks before being used for experiments. Test fraction and compounds were dissolved in DMSO (final concentration in culture, 0.1%). Cortical cell cultures were washed with DMEM and incubated with test compounds for 1 hr. The cultures were then exposed to 100 M glutamate and maintained for 24 hr. After the incubation, the cultures were assessed for the extent of neuronal damage by measuring the efflux of LDH (lactic dehydrogenase) which reflects the integrity of cellular membrane.

Statistical analysis: The results are expressed as means ±standard errors (S.E.). The data were statistically analyzed by one way ANOVA. Differences with $P < 0.05$ were considered significant.

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Results

As part of our continued study of the neuroprotective effects of *P. radix*, we have now isolated single compounds (platycodin A, C, D and deapioplatycodin D) from the methanol extract of *P. radix* and examined their inhibitory effects on glutamate-induced toxicity in primary cultured rat cortical cells. It is notable that the neuroprotective activities of both platycodin C, deapioplatycodin D and platycodin D were comparable to those of MK-801, APV and CNOX, all ineffective, and among the tested four triterpene saponins, only platycodin A displayed activity, significantly attenuatingglutamate-induced toxicity at concentrations ranging from 0.1 to 10 mMand exhibiting cell viabilities of 50-60 %.

Table 1. Neuroprotective effects of 4 triterpene saponin from *P. radix* against glutamate-induced toxicity in primary cultured rat cortical cells [a]

Compounds	Cell viability [b] (%)		
	0.1	1	10
Control [c]		100	
Glutamate-treated [c, e]		0	
Platycodin A	13.6 ◆ 0.4*	32.5 ◆ 4.8*	52.4 ◆ 3.8***
Platycodin C	11.6 ◆ 2.8	20.3◆ 2.5	16.3 ◆ 2.1
Platycodin D	16.5 ◆ 2.7	21.6 ◆ 2.1	26.5 ◆ 3.7
Deapioplatycodin D	22.3 ◆ 3.8	21.2 ◆ 1.3	26.5 ◆ 2.0
APV [f]	10.9 ± 2.2	23.9 ± 2.8	40.0 ± 3.8*
MK-801 [g]	51.8 ± 4.8**	63.8 ± 5.4***	72.8 ± 4.9***
CNQX [h]	28.3 ± 3.4*	41.5 ± 3.6*	51.5 ± 4.8***

[a] Rat cortical cell cultures were incubated with test compounds for 1h. The cultures were then exposed to 100 μM glutamate for 24 hrs. [b] After the incubation, the cultures were assessed for the extent of neuronal damage; [b] Cell viability was measured by the LDH assay; [c] LDH released from control and glutamate-treated cultures were 11.9 ± 1.2 and 48.3 ± 4.1 units/mL, respectively; [d] Cell viability was calculated as 100 x (LDH released from glutamate-treated-LDH released from glutamate+test compound-treated)/(LDH released from glutamate-treated-LDH released from control). The values shown are the mean ± STD of three experiments (3-4 cultures per experiment). Results differ significantly from the glutamate-treated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; [e] Glutamate-treated value differed significantly from the untreated control at the level of$p < 0.001$; [f] APV: *DL*-2-amino-5-phosphonovaleric acid, a competitive NMDA receptor antagonist; [g] MK-801: dizocilpine maleate, a noncompetitive NMDA receptor; [h] CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione, non-NMDA receptor antagonist.