A WATER EXTRACT OF *PUERARIA LOBATA* INHIBITED CYTOTOXICITY OF ENTEROVIRUS 71 IN A HUMAN FORESKIN FIBROBLAST CELL LINE

Fu-Min Su,[1,2] Jung-San Chang,[3,4] Kuo-Chih Wang,[5] Jih-Jin Tsai,[6,7] and Lien-Chai Chiang[8]
[1]Department of Pharmacy, [3]Division of Gastroenterology, Department of Internal Medicine, and [6]Division of Infectious Diseases, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, [2]Faculty of Pharmacy, College of Pharmacy, [4]Faculty of Renal Care, College of Medicine, [5]Graduate Institute of Natural Products, College of Pharmacy, [7]Faculty of Medicine, College of Medicine, and [8]Department of Microbiology, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Enterovirus 71 (EV71) can cause brain encephalitis and mortality. However, effective vaccines or chemotherapeutic agents are not yet available. We tested the hypothesis that *Pueraria lobata* could inhibit the cytotoxic effect of EV71 in a human foreskin fibroblast cell line by the XTT method. Our results showed that the water extract of *P. lobata* could inhibit cytopathy induced by EV71 when given before ($p < 0.0001$), simultaneously with ($p < 0.0001$), or after viral infection ($p < 0.0001$). Water extract of *P. lobata* was effective and its minimal concentration that inhibited 50% of the cytopathic effect (IC_{50}) was 0.028 μg/mL. *P. lobata* was also safe with a selectivity index greater than 107,000. Water extract of *P. lobata* appeared to inhibit viral attachment ($p < 0.0001$) and penetration ($p < 0.0001$). The anti-EV71 activity of the water extract of *P. lobata* was not mediated by interferons. In conclusion, the water extract of *P. lobata* was effective in the management of the disease induced by EV71 infection.

Key Words: chemoprevention, enterovirus, EV71, *Pueraria lobata*, therapy
(*Kaohsiung J Med Sci* 2008;24:523–30)

Enterovirus infection is a common disease encountered worldwide. It can infect without major symptoms [1]. However, it can also cause severe forms of disease, such as infection of the central nervous system (CNS), with mortality and neurologic sequelae [2]. Enterovirus 71 (EV71) is a common enterovirus that usually causes hand, foot and mouth disease (HFMD) [3]. Nevertheless, EV71 can cause brainstem encephalitis and death [3,4]. EV71 caused an outbreak with 78 mortalities in Taiwan in the early 1990s [4]. Most of the victims were younger than 5 years of age. They died of CNS involvement and cardiopulmonary failure [4]. Furthermore, survivors of EV71 CNS infection often have neurologic sequelae and delayed neurodevelopment [5]. Therefore, effective management is crucial. Unfortunately, effective agents and vaccines are not currently available [6]. Furthermore, EV71 is easily transmitted, mainly by the fecal–oral route [7] in a crowded environment with poor public hygiene. Therefore, a large outbreak will place a heavy burden on the Bureau of National Health Insurance. A cheap, readily available, effective therapeutic modality is urgently required.

Ge Gen Tang (GGT) has been used to manage upper respiratory airway diseases for thousands of years in ancient China. Most upper respiratory airway diseases are caused by viruses; therefore, GGT

Received: Aug 22, 2008 Accepted: Oct 22, 2008
Address correspondence and reprint requests to: Professor Lien-Chai Chiang, Department of Microbiology, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan.
E-mail: lcch@kmu.edu.tw

© 2008 Elsevier. All rights reserved.

appears to have antiviral activity. *Pueraria lobata*, one of the earliest medicinal plants used in traditional Chinese medicine, is the main ingredient of GGT. It has been used for thousands of years with several clinical applications, such as coronary dilatation, and possesses antispasmodic, antipyretic, prokinetic, antihypertensive and antihyperglycemic properties [8]. Recently, *P. lobata* has become famous for its use as a hangover/alcoholism remedy to treat alcohol-related problems [9–11]. *P. lobata* is still commonly used to manage flu-like syndromes. Therefore, *P. lobata* is believed to have antiviral activity. However, such antiviral activity has not been studied. The initial presentations of EV71 infection might mimic flu symptoms; therefore, we tested the hypothesis that *P. lobata* is effective on cytopathy induced by EV71.

Methods

Preparation of plant extracts

Pueraria lobata Ohwi (*Pueraria lobata*) was collected from markets in South Taiwan. The authenticity was confirmed by Professor M.H. Yen of the Graduate Institute of Natural Products of Kaohsiung Medical University. A water extract of *P. lobata* was prepared as previously reported [12]. Briefly, 100 g of *P. lobata* were shade-dried and decocted for 1 hour with 1 L of boiling reverse-osmosis-treated water three times. The decoctions were mixed, filtered, concentrated and lyophilized. The w/w (weight/weight) yield of *P. lobata* was 18.2%. The extract of *P. lobata* was dissolved in minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) into the final concentrations of 0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 30 μg/mL for bioactivity and up to 3,000 μg/mL for cytotoxicity tests before the experiments.

Reagents, cell and viruses

XTT (sodium 3′-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid) kits were purchased from Roche Diagnostics GmbH (Mannheim, Germany). Ribavirin (Sigma, St. Louis, MO, USA), a well-known wide-spectrum antiviral agent [13], was used as a positive control and 0.1% DMSO as a negative control. Reagents and medium for cell culture were purchased from Gibco BRL (Grand Island, NY, USA). A human foreskin fibroblast cell line (CCFS-1/KMC) [14] was used to culture clinically isolated EV71 (BrCr strain, GenBank accession no. U22521). Cells were propagated at 37°C under 5% CO_2 in MEM supplemented with 10% FCS, 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate and 0.25 μg/mL amphotericin B. The virus was propagated on a 90% confluent cell monolayer in MEM with 2% FCS and antibiotics as described above. Viral titer was determined by the cytopathic effect (CPE) and expressed as 50% tissue culture infective dose ($TCID_{50}$) per milliliter (mL). The virus was stored at –70°C until use.

Cytotoxicity assay

The cytotoxic effect of water extract of *P. lobata* on proliferating cells was assayed using an XTT-based method [15,16]. Briefly, cells (1×10^4 cells/well) were seeded into 96-well culture plates (Falcon; BD Biosciences, NJ, USA) and were incubated overnight at 37°C under 5% CO_2. Then, the medium was removed and different concentrations (100, 300, 1,000 or 3,000 μg/mL) of the extract of *P. lobata* were applied to triplicate wells. After incubation for 3 days, the cytotoxicity of the concentrations was determined using the XTT assay according to the manufacturer's instructions. The 50% cytotoxic concentration (CC_{50}) of the extract was calculated by regression analysis of the dose-response curve.

Antiviral effectiveness assay and selectivity index

The anti-EV71 activity of water extract of *P. lobata* was assayed by the XTT method, as described previously [15,16]. Briefly, cells (1×10^4 cells/well) were plated into 96-well culture plates for incubation of 72 hours. The medium was removed and cells were infected with 100 $TCID_{50}$ of EV71. Then, various concentrations of the extract were added immediately and samples were incubated for another 72 hours. Each experiment was tested in triplicate and performed at least three times separately. Then, the medium was aspirated and the cytoprotective activity was determined using the XTT assay according to the manufacturer's instructions. The absorbance at 492 nm (A_{492} nm) was determined with an enzyme-linked immunosorbent assay (ELISA) reader (Multiskan EX, Labsystems, Helsinki, Finland) with a reference of A_{690} nm. The cytoprotection rate (percent of control) was calculated as follows: $(A_{492}tv - A_{492}cv)/(A_{492}cd - A_{492}cv) \times 100\%$, where $A_{492}tv$ is the absorbance of the test compound

with virus-infected cells, A_{492}cv is the absorbance of viral control, and A_{492}cd is the absorbance of the cell control only. The antiviral activity of the *P. lobata* extract with its minimal concentration required to inhibit the 50% CPE of EV71 (IC_{50}) was estimated [15,16]. The selectivity index (SI) was calculated as the ratio of CC_{50} to IC_{50}.

Time course assay

The antiviral activity of the water extract of *P. lobata* was also examined at different times before and after viral infection [15,16]. Briefly, cells (1×10^4 cells/well) were seeded and incubated for 24 hours, as previously described. The cell monolayer was then infected with 100 $TCID_{50}$ of EV71. Different concentrations of the extract were added to the wells at –2 and –1 hour (2 and 1 hour before viral inoculation, respectively) and at 1 and 2 hours (1 and 2 hours after viral inoculation, respectively). They were incubated for a further 72 hours and assayed with the XTT reagent as described above.

Attachment assay

The water extract of *P. lobata* was evaluated for its effect on viral attachment [16]. Briefly, cells (1×10^4 cells/well) were seeded and incubated for 48 hours, as previously described. Cells were pre-chilled at 4°C for 1 hour and the medium was aspirated. The cell monolayer was then infected with 200 $TCID_{50}$ of EV71 with different concentrations of the extract. After incubation at 4°C for another 3 hours, the medium was aspirated to remove unadsorbed virus. The cell monolayer was then washed with phosphate-buffered saline (PBS) three times and incubated for a further 72 hours and assayed with the XTT reagent as described above.

Penetration assay

The effect of the water extract of *P. lobata* on viral penetration was also evaluated [16]. Briefly, the cell monolayer was grown in 96-well culture plates and pre-chilled at 4°C for 1 hour. The cell monolayer was then infected with EV71 of 200 $TCID_{50}$ and incubated at 4°C for another 3 hours to allow viral attachment to the cell surface. Then, various concentrations of the extract were supplemented. They were incubated at 37°C under 5% CO_2 for a further 1 hour to maximize the penetration of viruses. At 10-minute intervals, the infected cell monolayer was treated with alkaline PBS (pH 11) for 1 minute to inactivate unpenetrated virus. Then, acidic PBS (pH 3) was added immediately to neutralize the alkaline PBS (pH 11). The neutral PBS was removed immediately and the cell monolayer was supplemented with fresh viral culture medium (MEM plus 2% FCS). After incubation at 37°C for a further 72 hours, the cell monolayer was assayed with the XTT reagent as described above.

Interferon assay

After treatment of the cells described in the above section titled "Antiviral effectiveness assay and selectivity index", the culture medium was collected and assayed by the interferon (IFN)-beta ELISA kit (PBL Biomedical Laboratories, Piscataway, NJ, USA) according to the manufacturer's instructions. Briefly, 100 μL of culture medium was placed into a 96-well microplate precoated with IFN-β antibodies. This was incubated at room temperature for 1 hour. After clearance by washing buffer, the wells were incubated with 100 μL/well peroxidase-conjugated IFN-β antibodies for another 1 hour. Then, they were washed again and were reincubated with 100 μL/well TMB substrate at room temperature in the dark for another 15 minutes. Finally, 100 μL/well 2N H_2SO_4 was supplemented to terminate the reaction. The A_{450} nm was determined with ELISA reader (Multiskan EX, Labsystems).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). The IC_{50} and CC_{50} values were calculated by Microsoft Excel 2003. The percentage of controls (cytoprotection; %) was used for statistical analysis with ANOVA by JMP version 6.0 software (SAS Institute, Cary, NC, USA). Tukey's honestly significant difference (HSD) test was used to compare all pairs of groups in the ANOVA test. A p value < 0.05 was considered statistically significant.

Results

Cytotoxicity assay

The water extract of *P. lobata* did not show cytotoxicity against host cells at concentrations up to 3,000 μg/mL (Figure 1). The estimated CC_{50} was more than 3,000 μg/mL. The high CC_{50} indicates its safety for daily use.

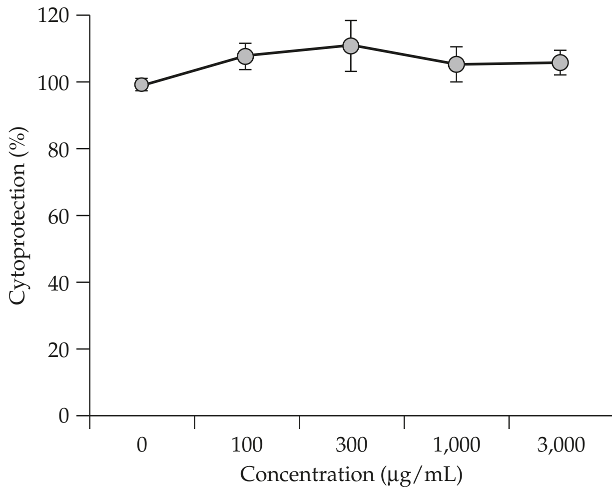

Figure 1. *Cytotoxicity assay of water extract of P. lobata. The extract of P. lobata did not show any cytotoxicity against host cells at concentrations up to 3,000 µg/mL. The estimated CC_{50} exceeded 3,000 µg/mL. Data are mean ± standard deviation of three independent experiments.*

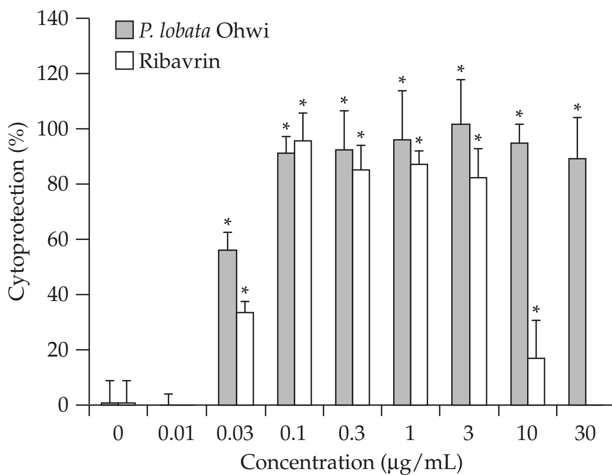

Figure 2. *Antiviral effectiveness of water extract of P. lobata against the BrCr strain of EV71. The extract of P. lobata inhibited the cytopathic effects induced by the EV71 BrCr strain at a concentration of 0.03 µg/mL ($p < 0.0001$). The estimated IC_{50} of the extract of P. lobata was 0.028 µg/mL, with an antiviral selectivity index > 107,000. Data are mean ± standard deviation of nine determinations. *$p < 0.05$ vs. control.*

Antiviral effectiveness assay and SI

The water extract of *P. lobata* was effective ($p < 0.0001$; Figure 2) against the BrCr strain of EV71 at a concentration of 0.03 µg/mL. The estimated minimal concentration required to inhibit the 50% CPE of EV71 (IC_{50}) with the extract of *P. lobata* was 0.028 µg/mL. This IC_{50} was far smaller than its cytotoxic concentration (> 3,000 µg/mL), giving a very high SI exceeding

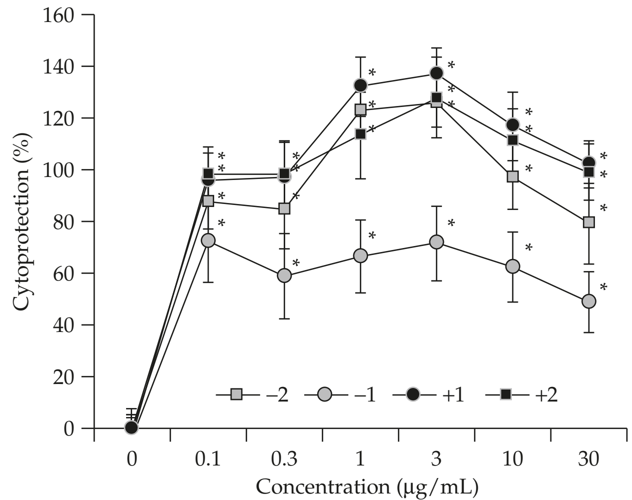

Figure 3. *Time course assay of the water extract of P. lobata against EV71 infection. The extract of P. lobata significantly inhibited the cytopathic effect of EV71 when administered before (–2 and –1 hours) or after (+1 and +2 hours) viral infection ($p < 0.0001$). The effectiveness was lower when given 1 hour before viral infection (–1 hour). Data are presented as mean ± standard deviation of nine determinations. *$p < 0.05$ vs. control.*

107,000. Therefore, the extract of *P. lobata* was an effective and safe agent.

Time course assay

After validating the anti-EV71 effectiveness of the water extract of *P. lobata* with simultaneous supplementation, it was of interest to know whether the extract of *P. lobata* could work before and/or after EV71 infection. Our results showed that the extract of *P. lobata* could significantly inhibit the CPE of EV71 when supplemented either before or after viral infection ($p < 0.0001$; Figure 3). The minimal effective concentration was 0.1 µg/mL when added either before or after viral infection. This potent effectiveness of the water extract of *P. lobata* against EV71 could be beneficial for prevention and/or treatment. There was no statistical difference in effectiveness between the different concentrations tested.

Attachment assay

Because the water extract of *P. lobata* had anti-EV71 activity before viral infection, we hypothesized that the extract of *P. lobata* might inhibit viral attachment and/or penetration to prevent viral entrance into cells. After performing the attachment assay, we found that the extract of *P. lobata* could significantly inhibit viral attachment to enter the host cell at concentrations

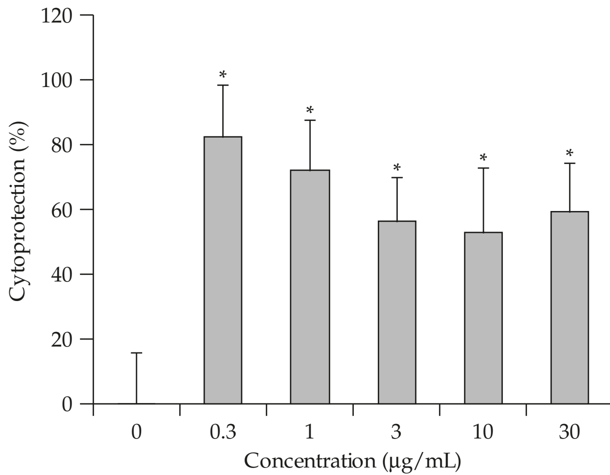

Figure 4. *Attachment assay of the water extract of P. lobata against EV71 infection. The extract of P. lobata significantly inhibited viral attachment and entry into the host cell at concentrations >0.3 μg/mL (p<0.0001). Data are mean ± standard deviation of nine determinations. *p<0.05 vs. control.*

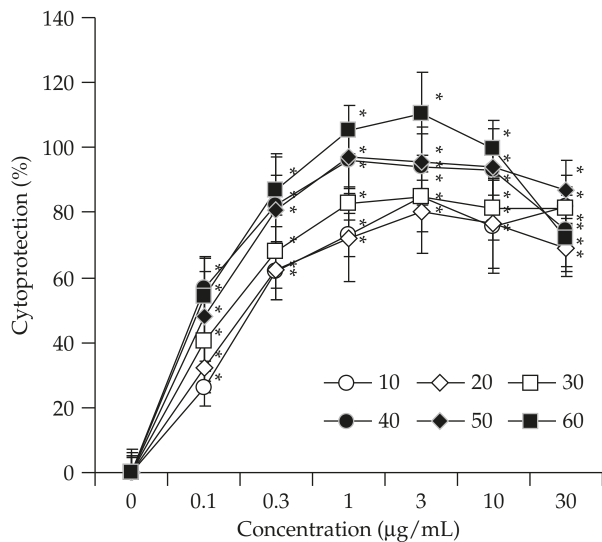

Figure 5. *Penetration assay of the water extract of P. lobata against EV71 infection. The extract of P. lobata significantly inhibited the penetration of EV71 at concentrations >0.1 μg/mL (p<0.0001). The effect developed rapidly within 10 minutes. Data are mean ± standard deviation of nine determinations. *p<0.05 vs. control.*

greater than 0.3 μg/mL ($p<0.0001$; Figure 4). The effectiveness showed a trend towards decreased effectiveness. However, there was no statistical significance.

Penetration assay

We also found that the water extract of *P. lobata* could effectively inhibit viral penetration at concentrations greater than 0.1 μg/mL ($p<0.0001$; Figure 5). The

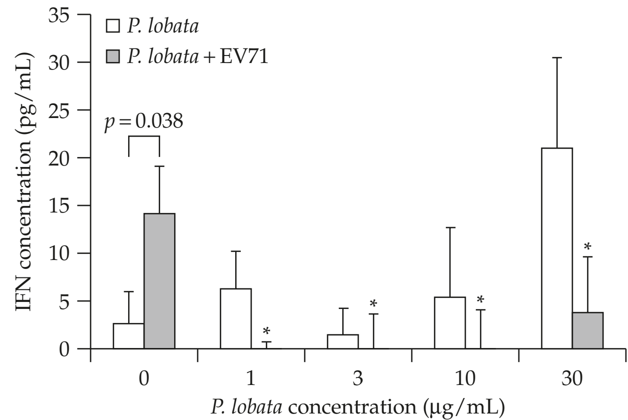

Figure 6. *Effect of the water extract of P. lobata on interferon (IFN) production induced by EV71 infection. EV71 infection (0 μg/mL P. lobata) significantly enhanced IFN production above the constitutive level (cells only). The extract of P. lobata decreased IFN production induced by EV71 infection (p<0.05). Data are mean ± standard deviation of three independent experiments. *p<0.05 vs. control.*

extract of *P. lobata* exerted its effect during the early stage of penetration. There was a trend that increasing the incubation time with extract of *P. lobata* increased its effectiveness. However, there was no statistical difference between the various treatment durations. This activity to inhibit viral penetration could be additive to the antiviral attachment effect to prevent viral entrance.

IFN assay

EV71 infection (0 μg/mL *P. lobata* + EV71; Figure 6) significantly increased IFN production above the constitutive level (0 μg/mL *P. lobata*; $p=0.037$). The water extract of *P. lobata* effectively decreased the IFN production induced by EV71 infection ($p<0.05$). Therefore, its anti-EV71 activity was not mediated by IFN.

Discussion

Our experiment has demonstrated that the water extract of *P. lobata* was highly effective against cytopathy induced by EV71 infection. The extract of *P. lobata* had anti-EV71 activity whether supplemented before or after viral infection. We have provided evidence of its effect on viral attachment and penetration. However, its effect on viral replication needs to be clarified in future studies after its lifecycle becomes better understood. The extract of *P. lobata* exerted its anti-EV71

activity at a concentration of 0.03 μg/mL. The low IC_{50} (0.028 μg/mL) with high SI (>107,000) means that the *P. lobata* extract is a good candidate for the treatment of disease induced by EV71 infection. Large quantities of effective agents are needed during an outbreak, and this would involve huge medical expenditure and heavy financial burden on the government. However, because the water extract of *P. lobata* is a simple to prepare, crude extract of a medicinal plant, it is relatively cheap. Therefore, it is estimated that the cost to protect a child from EV71 infection would be less than NT$1 per day. Considering its effectiveness without clear cytotoxicity, the water extract of *P. lobata* could be taken regularly during the endemic period. This high effectiveness of a cheap crude extract of a medicinal plant has offered an approach to prevent EV71 infection during an outbreak. However, EV71 is a genetically diverse, rapidly evolving virus [17]. Viral mutation with drug resistance could develop rapidly after introducing chemotherapeutic agents into disease control. It is still unknown whether the extract of *P. lobata* is effective against mutant strains. Furthermore, its safety in neonates is also unknown.

Fundamentally, viral infection could induce cellular IFN production to limit viral spread. Increases in IFN levels above the basal level are also found during EV71 infection. However, abnormal cytokine production during EV71 infection could be associated with fatality. Severely ill patients with pulmonary edema have significantly higher serum IFN levels [18,19]. Early administration of intravenous immunoglobulin can decrease IFN levels and were associated with lower mortality rates in patients with EV71 infection and pulmonary edema [20]. We found that the extract of *P. lobata* could counteract IFN production induced by EV71 infection (Figure 6). Therefore, the extract of *P. lobata* might have additional benefits to manage disease caused by EV71 infection. However, the impact of IFN on disease progress is still debated. Therefore, the beneficial effects of the water extract of *P. lobata* on IFN production need further testing and verification.

We have demonstrated that the water extract of *P. lobata* is highly effective against EV71 infection in a foreskin fibroblast cell line. However, neurotropic EV71 can be fatal after CNS involvement. Our experimental model does not support *P. lobata* as being effective for the management of EV71 encephalitis. Nevertheless, lipophilic substances, rather than aqueous extracts, can better access brain tissue; thus, scientists might doubt the usefulness of the water extract of *P. lobata.* However, the peak incidence of EV71 encephalitis occurs at around the same time as the peak of HFMD [4]. Therefore, as the number of patients infected with EV71 increases, the number of patients affected by EV71 encephalitis will also increase. A reduction in the EV71-infected population might decrease the incidence of EV71 encephalitis. Therefore, it is rational to use the water extract of *P. lobata* during an endemic period of HFMD to prevent further fatal brain stem encephalitis.

In conclusion, the water extract of *P. lobata* was effective for the inhibition of cytopathy induced by EV71. The water extract of *P. lobata* is cheap and readily available and could be beneficial to prevent and limit the spread of EV71 during endemic outbreaks.

Acknowledgments

We are grateful to Miss Merry Young for her technical assistance. We also thank the National Science Council of Taiwan for grant support (NSC 96-2320-B-273-002). The authors report no potential conflicts of interest relevant to this work.

References

1. Witso E, Palacios G, Cinek O, et al. High prevalence of human enterovirus infections in natural circulation of human enteroviruses. *J Clin Microbiol* 2006;44: 4095–100.
2. Pallansch MA, Roos RP. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe DM, Howley PM, Griffin DE, et al., eds. *Fields Virology*. Philadelphia: Lippincott Williams & Wilkins, 2001:723–75.
3. Wang SM, Liu CC, Tseng HW, et al. Clinical spectrum of enterovirus 71 infection in children in southern Taiwan, with an emphasis on neurological complications. *Clin Infect Dis* 1999;29:184–90.
4. Ho M, Chen ER, Hsu KH, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. *N Engl J Med* 1999;341: 929–35.
5. Chang LY, Huang LM, Gau SS, et al. Neurodevelopment and cognition in children after enterovirus 71 infection. *N Engl J Med* 2007;356:1226–34.
6. McMinn PC. An overview of the evolution of enterovirus 71 and its clinical and public health significance. *FEMS Microbiol Rev* 2002;26:91–107.

7. Li CC, Yang MY, Chen RF, et al. Clinical manifestations and laboratory assessment in an enterovirus 71 outbreak in southern Taiwan. *Scand J Infect Dis* 2002;34:104–9.
8. Lin CC. *Pueraria lobata*. In: *The Clinical and Application of Crude Drug*. Kaohsiung: Fuji Press, 1987:237–9.
9. McGregor NR. *Pueraria lobata* (Kudzu root) hangover remedies and acetaldehyde-associated neoplasm risk. *Alcohol* 2007;41:469–78.
10. Xu BJ, Zheng YN, Sung CK. Natural medicines for alcoholism treatment: a review. *Drug Alcohol Rev* 2005; 24:525–36.
11. Lin RC, Li TK. Effects of isoflavones on alcohol pharmacokinetics and alcohol-drinking behavior in rats. *Am J Clin Nutr* 1998;68:1512S–5S.
12. Yen MH, Lin CC, Chuang CH, et al. Evaluation of root quality of Bupleurum species by TLC scanner and the liver protective effects of "xiao-chai-hu-tang" prepared using three different Bupleurum species. *J Ethnopharmacol* 1991;34:155–65.
13. Sidwell RW, Huffman JH, Khare GP, et al. Broad-spectrum antiviral activity of Virazole: 1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science* 1972; 177:705–6.
14. Chiang LC, Chiang W, Chang SF, et al. Characterization of an immortalized human cell line derived from neonatal foreskin diploid fibroblasts. *J Dermatol* 1992;19:1–11.
15. Chiang LC, Chiang W, Chang MY, et al. Antiviral activity of Plantago major extracts and related compounds *in vitro*. *Antiviral Res* 2002;55:53–62.
16. Chang JS, Wang KC, Chiang LC. Sheng-Ma-Ge-Gen-Tang inhibited enterovirus 71 infection in human foreskin fibroblast cell line. *J Ethnopharmacol* 2008;119: 104–8.
17. Brown BA, Oberste MS, Alexander JP Jr, et al. Molecular epidemiology and evolution of enterovirus 71 strains isolated from 1970 to 1998. *J Virol* 1999;73: 9969–75.
18. Wang SM, Lei HY, Huang KJ, et al. Pathogenesis of enterovirus 71 brainstem encephalitis in pediatric patients: roles of cytokines and cellular immune activation in patients with pulmonary edema. *J Infect Dis* 2003; 188:564–70.
19. Lin TY, Hsia SH, Huang YC, et al. Proinflammatory cytokine reactions in enterovirus 71 infections of the central nervous system. *Clin Infect Dis* 2003;36:269–74.
20. Wang SM, Lei HY, Huang MC, et al. Modulation of cytokine production by intravenous immunoglobulin in patients with enterovirus 71-associated brainstem encephalitis. *J Clin Virol* 2006;37:47–52.

葛根 (*Pueraria lobata*) 熱水萃取物在人類包皮纖維母細胞株抑制腸病毒 71 型造成的細胞毒性

蘇富敏 [1,2] 張榮參 [3,4] 王國治 [5] 蔡季君 [6,7] 蔣連財 [8]

高雄醫學大學附設醫院 [1]藥劑部 [3]內科部 胃腸內科 [6]內科部 感染內科

高雄醫學大學 [2]藥學系 [4]腎臟照護學系 [5]天然藥物研究所 [7]醫學系 [8]微生物學科

腸病毒 71 型 (EV71) 可導致腦性腦炎和死亡。但是目前並無有效的疫苗，也沒有化療藥物可用於治療腸病毒 71 型造成的感染。我們利用人類包皮纖維母細胞株以及 XTT 方法檢定葛根熱水萃取物能有效抑制腸病毒 71 型的細胞毒性作用。我們的結果顯示，葛根熱水萃取物能有效抑制腸病毒 71 型感染產生的細胞毒性作用。在病毒感染前投與 ($p < 0.0001$)，在病毒感染時投與 ($p < 0.0001$)，或在病毒感染之後投與 ($p < 0.0001$)，皆能顯示葛根熱水萃取物有效抑制腸病毒 71 型感染造成的細胞毒殺作用。本實驗證實葛根熱水萃取物抑制腸病毒 71 型是有效 (IC_{50} 為 0.028 μg/mL) 而且安全 (選擇指數大於 107000)。葛根可能可以抑制病毒附著 ($p < 0.0001$) 和穿透 ($p < 0.0001$) 細胞的過程。葛根熱水萃取物不會增加腸病毒 71 型感染產生的干擾素，因此其作用機轉和干擾素無關。結論為葛根熱水萃取物將來可能可用於腸病毒 71 型感染的防治。

關鍵詞：化學預防，腸病毒，腸病毒 71 型，葛根，治療

(高雄醫誌 2008;24:523–30)

收文日期：97 年 8 月 22 日
接受刊載：97 年 10 月 22 日

通訊作者：蔣連財教授
高雄醫學大學醫學系微生物學科
高雄市三民區十全一路 100 號