J Huazhong Univ Sci Technol［Med Sci］
DOI 10.1007/s11596-013-1132-9
33(3):406-411,2013

Effect of Baicalein on the Expression of VIP in Extravillous Cytotrophoblasts Infected with Human Cytomegalovirus *In Vitro*[*]

Yuan QIAO (乔 媛)[1], Jian-guo FANG (方建国)[2], Juan XIAO (肖 娟)[1], Tao LIU (刘 涛)[3], Jing LIU (刘 静)[1], Yan-li ZHANG (张艳丽)[1], Su-hua CHEN (陈素华)[1#]
[1]*Department of Obstetrics and Gynecology,* [2]*Department of Pharmacy, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*
[3]*Department of Obstetrics and Gynecology, Tai'an City Central Hospital, Tai'an 271000, China*

Summary: This paper aimed to study the ability of baicalein to block human cytomegalovirus (HCMV) infection in extravillous cytotrophoblasts (EVT) and its effect on the vasoactive intestinal peptide (VIP) expression in HCMV-infected EVT *in vitro*. A human trophoblast cell line (HPT-8) was chosen in this study. HCMV with 100 $TCID_{50}$ was added into culture medium to infect HPT-8 cells, and then HCMV pp65 antigen was assayed by immunofluorescence staining. The infection status was determined by virus titration. Real-time quantitative polymerase chain reaction (qRT-PCR) was used to detect virus DNA load in the infected cells. The expression of VIP mRNA and protein in the infected cells was measured by qRT-PCR, immunocytochemistry and Western blotting. Concentration of VIP secreted in supernatants was determined by ELISA. Red-stained HCMV pp65 antigens were found in infected HPT-8 cells 48 h after infection. HCMV replicated in large quantity in infected HPT-8 cells 4 days after infection, reaching a peak at day 6 post-infection. After treatment with baicalein, virus DNA load in infected HPT-8 cells was decreased (P<0.05), and the levels of VIP mRNA and protein, and the concentration were raised to the normal (P>0.05). Our study suggested that baicalein exerts a positive effect on the VIP expression in HCMV-infected EVT at maternal-fetal interface.
Key words: baicalein; human cytomegalovirus; extravillous cytotrophoblasts; vasoactive intestinal peptide

Human cytomegalovirus (HCMV) is the most common cause of viral intrauterine infection. Upon entering the bloodstream, HCMVs cross the placenta to invade the fetus in uterine[1]. In the United States, the rate of primary infection in pregnant women stands between 2%–2.5%[2], and 40% of the infection was transmitted through intrauterine route[3]. Intrauterine transmission of HCMV can lead to pregnant abnormalities[4], such as abortion, preterm or still birth, intrauterine growth retardation *etc*, but the underlying mechanisms have not been fully understood yet.

Immune tolerance at maternal-fetal interface plays an important role in establishing and maintaining normal pregnancy. Once it is disrupted, pathological pregnant outcomes may result[5]. Vasoactive intestinal peptide (VIP)[6–8] is a neuropeptide or neurotransmitter that induces maternal immunological tolerance. Its low expression at the maternal-fetal interface was found to be closely associated with adverse pregnancy outcomes[9], but its relationship with HCMV-related adverse pregnancy outcomes is poorly understood.

Immunization and anti-viral treatment are important measures that prevent intrauterine transmission of HCMV. However, currently no vaccine has been approved for clinical use. Approved drugs for the treatment of HCMV infection, including ganciclovir, has been widely applied for HIV patients with HCMV infection but the drugs are not indicated for pregnant women since it belongs to Pregnancy Category C drugs as designated by FDA[10, 11]. *Radix scutellariae* is a common Chinese herbal medicine, which Chinese traditional medicine believes possesses functions of "*heat-clearing*, *fire-removing*, *detoxification*, and *fetus-protection*"[12]. *Radix scutellariae* given orally to pregnant mice at less than 5 g/kg/day had little teratogenic effect[13]. Several *in vitro* researches also found that baicalein, one of the major active ingredients of *Radix scutellariae*, could decrease the levels of HCMV immediate-early, early and late proteins, as well as DNA synthesis[14, 15].

Extravillous cytotrophoblasts (EVT), with both invasive and immuno-regulatory properties, play an important part at maternal-fetal interface for maintaining healthy pregnancy[16]. HPT-8 cells, an EVT cell line obtained from human placental villi of 6 to 9 gestational weeks, were found to have some endocrine functions[17]. We previously reported that HCMV could inhibit the invasion of HPT-8 cells[18].

In order to examine the ability of baicalein to prevent

Yuan QIAO, E-mail: qiaoyuan_505@hotmail.com
[#]Corresponding author: E-mail: chen_suhua@mail.hust. edu.cn
[*]This project was supported by grants from the National Natural Science Foundation of China (Nos. 39970769, 30371488, 30672243, and 81200354) and the Natural Science Foundation of Hubei Province (No. 2009CDD216).

HCMV infection and to recover maternal-fetal immune tolerance, HPT-8 cells were cultured *in vitro* to observe VIP expression in EVT after HCMV infection and the inhibitory effect of baicalein on HCMV infection and VIP expression in HCMV-infected EVT.

1 MATERIALS AND METHODS

1.1 Cells and Virus

Human lung fibroblasts (HEL), purchased from American Type Culture Collection (ATCC), were stored in Perinatology Laboratory in Department of Obstetrics and Gynecology, Tongji Hospital, Wuhan, China. HPT-8 cells were bought from the Department of Epidemiology, the Fourth Military Medical University, Xi'an, China. HCMV AD169 was obtained from the Institute of Virology, Wuhan, China, with $TCID_{50}$ being$10^{-4.8}$/0.1 mL.

1.2 Major Drugs, Reagents and Antibodies

Baicalein (purity>99%), provided by the Department of Pharmacy, Tongji Hospital, was dissolved in dimethylsulfoxide (DMSO) solution, with an initial concentration of 10 mg/mL and stored at –20°C. TIANamp genomic DNA kit was from Tiangen Biotech Co. Ltd, China.

β-actin and goat anti-human VIP antibody were bought from Santa Cruz Co., USA. Mouse anti-HCMV pp65 monoclonal antibody was purchased from Chemicon Co., USA. Human VIP ELLSA kit was from Phoenix Pharmaceuticals Co., Karlsruhe, Germany. HCMV PCR florescence quantitative detection kit was procured from Da An Gene, China.

1.3 Cytotoxicity Assay

A well-established method developed in our laboratory[19, 20] was used with some modifications. Serially diluted baicalein (from 1:10 to $1:10^8$) was added to culture medium after serum-starved culture of HPT-8 cells for 48 h. Afterwards, MTT assay was employed to detect cell viability 72 h after the incubation. The absorbance (*A*) values of three replication samples were obtained on an ELLSA plate reader. The largest non-toxic dose (TD_0) and maximum tolerance concentration (MTC) of baicalein were determined by 50% and 100% cell viability respectively by using the formula: Cell viability (%)=*A* (baicalein group)/*A* (blank control group)×100%.

1.4 Grouping

HPT-8 cells were randomly divided into three groups: blank control group, HCMV group and baicalein group. Serum-starved cells were incubated in 6-well plates for 48 h prior to HCMV infection, and HCMV with 100 $TCID_{50}$ were added to DMEM/high glucose containing 3% fetal calf serum (FCS). In baicalein group, both HCMV (100 $TCID_{50}$) and serially-diluted baicalein (from 1:10 to $1:10^4$) from TD_0 were added into culture medium. The cells in blank control group were cultured with equal amount of PBS. Following incubation for 2 h, the cells were washed intensively and further incubated in the same medium till the end of the experiment. HPT-8 cells were collected and stored under –70°C.

1.5 Virus Detection

1.5.1 Immunofluorescence Detection of HCMV pp65 Antigen After infection of HPT-8 cells with HCMV for 48 h, cells were fixed with 4% paraformaldehyde for 30 min at room temperature. They were made to react with mouse anti-HCMV pp65 monoclonal antibody (dilution: 1:1000) for 24 h at 4°C, and then with Cy3-conjugated secondary antibody for 1 h at 37°C. Mounting medium with Hoechst was used as a nuclear stain. Slides were observed under a fluorescence microscope.

1.5.2 HCMV Titration The supernatants from HCMV group were collected at different time points, and then used to inoculate monolayer HEL cells after repetitive freezing-thawing and centrifugation. Cytopathic effect was observed and $TCID_{50}$ in its supernatants was calculated by drawing a growth curve.

1.5.3 Quantification of HCMV DNA Load by Real-time Quantitative PCR (qRT-PCR) Viral DNA was extracted and purified by following kit instructions. The samples of purified DNA were subjected to qRT-PCR on an ABI 7500 Real-Time PCR system by using primers and probes derived from IE1 according to the manufacturer's instructions. The thermal cycle profile was: 2 min at 50°C, 15 min at 95°C, 40 cycles of 15 s at 94°C, 45 s at 55°C. Amplification curve of each group was analyzed at the end of the reaction, and was then converted to HCMV DNA load.

1.6 VIP Detection

1.6.1 Quantification of VIP mRNA qRT-PCR was performed to detect the expression of VIP mRNA, with β-actin serving as the internal control. Briefly, total RNA was extracted and reversely-transcribed into complementary DNA (cDNA) by using Prime Script RT reagent. The primers were designed by employing 5.0 primer design software package and were synthesized by Shanghai Biological Engineering Co. Ltd., Shanghai, China. Each primer was checked against NCBI BLAST database to ensure that it was unique for the target mRNA transcription. The primer sequences were as follows: VIP: forward 5'-CCT TCT GCT CTC AGG TTG GG-3', reverse 5'-CGT TTT CCC ATA AGA GAC TCA AG -3', β-actin: forward 5'-GTC CAC CGC AAA TGC TTC TA-3', reverse 5'-TGC TGT CAC CTT CAC CGT TC - 3'. For qRT-PCR, amplification was performed on an ABI 7500 real-time PCR system by using the SYBR Green kit. The cycling conditions were 2 min at 50°C, 40 cycles of 95 °C for 15 s，60°C for 50 s. Relative VIP mRNA expression was normalized to β-actin expression by using ΔCT method.

1.6.2 Immunocytochemical Detection of VIP Antigen Cells grown on coverslips were fixed with 4% paraformaldehyde for 30 min at room temperature. Normal rabbit serum (10%) was added to block non-specific binding, and then samples were incubated with goat anti-VIP polyclonal antibody (dilution: 1:500) at 4°C overnight. The following day, cells were then incubated for 60 min with biotinylated-conjugated IgG antibody at 37°C (S-ABC method). Proteins were visualized by incubating with DAB solution for 10 min. The positive expression presented as yellowish-brown or brown particles under light microscope. Ten randomly selected high-power visual fields were observed. The average *A* values were calculated and subjected to the semi-quantitative statistical analysis.

1.6.3 Western Blotting of VIP Cells were intensively washed with PBS. Proteins were extracted after

lysing cells by the mixture of 0.2 mg/mL PMSF (containing 1% NP40 and 0.1%SDS) and proteases inhibitor. Equivalent sample proteins were taken for 12% SDS-PAGE and then electro-transfered to wet PVDF membranes. After blockade with 5% powered milk, goat anti-human VIP antibody (dilution: 1:500) was added and incubated overnight at 4°C. The horseradish peroxidase (HRP)-conjugated donkey anti-goat IgG (dilution: 1:3000) was added and the samples were hatched for 1 h at room temperature. Finally, the blots were developed by using a digital imaging analysis system and the intensity of signals was analyzed by using Quantity One software.

1.6.4 Cytokine Quantification Supernatants were collected and measured for VIP concentration. Data were presented in pictogram per milliliter (pg/mL).

1.7 Statistical Analysis

All the experiments were repeated over three times. SPSS statistical software (Ver. 17.0) was adopted for statistical analysis. The results were expressed as $\bar{x}\pm s$. The one-way ANONA was used for comparison among different groups. A $P<0.05$ was considered to be statistically significant.

2 RESULTS

2.1 Baicalein Cytotoxicity

MTT assay showed that baicalein at high-concentration had direct cytotoxic effects on HPT-8 cells. The TD_0 of baicalein was 250 μg/mL and the MTC was 62.5 μg/mL.

2.2 Effect of Baicalein on HCMV DNA Load in HPT-8 Cells

Fig.1 showed that there existed plenty of red-stained HCMV pp65 antigen signals in cytoplasm, while no red signals were found in HPT-8 cells of blank control group or negative control group. The viral titration showed that HCMV replicated in large quantity in infected HPT-8 cells 4 days after infection, reaching a peak at day 6 post-infection. However, HCMV replicated in large quantity in infected HEL cells 2 days after infection, arriving at a peak 5 days after infection. The titer in the infected HPT-8 cultures was 2 logs lower than that in identically-infected HEL cultures (fig. 2).

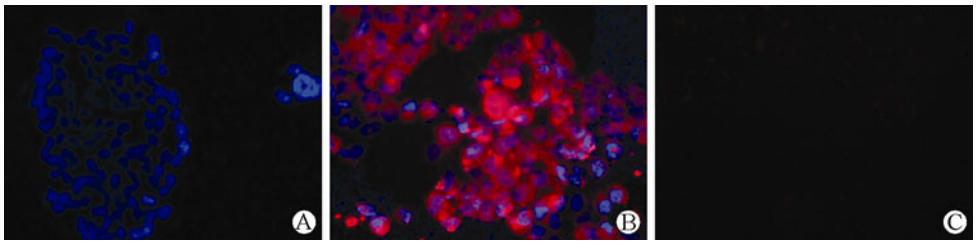

Fig. 1 Immunofluorescence detection of HCMV pp65 antigen in HPT-8 cells (×400)
A: blank control group. Nuclei are stained blue; B: HCMV group. Red-stained HCMV pp65 antigen signals are shown in cytoplasm; C: negative control group

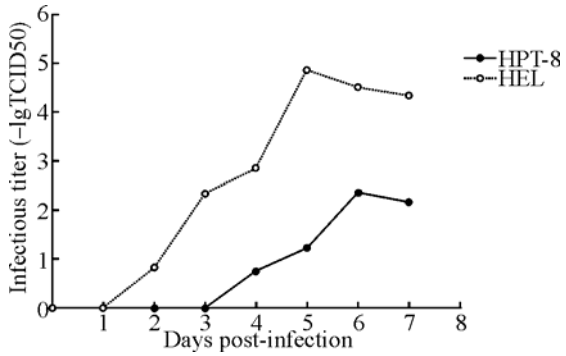

Fig. 2 Viral growth curve of HPT-8 and HEL cultures

As shown in fig. 3, compared with HCMV group, viral DNA loads in 1:10, 1:100 and $1:10^3$ T_0 baicalein groups were decreased significantly ($P<0.05$), whereas, there was no significantly statistical difference in viral DNA load between $1:10^4$ T_0 baicalein group and HCMV group ($P>0.05$).

The aforementioned results indicated that HCMV could infect HPT-8 cells and completely replicate in them, and baicalein could reduce HCMV DNA accumulation in infected HPT-8 cells at certain range of concentrations.

2.3 The Effect of Baicalein on the VTP Expression in HPT-8 Cells

The results of qRT-PCR showed that compared with blank control group and MTC baicalein group, VIP mRNA expression in HCMV group was decreased (P=0.002, 0.009), while there was no statistically significant difference in VIP expression between the MTC baicalein group and blank control group (P=0.207) (fig. 4).

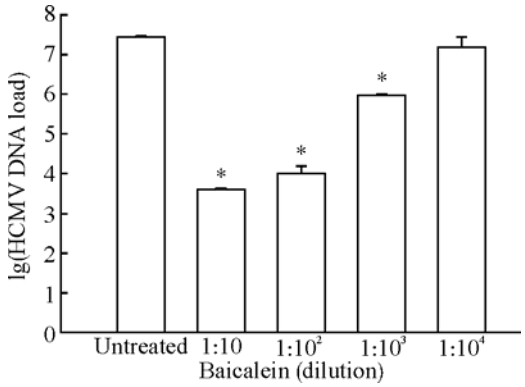

Fig. 3 Viral DNA load in infected HPT-8 cells
Compared with HCMV group (untreated), viral DNA loads in 1:10, $1:10^2$ and $1:10^3$ T_0 baicalein groups were decreased significantly ($P<0.05$).

Fig. 5 showed that VIP protein expressed in cytoplast and cytoplasm of HPT-8 cells in three groups, pre-

senting yellow-brownish color. The semi-quantitative evaluation indicated that the level of VIP in HCMV group was lower than that in blank control group and MTC baicalein group (P=0.001, 0.001). Western blotting further showed that the expression of VIP was decreased in HCMV group as compared with blank control group and MTC baicalein group (P=0.001, 0.009), while the difference in VIP expression between MTC baicalein group and blank control group revealed no statistical difference (P=0.192) (fig. 6).

The results of ELISA indicated that the concentration of VIP in HCMV group was significantly lower than that in blank control group and MTC baicalein group (P=0.001, 0.002), while there was no statistical difference in VIP concentration between MTC baicalein group and blank control group (P=0.750) (fig. 7).

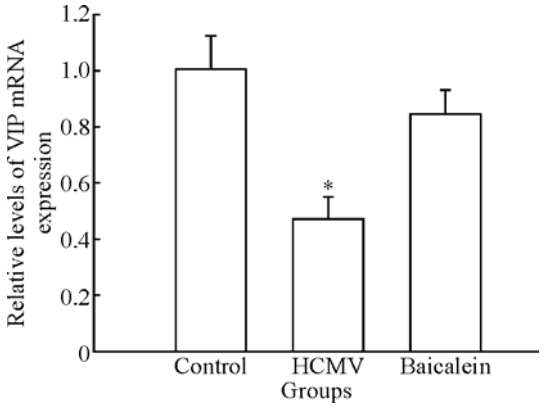

Fig. 4 VIP mRNA expression in HPT-8 cells of different groups
VIP mRNA expression in HPT-8 cells was normalized to β-actin. ($^*P$<0.05 *vs*. control group)

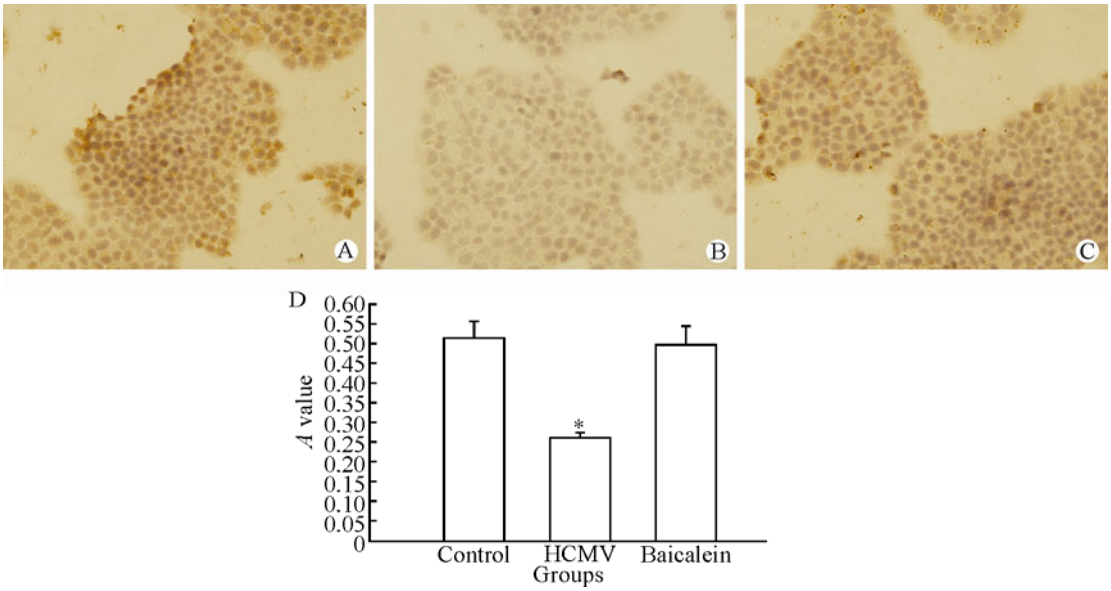

Fig. 5 Immunocytochemical images of VIP expression in HPT-8 cells (SABC method, ×400)
A: blank control group; B: HCMV group; C: MTC baicalein group; D: comparison of the average A value of VIP protein expression in HPT-8 cells of different groups ($^*P$<0.05 *vs*. control group)

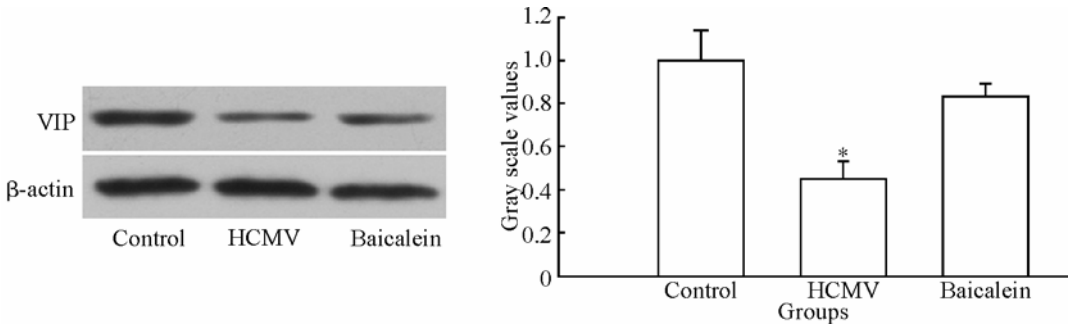

Fig. 6 Western blotting analysis of VIP protein expression in HPT-8 cells
A: representative images of Western blot bands of VIP and β-actin; B: VIP intensity was normalized to β-actin ($^*P$<0.05 *vs*. control group).

The above results suggested that the VIP expression in genetic transcription, protein synthesis and secretion were decreased after HCMV infection, while baicelein could, to some extent, raise them to the normal levels.

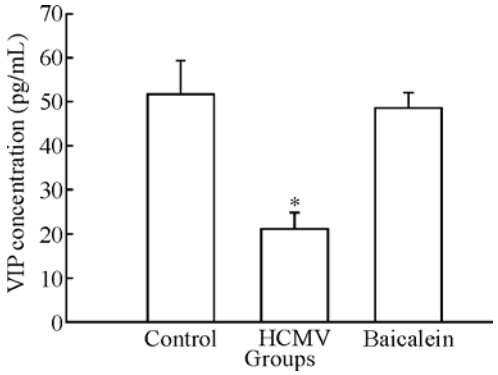

Fig. 7 VIP concentration in supernatants of HPT-8 cells
The results are presented as $\bar{x}\pm s$ ($P<0.05$ *vs*. control group).

3 DISCUSSION

The incidence of HCMV intrauterine transmission in live births is approximately 0.2%–2.2% worldwide[21]. HCMV represents the leading cause of adverse pregnancy[22], but so far the pathogenesis of HCMV infection has been poorly understood.

From the perspective of reproductive immunology, normal pregnancy is a process similar to allografting. Materno-fetal immune tolerance involves the identification of paternal antigen-carrying embryo by maternal immune system[8] and development of protective immunity, which is indispensable event for maintaining pregnancy. EVT are crucial to maternal-fetal immune tolerance[23]. During early trimester, EVT invade the decidualizd endometrium and its vessels to form feto-placental unit for material exchange[16]. Meanwhile, EVT synthetize and secrete immunoregulatory substances, which protect embryo from the attack of maternal decidual immune cells[24, 25]. We previously reported that HCMV could infect EVT and inhibited its invasiveness, but the studies concerning the effect of HCMV on their immune functions were scanty[18].

As a neuropeptide or neurotransmitter consisting of 28 amino acids, VIP was first identified and extracted by Said and Mutt[26] from tissue of swine small intestine. VIP is synthesized and secreted by EVT in the early trimester, and VIP receptor VPAC1 was also found to exist on the surface of EVT[9, 27, 28]. By inhibiting the expression of IL-6 and monocyte chemoattractant protein 1 (MCP-1), VIP could increase the number of Treg cells at maternal-fetal interface, thereby maintaining immune tolerance.

Both MTT and cytopathic effect assay were previously used to screen the effective component against CMV, and the results showed that baicalein had the highest therapeutic index (TI) among 18 kinds of Chinese medicine monomers, such as flavonoid, organic acid phenols, triterpenoid saponins *etc*[29]. Evers *et al*[14] also found that baicalein not only had the lowest inhibitory concentration (IC_{50}=0.4–1.2) among ten flavonoid Chinese medicine monomers, but could directly block virus penetration via inhibiting the activity of tyrosine kinase in epidermal growth factor receptor (EGFR) and cell cycle-related proteins in HCMV.

This experiment studied the effect of baicalein on HCMV-infected EVT and the VIP expression in the cells. HCMV pp65 antigen signals were detected in cytoplasm of HPT-8 cells and the virus replicated in large quantity in cells 4 days after infection, reaching a peak 6 days after infection. The pp65 expression suggested that HCMV had finished replication and viruses had completely dissolved in cells, which was an indicator of active infection[30]. Our results also showed that, with increasing concentration of baicalein, HCMV DNA load in infected cells declined gradually, indicating that baicalein could inhibit HCMV replication in HPT-8 cells in a dose-dependent manner.

Moreover, out study exhibited that the levels of VIP mRNA, protein were decreased in infected HPT-8 cells and its concentration in the supernatants of the infected cells were also reduced. This may be one of the mechanisms by which HCMV infection causes abnormal pregnancy such as abortion. After treatment with baicalein, viral DNA load was decreased and the expression of VIP mRNA and protein in infected HPT-8 cells, including the concentration of VIP in supernatant of the infected cells, was raised to the normal level.

To sum up, baicalein plays positive roles in blocking HCMV infection in EVT and reducing the infection-related impairment in maternal-fetal immune tolerance. However, the precise mechanism still warrants to be further studied.

REFERENCES

1 Ross SA, Boppana SB. Congenital cytomegalovirus infection: outcome and diagnosis. Semin Pediatr Infect Dis, 2005,16(1):44-49
2 Raynor BD. Cytomegalovirus infection in pregnancy. Semin Perinatol, 1993,17(6):394-402
3 Stagno S, Pass RF, Cloud G, *et al*. Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome. JAMA, 1986,256(14):1904-1908
4 Cordier AG, Guitton S, Vauloup-Fellous C, *et al*. Awareness of cytomegalovirus infection among pregnant women in France. J Clin Virol, 2012,53(4):332-337
5 Kumpel BM, Manoussaka MS. Placental immunology and maternal alloimmune responses. Vox Sang, 2012,102(1): 2-12
6 Fraccaroli L, Grasso E, Hauk V, *et al*. Defects in the vasoactive intestinal peptide (VIP)/VPAC system during early stages of the placental-maternal leucocyte interaction impair the maternal tolerogenic response. Clin Exp Immunol, 2012,170(3):310-320
7 Delgado M, Ganea D. Vasoactive intestinal peptide: a neuropeptide with pleiotropic immune functions. Amino Acids, 2011
8 Warning JC, Mccracken SA, Morris JM. A balancing act: mechanisms by which the fetus avoids rejection by the maternal immune system. Reproduction, 2011,141(6): 715-724
9 Fraccaroli L, Alfieri J, Larocca L, *et al*. VIP modulates the pro-inflammatory maternal response, inducing tolerance to trophoblast cells. Br J Pharmacol, 2009,156(1):116-126
10 Griffiths PD. The 2001 Garrod lecture. The treatment of cytomegalovirus infection. J Antimicrob Chemother, 2002, 49(2):243-253
11 Nassetta L, Kimberlin D, Whitley R. Treatment of congenital cytomegalovirus infection: implications for future

therapeutic strategies. J Antimicrob Chemother, 2009, 63(5):862-867
12 Gao Y, Gu ZL, Jiang XG, *et al*. The new research of baicalein pharmacology. Shizhen Guoyi Guoyao (Chinese). 2010(7):1765-1767
13 Yang LP, Yang YM, Yang J, *et al*. Observations of teratogenic effects of scutellaria baicalensis georgi on embryos of rats. Zhongguo Bijiao Yixue Zazhi (Chinese). (5).
14 Evers DL, Chao CF, Wang X, *et al*. Human cytomegalovirus-inhibitory flavonoids: studies on antiviral activity and mechanism of action. Antiviral Res, 2005,68(3): 124-134
15 Cotin S, Calliste CA, Mazeron MC, *et al*. Eight flavonoids and their potential as inhibitors of human cytomegalovirus replication. Antiviral Res, 2012,96(2):181-186
16 Tarrade A, Lai KR, Malassine A, *et al*. Characterization of human villous and extravillous trophoblasts isolated from first trimester placenta. Lab Invest, 2001,81(9):1199-1211
17 Zhang L, Zhang W, Shao C, *et al*. Establishment and characterization of a spontaneously immortalized trophoblast cell line (HPT-8) and its hepatitis B virus-expressing clone. Hum Reprod, 2011,26(8):2146-2156
18 Liu T, Zheng X, Chen J, *et al*. Effect of human cytomegalovirus on invasive capability of early pregnant extravillous cytotrophoblasts. J Huazhong Univ Sci Technolog [Med Sci], 2011,31(6):819-823
19 Zhao JJ, Chen JJ, Liu T, *et al*. Anti-viral effects of urosolic acid on guinea pig cytomegalovirus *in vitro*. J Huazhong Uni Sci Technolog [Med Sci], 2012 (6):883-887
20 Zhao JJ, Chen SH, Fang JG, *et al*. Antiviral effects of obeanolic acid on guniea cytomegalovirus *in vitro*. Lishizhen Med Materia Medica Res (Chinese). 2011(12): 2862-2864
21 Yinon Y, Farine D, Yudin MH, *et al*. Cytomegalovirus infection in pregnancy. J Obstet Gynaecol Can, 2010,32 (4):348-354
22 Mccarthy FP, Giles ML, Rowlands S, *et al*. Antenatal interventions for preventing the transmission of cytomegalovirus (CMV) from the mother to fetus during pregnancy and adverse outcomes in the congenitally infected infant. Cochrane Database Syst Rev, 2011(3): D8371
23 Stenqvist AC, Chen T, Hedlund M, *et al*. An efficient optimized method for isolation of villous trophoblast cells from human early pregnancy placenta suitable for functional and molecular studies. Am J Reprod Immunol, 2008, 60(1):33-42
24 Dong M, Ding G, Zhou J, *et al*. The effect of trophoblasts on T lymphocytes: possible regulatory effector molecules—a proteomic analysis. Cell Physiol Biochem, 2008, 21(5-6):463-472
25 Ramhorst R, Fraccaroli L, Aldo P, *et al*. Modulation and recruitment of inducible regulatory T cells by first trimester trophoblast cells. Am J Reprod Immunol, 2012,67(1): 17-27
26 Said S I, Mutt V. Polypeptide with broad biological activity: isolation from small intestine. Science, 1970,169 (3951):1217-1218
27 Spong C Y, Lee SJ, Mccune SK, *et al*. Maternal regulation of embryonic growth: the role of vasoactive intestinal peptide. Endocrinology, 1999,140(2):917-924
28 Marzioni D, Fiore G, Giordano A, *et al*. Placental expression of substance P and vasoactive intestinal peptide: evidence for a local effect on hormone release. J Clin Endocrinol Metab, 2005,90(4):2378-2383
29 Zhao JJ. Effective constituents of TCM anti-GPCMV infection experimental study *in vitro*[D]. Huazhong Uni, 2011
30 Cristea IM, Moorman NJ, Terhune SS, *et al*. Human cytomegalovirus pUL83 stimulates activity of the viral immediate-early promoter through its interaction with the cellular IFI16 protein. J Virol, 2010,84(15):7803-7814

(Received Jan. 21, 2013)