

Study on Baicalein and Genistein to Inhibit Human Cytomegalovirus Infection in Human Astrocyte

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Abstract: There are no specific ways to prevent and cure human cytomegalovirus (HCMV) infection to date. We investigate the effect and mechanism of baicalein (BAI) and genistein (GEN) used as a drug to inhibit HCMV infection in human astrocyte(AS). RT-qPCR was used to detect the expression changes of viral IE1,IE2 genes. Chromatin immunoprecipitation assay (ChIP) was used to detect the expression change of major immediate early promoter (MIEP). RT-qPCR results showed that compared with HCMV group, 20 $\mu\text{mol/L}$ BAI+HCMV,HCMV+BAI group, and 10 $\mu\text{mol/L}$ GEN+HCMV,HCMV+GEN group can down-regulate the IE1,IE2 genes expressions. ChIP results showed that compared with HCMV group, 20 $\mu\text{mol/L}$ BAI+HCMV,HCMV+BAI group, and 10 $\mu\text{mol/L}$ GEN+HCMV and HCMV+GEN group can reduce histone acetylation of MIEP. A certain concentration of BAI or GEN can inhibit the human astrocyte's IE1 and IE2 to some extent. The inhibiting mechanism may be that BAI and GEN can decrease the expression of IE1,IE2 genes and also inhibit histone acetylation of MIEP, thus decrease the expression of IE1,IE2 genes, which makes BAI and GEN inhibit viral proliferation.

Keywords: Human Cytomegalovirus; Human Astrocyte; Baicalein ; Genistein; ChIP

Introduction

Human cytomegalovirus (HCMV) is a β -herpesvirus and a ubiquitous pathogen that resides latently in the host for life once the host is infected. It exhibits a seroprevalence of about 60-100% in different human groups. Healthy individuals infected with HCMV are usually asymptomatic. But in newborns, immunocompromised adults and transplant recipients, the virus can reactivate to cause severe disease and often mortality[1, 2]. To date, there are no specific ways to prevent and cure HCMV infection. Baicalein(BAI), 5,6,7-Trihydroxyflavone, $\text{C}_{15}\text{H}_{10}\text{O}_5$, is a phenolic

flavonoid derived originally from the root of *Scutellaria baicalensis* Georgi and it exhibits various biological activities[3-8]. Genistein(GEN), 4',5,7-trihydroxyisoflavone, $\text{C}_{15}\text{H}_{10}\text{O}_5$, is a soy-derived biologically active isoflavone that possess various biological activities including antiviral effect[9, 10]. There are few reports about using BAI or GEN to inhibit HCMV infection in human astrocyte(AS). HCMV major immediate early promoter(MIEP) regulate the expression of UL122(encoding immediate early [IE] protein 2 [IE2]) and UL123(encoding IE1)[11]. Suppressing this

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promoter can inhibit HCMV's reactivation. This study investigated the effect and mechanism of inhibiting HCMV in AS using BAI or GEN in order to provide basis for HCMV's prophylaxis and treatment.

Materials and methods

Materials

Cells AS was derived from differentiation of human neural stem cell(NSC).Both NSC and human embryonic lung fibroblast(HELFL) were stored in our lab. HCMV AD169 strain was donated by Pasteur Institute in France, proliferated through HELFL, and collected when the cytopathic effect reached 80%.The virus titer was 10^8 PFU/mL tested by plaque formation assay and the virus was stored at -86°C .

Culture medium Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12,HyClone) with 10% fetal calf serum (FBS)(Gibco), DMEM/F-12 (HyClone) with 2% FBS(Gibco).

Drugs Baicalein(Sigma), $\text{C}_{15}\text{H}_{10}\text{O}_5$,Mr270.24,dissolved by DMSO, stored at -20°C .Genistein(Sigma), $\text{C}_{15}\text{H}_{10}\text{O}_5$,Mr270.24, dissolved by DMSO, stored at -20°C . According to previous test, we chose $20\ \mu\text{mol/L}$ BAI and $10\ \mu\text{mol/L}$ GEN prepared with DMEM/F-12 with 2% FBS as drugs.

Methods

Cell culture ASs were cultured in the medium of DMEM/F-12 with 10% FBS and in 37°C ,5% CO_2 . incubator. The medium was changed every 3d,and subcultured every 5~6d.The second generation ASs which grew in the logarithmic phase were chose to experiment.

Experimental groups When the ASs were cultured to the logarithmic phase, the medium was replaced with DMEM/F-12 including 2% FBS. Five groups were set up as follows: ①Contra(C) ②Drug(D) ③HCMV(V) ④HCMV+Drug(V+D) ⑤Drug+HCMV(D+V).”Drug” means BAI or GEN. The moment when the virus was added was set as a benchmark.4 hours before the

benchmark, The medium of C group, V group, V+D group was replaced with DMEM/F-12 including 2% FBS. Meantime the medium of D group, D+V group was replaced with the same volume of BAI or GEN solution prepared with DMEM/F-12 including 2% FBS. At the moment of benchmark, V group, V+D group, D+V group were added HCMV(MOI=5), C group, D group were added the same volume of DMEM/F-12.4 hours after the benchmark, the medium of C group, V group was replaced with DMEM/F-12 including 2% FBS. The medium of D group, V+D group, D+V group was replaced with the same volume of new BAI or GEN solution. The moment when HCMV was added was set as the start time point,6h group, 24h group,48h group and 72h group and so on were set based on different experimental needs and each time group included the 5 groups above-mentioned.

RT-qPCR RNA extraction. ASs were cultured in 6 cm culture dishes and divided into 5 groups according to methods mentioned above when they grew to the logarithmic phase. The volume of HCMV added was $100\ \mu\text{L}$. The cells which had been infected for 24 hours, 48 hours and 72 hours were collected. Trizol was used to extract the RNA. Transcript First Strand cDNA Synthesis Kit (Roche) was used to reverse transcribe RNA. LightCycler 96 (Roche) was used to run qPCR. β -actin F primer:5'-TGGAACGGTGAAGGTGACAG-3',R primer:5'-GGCTTTTAGGATGGCAAGGG-3'(154bp).IE1 F primer:5'-CAAGTGACCGAGGATTGCAA-3', R primer:5'-CACCATGTCCACTCGAACCTT-3'(85bp).IE2 F primer:5'-TGACCGAGGATTGCAACGA-3',R primer:5'-CGGCATGATIGACAGCCTG-3'(89bp).Real-time PCR condition: 95°C ,5 min; 95°C ,10 sec, 55°C ,20 sec, 72°C ,30 sec,40 cycles.The data were analyzed with the method of $2^{-\Delta\Delta\text{Ct}}$.

Chromatin immunoprecipitation(ChIP) ASs were cultured in 10 cm culture dishes and divided into 5 groups according to methods mentioned above when they grew to the logarithmic phase. The volume of HCMV added was $200\ \mu\text{L}$. The cells which had been

infected for 6 hours and 24 hours were collected. The experiment was performed with Acetyl-Histone H3 ChIP Assay Kit (Millipore #17-245) and then ran PCR. MIEP F primer:5'-TGGGACTTTCCTACTTGG-3',R primer:5'-CCAGGCGATCTGACGGTT-3'.PCR procedure:95 °C,5 min;94 °C,30 sec,55 °C,30 sec,72 °C,1 min,35 cycles. Gray values were analyzed with Gel-Pro Analyzer. The expression values of V group, V+D group,

D+V group, divided the input values in the same time group, and the input value was set to 1 at last.

Statistical analyses Every experiments were performed three times independently. Results were showed by means \pm standard deviations ($\bar{x} \pm s$). Data were analyzed by One-way ANOVA. Differences were considered to be significant when P values were less than 0.05

Results

RT-qPCR

Fig. 1 Relative expression of IE genes in different groups (BAI)

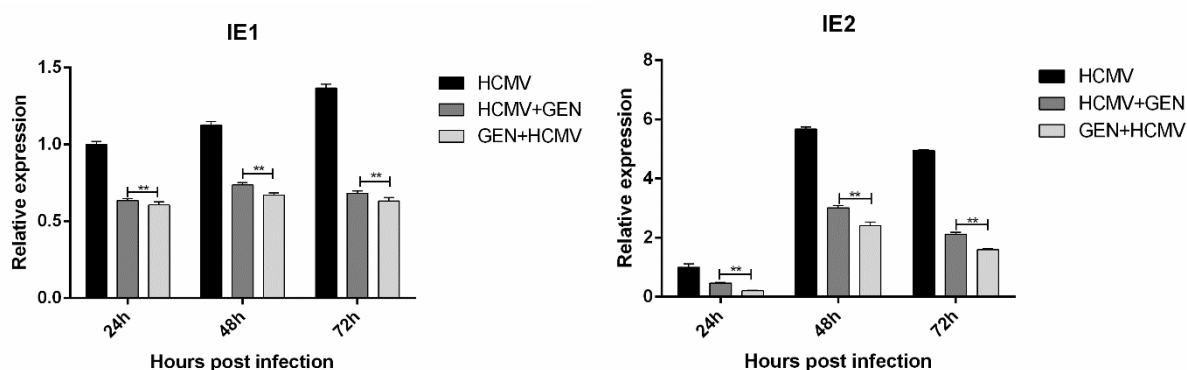
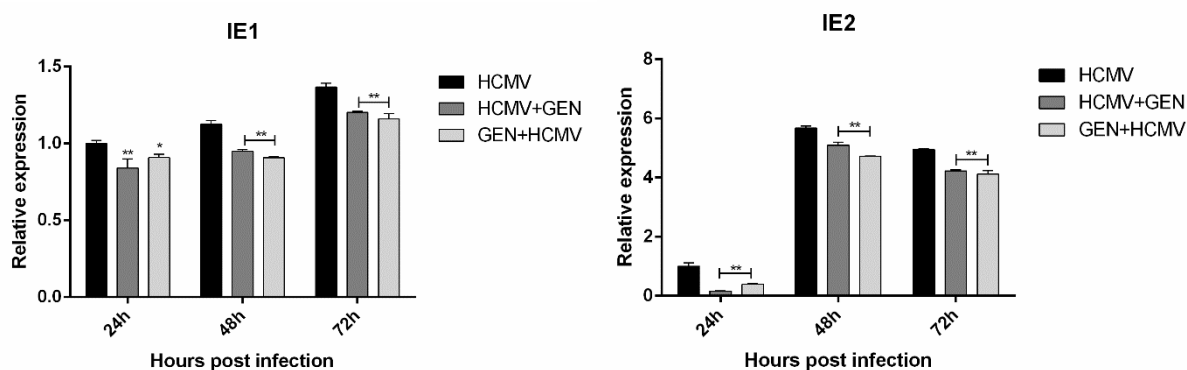


Fig. 2 Relative expression of IE genes in different groups (GEN)

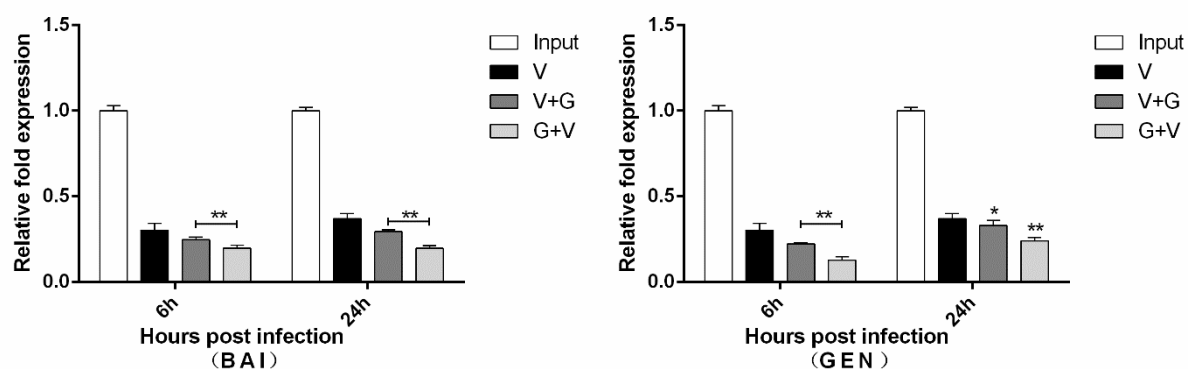


Results of gene expression were relative to levels measured at 24h post infection. Compared with HCMV group, *P<0.05, **P<0.01

Results showed, the expression of IE1 and IE2 genes in V+D group and D+V group was lower than that in V group at 24h,48h and 72h. The inhibiting effect in D+V group was stronger than that in V+G group. Also we can found the inhibiting effect on HCMV infection using BAI was stronger than that using GEN.

ChIP

Fig. 3 Relative expression of MIEP in different groups



Compared with HCMV group, * $P < 0.05$, ** $P < 0.01$

Results showed, The relative expression of MIEP in V+D group and D+V group was lower than that in HCMV group at 6h and 24h. The inhibiting effect on histone acetylation in D+V was stronger than that in V+G group.

Discussion

BAI has been studied that it exhibits various biological activities including anti-cancer[4], anti-virus[5, 6], and has effect on other diseases[7, 8]. GEN also have been confirmed it has a wide spectrum of activities including anti-cancer[12], anti-viruses[13-15] and treating diabetes[16, 17], etc. We found that, BAI and GEN have a certain effect on inhibiting HCMV infection in AS. The group adding drug first (D+V) and the group adding drug last (V+D) were set to investigate the effect of them on stopping virus from entering the cells. Results showed that drugs added last also had an obvious effect on inhibiting HCMV infection. Results showed 20 $\mu\text{mol/L}$ BAI and 10 $\mu\text{mol/L}$ GEN can inhibit HCMV IE1 and IE2 genes. By further research, we found these drugs can inhibit the histone-3 acetylation of MIEP. The inhibiting effect in D+V group was stronger than that in V+G group. This suggests BAI and GEN have a certain effect on preventing virus from entering the cells. And we also found the inhibiting effect on HCMV infection using BAI was stronger than that using GEN.

The study showed, 20 $\mu\text{mol/L}$ BAI and 10 $\mu\text{mol/L}$ GEN can inhibit HCMV IE1 and IE2 genes. The inhibiting mechanism may be that drugs can down-regulate the expression of IE1 and IE2 genes directly or inhibit MIEP histone acetylation, then down-regulate the expression

of IE1 and IE2 genes. However, further studies are needed to determine the effectiveness and exact mechanism of inhibiting HCMV infection in AS using BAI and GEN.

References

- [1] Britt W. Manifestations of human cytomegalovirus infection: proposed mechanisms of acute and chronic disease. *Current topics in microbiology and immunology*. 2008;325:417-70.
- [2] Griffiths P, Baraniak I, Reeves M. The pathogenesis of human cytomegalovirus. *The Journal of pathology*. 2015;235:288-97.
- [3] Huang Y, Tsang SY, Yao X, Chen ZY. Biological properties of baicalein in cardiovascular system. *Current drug targets Cardiovascular & haematological disorders*. 2005;5:177-84.
- [4] Li-Weber M. New therapeutic aspects of flavones: the anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin. *Cancer treatment reviews*. 2009;35:57-68.
- [5] Xu G, Dou J, Zhang L, Guo Q, Zhou C. Inhibitory effects of baicalein on the influenza virus in vivo is determined by baicalin in the serum. *Biological & pharmaceutical bulletin*. 2010;33:238-43.
- [6] Moghaddam E, Teoh BT, Sam SS, Lani R, Hassandarvish P, Chik Z, et al. Baicalin, a metabolite of baicalein with antiviral activity against dengue virus. *Scientific reports*. 2014;4:5452.
- [7] Yu X, He G, Du G. [Neuroprotective effect of baicalein in patients with Parkinson's disease]. *Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica*. 2012;37:421-5.

- [8] de Oliveira MR, Nabavi SF, Habtemariam S, Erdogan Orhan I, Daglia M, Nabavi SM. The effects of baicalein and baicalin on mitochondrial function and dynamics: A review. *Pharmacological research*. 2015;100:296-308.
- [9] Ganai AA, Farooqi H. Bioactivity of genistein: A review of in vitro and in vivo studies. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*. 2015;76:30-8.
- [10] Tarkowski M, Kokocinska M, Latocha M. [Genistein in chemoprevention and treatment]. *Polski merkuriusz lekarski : organ Polskiego Towarzystwa Lekarskiego*. 2013;34:54-7.
- [11] Stinski MF, Meier JL. Immediate-early viral gene regulation and function. In: Arvin A, Campadelli-Fiume G, Mocarski E, Moore PS, Roizman B, Whitley R, et al., editors. *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis*. Cambridge2007.
- [12] Spagnuolo C, Russo GL, Orhan IE, Habtemariam S, Daglia M, Sureda A, et al. Genistein and cancer: current status, challenges, and future directions. *Advances in nutrition (Bethesda, Md)*. 2015;6:408-19.
- [13] Andres A, Donovan SM, Kuhlenschmidt MS. Soy isoflavones and virus infections. *The Journal of nutritional biochemistry*. 2009;20:563-9.
- [14] Argenta DF, de Mattos CB, Misturini FD, Koester LS, Bassani VL, Simoes CM, et al. Factorial design applied to the optimization of lipid composition of topical antiherpetic nanoemulsions containing isoflavone genistein. *International journal of nanomedicine*. 2014;9:4737-47.
- [15] Sauter D, Schwarz S, Wang K, Zhang R, Sun B, Schwarz W. Genistein as antiviral drug against HIV ion channel. *Planta medica*. 2014;80:682-7.
- [16] Gilbert ER, Liu D. Anti-diabetic functions of soy isoflavone genistein: mechanisms underlying its effects on pancreatic beta-cell function. *Food & function*. 2013;4:200-12.
- [17] Behloul N, Wu G. Genistein: a promising therapeutic agent for obesity and diabetes treatment. *European journal of pharmacology*. 2013;698:31-8.