

Primary study on the application of Serum Pharmacology in Chinese traditional medicine

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Abstract

In the paper, two main methods, which are Serum Pharmacology and Traditional Pharmacology, were adopted to study Chinese traditional medicine, such as *Ginkgo biloba* extract (GBE), ginsenosides (GS) and compound GG (GBE + GS), pharmacology in vitro. The results showed that there were evident difference between the results of Serum Pharmacology and that of Traditional Pharmacology. There was no significant difference between the drug effect of crude GS on nitric oxide (NO) production in ECV304 and that of crude GBE, and the drug effect of GG was superior to that of GS and GBE, respectively. But, compared with GBE serum, the GS serum up-regulation of NO production in ECV304 increased significantly, and the GG serum up-regulation of the NO production in ECV304 was inferior to that of GS serum and GBE serum significantly. The results suggested that Serum Pharmacological study should be adopted in the pharmacological investigation on the Chinese traditional medicine and the drug screening of the Chinese traditional medicine.

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1. Introduction

In pharmacology study for Chinese traditional medicine, crude drugs are often added into the culture system of cells or organs in vitro directly for a long time. Whereas, the results of experimental study in vitro with crude drugs are often different from that of in vivo because the compositions of Chinese traditional medicine are complex. Chinese traditional medicine Serum Pharmacology, in which drug or drug compound are given to animals orally, and blood is collected to separate serum after definite time and then the drug serum is used for experimental study in vitro, has been developed recently for experimental study on Chinese traditional medicine pharmacology in vitro. Many researchers believe that Serum Pharmacology is more scientific and more befitting for Chinese traditional medicine than Traditional

Pharmacology in which crude drugs are directly added into the culture system of cells or organs in vitro.

Ginseng and *Ginkgo biloba* are traditional medicines in China and have pharmacological activeness in many aspects [1]. Researchers carried out a lot of study on their chemical components and relative pharmacology [2,3]. Many studies showed that *G. biloba* extract (GBE) and ginsenosides (GS) had drug effect on cardio-vascular diseases [4–8]. Nitric oxide (NO), which is produced mainly by vascular endothelial cells (ECV), is an important physiological messenger and effector molecule in many biological systems, including immunological, neuronal and cardiovascular tissues [9]. The abnormality of NO production in ECV resulted from lipid peroxidation injury has close relation to cardiovascular disease [10]. So, the two methods were adopted to study the effect of GBE, GS and compound GG (GBE + GS) on the nitric oxide NO production in vascular endothelial cells which were subjected to lipid peroxidation injury induced by diamide [11] in order to compare the difference of the results of this two methods.

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2. Material and methods

2.1. Cells

Human vascular endothelial cells strain ECV304 purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China).

2.2. Animals

Japanese flap-eared rabbits purchased from the Third Military Surgeon University (Chongqing, China).

2.3. Apparatus

TC232-2E CO₂ culture tank of Sheldon Manufacturing Inc., USA; ITM-2 inverted microscope of Olympus Optical Co. Ltd., Japan.

2.4. Reagent

GBE, which contains over 30% of total *G. biloba* flavones, was prepared by us. GS, which contains over 98% of ginsenosides, was a generous gift from Tecthmate Co., Beijing, China. Diamide, whose molecular weight is 50.06, was purchased from Easten Regent Co., Chongqing, China; F-12 DMEM culture medium (cat no.: SH30004.01) was purchased from Hyclone Co., USA; newborn calf serum was purchased from Biotechnology Development Center, China; NO assay kit (cat no.: S0021) was purchased from Beyotime Biotechnology Co., Jiangsu, China.

2.5. ECV304 culture

ECV304 stored by freezing were resuscitated and put into culture flask with DMEM culture medium, which include 10% newborn calf serum, and then incubated in 5% CO₂ culture tank under the condition of 37 °C and 80% relative humidity. When cells came into logarithmic growth, they were prepared into cell suspend solution with culture medium. And then the concentration of cells was adjusted to about 1×10^5 ml⁻¹.

2.6. Serum preparation

Rabbits were grouped to blank serum group (saline group), GBE serum group, GS serum group and GG serum group. Two adult female Japanese flap-eared rabbits were adopted in each group. The rabbits of blank serum group were treated with 30 ml saline orally one time each day for 3 days in succession each rabbit. Other groups were treated with relevant drugs orally one time each day for 3 days in succession each rabbit. After 2 h of treatment with drugs on third day, the blood was obtained germ freely from main ventral artery. And after the tubes containing bloods were allowed to stand

at 25 °C for about 5 h, the serums were acquired by centrifugation at 2500 rpm for 20 min. After two times filtration with 0.22 μm cellulose acetate membrane, the serums were bottled. Next, the serums were caled by 56 °C water for 30 min and then stored at -20 °C.

2.7. Experimental groups

Cells suspend solution whose concentration was 1×10^5 ml⁻¹ was inoculated into 96-well culture plate with 0.1 ml each well. After incubated in 5% CO₂ culture tank for 24 h under the condition of 37 °C and 80% relative humidity, the upper clear liquid was thrown away. Then, the experiments were carried out as the following grouping. The first one was the crude drugs group, in which drugs and diamide were added into culture system simultaneously. The second one was the serum drugs group, in which diamide was previously added into culture system, then diamide was thrown away and drugs were added into after 4 h. And each group had the following five subgroups: control group incubated without diamide and drugs, model group incubated with diamide and without drugs, GBE group with diamide, GS group with diamide and GG group with diamide. For the crude drugs group, the control group and the model group incubated without serum, and for the serum drugs group, the control group and the model group incubated with blank serum. At the same time, each subgroup had six parallel samples. After cells were incubated according to the grouping, NO production in VEC was detected.

2.8. NO determination

The transient and volatile nature of NO makes it unsuitable for most convenient detection methods, however two stable breakdown products, nitrate (NO₃⁻) and nitrite (NO₂⁻) can be easily detected by photometric means [12]. In this experimental, Griess method was adopted to detect NO, which is based on the chemical diazotization reaction that was originally described by Griess in 1879, which uses sulfanilamide and *N*-1-naphthylethylenediamine dihydrochloride (NED) under acidic (phosphoric acid) conditions [13]. After cells incubated according to the aforementioned grouping, 50 μl culture solutions of each well were collected and put into the counterpart well of another plates. Then, NO production in cells was measured by Griess method and according to the indication on the NO assay kit.

2.9. Statistical analysis

All values are represented as the means ± S.D. The differences of datum in mean values were analyzed by the Student's *t*-test using Statistical Program for Social Sciences and a *p*-value of less than 0.05 was considered significant.

3. Results

The NO production in ECV304 of each group was determined and the results are shown in Figs. 1 and 2.

As is shown in Figs. 1 and 2, the following points could be found out. NO production in ECV304 of model group decreased significantly compared with that of control group and crude drugs and drug serums also could up-regulate NO production in ECV304 subjected to lipid peroxidation injury. Compared with that of crude GBE group and crude GS group, NO production in ECV304 of crude GG group increased by 6.3% and 3.1%, respectively. But compared with that of GBE serum group and GS serum group, NO production in ECV304 of GG serum group decreased significantly by 29.8% and 66.7%, respectively. At the same time, NO production in ECV304 of crude GS group only increased by 2.4% compared with that of crude GBE group. But NO production in ECV304 of GS serum group increased significantly by 110.5% compared with that of GBE serum group.

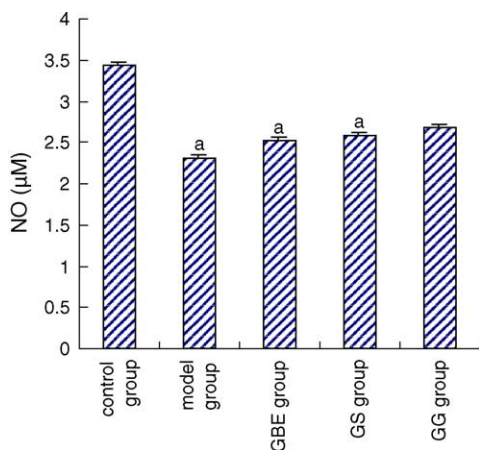


Fig. 1. Effect of crude drugs on NO production in ECV304. (a) Significantly different compared with the control group, *t*-test, $p < 0.05$.

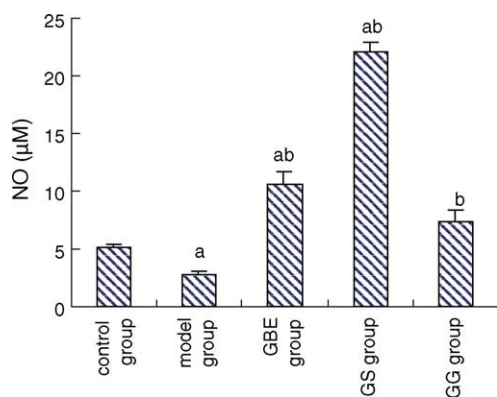


Fig. 2. Effect of drug serum on NO production in ECV304. (a) Significantly different compared with the control group, *t*-test, $p < 0.05$ and (b) significantly different compared with the model group, *t*-test, $p < 0.05$.

4. Discussion

In Traditional Pharmacology for Chinese traditional medicine, crude drugs or crude drug compounds are added into the culture system of cells or organs in vitro directly [14,15]. But the compositions of drug or drug compound of Chinese traditional medicine are complex and many of the compositions do not work until they undergo a series of biotransformation after digest and absorption in gastrointestinal tract. So, the effective compositions in crude drug could be not the real effective compositions working in vivo and it is difficult to achieve same results in experiment study in vitro with crude drugs with drug effect in vivo. Additionally, the pH, pervasion pressure, physical and chemical character of crude drugs of Chinese traditional medicine and the impurities in them are bound to result in the changes of physiology of reaction system in vitro and then affect the validity of experimental results [16]. So, the false positive results or the false negative results are easy to occur. Serum Pharmacology, which was put forward by Tashino [17], get over the interferences of crude drugs physical and chemical character on experiment results. And the experiment conditions of Serum Pharmacology are more similar with the environment in which drugs work in vivo than that of Traditional Pharmacology for Chinese traditional medicine. So, it could be believed that Serum Pharmacology sets up the bridge of experiment in vitro and experiment in vivo for Chinese traditional medicine pharmacology study.

The results of Traditional Pharmacology study (detailed to see Fig. 1) showed that crude GBE, GS and GG all could up-regulate the decrease of NO production in ECV304 in vitro that was induced by lipid peroxidation injury, the effect of crude GS was no significant difference from that of crude GBE and the effect of crude GG was superior to that of GBE and that of GS. But the results of Serum Pharmacology study (detailed to see Fig. 2) showed that the effect of GS serum on up-regulation of NO production in ECV304 was superior to that of GBE significantly and the effect of GG serum on up-regulation of NO production in ECV304 was inferior to that of GBE and that of GS significantly. It could be found easily that the results of Serum Pharmacology study are different from that of the results of Traditional Pharmacology study evidently.

The factors causing the difference may be that the drugs did not undergo metabolism and biotransformation in Traditional Pharmacology study so that there were not the interactions of drugs between each other, which led to the environment felt by cells was significantly different from that in Serum Pharmacological study. Because Chinese traditional medicine's process of metabolism cannot be learned clearly owing to its complicated components as that of western medicine, Serum Pharmacological study should be adopted, which could lead to scientific and exact results, in the pharmacological investigation on Chinese traditional medicine and drug screening of Chinese traditional medicine.

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