

## Exploring the standardization of serum pharmacology methods for Chinese herbal compound

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**Abstract:** Based on the discussion of the standardization of serum pharmacology methods of Chinese herbal compounds, this paper mainly analyzes four aspects, namely, serum pharmacological methods of Chinese herbal compounds, the current research status of serum pharmacology methods of Chinese herbal compounds, the difficulties in the research of serum pharmacological methods of Chinese herbal compounds, and several suggestions for the analysis of serum pharmacological methods of Chinese herbal compounds, hoping to contribute to the improvement and promotion of serum pharmacology methods of Chinese herbal compounds.

### 1. Introduction

Although serum pharmacology has appeared in traditional Chinese medicine for a relatively short time, its research value and significance cannot be ignored. Specifically, serum pharmacology overcomes the difficulty of directly using Chinese herbal compounds in *ex vivo* experiments. It is also possible to observe the comprehensive pharmacological effects of the compound *in vivo* after gastrointestinal absorption and biotransformation *in vitro* experiments. In addition, it is also beneficial for further studying the action law of Chinese herbal compounding and verifying many fundamental theories of traditional Chinese medicine. Thus, to promote traditional Chinese medicine's modernization more strongly, it is imperative to carry out a deeper discussion on the standardization of serum pharmacology methods for Chinese herbal compounds.

### 2. Overview of serum pharmacological methods of chinese herbal compounds

In 1948, Hiroko Iwama first proposed the concept of serum pharmacology in Japan. This method uses animals as objects, orally administers traditional Chinese medicine or Chinese herbal compound to them, waits for a while, and then performs blood collection and serum separation operations. Subsequently, the experimenters need to replace the crude extracts of traditional Chinese medicine with medicated serum and add new drug sources with *in vitro* reaction systems, supporting the study of pharmacological effects<sup>[1]</sup>. In other words, serum pharmacology can perform *in vitro* experiments using the medicated serum to characterize the complex chemical composition of Chinese medicine and its compound formulas. The experimental results obtained in this way are significantly more accurate and reliable than those obtained using crude preparations of Chinese medicine. The serum pharmacology experiment belongs to the semi-*in vivo* experiment category. It is also the primary method for Chinese medicine experts to study the pharmacological effects of traditional Chinese medicine in recent years.

The main advantages of the serum pharmacological method of Chinese herbal compounds can be summarized as follows.

Firstly, compared with the previous method of directly adding crude extracts of Chinese medicine,

the medicated serum is more closely related to the metabolism process of the Chinese herbal compound in the organism. On the one hand, it can more directly reflect the medicinal benefits of traditional Chinese medicine. On the other hand, it also gives us a more intuitive understanding of how traditional Chinese medicines and their compounds are processed in the stomach. Second, regarding screening and evaluation of drugs and their active ingredients, the medicated serum is more effective, precise, and relevant to reality. Third, serum pharmacology has promoted innovation in traditional medicine. The most representative of these is the reformulation of pharmacological mechanisms using cellular and molecular biology. It is conducive to observing and tracking the absorption and metabolism of drugs and also beneficial to discover the actual active ingredients and active sites in traditional Chinese medicine. Fourth, with the widespread promotion of serum pharmacology, many new subdisciplines of traditional Chinese medicine pharmacology, such as analytical chemistry of traditional Chinese medicine, serum pharmacochemistry, and pharmacokinetics of traditional Chinese medicine and its compounding, have been developed rapidly. All these have contributed significantly to the modernization of traditional Chinese medicine. After scholars' research at home and abroad in recent years, although the serum pharmacological methods of Chinese herbal compounds have been optimized and improved to a certain extent, there is still much room for progress. We should conduct more in-depth research on this so that this method can be more effective.

### **3. Current status of research on serum pharmacology methods of Chinese herbal compounds**

#### **3.1 Determine the administration dosage**

The most crucial task in serum pharmacology experiments is administering drugs to animals and conducting pharmacological observations on their serum. When determining the administration dosage, the experimenter must address the following issues. After adding the medicated serum to the *in vitro* reaction system, its concentration will decrease. In other words, if an equivalent dose is given to the subject, the blood concentration will be diluted to one-tenth of the original dose by adding one-tenth of the serum volume to the *in vitro* reaction system. If the reaction system subsequently shows a negative reaction, this false negative result may be caused by the drug concentration being far below the standard. Therefore, to ensure the accuracy of serum pharmacological experimental results, how to achieve approximately the same drug concentration in the *in vitro* reaction system under *in vivo* conditions must be considered before the experiments are carried out.

#### **3.2 Choose the method of administration**

##### **3.2.1 Method of administration**

Taking the serum freeze-dried powder method as an example, when designing the dosage, the technical requirements of the pharmacological research of new drugs should be taken as the basis. The experimenter first needs to make the medicated serum into the freeze-dried powder. After the serum is frozen in the freezer, it is placed in a vacuum at  $-70^{\circ}\text{C}$ . Under drying conditions, its water is evaporated and turned into a dry powder. The experimenter then adds freeze-dried powder to the reaction system so that the concentration of the drug is as close as possible to the standard under *in vitro* conditions, thus achieving the goal of controlling the concentration of traditional Chinese medicine in the reaction system as required. In this case, it is also essential to understand that "iso-concentration" means that the drug concentration in the *in vitro* response system must equal the blood concentration *in vivo*. After meeting this requirement of iso-concentration, the results of serum pharmacology experiments are the most comparable, and the drug's action *in vivo* can be most strongly described. They are of great importance for evaluating the efficacy of new drugs.

The serum freeze-dried powder method can achieve iso-concentration, while the other method cannot. Specifically, suppose the same volume of medicated serum freeze-dried powder is added to 100  $\mu\text{L}$  of culture medium. In that case, the drug concentration in the medium is not diluted from a theoretical point of view, so the requirement of equal concentration is met. On the contrary, if the medicated serum is directly added to the *in vitro* reaction system unless the reactants, such as cells or

tissues, are directly added to the serum without any nutrient solution, it is impossible to achieve iso-concentration anyway. In addition, since there is an upper limit on the amount of medicated serum added during actual serum pharmacology experiments, exceeding this limit will affect cell or tissue activity. The results obtained after using the serum freeze-dried powder method to observe the effects of traditional Chinese medicine in an iso-concentration environment were very considerable. It shows that the freeze-dried serum powder not only enhances the drug concentration in the in vitro reaction system as required but is also helpful for storing and preserving serum<sup>[2]</sup>.

### **3.2.2 Frequency and time interval of administration**

The most common current dosing regimen is administering the drug at 2-4h intervals, 1-3 times/d, with dosing lasting 3-10days. It has been experimentally proven that if the administration interval and dose are fixed, the blood concentration will be stabilized after several repeated administrations. In this state, even if the extraction time of the medicated serum was different, the pharmacological action was found to be high and similar in intensity after the experiment. Domestic researcher Li Meng has conducted experiments on the preparation of medicated serum based on the effect that compound Danshen serum would have in releasing reactive oxygen species, allowing the scientific validity of this drug delivery protocol to be demonstrated. Experiments show that this regimen is significantly different from the one-time administration regimen and is closely related to the drug-induced formation of endogenous bioactive substances in vivo; the industry has recognized this conclusion.

### **3.3 Determine blood collection time**

The peak of blood concentration is the most appropriate time to collect blood. Yichun Meng et al. suggested that the time range of blood collection could be determined based on the absorption and transformation characteristics of traditional Chinese medicine and the digestive and physiological aspects of the experimental subjects. In rats, the blood concentration usually peaks at 1h-2h, and the serum at this time is the medicated serum. Some drug groups will change over time and thus be converted into the drug-metabolizing serum. Multiple blood collection time should usually be concentrated between 30min-3h after the last administration. After the administration of the Chinese herbal compound, the time when the blood drug concentration enters the peak period is also different for different drugs. Therefore, there is no strict uniform standard for the time of blood collection. In actual experiments, blood can be collected separately for pre-experiments according to the corresponding time points. The medicated serum obtained at each end can be compared before deciding when to collect blood, which is relatively more reliable and scientific<sup>[3]</sup>.

### **3.4 Common blood collection methods**

Subjects may be given the drug to be tested twice a day for three consecutive days, with blood collected one hour after the final administration. At this time, the blood concentrations of the experimental subjects have been more significant than the C<sub>max</sub> of a single administration. If the accumulation is not appeared simultaneously, reaching the peak is about one hour. The possibility of the drug components whose blood concentrations are close to or exceed the C<sub>max</sub> of a single administration is as high as 80%. Following the above administration and blood collection can eliminate the standardized pre-testing process. Still, it is prone to the disadvantage of negative results for a few drug components due to blood concentrations not reaching peak blood concentrations. In actual operation, blood collection can be carried out according to this plan first. If the result fails to meet expectations, the problem can be found in the blood collection time, and the standard method can be used to obtain the optimal blood collection time<sup>[4]</sup>.

## **4. Difficulties in the research of serum pharmacological methods of Chinese herbal compounds**

Serum pharmacology of traditional Chinese medicine is still in the research and exploration stage. It cannot yet be fully used as a substitute for experimental pharmacological methods in traditional Chinese medicine. The difficulties in studying serum pharmacological methods of Chinese herbal compounds are rough as follows.

First, the serum has complex endogenous components, which is the first difficulty to overcome in the serum pharmacology study of Chinese herbal compounds. Secondly, animals of different ages and species have various absorption capacities, which may affect the drug content and amount of drugs in the serum, so the specification of the period and species of animals should also be considered. Third, differences in the administration and time of blood collection can result in differences in the composition and amount of drugs contained in the serum. Therefore, the control of administration and time of blood collection is also a problematic issue. Fourth, the medicated serum in each individual is not the same, which significantly increases the research difficulty<sup>[5]</sup>.

## **5. Suggestions for the analysis of serum pharmacological methods of Chinese herbal compounds**

First, in studying serum pharmacological methods of Chinese herbal compounds, the preparation of medicated serum should try to use animals of the same species as the cells, which can effectively avoid the problems of serum providers in terms of species. At the same time, the differences in the intestinal flora of different species and the possible influence of the active ingredient metabolism on the experimental results should be considered. Second, experimental studies on the quantitative and temporal impact should be conducted based on the drug and the observed indicators. Pharmacological experiments should be performed after the administration dosage, time of blood collection, and blood addition concentration has been determined. Third, the *in vitro* and *in vivo* tests can be conducted together. The conditions of the *in vitro* experiment are relatively less difficult to control, and the interfering factors *in vivo* can be ruled out. However, the two cell functions are not wholly consistent due to the difference between the *in vivo* and *in vitro*. Therefore, if the two can achieve scientific cooperation, the mechanism of action of the compound preparation can be elucidated at a deeper level, which is more consistent with clinical experiments and will help enhance the persuasiveness of the experimental design<sup>[6]</sup>. Fourth, the blood is not the channel through which all traditional Chinese medicines work, and the effects of some traditional Chinese medicines are directly reflected in the digestive tract. At the same time, the role of traditional Chinese medicine, four natures and five flavors in the blood, is not reflected, and serum pharmacology in this regard is powerless. Fifth, some experiments that require inactivated serum usually require the exclusion of non-pharmacological interfering factors. However, there are also some experiments where the serum cannot be inactivated or preserved. Otherwise, it will likely cause the loss of its active ingredients, which will fail the experiment. Therefore, the preservation or inactivation of the medicated serum should also be determined according to the situation. Sixth, any single element in the pharmacological mechanism of traditional Chinese medicine is challenging to play an essential role in optimizing compound efficacy. Still, we can accumulate it into biological effects<sup>[7]</sup>.

Nowadays, there is no technical tool for analyzing all single components of compounded serum, which seriously limits the application of serum pharmacology of traditional Chinese medicine in Chinese herbal compounding research and new drug development. The advantages of serum pharmacology in traditional Chinese medicine have placed it in an irreplaceable position. However, there are still many problems to be solved, and all researchers in the industry must continue their efforts.

## **6. Conclusion**

In conclusion, as the science of normal human physiology, pathology, health, and disease research, medicine is no different from other natural science research. Medical research is also the process of understanding objective things and exploring the unknown. The main task of medical research is to reveal the phenomena and mechanisms of the emergence and development of human diseases and the essence of life. The same is true for studying serum pharmacology methods of Chinese herbal compounds. We should base on respecting science and seeking truth from facts and make the serum pharmacology methods of Chinese herbal compounds more standardized through rigorous experiments and calculations. Thus, it can make a more significant contribution to the overall

development of the field of traditional Chinese medicine.

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