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The Development and Application of Methodology of Reverse Pharmacology Illustrated with the Research on Analgesic Effect of *Resina Draconis*

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Abstract

In reverse pharmacology, traditional drug that has a history of therapeutic activity is used as a starting point for drug discovery. However, documented natural-product drugs from traditional medicine are generally the mixtures of compounds having a variety of pharmacological effects. It is extremely difficult to identify their active components and clarify their pharmacological mechanism due to the complex relationship between the pharmacological effects of traditional drug itself and that of its components. The key idea used in solving this problem is that traditional medicine-inspired approaches to drug discovery should start with the relevance between identifying the material basis for the efficacy and clarifying the pharmacological mechanism of traditional drug.

A basic principle of the methodology of reverse pharmacology was proposed: Taking the pharmacological effect of traditional drug itself as reference, the effects of the components and/or the combinations of components were compared with traditional drug itself to select lead compound or combination which can produce the pharmacological effect as similar as original drug.

According to the principle above, the operational definition of the material basis for the efficacy of traditional drug was established. Searching for the material basis of the efficacy of traditional drug was converted to detecting, expressing and analyzing of the relationship between the pharmacological effects of the component and/or combination and the traditional drug itself. Thus the research framework of the pharmacological mechanism and the material basis of traditional drug was built up. The methodology of reverse pharmacology was applied to study the material basis and pharmacological mechanism of *Resina Draconis*. The analgesic effect of *Resina Draconis* was proved to be the synergistic effect of three components (cochinchinenin A, cochinchinenin B and loureirin B), and the methodology of reverse pharmacology was verified by this typical example.

Keywords: Reverse pharmacology; Methodology; Traditional medicine; Material basis; *Resina Draconis*

Introduction

Plant-based drugs from each nationality has been a potential abundant source for international pharmaceutical market, but few drugs have been in-depth researched and developed [1,2]. The previous pharmacological method begins with the study of disease-drug relationship and gradually turns to the research on the molecular pharmacological mechanism of the drug. But this traditional method in the research and development of new drug is inefficient [2]. Chemists have synthesized millions of compounds as the research objects for drug screening, yet it is difficult for us to research and develop new drug from the current libraries of compounds. However, the cost and time can be greatly reduced when the research framework of reverse pharmacology is used to research and develop new drug from traditional medicine [3-5]. The research and development starts with known traditional drug which has been applied to treat diseases for a long time in history and proved to be very safe and effective. And then such action targets of the traditional drug as receptor, ion channel and enzyme are identified on the basis of its pharmacological mechanism. Finally the biological active compounds of the traditional drug which have pharmacological effects on the above identified target are selected as r new drug candidates. Furthermore, traditional drugs can best embody the characteristics of traditional medicine cures and are regarded as their essence. It is of great importance for the development and further investigation of traditional medicine cures to seek the chemical component or the combination of the chemical components whose pharmacological effect could replace that of traditional drug itself and whose chemical structure is very clear because each traditional drug is the composition of various chemical components. However, the under-mentioned problems have emerged in the course of the research and development of traditional drugs using the research framework of reverse pharmacology. Some traditional drugs have been regarded as safe and effective in the clinical treatment, but their pharmacological mechanisms and action targets are not fully made clear. So far no method is satisfactory enough to be used to extract all the chemical components acting as material basis for the efficacy of the traditional drug. At present, there are lots of components extracted from traditional drugs and there will be more new components discovered as time goes by. Do the components extracted from traditional drugs have the same pharmacological effects as those of the original drugs? Are the interactions of the extracted components the same as those in original drugs? The answers to these questions are not certain. Sometimes, the pharmacological effects of the same component on various cells are different, whereas the pharmacological effects of various components

on the same cell are also different. The various pharmacological effects of the original traditional drug are not in perfect consistence with those of the components extracted from the traditional drug. Therefore, in the pharmacological research on the traditional drug and in the validation of the material basis for its efficacy, if the pharmacological effects of the traditional drug are not associated with those of its chemical components, it is impossible to exactly validate which components could produce the specific pharmacological effect of the traditional drug itself and to which degree this would happen. There is no appropriate approach to judge whether and how the chemical components extracted from the traditional drug constitute the material basis for the specific efficacy of the traditional drug according to one kind of pharmacological mechanism. Therefore, in order to find out the pharmacological mechanisms of the traditional drug and utilize the existing resource of chemical components of traditional drugs as much as possible, the correlation between clarifying the pharmacological effect and discerning the material basis for the efficacy of the traditional drug should be acquainted. The methodology for reverse pharmacology should be developed from this point. Thus, the physical and chemical properties and pharmacological characteristics of the extracted components could be utilized to search for the component or combination which has clear chemical structure and could produce the pharmacological effect of the traditional drug step by step.

Pain is considered as one of the major problem which heavily threatens the health of human beings. There is no lack of analgesic drugs in traditional medicine. *Resina Draconis* is yielded from resin of *Dracaena cochinchinensis* (Lour.) S.C. Chen [6]. *Resina Draconis* is one type of Dragon's Blood which is a renowned traditional drug and could be obtained from multi- species of four distinct plant genera [7]. Dragon's Blood from different species have similar effects such as anti-inflammation, relieving pain, hemostasis, promoting wound healing, etc [8]. *Resina Draconis* is a mixture of many ingredients including flavonoids, saponins, terpenes, phenols, lipids, etc [7]. Methodology of reverse pharmacology was developed by taking clarifying the analgesic mechanism of *Resina Draconis* and seeking its analgesic components as breakthrough point. According to physiological mechanism of pain signal generation and transmission, ion channels which are closely associated with the pain were selected as targets, and then the effects of *Resina Draconis* and its compounds on these targets were examined to screen out active components. Afterwards, compound or combination of some compounds which can produce the pharmacological effect as similar as *Resina Draconis* will be identified. Furthermore, the pharmacological mechanism of the combination of these components on the targets was studied. Traditional research approach study the effect of original drug or its component in isolation, which can't help to clarify the mechanism of the traditional medicine and the components producing the effect of original drug. This research strategy can overcome the disadvantage.

Developing the methodology for reverse pharmacology

Generally speaking, new situation was opened up for drug discovery when modern science has got a lot of progress in biotechnology, such as the high throughput screening, combinatorial synthesis, asymmetric synthesis, et al. But the pharmaceutical industry is still facing serious challenges because the drug discovery has become more expensive, more risk and lower efficiency. Therefore, a significant change that focus shift from single-target drugs to multi-target drugs have been occurring based on the knowledge of traditional medicine. Strategic choice which based on the drug discovery from natural products, ethnic pharmacology and traditional medicine is expected to overcome the main obstacle that caused by three factors of time, cost, and toxicity during the previous drug discovery. It has been coming to the fore as another drug discovery engine. New strategic choice spawned an interdisciplinary research field called reverse pharmacology [3,9] which provides a paradigm shift from random tracking accidental discovery to organizing a route for seeking lead compound in drug development.

In the study of reverse pharmacology, traditional drugs which have been proved curative in the long-term medical practice were selected for exploratory research aimed at standardization and safety. The confirmation of traditional drugs in the different biological levels, the clarification of its pharmacological mechanism and the identification of its corresponding active components or effective substance will provide scientific basis for the clinical application of traditional drugs, lay the foundation for safety assessment and quality control of traditional drugs, find new leads for creating new chemical drugs and turn the leads to drug candidate through preclinical and clinical research. In this process, safety is the most essential starting point and the effect becomes a thing to be confirmed. Previous "laboratory - clinical" mode in the drug discovery process was reversed to "clinical - laboratory" by reverse pharmacology inspired by the traditional knowledge. The innovativeness of this research mode is that combines the traditional knowledge and modern science and technology, to provide better and safer lead compounds more efficiently. Thus, the traditional herbal medicines which have appeared high efficacy and low side effects in long-term clinic practice got more attention.

The traditional medicine, a rich resource to obtain new lead compounds, generally has complex and diverse chemical composition. In case of analgesic drugs, based on past clinical experience and experimental observation, single herb analgesic are rhizoma ligustici wallichii [10], corydalis [11], panax notoginseng [12], radix angelicae pubesentis [13], clematis [14], etc. However, the analgesic activity component of these herbs has few developments so far. One reason for this situation is although these drugs and their ingredients have analgesic effect, the mechanism and targets have been not elucidated in the modern technological level. Another reason is which ingredients produce the analgesic effect of original drug has not been confirmed as the original drug is a mixture of a variety of chemical constituents and it usually has a variety of pharmacological effects.

Different from single chemical entity drugs, the pharmacological study of traditional medicine need not only clarify the pharmacological mechanism of the traditional drug but also identify the effective substances which produce specific pharmacological effects. The mechanism of the pharmacological effects was revealed by identifying the effective substances, while identifying the effective substances depend on clarifying the pharmacological mechanism of the traditional drug. This is a key issue that clarify scientific basis of clinical curative effect of traditional medicine through the correlation of effective substances and pharmacological mechanism. This is also a methodology question about how to get lead compounds which can reflect original drug's good performance.

Usual research method of traditional drug is the effects of the chemical composition of the original drug and those of the original drug itself were studied separately. This method do not link the effects of the original drug and its composition, and do ignore a problem that the effect of the original drug and its composition may not be completely consistent, which should give full attention in the research of traditional drug. Therefore, the past method does little help to

clarify the pharmacological mechanism of the original drug and to identify the compositions which have the specific pharmacological effect of the original drug and its degree. It is more difficult to determine whether the isolated compositions still retain the relationship between the ingredients in original drug when they produce specific pharmacological effect (Few studies on the combined effects of ingredients in original drug). Aiming at these unsolved questions in research and development of traditional medicine, based on the biological background of traditional medicine *Resina Draconis*, the basic principle of reverse pharmacology methodology that seeking the component/combinations as primer of new drug should compare the effects of the component/combination with those of the traditional medicine itself with the reference of the pharmacological effects of the traditional medicine were put forward.

In order to solve the problem, the operational definition of the material basis for the efficacy of traditional drug was established as follows [15]. If some component/combination extracted from the traditional drug could produce some pharmacological effect which could also be produced by the traditional drug, the component/combination can be regarded as the effective component/combination of the traditional drug corresponding to the pharmacological effect (We'd like to point out that the concept of active component is used particularly to refer to the component extracted from nature medicine with some pharmacological effects). When the quantity of the effective component/combination is correlative with the quantity of the component/combination contained in the traditional drug and when the pharmacological effect of the effective component/combination could replace that of the traditional drug, the effective component/combination can be named as the material basis for the efficacy of the traditional drug. Whether the traditional drug and its component/combination have pharmacological effects is determined by the values of their pharmacodynamic parameters. The effective degree of the component/combination is determined by the degree of agreement between the values of pharmacodynamic parameters of the component/combination and those of the traditional drug. According to the operational definition, a corresponding research strategy was designed: when the material basis for the efficacy of the traditional drug corresponding to some pharmacological effect is identified, the pharmacological research on the traditional drug should be combined with that on its component/combination. Besides, the study of material basis and the research on pharmacological mechanism of the traditional drug should also be combined. The pharmacological effect of the traditional drug as a whole should be identified first, and by using it as the reference, whether the examined component/combination of the traditional drug has similar effect should be subsequently identified. And then the values of pharmacodynamic parameters of the component/combination should be compared with those of the traditional drug. The comparison determines whether the component/combination is the effective component/combination or the material basis for the efficacy of the traditional drug. Conforming to the operational definition, the component/combination whose pharmacological effect could embody the pharmacological characteristics of the traditional drug and replace the pharmacological effect of the original traditional drug should be found out from various components of the traditional drug to act as the material basis for its efficacy.

The operational definition of the material basis for the efficacy of the traditional drug reflects the relationship between the pharmacological effect of original drug and that of the component/combination, and it helps to quantitatively characterize effectiveness of the component/combination. By the establishment of operational definition of effective substance, searching for the effective substance of traditional drug was converted to a problem that is how to detect, express and analyze the relationships between the pharmacological effects of the component/combination and the traditional drug itself, which could be solved by modern technology. This strategy makes the drug discovery based on traditional medicine resource can be really implemented. After the introduction of the operational definition of the material basis for the efficacy of the traditional medicine, the research model of reverse pharmacology is as follows. At first, based on the reliable data and clinical observations of the therapeutical effect of the traditional drug, the hypothesis of pharmacological mechanism of the traditional drug should be raised. Aiming at the target, exploratory study was carried out to confirm the effect of traditional drug at the molecular and cellular levels. According to the above operational definition and referring to the effects of the original drug, the component/combination having pharmacological activity were screened out. Through the qualitative and quantitative analysis of the pharmacological effects between the original drug and its component/combination, the material basis for the efficacy of the traditional drug which could replace (or approximation to replace) the original traditional drug can be identified. If the material basis for the efficacy consists of several components, the interaction between the components should also be analyzed. Afterwards, more extensive pharmacological and pharmacodynamic studies on the material basis for the efficacy should be carried out at the tissue, organ and individual levels.

Follow the methodology of reverse pharmacology above, a well-known traditional drug *Resina Draconis* which has been proved has good analgesic effect and no significant side effect in the long-term clinical practice was carried out a series of studies.

Practice of the methodology of reverse pharmacology

The pain's relationship with voltage-gated sodium channels and transient receptor potential vanilloid 1 (TRPV1)

Pain is a kind of subjective feeling which is from unpleasant to unbearable. This kind of feeling is due to some physical and chemical stimulation on peripheral nerve endings, such as high threshold of mechanical stimulation, too high or too low temperature stimulation, and variety of harmful chemical stimulation, etc. These kinds of stimulation are called nociceptive/noxious stimuli because they can lead to tissue damage. In the process of biological evolution some cells differentiation into sensory neurons. A part of neurons were classified as nociceptor which specifically receive nociceptive stimulation [16]. DRG neurons transmit nociceptive information from the peripheral region to central nervous system. The sensory nerve fibers derived from the abdominal region and the visceral nerve fibers transmitting pain gather together in DRG neurons. There are a lot of membrane protein (membrane receptor /channel protein) molecules in the membrane of the DRG neurons [17], such as Sodium channels, potassium channels, calcium channels, TRPV1, ASICs, nAChRs, etc.

Voltage-gated sodium channel, a kind of membrane glycoprotein, is widely distributed in excitable cells. Each channel comprises one α-subunit and two β-subunits. All sodium channel α-subunits consist of four homologous domains that form a single, voltage-gated aqueous pore [18]. Based on different sensitivity to tetrodotoxin (TTX), sodium channels in DRG neurons are classified into two types, one is TTX-S sodium channel and the other is TTX-R sodium channel.

TTX-S sodium channels are responsible for action potential initiation and propagation in excitable cells [19], including nerve, muscle, and neuroendocrine cell. Because the stimulus intensity inducing tactile allodynia could activate afferent Aβ fiber, there is a great possibility that TTX-S sodium channels are also involved in the transmission of nociceptive information [20]. It has been reported that the application of TTX at low doses (12.5-50 nM, far less than those needed for blocking action potential conduction) produced a significant elevation of mechanical threshold in the paw for foot withdrawals [21]. This change was a sign of reduced allodynic behaviors and suggested that TTX-S subtypes of sodium channels played an important role in maintaining allodynic behaviors in an animal model of neuropathic pain.

The involvement of TTX-R sodium channel has long been known in pain transmission, especially in chronic inflammatory pain and neuropathic pain. Hyperalgesic agents directly increased the magnitude of TTX-R sodium current, decreased its threshold for activation and increased its rate of activation and inactivation, which may underlie nociceptor sensitization [22]. In a model of neuropathic pain, drugs selectively decreased the expressions and the current amplitudes of TTX-R sodium channels, which could reverse the neuropathic pain induced by spinal nerve injury [23]. TTX-R sodium channel has been an attractive therapeutic drug target for inflammatory and neuropathic pain on the basis of its specific distribution in primary sensory neurons and its modulation induced by inflammatory mediators.

TRPV1 receptor is widely distributed in the central and peripheral nervous systems. It is activated not only by chemical factors such as capsaicin and acids, but also by a physical factor such as hot temperature (≥ 42°C) [24,25]. TRPV1 receptor is considered an integrator of noxious stimuli in peripheral nociceptor terminals and therefore may be at a crossroads for pain transmission pathways. The logical strategy for development of novel analgesics is to target the beginning of pain transmission pathway and aim potential treatments directly at nociceptors. The TRPV1 receptor antagonists may deliver broad spectrum efficacy in nociceptive pain via silencing pain signaling pathways [26]. Therefore, the discovery of new TRPV1 receptor antagonists becomes an attractive destination in the treatment of pain.

Identifying the material basis of Resina Draconis for modulating the voltage-gated sodium currents and inhibiting capsaicin-induced TRPV1 currents

Based on the understanding of physiology and pathological mechanisms of pain in modern medicine, TTX-S and TTX-R voltage-gated sodium channels and TRPV1 in rat dorsal root ganglion (DRG) neurons were selected as drug targets. By using whole-cell patch-clamp technique, the effects of *Resina Draconis* and its flavonoids on these targets were observed. *Resina Draconis*'s effects on TTX-S/TTX-R sodium currents and capsaicin-induced TRPV1 currents on rat DRG neurons were used as the reference for the evaluation of the pharmacological effects. The extracted components/combinations which have pharmacological activities were screened out, then the pharmacological parameters of the components/combinations and *Resina Draconis* were compared. According to the operational definition of the material basis for the efficacy of the traditional drug, it is to be determined that the material basis for the effects of *Resina Draconis* on TTX-S/TTX-R sodium currents and capsaicin-induced TRPV1 currents. The concentrations of various components in the combinations were determined by the mass ratio of these components in final products of *Resina Draconis*, which guaranteed that the quantity of each component existent in the combination was in agreement with that in *Resina Draconis*.

Cochinchinenin A, cochinchinenin B and loureirin B extracted from *Resina Draconis* which have significant pharmacological activity were screened out. Experimental results suggest that only loureirin B could produce approximate inhibition on the TTX-S sodium currents as *Resina Draconis*. Loureirin B could be regarded as the material basis of *Resina Draconis* for the inhibition on the TTX-S sodium currents [27]. Although cochinchinenin A, cochinchinenin B and loureirin B can inhibit the TTX-R sodium currents when they was used alone, none of them had approximate effect of Resina Draconis. The combination of cochinchinenin A, cochinchinenin B and loureirin B (molar ratio is 38:19:8) could produce approximate inhibition on the TTX-R sodium currents as *Resina Draconis*. So it is considered that the combination of cochinchinenin A, cochinchinenin B and loureirin B (38:19:8) was the material basis of *Resina Draconis* for the inhibition on the TTX-R sodium currents (Figure 1) [15].

None of the three components had approximate inhibition of *Resina Draconis* on the capsaicin-induced TRPV1 receptor currents on rat DRG neurons. But the combination of cochinchinenin A, cochinchinenin B and loureirin B (molar ratio is 38:19:8) could produce approximate inhibition on the capsaicin-induced TRPV1 receptor currents as *Resina Draconis* (Figure 1). Therefore it was considered that the combination containing cochinchinenin A, cochinchinenin B, and loureirin B (38:19:8) was also the material basis of *Resina Draconis* for inhibiting capsaicin-induced TRPV1 receptor current [28]. This inhibition could relief pain by intervening in the transmission of pain messages.

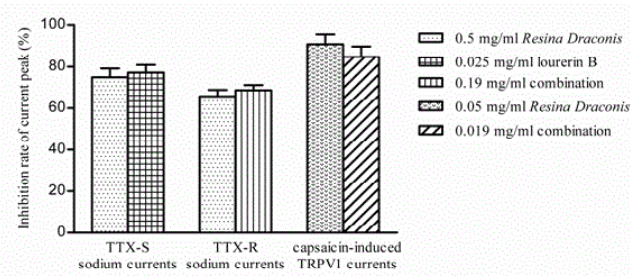

Figure 1: The pharmacological effects of *Resina Draconis* and its material basis on the voltage-gated sodium currents and capsaicin-induced TRPV1 currents. The molar concentration ratio of cochinchinenin A, cochinchinenin B, loureirin B in the combination is 38:19:8. $P > 0.05$, when compared with *Resina Draconis* by unpaired student's t-test [15,27,28]

Assessment of the interaction of the three components of Resina Draconis

The above results that the material basis for inhibition on the TTX-R sodium currents in DRG neuron of *Resina Draconis* was the effective combination of the three components showed that sometimes the pharmacological effect of the traditional drug was produced not only by one component but by the effective combination of several components. It is likely that the pharmacological effect of the single component in the combination was influenced by other components

in the combination, which required that the interaction of the components of the material basis for the efficacy of the traditional drug should be assessed if the material basis for the efficacy of the traditional drug was not a single component. This is also an indispensable step in making clear the pharmacological mechanism of the material basis for the efficacy of the traditional drug.

Drug interactions are usually divided into three types: antagonism, synergism and additivity. One mathematical model used to assess drug-drug interactions was developed by Greco et al [29,30] and could be used to characterize the interactions of two or three drugs when the dose-response curve of each drug follows Hill equation. All the dose-response curves of cochinchinenin A, cochinchinenin B and loureirin B could be fitted well with Hill equation. The interactions of cochinchinenin A, cochinchinenin B and loureirin B in modulating the TTX-R sodium currents in DRG neurons were analyzed by using the Greco model. The calculation suggested that the interaction of cochinchinenin A, cochinchinenin B and loureirin B was synergistic when the combination inhibited the TTX-R sodium currents on DRG neurons [15]. This marked synergistic interaction of the three components may explain the experimental phenomenon that the three components used in combination at lower dose could produce the same effects on the TTX-R sodium currents as the three components used alone at higher doses.

A visual model of the interaction of three components was established according to the equivalent surface formula derived by Guo et al. [31]. As shown in Figure 2, point Q1 was a mixed concentration point of combination (38 μmol/l of cochinchinenin A, 19 μmol/l of cochinchinenin B, and 8 μmol/l of loureirin B). The coordinate values of Q1 were statistically smaller than those of their corresponding theoretical additive points. Therefore, the point Q1 was below the respective corresponding three additive surfaces, which indicated that interactional effect of the three components in combination on capsaicin-induced TRPV1 currents were synergistic.

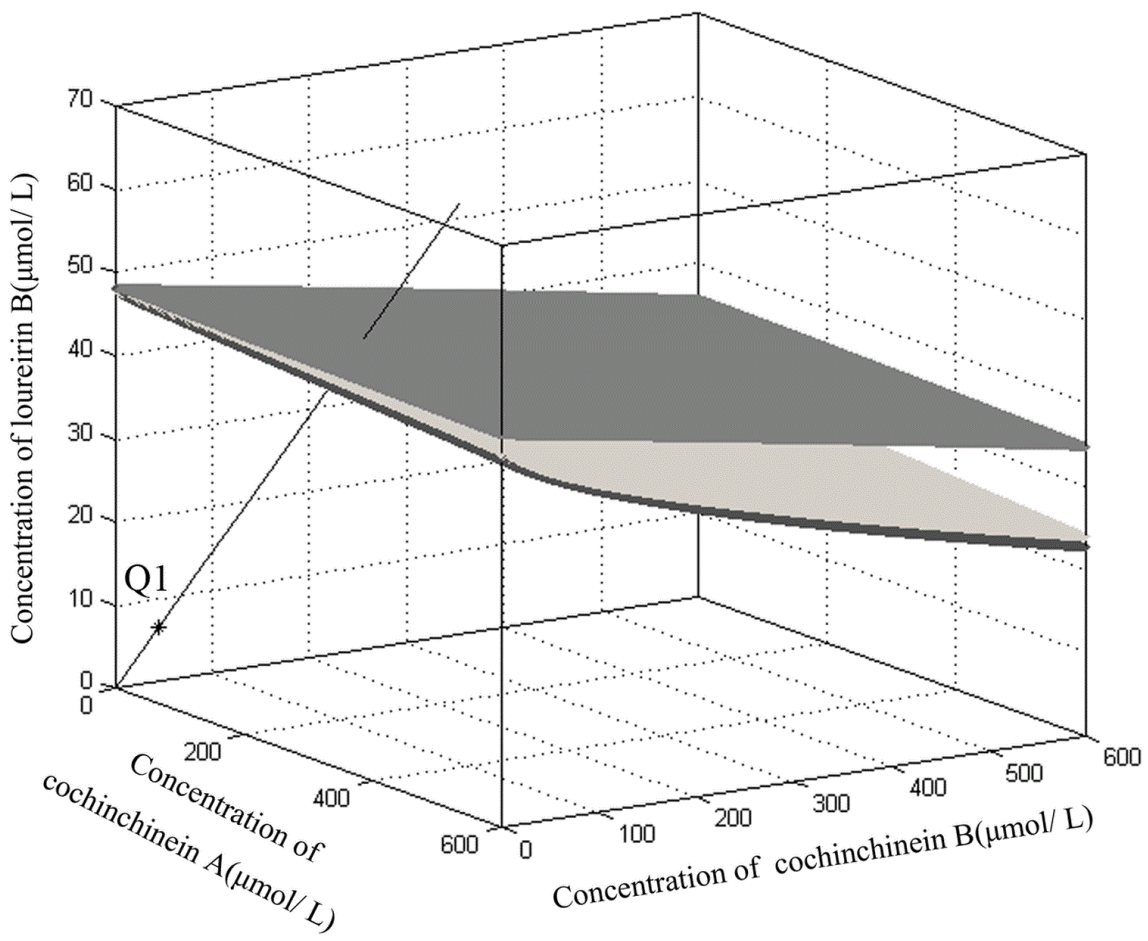

Figure 2: Interaction model of the three components inhibiting the capsaicin-induced TRPV1 currents [28]. Additive surfaces corresponding to the combined effects produced by cochinchinenin A, cochinchinenin B, and loureirin B. Point Q1 was a mixed concentration point of combination (38 μmol/l of cochinchinenin A, 19 μmol/l of cochinchinenin B, and 8 μmol/l of loureirin B). The coordinate values of Q1 were statistically smaller than those of their corresponding theoretical additive points

Verification of material basis for analgesic effect of Resina Draconis in vivo

The drug effectual procedure in vivo is very complex so that it is necessary to design the in vivo experiment to confirm the material basis for the analgesic effect of *Resina Draconis* at systemic level. In pain transmission pathways, spinal dorsal horn (SDH) is a pivotal center for integrating and relaying pain-sensing signals (nociceptive) from periphery to brain. In SDH neurons, it was wide dynamic range (WDR) neurons that play a fundamental role in the transmission and processing of pain signals and in the coding for the stimulus intensity [32].

Extracellular microelectrode recordings were used to observe the effects of *Resina Draconis* and its component/combination on the discharge activities of WDR neurous in SDH of intact male Wistar rats evoked by electric stimulation at sciatic nerve. The effect of 0.5 mg/mL *Resina Draconis* on the discharge activities of WDR neurons were taken as a reference for the comparison between the pharmacological effect of *Resina Draconis* and the effects of the component/combination. The concentration of each component in various component combinations was determined according to the percentage content of each component in final products of *Resina Draconis*, which ensured that the quantity of each component existent as a single or in combination was in agreement with that in *Resina Draconis*. The comparative results showed that although the evoked discharge of WDR neurons could be inhibited to different extent by the three components (cochinchinenin A, cochinchinenin B and loureirin B), none of them could produce the same inhibition as *Resina Draconis* when their quantities were correlative with their quantities contained in *Resina Draconis*. In the various combinations of the three components, only the combination of cochinchinenin A, cochinchinenin B and loureirin B (molar ratio is 38:19:8) could produce the approximate inhibition on the evoked discharge of WDR neurons as *Resina Draconis* (Figure 3).

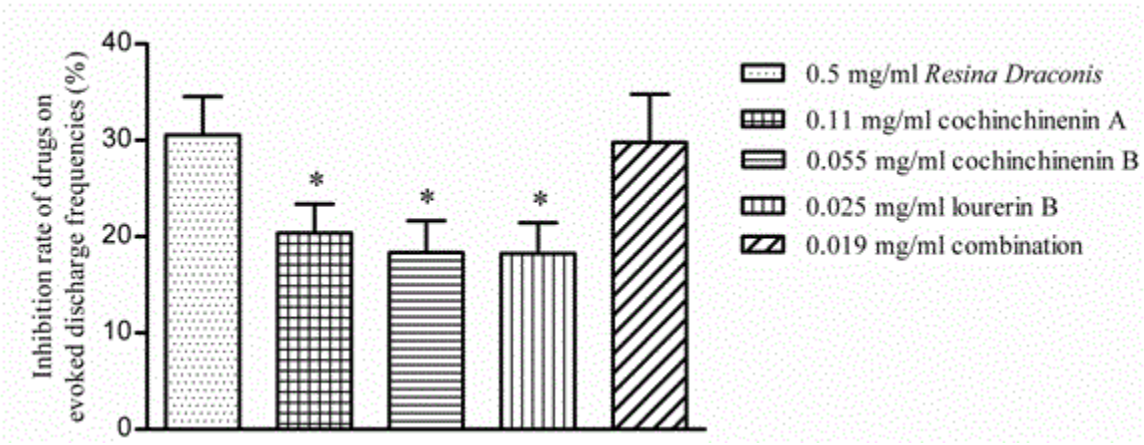

Figure 3: The inhibition rates of drugs on evoked discharge frequencies. The concentration ratio of cochinchinenin A, cochinchinenin B and loureirin B in the combination is 38:19:8. Each group represents the mean ± SEM of 10 animals. * $p < 0.05$, when compared with *Resina Draconis* by one-way ANOVA. Data in the diagram cited from reference [31].

Discussion

Reverse pharmacology is a new research idea relative to conventional pharmacology. In reverse pharmacology research, clinical experiences, observations or available data collected from the traditional drug therapy serve as the research clues for further exploration. Then the experiments in vitro or in vivo used to analyze in detail the effect of the drug on the cell or organism are carried out. However, this research framework is not adequate for the research on the pharmacological mechanism and the material basis for the efficacy of traditional drug because it lacks an operational approach. Different from the selection of active component from nature medicine or compounds with known molecular structure, the validation of the material basis for the specific efficacy of the traditional drug could not be separated from the research on the pharmacological mechanism of the traditional drug. The foremost problem of the research on the traditional drug using the research framework of reverse pharmacology is how to test, express and analyze the relationship between the pharmacological effects of traditional drug and its components. This problem has never emerged in the past research. Therefore, the experiments should be designed to observe which component/combination could produce the same pharmacological effects as the original traditional drug and to measure their extents. On the basis of their pharmacological characterization, it should be validated which component/combination could replace the original traditional drug and produce its specific pharmacological effect. If possible, the interaction of the components should be analyzed. We can refer to the established conceptual framework in the previous medicine research, but we cannot completely follow the framework in solving above problem. In the problem-solving process, a high demand is made for the advance of science and technology, besides, it requires that modern subject system should be adjusted to adapt to the research and development of the traditional medicine. So some core concepts in the research on the traditional medicine must be reinvestigated and redefined. The established operational definition of the material basis for the efficacy of the traditional drug places great emphasis on the corresponding relationship between the traditional drug and the material basis for its efficacy, as well as the corresponding relationship between their pharmacological effects, whereas the concept of active component confused with that of effective component is used particularly to refer to some components extracted from nature medicine with some pharmacological effects.

In summary, the modernization research of traditional medicine requires not only new object and new technology, but also the corresponding research strategy based on the appropriate methodology. Good luck is important, but it is likely to miss it without the guidance of methodology. We hope that our research work on the analgesic effect of *Resina Draconis* for the solution of the above problem could provide a good starting point to induce traditional medicine researchers to raise more appropriate questions in the research and development of the traditional medicine and find solutions to the questions.

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