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Purgative components in rhubarbs: Adrenergic receptor inhibitors linked with glucose carriers

Tian-Shi Feng [a], Zhi-Yi Yuan [a], Run-Qing Yang [a], Shuang Zhao [a], Fan Lei [a], Xin-Yue Xiao [b], Dong-Ming Xing [a], Wei-Hua Wang [c], Yi Ding [c], Li-Jun Du [a,*]

[a] MOE Key Laboratory of Protein Sciences, Laboratory of Molecular Pharmacology and Pharmaceutical Sciences, School of Medicine and School of Life Sciences, Tsinghua University, Beijing 100084, China
[b] National Institutes for Food and Drug Control, Beijing 100050, China
[c] Drug Discovery Facility, School of Life Sciences, Tsinghua University, Beijing 100084, China

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ABSTRACT

Rhubarbs and their extractives have been used as cathartic for many years. There have been numerous breakthroughs in the pharmacological research of the drug. However, as the key point of the mechanism, the targets of the effective components still remain unclear. In this paper, with an in vitro system of isolated intestine, we found that both the rhubarb extractives and the anthraquinone derivatives can antagonize the adrenaline effectively. Furthermore, computer based docking provided the binding model of the anthraquinone derivatives and adrenergic receptor. Then, based on the results of the small intestinal promotion and purgative effect experiments in vivo, we built an "inhibitor–carrier" hypothesis to elucidate the mechanism of rhubarb. This work provided key massages for the pharmacological research of rhubarb, such a common and active medicinal plant, and might be of help for the development of new purgative drugs.

1. Introduction

As an effective cathartic, the extractives of rhubarbs are widely used for a long period all over the world. In about 70 years, there are numerous papers about the active components and the purgative mechanisms of rhubarb extractives and some important breakthroughs have been made.

The anthraquinone derivatives are widely believed to play the most important role in stimulating the large intestine and leading to diarrhea in the extractives [1]. There are a number of anthraquinone derivatives in rhubarbs and they can be divided into two groups: free anthraquinone derivatives (chrysophanol, physcione, emodin, aloe-emodin, rhein) (Fig. 1) and their glycosides [2]. It was found that when the free anthraquinone derivatives were injected to the colon of rat, the re-absorption of water and electrolyte would be inhibited [3]. Similar results could be repeated on mice [4]. These experiments demonstrated that the anthraquinone derivatives are the active compounds.

However, when the free anthraquinone derivatives were administrated through oral ways, they showed no obvious purgative activity [1]. On the contrary, the anthraquinone glycosides could effectively lead to diarrhea when injected to the stomach [1]. Further pharmacokinetics study found that the free anthraquinone derivatives are easy to be absorbed by

* Corresponding author at: Laboratory of Molecular Pharmacology and Pharmaceutical Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China. Tel./fax: +86 10 62773630.
E-mail address: lijundu@mail.tsinghua.edu.cn (L.-J. Du).

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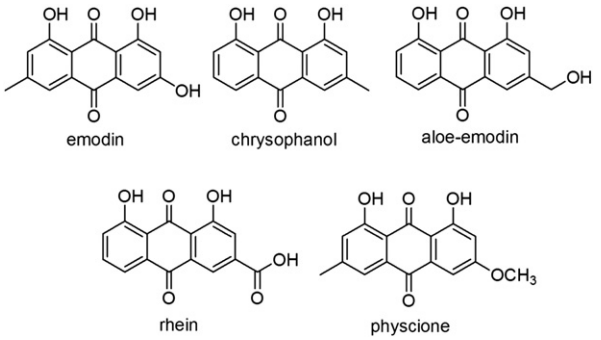

Fig. 1. Structures of the five anthraquinone derivatives in rhubarbs.

the small intestine and thus cannot reach the large intestine [5,6]. However, protected by glucose, the anthraquinone glycosides can reach large intestines and hydrolyzed by bacteria there [4,7]. Then, the released free anthraquinone derivatives show their functions.

Although it seems that the purgative mechanisms of rhubarbs have been clear, as one of the key point of the mechanism, the target of anthraquinone derivatives remains unknown.

The smooth muscle of intestine is regulated by both sympathetic nerve system and parasympathetic nerve system. The two systems have exactly opposite functions and if the balance of them is broken, the movement of small intestine will also be influenced. As one of the neurotransmitters of sympathetic nerve [8], adrenaline can inhibit the contraction of the smooth muscle [9]. Here, we found that the function of adrenaline could be effectively inhibited by both the extractives of rhubarbs and free anthraquinone derivatives, which means that the active components of rhubarbs are likely to be adrenergic receptor inhibitors. With the computer based molecular docking, the model of the interaction was built and analyzed. Furthermore, the pharmacological difference of hydrolyzed and unhydrolyzed extractives in vivo was observed. Based on these, a new hypothesis of "adrenergic receptor inhibitor–carrier" was proposed to explain the purgative effects of rhubarb.

2. Experimental procedures

2.1. Animals

Male ICR mice weighing 18–22 g were purchased from Vital River Laboratories (Beijing, China). The animals were housed in the Animal Center of Tsinghua University in temperature- and humidity-controlled rooms, kept on a 12 h light/dark cycle, and provided with rodent chow and drinkable water ad libitum. All procedures for animal experimentation were in accordance with national guidelines for animal care and approved by the Institutional Animal Care and Use Committee of Tsinghua University and Animal Welfare and Ethics Committee of Tsinghua University.

2.2. Hydrolysis of rhubarb extracts [10]

The extractives of *Rheum palmatum* L. (rhubarb) were provided by the National Institutes for Food and Drug Control and were prepared by 50% ethanol reflux extraction.

About 1 g rhubarb extractive was added into 100 ml 7% hydrochloric acid solution. Then the flask was put in the sonic washer for 2 min to break off the undissolved extractives into powder. The mixture was continuously stirred and kept in 60 °C for 4 h. The solvent was removed by reduced pressure distillation and then the hydrolyzed extractive was dissolved again with methanol. The solution was transferred into an evaporating dish and was kept in room temperature for about one day to evaporate the solvent.

2.3. HPLC system and methods [11]

HPLC system: Hypersil ODS-2 HPLC column, particle size: 5 μm, bought from Beijing Analysis Apparatus Factory, No. 210606. Agilent 1260 HPLC system (1260 Quat Pump, 1260 DAD, 1260 ALS). Mobile phase is methanol/0.1% formic acid water solution (75:25, v/v) at a flow rate of 1 ml/min. UV detector was set at 254 nm.

Aloe-emodin and physcione standard references were provided by Professor Xiuwei Yang who is in Peking University, China. Emodin standard reference was bought from the National Institutes for Food and Drug Control, No. 110756-200110. Chrysophanol standard reference was purchased from the National Institutes for Food and Drug Control, No. 110796-200716. Rhein standard reference was purchased from Chengdu Mansite Bio-Technology Co., Ltd., No. MUST-12031210. All of the standard references and samples are dissolved in methanol before injected into the HPLC system.

10 μl (10 μg/ml) emodin, aloe-emodin, rhein, chrysophanol and physcione respectively were injected into the system to confirm the retention time of each compound.

10 standard mixtures of emodin, aloe-emodin, rhein and chrysophanol were prepared. The concentrations of the 4 compounds were equal. The concentrations of each compound in the ten mixtures were 0.025 μg/ml, 0.05 μg/ml, 0.125 μg/ml, 0.25 μg/ml, 1.25 μg/ml, 2.5 μg/ml, 5 μg/ml, 7.5 μg/ml, 10 μg/ml and 12.5 μg/ml respectively.

10 standard mixtures of physcione and chrysophanol were prepared. The concentrations of physcione in the ten mixtures were 0.0966 μg/ml, 0.319 μg/ml, 0.437 μg/ml, 3.627 μg/ml, 7.349 μg/ml, 14.193 μg/ml, 22.375 μg/ml, 28.803 μg/ml and 36.194 μg/ml respectively.

1 mg of the hydrolyzed and unhydrolyzed rhubarb extractives was dissolved in 1 ml methanol respectively.

All of the samples above were injected into the system.

2.4. Isolated small-intestinal contraction experiment [12]

System: RM6240BD multi-signal collector (Chengdu Instrument Factory, China), JZJ01 muscle tension transducer, (Chengdu Instrument Factory, China), 25 ml thermostat bath (Kent Science Corporation, USA), type 210 thermostat peristaltic pump (PolyScience, USA).

Kreb's buffer (pH = 7.4) (mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 1.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 5.5, KH_2PO_4 1.2.

An ICR mouse was killed, and then the small intestine was immediately taken out and soaked in the Kreb's buffer. About 1–1.5 cm intestine was cut down and fixed in the 37 °C thermostat bath with a 20 ml Kreb's buffer. The other end of the intestine was linked with a muscle tension transducer