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Research paper

Pulmonary administration of resveratrol/hydroxypropyl-β-cyclodextrin inclusion complex: *in vivo* disposition and *in vitro* metabolic study

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ABSTRACT

Until today, the clinical application of phytochemicals is still limited largely due to poor oral bioavailability. Pulmonary administration is considered as an attractive alternative to oral delivery as it can improve the pharmacokinetic properties of compounds with extensive metabolism. In this study, resveratrol was selected as a study model to evaluate the feasibility of pulmonary administration in the delivery of phytochemicals, aiming to promote their therapeutic potential. The pharmacokinetic investigation on resveratrol was conducted in rats. Compared with oral delivery, resveratrol in rats after pulmonary administration showed superior absorption and bioavailability (92.95%). Directed delivery of resveratrol to lungs also showed potential usefulness in the treatment of lung diseases as drugs rapidly accumulated in lung tissues and concentrations were more than 100 times those of oral delivery at early time points. The metabolic study was carried out using rat microsomes and showed that metabolic activity of lung was much lower than that of liver. The overall results indicate that pulmonary administration is an effective strategy for both systemic and local delivery of resveratrol.

1. Introduction

Phytochemicals derived from plants is of great value for development as nutritional and pharmaceutical agents. Resveratrol (RES, Fig. 1) is one of the most extensively studied phytochemicals, being abundant in plant foods such as grapes, and peanuts [1]. The human health benefits of it have been widely investigated since the reveal of "the French Paradox" in the early 1990s [2]. To date, various *in vitro* and *in vivo* studies have reported a broad range of desirable activities of RES such as antioxidant, anti-inflammatory, cardioprotective, and anti-cancer [2,3].

Lung disease remains a worldwide public health issue with high morbidity and mortality. RES has shown the potential against some lung diseases including chronic obstructive pulmonary diseases (COPD) and lung cancer. Recent studies reported that RES can scavenge oxygen-derived free radicals and inhibit the releases of inflammatory cytokines from alveolar macrophages in COPD [4]. In the treatment of lung cancer, RES has shown the ability to inhibit cancerous cell initiation, promotion, and progression [5]. Besides, RES can also be used to treat pulmonary arterial hypertension because of its capacity to inhibit the production and secretion of MCP-1 in vascular endothelial cells [6]. In spite of these positive activities, the clinical translation of RES is largely hampered since only a small proportion of drugs could reach the lungs after conventional administration. Another problem with RES is limited bioavailability. Previous studies demonstrated that the oral bioavailability of RES in human is almost zero mainly due to the first-pass metabolism [7]. As a polyphenolic compound, RES is susceptible to phase II metabolism catalyzed by UDP-glucuronosyltransferase (UGT) and resveratrol-3-*O*-glucuronide (R3G) was reported as the most abundant metabolite in human and animals [8,9]. Because glucuronidation can reduce permeability of cell to drugs and contribute to their excretion [5], the elimination of RES from the body is extremely fast, and thus it is hard to maintain the therapeutically relevant level at action sites.

To tackle these limitations, we evaluated the pulmonary route of RES. Pulmonary administration is a non-invasive method considered as

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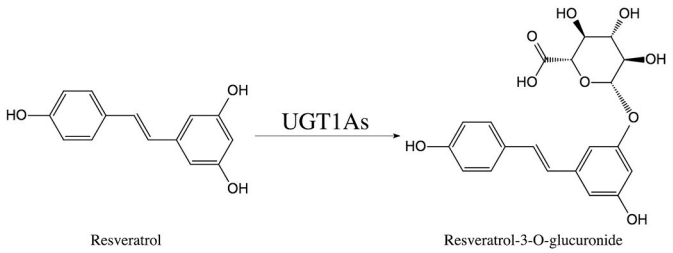

Fig. 1. Chemical structure of resveratrol (*trans*-3,5,4′-trihydroxystilbene, RES) and resveratrol-3-*O*-glucuronide (R3G). The glucuronidation was catalyzed by UDP-glucuronosyltransferase (UGT).

an alternative to oral and parenteral routs. As a local access, it is an effective approach for targeted delivery to lungs and is desirable for treating lung-specific disease states [10]. It can also provide a direct and quick access of drug into systemic circulation because of the tremendous surface area and extensive vascularization in lungs. Additionally, as the enzyme activity is relatively low in lungs, pulmonary route is a promising delivery route for compounds with extensive metabolism to circumvent hepatic and intestinal first-pass effect [11]. Benefited from these advantages, researchers have developed some inhalable formulations for delivery of RES. Valentina et al. prepared a respirable RES dry powder formulation, showing good antioxidant and anti-inflammatory activates *in vitro* [4]. A recent work from Gyeungyun et al. reported the preparation of an inhalable micelle loading RES and higher anti-inflammatory effect of that in the acute lung injury animal model [12]. Although pharmacological activities of inhalable RES have been investigated *in vitro* and *in vivo*, there is little work directed toward the pharmacokinetic characters of RES after pulmonary administration, which is also an important factor for the development of drug.

In this study, we investigated the pharmacokinetic behavior of RES after pulmonary administration to evaluate the potential of this route in the delivery of RES. The pharmacokinetic profile was evaluated in rats. RES/hydroxypropyl-β-cyclodextrin (HP-β-CD) inclusion complex was prepared and used as dosing formulation to resolve the problem of dissolution, since HP-β-CD can enhance the hydrophilicity of poorly soluble compounds through the formation of inclusion complex [13]. To further explore the metabolism mechanism of RES after pulmonary administration, metabolic profiles of it were investigated *in vitro* using rat liver microsomes and lung microsomes. Hopefully, the information obtained in this study will contribute to the further development and clinical application.

2. Materials and methods

2.1. Chemicals and regents

Resveratrol ($C_{14}H_{12}O_3$, purity>95%) and resveratrol-3-*O*-glucuronide ($C_{20}H_{20}O_9$, purity>95%) were purchased from Keweichem (Shanghai, China) and Glpbio (Montclair, America), respectively. Chlorzoxazone ($C_7H_4ClNO_2$, purity≥99%) was obtained from Sigma (Munich, Germany). Hydroxypropyl-β-cyclodextrin (HP-β-CD) was provided by J&K® (China). Lactic acid was of analytical grade and purchased from Nanjing Chemical Reagent Co., Ltd (Nanjing, China). Uridine 5′-diphosphoglucuronic acid (UDPGA, trisodium salt) was purchased from Sigma (Munich, Germany). Methanol and acetonitrile of HPLC grade were purchased from Tedia company (Fairfield, USA). Purified water used for UPLC-MS/MS analysis was produced by a Milli-Q water purification system (Millipore, Bedford, USA).

2.2. Animals

Sprague-Dawley rats were purchased from the animal center of Qinglong mountain (Nanjing, China). Rats were housed under standard conditions with a 12 h on/12 h off light cycle. During the acclimatization period, animals were raised with free access to water and chow. All experimental procedures and protocols were approved by the Animal Ethics Committee of China Pharmaceutical University (Nanjing, China).

2.3. Pharmacokinetics study

To access pharmacokinetic characters of RES after pulmonary or gastric administration, four different dosing routes were employed including intravenous (i.v), intragastric (i.g), intratracheal (i.t), and inhalable (i.h) administration. The dosing formulation of RES was prepared with 0.3 M HP-β-CD and 1% lactic acid. In brief, the excess amounts of RES were added to the aqueous solution containing 0.3 M HP-β-CD and 1% lactic acid. The mixture was continuously shaken at room temperature for 24 h to reach complete solubilization. After filtering to remove the undissolved RES, the resulting solution was freeze-dried. The obtained lyophilized complex powders were dissolved in distilled water to obtain dosing solution at 20 mg/mL of RES before animal experiments.

Animals were fasted for 12 h before the experiment and were randomly allocated to four groups (n = 5). Rats in Group I and II received RES via bolus tail vein injection (10 mg/kg) and gavage (50 mg/kg), respectively. The pulmonary administration was carried out through two different ways in the other two groups. For Group III, i. t instillation was conducted through orotracheal intubation at 20 mg/kg. Rat was anesthetized first and kept at approximately 60° angle of tilt on restraining board. After that, the tongue of animal was pulled out using forceps, and then solution was instilled into the trachea using a syringe equipped with a gavage needle followed by insufflation of an air bolus. While in Group IV, a small animal nose-only inhalation exposure system (HRH-MNE3026, Huironghe Technology) was used to achieve i. h administration of RES. That is a computer-assisted inhalation device, in which aerosol is produced through interplay between a vacuum and pressurized air gas by a controlled valve system. Each rat was placed in a restraining tube and held in place by a plunger. During the inhalation, tubes containing unanesthetized animals were connected to exposure ports of the device. Rats inhaled the aerosols at an air flow rate of 12 L/min for 100 min continuously. The concentration of RES aerosols in exposure system was measured using a filter connected to the exposure port. The amount of drug on filter membrane was quantified using UPLC-MS/MS. The following equation determined the actual dose for rats:

$$Dose(mg) = Concentration(mg/L) \times Minute\ ventilation\ of\ rat(L/min) \times Inhalation\ time(min)$$

Serial blood samples from each rat were collected from the orbital sinus at predetermined time intervals until 10 h post-dose. After centrifugation, plasma samples were harvested and stored at −70 °C. Because of the photolabile nature of RES, all these procedures were carried out under dim light conditions.