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Research paper

Pharmacokinetics and tissue distribution of emodin loaded nanoemulsion in rats

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ABSTRACT

Emodin, a natural product originated from radix et rhizoma Rhei, is a potential agent for anti-constipation, anti-inflammation, anti-cancer and so on. In this paper, a simple, economic and sensitive HPLC-FD method was developed and validated for the determination of emodin in rat plasma and tissue homogenates after oral administration of emodin loaded nanoemulsion (EMO-NE). Simultaneously, the pharmacokinetics of EMO-NE and emodin suspension were investigated so as to embody advantages of EMO-NE. $AUC_{0-\infty}$, C_{max}, $t_{1/2}$ and $MRT_{0-\infty}$ of emodin-loaded nanoemulsion were respectively 2.37, 1.62, 3.99 and 2.39-fold higher than those of emodin suspension. Meanwhile, EMO-NE decreased the clearance rate of emodin more than double that of emodin suspension. Thus, it can be assumed that EMO-NE can effectively improve the bioavailability of emodin and prolong *in vivo* mean residence time *via* oral administration. All tested tissues of rats were found to retain parent drug for 48 h following a single oral dose of EMO-NE. The amount of emodin was the highest in the liver, and then lung, kidney, heart or spleen, and lowest in the brain. However, the mean residence time of emodin in the brain was the longest, almost two-fold longer than that in the other tissues.

Emodin (1,3,8-trihydroxy-6-methylanthraquinone, PubChem CID:3220) is one of the major bioactive anthraquinones existed in the radix et rhizoma Rhei and other medical plants. Recent evidences indicated that emodin showed mild laxative property [1], significantly inhibited the weight gain of high-fatted rats, regulated blood lipids and blood glucose metabolism disorders, enhanced anti-lipid peroxidation and protected the liver function [2]. Emodin mediated protection from myocardial cell injury by suppressing TNF-α expression, and inhibited caspase-3 and NF-κB activation in the local myocardial infarction area [3]. Emodin induced apoptosis in several types of cancer cells, such as lung cancer cells [4], colon cancer cells [5], hepatocellular carcinoma cells [6], human cervical cancer hela cells [7], and possessed strong inhibitory effects on cancer cells proliferation, migration and invasion [8–10]. More recent studies demonstrated that emodin had anti-inflammatory [11,12], anti-bacterial [13,14], anti-virus [15], anti-allergic [16], anti-diabetic and anti-epileptic activities [17,18], neuroprotective and immunosuppressive effects [19–21], and enhanced osteogenesis [22].

However, there are obvious obstacles to the development of emodin as a viable therapeutic dosage form owing to its low aqueous solubility and first-pass effect. The major metabolites in rat plasma were found to be glucuronides and sulfates, chiefly identified to be emodin 3- O-β-D-glucuronide by a UPLC-MS/MS method, which might be a major reason why emodin had very low bioavailability in rats following an oral administration [23]. Parenteral administration, chemical structure modification or new drug delivery system may overcome these disadvantages to some degree.

In recent years, nanotechnology-based drug delivery systems, including biodegradable polymeric nanoparticles, smart polymeric micelles, nanoemulsion and lipid nanoparticles so on, are being aimed to improve low aqueous solubility or oral bioavailability of some substances with good druggability so as to bring about satisfactory therapeutic efficacy [25]. Among them, nanoemulsion is non-equilibrium system and possess kinetic stability in a long period with a remarkable small droplet size (between 10 and 100 nm). It is one of the most concerned delivery systems in

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pharmaceutical applications because of the ability of solubilizing water-insoluble drugs, intensifying bioavailability of drugs by dispersing them into extremely small particles to greatly increase the solubility and *in vivo* permeability. Besides, improved drug stability and mobility of emulsion membrane also contribute to the increased bioavailability. Drug enclosed in nanoemulsion droplet is free from air, light, and hard environment *in vitro*, and avoid fisrt-pass effect *in vivo* as it is orally administrated. Notably, drug-loaded nanoemulsion can be directly absorbed into blood and distributed in tissues, and drug in internal phase can effectively keep from unexpected biotransformation. Therefore, as a new drug delivery system, nanoemulsion can not only improve the bioavailability of drugs, but also protect them from oxidation, hydrolysis and other possible metabolisms [26]. Generally, nanoemulsion possesses an ability of sustained drug release and long residence time *in vivo*.

In our previous work, emodin has been successfully prepared into o/w type nanoemulsion [24]. Briefly, emodin-loaded nanoemulsion (EMO-NE) was formulated using capryol 90 served as dispersed phase, deionized water as continuous phase, cremophor RH 40 as a surfactant, transcutol HP as a cosurfactant, and oleic acid as a stabilizer. The new dose form can be easily diluted in water, and demonstrated notably sustained-release profile in the *in vitro* drug release experiment as compared to that of emodin suspension [24]. Based on these results, it can be speculated that the EMO-NE may improve the bioavailability of emodin after oral administration.

This paper aims to investigate the pharmacokinetics of EMO-NE and emodin suspension to verify the advantages of emodin loaded nanoemulsion mentioned above. The distribution and pharmacokinetics of EMO-NE in the main tissues including brain, heart, liver, spleen, lung and kidney were also studied after it was orally administered. Concomitantly, a simple, economic and sensitive high performance liquid chromatography with fluorescence detection (HPL-FD) method was established, and successfully applied to detect emodin in biological samples.

1. Materials and methods

1.1. Materials

Emodin reference (Batch No.110757, Fig. 1A) and 1,8-dihydroxy (Batch No.110829, Fig. 1B) were purchased from National Institutes for Food and Drug Control (Beijing, China). Emodin used for drug loaded nanoemulsion was isolated from root and rhizome of *Rheum palmatum* L. with a purity above 98.0%, authenticated by thin layer chromatography and quantitatively determined by high performance chromatography in comparison with emodin reference. Methanol and acetonitrile purchased from Mreda Technology Inc., USA were of chromatographic grade. Chloral hydrate was donated by The Second Hospital of Lanzhou University. Heparin sodium was bought from Shanghai Xinxing Chemical Reagent Research Institute (Shanghai, China). Capryol™ 90 (Batch No.146642) and Transcutol® HP (Batch No.139125) were donated by GATTEFOSSE Trade Co., Ltd (Shanghai, China). Cremophor® RH 40 (Batch No.81088756P0) was purchased from BASF SE (Germany). Deionized water was obtained from Hangzhou Wahaha Group Co., Ltd (Hangzhou, China). Other chemicals were obtained from Tianjin Yuanli Chemical Co., Ltd (Tianjin, China).

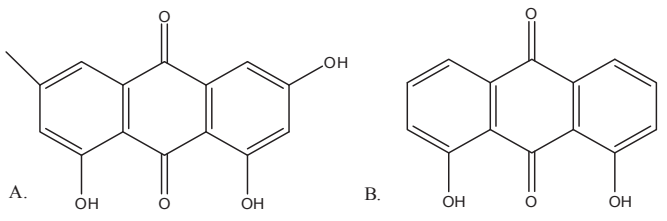

Fig. 1. Chemical structures of emodin (A) and 1,8-dihydroxy anthraquinone (B).

1.2. HPLC-FD and chromatographic conditions

A LC-10ATvp series high performance liquid chromatography system (Shimadzu Corporation, Japan) was equipped with LC-10ATvp binary pump, CTO-10ASvp column oven, 7725i manual injector, and SCL-10Avp fluorescence detector (HPLC-FD). A Diamonsil® column (250 × 4.6 mm, 5 μm, C18, Dikma) with a Securityguard™ guard cartridge (Phenomenex, USA) was applied to separate all samples. The mobile phase was selected as acetonitrile-methanol-0.1% phosphoric acid (66:22:12, v/v/v) with an isocratic elution, and the flow rate was adjusted to 1.0 ml/min. Fluorescence detection was employed with an excitation wavelength of 440 nm and an emission wavelength of 515 nm. The column temperature was maintained at 40 °C. The injection volume of all samples was 20 μL, and each sample was analyzed in duplicate.

1.3. Animals

Sprague–Dawley rats (half male and half female), ageing 9–10 weeks and weighing 200–250 g, were obtained from the Experimental Animal Center of Lanzhou University (Lanzhou, China). Animal welfare and experimental procedures were strictly in accordance with the Guide for the Care and Use of Laboratory Animals (US National Research Council, 1996) and the related ethic regulations of Lanzhou University. All animals were kept in an environmentally controlled breeding room for at least one week before starting the experiments, fed with standard laboratory food and had free access to water. Prior to each experiment, the rats were fasted for 12 h with water *ad libitum*.

1.4. Preparation of EMO-NE and emodin suspension

The optimization and preparation of EMO-NE has been reported [24]. In short, emodin loaded nanoemulsion was prepared by mixing cremophor RH 40/transcutol HP at a fixed mass ratio of 2:1 (20.16%, w/w), oleic acid (1%, w/w) and capryol 90 containing emodin (11.84%, w/w), followed by adding distilled water into the above mixture dropwise. The whole preparation process was made under continuous magnetic stirring. The coarse emulsion was then subjected to ultrasonic emulsification for 20 min. Finally, pH of nanoemulsion was adjusted to 6.5 with sodium hydroxide solution. Emodin suspension was prepared by mixing 1.0 mg emodin and 40 mg Tween-80 using mortar and pestle, and then slowly adding 2 mL 0.5% (w/v) sodium carboxymethyl cellulose with continuous stiring to obtain the tested suspension. The EMO-NE and emodin suspension were stored in dark place at room temperature before use.

1.5. Animal experiments

Rats were randomly assigned to EMO-NE group and emodin suspension group. EMO-NE group was further divided into 12 subgroups (corresponding to 12 sampling time points), and 4 rats were for each sub-group. EMO-NE was orally administrated by a single dose of 10 mg/kg. Blood samples were collected into heparinized micro-centrifuge tubes by the heart puncture at 0, 0.17, 0.25, 0.33, 0.75, 1, 3, 6, 8, 12, 24, and 48 h after being taken medicine, followed by centrifuging at 10625 ×*g* (12,000 rpm) for 10 min. The resulting plasma layers were separated and stored in micro-centrifuge tubes