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Original Research Paper

Influence of pretreatment of piperazine ferulate on pharmacokinetic parameters of methotrexate in methotrexate-induced renal injury model rats by HPLC-MS

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ARTICLE INFO

Article history:
Received 11 July 2016
Received in revised form 25 August 2016
Accepted 30 August 2016
Available online 21 September 2016

Keywords:
Methotrexate
Piperazine ferulate
Pharmacokinetic parameters
HPLC-MS

ABSTRACT

The present study was designed to investigate the influence of the pretreatment of piperazine ferulate on pharmacokinetic parameters of methotrexate in methotrexate-induced renal injury rats. A simple and efficient high performance liquid chromatography coupled with mass spectrometry (HPLC-MS) method was developed to determine methotrexate in rat plasma. Methotrexate and syringic acid (internal standard) were extracted from rat plasma samples by protein precipitation with acetonitrile. The analysis was performed on a CAPCELL PAK C_{18} column (150 mm × 4.6 mm, 5 μm) with acetonitrile and 5 mmol/l ammonium acetate aqueous (10:90, *v*/*v*). The linear range was 5.0×10^{-2} to 100.0 μg/ml for methotrexate. Other parameters were all within the acceptance criteria. The validated method was successfully applied the pharmacokinetic study of methotrexate between two methotrexate treated groups (with and without the pretreatment of piperazine ferulate). Compared with the methotrexate treated alone group, the pharmacokinetic parameters in the methotrexate with the pretreatment of piperazine ferulate group showed significantly lower $MRT_{(0\text{-}t)}$, $MRT_{(0\text{-}\infty)}$ and $T_{1/2}$. Results suggested that methotrexate can be rapidly eliminated, cleared or metabolized in rat blood, which might be related to the pretreatment of piperazine ferulate. The method provided deeper insights into rational clinical use of methotrexate with the pretreatment of piperazine ferulate on cancer patients with renal dysfunction.

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Peer review under responsibility of Shenyang Pharmaceutical University.
http://dx.doi.org/10.1016/j.ajps.2016.08.010

1. Introduction

As a dihydrofolate reductase inhibitor, methotrexate has been widely used to treat cancer [1–3]. However, nonoliguric and oligoanuric acute renal failure has been reported following administration of low- or high-dose methotrexate in many clinical cases [4–7], which is a major limiting factor in clinical therapy. Some regimens, such as folic acid or leucovorin, were reported during treatment with methotrexate, so as to relieve the toxicity induced by methotrexate in the clinical therapy [8,9]. However, it was supposed that folic acid supplementation may cause a reduction in efficacy of methotrexate [10]. Meanwhile, leucovorin may result in the delayed clearance of methotrexate and this incidence can be higher in older patients and during the first cycles of treatment [11]. Renal dysfunction is a common occurrence in patients with gynecologic cancer [12], metastatic renal cell carcinoma and other kinds of caner [13]. Renal impairment is especially frequent in elderly patients with cancer [14]. Thus, it is meaningful to find renal protective regimens when using methotrexate to treat cancer patients with kidney injury.

Piperazine ferulate is the substrate in the extract of Traditional Chinese Medicine – ligustrazine, which has been widely used in clinical practice in China to treat patients with chronic glomerulonephritis [15]. Piperazine ferulate can partly inhibit the synthesis of extracellular matrix and connective tissue growth factor in rat glomerular mesangial cells, and may be in connection with the prevention of glomerulosclerosis [16]. So piperazine ferulate may attenuate the potential renal injury caused by methotrexate. Although piperazine ferulate is believed to have the effects on improving renal functions [16–18], there is little study about the influence of pretreatment of piperazine ferulate on pharmacokinetic parameters of methotrexate in rats, and that was the reason why piperazine ferulate was chosen in this study.

Pharmacokinetics is reported to be a useful proof to reveal the reasonable drug compatibility in combination treatment [19–21]. In this study, pharmacokinetics had been used to assess the influence of piperazine ferulate on pharmacokinetics profile of methotrexate with HPLC-MS method. Although there were other advanced analytical methods to quantitatively determine methotrexate [22–24], this HPLC-MS method possessed the property of being easy to operate with high recovery, accuracy and precision. The pharmacokinetic results were expected to be useful for improving clinical use of methotrexate through the pretreatment of piperazine ferulate.

Renal histopathology was conducted to evaluate the renal failure induced by methotrexate. The histopathological differences between methotrexate with and without the pretreatment of piperazine ferulate groups could be regarded as another evidence of clinical application of methotrexate with the pretreatment of piperazine ferulate.

2. Materials and methods

2.1. Materials and reagents

Methotrexate and physiological saline (0.9%) were purchased from Jiangsu Hengrui Medicine Co., Ltd (Jiangsu, China) and Cologne Heilongjiang Pharmaceutical Co., Ltd (Heilongjiang, China), respectively. Piperazine ferulate tablets were supplied by Shandong Hill Kangtai Pharmaceutical Co., Ltd (Shandong, China). The reference standards of methotrexate and syringic acid (IS, purity >98.0%) (Fig. 1) were both obtained from Aladdin Ltd (Shanghai, China). Acetonitrile and methanol (HPLC grade) were provided by Fisher Scientific (Pittsburgh, PA, USA), and ammonium acetate was supplied by Kermel Chemical Reagent Co., Ltd (Tianjin, China). Distilled water was provided by Wahaha Co., Ltd (Hangzhou, China). All other reagents were of analytical grade.

2.2. Animals

In this study, all male Sprague-Dawley rats (220–250 g) were kindly provided by Experimental Animal Center of Shenyang Pharmaceutical University and bred with unlimited access to food and water in an air-conditioned animal center at a temperature of 22 ± 2 °C and a relative humidity of 50 ± 10%, with a natural light-dark cycle for 3 days. Before the drug administration, they were fasted overnight with free access to water. Animal study was carried out following the Guidelines of Animal Experimentation of Shenyang Pharmaceutical University, and the protocol was approved by the Animal Ethics Committee of the institution.

Six rats were randomly divided into two groups (3 rats per group) for histopathology study: methotrexate treated alone group (Group A1) and piperazine ferulate pretreated group (Group B1). Rats in Group B1 and Group A1 were orally given piperazine ferulate (at 50 mg/kg/d) and physiological saline (the same volume of vehicle) for 14 days, respectively. Then all rats were given intravenous injection of methotrexate at a dosage of 50 mg/kg at 14th day. At the end of the histopathology period, rats were sacrificed. Kidney tissues were immediately dissected out and preserved in neutral buffered formalin. Kidney samples were sliced to 3 μm wax sections, and the tissue sections were stained with hematoxylin and eosin (H&E) dye for microscopic examinations after gradual rehydration in a series of graded alcohols.

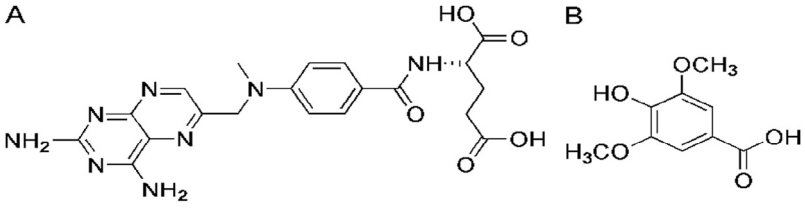

Fig. 1 – Chemical structures of methotrexate (A) and IS (B).