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Simultaneous quantitative determination of celecoxib and its two metabolites using liquid chromatography–tandem mass spectrometry in alternating polarity switching mode

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ABSTRACT

A simple and rapid quantitative analytical method for the simultaneous detection of celecoxib and its two main metabolites, hydroxycelecoxib (celecoxib-OH) and celecoxib carboxylic acid (celecoxib-COOH), in rat plasma using liquid chromatography–tandem mass spectrometry (LC–MS/MS) was developed. The plasma sample was prepared through simple protein precipitation, and the reconstitution solution (0.1% formic acid in 50% methanol) was optimized to achieve the best peak shape and recovery. The analytes were separated using an Atlantis T3 column (2.1 mm × 100 mm, 3 μm), and the mobile phase was composed of 10 mM ammonium formate in either 5% acetonitrile or 95% acetonitrile. The detection of the analytes was performed in alternating polarity switching mode using electrospray ionization. As celecoxib-OH and celecoxib-COOH were slightly unstable following freeze–thaw cycles and long-term storage at −80 °C in stability tests, every analysis was carefully conducted with one-freeze thaw cycle and a short storage duration (<1 week). Acceptable accuracy (<15%) and precision (<15%) were obtained in intra- and inter-day validations.

The method was successfully applied to the pharmacokinetic study of celecoxib, celecoxib-OH and celecoxib-COOH following the oral administration of celecoxib in rats at a dose of 10 mg/kg. Comparing the related pharmacokinetic parameters of celecoxib and its metabolites, celecoxib was quickly metabolized into celecoxib-OH and subsequently converted to celecoxib-COOH in short intervals. The AUCs for the two metabolites were less than 10% of that for celecoxib, indicating that the rate of celecoxib metabolism was low.

1. Introduction

Celecoxib, a new non-steroidal anti-inflammatory drug (NSAID), is a highly selective inhibitor of cyclooxygenase-2 (COX-2) [1] and is used for the symptomatic treatment of osteoarthritis, rheumatoid arthritis and pain, with a distinct safety advantage over conventional NSAIDs [2]. Celecoxib has also been shown to have potential for the prevention of cancer through both COX-2-dependent and -independent mechanisms [3] and has been considered to reduce the number of adenomatous colorectal polyps in the treatment of Familial Adenomatous Polyposis (FAP), a hereditary colon cancer susceptibility syndrome [4]. Based on this mechanism of action, celecoxib is recognized as a promising anticancer drug [5], which is an example of drug repositioning [6]. Accordingly, information on celecoxib metabolism under various physiological or pathophysiological conditions can provide valuable ideas for the innovative strategy of celecoxib repositioning because drug repositioning is based on unknown facets of drugs. Celecoxib is extensively metabolized to the hydroxylated metabolite, mainly by CYP2C9, and is further oxidized to the corresponding carboxylated form by alcohol dehydrogenase (ADH). Glucuronide conjugates of the

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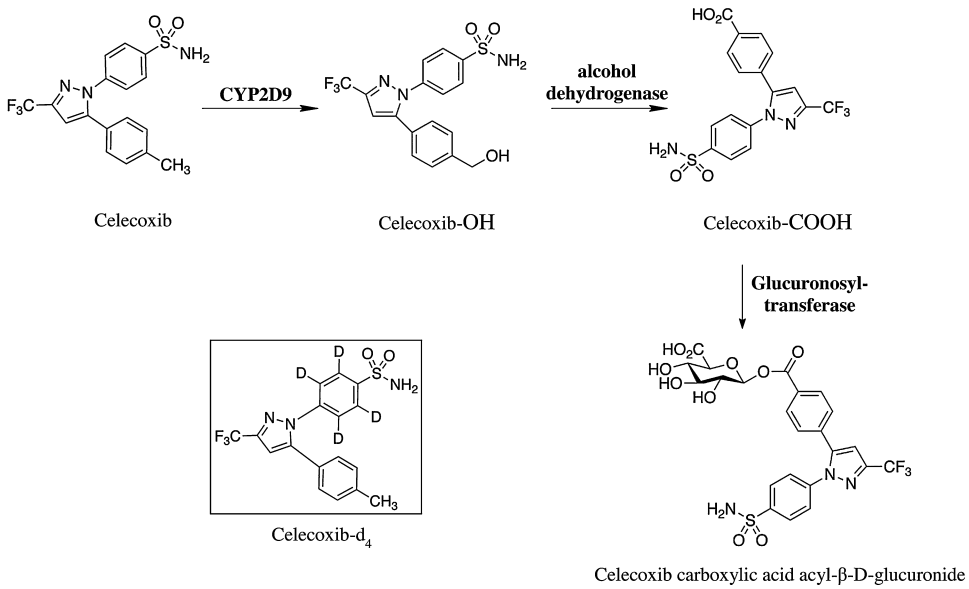

Fig. 1. The celecoxib metabolic pathway and the chemical structures of celecoxib, its metabolites and celecoxib-d_4.

carboxylated metabolites are also generated from the carboxylated form by glucuronosyltransferase (UGT) in humans and in rats, but these metabolites are found in very low concentrations in plasma (Fig. 1) [7–9]. So far, no study has confirmed that the metabolites of celecoxib are pharmacologically active [10]. Many studies have reported that marked interindividual variability in the response to celecoxib can influence its efficacy and safety, which is associated with a high incidence of cardiovascular risk [11]. Although its biological mechanism remains unclear, variations in several genes, including an SNP of cytochrome P450 2C9, may play significant roles [12,13]. Therefore, the pharmacokinetic properties of the parent drug and its major metabolites, hydroxycelecoxib (celecoxib-OH) and celecoxib carboxylic acid (celecoxib-COOH), can be good indicators of individual differences in drug response, especially as related to the metabolism of the drug [14,15]. Therefore, the pharmacokinetics of celecoxib itself and of its metabolites should be considered to clarify the pharmacokinetic characteristics of celecoxib and the related biological response.

For pharmacokinetic studies, several analytical methods have been reported for evaluating celecoxib in biological fluids such as plasma, serum, urine or feces, but most of these techniques have focused only on the quantitation of the parent drug or on the identification of its metabolites [7,16–19]. In a previous publication, a simultaneous quantitative analytical method for celecoxib, celecoxib-OH and celecoxib-COOH in plasma was reported using high-performance liquid chromatography-ultraviolet (HPLC-UV) detection [20]. However, a large amount of plasma (0.5 mL) was needed for the solid-phase extraction, and the method required a long run time (75 min). Thus, in the present study, we developed a reliable and high-throughput method for the simultaneous quantitative analysis of celecoxib and its two major metabolites, celecoxib-OH and celecoxib-COOH, in plasma using liquid chromatography–tandem mass spectrometry (LC–MS/MS) to study the pharmacokinetic characteristics of celecoxib.

In this method, simple protein precipitation was used for the sample preparation with a small volume of plasma (50 μL), and the total time of the analytical run was relatively short compared to that of the previously reported method. This method was successfully applied to the pharmacokinetic study of the oral administration of celecoxib in rats.

2. Materials and methods

2.1. Materials

Celecoxib (certified purity ≥98%), celecoxib-COOH (certified purity ≥98%) and celecoxib-d_4 (certified purity ≥98%) were purchased from Toronto Research Chemicals Inc. (North York, Ontario, Canada). Celecoxib-OH (certified purity ≥95%) was purchased from Clearsynth Inc. (Mumbai, India). Ammonium formate and formic acid were supplied by Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were obtained from SK Chemicals (Ulsan, Republic of Korea). Ultrapure water (18.2 MΩ) was obtained using a Milli-Q apparatus from Millipore (Milford, MA, USA).

2.2. Instruments

An Agilent 1200 Infinity Series HPLC (Agilent, Palo Alto, CA, USA) was used for the separation, and an AB Sciex API 3200 triple quadruple mass spectrometer (AB Sciex, Foster City, CA, USA) with an electrospray ionization probe was used for the detection. Analyst 1.5.2 system software (AB Sciex, Foster City, CA, USA) was used for the data analysis.

2.3. Liquid chromatography and mass spectrometric conditions

An Atlantis T3 column (2.1 mm × 100 mm, 3 μm, Waters, Milford, MA, USA) was used for the separation of celecoxib and its metabolites, which were measured during a 17 min run using a tandem triple quadruple mass spectrometer. The mobile phase was composed of 10 mM ammonium formate in either 5% acetonitrile (v/v, mobile phase A) or 95% acetonitrile (v/v, mobile phase B). At a flow rate of 0.2 mL/min, the elution gradient was as follows: 90% mobile phase A for 1 min, followed by a linear increase to 50% mobile phase B from 1 to 2 min; then, mobile phase B was increased from 50% to 100% for 5 min and retained until 9.5 min; the column was re-equilibrated with 90% mobile phase A from 9.5 to 17 min. The column was maintained at 35 °C, and the injection volume was 5 μL for each sample. The samples were kept at 4 °C in an auto-sampler during the analysis.

Each analyte was detected with multiple reaction monitoring (MRM) in alternating positive/negative polarity switching mode