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Short communication

Determination of irinotecan and SN38 in human plasma by TurboFlow™ liquid chromatography–tandem mass spectrometry

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ABSTRACT

Irinotecan is a cytotoxic agent used in the treatment of metastatic colorectal cancer. Irinotecan is a prodrug when is converted in vivo to an active metabolite SN38, which has potent pharmacological activity. SN38 is then inactivated and excreted as SN38-glucuronide. High-performance liquid chromatography–mass spectrometry is a widely used bioanalysis technique that can be coupled to the turbulent-flow extraction line to shorten preparation time. A technique was developed to quantify irinotecan and its metabolite by liquid chromatography–tandem mass spectrometry coupled with a turbulent-flow online extraction method.

Assays were performed on 100 μL of plasma after protein precipitation. The supernatant is injected directly into the extraction column, transferred to the chromatographic column, and analyzed by tandem mass spectrometry.

Linearity, reproducibility and repeatability of the method were validated on a concentration range of 25–2500 ng/mL for irinotecan and 5–500 ng/mL for SN38. For the low limit of quantification of irinotecan and SN38, precision is 6.31% and 8.73%, and accuracy is 84.0% and 91.8%, respectively. The SN38-glucuronide determination protocol included a hydrolyzation step.

This method was successfully used to quantify irinotecan, SN38 and SN38-G in human plasma in a clinical trial.

1. Introduction

Irinotecan is a semi-synthetic derivative of camptothecin that achieves good anti-tumor activity by inhibiting topoisomerase I [1], inducing DNA transcription termination and leading to cell death. This molecule is a prodrug which is used in to the treatment of metastatic colorectal cancer (mCRC), where it is generally used in combination with 5-fluorouracil and targeted therapies (cetuximab or bevacizumab).

The metabolism of irinotecan is mainly hepatic with biliary elimination. It is oxidized by cytochrome P450 3A (CYP3A) to inactive metabolites 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin (APC) and 7-ethyl-10-[4-amino-(1-piperidino)-carbonyloxycamptothecin (NPC) [2]. Irinotecan is cleaved by carboxylesterase (CES) to 7-ethyl-10-hydroxycamptothecin (SN38), a pharmacologically active metabolite 100–1000 times more active than the parent drug [3]. SN38 plays a major role in the inhibition of topoisomerase I [4,5] but it also causes major side effects (diarrhea and neutropenia) following irinotecan administration. SN38 is removed by glucuronidation in the liver, mainly by uridine diphosphate glucuronosyltransferases including UGT1A1, which transforms it into an inactive SN38-glucuronide (SN38-G). This combination renders SN38 both inactive and hydrophilic, thus facilitating biliary

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elimination [6]. Different patients show different rates of SN38 glucuronidation, which explains the interindividual variation in pharmacokinetic parameters of SN38 and toxicities observed after administration of irinotecan. Indeed, genetic polymorphism in the gene promoter (UGT1A1*28) causes stronger downregulation of the enzyme associated with circulating SN38 concentrations lowest than in patients carrying UGT1A1*1 [7]. Prevalance of this mutation is 7.7–8.8% for genotype *28/*28, 41.9–45.6% for genotype *1/*28 and 45.6–50.5% for genotype *1/*1 [8]. A pharmacokinetic study led on 20 patients treated with irinotecan at a dose of 300 mg/m^2 every 3 weeks found that circulating SN38 rates are higher for carriers of allele *28 [9]. The area under curve (AUC) ratio SN38-G/SN38 indicates low glucuronidation rates for *1/*28 and *28/*28 patients ($p = 0.001$). These molecules need to be measured to yield information on the patient's metabolism and the potential interindividual variability involved.

Therapeutic drug monitoring of irinotecan can be divided into two strands, genetic tests to determine the adapted dose, and pharmacokinetic assays to validate the effective circulating concentrations of irinotecan and SN38.

Several quantification methods have been developed using high-performance liquid chromatography (HPLC) with fluorimetric/UV detection [10–18] or mass spectrometry/tandem mass spectrometry (LC–MS or LC–MS/MS) after solid or liquid extraction [1,2,5,19–24], and one with online extraction [25], but never with TurbulentFlow® technology. This system is an application of turbulent-flow chromatography columns and automated online sample preparation that enables dramatically shorter sample pretreatment times. The extraction column is composed by a macroporous structure and an adsorbent with large particle size to binds small molecule like drugs, and adapted to high flow rate [26]. A designed loop (focus mode), whose composition and transfer time have been optimized, enables the retained analytes to subsequently get eluted from the extraction column onto the analytical column for chromatographic separation. This online extraction method eliminates the need for time-consuming off-line sample extraction procedures, thus significantly increasing productivity. This technology is already used for other kinds of application like pharmaceuticals drugs [27,28].

The aim of this study was to develop, validate and apply an LC–MS/MS method coupled with TurboFlow™ technology to quantify irinotecan, SN38 and SN38-G for therapeutic drug monitoring. The optimal circulating concentration of irinotecan established by this method will then be studied in pharmacokinetic assays on human plasma.

2. Material and methods

2.1. Chemicals and reagents

All chemical were analytical-grade or high-quality standard. Irinotecan (purity ≥ 97%), SN38 (purity ≥ 98%) and camptothecin (purity ≥ 90%) were from Sigma–Aldrich (Saint-Quentin-Fallavier, France). SN38-glucuronide (purity = 98%) was from Toronto Research Chemicals (North York, ON, Canada). Acetonitrile and acetone were from Carlo Erba Reagents (Val-de-Reuil, France). Formic acid, dimethylsulfoxide, ammonium acetate, ammonium formate, perchloric acid and Tris–HCl were from Fluka Analytical (Sigma–Aldrich). β-glucuronidase from *Helix pomatia* was from Sigma–Aldrich. Ammonia, isopropanol and methanol were from VWR International (Pessac, France). High-performance aqueous mobile phases were prepared on a Maxima station (Millipore, Molsheim, France). Hemoglobin, bilirubin and intralipid used for matrix effect were from Sigma–Aldrich.

The plasma used for the development of this method was provided from Etablissement Français du Sang (Saint Etienne, France).

2.2. Preparation of stock solutions, calibration standards and quality control samples

Standard stock solutions of irinotecan and camptothecin were prepared in methanol at a concentration of 1 g/L and stored at −20 °C. Standard stock solution of SN38 was prepared at 1 g/L in MeOH/dimethylsulfoxide (65:35, v/v) and stored at −20 °C [1]. Working solutions were prepared by diluting standard stock solution in methanol. For calibration standards, working solutions were freshly spiked in plasma to achieve 6 calibration standards at concentration of 25, 50, 100, 500, 1000 and 2500 ng/mL for irinotecan and 5, 10, 20, 100, 200 and 500 ng/mL for SN38. Four quality-control samples (QC) were freshly and separately prepared in plasma and assayed each day samples were analyzed. QC1 (lower limit of quantification, LLOQ) is at 25 and 5 ng/mL, QC2 at 75 and 15 ng/mL, QC3 at 750 and 150 ng/mL and QC4 (upper limit of quantification, ULOQ) at 2500 and 500 ng/mL for irinotecan and SN38, respectively. For the calibration curve used to determine SN38-G after the step of hydrolization, working solutions were freshly spiked in plasma to obtain 6 calibration standards at concentration of 5, 10, 20, 100, 200 and 500 ng/mL for SN38. Four QC samples were also prepared, QC1 at 5 ng/mL, QC2 at 15 ng/mL, QC3 at 150 ng/mL and QC4 at 500 ng/mL.

2.3. Sample preparation

Fresh blood samples were centrifuged at 3400 rpm for 10 min at 4 °C and plasma was frozen at −20 °C until analysis [1]. For the determination of irinotecan and SN38, protein precipitation is performed on 100 μL of plasma by adding 10 μL of camptothecin (IS) working solution (100 μg/mL) and 10 μL of perchloric acid 30% for deproteinization in polypropylene microtubes. After vortex-mixing for 1 min followed by centrifugation at 11,000 rpm for 10 min at 4 °C, the supernatant was transferred to insert and 20 μL was analyzed by LC–MS/MS. For the determination of SN38-G, enzymatic hydrolysis with β-glucuronidase is carried out just before the deproteinization. The same sample is analyzed hydrolyzed and non-hydrolyzed in order to identify the amount of free SN38 and the glucuronide form using specific calibration curves. The hydrolysis step was based on Zhang's protocol [5]. 10 μL of IS solution is added to 100 μL of plasma sample. After addition of 95 μL of Tris–HCl buffer and 5 μL of β-glucuronidase, the sample is incubated for 1 h at 56 °C then deproteinized and analyzed.

2.4. LC–MS/MS system and conditions

The LC–MS/MS set-up consisted of a TSQ Quantum Ultra™ (Thermo Fisher Scientific, San Jose, California) coupled to a Transcend TLX-1 system (Thermo Fisher Scientific). The TurboFlow™ method with automated online sample preparation was performed on a 0.5 × 50 mm TurboFlow Cyclone P column (Thermo Fisher Scientific). The analytical column was a 50 × 2.1 mm, 3 μm particle-size Thermo Scientific Hypersil GOLD column.

The TLX-1 system is composed of a CTC PAL autosampler at 8 °C with an injection DLW system, two binary pumps (Agilent Technologies® 1200 series binary pump SL) and two selective mobile phases (CTC Analytics, Zwingen, Switzerland). One loading pump is dedicated to the extraction line and the other eluting pump is used for the chromatographic separation. During the loading phase (step 1–2), the sample is injected onto the extraction column using ammonium acetate buffer (pH 9; 10 mM). After 0.58 min, the compounds are eluted from the extraction column by a focus mode (step 3) with H_2O 0.1% formic acid-acetonitrile 0.1% formic