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ORIGINAL ARTICLE

Validated LC–MS/MS method for simultaneous determination of SIM and its acid form in human plasma and cell lysate: Pharmacokinetic application

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Abstract Simvastatin (SIM) is a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor widely used in hyperlipidemia therapy. SIM has recently been studied for its anticancer activity at doses higher than those used for the hyperlipidemia therapy. This prompted us to study the pharmacokinetics of high-dose SIM in cancer patients. For this purpose, an LC–MS/MS method was developed to measure SIM and its acid form (SIMA) in plasma and peripheral blood mononuclear cells (PBMCs) obtained from patients. Chromatographic analyte separation was carried out on a reverse-phase column using 75:25 (% v/v) acetonitrile:ammonium acetate (0.1 M, pH 5.0) mobile phase. Detection was performed on a triple quadrupole mass spectrometer, equipped with a turbo ion spray source and operated in positive ionization mode. The assay was linear over a range 2.5–500 ng/mL for SIM and 5–500 ng/mL for SIMA in plasma and 2.5–250 ng/mL for SIM and 5–250 ng/mL for SIMA in cell lysate. Recovery was >58% for SIM and >75% for SIMA in both plasma and cell lysate. SIM and SIMA were stable in plasma, cell lysate and the reconstitution solution. This method was successfully applied for the determination of SIM and SIMA in plasma and PBMCs samples collected in the pharmacokinetic study of high-dose SIM in cancer patients.

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1. Introduction

Simvastatin (SIM) is a well-established drug for the treatment of hyperlipidemia. SIM is a prodrug administered in the lactone form, which is converted in the liver into the active acid form (Fig. 1). It is this active carboxylate form that reduces cholesterol biosynthesis by competitively inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)

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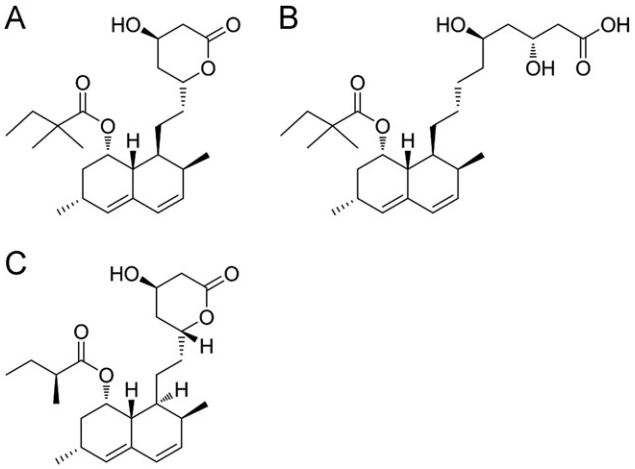

Figure 1 Chemical structures of (A) simvastatin, (B) simvastatin acid and (C) lovastatin.

reductase, the rate limiting enzyme in the mevalonate pathway [1,2]. Additionally, statins inhibit the synthesis of other downstream products in the mevalonate pathway, such as the isoprenoids [1,2]. Isoprenoids, including farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), are known to be involved in important cellular processes such as proliferation and apoptosis [3]. Thus, statins have recently been tested for their potential use as anticancer agents. As with all agents in this class, in vitro studies have shown that SIM displays anticancer activity, but only at concentrations that are higher than those observed in plasma of patients being administered typical doses associated with the hyperlipidemia therapy [4].

Several clinical trials were subsequently conducted to study the safety and tolerability of high dose statin analogs, including simvastatin, in cancer patients [5–7]. Oral statins were found to be well tolerated at high doses with minor side effects. In a phase I study, lovastatin (LOV) given orally at a dose of 25 mg/kg daily was well tolerated and safe in patients with solid tumor [6]. In the case of SIM, a phase I study in patients with myeloma or lymphoma has shown that the maximal tolerated dose (MTD) of SIM, given orally, is 7.5 mg/kg twice a day, which is 25-fold higher than typical dose. The most common side effects of high dose SIM were nausea, diarrhea, muscle weakness and myalgia [7]. However, pharmacokinetics (PK) was not defined and it is not known if SIM plasma concentrations can reach the levels necessary for the antitumor activity observed in vitro. In this context, we initiated a clinical study to characterize the pharmacokinetics of simvastatin lactone and its acid form (simvastatin acid, SIMA) in plasma and peripheral blood mononuclear cells (PBMCs) after oral administration of SIM at 7.5 mg/kg twice daily in patients with recurrent and refractory chronic lymphocytic leukemia (CLL).

SIM has low systemic bioavailability which is attributed to the high extraction by the liver, the main site of action for treating hyperlipidemia. Therefore, sensitive analytical methods have previously been developed to assay both SIM and SIMA in plasma [8–11]. The first analytical method developed was an LC coupled with UV detection (238 nm); nonetheless, low sensitivity for quantitation of SIM and SIMA in biological fluids was reported [12]. Better sensitivity using UV detection was achieved later with an LOQ of 0.5 ng/mL but with run time >28.7 min [13]. A more sensitive HPLC-FD method using 1-bromoacetylpyrene for derivatization has been reported with an LOQ of 0.1 ng/mL for both analytes [14]. Although this LC-FD method is highly sensitive, sample preparation using solid phase extraction and analyte derivatization is inconvenient and time consuming. On the other hand, several LC–MS/MS methods have been developed for the determination of SIM and SIMA in biological fluids which are more sensitive and specific [8–11]. These methods are coupled with either solid phase extraction (SPE) or liquid–liquid extraction (LLE) procedures. Solid phase extraction has yielded good recoveries for SIM but SIMA recovery was low [15]. LLE showed better recoveries for both SIM and SIMA compared to SPE [8,10,11]. Current analytical methods have not been validated for the analyses of SIM and SIMA in cell lysates. Moreover, few assays have been validated to measure plasma concentration of SIM and SIMA at higher levels [16–18]. Here we report the development and validation of an LC–MS/MS method for the analysis of SIM and SIMA human plasma and PBMCs.

2. Experimental

2.1. Chemicals and reagents

SIM was purchased from Toronto Research Chemicals Inc. (North York, Canada). Ammonium acetate (Mallinckrodt Baker, Philipsburg, NJ, USA) and sodium hydroxide (EM Science, Gibbstown, NJ, USA) were purchased from VWR (West Chester, PA, USA). HPLC grade acetonitrile and diethyl ether were obtained from Sigma-Aldrich (St Louis, MO, USA). LOV (Alexis Biochemicals, San Diego, CA, USA), hydrochloric acid and glacial acetic acid were from Fisher Scientific (Fair Lawn, NJ, USA). Anhydrous ethanol was obtained from IBI Scientific (Peosta, IA, USA). K562, a chronic myelogenous leukemia (CML) cell line, was purchased from ATCC (Manassas, VA, USA).

2.2. LC–MS/MS instrumentation and conditions

All analyses were performed using an HPLC system consisting of a Shimadzu LC-20AD pump and a Shimadzu SIL-20AC VP autosampler (Shimadzu, Columbia, MD, USA). The LC system was interfaced to an API 2000 ESI-MS/MS (Applied Biosystems, Foster City, CA, USA). The analytical column used was a Phenomenex Luna C_{18} (2.0 mm $\times$ 100 mm i.d.; 2.5 µm particle size), connected to a C_{18} guard column (Phenomenex C_{18}, 2.0 mm $\times$ 4 mm; 5 µm particle size). An isocratic mobile phase was used consisting of 75:25 (% v/v) acetonitrile:ammonium acetate (0.1 M, pH 5.0 adjusted with acetic acid). The flow rate was 0.15 mL/min under ambient temperature. The autosampler temperature was maintained at 4 °C and the injection volume was 20 µL. The run time was 10 min. All analytes and internal standard were detected on a triple quadrupole mass spectrometer (API 2000), equipped with a turbo ion spray source (MDS SCIEX, Toronto, Canada) and operating in the positive ion mode. LOV was used as an internal standard (IS). Quantitation was performed using multiple reaction monitoring (MRM) of precursor/