ELSEVIER

Prostaglandins & other Lipid Mediators 81 (2006) 162–170

PROSTAGLANDINS & other LIPID MEDIATORS

LC–MS/MS-analysis of sphingosine-1-phosphate and related compounds in plasma samples

Helmut Schmidt*, Ronald Schmidt, Gerd Geisslinger

Pharmazentrum Frankfurt/ZAFES, Institut für Klinische Pharmakologie, Klinikum der Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

Received 28 June 2006; received in revised form 4 September 2006; accepted 7 September 2006
Available online 10 October 2006

Abstract

Sphingosine-1-phosphate (S1P) and related compounds are important signaling molecules and are normal constituents of human plasma. So far, only a few methods exist for their determination specifically in plasma demanding radioactive agents, more or less time consuming extraction or derivatization procedures. Here, we describe a very simple, reliable, sensitive standard-addition method for the simultaneous determination of S1P, sphingosine (SPH), sphinganine (SAPH) and sphinganine-1-phosphate (SA1P) in human and rat plasma samples. After methanol precipitation of plasma samples the supernatants were directly assessed by liquid chromatography–electrospray ionisation-tandem mass spectrometry (LC–ESI-MS/MS). HPLC analysis was done under gradient conditions using a C18 reversed phase column. The lower limit of quantification (LLOQ) was <10.2, <4.6, <1.9 and 0.57 ng/ml for S1P, SPH, SAPH and SA1P, respectively. Variations in accuracy and intraday and interday precision were <15% over the range of calibration. All analytes were normal constituents both in human and rat plasma although the SA1P concentrations in a few rat plasma samples were below the lower limit of quantification.

This validated method is suitable to generate new pharmacological findings by monitoring plasma concentrations of S1P and related compounds especially when low amounts of plasma samples are present (e.g. plasma samples from rodents).

Keywords: Sphingosine-1-phosphate; Sphingosine; Sphinganine; Sphinganine-1-phosphate; Chromatography; Tandem mass spectrometry; ESI; Human and rat plasma

1. Introduction

Sphingolipids such as sphingosine-1-phosphate (S1P) and related compounds are bioactive lipid mediators.

S1P which is synthesized from sphingosine (SPH) by sphingosine kinases was initially classified as an intracellular second messenger regulating a wide range of biological responses such as cell growth, cell death, differentiation and migration. However, identification of plasma membrane receptors demanded to reevaluate the functions of S1P and revealed that S1P also plays a role as extracellular mediator [1]. Indeed, S1P which is abundantly expressed in platelets due to high activity of sphingosine kinases and lack of S1P-lyase [2] is released from activated platelets [3] and is a normal constituent of human plasma and serum [4] with concentrations of approximately 200–1000 nmol/l [4–7] being lower in plasma than in serum. In plasma and serum, S1P is particularly accumulated in the high-density lipoprotein

* Corresponding author. Tel.: +49 69 6301 7618; fax: +49 69 6301 7636.
E-mail address: Helmut.Schmidt@em.uni-frankfurt.de (H. Schmidt).

1098-8823/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.prostaglandins.2006.09.003

(HDL) fraction [7]. The atheroprotective effect of HDL may be in part due to the presence of lysosphingolipids such as S1P in this lipoprotein fraction [8]. Furthermore, it was shown that serum S1P is a remarkably strong and robust predictor of both the occurrence and severity of coronary stenosis [5].

Sphingosine (SPH) which is synthesized by deacylation of ceramide by the enzyme ceramidase and sphinganine (SAPH) which is the key intermediate in the *de novo* synthesis of ceramide from serine and palmitoyl-CoA are also biologically active mediators. Both are present in human plasma and serum [9,10]. It was shown that concentrations of SPH and SAPH in plasma of patients with type 2 diabetes were elevated indicating that metabolism of ceramide is augmented [9].

Although a lot of various methods have been published to quantify S1P and related compounds, only a few of them assessed these analytes specifically in plasma or serum. One assay measured S1P in horse, fetal bovine and calf serum by an enzymatic and radioactive approach [11], another one determined S1P in human serum by radioreceptor-binding assay [12], a third one produced derivatives of S1P and related compounds to quantify them in human plasma and serum [13], a fourth one produced derivatives of S1P and the internal standard SA1P to quantify S1P in human serum [14] and another one used derivatives of S1P and SA1P to quantify them in human platelet poor plasma [15]. All methods employed more or less time consuming extraction procedures.

Here we present a validated and very simple, sensitive, reliable and fast method for determination of S1P and related compounds in human and rat plasma by methanol precipitation and direct determination of the analytes by liquid chromatography–electrospray ionisation-tandem mass spectrometry (LC–ESI-MS/MS).

2. Materials and methods

2.1. Materials

Acetonitrile, ethanol and methanol (all gradient grade for liquid chromatography), tetrahydrofuran (for liquid chromatography), formic acid (89–91%, GR for analysis) and hydrochloric acid 32% (GR) were purchased from Merck KGaA (Darmstadt, Germany). Water (LC–MS grade) and dimethyl sulfoxide (analytical reagent grade) were obtained from Carl Roth GmbH + Co. KG (Karlsruhe, Germany) and Fisher Scientific GmbH (Schwerte, Germany), respectively. D-*erythro*-Sphingosine-1-phosphate (S1P), D-*erythro*-sphingosine (SPH), D-*erythro*-sphinganine (SAPH), D-*erythro*-sphinganine-1-phosphate (SA1P) and the internal standards D-*erythro*-C17-sphingosine-1-phosphate (C17-S1P) and D-*erythro*-C17-sphingosine (C17-SPH) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA).

Human and rat plasma were gifts from the Blutspendedienst Hessen (Deutsches Rotes Kreuz, Frankfurt am Main, Germany) and the Tierversuchsanstalt (TVA, Universitätsklinikum Frankfurt am Main, Germany), respectively. Human plasma was prepared with a blood bag system (four bags, article number T 3989, Fresenius HemoCare, HM Emmer-Compascuum, Netherlands). About 500 ml of blood was added to 70 ml anticoagulant solution which contained 26.3 g/l sodium citrate dihydrate, 3.27 g/l citric acid hydrate and 2.51 g/l sodium dihydrogen phosphate dihydrate resulting in final concentrations of 3.23 mg/ml sodium citrate dihydrate, 0.4 mg/ml citric acid hydrate and 0.31 mg/ml sodium dihydrogen phosphate dihydrate in the (diluted) blood. Rat plasma was prepared by adding 5 ml blood to 20 μl of Liquemin® N (25,000 units heparin/ml, Roche Pharma AG, Grenzach-Wyhlen, Germany) resulting in a final concentration of 100 units heparin per ml blood.

2.2. Instrumentation

Sample analysis was performed by using liquid chromatography–electrospray ionisation-tandem mass spectrometry (LC–ESI-MS/MS). The HPLC equipment consisted of an Agilent 1100 Series binary pump (G1312A) and degasser (G1379A) connected to an HTC PAL autosampler (Chromtech, Idstein, Germany). A triple quadrupole mass spectrometer 4000 Q TRAP equipped with a Turbo V source ion spray operating in positive ESI mode was used for detection (Applied Biosystems, Darmstadt, Germany). High purity nitrogen for the mass spectrometer was produced by the nitrogen generator NGM 22-LC/MS (cmc Instruments, Eschborn, Germany).

2.3. LC–MS/MS conditions for plasma samples

Chromatographic separations were obtained under gradient conditions using a Luna-RP column (150 cm L × 2 mm i.d., 5 μm particle size and 100 Å pore size) with a C18 guard column (4 mm L × 2 mm i.d.) (Phenomenex, Aschaf-