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Evaluation of the change in sphingolipids in the human multiple myeloma cell line U266 and gastric cancer cell line MGC-803 treated with arsenic trioxide

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ABSTRACT

Arsenic trioxide (As_2O_3) has been found to display anticancer activity against many types of tumors and has been developed into an anticancer drug in clinical treatments. Sphingolipids are membrane lipids that participate in many signal transduction pathways. In this paper, the changes in sphingolipids of the human multiple myeloma cell line U266 and the gastric cancer cell line MGC-803 treated with arsenic trioxide were investigated using an HPLC-ESI–MS/MS method. Analytes were separated by an XBridge BEH C8 column used for Cer, HexCer, LacCer and SM chromatographic separation, and a Capcell PAK MG II C18 column was used for Sph, dhSph, S1P and dhS1P chromatographic separation and gradient elution with acetonitrile–water containing 0.1% formic acid as a mobile phase. A tandem mass spectrometer QTrap in SRM mode was employed in combination with RPLC as a detector for quantitative analysis. The ceramide/sphingolipid internal standard (IS) mixture was used to quantify the levels of sphingolipids. The distributions of sphingolipids were found to be different in the human multiple myeloma cell line U266 and the gastric cancer cell line MGC-803. Ceramide (Cer), hexosylceramide (HexCer) and dihexosylceramide (Hex2Cer) levels in U266 cell line are higher than those in MGC-803 cell line. Additionally, sphingomyelin (SM), sphingosine-1-phosphate (S1P) and sphinganine-1-phosphate (dhS1P) levels in the MGC-803 cell line are higher than those in the U266 cell line. When treated with arsenic trioxide (1–5 μM iAs^{III}(As^{III} ions)), the levels of Hex2Cer in the human multiple myeloma cell line U266 decreased, and the levels of S1P and dhS1P in the human gastric cancer cell line MGC-803 decreased. The decrease of Hex2Cer, S1P and dhS1P in the human multiple myeloma cell line U266 and gastric cancer cell line MGC-803 were observed when the concentration of iAs^{III} is 1.0 μM. Therefore, arsenic trioxide exhibits anti-cancer activity by altering the sphingolipid pathway in the human multiple myeloma cell line U266 and the gastric cancer cell line MGC-803.

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1. Introduction

Arsenic trioxide (As_2O_3) has shown anticancer activity against many types of tumors and has been developed into an anticancer drug in promyelocytic leukemia clinical treatment [1–4]. Arsenic trioxide has been reported to exhibit its anti-cancer activity by inducing apoptosis and autophagy, promoting differentiation and inhibiting invasion of the cell [5–8].

Sphingolipids are the second largest group of membrane lipids and major components of lipid rafts. Sphingolipids and their metabolites are involved in many important signal transduction pathways that regulate diverse cellular events such as cell cycle arrest or apoptosis, proliferation, cancer development, and multidrug resistance [9–10]. Ceramide is at the core of the sphingolipid metabolism pathway and can be generated from simple molecules by a de novo synthesis pathway or from the hydrolysis of more complex sphingolipids (Fig. 1) [9].

Abbreviation: DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential; iAsIII, AsIII ions; QTrap, quadrupole linear-ion trap; SRM, selected reaction monitoring; Cer, ceramide; HexCer, hexosylceramide; Hex2Cer, dihexosylceramide; SM, sphingomyelin; Sph, sphingosine; dhSph, sphinganine; S1P, sphingosine 1-phosphate; dhS1P, sphinganine 1-phosphate.

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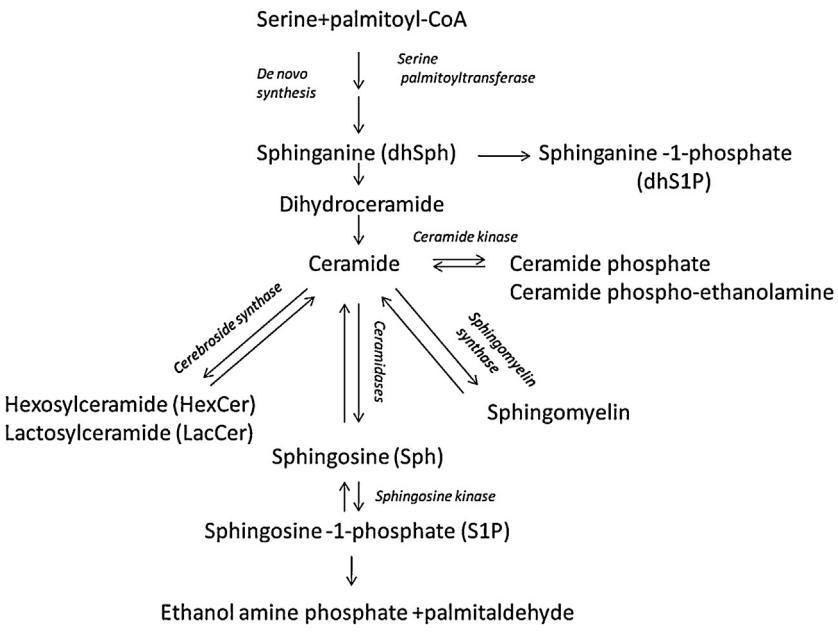

Fig. 1. The metabolic pathways of sphingolipids.

Multiple categories of sphingolipids are noted because of their numerous variations in sphingoid bases, *N*-acyl linked fatty acids, and head groups [11]. Therefore, sphingolipids were difficult to analyze until the advent of mass spectrometry technologies [12–15]. Among the different mass spectrometry techniques, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS has been widely used in the determination and analysis of different classes of lipids in various tissues and disease states [16–20]. Because the characteristic product ions can be distinguished in HPLC–MS/MS, HPLC–MS/MS can identify more molecular species of each class of lipids and provide more accurate results than MALDI-TOF-MS. Multiple papers report methods for determining the quality and quantity of different classes of sphingolipids in blood, cells and the tissues using HPLC–MS/MS [21–31]. However, more application research is required for sphingolipids when using HPLC–MS/MS techniques.

In 2007, arsenic trioxide was reported to be able to induce the accumulation of ceramide in acute promyelocytic leukemia and adult T-cell leukemia/lymphoma cells through de novo ceramide synthesis and the inhibition of glucosylceramide synthase activity. The ceramide was measured with a modified diacylglycerol kinase assay using external ceramide standards [32]. In 2012, arsenic trioxide was reported to inhibit HCCLM3 cells invasion through de novo ceramide synthesis and the hydrolysis of sphingomyelin; the ceramide antibody was used to evaluate the change of ceramide on the membrane of cells under the microscope [33]. Until now, no detailed research was available on the direct determination of sphingolipids in cells treated with arsenic trioxide using HPLC-MS/MS techniques.

Clinically achievable levels ($iAs^{III} \leq 5\ \mu M$) of As_2O_3 could induce apoptosis in cell lines or primary patient cells [34–35]. In this paper, therefore, we used HPLC–MS/MS techniques to investigate the change in sphingolipids in the human multiple myeloma cell line U266 and the gastric cancer cell line MGC-803 treated with clinical levels of arsenic trioxide (1–5 μM iAs^{III}). The changes in sphingolipids were found to be different in the human multiple myeloma cell line U266 and the gastric cancer cell line MGC-803 treated with arsenic trioxide.

2. Materials and methods

2.1. Chemicals

Arsenic trioxide was purchased from Sigma (St. Louis, MO, USA) and dissolved in a 0.1 M sodium hydroxide solution to produce a trivalent arsenite (iAs^{III}) stock solution. Sphingolipid quality standards (14:0-Cer, 16:0-Cer, 18:0-Cer, 20:0-Cer, 22:0-Cer, 24:0-Cer, 24:1-Cer, 16:0-HexCer, 24:1-HexCer, 16:0-Hex2Cer, 16:0-SM, Sph, dhSph, S1P and dhS1P) and sphingolipid quantity IS (Ceramide/Sphingoid Internal Standard Mixture II including 17:0-Sph, 17:1-dhSph, 17:0-S1P, 17:1-dhS1P, 12:0-LacCer, 12:0-SM, 12:0-HexCer, 12:0-Cer and 12:0Ceramide-1-P) were purchased from a commercially available source (Avanti Polar Lipids, Alabaster, AL, USA) at purities ≥98%. HPLC-grade acetonitrile was purchased from Merck KGaA (Darmstadt, Germany). HPLC-grade formic acid was purchased from Sigma–Aldrich (St. Louis, MO, USA). The water was distilled and purified using a Milli-Q Water Purification System (Millipore, Bedford, MA, USA). Penicillin, streptomycin, RPMI 1640, and fetal bovine serum (FBS) used in cell culturing were purchased from Invitrogen (Grand Island, USA). All other chemicals were of analytical grade.

2.2. Cell culture

Because the human multiple myeloma U266 cells were suspended, the cells were seeded at a density of 1.0×10^6 in a T25 flask and treated with different levels of iAs^{III} (0.0 μM, 1.0 μM, 2.0 μM, 3.0 μM, 4.0 μM, and 5.0 μM) and maintained in logarithmic growth phase RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin at 37 °C in a 5% CO_2 atmosphere for 24 h.

Because the human gastric cancer MGC-803 cells were adherent cells, the arsenic trioxide was added after the cells adhered to the wall. The human gastric cancer MGC-803 cells were seeded at a density of 1.0×10^6 in a T25 flask and were cultured for 12 h to allow the cells to adhere to the wall. After the cells adhered to the wall, the cultures were washed twice with PBS, fresh medium was added, and the cells were then treated with different levels of iAs^{III} (0.0 μM,

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