International Journal of Mass Spectrometry xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Full Length Article

Quantification of *S*-adenosylmethionine and *S*-adenosylhomocysteine in human placenta and placental explants under homocysteine treatment

R. Rodriguez [a], O. Vakulenko [a], S. Ralchenko [a], A. Kostiuk [d], L. Porublyova [a], I. Konovets [b], I. Voronina [c], M. Obolenskaya [a,*]

[a] *Institute of Molecular Biology & Genetics, NAS of Ukraine, Ukraine*
[b] *Institute of Hydrobiology, NAS of Ukraine, Ukraine*
[c] *Kyiv State Maternity Hospital №2, Ukraine*
[d] *Institute of Biology & Medicine, Taras Shevchenko National University of Kyiv, Ukraine*

ARTICLE INFO

Article history:
Received 1 May 2017
Received in revised form 31 July 2017
Accepted 4 August 2017
Available online xxx

Keywords:
Human placenta
S-Adenosylmethionine
S-Adenosylhomocysteine
Homocysteine

ABSTRACT

S-Adenosylmethionine (SAM) and *S*-adenosylhomocysteine(SAH) are essential compounds in methionine cycle. SAM is a universal donor in majority of methyltransferase reactions while SAH is a universal product and a potent inhibitor of methyltransferase reactions. The reactions of methionine cycle and connected with them folate-related reactions and transsulfuration pathway are implicated in various complications of pregnancy. Their role in placental metabolism and responsiveness to hyperhomocysteinemia is scarcely investigated though hyperhomocysteinemia is a risk factor in several obstetric pathologies. In this paper, a simple and fast method based on LC/MS technology is applied for simultaneous SAM and SAH quantification in human placenta and placental explants. The developed method proved to be highly sensitive, inter-day repeatable and intra-day reproducible. The content of SAM and SAH in term human placenta is in the range 8.33–18.73 and 5.20–8.74 nmol/g wet tissue, respectively. SAM concentration doubles while SAH decreases under treatment of cultivated placental explants with clinically relevant 20 μM homocysteine in comparison with placental tissue with ultimate impact on velocity of numerous methyltransferase reactions.

1. Introduction

Many pregnancy-related disorders are associated with the disturbances in folate-related one-carbon unit metabolism [1–4]. The folate-related metabolism includes folate and methionine cycles and transsulfuration pathway. The folate cycle harbors the de novo biosynthesis of purine nucleotides and deoxythymidine monophosphate, the precursors of nucleic acids and energy carriers [5]. It is connected with the methionine cycle via remethylation of homocysteine to methionine. Methionine is adenosylated by methionine adenosyltransferase [EC 2.5.1.6] to *S*-adenosylmethionine (SAM), which functions as an intermediate for polyamine synthesis, a cofactor and methyl group donor for the overwhelming majority of methylation reactions and a potent regulator of enzymatic activities [5,6]. The demethylated derivative of SAM is *S*-adenosylhomocysteine (SAH) that is cleaved into homocysteine and adenosine by SAH hydrolase [EC 3.3.1.1]. The thermodynamic equilibrium reaction catalyzed by SAH hydrolase favors the reverse reaction of homocysteine and adenosine making SAH [7]. In majority of tissues, about 50% of homocysteine is conserved by remethylation to methionine. Nearly the same amount of homocysteine is irreversibly converted by the transsulfuration pathway to cysteine, the precursor of glutathione, taurine and hydrogen sulfide [7–9]. Some amount of homocysteine is released into extracellular fluids (e.g. plasma, urine). The reactions of one-carbon unit metabolism provide support for the basic needs of dividing cells: rapid generation of ATP and GTP to maintain energy status; increased biosynthesis of macromolecules; epigenetic regulation of gene expression; and tightened maintenance of the appropriate cellular redox status [5,8,10,11]. Hence, the disturbances in one-carbon unit metabolism in growing placenta and fetus may have severe consequences for mother and baby during pregnancy and after it.

Among different markers of one-carbon metabolism disorders, homocysteine concentration in maternal blood was for a long time of specific interest. A systematic review of the literature reveals an

* Corresponding author at: 150, Zabolotnoho str., Kyiv, 03680, Ukraine.
E-mail address: m.obolenska@gmail.com (M. Obolenskaya).

http://dx.doi.org/10.1016/j.ijms.2017.08.002
1387-3806/

overall association between higher homocysteine concentrations in maternal blood and preeclampsia, pregnancy-induced hypertension, intrauterine growth restriction and spontaneous abortion [4,12–17]. In normal pregnancy plasma homocysteine concentration is significantly lower than in non-pregnant women, being at average 5 μM vs. 8 μM, respectively. It is slightly increased in normotensive pregnancies that later develop preeclampsia and reaches 18.4 ± 6.59 μM in established preeclampsia [13]. Plasma homocysteine concentration is an integrative characteristic. Up to 50% of plasma homocysteine is formed in the liver [18]. The characteristics of placental one-carbon metabolism and its responsiveness to elevated maternal homocysteine concentration are scarcely known though the disturbances in this metabolism may cause multivarious adverse consequences for the fetus and mother [15,19,20].

Over the past decade, the primary focus on homocysteine as a marker has shifted towards methionine cycle intermediates, in particular SAM, SAH and SAM/SAH ratio. In many cases (e.g. vascular inflammation, lack of cystathionine beta synthase activity, osteoporosis) SAH is a more sensitive marker than homocysteine [21–23]. Interestingly, lowering of homocysteine with B vitamins does not necessarily correlate with lowering of SAH. SAH could still be elevated due to SAH hydrolase SNPs, NAD deficiency or elevated levels of adenosine. SAH is a potent inhibitor of most SAM-dependent methyltransferases with Ki values in the submicromolar to low micromolar range [24]. SAM level also promises to be an independent marker from homocysteine, as increased SAM does not inevitably result in homocysteine level increase [25]. Moreover, SAM/SAH ratio serves as a marker of "methylation potential". The decrease in the SAM/SAH ratio have been observed in patients with end-stage renal failure, cardiovascular disease, in cancer cells suggesting a link between disturbed transmethylation reactions and vascular dysfunction, impaired renal function, and proliferation [21,26].

Different analytical methods reporting the SAM and SAH determination in body tissues and fluids were developed. They include methods requiring tedious sample preprocessing (e.g. derivatization) and using LC coupled with MS or MS/MS [27,28].

In this study, we quantified SAM and SAH in human placenta and placental explants cultivated with clinically relevant 20 μM homocysteine. For this purpose we modified the methods based on HPLC-PBA-MS which was approved for SAM and SAH quantification in plasma and blood [27].

The developed method proved to be highly sensitive, inter- and intra-day reproducible The content of SAM and SAH in term human placenta is in the range 8.33–18.73 and 5.20–8.74 nmol/g wet tissue, respectively; the concentration of SAM doubles while SAH decreases in placental explants cultivated with 20 μM homocysteine, a risk factor for pregnancy complications, with ultimate impact on velocity of multiple methyltransferase reactions.

2. Methods

2.1. Reagents and reference materials

Solvents and reagents (HPLC grade acetonitrile, methanol, acetic acid, ammonium acetate and perchloric acid) used for sample preparation and as mobile phases were from RSI Labscan LTD (Bangkok, Thailand) and used without further purification. SAM, SAH standards, DL-homocysteine and DMEM-F12 culture medium were from Sigma Aldrich (Taufkirchen, Germany). Polystyrene tissue culture plates, Cat. #662102, were from GREINER BIO ONE (Kremsmünster, Austria). Bond Elut PBA solid phase extraction columns (Part No 12102018) were from Agilent Technologies, Santa Clara (California, USA).

2.2. Sample collection

This study was carried out according to the Declaration of Helsinki. The ethics committee of the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine (Kyiv) approved the study protocol and the use of human tissues. Placental samples from women with normal pregnancies (n = 5; 40 weeks of gestation) were collected immediately after delivery in the Kyiv city clinical hospital # 2, Ukraine. All women who participated in the study signed the informed consent form.

2.3. Explant culture of placental tissue

Placental villous tissue was cut out from the central part of placenta and used for quantification of SAM and SAH content and for preparation of explants. Explants were cultivated in serum-free DMEM-F12 culture medium at 37 °C for 24 h as described in the literature [29]. Method was applied with some modifications, namely, cultivation in a humidified atmosphere of 20% O_2 and 5% CO_2 in the absence and presence of 20 μM homocysteine added after 4 h from the beginning of cultivation. Serum-free DMEM-F12 culture medium was prepared according to the manufacturer recommendations.

2.4. SAM/SAH extraction

Extraction protocol was based on procedures described in the literature [27,28,30,31] and developed with several modifications. To reduce the ion suppression from the sample matrix and for sample cleanup, the sampling was carried out by protein precipitation with perchloric acid and phenylboronic acid (PBA) solid-phase extraction (SPE) to bind SAM and SAH. Placental tissue and explants (ca. 0.5 g) were ground with a mechanical homogenizer in 2.5 ml of 0.5 M perchloric acid, centrifuged at 11,000*g*, 4 °C; the supernatant was neutralized to pH 7.0 with 10% NH_4OH and applied on the Bond Elut PBA solid phase extraction column. The column was previously conditioned with 0.1 M acetic acid and 0.4 M ammonium acetate. The column was washed with 2 ml of 0.4 M ammonium acetate, and eluted with 1.0 ml of 0.1 M acetic acid. The eluate was frozen in liquid nitrogen until analysis.

2.5. LC–MS assay for the quantification of SAM and SAH

Samples were analyzed using an Agilent 1200 series HPLC system consisting of a G1312 binary pump, a G1322A vacuum degasser, and a G1316A thermostated column compartment (Agilent Technologies, Palo Alto, CA) in combination with an Agilent 1200 G1329A auto sampler (Carrboro, NC). The HPLC system was interfaced with an Agilent 6130 single quadrupole mass-spectrometer (Agilent Technologies, Palo Alto, CA) operating with an electrospray ionization source (ESI) using nitrogen (purity: 99.99%). Fifty microliters of the PBA solid phase eluate were injected onto a rapid resolution 4.6 × 150 mm, 3.5 μm C18 column (Zorbax, Agilent, Palo Alto, CA). Mobile phase was 10% methanol solution in water brought to pH 5.0 with acetic acid. Isocratic elution was run over 12 min at a flow rate of 1 ml/min. The mass-spectrometer was operated in SIM (selected ion monitoring) mode set to detect protonated molecular ions $[M+H]^+$ of each compound, SAM (m/z = 399.0) and SAH (m/z = 385.1).

2.6. Assay validation

The assay was validated following the FDA guidelines on Bioanalytical Method Validation [32] as considered fit-for-purpose. The calibration curves were obtained from the mean values of triplicate measurements for seven different concentrations of SAM and

Please cite this article in press as: R. Rodriguez, et al., Quantification of *S*-adenosylmethionine and *S*-adenosylhomocysteine in human placenta and placental explants under homocysteine treatment, Int. J. Mass Spectrom. (2017), http://dx.doi.org/10.1016/j.ijms.2017.08.002