Identification of a Novel Human Alkyl Thiol-Methyltransferase

Introduction:

Currently, S-methylation of exogenous thiols in humans is known to be catalyzed by thiopurine methyltransferase (TPMT) and thiol methyltransferase (TMT). Purified TPMT has been well characterized and is known to metabolize a number of drugs, including the chemotherapeutic 6-mercaptopurine and the immunosuppressant azathioprine. Additionally, TPMT has been shown to possess a number of polymorphisms which can impact the rate of clearance of TPMT-metabolized drugs. Due to the serious conditions treated with TPMT-metabolized drugs, randomized clinical trials have been developed to test whether genetic testing for TPMT polymorphisms may allow better dosing regimens.

On the other hand, TMT has only been identified at the activity level despite being enriched to high specific activity. Previous works have shown that TMT is capable of metabolizing a number of thiol-containing drugs such as 7α-thio-spironolactone, captopril, ziprasidone, the active metabolites of clopidogrel and prasugrel and several others. Captopril is known to cause idiosyncratic toxicity via conjugation to intracellular glutathione as well as plasma proteins and clopidogrel also causes hepatotoxicity upon activation and exposure of an aliphatic thiol. The shared route of metabolism via thiol methylation suggests a potential enzymatic or genetic disposition to idiosyncratic toxicity.

TMT has been shown to display pH-dependent biphasic kinetics with a high affinity form existing at pH 7.2-7.6 and a low affinity form at pH 8.8 as well as normal Mendelian patterns of inheritance. It is predicted to have a molecular weight of around 28 kDa and is localized to the endoplasmic reticulum. TMT activity has been reported in lung, kidney and liver tissue as well as red blood cell membranes. So far, TMT has not yet been purified and identified at the gene level which has prevented expression of recombinant protein and inhibited further investigation in the role of metabolism of potentially toxic thiol-containing drugs.

In this work, we have identified human methyltransferase-like protein 7B (METTL7B) as a novel enzyme capable of catalyzing the S-methylation of captopril. METTL7B was originally identified from partially purified rat liver microsomes using a combination of a specific LC-MS/MS captopril methylation assay, high-mass accuracy proteomics and computational analysis. Alteration of gene and protein expression in vitro confirmed the role of METTL7B in the formation of the captopril S-methyl metabolite in human HepG2 and HeLa cells. Finally, METTL7B was recombinantly expressed, purified from human HEK293F cells and shown to catalyze captopril methylation in vitro. For the first time, S-methylation of captopril has been demonstrated in vitro using METTL7B, a novel enzyme capable of catalyzing a TMT-specific reaction.
Methods and Materials:

Rat Liver Microsome Solubilization:

All procedures were performed at 4 °C unless stated otherwise stated. Pooled rat liver microsomes were centrifuged at 13,000 g for 15 minutes. The supernatant was aspirated and the pellet resuspended via sequential addition of two volumes of solubilization buffer (10 mM potassium phosphate (KP) buffer pH 8.0, 0.3% Zwittergent 3-14, 20% glycerol). Samples were placed on a rotator for 30 minutes after each addition of solubilization buffer. The samples were then centrifuged at 100,000 g for 1 hour and the supernatant was added to a 2 mL DEAE column and washed with 10 column volumes of solubilization buffer. A five-step discontinuous ionic strength gradient consisting of 50 mM, 100 mM, 200 mM, 350 mM and 500 mM KP solubilization buffer was used to elute protein from the column. One column volume fractions were collected during the wash and elution steps and assayed for activity using the method described below.

TMT activity assay:

TMT activity was determined using a specific LC-MS/MS assay that measures the formation of the S-methyl metabolite of captopril. The activity assay was conducted with a total sample volume of 250 µL. The reaction mixture containing column fractions were mixed with captopril for a final concentration of 500 µM. The mixture was pre-incubated at 37 °C for three minutes before initiation via addition of S-adenosylmethionine for a final concentration of 25 µM. The mixture was incubated at 37 °C with light shaking (30 rpm) for one hour before being quenched via addition of ice cold 15% (w/v) zinc sulfate in a 1:5 dilution and placed on ice. An internal standard of 15 ng d3-6-methyl-mercaptopurine was added to each sample which were then centrifuged at 2,500 g for 5 minutes and the supernatants were analyzed via LC-MS/MS.

Mass spectral data were acquired using an AB Sciex 4000 Qtrap mass spectrometer and an Acquity UPLC BEH C18 1.7 µm 2.1 x 100 mm column fitted with an in-line C18 guard column. S-methyl captopril was measured using transitions from the m/z 232.1 parent ion to the m/z 89.0 daughter ion. The d3-6-methyl-mercaptopurine internal standard was measured using transitions from the m/z 170.1 parent ion to the m/z 126.2 daughter ion.
Citations:


