CURRICULUM VITAE

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PRESENT POSITION AND ADDRESS: 11/2012-present PhD student at University of Patras, Greece

BIOGRAPHICAL: 21/12/1983 Patras, Achaia, Greece, Greek citizenship, Dios 16 street, Nafpaktos, 30300, Greece

EDUCATION: 10/2001-12/2006 Basic Degree in Chemistry Department, University of Patras, Greece
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RESEARCH ACTIVITIES:

A. Molecular Pharmacology
B. H_2S biology

ADDITIONAL INFORMATION: Expression and purification of proteins involved in H_2S production. Measurment of H_2S production (methylene blue assay). High throughput screening of inhibitors on human purified enzymes.and in cell cultures.

PUBLICATIONS:

1. Coletta C. et al., Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation. Proc Natl Acad Sci U S A. 109(23):9161-6; 2012
2. Módis K, Asimakopoulou A, Coletta C, Papapetropoulos A, Szabo C. Oxidative stress suppresses the cellular bioenergetic effect of the 3-mercaptopyruvate sulfurtransferase/hydrogen sulfide pathway. Biochem Biophys Res Commun. 2013 Apr 19;433(4):401-7.
3. Asimakopoulou Antonia; Panopoulos Panagiotis; Chasapis Christos; Coletta Ciro; Zhou Zongmin; Cirino Giuseppe; Giannis Athanassios; Szabo Csaba; Spyroulias Georgios; Papapetropoulos Andreas, Selectivity of commonly used pharmacological inhibitors for cystathionine beta synthase (CBS) and cystathionine gamma lyase (CSE), Br J Pharmacol. 2013 Jun;169(4):922-32.

PUBLISHED ABSTRACTS:

A. SELECTIVITY OF COMMONLY USED PHARMACOLOGICAL INHIBITORS FOR CYSTATHIONINE BETA SYNTHASE (CBS) AND CYSTATHIONINE GAMMA LYASE (CSE)

Asimakopoulou A., Panopoulos P., Chasapis C., Giannis A., Spyroulias G. A., Papapetropoulos A.

Hydrogen sulphide (H_2S) is a colorless, flammable gas with the characteristic smell of rotten eggs. H_2S is now recognized as an important signalling molecule in the cardiovascular and nervous systems and is the third member of the gasotransmitter family. H_2S is synthesized via two pyridoxal-5′-phosphate-dependent enzymes responsible for the metabolism of L-cysteine: cystathionine beta synthase (CBS) and cystathionine gamma lyase (CSE), as well as by a recently identified third pathway that catalyzes the production of H_2S from L-cysteine via the combined action of 3-mercaptopyruvate sulphurtransferase and cysteine aminotransferase (3MST/CAT). In the present study we examined the selectivity of commonly used pharmacological inhibitors of H_2S biosynthesis towards CSE and CBS. To address this question human CSE or CBS enzymes were expressed and purified from E. coli as fusion proteins with Glutathione-S-Transferase (GST). After purification the activity of the recombinant enzymes was tested using the methylene blue method. We found that β-cyanoalanine (BCA) was a more potent CSE inhibitor than DL-propargylglycine (PAG) for CSE (IC_{50} 14μM and 750μM, respectively), and that aminooxyacetic acid (AOAA) was even more potent that BCA and PAG towards CSE (IC_{50}1.1μM), although it is claimed to be a CBS-selective inhibitor; AOAA inhibited CBS with an IC_{50} 8.5μM.

Trifluoroalanine and hydroxylamine inhibited both enzymes with the former being a more potent inhibitor of CBS, while the later of CSE. In conclusion, although PAG and BCA exhibit selectivity in inhibiting CSE vs CBS, no selective CBS inhibitor is currently available.
THE FIRST EUROPEAN CONFERENCE ON THE BIOLOGY OF H_2S, Smolenice Castle, Slovakia June 15 - 18, 2012.

B. EFFECT OF S-ADENOSYL-L-METHIONINE (SAM), AN ALLOSTERIC ACTIVATOR OF CYSTATHIONINE BETA SYNTHASE (CBS) ON COLORECTAL CANCER CELL PROLIFERATION AND BIOENERGETICS *IN VITRO*.

Antonia Asimakopoulou, Katalin Módis, Ciro Coletta, Celia Chao, Andreas Papapetropoulos, Mark Hellmich and Csaba Szabo.

Introduction: Several series of *in vitro* and *in vivo* data presented at the current Meeting support the conclusion that colon cancer cells selectively overexpress CBS, which produces H_2S, to maintain cellular bioenergetics, thereby supporting tumor growth and to promote angiogenesis and vasorelaxation. These studies, taken together, identify CBS-derived H_2S as a tumor promoting factor and a potential future anticancer drug target. The purpose of the current study was to investigate the effect of S-adenosyl-L-methionine (SAM) on the proliferation and bioenergetics of the colon cancer cell line HCT116 *in vitro*. The non-transformed, the non-tumorigenic colon epithelial cell line NCM356, derived from the normal margin of a rectal cancer specimen, was used as a control.
Methods: HCT116 cells were cultured in McCoy's 5A medium, while NCM356 cells were cultured in DMEM supplemented with 10% fetal bovine serum. For assessment of cell proliferation, the xCELLigence system (Roche) was used. For the measurement of bioenergetic function, the XF24 Extracellular Flux Analyzer (Seahorse) was used. Oxygen consumption rate (OCR) after oligomycin (1.5 μg/ml) was used to assess ATP production rate and OCR after FCCP (0.5 μM) to assess maximal mitochondrial respiratory capacity. 2-deoxyglucose (100 mM) was used to estimate cellular glycolytic dependency and antimycin A (2 μg/m) and rotenone (2 μM) were used to inhibit the flux of electrons through complex III and I, to detect residual non-mitochondrial activity.
Results: Addition of SAM (0.1 - 1 mM) to HCT116 cells (which show a high expression of CBS) induced a concentration-dependent increase in cell proliferation between 0-50 hours, while higher concentrations of SAM (3 mM) inhibited cell proliferation. However, at time periods between 50-100 hours, the cells treated with intermediary concentrations of SAM (0.3-1 mM) showed a suppression of cell proliferation compared to the vehicle control; the proliferation-enhancing effect of SAM was only maintained throughout the entire experimental period with the 0.1 mM concentration of SAM. In contrast to HCT116 cells, NCM356 cells (which have only a low-level expression of CBS) were proliferating at a substantially slower rate, and SAM failed to stimulate their proliferation. Extracellular Flux Analysis of the effect of acute exposure of HCT116 cells to SAM (0.1-1 mM) induced a concentration-dependent increase in their oxygen consumption and bioenergetic function, while in NCM356 cells SAM failed to enhance oxygen consumption and cellular bioenergetics. Longer-term exposure of HCT116 cells to both low concentrations of SAM (0.1 mM) and high concentrations of SAM (1 mM) suppressed cellular bioenergetic responses. Measurements of LDH release showed no cytotoxicity (LDH release) with SAM (0.1 mM-1 mM) either at short-term nor at long-term incubations/
Conclusions: Studies presented at the current meeting demonstrate that the H_2S-producing enzyme CBS is selectively overexpressed in human colorectal cancer cells, and significantly contributes to their proliferation, migration and invasion *in vitro*. The present data, demonstrating that short-term exposure of SAM to HCT116 cells exerts proliferative and positive bioenergetic effects that are in line with the effects of exogenously administered H_2S in the same experimental system further support the view that CBS in colon cancer cells produces H_2S in a regulated fashion, and that this H_2S serves as an endogenous cancer cell proliferation and bioenergetic factor. Higher local concentrations of H_2S are known to be antiproliferative, at least in part due to their inhibitory on mitochondrial function. Therefore, the longer-term inhibition of cell proliferation and bioenergetic function noted with SAM in the present experiments may either be attributed to the adverse autocrine effects of high concentrations of H_2S, produced when CBS is 'over-activated' by SAM, or, alternatively, may be related to additional, CBS-independent pharmacological effects of SAM.
THE SECOND EUROPEAN CONFERENCE ON THE BIOLOGY OF H_2S, SEPTEMBER 8-11th 2013, EXETER, UK.