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TIPOLOGIA DI BORSA RICEVUTA: Borsa SIF per brevi soggiorni all' estero

TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale): Relazione di fine periodo

TITOLO DELLA RELAZIONE: Application of biochemical/molecular biology techniques for the evaluation of the biological effects of gasotransmitters

RELAZIONE:

Introduction

In the past two decades, an increasing number of reports have indicated the significant role of gasotransmitters in biology and medicine. The term gasotransmitter was first conied by Wang in 2002 and indicates a group of small endogenously synthesized gaseous molecules, including nitric oxide (NO) and hydrogen sulfide (H_2S). This molecules have high lipid solubility and can easily cross cell membranes without requiring a specific transporter or receptor. Physiologically, gasotransmitters exert various beneficial effects by acting on specific cellular and molecular targets [1].

NO is the first acknowledged and best characterized gasotransmitter. In the cardiovascular system, NO is consitutively produced within the endothelium from the enzymatic conversion of L-arginine into L-citrulline by the type 3 isoform of the nitric oxide synthase (NOS3). Since dysregulation in NO production is implicated in cardiovascular diseases (such as hypertension and myocardial infarction), NO-related drugs have been employed to therapeutic effect in a range of cardiovascular diseases where loss of NO signaling is believed to play a contributory role [4]. Together with NO, H_2S is a gasotransmitter which exerts its biological role mantaining the cardiovascular homeostasis and inducing vasodilation and cardioprotective effects. Moreover, H_2S plays significant antioxidant, antinflammatory and citoprotective properties. Therefore, the mantainance of physiological concentrations of H_2S is essential in the prevention of cardiovascular diseases, such as atherosclerosis, hypertrophy, hypertension and myocardial infarction [5]. The growing evidence about a protective role played by H_2S, led to investigate several molecules able to release H_2S (H_2S-donor) as possibile innovative cardioprotective agents [6]. Interestingly, many experimental evidences suggest that NO and H_2S signaling pathways are intimately intertwined, with mutual attenuation or potentiation of biological responses in the cardiovascular system [7]. So, a deeper investigation of the signaling pathways regulated by this gasotrasmitters may allow a more rational use of natural and/or synthetic molecules capable of modulating the same intracellular pathways.

Aim of the project

In this context, the learning of different biochemical/molecular biology techniques can be useful for the investigation of the mechanism of action of a lot of compounds, including those that are subject of my PhD project (i.e. isothiocyanates present in many edible plants of the *Brassicaceae* botanical family, which are naturally occurring H_2S-donors [8]).

Methods and results

During the first period at the Heinrich-Heine Üniversitat (Düsseldorf), I evaluated the expression of eNOS in transgenic mice with Real Time Quantitative Polymerase Chain Reaction (RT-qPCR) to define a possible reliable model for studying the role of the gasotransmitter NO in several tissues and organs. In particular, I selected three different C57Bl/6 mice strains:

1) Endothelial specific eNOS knock out
2) Red blood cells specific eNOS knock out
3) Global eNOS knock out

Total RNA was extracted from aortae, heart, skeletal muscle and bone marrow of these mice using the RNeasy Mini Kit (Qiagen); then, the RNA was reverse transcribed with the QuantiTect Reverse Transcription Kit (Qiagen) and RT-qPCR was performed on QuantStudio7 Flex (Applied Biosystems) in order to quantify the expression of NOS3 gene, comparing the results with those obtained on wild type mice. Moreover, NOS3 expression was evaluated with the semi-quantitative method of Western Blot in the same tissues to strengthen the results on gene expression analysis. Data showed that tissue specific eNOS knock out mice were successfully created, suggesting a possible use of these mice strains for further investigations.

One of the main objectives of my PhD project is the evaluation of the cardiovascular beneficial effects of compounds able to slowly release the gasotransmitter H_2S. In the laboratories directed by Prof. Vincenzo Calderone, I investigated the effects of an Eruca sativa Mill. (*Brassicaceae*) seed extract titled in glucosinolates, which are precursors of sulfur H_2S-donors molecules. Briefly, young male mice were fed for 10 weeks with a control diet or with an high-fat diet (45% fat of calories from fats) eventually enriched with the Eruca sativa Mill. extract. As riported in the literature, high-fat diet induces metabolic syndrome in mice and increases the risk of developing associated cardiovascular diseases. At the end of the experiment, mice were sacrified and organs were collected and stored at -80°C for further investigations.
During the period spent at the Heinrich-Heine Üniversitat (Düsseldorf), I evaluated the potential antioxidant effects of the extract in the liver, investigating also its mechanism of action. Considering that several cardiovascular diseases are associated with perturbations in redox signaling and impaired H_2S metabolism, I measured the levels of sulfur-containing species in the liver (i.e. cysteine, cystine, homocystein, glutathion and oxidized glutathione) with an high-sensitive analytic method. As described by Sutton et al. (2018), thiols were isolated from liver homogenates and they were separed using a Waters Aquity ultrahigh performance liquid chromatography (UPLC) system with a thermostated autosampler (kept at 5°C) and an ultrahigh performance binary pump, coupled to a triple-quadrupole mass spectrometer (Xevo TQ-S, Waters) equipped with a heated electrospray ionization source (ESI). Chromatographic separation of the target analytes was achieved using an UPLC column (Chromatography Direct, Runcorn, UK) kept at a temperature of 30°C. Signals were captured and quantification of the compounds of interest was accomplished by comparison of peak areas to representative freshly prepared and diluted external standards. Results showed that high-fat diet modified the redox signaling in the liver, inducing a reduction of endogenous antioxidant molecules (such as glutathione). Unfortunately, the chronic treatment with Eruca sativa Mill. extract didn't significantly improved the redox state of the liver.

Finally, I investigated the effects of Eruca sativa Mill. extract on antioxidant gene expression in the liver by RT-qPCR. In particular, I assessed the ability of the extract to modulate the expression of glutamate-cysteine ligase catalytic subunit (Gclc), glutahione peroxidase-1 (Gpx1), quinone oxidoreductase-1 (NQO1), heme oxigenase-1 (HO-1), superoxide dismutase-1 (SOD1) and xanthine oxidoreductase (XOR). According to the literature, data showed that high-fat diet reduced mRNA levels of Gclc, HO-1 and XOR; conversely, high-fat diet increased the expression of Gpx1 in the liver. Interestingly, Eruca sativa Mill. extract markedly maintained standard mRNA levels of Gclc, XOR and Gpx1 without affecting HO-1 expression.

Taken together, this results suggest that a deeper investigation of the mechanism of action of this extract is necessary to confirm that Eruca sativa Mill. is a possible candidate for the prevention and the treatment of several cardiovascular diseases associated with metabolic syndrome.

References

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Data, 30/04/2019

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