VASCULAR EFFECTS OF ERUCIN, A H_2S-DONOR FROM ERUCA SATIVA MILL., AGAINST OXIDATIVE AND PRO-INFLAMMATORY STIMULI

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Introduction: Vascular inflammation is a pathological condition that underlies several cardiovascular (CV) and non-CV diseases such as hypertension, diabetes, atherosclerosis, neurodegeneration and so on. The research of novel compounds, and in particular nutraceutical products based on botanicals, able to preserve the vascular wall from oxidative and pro-inflammatory stimuli, represents an important challenge for pharmacology. Among the several classes of natural compounds, we selected erucin, an isothiocyanate deriving from the hydrolysis of the glucosinolate glucoerucin present in Eruca sativa Mill. (rocket salad or arugula), as potential nutraceutical compound able to exert vascular protection. In previous works we demonstrated that erucin was a "smart" H_2S-donor, able to release hydrogen sulfide (H_2S) in a slow and gradual way. Accordingly, we demonstrated also that it was a vasorelaxant and anti-hypertensive agent and hence useful to be administered in case of endogenous H_2S deficiency. Indeed, H_2S plays a fundamental role in the maintenance of homeostasis in several districts and in particular at CV level where it exhibits anti-oxidant, anti-inflammatory and vasorelaxant properties. In this study, in particular, we investigated the potential protective effects of erucin at vascular level on both the components of the vascular wall: the endothelial and the smooth muscle ones.

Methods and materials: The experiments were performed on both Human Umbilical Vein Endothelial Cells (HUVECs) and Human Aortic Smooth Muscle Cells (HASMCs), and evaluated the ability of erucin to release H_2S-inside cells and to exert anti-oxidant and anti-inflammatory activities. For the evaluation of intracellular H_2S-release a fluorescent dye (WSP-1), able to detect selectively H_2S, was incubated in HUVECs and HASMCs for 30 minutes to allow cells capitation; then the extracellular remaining dye was removed and erucin at several concentration as well as the reference drug diallyldisulfide were added to record the intracellular release of H_2S for about 40 minutes. Instead, to investigate the protective effects, both HUVECs and HASMCs were incubated with erucin at several concentrations for 1hour and then HUVECs and HASMCs were treated with oxidative stimuli (i.e. $H_2O_2$100 or 200μM respectively) for 2hours or pro-inflammatory stimuli (serum-free medium medicated with high-glucose concentration, 25mM) for 24or 72h respectively to test the ability of erucin to protect cells from death, increase of reactive oxygen species (ROS) and apoptotic events (caspase 3/7levels).

Results: The results showed that erucin released H_2S inside HUVECs and HASMCs in a concentration dependent manner. Against the oxidative damage Erucin protected both cell lines from in a concentration dependent manner and, in particular, prevented ROS production after H_2O_2exposure in HASMCs. As concerns, the cellular model of vascular inflammation, erucin again preserved cell viability in a concentration dependent manner and prevented caspase 3/7release as a marker of apoptosis at the highest tested concentration (3μM).

Discussion and conclusions: In conclusion, this preliminary study paves the way for the use of erucin as nutraceutic agent deriving from Brassicaceae edible plants, able to act as a protector of the vascular tree integrity both on the endothelian and on the smooth muscle components. Further experiments will be addressed to evaluate erucin influence on inflammation markers (e.g. TNF-α, IL-6, IL-1β) and to set up other models of vascular inflammation (i.e. lipopolysaccharide, LPS).