THE NOVEL BUTYRATE DERIVATIVE PHENYLALANINE-BUTYRAMIDE PROTECTS FROM DOXORUBICIN-INDUCED CARDIOTOXICITY

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Introduction: Butyric acid (BUT), a short chain fatty acid produced daily by the gut microbiota, has proven beneficial in models of cardiovascular diseases. With advancements in cancer survival, an increasing number of patients are at risk of anticancer drug cardiotoxicity. Here we assess whether the novel BUT derivative phenylalanine-butyramide (FBA), with odorless and tasteless properties, is able to protect from doxorubicin (DOXO) cardiotoxicity, by decreasing oxidative stress and improving mitochondrial function.

Materials and methods: C57BL/6mice were injected with a cumulative dose of 7mg/kg DOXO via seven daily intraperitoneal injections (1mg/kg/die i.p.). Another group of mice was treated with oral gavage with FBA (30mg/kg/day) daily for 21days (FBA group). A third group of mice, after14days of FBA, received co-administration of FBA (1h before i.p. administration of DOXO) and DOXO, at the same doses used in the DOXO group (FBA+DOXO group). In vivo, cardiac function was assessed by transthoracic echocardiography. Then, immunohistological and interstitial fibrosis analysis, TUNEL assay, real time PCR, Western blot and mitochondrial analysis were performed. The protective effect of FBA was eventually tested in various normal and transformed human cell types relevant to cardioncology (hiPSC-derived cardiomyocyte, HUVEC and MCF-7cell lines) and lactate dehydrogenase and MTT assays were carried out.

Results: In C57BL6mice, DOXO produced left ventricular dilatation. FBA prevented left ventricular dilatation, fibrosis and cardiomyocyte apoptosis when co-administered with DOXO. DOXO increased atrial natriuretic peptide, brain natriuretic peptide, connective tissue growth factor, and matrix metalloproteinase-2mRNAs, which were not elevated on co-treatment with FBA. DOXO, but not FBA + DOXO mice, also showed higher nitrotyrosine levels, and increased inducible nitric oxide synthase expression. Accordingly, DOXO hearts showed lower levels of intracellular catalase vs. sham, while pre-treatment with FBA prevented this decrease. We then assessed for reactive oxygen species (ROS) emission: DOXO induced increased activity of mitochondrial superoxide dismutase and higher production of H_2O_2, which were blunted by FBA pre-treatment. FBA also ameliorated mitochondrial state 3and state 4respiration rates that were compromised by DOXO. Furthermore, in DOXO animals, the mitochondrial degree of coupling was significantly increased vs. sham, while FBA was able to prevent such increase, contributing to limit ROS production, Finally, FBA reduced DOXO damage in human cellular models, and increased the tumour-killing action of DOXO.

Discussion and conclusions: To date, there are no specific treatments for Doxo induced cardiotoxicity. The perspective of using a diet-derived molecule, with great compliance and an optimal cost:yeld ratio is very attractive. Thanks to its odorless and tasteless properties, FBA could be safely administered to oncological patients. Our studies show for the first time that FBA, a novel synthetic derivative of BUT, is able to blunt doxorubicin experimental cardiotoxicity *in vivo*, via a mechanism that involves lowering of Oxidative and nitrosative stress and improvement of mitochondrial function. Further investigations are necessary to better establish the potentiality of FBA in cardio-oncology.