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A1

EFFECT OF PALMITATE AND OLEATE ON MITOCHONDRIAL DYNAMICS PROCESSES IN HEPATIC CELLS HEPG2

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Introduction: Mitochondria are organelles constantly submitted to fusion and fission processes (mitochondrial dynamics). Fission process is associated with mitochondrial disfunction, oxidative stress and apoptosis, and therefore with insulin resistance onset. On the other hand, fusion process is associated with the prevention of this metabolic alteration, being able to protect cells against insulin resistance.
Objectives and Methods: This work aims to evaluate the dose-dependence effect of saturated (palmitate) and monounsaturated fatty acids (oleate), carried out in a cell culture model (hepatic cells HEPG2), treated for 24 hours. Five different doses (10, 50, 100, 250 – 500 μM) were chosen based on the literature. The content of proteins involved in the mitochondrial dynamics processes were analysed. The content of Dynamin-related protein 1 (DRP1) a mitochondrial fission marker, Mitofusin 2 (Mfn2), an outer mitochondrial membrane fusion marker and Optic Atrophy 1 (Opa 1), an inner mitochondrial membrane fusion marker which also controls the cristae integrity, were quantified by Western Blot.
Results: Palmitate induced an increase in DRP1 content (doses of 10 – 500 μM) whereas any difference was observed in Mfn2 content; the content of Opa1 increased from the dose of 50 μM, indicating a possible disorganization of mitochondrial cristae. All the oleate doses promoted an increase in Mfn2 content whereas only the three highest doses were able to induce an increase in Opa 1 and DRP1 contents.
Conclusion: Palmitate induced mitochondrial fission process, whereas oleate promoted fusion and, therefore, may protect the cells against insulin resistance onset. However, additional experiments are required to confirm this hypothesis.

A2

ACTIVATION OF CYTOSTATIC AUTOPHAGY BY POLAR EXTRACT OF HEMPSEED OIL (CANNABIS SATIVA L.) IN A COLORECTAL ADENOCARCINOMA CELL LINE

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Introduction: The hempseed oil, suitable for human feeding, can be obtained by cold pressing of seed belonging to a non-pharmaceutical hemp variety (Cannabis sativa L.). Beyond its nutritional value due to the ideal ratio (3:1) of essential polyunsaturated fatty acids, the presence of biomolecules, such as phytocannabinoids and polyphenols, has been also associated with potential beneficial effects.
Objectives: We evaluated the anti-proliferative effect of an oil polar extract (OPE) obtained from cold pressed hempseed of Codimono cultivar (Cannabis sativa L.) on human colon adenocarcinoma (HT-29) cell line.
Results: OPE was not cytotoxic, but induced a significant 50%, dose-dependent (70–150 ug/ml, w/v) delay in cell growth. OPE-dependent (130 ug/ml, w/v) autophagy after 24 h treatment was detected by measuring autophagosome formation and increased expression of LC3-II. These effects were associated with a 50% reduction in intracellular ATP concentration and activation of AMPK (increased expression of its phosphorylated form on Thr172). OPE also affected cell cycle progression blocking cells in G0/G1 (20% increase) after 72 h of treatment. Furthermore, the chemical analysis of OPE components by means of UHPLC-HR MS and MS/MS techniques, evidenced the presence of phytocannabinoids, as the cannabidiolic acid and non-cannabinoids polyphenols, including cannflavins.
Conclusions: The results obtained will be discussed at the light of the multiple mechanisms triggered by the bioactive components of OPE and resulting in a delay of cell proliferation in cancer cells associated with cytostatic autophagy and cell cycle arrest.

A3

A PHENOLIC EXTRACT FROM EXTRA VIRGIN OLIVE OIL INDUCES AUTHOPHAGY AND APOPOTOSIS IN HUMAN BLADDER CANCER CELL LINES DEPENDING ON TUMOR PROGRESSION

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Introduction: The regular consumption of olive oil has associated with a protection against several diseases, including cancer. Epidemiological evidence indicate an inverse association between olive oil intake and bladder cancer risk. The phenolic fraction of olive oil plays a key role in this beneficial effect. Bladder cancer is one of the most common cancer in Europe, United States and Nord Africa. In particular, the transitional cell carcinoma histotype shows an aggressive behavior and the current therapies are ineffective.
Objectives: We investigated the anti-proliferative effects of an extra virgin olive oil phenolic extract (EVOOE) on two human bladder cancer cell lines: RT112 and J82, selected for their features to represent the progression from well to poorly differentiated phenotypes, respectively. These cell lines have been used to investigate the different response of superficial vs invasive bladder cancer to treatment.
Results: The EVOOE activated different pathways in the two cell lines employed. In RT112, the EVOOE triggered a non-protective autophagic response (high dose, 132 microg/ml, delayed cell growth of about 30%), evidenced with the vacuoles formation and the increase of LC-3 lipidation (about 45%). In J82, the invasive transitional cell carcinoma, the induction of apoptosis was rapid and remarkable with 40% decrease of cell viability after 24 h of treatment at 33 microg/ml EVOOE concentration, as shown by the significant increase of Annexin V positivity and caspases-3 and -9 activities.
Conclusions: Data obtained suggest that the mixture of phenolic compounds in EVOO activates different anti-proliferative pathways. Interesting hypotheses can be formulated to explain, from a molecular point of view, the switch from autophagy to apoptosis depending on bladder cancer stage.

A4

RED WINE POLYPHENOLS INDUCE ADAPTIVE RESPONSE IN A MONOCYTE/MACROPHAGE MURINE CELL LINE (J774)

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Introduction: Recent studies confirmed that a moderate amount of wine may have health benefits. Experimental evidence suggested that these beneficial effects could be due to the polyphenol content of red wine.
Objectives: In this study, we evaluated the effect of red wine polyphenols (RWP) in J774, a cellular model widely used for studies on oxidative stress and redox capacity of plant extracts and molecules.
Results: In the presence of RWP, J774 cells showed an opposite effect on cell viability. Increased cell growth of about 20% was measured at low concentration (4 μg/ml GAE [gallic acid equivalents]), while 50% cytotoxicity was observed at higher concentrations (40 μg/ml GAE). We also detected a rapid (15 min) and dose-dependent increase of superoxide anions (15%—and 180% at 4 and 40 μg/ml GAE, respectively compared to untreated controls). However, while at the lower concentration applied GSH and H_2O_2 production did not change significantly, at the higher RWP value, GSH and H2O2 contents decreased significantly of 38% and 15%, respectively. To counteract the intracellular ROS production, cells triggered a rapid dose-dependent activation of all the antioxidant enzymes investigated at 4 μg/ml GAE (41% SOD; 18% GSH peroxidase; 24% GSH reductase; 23% G6PD) and 40 μg/ml GAE (150% SOD; 97% GSH peroxidase; 100% GSH reductase and G6PD).
Conclusions: Our preliminary results indicate a pro-oxidant activity of RWP, which may promote cell proliferation at low doses and cell death at higher ones.

A5
VITAMIN D ANALOGUE EB1089 SENSITIZES HUMAN TRANSFORMED CELL LINES TO GAMMA RADIATIONS BY INDUCING LETHAL AUTOPHAGY AND APOPTOSIS

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Introduction: The gold standard for unresectable solid tumours and advanced forms of leukemia is represented by chemo-radiation. However, therapeutic options are limited and patients undergo systemic cytotoxicity. Vitamin D insufficiency is a widespread problem and its low serum levels are linked to higher cancer incidence. Previous studies showed a strong correlation between serum concentration of vitamin D and time of first treatment in chronic lymphocytic leukemia (CLL). Other studies demonstrated that vitamin D and its ipocalcemic analogue EB1089 are able to bypass radio-resistance in breast and lung cancer cell lines by activating cytostatic/cytotoxic forms of autophagy. However, only few data are available on the intracellular and molecular effects of vitamin D in osteosarcoma (OS) and CLL-derived cells and on its association with gamma-radiation in sensitizing these cancer types.
Objectives: We studied the antiproliferative effects of an active form of vitamin D, the analogue EB1089, in two cells lines U2Os and HG3 derived from a human OS and a CLL, respectively, and its efficacy after treatment with gamma-radiations in terms of cytotoxicity, autophagic and apoptotic effects.
Results: EB1089, used at physiological concentration (100 ng/ml), is able to bypass gamma-radiations resistance in U2Os and HG3 cell lines by activating cytotoxic autophagy and apoptosis. The co-treatment resulted highly synergic in terms of combination index (C.I. <1) inducing 85% of cell death at higher doses of radiation after 24h of co-treatment.
Conclusions: The results obtained will be discussed at the light of the cytostatic/cytotoxic function of autophagy mediated by vitamin D and involving MAPK/ERK and AMPK pathways in enhancing the therapeutic response to gamma-radiations.

A6
AUTOPHAGY FLUX MODULATION BY A CAROTENOID-ENRICHED EXTRACT FROM THE PUMPKIN CUCURBITA MOSCHATA ON HUMAN CHRONIC LYMPHOCYTIC LEUKEMIA CELL LINE

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Introduction: Chronic lymphocytic leukemia (CLL) is the most frequent form of leukemia in adult population and chemotherapy resistance occurs in 15—30% of patients with elevated genomic complexity. Apoptosis resistance and induction of a protective form of autophagy are possible explanations of the poor responsivity of CLL to conventional and novel therapeutic drugs. Given the difficulties to maintain in culture B-CLL lymphocytes, the HG3 cell line represents an interesting preclinical model to study the effects of natural bioactive molecules or extracts derived from food matrix as potential, chemo-sensitizers in CLL.
Objective: A previous study demonstrated the induction of "not-protective" autophagy on osteosarcoma and colon adenocarcinoma cell lines after prolonged treatment with a carotenoid-enriched extract (CE) obtained from the pumpkin Cucurbita moschata, variety "long of Naples". To extend and confirm these data, the present communication focuses on the anti-proliferative effect of the same extract in HG3 cell line derived from EBV immortalization of B-CLL cells.
Results: CE was obtained from pumpkin by supercritical CO2 extraction and delivered to HG3 cells in combination with foetal bovine serum. After 96 h, we detected a 40% delay in cell proliferation compared to untreated cells, without signs of cytotoxicity. This delay was due to p27/KIP1 over-expression and modulation of autophagic flux, measured by different autophagy markers (LC3II; p62) and 30% autophagosome intracellular increase.
Conclusions: The results obtained will be discussed at the light of the functional cross-talk between the modulation of the autophagy flux by the CE extract and the retard in cell growth observed in HG3 cells, as an opportunity to prolong the asymptomatic phase of CLL before disease occurrence.

A7
ANTI-INFLAMMATORY EFFECTS OF BLUEBERRY EXTRACT IN MICROGLIAL CELLS

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Background: Microglia (MG), the immunocompetent cells of the CNS, respond to brain injury activating and modifying their morphology. Microglia can exist broadly between two different activation states, namely the classical (M1) and the alternative activated (M2) phenotype. The first one is characterized by the production of pro-inflammatory cytokines, in contrast, the latter is characterized by the production of anti-inflammatory cytokines (Kettenmann et al., Neuron. 2013; 77:10—18). Blueberry is involved in the control of the redox state of the cell, cooperating with antioxidant mechanisms, whereas its anti-inflammatory activity is still poorly understood (Businaro et al., Curr. Alzheimer Res. 2018; 15: 363— 380). The aim of the present study is to determine the effect of blueberry extract in resting form or lipopolysaccharide (LPS)-stimulated BV-2 murine MG cells.
Methods: The hydroalcoholic extract obtained from fresh blueberries was analyzed by UHPLC/MS. The cellular viability was evaluated by MTT test and Trypan blue assay. Cellular migration was determined by Boyden chamber and Scratch assay. Cytokines mRNA levels were determined by qPCR. Actin cytoskeletal organization and M1/M2 marker expression were analyzed by immunofluorescence.
Results: Isomers of the chlorogenic acid, a powerful antioxidant, were detected in the blueberry extract, which, added to the cultures, had no cytotoxic effect, but induced increased cell viability and reduced LPS-driven migration. mRNA expression of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α and that of iNOS (M1 marker) was decreased, whereas Arg-1 expression (M2 marker) was increased.
Conclusion: Our results suggest that blueberry may promote MG polarization towards the M2 phenotype, and therefore may be used as a nutraceutical in the treatment of neuroinflammatory diseases.

A8
INTESTINAL EPITHELIUM RESPONSES TO TITANIUM DIOXIDE NANOPARTICLES

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