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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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