Involvement of the PI3K/Akt pathway in the antiproliferative effects of *Salvia* extracts in HCT15 colon cells

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Introduction

The phosphatidylinositol-3-kinase (PI3K)/Akt signalling pathway plays a critical role in cell proliferation and survival. This pathway has been found to be altered in a variety of tumor cells, including colon cancer cells (1). Constitutive activation of PI3K/Akt pathway in colon cancer occurs frequently due to mutations in the PI3KCA and Akt genes, or as a result of inactivation of PTEN. Some natural compounds are known to induce apoptosis and have anti-proliferative effects in cancer cells via this pathway (2-4). Compounds that specifically inhibit PI3K and Akt or activate PTEN could provide good candidates for target cancer therapy and help define adequate dietary strategies for people at risk.

In this study we aim to identify natural compounds and plant extracts with potential anti-carcinogenic effects through the PI3K/Akt pathway in colon cancer. To this purpose, we investigated the effects of *Salvia fruticosa* (SF) and *Salvia officinalis* (SO) aqueous extracts, in HCT15 colon cell line, as well as individual phenolic compounds such as luteolin (Lut), quercetin (Que) and ursolic acid (UA). The inhibitors of PI3K, wortmannin (Wort) and quercetin were used to demonstrate the involvement of PI3K/Akt pathway on the effects produced by the test compounds and *Salvia* extracts. Western blotting analysis was performed to determine phospho-Akt levels in HCT15 and CO115 colon cell lines.

**Results and Discussion**

- **MTT**

  - Effect of compounds and *Salvia* extracts (48h) on HCT15 colon cell viability/growth using the MTT assay. Results are expressed as mean ± SD of four independent experiments. Statistical significances were evaluated by the one-way ANOVA followed by the Student-Newman-Keuls post-test. *** P ≤ 0.001, compared to control cells.

- **BrdU**

  - In HCT15 colon cell line, SF and SO significantly inhibit cell proliferation and induce apoptosis at 50µg/ml. At higher concentrations all compounds (Lut, Que and UA) inhibit cell proliferation and induce apoptosis.

**Material and Methods**

- **Cell Viability/Growth by MTT reduction test**

  HCT15 colon cells seeded onto 12-well plates for 24h were treated with different concentrations of test compounds and *Salvia* extracts during 48h. Cell proliferation was assessed by the MTT assay. In order to determine the percentage of MTT reduction, relative to the control, the following formula and scheme were used.

- **Apoptosis by TUNEL assay and Cell Proliferation by BrdU assay**

  In HCT15 colon cells DNA cleavage was assessed by enzymatic end-labeling of DNA strand breaks using the TUNEL assay (Roche).

  To assess proliferation we used the BrdU assay. Briefly, BrdU (5-bromo-2'-deoxyuridine; Sigma) was added to cell cultures for 3h and the cells fixed and incubated with anti-BrdU antibody (1:20; DakoCytomation) first and subsequently with anti-mouse Ig FITC conjugate antibody (1:100; Sigma). Nuclear counterstaining with Hoechst was used to quantify total cell numbers. Cells were analyzed under a fluorescent microscope.

- **Protein expression Western Blotting**

  In HCT15 and CO115 colon cells, the abundance of Akt and phospho-Akt proteins was measured by western blotting (WB) using the phospho-Akt (Ser473) monoclonal antibody (1:2,000; Cell Signaling). Akt antibody (1:2,000; Cell Signaling), monoclonal anti-β-Actin (1:5,000; Sigma) and the secondary antibody (1:30,000; GE Healthcare). Cross-reactive bands were detected by ECL. The resulting images were analyzed using the Quantity One, ChemiDoc XRS program.

- **Results and Discussion**

  - In HCT15 colon cell lines the expression of phospho-Akt was not detected by WB (Fig.5A)
  - In CO115 colon cell line, Wort (PI3K inhibitor), Lut and Que significantly inhibited the expression of phospho-Akt and SO seemed also to reduce this expression (Fig.5B)
  - Although the total Akt was also decreased by the above compounds/SO, the ratio p-Akt/Akt also decreased, when compared to the control (Fig.5C)

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**References**