DIOSGENINA, PRINCIPIUL ACTIV AL EXTRACTELOR DE TRIGONELLA SP. AR PUTEA INDUCE APOPTOZA CELULELOR CANCEROASE MCF7 PRIN ACTIVAREA CASPAZELOR

DIOSGENIN, THE ACTIVE PRINCIPLE OF TRIGONELLA SP. EXTRACTS MAY INDUCE APOPTOSIS ON MCF7 CANCER CELLS THROUGH CASPASE ACTIVATION

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Abstract

Recent studies suggest that fenugreek and its active constituents may possess anticarcinogenic potential. The methods used were those of culture and measurement of MCF7 cell line growth using MTT methods. Apoptosis was detected using agarose gel electrophoresis. The ratio of apoptotic cell was measured using APO-BRDU kit. The distribution of cell cycle and of mithocondrial membrane potential was investigated by flow-cytometry. Caspase activity was evaluated using caspase-induced apoptosis detection kit. Western blot analysis was used to evaluate the level of mithochondrial Bcl-2 expression. The results observed were those of MCF7 cells growth inhibition due to diosgenin. However, the precise mechanism of diosgenin-induced apoptosis is still unclear. Concluding, diosgenin, obtained from Trigonela foenum-graecum extract may induce apoptosis of MCF7 cancer cells by caspase pathway activation.

Keywords: *diosgenin, MCF7 cells, apoptosis, Trigonella sp.*
Cuvinte cheie: *diosgenina, celule MCF7, apoptoza, Trigonella sp.*

Introduction

Cancer is the second leading cause of death worldwide. Conventional therapies cause serious side effects and, at best, merely extend the patient's lifespan by a few years. Cancer control may therefore benefit from the potential that resides in alternative therapies. There is thus an increasing demand to use alternative concepts or approaches to the prevention of cancer.

Trigonella foenum graecum (fenugreek) is traditionally used to treat disorders such as diabetes, high cholesterol, wounds, inflammation, and gastrointestinal ailments. Recent studies [1,2,3,4] suggest that fenugreek and its active constituents may possess anticarcinogenic potential. We evaluated the preventive efficacy of dietary fenugreek seed and its major steroidal saponin constituent, diosgenin.

The objective of this study was to investigate the mechanism of apoptosis induced by active principles of *Trigonella* sp. extracts on MCF7 cell line. The methods used were those of culture and measurement of MCF7 cell line growth using MTT methods. Apoptosis was detected using agarose gel electrophoresis. The ratio of apoptotic cell was measured using APO-BRDU kit. The distribution of cell cycle and of mithocondrial membrane potential was investigated by flow-cytometry. Caspase activity was evaluated using caspase-induced apoptosis detection kit. Western blot analysis was used to evaluate the level of mithochondrial Bcl-2 expression [5,6]. Treated cells were also investigated using the transmission electron microscope. The results observed were those of MCF7 cells growth inhibition due to diosgenin.

Material and methods

Diosgenin was extracted from Trigonella foenum-graecum plant. Stock solution of 1.0 × 10–2 M diosgenin was prepared with ethanol and diluted to desired concentrations with water. Phosphate buffer solution (PBS) composed of 136.7 mM NaCl, 2.7 mM KCl, 9.7 mM Na2HPO4 and 1.5 mM KH2PO4 was used as the background electrolyte in all experiments.

Breast cancer cell line MCF-7 was routinely cultured in RPMI-1640 medium (Invitrogen) + 10% fetal bovine serum (Sigma) + 0.05 mg mL–1 gentamicin (Sigma) + 100 U mL–1 penicillin (Sigma) at 37°C in a CO2 incubator containing 5% CO2.

After the breast cancer cells were cultured for five days, they were collected by trypsinization with 0.05% (v/v) trypsin (Sigma) and centrifugation at 1200 rpm for 10 min, and then suspended in PBS. It should be mentioned that PBS-containing cells should be added to the detection chamber for the experiments immediately after the cell harvest.

The density of the cells was determined by standard pour plate count (PPC) technique. For drug experiments, cells were allowed to grow and adhere for 24 h in culture medium before exposure to diosgenin and total saponins. The same amount of alcohol was added to the control cells to eliminate the effect of ethanol on the breast cancer cells.

Results and Conclusions

Diosgenin, a plant steroid (5a-spirosten-3ß-ol) was first isolated from Dioscorea tokoro in 1930s. It has been reported that diosgenin induces differentiation of human erythroleukemia cell line (HEL TIB 180) through changing lipoxygenase activities. In addition, diosgenin was used to treat osteoporosis in the ovariectomized adult rat model. Recently, diosgenin has been reported to induce apoptosis and cell cycle arrest in human osterosarcoma 1547 cell line. However, the precise mechanism of diosgenin-induced apoptosis is still unclear.

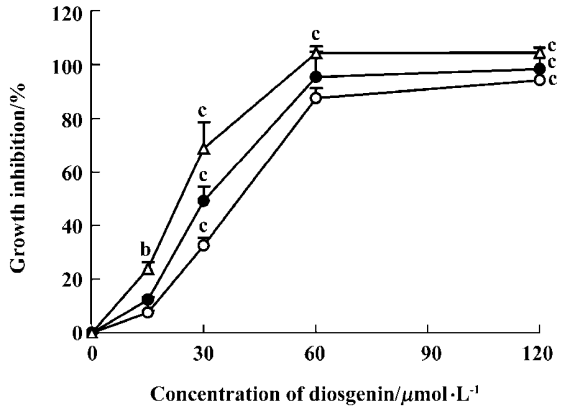

Fig. 1 Inhibitory effect of diosgenin on MCF7 cell growth. The cells (1.5×108 cells/L) were incubated with various concentrations of diosgenin for 12 h, 24 h, 36 h

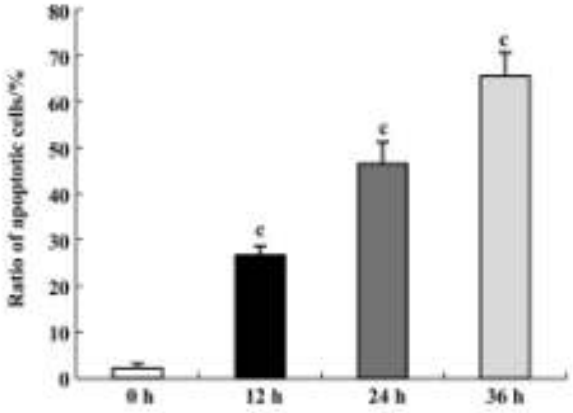

Fig. 2 Detection of apoptotic cells by flow cytometry. MCF7 cells were treated with diosgenin 30 μmol/L for 0, 12, 24, and 36 h

The caspase family of aspartate-specific cysteine proteases have been demonstrated to be important mediators in apoptotic pathway. Caspases, a family of at least 14 cysteine proteases, are synthesized as proenzymes, which are proteolytically cleaved into active heterodimers. Caspases can be grouped according to their substrate specificities, which are mainly defined by the amino acids preceding the cleavage site of aspartic acid residue.

Mitochondria plays a key role in apoptotic process. In apoptosis, there is an alteration of mitochondrial membrane permeability, which causes the loss of mitochondrial membrane potential and translocation of the cytochrome c into cytoplasm. The cytochrome c in turn activates caspase cascade. Bcl-2 is a membrane protein located mainly at the outer membrane of mitochondria and it appears antiapoptotic function. Many reports have demonstrated that one possible role of Bcl-2 in prevention apoptosis is to block cytochrome c release from mitochondria [7,8,9].

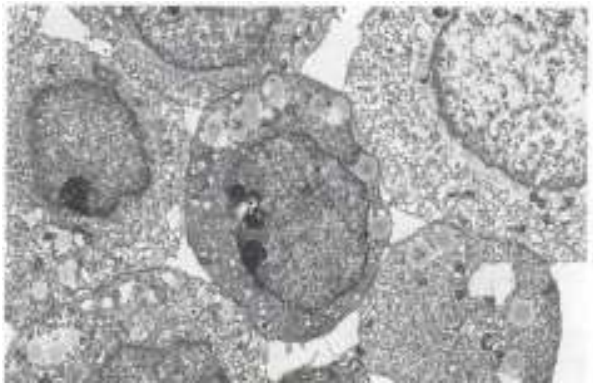

Fig. 3 Signs of membrane deformation; cytoplasmic and nuclear material became much less dense (X4,000; 600 ppm diosgenin)

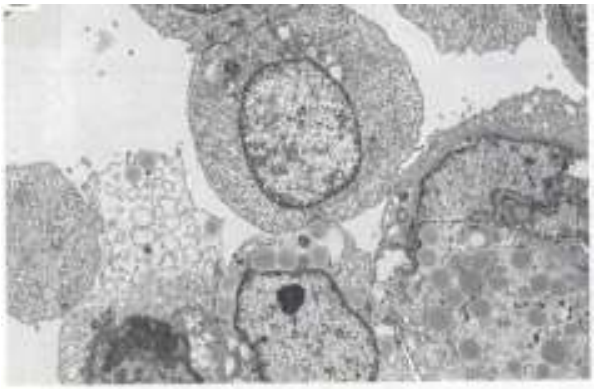

Fig. 4 Formation of vesicles are evident occupying most of the area of the cytoplasm. Membrane rupture has occurred (X4,000; 1,200 ppm diosgenin)

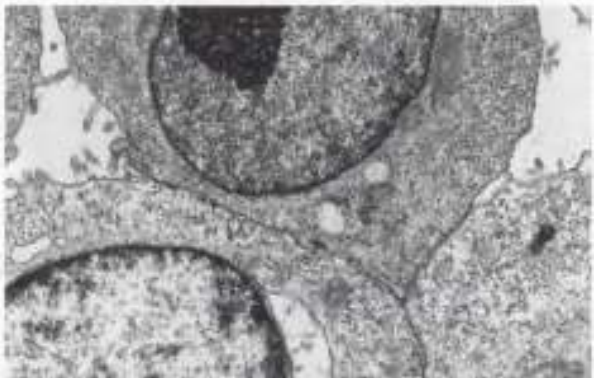

Fig. 5 Cells treated with 40 ppm *Trigonella* diosgenin without noticeable changes (X8,000)

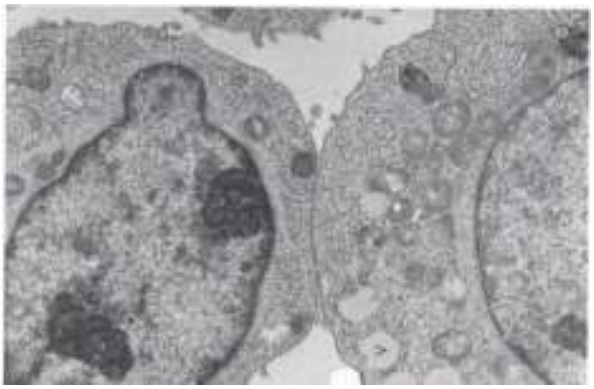

Fig. 6 Cells treated with 80 ppm *Trigonella* diosgenin. Newly formed vesicles appeared without changes in density of cellular organelles (X8,000)

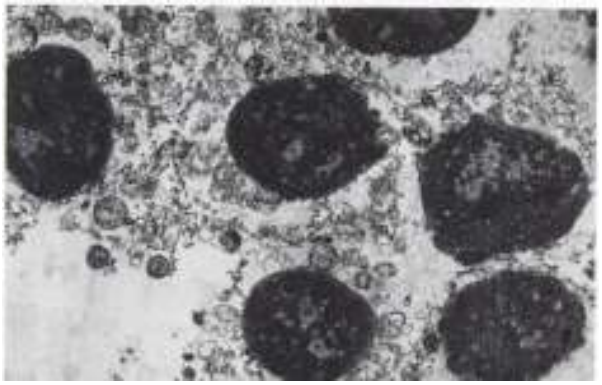

Fig. 7 Cells treated with 160 ppm *Trigonella*

diosgenin. Plasma membranes are completely ruptured with electron-dense nucleus (X5,300)

MCF7 cells treated with diosgenin presents typical characteristics of apoptosis including morphologic alterations and DNA damage. Caspase-inhibitor family proteins, especially the caspase-9 and caspase-3 inhibitors can partially prevent the diosgenin-induced apoptosis. Diosgenin can induce the reduction of mithochondrial membrane potential and Bcl-2 expression control [9,10].

Concluding, diosgenin, obtained from Trigonela foenum-graecum extract may induce apoptosis of MCF7 cancer cells by caspase pathway activation.

References

1.Yang, M.H., *Steroidal sapogenins from plants of Dioscorea*. Chin Trad Herb Drugs 1981; 12, p. 41.

2. Beneytout, JL, Nappez, C, Leboutet, MJ, Malinvaud, G., *A plant steroid, diosgenin, a new megakaryocytic differentiation induced of HEL cells*. Biochem Biophys Res Commun 1995; 207, pp. 398-404.

3. Nappez, C., Liagre, B., Beneytout, JL., *Changes in lipoxygenase activities in human erythroleukemia (HE) cells during diosgenin-induced differentiation*. Cancer Lett 1995; 96: 133, p. 40.

4. Moalic, S., Liagre, B., Corbiere, C., Bianchi, A., Dauca, M., Bordji, K., et al., *A plant steroid, diosgenin, induces apoptosis, cell cycle arrest and COX activity in osteosarcoma cells*. FEBS Lett 2001; 506: 225, p. 30.

5. Kirsch, DG., Doseff, A., Nelson-Chau, B., Lim, DS., de Souza-Pinto, NC., Hansford R., et al. *Caspase-3-dependent cleavage of bcl-2 promotes release of cytochrome c.* J Biol Chem 1999; 274: 21155, p. 61.

6. Green, DR., Reed, JC., *Mitochondria and apoptosis*. Science 1998; 281: 1309-12.

7. Wolf, BB., Schulert, M., Echeverri, F., Green, DR., *Caspase-3 is the primary activator of apoptotic DNA fragmentation via DNA fragmentation factor-45/inhibitor of caspase-activated DNase inactivation.* J Biol Chem 1999; 43: 30651, p. 6.

8. Wang, J., Chun, HJ., Wong, W., Spencer, DM., Lenardo, MJ., *Caspase-10 is an initiator caspase in death receptor signaling*. Proc Natl Acad Sci USA 2001; 98: 13884, p. 8.

9. Hengartner, MO., *The biochemistry of apoptosis*. Nature 2000; 407: 770-6.

10. Cai, J., Liu, M., Wang, Z., Ju, Y., *Apoptosis induced by Dioscin in HeLa cells*. Biol Pharm Bull 2002; 25: 193-6.