

Evaluation of α -tomatine effects on the RNAm of MARK2, HSPA5, HSPA14 and GADD153 genes expression on HepG2/C3A cells

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Introduction: The most abundant glycoalkaloid present on the tomato plant (*Lycopersicon esculentum*) is the α -tomatine. It is found specially on the green fruit and has been described as a compound with antimutagenic, antiinflammatory, immunomodulator, fungicide and bactericide properties. Its inhibitory activity is highly effective against many human cell lines, including the neoplastic ones. Some studies have revealed that this glycoalkaloid can react and destabilize cell membranes, and it also induces apoptosis, but is known that the classical apoptotic pathways are not involved. The mechanism involved in the cell death by α -tomatine could be related to the endoplasmic reticulum (ER) stress, in which the accumulation of misfolded proteins on the ER - a common situation in tumor cells - activates a cellular response that attempts to maintain homeostasis. However, in some situations, the response is not sufficient to restore normal ER function and cells die by apoptosis. **Objective:** The present study aimed to evaluate the cytotoxicity of α -tomatine in human hepatoma cells (HepG2/C3A) by the MTT assay and evaluate by qRT-PCR the expression of ER stress involved genes (*MARK1*, *HSPA5*, *HSPA14* and *GADD153*) in HepG2/C3A cells treated with alpha-tomatine. **Methods:** In order to determine the cytotoxicity of the compound, we performed the MTT assay. The cells were treated with 5 concentrations of α -tomatine (1, 2, 2.5, 3 and 4 $\mu\text{g/mL}$) during 24 hours and the values of measured parameters were compared by ANOVA followed by Dunnett's test ($p < 0.05$). For the qRT-PCR, 2×10^6 cells were seeded on 25cm^2 culture flasks and after 24 hours the treatment flasks received α -tomatine (2 $\mu\text{g/mL}$) for 1 hour. The RNA extraction was performed using the TRIZOL® Invitrogen protocol. After the cDNA synthesis, the qRT-PCR was performed in the LightCycler® Nano System (Roche) using Platinum® SYBR® Green qRT-PCR Supermix-UDG (Invitrogen) as detection system. The relative levels of mRNA expression were determined by Pfaffl et al. (2002), with statistical analysis performed using the software REST-2009, with $p < 0.05$. **Results:** Only the lower concentration on the MTT assay (1 $\mu\text{g/mL}$) was not considered cytotoxic, having the 2 $\mu\text{g/mL}$ concentration the same cytotoxicity as the positive control (Doxorubicin [10 $\mu\text{g/mL}$]). In gene relative expression, *MARK2*, *HSPA5* and *HSPA14* showed to be down-regulated when compared to the control cells (0,421; 0,610 and 0,499, respectively). *GADD153* had a 1,230 times up-regulated relative expression. **Conclusion:** At a 2 $\mu\text{g/mL}$ concentration, α -tomatine cytotoxicity can be compared to current used anti-cancer drugs, such as Doxorubicin and its HepG/C3A cell death mechanism appears to be related with a ER stress response. HSPA5 serves as an ER stress signaling regulator and in cancer cell lines it promotes survival and chemoresistance. Its reduced expression leads to specific induction of GADD153, a protein synthesis and oxidation promoter that induces apoptosis during ER stress. HSPA5 and HSPA14 depletion activates Caspase 3 and 7, and MARK2 suppression leads to the loss of cell polarity. Taking together, the results indicate that α -tomatine death cell mechanism may be related to ER stress induced apoptosis.

Keywords: α -tomatine, apoptosis, ER stress, qRT-PCR.

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