Datura Aqueous Leaf Extract Enhances Cytotoxicity via Metabolic Oxidative Stress on Different Human Cancer Cells

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Abstract

This study was designed to evaluate the cytotoxic effect of aqueous \textit{Datura stramonium} leaf extract on different human cancer cell lines \textit{in vitro}. Breast (MDA-MB-231), head, neck (FaDu), and lung (A549) cancer cell lines were treated with 1 mg/mL of \textit{Datura} aqueous extract for 24 and 48 hours. Exposure of MDA-MB-231 and FaDu cells to the extract for 24 hours resulted in a significant decrease in cell survival. Same effect was seen with all cell lines exposed to the \textit{Datura} aqueous extract for 48 hours. Treatment with \textit{Datura} aqueous extract also caused perturbations in parameters indicative of oxidative stress, including increased glutathione disulfide (GSSG) in FaDu cells treated for 48 hours. Additionally, an increase on the redox sensitive enzymes seen with all cell lines exposed to the \textit{Datura} aqueous extract for 24 and 48 hours. The results may suggest therapeutic potential of \textit{Datura} aqueous leaf extract for the treatment of different types of cancer. Further investigations are needed to verify whether this cytotoxic effect occurs \textit{in vivo}.

Keywords: \textit{Datura} Stramonium; Glutathione; MnSOD; HO-1.

1. Introduction

1.1. Plant

\textit{Datura stramonium}, more commonly known as jimson weed or thorn apple, is a wild-growing flowering plant belonging to the family Solanaceae and is a medicinal plant with antioxidant (Abdallali et al., 2003) antioxidant (Coxidis et al., 2003), hypolipidemic (Rasekh et al., 2001), anti-inflammatory, anti-rheumatoid (Tariq et al., 1989), and hypoglycemic (Gharabeh et al., 1988) properties. Therefore, this study was carried out to evaluate the therapeutic potential of the aqueous \textit{Datura stramonium} leaf extract in the treatment of different types of cancer.

1.2. Oxidative Stress

Manumal cells continuously produce reactive oxygen species (ROS) through various metabolic pathways. Reactive oxygen species are molecules that contain oxygen and have higher reactivity than ground-state molecular oxygen. These species include not only the oxygen radicals (like O$_2^\cdot$), "OH, and peroxyl radicals), but also non-radical molecules such as H$_2$O$_2$ and O$_2$. Superoxide is formed during the reduction of O$_2$ by the mitochondrial electron transport system (Boveris and Cadenas, 1982). Eukaryotic cells are equipped with an antioxidant system capable of converting ROS to H$_2$O via different cytosolic enzymes. Oxidative stress results when the balance between the production of ROS exceeds the antioxidant capability of the target cell. It is generally thought that low levels of ROS are not harmful to cells, and indeed even perform useful signaling functions, whereas high levels of ROS are detrimental through covalent reactions with cellular proteins, lipids, and DNA that results in altered target molecule function. The accumulation of oxidative damage has been implicated in
both acute and chronic cell injury, including possible participation in the formation of cancer. Acute oxidative injury may produce selective cell death or sublethal injury, such as mutations, chromosomal aberrations or carcinogenesis (McConahey, 1971; Klaunig, et al., 1998).

In contrast, chronic oxidative injury may lead to a non-lethal modification of normal cellular growth control mechanisms. Cellular oxidative stress may modify intracellular communication, protein kinase activity, membrane structure and function, and gene expression, and it may result in modulation of cell growth (Klaunig et al., 1998).

Cells are protected against oxidative stress by different intracellular antioxidant compounds, mainly Glutathione (GSH) and thioredoxin, and by other antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1) (Tsan, 1989; Guo et al., 2001). These antioxidant enzymes were shown to be up-regulated by various physical, chemical, and biological agents and oxidative stress (Tsan, 1989; Wong et al., 1989; Bianchi et al., 2002).

Little information is available on the antioxidant or pro-oxidant properties of the herbal preparations of *Datura stramonium*. The purpose of this study was to evaluate the therapeutic potential of aqueous leaf extract of this plant in the treatment of cancer in vitro.

2. Material and Methods

2.1. Cell Culture

Breast (MDA-MB231) cells were routinely kept in RPMI 1640 medium supplemented with 10% fetal bovine serum, and head, neck (FaDu), and lung (A549) cancer cells lines were routinely kept in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. All cells were obtained from the European Collection of Cell Cultures (ECACC) and were kept at 37 °C in a humidified 5% CO2 incubator.

2.2. Preparations of Extract

*Datura stramonium*, a wild-growing flowering plant belonging to the family Solanaceae, was collected during the flowering period in August 2005 in Jordan. The leaves were separated and dried in the shade in green house for several days; and was deposited in the Herbarium of the Department of Biology at the Hashemite University. The procedure was as follows: Leaf part of *Datura stramonium* (150 g) of dried plant was ground and the obtained powder was mixed with 1 L of boiling distilled water for 1 hour. The obtained mixture was filtered twice through a funnel by using suction pump. Water was concentrated under vacuum by using a rotary evaporator at a temperature of 50°C. The extract was evaporated under a reduced pressure till it dried by using a lyophilizer (or by using fume hood). The extract was stored in glass flasks to protect them from humidity and light 1 mg/ mL of the extract was prepared by dilution of the stock with sterile phosphate-buffered saline (PBS) solution.

2.3. Cell Survival Experiments

Cells were plated in 60 mm tissue culture dishes at low density (300 per dish) and grown for 3 days in the presence of antibiotics (Gentamycin). At the beginning of each experiment, the cells were placed in DMEM or RPMI-1640 supplemented with 10% fetal bovine serum. Control cultures were treated identically. Cells were then treated with *Datura aequosus* extract (1 mg/mL). Cultures were then placed in an incubator. At each time point (24 and 48 hours), cells were trypsinized, counted, dihydro, and plated at low density (300-1000 per plate) for clonogenic cell survival assay as previously described (Spitz et al., 1990). Surviving colonies were fixed and stained with Coomassie Blue stain after 14 days of incubation, and were counted under a dissecting microscope. Colonies containing 50 cells or more were scored.

2.4. Measurement of Glutathione Levels

The intracellular levels of reduced glutathione (GSH) and GSSG in cancer cells were measured. Total glutathione content was determined according to (Anderson, 1985). The total intracellular GSH was determined by the colorimetric reaction of DTNB (5,5-dithiobis-2-nitrobenzoic acid) with GSH to form TNB (5-thio-2-nitrobenzoic acid). The rate of formation of TNB, which is proportional to the total GSH concentration (GSH + GSSG), was measured spectrophotometrically at 412 nm. Cellular GSSG is reduced to GSH by glutathione reductase (GR), using NADPH as a cofactor. Briefly, cell pellet was lysed in 5% 5-sulfosalicylic acid (SSA); the pellet was lysed in 5 % 5-sulfosalicylic acid (SSA); the extract was centrifuged. Cells were lysed by sonication in 10 mM phosphate-buffered saline (PBS) solution. After separation, the proteins were electrophoretically transferred to nitrocellulose membranes (Bio Rad, Hercules, CA). The membrane was blocked with 5% skim milk in TBST (Tris-
buffered saline with 0.1% Tween for 1 hr, and then incubated with the primary antibody for 1–2 hr. The blot was washed with TBST and incubated with secondary antibody (horseradish peroxidase-conjugated anti-IgG (Amersham Pharmacia Biotech, Inc., Piscataway, NJ). The immunoreactive protein was detected using an enhanced chemiluminescence (ELC) detection kit (Amersham Pharmacia Biotech, USA). Primary antibodies were anti-Heme Oxygenase-1 (Stressgen Biotech Serologies, USA), and Rabbit anti-MnSOD (kind gift from Dr. Larry Obesley, University of Iowa, USA).

2.6. Statistical Analysis

All results are expressed as mean ± 1 standard deviation (S.D). Student’s t test was employed (p<0.05) for two groups analysis.

3. Results

3.1. Datura Aqueous Leaf Extract -Induced Cytotoxicity and Oxidative Stress in Human Cancer Cell Lines

A variety of human cancer cells derived from breast (MDA-MB231), head and neck (FaDu), and lung (A549) human cancer cell lines were exposed for 24 and 48 hours to Datura aqueous leaf extract and clonogenic cell survival as well as parameters indicative of oxidative stress were assayed. MDA-MB231 and FaDu cells significantly enhanced (p<0.05) clonogenic cell killing following 24 hours exposure to the extract, relative to each respective control (Figure 1). However, A549 cells were found to be resistant to cell killing induced by the extract for 24 hours. Some variability in responses between the cell lines was also noted with MDA-MB231 showing 40% killing, and FaDu showing 65% cell killing during 24 hours of Datura aqueous leaf extract exposure (Figure 1). Exposure of these cells to the extract for 48 hours showed that all cancer cell lines were sensitive to cell killing induced by Datura aqueous leaf extract exposure with some variability (p<0.05) (Figure 2). MDA-MB231 showing 61% killing, FaDu showing 63% cell killing, and A549 showing 22% cell killing during 48 hours of Datura aqueous leaf extract exposure. Figure 3 shows the results of the glutathione analysis done on co-cultures obtained from the same experiments, shown in Figure 1 and 2. Glutathione is a major intracellular redox buffer such that the ratio of GSH to GSSG can be used as a reflection of intracellular redox status (Schaffer et al., 2001). Exposure of FaDu cells for 24 and 48 hours to Datura aqueous leaf extract caused a ~2-fold increase in total GSH and GSSG as well, whereas a minimal change was seen in MDA-MB231 exposed to Datura aqueous leaf extract for 24 hours. However, A549 cells exposure to Datura aqueous extract for 24 or 48 hours did not seem to significantly alter GSSG level (Figure 3A, B).

4. Discussion

It has been reported that all parts of the plant Datura stramonium are poisonous if ingested by humans or livestock (Radford et al., 1964). However, it could be used for medicinal purposes (King 1984, Mann 1992). The effect of Datura extracts on the oxidative stress has not been studied well. In this study, we investigated the toxic effect of Datura stramonium aqueous leaf extract on different cancer cells and how this exposure might affect the oxidative/antioxidant status of the cells.

Using clonogenic assay, and after incubation for 24 hours, both MDA-MB231 and FaDu cells showed significantly reduced growth (p<0.05) when compared to
aqueous leaf protecting cells from oxidative stress induced by apoptotic characteristics). Further studies are still to be induced different mechanism of cytotoxicity (pro-
regulated. This indicates that the extract might have cytotoxicity, the level of GSSG was not highly up-
exposure. This can be seen cl early by the level of GSSG oxidant/antioxidant capability of the cells caused by this extract could be attributed to the imbalance of the internal metabolism consistent with oxidative stress. Although, the FaDu cells was mediated by disruptions in thiol and 2 suggest that the cytotoxic effect of the extract in changes in FaDu cells. Taken together, the data in Figure 1 (Figure 1). The killing ability of the Datura aqueous leaf extract for 24 and 48 hours. Cells were then harvested for glutathione analysis, using the spectrophotometric recycling assay. For more details, see legend of Figure 1.

control. The situation was different with A549 cells (Figure 1). The killing ability of the Datura aqueous leaf extract could be attributed to the imbalance of the internal oxidant/antioxidant capability of the cells caused by this exposure. This can be seen clearly by the level of GSSG changes in FaDu cells. Taken together, the data in Figure 1 and 2 suggest that the cytotoxic effect of the extract in FaDu cells was mediated by disruptions in thiol metabolism consistent with oxidative stress. Although, the extract exposure in MDA-MB231 induced significant cytotoxicity, the level of GSSG was not highly up-regulated. This indicates that the extract might have induced different mechanism of cytotoxicity (pro-apoptotic characteristics). Further studies are still to be done. The higher levels of GSH in A549 cells could be protecting cells from oxidative stress induced by Datura aqueous leaf extract exposure for 24 hours (Figure 3).

Upon 48 hours exposure, the picture was different. The level of oxidative stress induced by exposure conditions was causing more killing in the three cell lines. Again looking at the GSH levels, it is clear that exposure to the extract induced GSH response to lesser extent this time. The inability of cells to induce more GSH production could be due to the toxic effect of the Datura aqueous leaf extract.

GSH and GSSG are the major redox pair involved in cellular redox homeostasis. A change in the cellular GSH or GSSG is regarded as a representative marker for oxidative stress, and is directly responsible for the

perturbation of cellular function (Schafer et al., 2001). This includes activation of antioxidant defense pathways, as well as induction of cytotoxic responses.

Our results above motivated us to study the expression of certain antioxidant enzymes such as MnSOD and HO-1. As we can see the levels of MnSOD or HO-1 were not changed upon exposure to the Datura aqueous leaf extract in both MDA-MB231 and FaDu cells, whereas A549 cells showed clear up-regulation on both 24 and 48 hours exposure (Figure 4). This interesting result shows that different cancer cells have different inherent response to oxidative stress. This response will affect the ability of different cancer cells to respond to compounds and chemicals that can induce oxidative stress. Studying signal pathways, involved in different activation processes, could evolve and explain the different responses seen.

In this study, we have demonstrated that Datura stramonium aqueous leaf extract induced oxidative stress in different human cancer cell lines. In response, these cells exhibit up-regulating the expression of certain antioxidant compounds and enzymes such as GSH, HO-1 and SOD. Further studies are still needed to explore the effect of Datura aqueous leaf extract on the signaling pathways involved.

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References


