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Cytotoxic effect of inositol hexaphosphate and its Ni(II) complex on human acute leukemia Jurkat T cells

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ABSTRACT

Inositol hexaphosphate ($InsP_6$) is present in cereals, legumes, nuts and seed oils and is biologically active against some tumor and cancer cells. Herein, this study aimed at evaluating the cellular toxicity, antiproliferative activity and effects on cell cycle progression of free $InsP_6$ and $InsP_6$–Ni(II) of leukemic T (Jurkat) and normal human cells. Treatments with $InsP_6$ at concentrations between 1.0 and 4.0 mM significantly decreased the viability of Jurkat cells, but showed no cytotoxic effect on normal human lymphocytes. Treatment with $InsP_6$–Ni(II) complex at concentrations between 0.05 and 0.30 mM showed an anti-proliferative dose and a time-dependent effect, with significantly reduced cell viability of Jurkat cells but showed no cytotoxic effect on normal human lymphocytes as compared to the control. Ni(II) free ion was toxic to normal cells while $InsP_6$–Ni(II) had no cytotoxic effect. The $InsP_6$–Ni(II) complex potentiated (up to 10×) the antiproliferative effect of free $InsP_6$ on Jurkat cells. The cytometric flow assay showed that $InsP_6$ led to an accumulation of cells in the G0/G1 phase of the cell cycle, accompanied by a decrease in the number of cells in S and G2/M phases, whereas $InsP_6$–Ni(II) has led to an accumulation of cells in the S and G2/M phases. Our findings showed that $InsP_6$–Ni(II) potentiates cytotoxic effects of $InsP_6$ on Jurkat cells and may be a potential adjuvant in the treatment of cancer.

1. Introduction

Inositol hexaphosphate, $InsP_6$, also known as phytic acid, is present in cereals, legumes, nuts and seeds, accounting for up to 85% of total phosphorus in cereals and leguminous plants (Graf and Eaton, 1990; Tsao et al., 1997). $InsP_6$ can also be found in humans at a physiological concentration that can reach up to 100 μM (Vucenik et al., 2005). *In vitro* studies have reported the anti-carcinogenic activity of $InsP_6$ on different types of tumors and cancer cell lines as it inhibits cell proliferation and differentiation of malignant cells, restoring the normal phenotype. $InsP_6$ acts specifically on the p21 proteins involved in the cell cycle of cancer cells (Kim et al., 2007). In addition, it induces G1 phase arrest and causes a significant decrease of the S phase of human cancer cell lines by modulating cyclins and cdks, up-regulating p27Kip1 and p21WAF1/CIP1, and decreasing the retinoblastoma (Rb) protein phosphorylation (Vucenik and Shamsuddin, 2006). Exposing MCF-7 breast cancer cells to $InsP_6$ has shown to block the proliferation of such cells through a PKCδ-dependent increase in $p27^{Kip1}$ and a significant decrease in retinoblastoma protein (pRb) phosphorylation (Vucenik et al., 2005). $InsP_6$ has potent inhibitory effects on the proliferation of HT-29 cells by modulating the expression of special cell cycle regulators (Tian and Song, 2006). Different concentrations of $InsP_6$ in HT-29 cells increased the transcription of p53 and p21 Waf1 genes. The ability of $InsP_6$ to arrest the cell cycle may be mediated by the transcriptional up-regulation of the p53-responsive p21(WAF1) gene (Węglarz et al., 2006).

Inositol tetra and pentaphosphate, $InsP_4$ and $InsP_5$, respectively, also induce the apoptosis of cancer cells; however, $InsP_6$ is more efficient and has shown to promote apoptosis of some cancer cells through different mechanisms (Agarwal et al., 2009). T98G glioblastoma cells treated with $InsP_6$ presented an increased expression of Bax (a proapoptic gene) and a decrease in the expression of Bcl-2 (an antiapoptic gene), caspase-3 activation and cellular death induced by apoptosis (Karmakar et al., 2007). Another mechanism of $InsP_6$ has been demonstrated using a colon cancer cell line (Caco-2) stimulated by interleukin-1β. The $InsP_6$ influences constitutive expression of metalloproteinase, MMP and TIMP (tissue inhibitors of MMPs) genes and downregulates IL-1β stimulated transcription of some of these genes. $InsP_6$ exerts its anti-metastatic activity through modulation of MMP and TIMP genes expression to prevent cancer cell migration and invasion (Kapral et al., 2012). Physiological intestinal concentrations of $InsP_6$ may have an

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inhibitory effect on IL-8 secretion by Caco-2 cells and one of the mechanisms of its action is the inhibition of PTK signaling cascade. The PTKs could be one of the molecular targets for $InsP_6$ effects in the intestinal epithelial cells (Wawszczyk et al., 2013).

In vivo studies have shown that $InsP_6$ combined with basic fibroblast growth factor, completely inhibited the formation of new blood vessels in mice. The mechanism of action of $InsP_6$ is also related to the regulation of angiogenesis. New blood vessels are responsible for providing nutrients and oxygen to the tumor, in addition to promoting the spread of metastatic cells (Vucenik et al., 2004). When topically applied, $InsP_6$ induces the apoptotic machinery by modulating the expression of mt p53, Bcl-2, and caspase activity (Singh and Gupta, 2008). According to Williams et al. (2011), the topical application of $InsP_6$ followed by exposure to UVB irradiation on SKH1 hairless mice decreased the tumor incidence and proliferation when compared with the control group. A recent study using quantitative high-resolution ^{1}H-NMR on prostate tissue extracts showed that $InsP_6$ significantly decreased the glucose metabolism and the synthesis of membrane phospholipid, in addition to causing an increase on myo-inositol levels in the prostate (Raina et al., 2013).

Several studies have related the ability of $InsP_6$ to reduce the risk of developing cancer and also to inhibit cell proliferation and tumor progression in animal models (Ferry et al., 2002; Deliliers et al., 2002; Rizvi et al., 2006; Karmakar et al., 2007; Agarwal et al., 2009; Kolappaswamy et al., 2009; Wawszczyk et al., 2013). The activity of some metal complexes against tumors using both *in vitro* and *in vivo* protocols is already known and recognized worldwide (Immel et al., 2011; Côrte-Real et al., 2013; Palanimuthu et al., 2013; Tan et al., 2010; Milenković et al., 2013). Studies have reported the characterization and stability of some $InsP_6$ complexes with transition metal ions, including Ni(II) (De Carli et al., 2006, 2009; Quirrenbach et al., 2009). However, no studies were found in the literature relating the bioactivity of $InsP_6$ coordination compounds.

Based on these facts, this study aimed at evaluating the cellular toxicity, antiproliferative activity and effects on cell cycle progression of free $InsP_6$ and $InsP_6$–Ni(II) in leukemic T cells (Jurkat) as well as in normal human cells.

2. Materials and methods

2.1. Chemicals

All analytical grade reagents were used without further purification. Milli-Q (Millipore) water was used to prepare all solutions. $InsP_6$ (dodecassodium salt), 3-(4.5-dimethylthiazol-2yl)-2-5-diphenyl-2H tetrazolato bromine (MTT), propidium iodide (PI) and trypan blue were obtained from Sigma Chemical, Co. (St. Louis, MO, USA). $NiCl_2 \cdot 6H_2O$ and vincristine sulfate (Tecnocris®), were obtained from Merck (Darmstadt, Germany) and Eurofarma Ltda (São Paulo, SP, Brazil), respectively. The $InsP_6$–Ni(II) complex was synthesized and characterized as previously described (De Carli et al., 2006, 2009).

2.2. Cell culture

The Jurkat cells were routinely cultivated in RPMI 1640 (Cultilab) at pH 7.4 supplemented with 10% fetal bovine serum (FBS, Vitrocell), 0.1% of antibiotic mix (10,000 units penicillin and 10 mg mL^{-1} streptomycin, Vitrocell) and sodium bicarbonate (2 mg mL^{-1}). They were placed in an incubator humidified at 37 °C with 5% CO_2. All the solutions used in the cell culture treatment were prepared in FBS-free medium, filtrated using a 0.22 μm membrane (Milex®GP, Millipore) and stored at 4 °C until use. The leukemic cell line was chosen to be the experimental model in the current study because it is highly susceptible to $InsP_6$ (Matejuk and Shamsuddin, 2010).

2.3. Isolation of normal human lymphocytes

In accordance with authorization from the Institute's Ethics Commission (Opinion Nº 66/2011, Protocol: 06065/11), peripheral blood samples were collected from healthy, non-smoking male volunteers, who had fasted for 12 h. Mononuclear cells (lymphocytes and monocytes) were separated with Histopaque reagent 1077. The layer of mononuclear cells was removed and washed twice with phosphate buffered saline solution (PBS). The cells were re-suspended in RPMI 1640 pH 7.4 culture supplemented with 10% FBS, 10,000 units penicillin, 10 mg mL^{-1} of streptomycin and sodium bicarbonate (2 mg mL^{-1}). They were then seeded in culture bottles (75 cm^2), and incubated for 2 h at 37 °C. After 2 h of culture, the lymphocytes present in the supernatant were separated from the monocytes and used in the experiments.

2.4. In vitro biological tests

2.4.1. Evaluation of the cytotoxic effect of $InsP_6$, $NiCl_2$ and $InsP_6$–Ni(II) complex on Jurkat cells

The Jurkat cells (1×10^5 cells mL^{-1}) were treated with $InsP_6$ at concentrations ranging from 0.250 to 4.00 mM; $InsP_6$–Ni(II) complex at concentrations from 0.055 to 0.220 mM; or $NiCl_2$ at concentrations from 0.10 to 0.60 mM, in RPMI medium containing 2.5% FBS. Vincristine sulfate (40 nM) was used as positive control. Cell viability was determined by means of two different assays: (1) trypan blue dye exclusion test, in which the cells were counted using a Neubauer chamber after 24 and 48 h; (2) MTT reduction assay. After 24 and 48 h of treatment, the cell suspension was transferred to 2 mL vials and centrifuged at 138 *g* for 5 min. The supernatant was discarded and 400 μL of a 1:10 mixture of MTT (5 mg mL^{-1}) was added to each vial. The cells were incubated at 37 °C for 30 min and centrifuged again at 800 *g* for 10 min. The supernatant was discarded and 200 μL of dimethyl sulfoxide were added to solubilize the purple precipitate (formazan) and the absorbance was measured at 550 nm (Côrte-Real et al., 2013). The IC_{50} value was calculated for $InsP_6$ and in order to compare the cytotoxicity of each compound tested, in which $InsP_6$–Ni(II) complex was used as a parameter for comparison purposes. Consequently, the IC_{50} was defined as the drug concentration (mM) inducing a 50% reduction in cellular viability through the exclusion assay performed with trypan blue dye. The IC_{50} was calculated by the Probit regression approach.

2.4.2. Evaluation of the antiproliferative effect of $InsP_6$, $NiCl_2$ and $InsP_6$–Ni(II) complex on Jurkat cells

The Jurkat cells (1×10^5 cells mL^{-1}) were treated with $InsP_6$ at concentrations ranging from 0.250 to 4.00 mM, $NiCl_2$ 0.10 to 0.60 mM and $InsP_6$–Ni(II) complex at concentrations ranging from 0.055 to 0.220 mM in RPMI medium with 2.5% FBS. The proliferation of Jurkat cells was performed by quantifying the DNA content (Sellitti et al., 2001). After 48 h of cultivation, the cell suspension was placed in 2 mL vials and centrifuged at 550 *g* for 10 min at room temperature. The supernatant was removed and 0.5 mL of DPA was added to the precipitate. This solution was incubated for 24 h and the absorbance was recorded at 575 nm. The DPA solution consisted of 8 mL of deionized water, 0.1 mL of 1% acetaldehyde and 20 mL of stock solution (1 g of diphenylamine, 90 mL of glacial acetic acid and 2 mL of sulphuric acid).

2.4.3. Effect of $InsP_6$ and $InsP_6$–Ni(II) complex on the cell cycle progression of Jurkat cells

The cell cycle progression assay was performed according to a protocol previously described by Nicoletti et al. (1991). Briefly, 10^6 mL^{-1} of Jurkat cells were seeded into each well of a 96-well plate and after 24 h of incubation, either $InsP_6$ (1.00, 2.00 and 4.00 mM) or $InsP_6$–Ni(II) complex (0.070, 0.175 and 0.300 mM) was added. The plate was further incubated for 24 h and 48 h. Subsequently, the cells were centrifuged at 550 ×*g* for 20 s. The pellet was washed with PBS buffer and after