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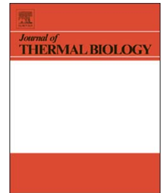

Hyperthermia enhances bortezomib-induced apoptosis in human white blood cancer cells

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ABSTRACT

At present, the current therapeutic strategy for apoptosis induction mainly relies on the administration of pharmacological apoptotic modulators. Apart from that, apoptosis can be induced by various external stimuli such as hyperthermia, ionizing radiation, and electric fields. Despite advantages, both physical and pharmacological approaches bear some limitations as well. The rationale of this study was to overcome the limitations by combining hyperthermia and apoptotic modulator 'bortezomib' (Velcade). Two types of human blood cancer cell lines were utilized: human leukemic monocyte lymphoma cell U937 line and peripheral blood mononuclear cells (PMBCs) derived from the patient diagnosed with acute myeloid leukemia. Prior to apoptosis experiments, cytotoxicity tests were performed at three types of temperature regimes (40 °, 42 ° and 44 °C). We observed a gradual inhibition of cell viability correlating with an increase of temperature and drug concentration in both cell lines. However, there was no significant difference between sham group and groups of leukemic PMBCs treated by high temperature (44 °C) and bortezomib. In U937 cells, combined treatment by heat shock and bortezomib led to an increase the number of cells underwent the late apoptosis stage. At the same time, similar treatment of PMBCs resulted in the stimulation of early apoptosis. Our data suggest that combination of bortezomib and hyperthermia enhances apoptosis induction in human cancer white blood cells, indicating a therapeutic potential for blood cancer therapy.

1. Introduction

Apoptosis is a widespread biological process, which is essential for the maintenance and correct development of an organism (Elmore, 2007; Kerr et al., 1972; Papathanassoglou et al., 2000). The fundamental role in the initiation of apoptotic reactions belongs to caspases. The recruitment of caspases happens either through the ligation of Fas receptors (tumor necrosis factor death receptors) to the cell surface (extrinsic pathway) or as a response to inner signals (intrinsic pathway) (Call et al., 2008; Gewies, 2003).

Apoptosis ('programmed cell death') can be triggered by various stimuli such as chemical agents (Hassan et al., 2014), ionizing radiation (Murakami et al., 2004), hyperthermia (Cheng et al., 2015; Luchetti et al., 2003), ultrasound (Furusawa et al., 2010) and electric pulses (Tang et al., 2009).

Hyperthermia has also been extensively studied as a potential candidate for induction of apoptosis in cancer cells (Cui et al., 2014b; Yu et al., 2008). It was demonstrated that hyperthermia is capable of enhancing cytotoxic effects of anti-cancer drugs by promoting permeability of cellular membrane (Cui et al., 2014a).

On the other hand, pharmacological approach offers a broad range of chemical agents targeting the apoptosis signaling pathways (Fulda and Debatin, 2006; Hickman, 1992; Taura et al., 2010). These agents are capable of inhibiting or promoting the key elements of apoptosis machinery thus providing an opportunity to modulate apoptosis processes, particularly in tumor tissues. For example, some drugs have been designed to specifically target TNF-related apoptosis-inducing ligand (TRAIL) pathway (Fischer and Schulze-Osthoff, 2005). Other

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efforts have been focused on developing a new range of caspase inhibitors.

One of current therapeutic approaches for cancer management is targeting the 26S proteasome, which is responsible for protein degradation (Voges et al., 1999). There are a few proteasome inhibitors at the market, including bortezomib, MLN519, salinosporamide, carfilzomib, epoxomicin, CEP-18770 and ritonavir. It has been demonstrated that proteasome inhibitors possess a broad spectrum of anti-proliferative and pro-apoptotic activities against different malignancies (Crawford et al., 2011). Bortezomib, dipeptidyl boronic acid proteasome blocker, has shown a promising results when it was tested against 60 cancer cell lines (Fischer and Schulze-Osthoff, 2005). Bortezomib is the first proteasome inhibitor approved by FDA that can cure multiple myeloma and mantle-cell lymphoma (Kane et al., 2007). This apoptosis modulator has been already tested in the combination with a wide range of drugs such as thalidomide, melphalan, dexamethasone, and other anticancer agents, including doxorubicin.

Despite advantages, both physical and pharmacological approaches bear some limitations too. Hyperthermia cannot provide a high degree of therapeutic selectivity to tumor tissues. Meanwhile, administration of bortezomib can cause a range of side effects, including peripheral neuropathy, hypotension, heart and lung problems, decrease of the level of neutrophils and leucocytes, etc. (Dou and Zonder, 2014).

The rational of this study was to overcome the above-mentioned limitations by combining hyperthermia and bortezomib. We used two human cancer cell lines: commercially available human leukemic monocyte lymphoma cell U937 line and peripheral blood mononuclear cells (PMBCs) derived from the patient diagnosed with acute myeloid leukemia.

2. Materials and methods

2.1. Cell culture

2.1.1. The U937 cells

Cells of human histiocytic lymphoma line U937 (Japanese Cancer Research Resource Bank, Japan) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) at 37.0 °C in incubator with humidified air with 5% CO_2.

2.1.2. The leukemic cells

Peripheral blood mononuclear cells (PMBCs) were derived from the patient (male, 28 years-old) diagnosed with acute myeloid leukemia (type: M2/ myeloblastic with maturation). Data of analysis of peripheral blood: haemoglobin level 6.9 g/dL, white blood cell (WBC) count 65×10^9 / L, platelets 50×10^9/L, lymphocytes 0.5×10^9/L, myelocytes 5%, blast cells 15% and 21% myeloblasts in bone marrow. A written and signed consent form was obtained from the participant. The patient did not receive chemotherapy or radiotherapy prior to the study. The method of obtaining and isolation of PMBCs was previously described by Heissig *et al.* (Heissig et al., 2000).

2.1.3. Ethical approval

All protocols pertaining to human subjects were first approved by Nazarbayev University's Institutional Research Ethics Committee, Astana, Kazakhstan. All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

2.2. Hyperthermia

Hyperthermia treatment was conducted by using ‘SubAqua 18 Plus’ water bath system (Grant Instruments, USA) according to protocol previously described by Cui et al. (2014a).

2.3. Preparation of bortezomib (Velcade)

The bortezomib was reconstituted in 0.9% of Sodium Chloride solution according to instructions of the manufacturer (Velcade, Janssen-Cilag Pty Ltd, Australia). The prepared solution was kept in dark and cold conditions (4 °C).

2.4. Studies of cytotoxicity

Cells were cultivated in 96 multi-well plate (Greiner Bio-One GmbH, Germany) in the concentration 4×10^{5} cells / ml (both cell lines). The insulated 96-well plate was placed into a water bath at 40, 42 and 44 °C for 10 min. Bortezomib was added to the cell culture in the following concentrations: 1, 2, 3, 4 and 5 ng/ml prior to the hyperthermia treatment. After 48 h of incubation (5% CO_2, 37 °C) the cells were collected and analyzed using the CellTiter 96® AQ_{ueous} One Solution Reagent on spectrophotometer Thermo Scientific™ Multiscan 60 (Thermo Fisher, USA). All data of cytotoxicity studies are present as normalized ratio to the values of control group (kept at a room temperature).

2.5. Detection of apoptosis

Cells were grown in 96 multi-well plates (Greiner Bio-One GmbH, Germany) (4×10^5 cells per ml). Then cells were placed into a water bath with 3 temperature regimes: 40, 42 and 44 °C. There were two control groups (without thermal exposure): untreated and treated with bortezomib.

Bortezomib was added to cell culture in the concentration of 5 ng/ml. After this, cells were put into the incubator (5% CO_2, 37 °C) for 48 h. Then, the cell culture was subjected to hyperthermia (40, 42 and 44 °C). Treated cells were collected and centrifuged for 15 min. Apoptosis detection was performed by using Annexin V Alexa Fluor 488 (Component A) and Tali Propidium Iodid (Component B) on TALI Image-based Cytometer™ (Life Technologies, USA).

2.6. Determination of cell viability

Trypan blue exclusion test was performed by mixing 100 µl cell suspension with an equal amount of 0.3% trypan blue solution (Sigma, St. Louis, Missouri, USA) in phosphate buffered saline (PBS). After 5 min incubation at room temperature unstained cells were counted with Automatic hemocytometer LUNA (Automate Cell Counter, LOGOS, Republic of Korea).

2.7. Statistical analysis of cytotoxicity studies

The one-factor analysis of variance with repetition (one-way ANOVA) was utilized in order to analyze the impact of temperature and bortezomib on cell viability and apoptosis (for two cell lines separately). Each experiment was conducted at least in triplicate. Statistical analysis was performed using SigmaPlot 11.0 software (Systat Software Inc., California, USA). Results are presented as mean ± SD.

3. Results

3.1. Effect of high temperatures and bortezomib on cell viability

Results demonstrated that bortezomib at 5 concentrations was capable of suppressing viability of U937 cells. An increase of drug's concentration led to gradual decrease of U937 cells viability in all experimental groups (exposed and non-exposed to hypethermia) (Fig. 1a). The maximal impact was observed in the group “bortezomib + 40 °C”. However, the further elevation of temperature up to 42° and 44 °C in combination with bortezomib hampered the suppressing effect

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