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CYTOTOXICITY STUDIES OF COMBINATION OF PROTEASOME INHIBITOR VELCADE AND HYPERTHERMIA

Timur Saliev,[1] Loreto B. Feril Jr., [2] Dinara Begimbetova, [1] Dinara Baiskhanova, [1] Xenya Bobrova, [1] Mars Akishev, [1] Katsuro Tachibana [2]

1) NLA, Nazarbayev University, Astana, Kazakhstan; 2) Department of Anatomy, Fukuoka University School of Medicine, Fukuoka, Japan

Introduction: One of perspective methods of cancer treatment is the induction of apoptosis ('programmed cell death') in malignant cells. The apoptosis can be stimulated by various factors: biological, chemical and physical. The current clinical strategy mainly relies on administration of apoptotic modulators such as Velcade (Janssen-Cilag Pty Ltd) which has been proven effective against blood cancers [1,2]. However, administration of Velcade can cause a range of side effects. Hyperthermia has been known as an alternative method for apoptosis induction in blood cancer cells [3-4]. We hypothesized that the combination of heat shock and apoptotic modulator Velcade might be used for elimination of malignant cells.

Aims of the study: To investigate cytotoxic effect of combination of pharmacological apoptosis modulator Velcade (Janssen-Cilag Pty Ltd) and hypethermia**.**

Methods: The cytotoxicity was studied for 5 concentrations of Velcade (1, 2, 3, 4 and 5 ng/ml), three types of temperature (40, 42 and 44^0C), and two types of cancer cells (human melanoma C-32 and human monocytic leukaemia U937 lines). The U937 cells (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 (5% CO_2). The C-32 cells were cultured in minimum essential medium (MEM) with Earle's salts and l-glutamine supplemented with 1% essential amino acids and 10% fetal bovine serum (Gibco, Invitrogen Corporation) under humidified air and 5% CO_2. Cells viability of both lines was always over 95% prior to the treatment. cells were cultivated in 96 multi-well plate (Greiner Bio-One GmbH) in concentration 400 000 cells / ml. The insulated 96-well plate was placed into a water bath (at 40, 42 and 44^0C) for 10 minutes. Prior to this, bortezomib was added to the cell culture in the following concentrations: 1, 2, 3, 4 and 5 ng/ml. After 2 days of incubation (5% CO2, 370C) the cells were collected and analyzed using the CellTiter 96® AQueous One Solution Reagent on spectrophotometer Thermo ScientificTM Multiscan 60 (Thermo Fisher, Japan).

Results: The results demonstrated that Velcade in all 5 concentrations was able of suppressing the viability of U937 cells (Fig.1). The decrease in cell survival rate was in a direct correlation with the increase of drug's concentration. The maximal level of dead cells was detected for 40^0C for all concentrations of Velcade. However, the acquired data showed that the augmentation of temperature up to 42^0C and 44^0C resulted in the rise of number of viable cells. These findings indicate that high temperatures have a cyto-protective effect. The possible mechanism may lay in the ability of mild hyperthermia to promote cell growth. The second feasible explanation is a negative impact of high temperature on Velcade's pharmacodynamics. The data of cytotoxicity studies of C-32 cells showed a similar picture (Fig.4). The elevation of temperature led to the decrease of cells viability. Notably, the highest temperature (44^0C) had a cyto-protective effect too.

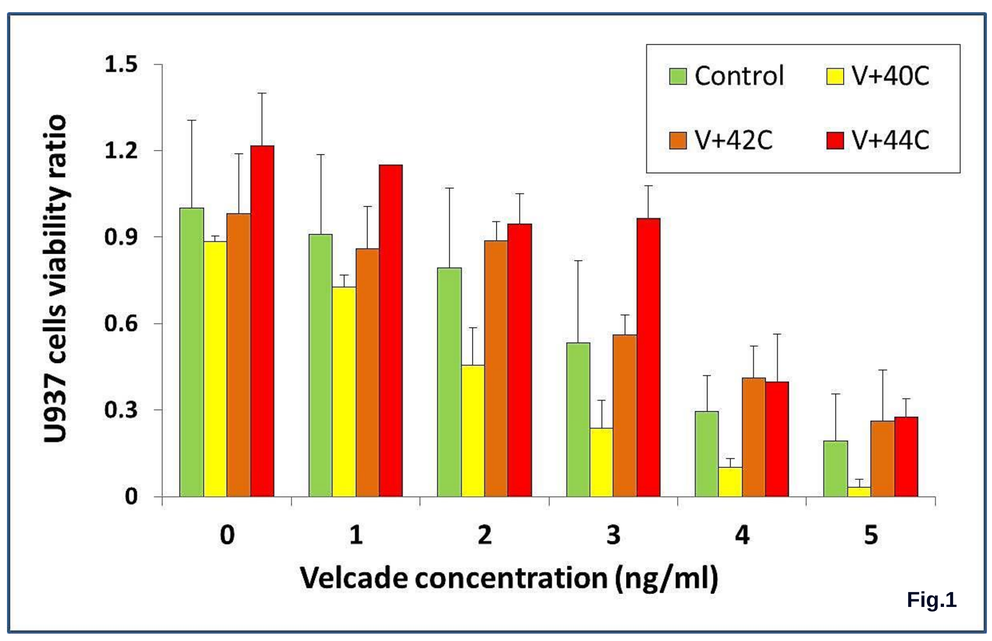

Fig.1

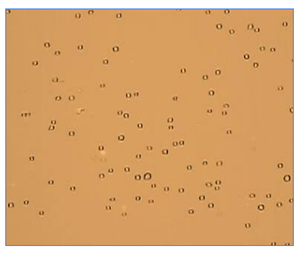
Fig.2

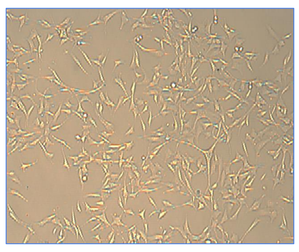
Fig.3

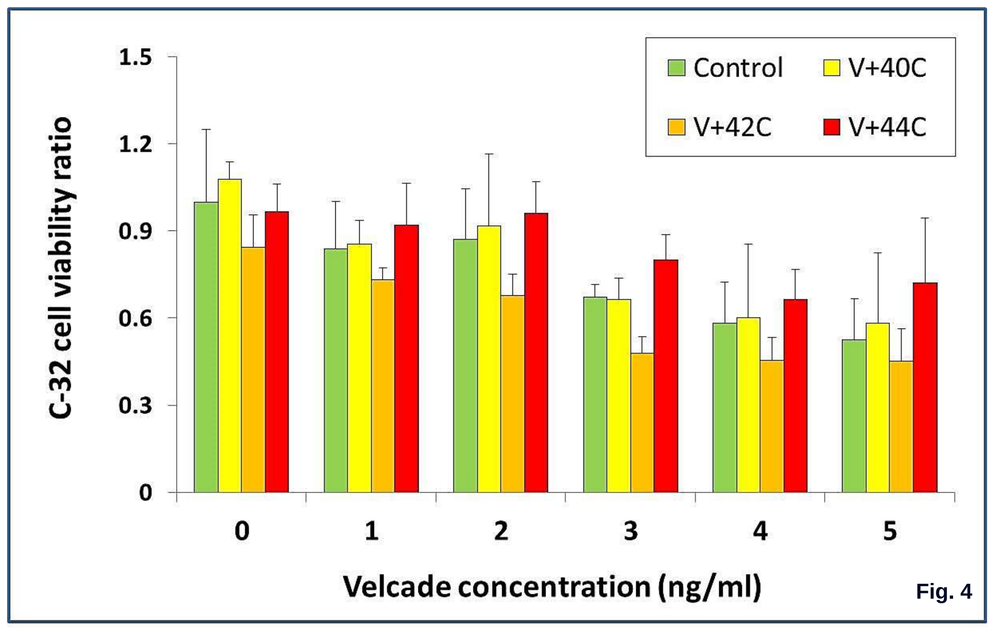

Fig. 4

Figures: 1) Normalized viability ratio of leukaemia U937 cells at different temperatures. 2) Microscopic image of U937 cells (x 600). 3) Microscopic image of C-32 cells (x 600). 4) Normalized viability ratio of melanoma C-32 cells at different temperatures.

Conclusion: We observed a direct correlation between cytotoxic effect of Velcade and an increase of temperature. The optimal temperature level for elimination of leukaemia U937 was 40^0 C for drug's doses. At the same time, for skin cancer cells C-32 the most effective in terms of destroying cells was 42^0 C. Here we demonstrated the dependence of Velacde-induced ctotoxic effect on the type of cell line. Our findings indicate that combination of apoptosis modulator Velcade and mild hypethermia can be effectively employed for the elimination of cancer cells.

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