Effect of cortisol on K562 leukemia cells

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Abstract

Numerous studies describe effects caused by stress on the development, progression and poor prognosis of various pathologies, such as cancer. In recent decades, researchers have investigated the role of stress-associated hormones and cancer progression. Cortisol is described as a primary stress hormone in the human body. Studies show a positive correlation of elevated cortisol levels and cancer progression. Increased cell proliferation and increased reactive oxygen species that contribute to DNA damage, dysplasia, and neoplasms are the result of prolonged stress where tissue becomes insensitive to cortisol, the primary human stress hormone. This study explores the influence of cortisol, an important hormone involved in stress, on tumor cell development, particularly in human cells of chronic myeloid leukemia (K562). K562 cells were exposed to increasing cortisol (hydrocortisone) concentrations for 24 or 48 hours and cytotoxicity (MTT assay [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazio bromide] and cell death processes (fluorescence microscopy) were investigated. Our data show a considerable role of cortisol not only in mitochondrial activity, but also in the processes of proliferation and apoptotic and necrotic death in K562 cells. These results demonstrate the possible influence of stress on tumor development and demonstrate that K562 cells can be adapted to cortisol levels over time.

Keywords: Stress. Hormone. Cell culture. Cancer. Apoptosis.

INTRODUCTION

Physiological levels of cortisol in the bloodstream impair chemotherapy for prostate, lung, bladder, renal, ovarian cancers and triple negative breast cancer (TNBC)[1]. Clinical studies attempt to establish the interaction between cancer diagnosis and treatment indicating that suppression of negative emotions such as depression, rejection and social support deficiency are associated with shorter survival[2,3,4]. Clinical

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data have also shown that induced stress modulates cancer progression[5]. In addition, neurohormonal products derived from chronic stress influence the progression of skin, breast, lung and colon cancer. Several studies suggest that human tumorigenesis is part of a multistep process[6,7,8]. These sequential stages (chemical and physical mutagens, infection by certain viral or bacterial pathogens, non-genotoxic dietary constituents, and stress) lead to genetic alterations that cause progressive alteration of normal human cells to malignant cells. The conversion of normal cells to a cancerous state seems to require genetic and epigenetic changes[9].

Interleukin-6 (IL-6), a cytokine that plays an important role in angiogenesis and tumor progression[10], increases its production through stress-related mediators[11]. However, studies correlating cortisol concentration in K562 cells (chronic myeloid leukemia) are absent. In the present study, the effect of cortisol on myeloid leukemia cells was evaluated by analyzing the cell viability and the type of death induced by this hormone.

In the present study, we propose to evaluate the influence of cortisol on tumor cells, based on the influence of stress as a positive element for the development or resistance of tumor cells to antineoplastic treatments, particularly in human cells of chronic myeloid leukemia (K562).

MATERIALS AND MÉTHODS

Cell culture and hydrocortisone treatment

K562 (chronic myeloid leukemia - ATCC-CCL 243) cells obtained from the cell bank of the Paul Ehrlich Scientific Technical Association - Rio de Janeiro, RJ) were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics and antimycotics (Life Technologies) at 37°C in a 5% CO_2 atmosphere.

Suspended cells were plated at 5x104 cells/mL per well in 96-well plates with DMEM supplemented with 10% FBS. The plates were incubated for 24 h at 37°C and 5% CO_2 for cell adhesion. After this, cells were treated with different concentrations (0 µM, 0.25 µM, 0.5 µM, 1.0 µM, 2.5 µM, 5.0 µM, 10 µM, 15 µM and 20 µM) of cortisol (Sigma-Aldrich hydrocortisone) for 24 or 48 h. All assays were performed in triplicate.

MTT Assay

This assay quantifies mitochondrial activity by analyzing the formazane crystals formed by reducing the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazol bromide salt (MTT- Sigma-Aldrich, Steinheim, Germany). The reduction of MTT occurs mainly in mitochondria through mitochondrial dehydrogenase actions present only in metabolically active cells (living cells), providing mitochondrial function measurement[12]. After exposure to hydrocortisone, cells were washed with PBS and incubated with 0.5 mg/mL MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazol bromide) for 1 h at 37°C in an atmospheric of 5% CO_2. To the formazane precipitate, 50 µl DMSO (dimethyl sulfoxide - Sigma) was added to each well and the plate was stirred for 30 minutes to solubilize the formazane crystals. The absorbance reading was performed on the Spectra Count-Packard ELISA reader, wavelength 570 nm.

Hoechst-Propidium Iodide Marking for Apoptotic Cells

Hoechst 33342, trihydrochloride, trihydrate (Thermo Fisher Scientific) nucleic acid dye, is a permeable marker that emits blue fluorescence when attached to double stranded DNA. This dye is often used to distinguish pyknotic nuclei in apoptotic cells[13]. After exposure to hydrocortisone, cells were washed with PBS and incubated with 1 µL Hoechst 33342 and 1 µL propidium iodide per 1 mL cell suspension and incubated on ice for 20-30 min. After incubation, labeled cells were analyzed by

fluorescence microscopy (Leica DMIL) using a UV/488 nm filter for dual excitation and fluorescence emission measured at ~460 nm and >575 nm. The cell population was divided into three groups: living cells (marked blue), cells in early apoptosis (fragmented nucleus marked blue), late apoptosis (fragmented nucleus characterized in pink and necrotic cells marked with an all red nucleus).

Statistical analysis

Statistical calculations were performed using the GraphPad Prism® version 6.0 statistical data analysis software. Data were compared by analysis of variance followed by Tukey test, defining the significance level at 5% ($p \leq 0.05$). All experiments were performed in triplicate.

RESULTS

In the characterization of the sample no statistical differences were found for age and cognitive status between groups ($p > 0.05$). The data are shown in Table 1.

Table 2 shows the results of the clinical variables. Group A presented a smaller number of falls compared to group S. For balance, group A had a higher score on the BBS compared with group S, indicating a better balance and lower risk of falls for group A.

For mobility, group A demonstrated a shorter TUG time compared with the S group, indicating that the elderly in group A were more agile. The results of falls, balance and mobility displayed statistically significant differences between the groups.

For handgrip strength, group A demonstrated greater strength than the S group. However, there were no statistically significant differences between the groups.

For the correlation between the HGS measurements and the balance and mobility variables, no relationship was found. The highest correlation found was 0.222, for HGS and BBS, and was considered a weak correlation. The values of the correlations between HGS and balance and mobility are presented in Table 3.

When incubation periods are compared, significant mitochondrial activity is observed in 0.25, 0.5, 1.0, 2.5 and 5 µM 48-hour groups when compared to 24-hour groups.

Tumor growth displays a balance between proliferation and cell death involving apoptotic mechanisms. To determine the type of cortisol-induced death, in cells incubated with increasing hormone concentrations over 24 and 48 hours, cells were labeled with Hoechst 33342 and propidium iodide. When a concentration of 0.25 µM (Figure 2b) was compared with the control group within 24 hours (Figure 2a), the results show that this concentration did not cause damage to the cell nucleus; however, at the same concentration after 48 hours (Figure 3b), the onset of apoptosis in the cells was detected. At 0.5 µM concentration, K562 cells exhibited early and late apoptosis in both incubation periods (Figure 2c-3c). At 2.5 µM and above, early and late apoptosis were observed at 24 and 48 h (Figure 2d-3d).

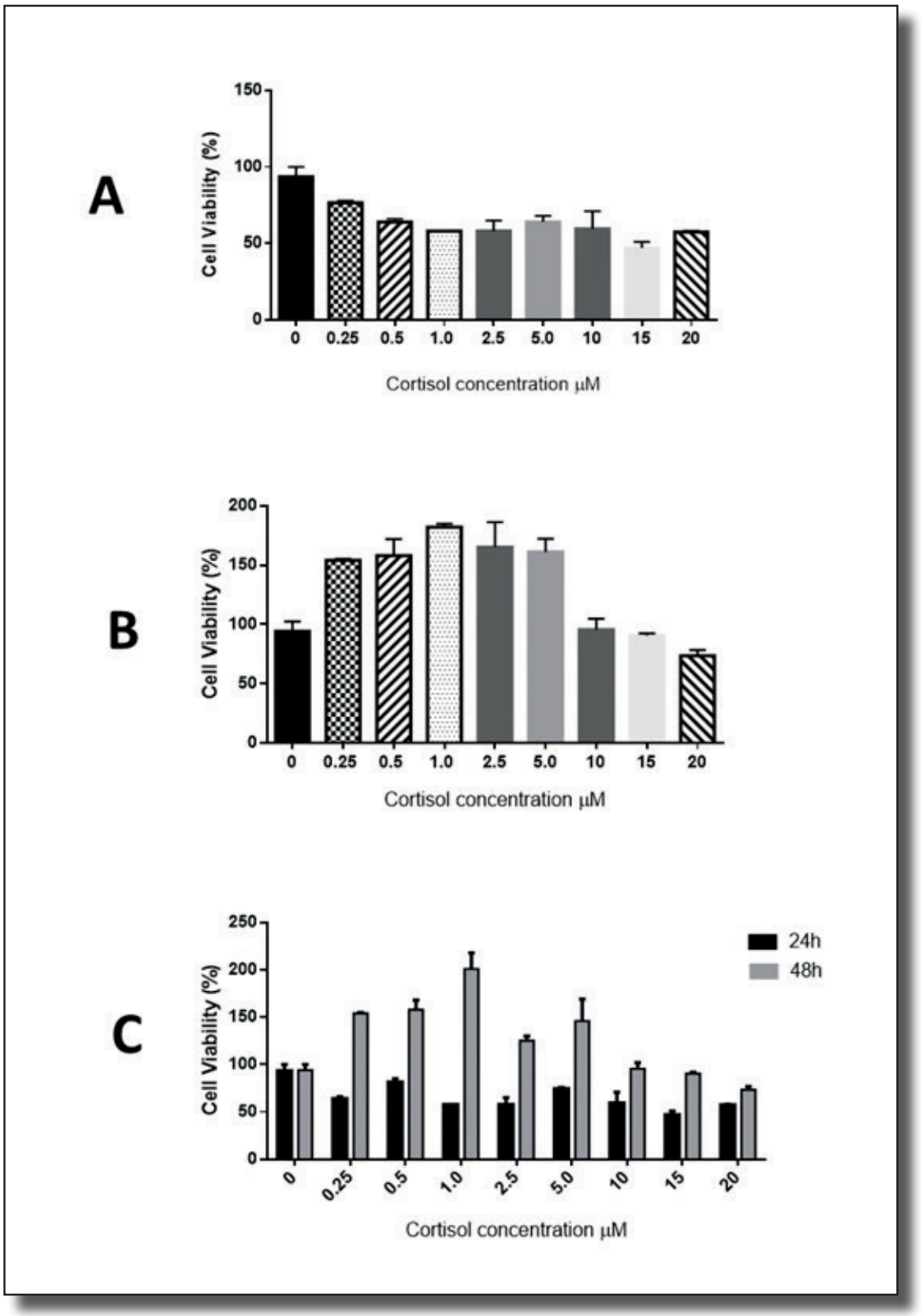

Figure 1– Metabolic activity in K562 after incubation with cortisol. Metabolic activity was assessed after 24 (A) and 48 hours (B) of incubation with increasing concentrations of cortisol's metabolic activity (A) in the MTT assay in K562 cells. Comparative metabolic activity of cells after 24 and 48 hours of incubation with increasing cortisol concentration (C).

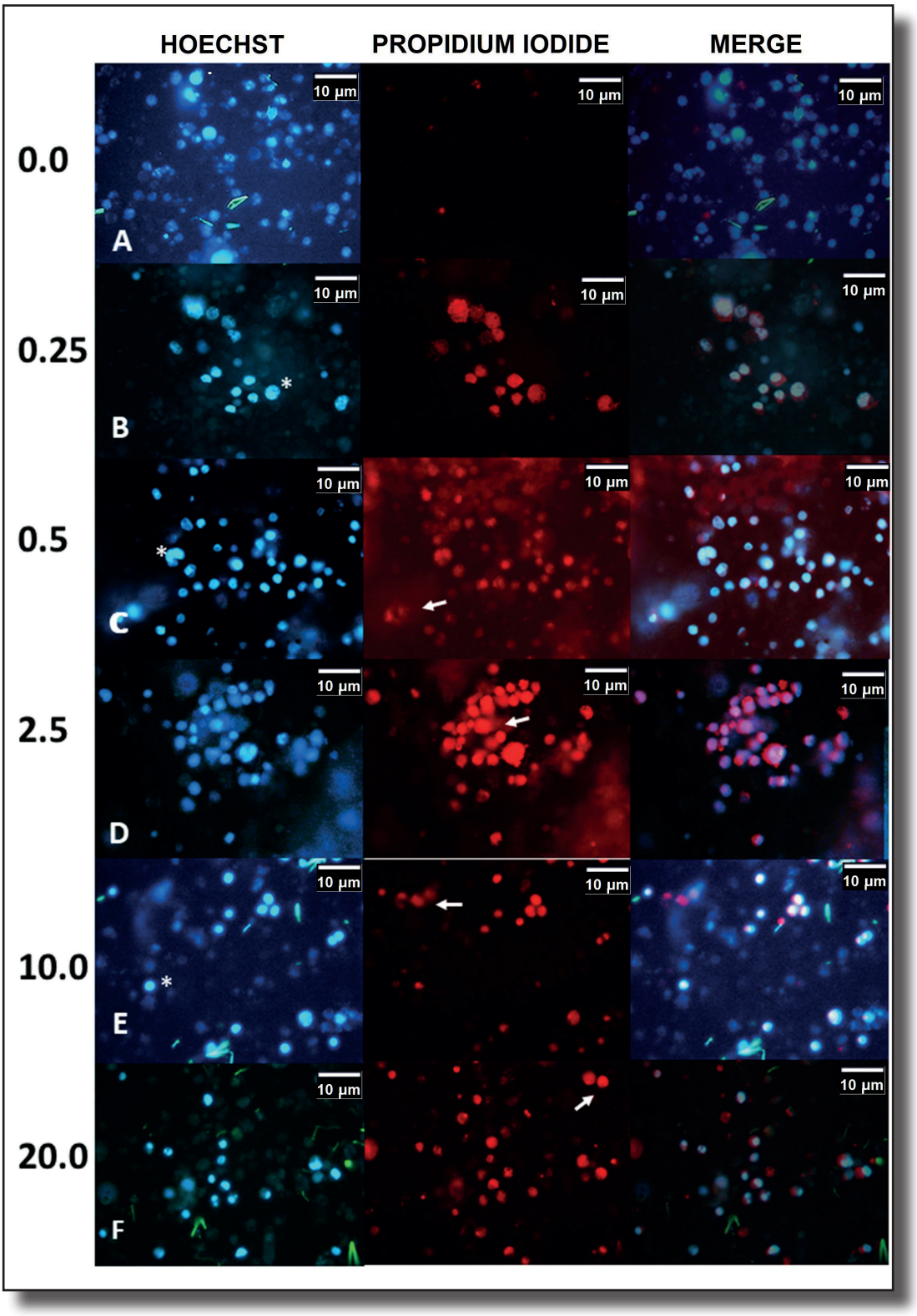

Figure 2– Staining K562 cells with Hoechst and propidium iodide. K562 cells were treated for 24 h with increasing cortisol concentrations. The nuclei marked with Hoechst are blue. Propidium iodide marks the necrotic cells red. Untreated cells demonstrated normal nuclei with Hoechst staining and no induction of necrosis with propidium iodide staining. In apoptotic cells, nuclei are generally fragmented and stained more intensely because of DNA condensation. (*). The necrotic cells are stained red (arrow).

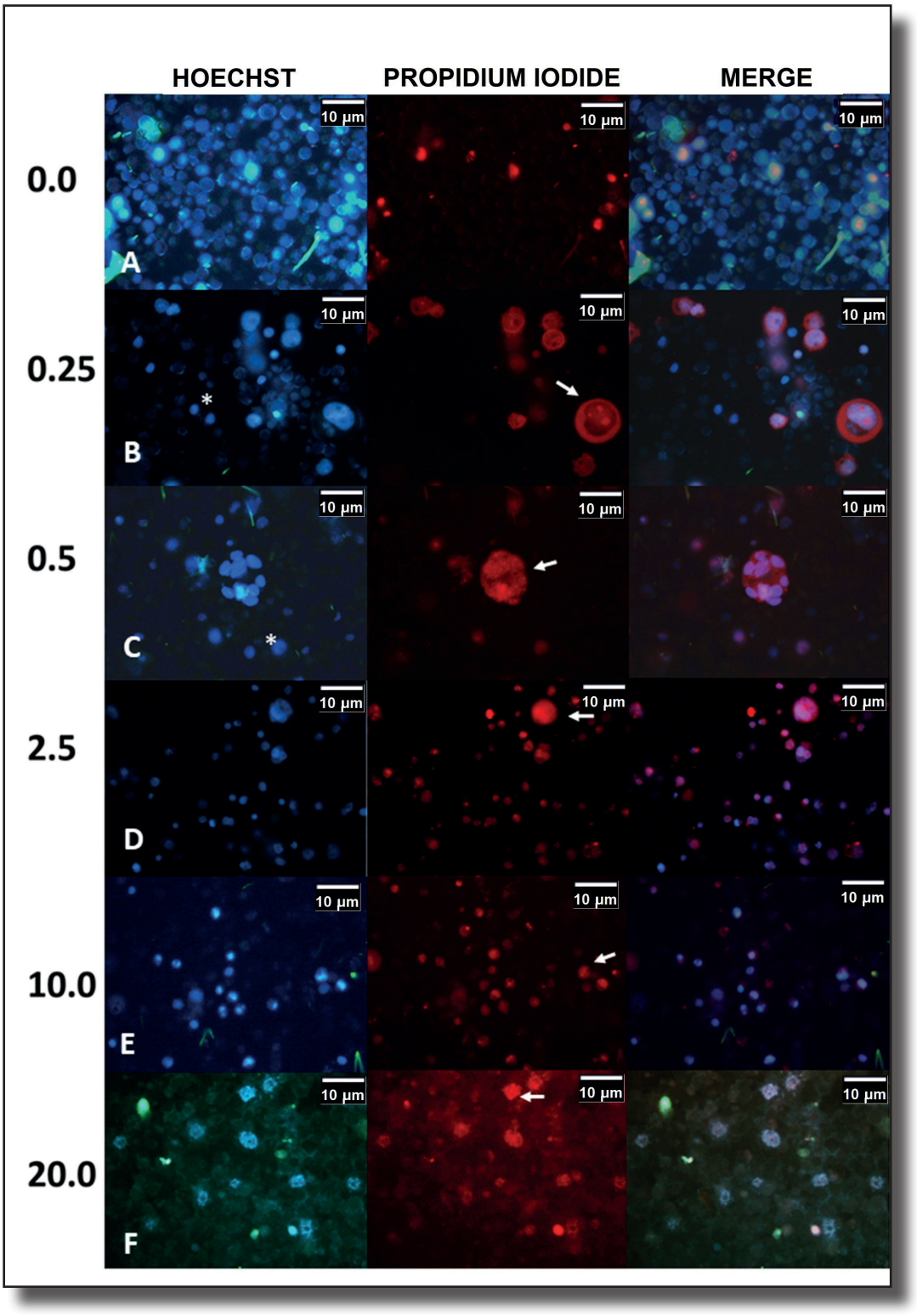

Figure 3– Staining K562 cells with Hoechst and propidium iodide. K562 cells were treated for 48 h with increasing cortisol concentration. The nuclei marked with Hoechst are blue. Propidium iodide labeled the red necrotic cells. Untreated cells demonstrated normal nuclei with Hoechst staining and no induction of necrosis with propidium iodide staining. In apoptotic cells, nuclei are usually fragmented and stained more intensely because of DNA condensation. (*) Cells in necrosis are stained red (arrow).

DISCUSSION

Cortisol action on K562 cells was evaluated by MTT test for cell viability and labeling with Hoechst 33342 and propidium iodide for cell death. The results obtained indicate that K562 cells incubated with cortisol for 24 hours showed reduced mitochondrial activity, indicating a cytotoxic effect on tumor cells. However, many cells survived cortisol action and showed increased mitochondrial activity as observed within 48 hours of incubation. Cell labeling for cell death assessment indicates cell death by mitochondrial activity-mediated intrinsic apoptosis[9], suggesting that, among other causes, cell death by mitochondrial may be initiated by increased corticosteroid concentrations within cells.

K562 cells were significantly resistant to low doses of cortisol, while concentrations greater than 5 μM were considered cytotoxic, corroborating the results obtained in neural stem cells[10.] The cell population remained virtually unchanged despite a 20-fold increase in concentration. Cortisol is an immunomodulator that reduces the ability of the immune system to detect and respond to tumor cells[11,14], acts on DNA repair mechanisms and modulates apoptosis[11,15,16].

Several studies have described the importance of glucocorticoids in tumor cell progression, activating pro-apoptotic effects or inhibiting the protective action of apoptotic events. These properties depend on variations in glucocorticoid concentration, exposure time and tissue characteristics[17,18 19]. It should be noted that studies with the K562 cell line had the same characteristic adaptability to injury-causing agents, and that the analysis in question may relate to the probable adaptability observed at the higher cortisol concentrations[20]. As described for neural stem cells, high cortisol concentrations are inhibitory, resulting in the loss of cell viability and proliferation through the apoptosis and necrosis processes10. It is known that glucocorticoids inhibit cell differentiation and the development of various brain synaptic regions[21,22]. The data presented in the fluorescence and brightfield images of K562 cells demonstrated that after 24 and 48 h of exposure, it is possible to see late apoptosis and necrosis. Similarly, observed cell death may be related to increasing cortisol concentrations and their exposure time. Necrosis and late apoptosis are occur starting from 5 μM and at 48 hours.

CONCLUSION

This study demonstrates that K562 cells can adapt to cortisol levels over time and concentration, and this plays an important role in the action of treatments to counteract the action of stress on tumor development.

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