Detection the Nitric Oxide Levels upon Exposure to Interferon Gamma and Dexamethasone in Chicken Lymphoid Cancer Cells

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ABSTRACT
Nitric oxide (NO) was linked to disease inflammation severity; however, it has been shown to play roles in tumor growth and tumor suppression. The aim of this study was to investigate NO regulation upon exposure of chicken lymphoid cancer (DT40) cells to interferon gamma (INFγ) and dexamethasone (DEX) at different concentrations. Pretreated-DT40 cells with 1μg INFγ/ml of the nutrition media were managed by adding DEX concentrations at 0.1, 1, 10, 50μM, INF+DEX group. Moreover, one group was left with no INFγ and DEX treatments, (NT) group, and other was received INFγ only, (INFγO) group. After 24hrs of the treatment, the levels of NO were measured in the cells by using Griess System, and the cell proliferation rate was also recorded. The findings recorded significantly (p<0.05) the lowest levels of NO in the INFγO cells in a comparison with the NT and INFγ+DEX cells. Moreover, the results displayed significant (p<0.05) lower levels of NO in the INFγ+DEX cells when only compared to those identified from the NT cells. However, INFγ+DEX cells at the DEX concentration 10μM showed no change in the NO levels when only compared with those from the NT cells. For the proliferation of the cells, the outcomes unveiled significant (p<0.05) lower proliferation rates in the INFγ+DEX cells when compared to those identified from the NT and the INFγO cells. On the other hands, the proliferation rate of the INFγO cells was significantly (p<0.05) higher than that recorded in the NT and INFγ+DEX cells. Thus, interferon gamma and dexamethasone play important roles in regulating the levels of nitric oxide in the cells, and that high levels of NO may suppress the progression of the tumor cells.

INTRODUCTION
The nitric oxide (NO), in different physiological and pathological activities, is a widespread water-soluble free-radical gas of a short-life period that is produced endogenously. NO has recently become an agent of concern for carcinogenesis and development of tumor growth. Nevertheless, the definition of its function in cancer biology is complicated and confusing. Tumor-progressing and inhibitory properties, depending on the time, location, and dosage, are believed to be parts of the NO activities. NO was proposed for the control of various events associated with cancer, such as angiogenesis, invasion and metastasis, and apoptosis and works as an anti-oncogenic agent (Shang, Li and Li, 2002; Harada et al., 2004; Ying and Hofseth, 2007).

Interferon game (INFγ), through the transcriptional regulation of related genes of the immune system, controls a wide range of cellular pathways. In the beginning of their discovery, the INFγ secretion was linked to viral infection and replication in the affected cells classified originally by the cell types responsible of their secretion; however, now, they are known, based on the sequence homological characteristics and their types of receptors, as type I and type II. The type I has the basic types of INFα (of 14 to 20 subtypes), INFβ, INFω, and INFγ, which have a shared heterodimeric receptor (IFNAR of 1 and 2 chains). INFs are low-level-secreted by all types of cells; however, cells of hematopoietic types are well known for high production of INFα and INFω, while INFβ are highly secreted by fibroblasts and with certain amounts from macrophages after stimulation. INFγ is the major component of INF type I. It is not related to INF type I due to structural differences and has a completely different chromosomal locus and receptor that binds to. It can be secreted by various cell types such as CD4+ T helper cell type 1 (Th1) lymphocytes, CD8+ cytotoxic lymphocytes, and natural killer (NK) cells, NKT cells, B cells, antigen-presenting cells (APCs) (Flaishon et al., 2000; Harris et al., 2000; Frucht et al., 2001; Jonasch and Haluska, 2001; Sen, 2001; Schroder et al., 2004).

The glucocorticoid receptor (GR) has a non-oncogenic activity compared to that from other hormone receptors such as the estrogen receptor (ER) and the androgen receptor (AR) that play important actions in breast and prostate cancer progression, respectively. Dexamethasone has been used for fighting lymphoid cancer cells; however, resistance has been developed in some cancer cells. For that, thorough studies were performed to understand the mechanisms behind this resistance and how to potentiate the work of DEX (Jinaba and Pui, 2010; Teuffel et al., 2011; Puaf, 2015).

The aim of this study was to investigate NO regulation upon exposure of chicken lymphoid cancer (DT40) cells...
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to interferon gamma (INFG) and dexamethasone (DEX) in different concentrations.

MATERIALS AND METHODS

Cell line and experiment

Chicken lymphoid cancer (DT40) cell lines were used that were exposed to INFG and DEX at different concentrations. Pretreated-DT40 cells with 1µg INFG/ml of the nutrition media were managed by adding DEX concentrations at 0.1, 1, 10, 50µM, INF+DEX group. Moreover, one group was left with no INFG and DEX treatments, (NT) group, and other was received INFG only, (INFGO) group. The exposure was continued for 24hrs.

Levels of NO and cell proliferation measurement

After 24hrs of the treatment, the levels of NO were measured in the cells by using Griess System, and the cell proliferation rate was also recorded.

RESULTS

The findings recorded significantly (p<0.05) the lowest levels of NO in the INFGO cells in a comparison with the NT and INF+DEX cells. Moreover, the results displayed significant (p<0.05) lower levels of NO in the INF+DEX cells when only compared to those identified from the NT cells. However, INF+DEX cells at the DEX concentration 10µM showed no change in the NO levels when only compared with those from the NT cells, figure 1.

![Figure 1: Nitric oxide regulation by GR upon tested compounds in DT40 cells. The findings recorded significantly (p<0.05) the lowest levels of NO in the INFGO cells in a comparison with the NT and INF+DEX cells. Moreover, the results displayed significant (p<0.05) lower levels of NO in the INF+DEX cells when only compared to those identified from the NT cells. However, INF+DEX cells at the DEX concentration 10µM showed no change in the NO levels when only compared with those from the NT cells.](image)

For the proliferation of the cells, the outcomes unveiled significant (p<0.05) lower proliferation rates in the INF+DEX cells when compared to those identified from the NT and the INFGO cells. On the other hands, the proliferation rate of the INFGO cells was significantly (p<0.05) higher than that recorded in the NT and INF+DEX cells, figure 2.

![Figure 2: Effects of different doses of DEX and IFNG on DT40 cells. For the proliferation of the cells, the outcomes unveiled significant (p<0.05) lower proliferation rates in the INF+DEX cells when compared to those identified from the NT and the INFGO cells. On the other hands, the proliferation rate of the INFGO cells was significantly (p<0.05) higher than that recorded in the NT and INF+DEX cells.](image)
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DISCUSSION
The function of NO is generally beneficial as a vasorelaxant and its presence in physiological conditions determines the effect of NO. NO favors and functions as either an anti-tumor or tumor promoter. There is a big debate going on about those two activities of this gas (Vahora et al., 2016).

The results identified important changes in the levels of NO in study groups. The findings recorded significantly (p<0.05) the lowest levels of NO in the INF+G cells in a comparison with the NT and INF+DEX cells. This indicates that INF may interfere with the production of NO leading to substantial decreases in the levels of this gas in the treated cells. Interestingly, the cells treated with INF only showed the highest proliferation rate which may approve that NO high levels are important in suppressing the cancer cell growth. The data, here, agree with Javanmard and Dana, 2012 (Javanmard and Dana, 2012) who found that high levels of INF encouraged decreases in the levels of NO production in the human umbilical vein endothelial cells. However, a disagreement with the current study findings can be shown when reviewing the work of these authors (Javanmard and Dana, 2012) who provided significant evidence that this reduction in the levels of NO had led to the highest levels of apoptosis in the treated cells. The physiological work of NO as an apoptotic agent may be disturbed due to timing and location which why the results, here, showed differences with the above mentioned study (Shang, Li and Li, 2002; Harada et al., 2004; Ying and Hofseth, 2007).

Moreover, the results displayed significant lower levels of NO in the INF+DEX cells when only compared to those identified from the NT cells. However, all those levels of NO resulted in higher apoptosis rates in the treated cells when compared to those from the NT and INFGO cell groups. This indicates restored functions of DEX via GR in the presence of INF (Inaba and Pui, 2010; Teuffel et al., 2011; Pufall, 2015).

CONCLUSION
Thus, interferon gamma and dexamethasone play important roles in regulating the levels of nitric oxide in the cells, and that high levels of NO may suppress the progression of the tumor cells.

REFERENCES