Journal of Cancer Science & Therapy
ISSN: 1948-5956

Matalka et al., J Cancer Sci Ther 2012, S18
DOI: 10.4172/1948-5956.S18-003

Research Article Open Access

Enhancing Doxorubicin-Induced MCA-Fibrosarcoma Cytotoxicity by an *Eriobotrya japonica* Hydrophilic Butanol-Treated Extract through Natural Killer Cells Activation

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Abstract

The combination of cytotoxic drugs with immunotherapy should be more effective than monotherapy alone since both therapeutic modalities may target different mechanisms. In addition, combination therapy may reduce adverse events associated with cytotoxic drugs. *Eriobotrya japonica* hydrophilic butanol-treated extract (EJWR) was found to modulate cytokines by enhancing IL-12, IFN-γ and TNF-α *in vitro* and *in vivo* and within tumor microenvironment. This was associated with enhancing survival time of mice bearing intra-peritoneal MCA fibrosarcoma (MCA FS). In the present work, we evaluated the combination of EJWR with doxorubicin (Dox) on MCA FS cytotoxicity using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction assay in the absence and presence of spleen cells or Natural Killer (NK) lymphocytes from tumor bearing mice. The results showed that Dox exhibited mild cytotoxicity to healthy spleen cells and EJWR reversed such cytotoxicity. In addition, increasing concentrations of Dox induced 40% ($p<0.01$) MCA FS cytotoxicity. This percent increased significantly to 60% at Dox 5 μM when co-cultured with NK cells from tumor bearing mice and increased further to 80% ($p<0.01$) when Dox was combined with EJWR. The latter increase in cytotoxicity was significantly ($p<0.01$) higher than each agent alone. This enhancement was associated with significant production of TNF-α and retaining IFN-γ levels from NK cells lysates. This concluded that the immunomodulator, EJWR, mediates NK activation and enhances Dox- induced MCA FS cytotoxicity.

Keywords: *Eriobotrya japonica;* NK cells; Cytotoxicity

Introduction

Despite recent advances in cancer therapy, treating solid cancer is still very difficult. This difficulty can be explained by the heterogeneity of cancer cells within the tumor microenvironment, the down regulation of the patient's immune system and the timing of diagnosis is usually late [1]. Thus a single-treatment modality is usually insufficient for the complete elimination of cancer cells and new therapeutic strategies from various aspects are needed.

Doxorubicin (Dox) is a potent chemotherapeutic drug and is widely used as a treatment of choice for solid tumors as well as lymphomas and some leukemias. Dox is known to interact with DNA by intercalation and thus inhibits macromolecular biosynthesis [2,3]. In addition, Dox was found to inhibit the progression of the enzyme topoisomerase and thus inhibiting DNA replication, and transcription [2-4]. Later, it has been proposed that Dox exerts an immunomodulatory activity since it has been shown that spleen cells and/or macrophages from Dox-treated mice have higher cell-mediated toxicity to tumor cell lines [5,6]. Furthermore, Dox administration resulted in increasing TNF, IL-1 and IFN-γ production in association with higher antitumor activity of peritoneal cells [7]. Recently, injection of Dox with plasmid-containing IL-12 gene enhanced the accumulation of IFN-γ in solid tumors [8]. In addition, Dox was found to inhibit TGF-β signaling in human lung carcinoma cell line [9].

The treatment of cancer patients with Dox, however, has several acute and chronic side effects. The acute effects are mainly myelosuppression, nausea, vomiting, weight loss, and arrhythmias, whereas the main chronic effect of Dox is severe cardiomyopathy with high risk of congestive heart failure [2-4]. Furthermore, Dox treated lymphoma and myeloma cell lines produces higher levels of IL-6 and IL-10 which were thought to induce chemoresistant [10].

Eriobotrya japonica (LINDL is a member of Rosaceae family and found in many countries. It has shown to have antimutagenic and antitumor effects in mouse tumor models and human oral tumor cell lines [11]. In addition, *Eriobotrya japonica* hydrophilic extract has been shown to induce *in vitro* and *in vivo* proinflammatory (IL-12, IFN-γ, TNF-α) cytokines production more than anti-inflammatory cytokines (IL-10) [12]. Moreover, the latter extract has been modified by butanol treatment to further modulate inflammatory and anti-inflammatory cytokines within tumor microenvironment, and shown to prolong survival of mouse bearing subcutaneous fibrosarcoma [12,13]. The induction of IFN-γ in the tumor microenvironment and spleen of MCA fibrosarcoma (MCA FS)-bearing mice by triple i.p. injection of such extract enhanced the activity of antitumor effectors cells [13].

In the present work, we evaluated the combination of *Eriobotrya japonica* hydrophilic butanol-treated extract (EJWR) with Dox on MCA FS cytotoxicity in the absence and presence of spleen cells or Natural Killer (NK) lymphocytes from tumor bearing mice. In addition, the latter effects were studied in association with the ability to modulate cytokines production such as IFN-γ and TNF-α. By

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Received November 05, 2012; **Accepted** December 12, 2012; **Published** December 14, 2012

Citation: Matalka KZ, Alsaadi MT, Qinna N, Mallah E, Awad R, et al. (2012) Enhancing Doxorubicin-Induced MCA-Fibrosarcoma Cytotoxicity by an *Eriobotrya japonica* Hydrophilic Butanol-Treated Extract through Natural Killer Cells Activation. J Cancer Sci Ther S18: 003. doi:10.4172/1948-5956.S18-003

utilizing both, the long term goal is to enhance the therapeutic activity of Dox via enhancing the immune system mainly the tumor infiltrating lymphocytes within the tumor microenvironment of solid tumors, and thus reducing Dox-induced adverse events and cancer cell resistance.

Materials and Methods

Materials

RPMI-1640, Fetal Bovine Serum (FBS), penicillin-streptomycin, and endotoxin-free Dulbecco's PBS without calcium and magnesium were purchased from Euroclone (Siziano, Italy). Igepal CA-630, Meth-A 3-methylcholanthrene (MCA; 98%) and Dox hydrochloride were products of Sigma (St.Louis, MO, USA). Trypsin and HEPES buffer were purchased from PAA Laboratories Gmbh (Linz, Austria) and T75 flasks were obtained from Costar (Cambridge, MA, USA). Mouse erythrocytes lysing kit and MagCellect mouse NK cell biotinylated antibody cocktail were products of R and D systems (UK). 3-(4,5-dimethylthiazolyl)-2,5 diphenyl-tetrazolium bromide (MTT reagent) and sodium dodecyl sulfate (MTT detergent) were purchased from Trevigen. Bovine serum albumin (BSA) was purchased from Research Organics (Cleveland, OH, USA). Tissue culture and Maxisorb 96-well flat plates were products of Nunc International (Denmark).

Plant material and extraction

Fresh leaves of EJ LINDL (Rosaceae) were collected, washed, grounded, and identified as described elsewhere [12,13]. Similarly, the extraction procedure is described in details elsewhere [12,13]. Before utilization in cell culture, EJWR was dissolved in endotoxin-free Phosphate Buffered Saline (PBS) and sterilized by filtration with 0.2 μm sterile filters and used fresh for each experiment. It has been noted that bacterial endotoxin levels from processed plants are variable but those plants that were cleaned have very low levels of endotoxins [12-14]. Furthermore, further extraction with two-phase partitioning such as in butanol, reduces endotoxin levels [15].

Animals

BALB/c males and females mice were utilized throughout the experiments as indicated. BALB/c mice were purchased from Yarmouk University (Irbid, Jordan). All mice were housed in a pathogen free environment at 22°C with 12 hours light/dark cycle with food and tap water *ad libitum*. All animal experiments were performed in compliance with FELASA guidelines (Federation of European Laboratory Animal Science Association) and the study protocol was approved by the Deanship of Scientific Research at Petra University.

MCA-induced tumors and cell culture

MCA fibrosarcoma (MCA FS) was induced in mice by injecting mice subcutaneously with 0.1 mg MCA/mouse dissolved in olive oil [13]. After 10-12 weeks, when the tumor size reached 1-2 cm in diameter it was removed aseptically, cut in small pieces, minced and cultured in T75 flask with RPMI-1640 and incubated at 37°C in humidified atmosphere and 5% CO_2. Media was changed every two days, and cells were split when they reached confluency.

Spleen and NK cells isolation

Mice were sacrificed by cervical dislocation and spleen tissues were collected, squeezed between two slides to generate a single cell suspension. Red blood cells were lysed using M-lyse buffer and then the cells were washed with washing buffer and centrifuged for 10 mins and the supernatant was discarded. The spleen cells were counted and added to a 96-well plate at density of 10^5/100 μl/well, incubated at 37°C in a humidified atmosphere with 5% CO_2.

For NK cells isolation, spleen cells as prepared above were re-suspended with 0.5 ml of cold MagCellect plus buffer and transferred to a polystyrene tube. MagCellect Mouse NK Cell biotinylated antibody cocktail was added mixed and then incubated for 15 minutes at 2-8°C. After the incubation, 100 μL of MagCellect streptavidin ferrofluid was added, mixed, and incubated for another 15 minutes at 2-8°C. The tube was put in the MagCellect Magnet for 6 minutes at room temperature, the unwanted cells migrated toward the magnet, and the desired cells were left in the suspension, removed by sterile Pasteur pipette into another polystyrene tube and repeated again. The purity of NK cells ranges between 80-90%. NK cells were counted, diluted to the proper concentration, and added to the 96 wells culture plate.

Cytotoxicity assay

MTT assay was performed to test the cytotoxicity of the Dox, EJWR, or their combination to cultured spleen cells or MCA FS tumor cells in 96-well plate at density of 10^5/100 μl/well incubated at 37°C in a humidified atmosphere with 5% CO_2. Spleen cells were cultured for 2 hrs whereas MCA FS cells were cultured for 24 hrs before Dox, EJWR, or their combination in a 100 μl volume/well was added and incubated for 48 hrs. Then MTT reagent was added to form a purple precipitate followed 4 hrs later with the addition of MTT detergent. When spleen or NK cells were co-cultured with the adherent MCA FS and before adding the MTT reagent, the supernatant content of each well was removed carefully leaving the adherent MCA FS in the wells. The absorbance was read at 490 nm by Microplate Reader SCO GmbH (Dingelsadt, Germany).

Spleen or NK cells culture for cytokine analysis

Spleen cells from healthy or tumor-bearing mice were treated as above and incubated with Dox, EJWR or their combination. In case of MCA FS stimulation, MCA FS cells were seeded into 96 well plates at a density of 10^5/well for 24 hrs, followed by the addition of spleen cells or NK cells at a similar density followed by the addition of Dox, EJWR, or both for another 24 hrs. After the second incubation, the content of the wells was harvested and added to a tube containing 50 μl of 0.1 % igepal CA-630 nonionic detergent diluted in endotoxin-free PBS for ten minutes at 4°C. The tubes were stored at -30°C for cytokine analysis.

Cytokines analysis

Measurement of NK cell-extracted IFN-γ and TNF-α level were performed using sandwich ELISA according to the manufacturer instructions (mouse Duoset cytokines; R& D Systems, UK).

Data analysis

The cytotoxicity data are presented as percent of survival of the target cells. The percent survival was calculated based on the control absorbance. The control setup (i.e. without or with spleen cells of NK cells) depends on each experiment. As for the cytokines analysis, the data are presented as pg/10^5 NK cells and control or the zero concentration was applied for each cytokine assay for each condition.

All the data in the figures are depicted as the mean ± standard error of the mean and assessed by using one way ANOVA analysis followed by a Tukey's test (95% confidence) for multiple comparisons (SPSS version 17). P value of <0.05 is considered statistically significant.

Results

EJWR reverses Dox-induced spleen cells cytotoxicity

Dox induced mild but significant ($p<0.05$) spleen cells cytotoxicity. This cytotoxicity reached 20% at 5-10 μM of Dox (Figure 1). On the other hand, EJWR (1-100 μg/ml) did not show significant cytotoxicity on mouse splenocytes. In addition, when EJWR was mixed with 5 μM Dox, it reversed Dox-induced splenocytes cytotoxicity (Figure 1).

Enhancing Dox and EJWR-induced MCA FS cytotoxicity by spleen cells

Dox induced significant MCA FS cytotoxicity in a concentration-dependent manner reaching 40% at 10 μM of Dox (Figure 2). Similarly, EJWR induced a mild, but significant MCA FS cytotoxicity reaching 20% at 10 and100 μg/ml of EJWR (Figure 3). When splenocytes from Tumor Bearing Mice (TBM) were added to Dox or EJWR culture system, MCA FS cytotoxicity increased significantly and in a concentration-dependent manner (Figures 2 and 3). Furthermore, the increase in MCA FS cytotoxicity was more evident in case of EJWR. However, the combination of EJWR (0.1-100 μg/ml) and Dox (5 μM) with or without spleen cells did not enhance MCA FS cytotoxicity (data not shown).

Enhancing NK cells-induced MCA FS cytotoxicity by Dox, EJWR and their combination

It was thought that the indirect cytotoxicity of Dox or EJWR was due to NK cells activation [12,16]. The idea is that EJWR stimulates NK cells non-specifically and thus enhances their cytotoxic function. Thus NK cells were isolated and introduced to MCA FS cells. Both healthy and TBM NK cells induced significant MCA FS cytotoxicity (Figure 4). However, healthy NK cells induced higher MCA FS cytotoxicity ($p<0.01$) than NK cells from TBM (Figure 4). Furthermore, Dox at 0.5 μM and above enhanced significantly ($p<0.05$-0.01) healthy NK cells-induced MCA FS cytotoxicity. However, the effect of higher concentrations of Dox (5 or 10 μM) on TBM NK cells-induced MCA FS cytotoxicity was not significantly different than that of healthy NK cells (Figure 4).

EJWR at 1 and 10 μg/ml enhanced significantly TBM NK cells-induced MCA FS cytotoxicity (Figure 5). In addition, when EJWR was added to Dox (5 μM) cultures, the TBM NK cells-induced MCA FS cytotoxicity increased significantly ($p<0.01$) more than each agent alone (70-80% versus 60%) (Figure 5). This latter effect was observed at 1 and 10 μg/ml of EJWR.

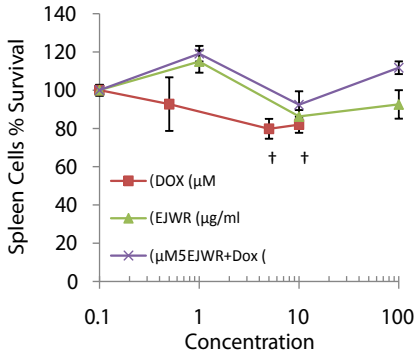

Figure 1: Cytotoxicity of Dox, EJWR and EJWR + Dox (5 μM) on healthy mouse spleen cells (10^5/well): Dox at 5 and 10 μM induced mild but significant spleen cells cytotoxicity (†$p<0.05$). EJWR, on the other hand, reversed such cytotoxicity.

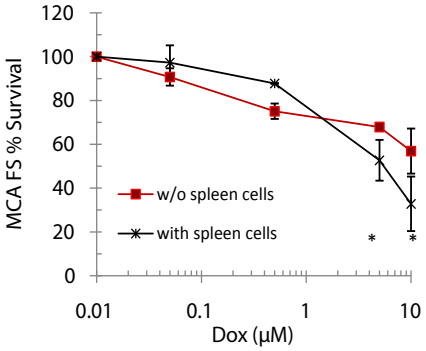

Figure 2: Effect of Dox without and with spleen cells from TBM on the survival of MCA FS: The ratio of cultured MCA FS to spleen cells is 1:1 (10^5 cells each). With spleen cells from TBM, Dox at 5 and 10 μM induced significantly higher MCA FS cytotoxicity than without spleen cells (*$p<0.01$).

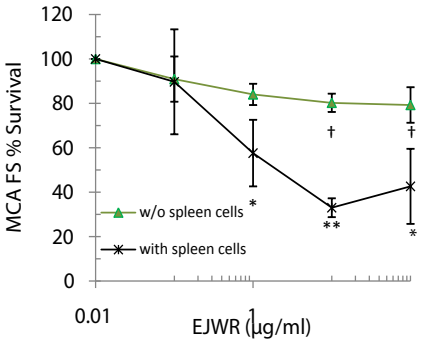

Figure 3: Effect of EJWR without and with spleen cells from TBM on the survival of MCA FS: The ratio of cultured MCA FS to spleen cells is 1:1 (10^5 cells each). EJWR induced a mild but significant MCA FS cytotoxicity (†$p<0.05$). With spleen cells from TBM, EJWR induced significantly higher MCA FS cytotoxicity than without spleen cells (*$p<0.01$; **$p<0.001$).

Modulation of NK cytokines production by Dox, EJWR and their combination

To investigate whether the increase in NK cells-induced MCA FS cytotoxicity was accompanied with cytokines modulation induced by Dox, EJWR and EJWR + Dox, IFN-γ and TNF-α were measured following such exposure. Dox (5 and 10 μM) increased significantly ($p<0.01$-0.001) IFN-γ production from healthy NK cells stimulated with MCA FS cells. This increase in IFN-γ production was not observed from TBM NK cells (Figure 6a). On the other hand, Dox at 0.05, 0.5 μM increased TNF-α production levels from TBM NK cells stimulated with MCA FS cells ($p<0.01$) (Figure 7a).

Regarding EJWR, 10 μg/ml increased the production of IFN-γ from healthy as well as TBM NK cells stimulated with MCA FS cells (Figure 6b). At 100 μg/ml, EJWR continued stimulating IFN-γ production from healthy NK cells only (Figure 6b). In addition, EJWR increased TNF-α production from healthy as well as TBM NK in a concentration dependent manner (Figure 7b). Furthermore, the combination of EJWR (1-10 μg/ml) and Dox increased significantly TNF-α production from TBM NK cells stimulated with MCA FS cells but it did not modulate IFN-γ production from such cells (Figures 6c and 7c). However, Dox + EJWR enhanced significantly the production of IFN-γ from healthy NK cells stimulated with MCA FS cells (Figure 6c).

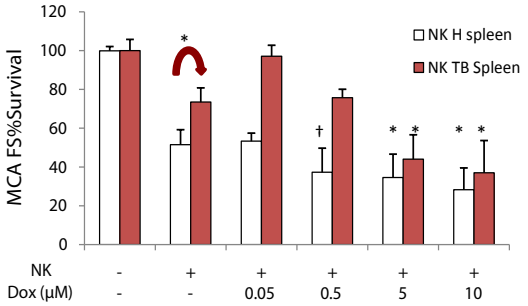

Figure 4: Effect of spleen NK cells isolated from healthy or TBM without and with Dox on the survival of MCA FS. The ratio of cultured MCA FS to NK cells is 1:1 (10^5 each). Healthy NK cells induced higher MCA FS cytotoxicity than NK cells obtained from TBM (*p<0.01). Dox at 0.5 μM and above enhanced significantly healthy NK cells induced MCA FS cytotoxicity (†p<0.05, p<0.01). However, the effect of higher concentrations of Dox (5 or 10 μM) on TBM NK cells induced MCA FS cytotoxicity was not significantly different than that of healthy NK cells.

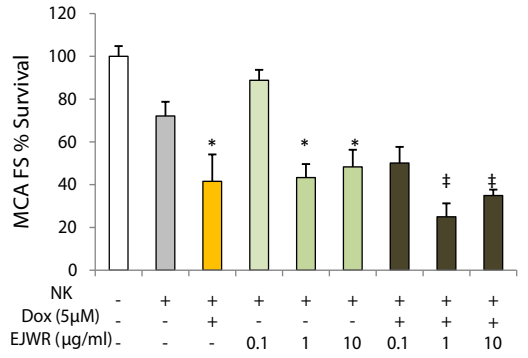

Figure 5: Effect of spleen NK cells isolated from TBM with Dox, EJWR and EJWR + Dox (5 μM) on the survival of MCA FS. Dox (5 μM), EJWR (1 and 10 μg/ml), and EJWR + Dox (5 μM) enhanced significantly NK-induced MCA FS cytotoxicity (*p<0.01). In addition, EJWR + Dox (5 μM) induced higher MCA FS cytotoxicity than single agent alone (‡p<0.01).

Discussion

It has been suggested that Dox has an immunomodulatory activities that could be related to its effectiveness in treating solid tumors and lymphomas [5-9]. Furthermore, it has been shown that Dox cytotoxicity can be enhanced by IFN-γ [17] and Dox induces interferon responsive genes via IFNγ-JAK-STAT1 signaling which enhances its cytotoxicity to HeLa cells [18]. Thus, in the present study, the aim was to enhance Dox cytotoxicity to MCA FS cells by combining it with an immunomodulator, EJWR [12,13]. Dox showed mild spleen cells cytotoxicity while EJWR reversed Dox-induced cytotoxicity. Furthermore, Dox induced moderate MCA FS cytotoxicity but this cytotoxicity was not modulated by EJWR. With the presence of spleen cells, Dox and EJWR exhibited higher MCA FS cytotoxicity indicating their immunomodulatory role in enhancing the function of cytotoxic immune cells to kill cancer cells. Such enhancing cytotoxicity is related to cytokine modulation by Dox and EJWR. Previous studies have shown that Dox treatment to tumor-bearing mice increased serum IL-12 and TNF-α [19,20] and IFN-γ [8]. In addition, EJWR stimulated the production of proinflammatory cytokines such as IFN-γ, TNF-α and IL-12 from human peripheral blood as well as in healthy mice [12], and reduced TGF-β from healthy mice spleen [13].

Since NK cells induce apoptosis to tumor cells without recognizing tumor specific antigens, they are considered major cells in the innate immune function [21]. In addition, since the present work depends on EJWR extract which is concentration dependant cytokines specific modulator; mainly enhance IFN-γ and TNF-α [12], we thought of studying its activity on NK cells functions. The MCA FS cytotoxicity was significantly higher when Dox or EJWR was co-cultured with NK cells obtained from healthy or TBM than either one alone indicating that Dox and EJWR do exhibit a positive effect on NK cells activity. In addition, the combination of Dox and EJWR increased significantly MCA FS cytotoxicity induced by NK cells more than single agent alone. This could be attributed to the induction of TNF-α from EJWR-induced NK cells that facilitate Fas ligand killing to MCA FS [22,23]. Furthermore, it has been shown that Dox can enhance the expression of Fas receptors on the tumor cells [24]. Thus the data presented herein could be due to enhancing the expression of Fas receptors on the MCA FS by Dox and the expression of FASL on NK cells via the TNF-α-mediated by EJWR in which both increase the interaction between tumor and NK cells and thus tumor cell apoptosis increases.

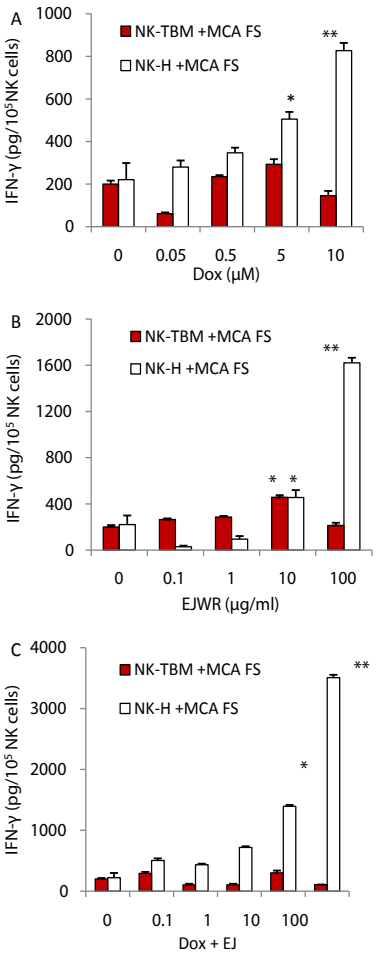

Figure 6: Effect of Dox, EJWR and EJWR + Dox (5 μM) on IFN-γ production from MCA FS-stimulated NK cells isolated from healthy and TBM. A) Dox at 5 and 10 μM increased significantly IFN-γ production only from healthy NK cells, B) EJWR at 10 μg/ml increased significantly IFN-γ production form NK-H as well as NK-TBM (*p<0.01 and **p<0.001 compared with its 0 counterpart). C) The combination EJWR+Dox (5 μM) only increased IFN-γ production from NK obtained from healthy mice (*p<0.01 and **p<0.001 compared with Dox counterpart).

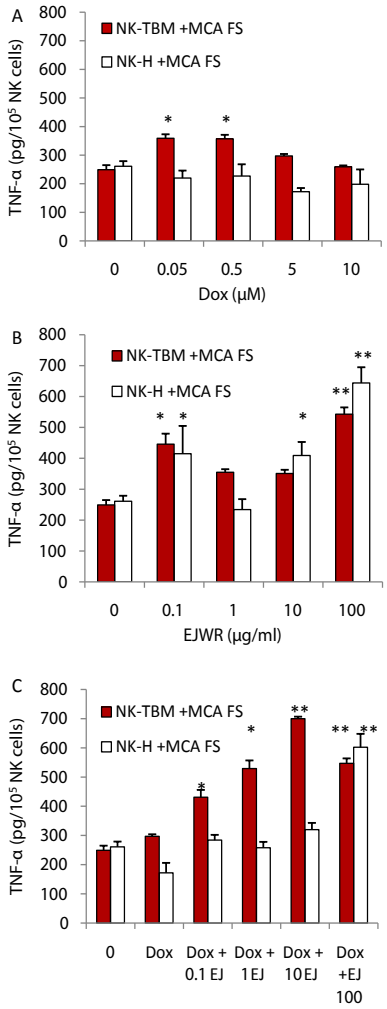

Figure 7: Effect of Dox, EJWR and EJWR + Dox (5 μM) on TNF-α production from MCA FS-stimulated NK cells isolated from healthy and TBM. A) Dox at 0.05 and 0.5 μM from NK-TBM increased the production of TNF-α (*p<0.01 compared with its 0 counterpart), B) EJWR increased TNF-α production from both NK cells (*p<0.01 compared with its 0 counterpart). C) The combination EJWR + Dox (5 μM) also increased TNF-α production from NK-TBM at 1-100 μg/ml and at 100 μg/ml from NK-H cells (**p<0.001 compared with Dox counterpart).

It is worth noting that the effect of Dox or EJWR on TNF-α and IFN-γ production from NK obtained from healthy versus TBM spleen is evident. TNF-α production was more pronounced in NK from TBM whereas IFN-γ production was not modulated as in healthy NK cells. It has been shown that Dox can induce NF-κB activation [25,26] and this activation was found to be essential in the cytotoxicity of Dox in several cancer cell lines [26]. On the other hand, the latter activation may also inhibit NK cells cytotoxicity if it is associated with IFN-γ depletion [27,28]. In addition, it has been shown that both TNF-α and IFN-γ signaling are required for T cells to inhibit tumorogenesis [29]. Since EJWR was found herein that it enhanced the production of both TNF-α and maintained IFN-γ secreting levels from NK; NK cell-induced cytotoxicity is enhanced by the combination of Dox + EJWR.

In conclusion, the combination of Dox and EJWR resulted in a significantly higher MCA FA cytotoxicity induced by NK cells more than each agent alone. This enhancement is associated with an increase in the production of TNF-α from NK cells from TBM. Further studies in blocking pro-tumor specific signaling pathways in the tumor microenvironment and secondary lymphoid organs of tumor-bearing host while sustaining Dox and EJWR anti-tumor immunomodulatory effects should increase tumor cells cytotoxicity, reduce Dox-induced adverse events and cancer cell resistance.

Acknowledgement

This work was supported by grants (#6-4-2010 and 3-4-2011) from the Deanship of Scientific Research at Petra University, Amman, Jordan.

References

1. Alshaker HA, Matalka KZ (2011) IFN-γ, IL-17 and TGF-β involvement in shaping the tumor microenvironment: The significance of modulating such cytokines in treating malignant solid tumors. Cancer Cell Int 11: 33.
2. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 56: 185-229.
3. Muller I, Niethammer D, Bruchelt G (1998) Anthracycline-derived chemotherapeutics in apoptosis and free radical cytotoxicity (Review). Int J Mol Med 1: 491-494.
4. Swift LP, Rephaeli A, Nudelman A, Phillips DR, Cutts SM (2006) Doxorubicin-DNA adducts induce a non-topoisomerase II-mediated form of cell death. Cancer Res 66: 4863-4871.
5. Ehrke MJ, Ryoyama K, Cohen SA (1984) Cellular basis for adriamycin-induced augmentation of cell-mediated cytotoxicity in culture. Cancer Res 44: 2497-2504.
6. Maccubbin DL, Wing KR, Mace KF, Ho RL, Ehrke MJ, et al. (1992) Adriamycin-induced modulation of host defenses in tumor-bearing mice. Cancer Res 52: 3572-3576.
7. Ujhazy P, Zaleskis G, Mihich E, Ehrke MJ, Berleth ES (2003) Doxorubicin induces specific immune functions and cytokine expression in peritoneal cells. Cancer Immunol Immunother 52: 463-472.
8. Zhu S, Waguespack M, Barker SA, Li S (2007) Doxorubicin directs the accumulation of interleukin-12 induced IFN gamma into tumors for enhancing STAT1 dependent antitumor effect. Clin Cancer Res 13: 4252-4260.
9. Filyak Y, Filyak O, Souchelnytskyi S, Stoika R (2008) Doxorubicin inhibits TGF-beta signaling in human lung carcinoma A549 cells. Eur J Pharmacol 590: 67-73.
10. Gougelet A, Mansuy A, Blay JY, Alberti L, Vermot-Desroches C (2009) Lymphoma and myeloma cell resistance to cytotoxic agents and ionizing radiation is not affected by exposure to anti-IL-6 antibody. PLoS One 4: e8026.
11. Ito H, Kobayashi E, Li SH, Hatano T, Sugita D, et al. (2002) Antitumor activity of compounds isolated from leaves of Eriobotrya japonica. J Agric Food Chem 50: 2400-2403.
12. Matalka KZ, Ali D, Khawad AE, Qa'dan F (2007) The differential effect of Eriobotrya japonica hydrophilic leaf extract on cytokines production and modulation. Cytokine 40: 235-240.
13. Alshaker H, Qinna N, Qadan F, Bustami M, Matalka K (2011) Eriobotrya japonica hydrophilic extract modulates cytokines in normal tissues, in the tumor of Meth-A-fibrosarcoma bearing mice, and enhances their survival time. BMC Compl Alter Med 11: 9.
14. Skorska C, Sitkowska J, Krysinska-Traczyk E, Cholewa G, Dutkiewicz J (2005) Exposure to airborne microorganisms, dust and endotoxin during processing of peppermint and chamomile herbs on farms. Ann Agric Environ Med 12: 281-288.
15. Magalhães PO, Lopes AM, Mazzola PG, Rangel-Yagui C, Penna TCV, et al. (2007) Methods of endotoxin removal from biological preparations: a review. J Pharm Pharm Sci 10: 388-404.
16. Soriani A, Zingoni A, Cerboni C, Iannitto ML, Ricciardi MR, et al. (2009) ATM-ATR-dependent up-regulation of DNAM-1 and NKG2D ligands on multiple myeloma cells by therapeutic agents results in enhanced NK-cell susceptibility and is associated with a senescent phenotype. Blood 113: 3503-3511.
17. Eppstein DA, van der Pas MA, Marsh YV, Bruno NA, Kurahara CG (1988) Enhanced antitumor efficacy of adriamycin in combination with interferon-gamma: effects on macrophages. J Interferon Res 8: 263-273.

18. Hussner J, Ameling S, Hammer E, Herzog S, Steil L, et al. (2012) Regulation of interferon-inducible proteins by doxorubicin via interferon γ-Janus tyrosine kinase-signal transducer and activator of transcription signaling in tumor cells. Mol Pharmacol 81: 679-688.

19. Ehrke MJ, Verstovsek S, Maccubbin DL, Ujhazy P, Zaleskis G, et al. (2000) Protective specific immunity induced by doxorubicin plus TNF-alpha combination treatment of EL4 lymphoma-bearing C57BL/6 mice. Int J Cancer 87: 101-109.

20. Ewens A, Luo L, Berleth E, Alderfer J, Wollman R, et al. (2006) Doxorubicin plus interleukin-2 chemoimmunotherapy against breast cancer in mice. Cancer Res 66: 5419-5426.

21. Sun JC, Lanier LL (2009) Natural killer cells remember: an evolutionary bridge between innate and adaptive immunity? Eur J Immunol 39: 2059-2064.

22. Russell JH, Ley TJ (2002) Lymphocyte-mediated cytotoxicity. Annu Rev Immunol 20: 323-370.

23. Alagkiozidis I, Facciabene A, Carpenito C, Benencia F, Jonak Z, et al. (2009) Increased immunogenicity of surviving tumor cells enables cooperation between liposomal doxorubicin and IL-18. J Transl Med 7: 104.

24. Micheau O, Solary E, Hammann A, Martin F, Dimanche-Boitrel MT (1997). Sensitization of cancer cells treated with cytotoxic drugs to Fas-mediated cytotoxicity. J Natl Cancer Inst 89: 783-789.

25. Mi J, Zhang X, Rabbani ZN, Liu Y, Reddy SK, et al. (2008) RNA aptamer-targeted inhibition of NF-kappa B suppresses non-small cell lung cancer resistance to doxorubicin. Mol Ther 16: 66-73.

26. Ashikawa K, Shishodia S, Fokt I, Priebe W, Aggarwal BB (2004) Evidence that activation of nuclear factor-kappaB is essential for the cytotoxic effects of doxorubicin and its analogues. Biochem Pharmacol 67: 353-364.

27. Tseng HC, Arasteh A, Paranjpe A, Teruel A, Yang W, et al. (2010) Increased lysis of stem cells but not their differentiated cells by natural killer cells; de-differentiation or reprogramming activates NK cells. PLoS One 5: e11590.

28. Jewett A, Tseng HC (2011) Tumor induced inactivation of natural killer cell cytotoxic function; Implication in growth, expansion and differentiation of cancer stem cells. J Cancer 2: 443-457.

29. Muller-Hermelink N, Braumuller H, Pichler B, Wieder T, Mailhammer R, et al. (2008) TNFR1 signaling and IFN-gamma signaling determine whether T cells induce tumor dormancy or promote multistage carcinogenesis. Cancer Cell 13: 507-518.