# Effect of recombinant human erythropoietin and doxorubicin in combination on the proliferation of MCF-7 and MDA-MB231 breast cancer cells

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Received March 8, 2015; Accepted August 19, 2015

DOI: 10.3892/mmr.2016.4989

**Abstract.** Patients with cancer often exhibit signs of anemia as the result of the disease. Thus, cancer chemotherapies often include erythropoietin (EPO) in the regime to improve the survival rate of these patients. The aim of the present study was to determine the effect of EPO on doxorubicin-treated breast cancer cells. The cytotoxicity of doxorubicin alone or in combination with EPO against the MCF-7 and MDA-MB-231 human breast cancer cells were determined using an MTT cell viability assay, neutral red (NR) uptake assay and lactate dehydrogenase (LDH) assay. The estimated half maximal inhibitory concentration values for doxorubicin and the combination of doxorubicin with EPO were between 0.140 and 0.260 $\mu$g/ml for all cells treated for 72 h. Treatment with doxorubicin in combination with EPO led to no notable difference in cytotoxicity, compared with treatment with doxorubicin alone. The antiproliferative effect of doxorubicin at a concentration of 1 $\mu$g/ml on the MDA-MB-231 cells was demonstrated by the decrease in viable cells from $3.6x10^5$ at 24 h to $2.1x10^5$ at 72 h of treatment. In order to confirm apoptosis in the doxorubicin-treated cells, the activities of caspases-3/7 and -9 were determined using a TBE assay. The results indicated that the activities of caspases-3/7 and -9 were significantly elevated in the doxorubicin-treated MDA-MB-231 cells by 571 and 645%, respectively, and in the MCF 7 cells by 471 and 345%, respectively, compared with the control cells. EPO did not modify the effect of doxorubicin on these cell lines. The results of the present study suggested that EPO was safe for use in combination with doxorubicin in the treatment of patients with breast cancer and concurrent anemia.

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*Key words:* erythropoietin, doxorubicin, proliferation, MCF-7, MDA-MB231, breast cancer

## Introduction

Patients with cancer often develop anemia as a result of the complex interaction of various factors, which renders treatment of the disease somewhat unpredictable (1,2). Among the factors contributing to anemia include hemodilution, bleeding, hypersplenism and hemophagocytosis, hemolysis, nutritional deficiencies, bone marrow damage, chemotherapy, radiotherapy and the anemia of the cancer itself (3-6). One of the causes of anemia in these patients may be due to the frequently lower than expected levels of circulating erythropoietin (EPO) for the degree of anemia (7-9).

EPO is a heavily glycosylated glycoprotein produced in the peritubular cells of the kidneys in response to hypoxia (10) and is vital as a hematopoietic hormone regulating erythrocyte production. The hormone binds to the EPO receptor to cause proliferation, differentiation and survival of erythoid progenitors. Each EPO binds to two EPO receptors on the erythroid cell surface to cause an effect (11-13).

Doxorubicin is the optimal known systemic chemotherapy, which may be used alone or in combination with a variety of agents, including, epirubicin, mitoxantrone, cisplatin and etoposide, in the treatment of breast cancer (14,15). However, doxorubicin frequently induces anemia in patients with breast cancer (16,17) by causing systemic changes, primarily through hemolysis or other conditions that reduce hemoglobin concentration (18-20). In patients with breast cancer, the management of anemia is beneficial for improvement of the survival rates of these patients.

Previously, EPO was found not to interfere with tamoxifen or Taxol in the treatment of MCF-7 or MDA-MB231 cells (21). Whether EPO can similarly affect the efficacy of doxorubicin in the treatment of breast cancer remains to be elucidated.

Therefore, the present study investigated the effect of EPO treatment in combination with doxorubicin on MDA-MB231 and MCF-7 cells. The present study also examined the mechanism underlying the cytotoxicity of this combination in these cancer cell lines. The present study aims to improve cancer therapeutics and provide potential insights to possible application of the recombinant human erythropoietin and doxorubicin combination in cancer therapy.

## Materials and methods

*Cell culture*. The three cell lines used in the present study were obtained from American Type Culture Collection (Rockville, MD, USA). These cell lines comprised the estrogen receptor-positive MCF-7 and estrogen receptor-negative MDA-MB-231 human breast cancer lines, and the normal MCF-10A breast cell line, which were characterized to be virus negative. These cells grow as an adherent monolayer of tightly knit epithelial cells.

*Cytotoxicity MTT assay*. The MCF-7, MDA-MB 231 and MCF-10A cells were seeded at $1x10^4$ cells/well by adding 200 $\mu$l of a $5x10^4$ cells/ml suspension to each well of a 96-well tissue culture plate. The cells were cultured at 37˚C for 24 h in the presence of 5% $CO_2$ until cell density of 50% confluence was obtained. The cells were then treated with either 1 $\mu$g/ml doxorubicin (Sigma-Aldrich, St. Louis, MO, USA), 1 IU EPO (Sigma-Aldrich) or a combination of 1 $\mu$g/ml doxorubicin and 1 IU EPO. Following 72 h incubation at 37˚C, 20 $\mu$l MTT solution (Sigma-Aldrich; 5 mg/ml) was added to each well and the plates were re-incubated for 4 h at 37˚C. The microplates were swiftly turned to discard the medium and the formazan precipitate was dissolved in dimethyl sulfoxide (100%; Ajax Finechem PTY Ltd., Sydney, Australia). The microplates were then gently agitated in the dark for 30 min and the absorbance was determined using a microtiter plate reader at 570 and 630 nm for background (Model 550; Bio-Rad Laboratories, Inc., Hercules, CA, USA). All experiments were performed in triplicate. The half maximal inhibitory concentration ($IC_{50}$) was determined from dose-response curves constructed for each cell line.

*Neutral red (NR) uptake*. The cells were seeded at $1x10^4$ cells/well into 96-well plates until they reached 40-60% confluence, and were subsequently incubated overnight at 37˚C in the presence of 5% $CO_2$ for cell attachment. After 24 h, the medium was removed and replaced with 200 $\mu$l fresh growth medium, containing either 1 $\mu$g/ml doxorubicin, 1 IU EPO or a combination of 1 $\mu$g/ml doxorubicin and 1 IU EPO. The plates were incubated at 37˚C, in 5% $CO_2$ for 72 h, following which the cells were washed three times with 200 $\mu$l phosphate-buffered saline (PBS) followed by the addition of 200 $\mu$l NR solution (Sigma-Aldrich). The cells were then incubated for 3 h at 25˚C. The NR solution was removed and the cells were exposed to fixing solution (Promega Corporation, Madison, WI, USA; 1% $CaCl_2$ and 0.5% formaldehyde in milliQ water) for 1-2 min, followed by two washing steps. The washing solution (Promega Corporation) consisted of 1% acetic acid and 50% ethanol in milliQ water. Following the second wash, the plates were incubated for 10 min, following which the plates were read using a microplate reader at 540 nm. A control experiment was performed on untreated cells under the same conditions. The intensity of NR staining was directly proportional to the number of viable cells.

*Lactate dehydrogenase (LDH) assay*. To determine the effect of doxorubicin on the membrane permeability of MCF-7 and MDA MB231 cells, an LDH release assay was used. The cells were seeded in 96-well culture plates at a density of $2x10^4$ cells/well in 100 $\mu$l and allowed to grow for 18 h prior to treatment. Treatments were performed, as described above for the MTT assay. Following incubation for 72 h, 40 $\mu$l of the supernatants were removed and placed in a fresh 96-well for the determination of LDH release. The original plate was replenished with 40 $\mu$l 6% Triton X-100 (Sigma-Aldrich) for determination of the total LDH concentration. An aliquot of 100 $\mu$l 4.6 mM pyruvic acid (Sigma-Aldrich) in 0.1 M potassium phosphate buffer (pH 7.5; Sigma-Aldrich) was dispensed into each well of the plate containing the supernatant only, and was mixed via repeated pipetting. Subsequently, 100 $\mu$l of 0.4 mg/ml reduced β-NADH (Sigma-Aldrich) in 0.1 M potassium phosphate buffer (pH 7.5) was added to the wells, and the kinetic change, based on the loss of NADH due to its oxidation to $NAD^+$ as pyruvate is converted into lactate, was determined. The change in absorbance at 340 nm was read for 1 min using an ELISA microplate reader (Model 550). This procedure was repeated using 40 $\mu$l of the total cell lysate from the original plate containing cells to determine the total LDH concentration. A change of 0.001 absorbance U/min was equivalent to 1 U/l of LDH activity (22). The percentage of LDH release was determined by dividing the LDH released in the supernatant by the total LDH in the respective cell lysate. Untreated cells retained LDH and exhibited minimal loss over the time.

*Trypan blue exclusion*. The MCF-7 and MDA MB231 cells were first seeded at $1x10^4$ cells/well into 6-well plates until they reached 40-60% confluence. Following 24 h of incubation to allow for cell attachment, the cells were exposed to 1 $\mu$g/ml doxorubicin or a combination of 1 $\mu$g/ml doxorubicin and 1 IU EPO. The plates were then incubated at 37˚C in 5% $CO_2$ for 24, 48 and 72 h. Following incubation, the media was removed and the cells washed with cold PBS to remove dead cells. Subsequently, 1 ml 0.05% (2 mg/ml) trypsin-EDTA (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was added to each well. The plates were re-incubated at 37˚C for 10-15 min, until the majority of the cells had detached. The cells were harvested, and the suspension was centrifuged at 2,000 x g for 10 min at 4˚C and the supernatant discarded. The cell suspension (20 $\mu$l) was mixed with 20 $\mu$l 0.4% trypan blue solution (Sigma-Aldrich). The cells were re-suspended and dye-excluded viable cells were microscopically counted using a Neubauer hemocytometer (Hirschmann Laborgeräte GmbH and Co. KG, Eberstadt, Germany).

*Microscopic examination of cell morphology*. The MCF-7 and MDA MB231 cells ($1x10^4$ cells/well) were seeded into 6-well plates. Following incubation for 24 h with Dulbecco's modified Eagle's medium (Sigma-Aldrich), the cells were treated

Table I. $IC_{50}$ values of DOX-EPO treatment of breast cancer cells lines for 72 h.

| | $IC_{50}$ ($\mu$g/ml) | | |
|---|---|---|---|
| Treatment | MCF-7 | MDA-MB231 | MCF-10a |
| DOX (1 $\mu$g/ml) | 0.217 | 0.121 | 0.149 |
| DOX-EPO (1 $\mu$g/ml-1 IU/ml) | 0.258 | 0.125 | 0.145 |

$IC_{50}$, half maximal inhibitory concentration; DOX, doxorubicin; EPO, erythropoietin.

either with 1 $\mu$g/ml doxorubicin (1 $\mu$g/ml), 1 IU/ml EPO or a combination of 1 $\mu$g/ml doxorubicin and 1 IU/ml EPO for 72 h. The untreated cells were used as a negative control. General morphological and membrane changes were examined under an inverted microscope (CMM 214; Nikon Corporation, Tokyo, Japan).

*Caspase-3/7 and -9 assays*. The extent of caspases-3/7 and -9 activation in the MDA-MB231 and MCF-7 cells were treated using the same formulations in the assays described above, and were assessed using a commercially available colorimetric assay kit, according to the manufacturer's instructions (CaspACE™ assay system; Promega Corporation). The caspase activity in a sample is proportional to the quantity of para-nitroaniline (pNA) product detected spectrophotometrically (Lambda 35; PerkinElmer, Inc., Waltham, MA, USA). This assay uses the caspase-specific substrate L-aspartic-L-glutamic-L-valyl-L-aspartic acid para-nitroaniline (DEVD-pNA) and L-leucine-L-glutamyl-L-histidyl-L-aspartic-p-nitroaniline acid amide (LEHD-pNA), labeled with pNA for caspase-3/7 and-9, respectively. Cleavage of the substrate by the specific cellular caspase yields free pNA, which can be detected spectrophotometrically at 405 nm. The cells were plated at a density of $1x10^6$ cells/culture dish. Following treatment, the cells were harvested by centrifugation at 2,000 x g for 10 min at 4˚C. The pellets were washed with PBS and lysed in 50 $\mu$l chilled cell lysis buffer (Promega Corporation) and maintained on ice for 10 min. The lysate was centrifuged at 10,000 x g for 1 min at 4˚C, and the supernatant was used to determine the caspase activities, which were measured colorimetrically at 405 nm by the production of pNA from the cleavage of DEVD-pNA and LEHD-pNA.

*DNA fragmentation*. DNA fragmentation was quantitatively determined using diphenylamine reagent. The cells were treated with doxorubicin alone or in combination with EPO at different time-points, and then harvested 12 and 24 h following treatment. Subsequently, 108 $\mu$l of 5 M perchloric acid (Sigma-Aldrich) was added to the samples, which were heated at 70˚C for 15 min, followed by the addition of two volumes of a solution containing diphenylamine reagent (Flinn Scientific, Batavia, IL, USA). The samples were stored at 4˚C for 48 h. The colorimetric reaction was quantified at 575 nm using an ultraviolet-visible thermo smart orbit spectrophotometer (PerkinElmer, Inc.). DNA from the pellet and supernatant were quantified. The degree of DNA fragmentation was determined according to the following equation: Degree of DNA fragmentation = $(DNA_{supernatant} / DNA_{(pellet+supernatant)}) \times 100\%$ (1).

Table II. $IC_{50}$ values of DOX-EPO treatment of breast cancer cells lines for 72 h, determined by neutral red exclusion assay.

| | $IC_{50}$ ($\mu$g/ml) | |
|---|---|---|
| Treatment | MCF-7 | MDA-MB231 |
| DOX (1 $\mu$g/ml) | 0.127 | 0.118 |
| DOX-EPO (1 $\mu$g/ml-1 IU/ml) | 0.143 | 0.120 |

$IC_{50}$, half maximal inhibitory concentration; DOX, doxorubicin; EPO, erythropoietin.

*Statistical analysis*. All experiments were completed in triplicate. The data were expressed as the mean ± standard deviation and analyzed using Minitab statistical software (version 15; Minitab Inc., State College, PA, USA). Treatment effects were determined using one-way analysis of variance followed by Tukey's post hoc analysis. A value of $P<0.05$ was considered to indicate a statistically significant difference.

## Results and Discussion

*Cell sensitivity to combination of doxorubicin and EPO*. The present study determined the sensitivity of the MDA-MB-231, MCF-7 and MCF-10A cells to doxorubicin by evaluating their survival following exposure for 72 h. Doxorubicin reduced the survival of all three cells in a dose-dependent manner (Fig. 1). Notably, the effect of doxorubicin was not selective towards breast cancer cells and also targeted the rapidly dividing normal cells. To investigate the effect of the combination treatment of doxorubicin and EPO, an equal combination of the two drugs were used. The combination treatment affected the cytotoxicity of doxorubicin. The estimated $IC_{50}$ values for doxorubicin and the combination of doxorubicin with EPO were between 0.140 and 0.260 $\mu$g/ml following 72 h treatment (Tables I and II).

*Lysosomal membrane activity*. Lysosomes function as digestive system cells (23), where degrading enzymes are located (24). In the NR cell uptake assay, the uptake of dye is considered to occur by passive diffusion across the viable cell membrane through proton pumps (25,26). The present study demonstrated that, in the MCF-7 cells, the lysosomal activity was proportionally lower, compared with the levels observed in the human breast cancer cells following treatment with doxorubicin or a combination of doxorubicin

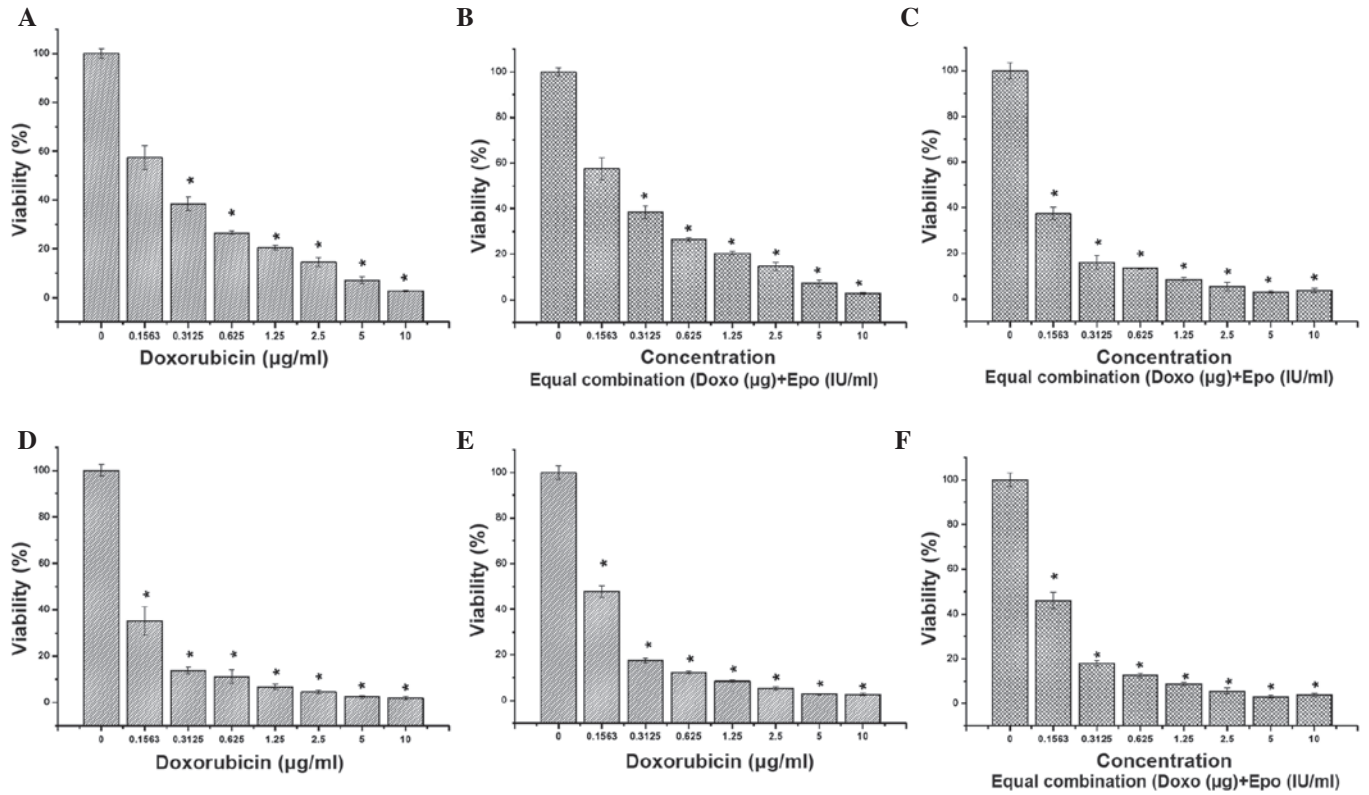


Figure 1. Treatment with Doxo, alone or combined with Epo decreases cell viability. Viability of the (A and B) MCF-7, (C and D) MDA-MB-231 and (E and F) MCF-10A cells after 72 h treatment with Doxo, alone or in combination with Epo. Data are expressed as the mean ± standard deviation (n=3 wells/treatment). *P<0.05, compared with the untreated cells. Doxo, doxorubicin; Epo, erythropoietin.

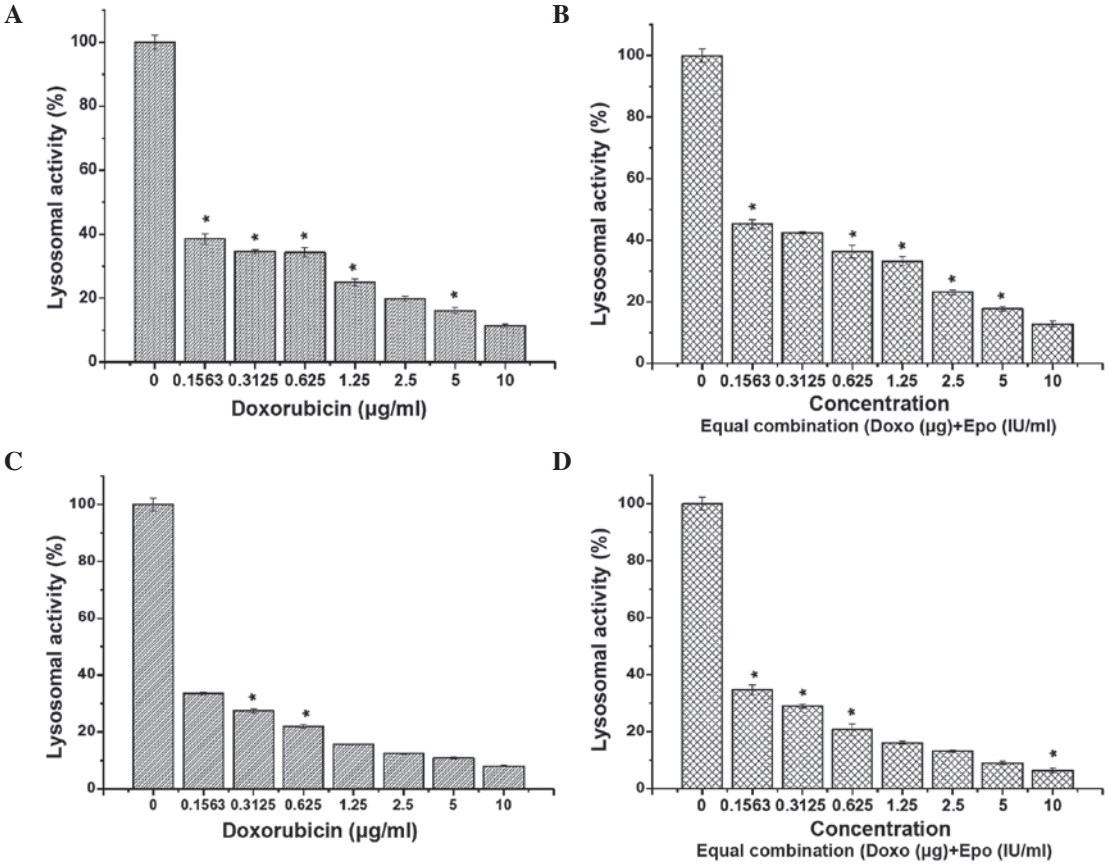


Figure 2. Treatment with Doxo, alone or combined with Epo decreases lysosomal activity. Lysosomal activity of the (A and B) MCF-7 and (C and D) MDA-MB-231 cells after 72 h treatment with Doxo, alone or in combination with Epo. Data are expressed as the mean ± standard deviation (n=3 wells/treatment). *P<0.05, compared with the untreated cells. Doxo, doxorubicin; Epo, erythropoietin.

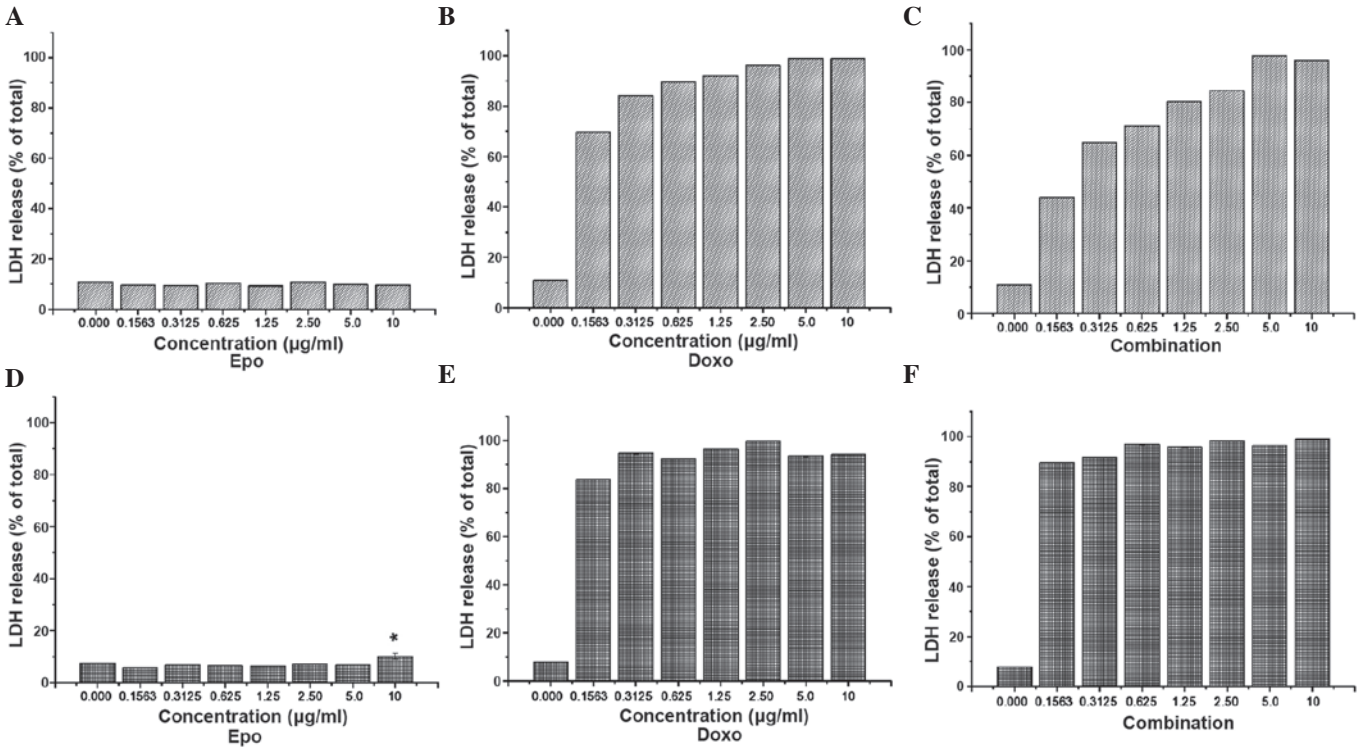


Figure 3. Treatment with Doxo, alone or combined with Epo increases LDH release. LDH release of the (A and B) MCF-7 and (C-F) MDA-MB-231 cells after 72 h treatment with Doxo, alone or in combination with Epo. Data are expressed as the mean ± standard deviation (n=3 wells/treatment). *P<0.05, compared with the untreated cells. Doxo, doxorubicin; Epo, erythropoietin; LDH, lactate dehydrogenase.

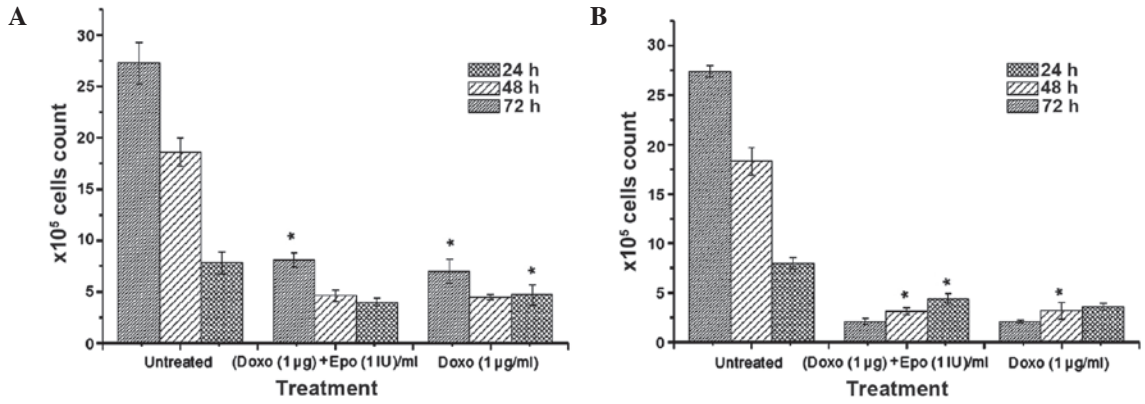


Figure 4. Treatment with Doxo, alone or combined with Epo has an antiproliferative effect. Antiproliferative effect of Doxo and Doxo-Epo combination on (A) MCF-7 and (B) MDA-MB231 cells. Data are expressed as the mean ± standard deviation (n=3 wells/treatment). *P<0.05, compared with the untreated cells. Doxo, doxorubicin; Epo, erythropoietin.

and EPO. This suggested that certain cells that appeared viable may have lost the ability to accumulate the NR dye, possibly through the loss of lysosomal membrane stability (Fig. 2). However, in the MDA-MB-231 cells, this effect was not evident, suggesting that the effect of doxorubicin and its combination with EPO differed between these human breast cancer cell lines.

*LDH release*. LDH is a leakage enzyme, which is released by dead cells. Thus, the measurement of LDH activity is another indicator of cell viability. As shown in Fig. 3, LDH release into the culture medium was examined following 72 h exposure to doxorubicin or the combination of doxorubicin with EPO. The exposure of MCF-7 to 0.31 $\mu$g/ml doxorubicin increased LDH leakage by up to 84%, compared with the increase of 68% observed following treatment with 0.15 $\mu$g/ml doxorubicin. However, increasing the doxorubicin concentration between 1.25 and 10 $\mu$g/ml caused only a marginal increase in LDH leakage, between 92 and 98%. Following treatment with 0.15 $\mu$g/ml doxorubicin and 0.15 IU/ml EPO, MCF-7 cell death decreased by 43%, which was lower than that observed in the Triton X-treated cells treated with 0.15 $\mu$g/ml doxorubicin (68%). Therefore, the MDA-MB-231 cells were more sensitive to doxorubicin, compared with the MCF-7 cells. In the MDA-MB-231 cells, LDH leakage was 84% following treatment with ~0.15 $\mu$g/ml doxorubicin for 72 h, suggesting

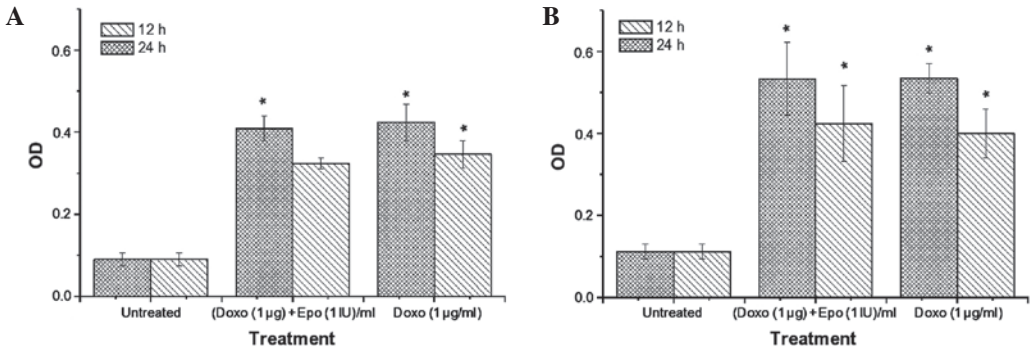


Figure 5. Treatment with Doxo, alone or combined with Epo increases the activity of caspase-3/7. Caspase-3/7 activity of the (A) MCF-7 and (B) MDA-MB231 cells following treatment with doxorubicin, alone or in combination with. Data are expressed as the mean ± standard deviation (n=3 wells/treatment). *P<0.05, compared with the untreated cells. Doxo, doxorubicin; Epo, erythropoietin; OD, optical density.

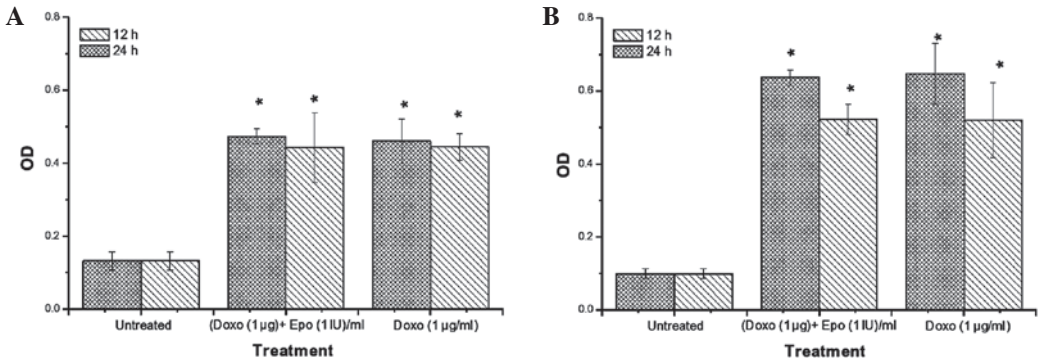


Figure 6. Treatment with Doxo, alone or combined with Epo increases the activity of caspase-9. Caspase-9 activity of the (A) MCF-7 and (B) MDA-MB231 cells following treatment with Doxo, alone or in combination with Epo. Data are expressed as the mean ± standard deviation (n=3 wells/treatment). *P<0.05, compared with the untreated cells. Doxo, doxorubicin; Epo, erythropoietin; OD, optical density.

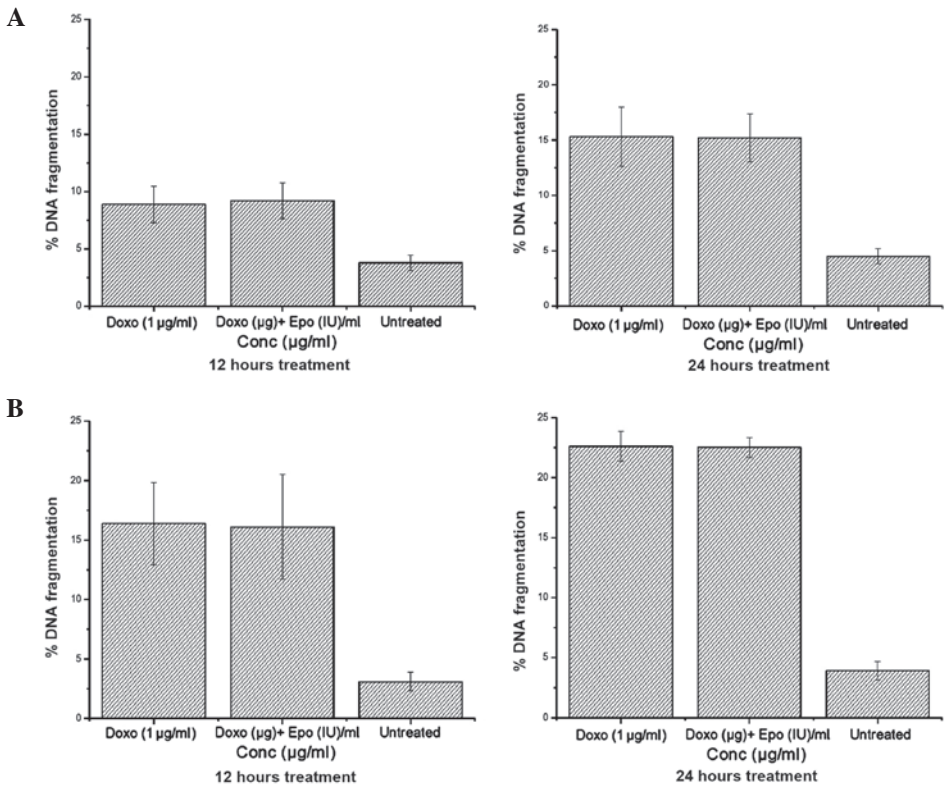


Figure 7. DNA fragmentation of the (A) MCF-7 and (B) MDA-MB231 cells treated with Doxo, alone or in combination with Epo. The percentages of DNA fragments are compared with the total DNA. Data are expressed as the mean ± standard deviation (n=3 wells/treatment). Doxo, doxorubicin; Epo, erythropoietin.

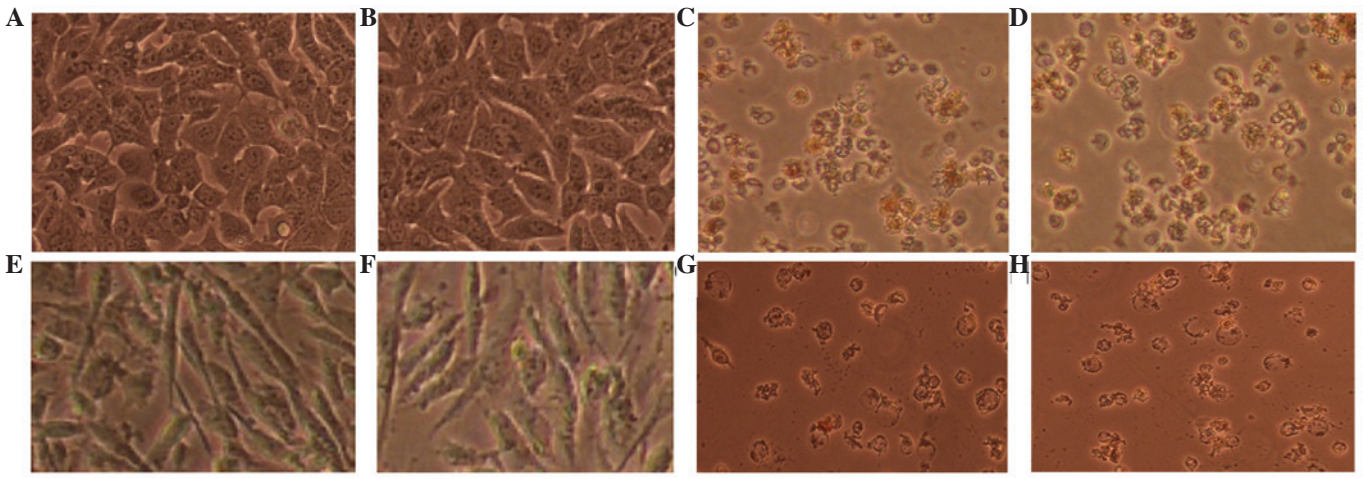


Figure 8. Photomicrographs (light microscopy; magnification, x200) showing morphological and growth inhibitory changes of (A-D) MCF-7 and (E-H) MDA-MB 231 cells following exposure to DOX, EPO or a combination of DOX and EPO for 72 h. (A and E) Untreated cells; (B-D) EPO, DOX and combination treated MCF-7 cells; (F-H) EPO, DOX and combination treated MDA-MB 231 cells. EPO, erythropoietin; DOX, doxorubicin.

that this cell line was more sensitive to the cytotoxic effect of doxorubicin, compared with the MCF-7 cells. None of the concentrations of the doxorubicin-EPO combination treatment suppressed the cytotoxicity of doxorubicin alone in the MDA-MB-231 cell line.

*Antiproliferation assay*. The results of the antiproliferative effect of doxorubicin either alone or in a combination with EPO on the MCF-7 and MDA-MB-231 cells following 24, 48 and 72 h of incubation are shown in Fig. 4. Consistent with the findings from the MTT assay, the combination of doxorubicin and EPO was marginally less cytotoxic to the MCF-7 cells, compared with doxorubicin treatment alone. The results also demonstrated that the MDA-MB-231 cells were more sensitive to doxorubicin, compared with the MCF-7 cells. The viable cell counts for the untreated MDA-MB-231 following 24, 48 and 72 h incubation were 8.0, 18.3 and 27.4x$10^5$/ml, respectively. As expected, the percentage survival of the doxorubicin-treated MDA-MB-231 cells decreased markedly with increase in exposure duration, which was in contrast to the untreated cells. The ratio of the viable cell to untreated cell counts at 24 and 72 h in the doxorubicin-EPO combination treatment group did not differ significantly to that in the doxorubicin alone treatment group.

*Caspase-3/7 and -9 activities*. Caspase-9 is an initiator of the mitochondria-mediated (intrinsic) apoptotic pathway (27), whereas caspase-3 is a major enzymatic marker of apoptosis (28). In the present study, the activities of caspase-3/7 (Fig. 5) and -9 (Fig. 6) increased significantly in the MCF-7 and MDA-MB-231 cells, compared with the untreated cells. Notably, the increase in caspase-3/7 activity appeared to be time-dependent. The highest caspase activities were observed in the MDA-MB-231 cells treated with doxorubicin. Doxorubicin-EPO combination treatment caused no significant difference in caspase activities, compared with the Dox alone group. After 24 h, the activity of caspase-3/7 in the MDA-MB-231 were 571 and 476% higher, compared with the untreated cells following doxorubicin and doxorubicin-EPO combination treatments respectively. By contrast, the same treatments produced 471 and 571% increases in caspase-3/7 activities, respectively, in the MCF-7 cells. Doxorubicin and its combination with EPO appeared to induce a more marked increase in caspase-9 activity in the MDA-MB-231 cells, compared with the MCF-7 cells. Following treatment for 24 h, the caspase activity in the MDA-MB-231 cells following doxorubicin and doxorubicin-EPO combination treatments were 645 and 635% higher than in the untreated cells, whereas the activities of this enzyme were 345 and 356% for the respective treatments in the MCF-7 cells. These results suggested that EPO did not alter the stimulatory effect of doxorubicin on the activities of caspases-3/7 and -9 in the breast cancer cell lines.

*DNA fragmentation*. The relative quantity of small DNA fragments in the cells treated with 1 $\mu$g/ml doxorubicin is shown in Fig. 7. In the two breast cancer cell lines, doxorubicin treatment increased DNA fragmentation in a time-dependent manner. No difference in DNA fragmentation was observed between the cells treated with doxorubicin alone or with doxorubicin in combination with EPO, however, these treatments caused 338 and 573% increases in DNA fragmentation in the MCF-7 and MDA-MB-231, respectively, compared with the untreated cells. These results suggested that doxorubicin was more sensitive to the estrogen-negative MDA-MB-231 cells than the estrogen-positive MCF-7 cells, and EPO did not modify the effect of doxorubicin.

*Morphological alterations*. The detachment of dead cells following exposure to doxorubicin was monitored using microscopic technique. Healthy cells remain elongated, whereas dying or dead cells are rounded and lose their adhesion to the culture plate. In the present study, inverse and fluorescence microscopy was performed following 72 h treatment of the MCF-7 and MDA MB231 cells with doxorubicin, which revealed marked morphological changes (Fig. 8). Cellular extensions were detected, cells were rounded and partially detached from the culture flask, and cellular membranes exhibited extensive blebbings.

Following 72 h incubation with 1.0 $\mu$g/ml doxorubicin and its combination with EPO, the majority of the adhered

MCF-7 cells were spherical and exhibited a markedly different morphology. The morphological changes caused by doxorubicin in the MCF-7 cells included detachment and floating of the cells, the presence of shrunken and dispersed cells, and a reduction in the formation of a monolayer. After 72 h, 1 $\mu$g/ml doxorubicin caused substantial morphological changes when added to the MDA MB231 cells, with cells being detached, shrunken and dispersed, and membrane blebbing and cytoplasmic shrinkage observed. EPO (1 IU/ml) did not exhibit any changes in either of these cell lines. The above-mentioned changes were more apparent in the MDA MB231 cells, compared with the MCF-7 cells when subjected to the doxorubicin treatment for 72 h.

The effect of doxorubicin or its combination with EPO were not selective for breast cancer cells as it also affected rapidly dividing normal breast cancer cells. However, the results of the cell viability investigations demonstrated that the MDA MB231 cells were more sensitive to the effect of doxorubicin or doxorubicin-EPO combination, compared with the MCF-7 cells. The results of the present study suggested that treatment with doxorubicin, either alone or in combination with EPO, induced apoptosis of the MCF-7 and MDA-MB-231 cells in a time-dependent manner, more through the caspase-9 than the caspase-3 pathway. This finding correlates with the observed increase in DNA fragmentation in the breast cancer cell lines following treatment. Of note, EPO did not modify the cytotoxicity of doxorubicin in the breast cancer cell lines, suggesting that these drugs can be safely used in combination in patients with breast cancer exhibiting symptoms of anemia.

## Acknowledgements

The authors would like to thank the Institute of Bioscience and the University Putra Malaysia (Serdang, Malaysia) for supporting the present study.

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