

Effects of Administered Cardioprotective Drugs on Treatment Response of Breast Cancer Cells

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Abstract. *Background:* Anticancer drug treatment, particularly with anthracyclines, is frequently associated with cardiotoxicity, an effect exacerbated by trastuzumab. Several compounds are in use clinically to attenuate the cardiac-damaging effects of chemotherapy drugs, including angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, the anti-diabetic drug metformin, and dexrazoxane. However, there is concern that the cardiac-preserving mechanisms of these drugs may also limit the anticancer efficacy of the chemotherapeutic agents. *Materials and Methods:* Herein two breast cancer cell lines, SKBr3 and BT474, overexpressing human epithelial receptor 2 (HER2), the target of the humanised antibody trastuzumab, were treated with a range of concentrations (20-2000 nM) of doxorubicin with and without trastuzumab in the presence of clinically relevant doses of the ACE inhibitor enalapril, the beta-blocker carvedilol, metformin or dexrazoxane, and cell survival determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. *Results:* None of the drugs reduced the anticancer effect of doxorubicin or trastuzumab (nor of the two drugs combined). Using Chou and Talalay's combination index, dexrazoxane and doxorubicin were found to act synergistically on the SKBr3 cells. ^{18}F -Fluoro-2-deoxy-D-glucose (^{18}F -FDG) incorporation was reduced by treatment of SKBr3 cells with doxorubicin and this was shown to be due to reduced phosphorylation of ^{18}F -FDG in doxorubicin-treated cells. Treatment of SKBr3 cells with doxorubicin and dexrazoxane further reduced ^{18}F -FDG incorporation, indicating that the synergy in the cytotoxicity of these two drugs was reflected in their combined effect on ^{18}F -FDG incorporation. *Conclusion:* Commonly administered cardioprotective drugs do not interfere with anticancer

activity of doxorubicin or trastuzumab. Further studies to establish the effect of cardioprotective drugs on anticancer drug efficacy would be beneficial.

Breast cancer is the most common neoplasia in European women, with an incidence in Europe of almost half a million cases in 2012 (1). Advances in patient treatment regimens have greatly improved breast cancer survival rates so that many patients live out their natural life. Unfortunately, anticancer drugs, especially anthracyclines, such as doxorubicin, can induce cardiotoxicity, including reduction in left ventricular ejection fraction and congestive heart failure (2). The humanized monoclonal antibody to the human epithelial receptor 2 (HER2), trastuzumab is used as part of the treatment course along with chemotherapy drugs to treat patients with HER2-overexpressing breast cancer as it can greatly improve outcome. However, trastuzumab can also exacerbate the cardiotoxic effects of chemotherapeutic drugs (3).

The anticancer effect of doxorubicin is mediated through its inhibition of topoisomerase II, preventing re-ligation of DNA during replication (4). However, doxorubicin can also induce the generation of reactive oxygen species (ROS), by iron cycling between Fe^{2+} to Fe^{3+} (5), which is believed to be its main cause of cardiotoxicity. Morphological changes induced by doxorubicin include heart chamber dilation, decreased ejection fraction, interstitial fibrosis, microfibril loss and degeneration of cardiomyocytes (6).

Attempts to counter the cardiotoxicity of anticancer treatments involving anthracyclines include co-administration of cardioprotective drugs, of which there are several, including angiotensin-converting enzyme (ACE) inhibitors such as enalapril (7), β -blockers such as carvedilol (8), metformin (9), and dexrazoxane (5).

The cardiac renin-angiotensin system plays a role in the development of doxorubicin-induced cardiomyopathy that may be attenuated by the use of ACE inhibitors (7, 10). ACE inhibitors have a number of beneficial effects, including reducing systolic ventricular wall stress, increasing cardiac output and reducing aldosterone-induced cardiac fibrosis (7). ACE inhibitors also reduce ROS levels (11) and apoptosis (12).

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Beta-blockers, including carvedilol (8), are cardioprotective by preventing catecholamine stimulation (which results in increased blood pressure and heart rate, and Ca^{2+} overload in cardiac cells) (13), maintaining the β -arrestin transactivation of the epidermal growth factor receptor pathway and preventing remodelling in a failing heart (14-16). Carvedilol also acts as an anti-oxidant and attenuates apoptosis (14, 17, 18).

The anti-diabetic drug metformin protects the heart (9) by activation of AMP-activated kinase (AMPK), which reduces the level of ROS in cardiac cells and cardiomyocyte apoptosis. Pre-treatment of cardiomyocytes with metformin prevented the drop in the level of anti-oxidant enzymes induced by doxorubicin and reduced doxorubicin-associated apoptosis (19).

Dexrazoxane protects the heart from doxorubicin by chelation of mitochondrial Fe (5), thereby preventing Fe-doxorubicin complex-induced ROS formation. Dexrazoxane also interferes with the formation of doxorubicin-topoisomerase II complexes, reducing doxorubicin-induced DNA damage (20).

In view of the importance of the induction of DNA damage and apoptosis in the anticancer action of doxorubicin, its attenuation in cancer cells could result in reduced cancer cell killing efficacy of doxorubicin. To examine such possible effects, HER2-overexpressing tumour cells were exposed to doxorubicin with/without trastuzumab in the absence or presence of clinically relevant concentrations of administered cardioprotective drugs. Positron-emission tomography (PET) with ^{18}F -fluoro-2-deoxy-D-glucose (^{18}F -FDG) is a very sensitive technique for measuring the effects of chemotherapeutic drugs on tumors. The incorporation of ^{18}F -FDG was examined in cells responding to doxorubicin in the presence of dexrazoxane.

Materials and Methods

Materials. SKBr3 and BT474 breast cancer cells were obtained from the LGC-Standards, Porton Down UK). Tissue culture medium was obtained from Invitrogen (Paisley, Scotland UK), Foetal bovine serum (FBS), streptomycin/penicillin and trypsin were produced by Gibco and obtained from Fisher-Scientific (Paisley UK). ^{18}F -FDG was obtained from the John Mallard PET Centre (Aberdeen UK). Unless otherwise stated all chemicals were obtained from Sigma-Aldrich (Poole, Dorset, UK). Tissue culture plastics were Greiner or Corning and obtained from Fisher-Scientific (UK). Radioactive measurements were made in a well counter with an Oakfield Instruments Ltd. (Oxford, UK) interface.

Tissue culture. SKBr3 and BT474 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and penicillin/streptomycin (5,000 unit/100 ml) in 75 cm^2 tissue culture flasks until confluent. Cells were passaged when confluent (1:10).

Cytotoxicity assay. The cytotoxic effect of doxorubicin and trastuzumab in the presence of clinically relevant doses of cardioprotective drugs on cancer cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After trypsin treatment, suspensions of SKBr3 (50,000 cells/ml) and BT474 (75,000 cells/ml) cells were prepared and seeded into nine columns of 96-well plates (flat-bottomed) at 100 μl per well. Water (100 μl) was placed in the outer-most wells to prevent evaporation of media, column 11 was kept free for background measurements. The plates were placed into a CO_2 incubator with a humidified atmosphere of CO_2 :air (5%:95%). The following day, 100 μl of medium was added to the first column of cells and 200 μl to the empty column (background wells). The cardioprotective drugs, metformin, carvedilol, enalapril or dexrazoxane were added to the lower three rows of wells with cells to give clinically relevant final concentrations of 5 μM (21), 25 ng/ml (22), 250 ng/ml (23) and 5 $\mu\text{g/ml}$ (24), respectively. An equivalent volume of medium was added to the three upper rows of cells. Doxorubicin was then added (either with or without 5 $\mu\text{g/ml}$ trastuzumab) to all the cells in ascending order of concentration to produce final concentrations of between 20 and 2,000 nM . The plates were returned to the incubator for 72 h. The medium was then removed from the plates by inversion and blotting on a paper towel. To each well, medium containing 0.5 mg/ml MTT was added and the plates were returned to the incubator. When the cells had taken up and metabolised sufficient MTT to appear dark (typically 1-2 h), the medium was removed and 100 μl dimethylsulfoxide added and the plates were read on a multiwell plater reader (Multiskan Ascent plate reader; Thermo Lab Systems, Loughborough, UK) with the wavelength set at 540 nm. Ascent software (Thermo Lab Systems) was used to process the raw data.

Combination index. Drug combination effects were determined using the combination index (CI) of Chou and Talalay (25): $\text{CI} = \text{Xa}/\text{Xb} + \text{Ya}/\text{Yb}$. Suitable drug concentrations (Xa, Ya) are used such that their combined effect reduces cell viability to about 30-60% that of the control. Xb and Yb are the concentrations of each drug which when used alone produce the same level of growth inhibition as the combination. A CI value of <0.9 is considered indicative of synergism.

^{18}F -FDG incorporation. SKBr3 cells were seeded (1×10^6 cell/flask) into 25 cm^2 tissue culture flasks. After 24 h incubation at 37°C , the medium was replaced with fresh containing no drugs (control flasks), dexrazoxane (5 $\mu\text{g/ml}$), doxorubicin (100 nM) or doxorubicin (100 nM) and dexrazoxane (5 $\mu\text{g/ml}$). The flasks were returned to the incubator for 72 h. The medium was then replaced with fresh-containing ^{18}F -FDG (37 kBq/ml) and the flasks incubated for 20 min at 37°C (uptake of ^{18}F -FDG is linear for at least 30 min in SKBr3 cells (26)). The cells were then washed rapidly five times with ice-cold phosphate-buffered saline (PBS). One millilitre of PBS was then added and the cells incubated for 5 min to release non-trapped (non-phosphorylated) ^{18}F -FDG (we have previously shown the remaining ^{18}F -FDG to be phosphorylated (27)). The PBS was removed and the radioactivity counted. The cells were detached with trypsin and radioactivity in the cells counted. The cells were then dissolved in NaOH (1 M) and after neutralisation with HCl, the protein content was determined using the bicinchoninic assay. The protein measurement was used to normalise the ^{18}F -FDG uptake data. The percentage phosphorylated

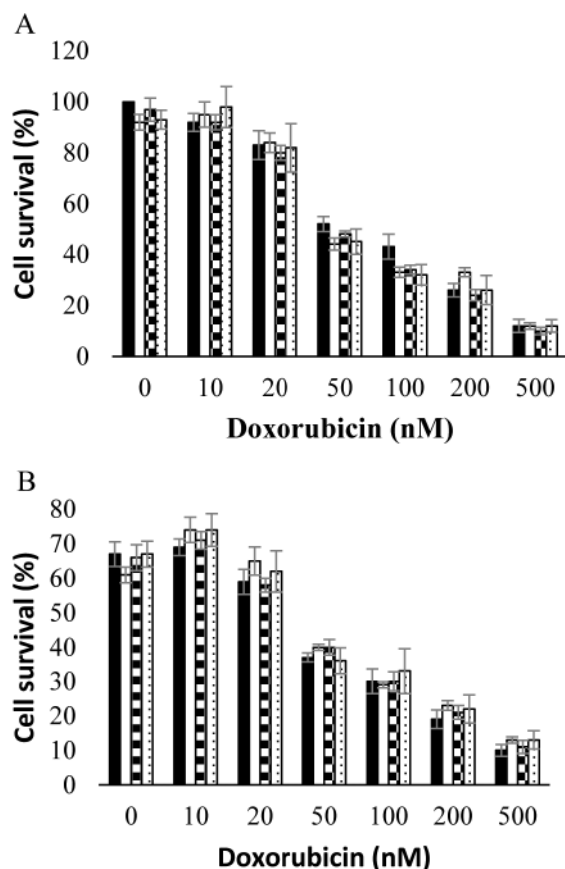


Figure 1. Mean (\pm standard deviation) survival (as a percentage that of untreated SKBr3 cells treated for 72 h with doxorubicin alone (A) or with trastuzumab (B) control (black) or co-incubated with 5 μ g/ml metformin (white), 25 ng/ml carvedilol (check) or 250 ng/ml enalapril (speckle).

^{18}F -FDG was determined by dividing the radioactivity in the cells by the total activity taken up by the cells (cell radioactivity + non-trapped ^{18}F -FDG) and is a measure of the phosphorylation rate. The procedure was also carried out using a 1-min incubation with ^{18}F -FDG to confirm differences in phosphorylation rates between treated and control cells.

Data are the mean [\pm standard deviation (SD)] from 3-5 separate experiments.

Statistical analysis. Statistically significant differences between means was determined using Student's *t*-test and the *p*-values are reported with the level of significance.

Results

Doxorubicin has a range of modes of action and it could be reasoned that the contribution of each to the cytotoxic effect could differ with doxorubicin concentration. Therefore a range of concentrations commensurate with levels in plasma of patients receiving doxorubicin was used.

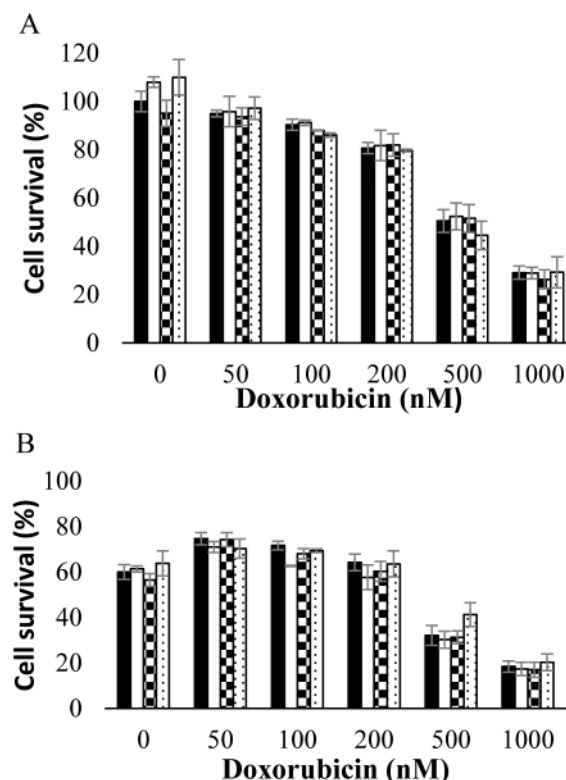


Figure 2. Mean (\pm standard deviation) survival (as a percentage that of the untreated BT474 cells treated for 72 h with doxorubicin alone (A) or with trastuzumab (B) control (black) or co-incubated 5 μ g/ml metformin (white), 25 ng/ml carvedilol (check) or 250 ng/ml enalapril (speckle).

Figures 1 and 2 show the effect of increasing doxorubicin concentration (with and without trastuzumab) on the growth of SKBr3 and BT474 cells, respectively, in the presence of clinically relevant doses of metformin, carvedilol or enalapril. SKBr3 cells were more sensitive than BT474 cells to doxorubicin treatment (Figures 1 and 2) and the growth-inhibitory effect was increased when combined with trastuzumab at all but the lowest concentration of doxorubicin. Trastuzumab also inhibited the growth of BT474 cells, but when combined with the lower concentrations (50-200 nM) of doxorubicin (Figure 1B and 2B), the growth-inhibitory effect was not as great as that with trastuzumab alone; however, at higher doxorubicin concentrations (>200 nM), growth inhibition was greater than at either alone. Incubation with metformin, carvedilol or enalapril did not interfere with the growth-inhibitory effect of doxorubicin neither alone nor in combination with trastuzumab.

The modulatory effect of dexrazoxane on the growth-inhibitory effect of doxorubicin with/without trastuzumab was carried out in a separate series of experiments (Figure 3) using a further batch of each cell line. Again, the inclusion

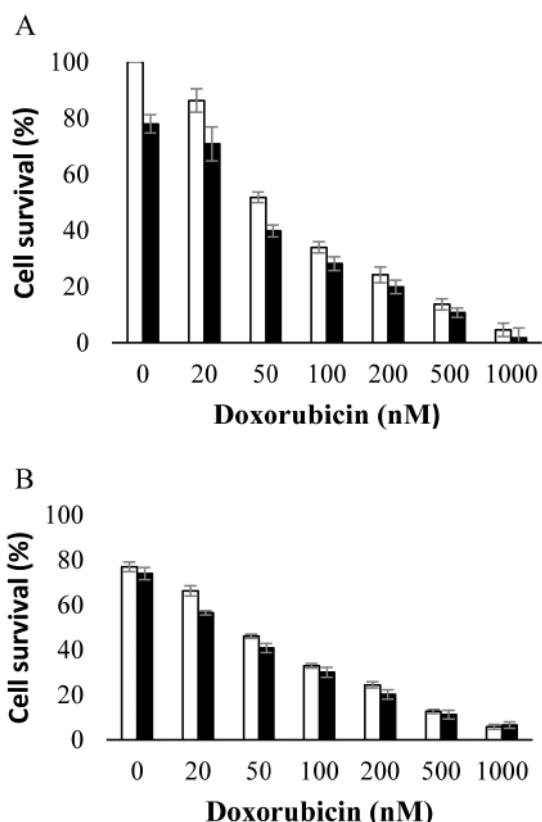


Figure 3. Mean (\pm standard deviation) survival (as a percentage that of the control) of SKBr3 (A, B) and BT474 (C, D) cells treated with doxorubicin alone (A, C) or with trastuzumab (B, D) control (black) or co-incubated 5 μ g/ml dexrazoxane (white).

of trastuzumab enhanced the growth-inhibitory effect of doxorubicin, but in the case of the BT474 cells, the effect of trastuzumab alone was greater than that with the lower doses of doxorubicin, suggesting that doxorubicin antagonizes the action of trastuzumab.

The inclusion of dexrazoxane at a concentration of 5 μ g/ml appeared to increase the growth-inhibitory effect of doxorubicin and to a lesser extent that of doxorubicin and trastuzumab combined especially in SKBr3 cells. However, this trend did not achieve statistical significance, therefore a more accurate analysis was applied to examine the possibility of synergy between doxorubicin and dexrazoxane on SKBr3 cells – the CI test, for which a dexrazoxane growth-inhibitory curve was required (Figure 4). The mean (\pm SD) CI of four separate experiments was 0.645 (\pm 0.084), indicating synergism between the two drugs in growth inhibition of SKBr3 cells.

Changes in the uptake of 18 F-FDG, determined clinically using PET, is a sensitive indicator of cancer cell drug response. 18 F-FDG incorporation was found to decrease in cells treated for 72 h with 100 nM doxorubicin ($t=8.8$,

$p<0.001$) and the presence of dexrazoxane further reduced ($t=2.5$, $p<0.05$) the incorporation of FDG in doxorubicin-treated cells (Figure 5A). Out of the 18 F-FDG that entered cells, the percentage that was phosphorylated was significantly ($t=2.97$, $p<0.05$) lower in doxorubicin-treated cells than in control cells (Figure 5B). To confirm the slower rate of phosphorylation in doxorubicin-treated cells, a 1-min incubation with 18 F-FDG (Figure 5C) was used where the difference ($t=3.76$, $p<0.01$) in the phosphorylated 18 F-FDG became even more apparent.

Discussion

Cardiomyopathy is a major side-effect of doxorubicin-based anticancer therapies, especially when this drug is combined with trastuzumab. However, several classes of cardioprotective drugs are helping prevent the development of cardiac problems including congestive heart failure. The findings of the present study suggest that administered cardioprotective drugs do not interfere with the cytotoxic effect of doxorubicin and trastuzumab and, in the case of dexrazoxane, may in fact enhance their anticancer efficacy.

Some cardioprotective drugs are activated by hepatic metabolism which is true for enalapril and dexrazoxane. Dexrazoxane is converted to ADR925 which is a chelator that can bind Fe, preventing redox cycling. However, like anthracyclines, dexrazoxane itself can inhibit the action of topoisomerase II (28), although by a different mechanism, which could account for the synergy in the growth-inhibitory effect of doxorubicin and dexrazoxane in SKBr3 cells. SKBr3 cells were shown to be more sensitive to doxorubicin than were BT474 cells, reflecting the overexpression of topoisomerase II in this cell type (29).

Inclusion of trastuzumab increases the response and survival of patients with breast cancer receiving anthracyclines (30). The mechanism of action of trastuzumab, induced by binding to HER2 on the tumour cell surface, includes inhibition of cell signaling, particularly the protein kinase B (AKT) pathway (31), down-regulation of HER2 expression and IgG receptor mobilisation of immune cells, including antibody-directed cellular cytotoxicity (32). Herein lower concentrations (up to 200 nM) of doxorubicin reduced the growth-inhibitory effect of trastuzumab on BT474 cells but this effect was less apparent in SKBr3 cells. Treatment of breast cancer cells with doxorubicin has been shown to modulate (sometimes increasing) the activation of AKT and this effect has been shown to vary from one cell line to another (33). The doxorubicin-attenuating effect on the growth inhibition induced by trastuzumab in BT474 cells may reflect opposing effects in the action of the two drugs on the AKT pathway. However, *in vivo* this may not be a problem due to the immune-mediated mechanisms by which trastuzumab also inhibits tumour growth.

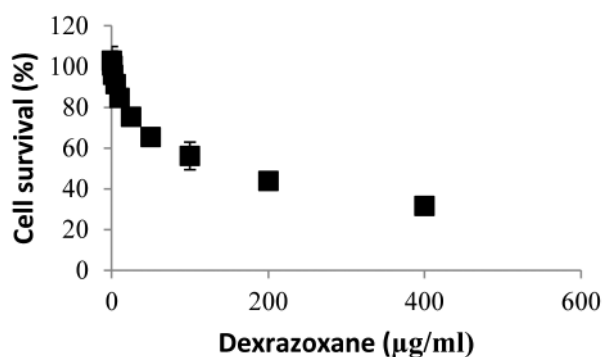


Figure 4. Mean (\pm standard deviation) survival (as a percentage that of the control) of SKBr3 cells treated for 72 h with dexrazoxane.

^{18}F -FDG-PET is a very sensitive method for monitoring response of cancer to chemotherapy including anthracycline-based regimens (34) that reduced ^{18}F -FDG incorporation. Changes in the incorporation of tracers by tumours *in vivo* can reflect therapy-induced changes in blood flow rather than differences in incorporation at the tumour cell level. Herein reduced ^{18}F -FDG incorporation in breast cancer cells treated with doxorubicin occurred at the tumour cell level. Dynamic ^{18}F -FDG-PET studies can establish rate-constants for each component of ^{18}F -FDG incorporation (35) *in vivo* and phosphorylation of ^{18}F -FDG has been showed to be rate limiting in breast cancer. Correspondingly, we show that the decrease in ^{18}F -FDG was associated with a slower rate of ^{18}F -FDG phosphorylation in doxorubicin-treated cells, supporting *in vivo* evidence that this step is rate-limiting in ^{18}F -FDG incorporation in breast cancer cells.

In summary, administered cardioprotective drugs were found not to reduce the cytotoxic effect of doxorubicin or trastuzumab. Decreased ^{18}F -FDG uptake induced by treatment with doxorubicin reflects a reduced rate of ^{18}F -FDG phosphorylation

Acknowledgements

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References

- 1 <http://www.europadonna.org/breast-cancer-facs/> accessed 8 Sept 2015.
- 2 Swain SM, Whaley FS and Ewer MS: Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer* 97: 2869-2879, 2003.
- 3 Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M, Mackey J, Glaspy J, Chan A, Pawlicki M, Tamas P, Valero V, Liu MC, Sauter G, von Minckwitz G, Visco F, Bee V, Buyse M, Bendahmane B, Tabah-Fisch I, Lindsay MA, Riva A

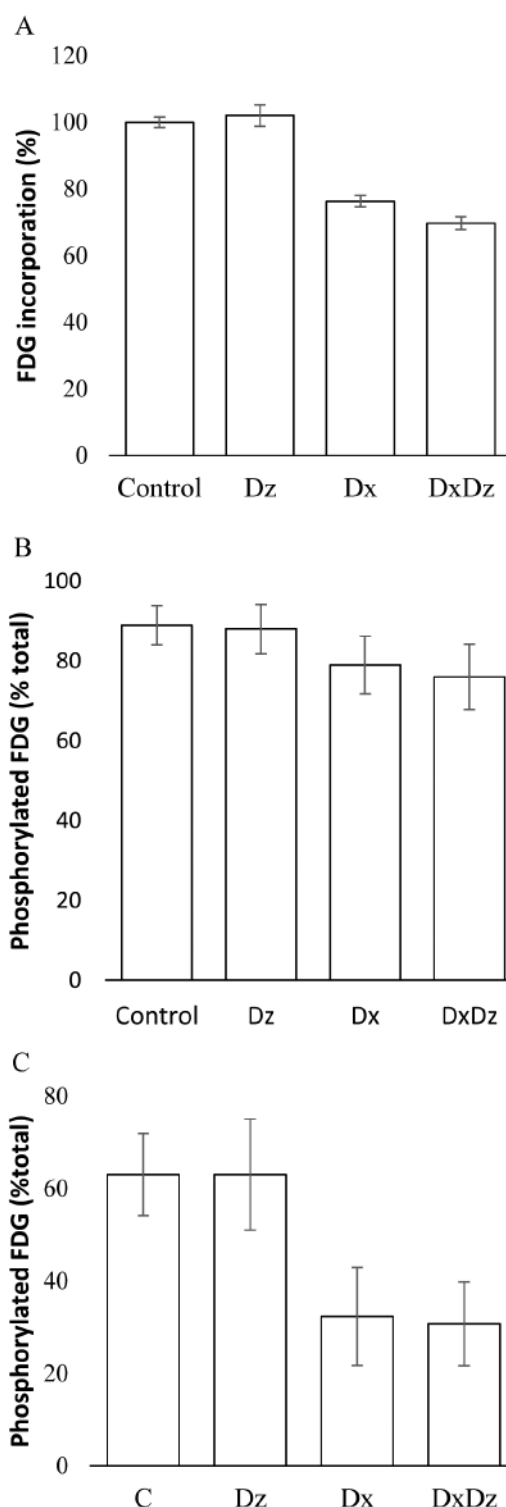


Figure 5. 2- ^{18}F -Fluoro-2-deoxy-D-glucose (^{18}F -FDG) incorporation per mg protein (as a percentage that of the control) in control, dexrazoxane (DZ), doxorubicin (Dx) and doxorubicin plus dexrazoxane treated cells (A). Percentage of phosphorylated ^{18}F -FDG in control, dexrazoxane, doxorubicin and doxorubicin plus dexrazoxane treated cells incubated for 20 min (B) or 1 min (C) with ^{18}F -FDG.

- and Crown J: Adjuvant trastuzumab in HER2-positive breast cancer. *New Eng J Med* 365: 1273-1283, 2011.
- 4 Binaschi M, Bigioni M, Cipollone A, Rossi C, Goso C, Maggi CA, Capranico G and Animati F: Anthracyclines: selected new developments *Curr Med Chem Anticancer Agents* 1: 113-130, 2001.
- 5 Ichikawa Y, Ghanefar M, Wu R, Khechaduri A, Prasad SVN, Mutharasan RK, Naik TJ and Ardehali H: Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *J Clin Invest* 124: 617-630, 2014.
- 6 Chatterjee K, Zhang J, Honbo N and Karliner JS: Doxorubicin cardiomyopathy. *Cardiol* 115: 155-162, 2010.
- 7 Cardinale D, Colombo A, Sandri MT, Lamantia G, Colombo N, Civelli M, Martinelli G, Veglia F, Fiorentini C and Cipolla CM: Prevention of high risk induced cardiotoxicity in high-risk patients by angiotensin-converting enzyme inhibition. *Circulation* 114: 2474-2481, 2006.
- 8 Kalay N, Basar E, Ozdogru I, Er O, Cetinkaya Y, Dogan A, Inanc T, Oguzhan A, Eryol NK, Topsakal R and Ergin A: Protective effects of carvedilol against anthracycline-induced cardiomyopathy. *J Am Coll Cardiol* 48: 2258-2262, 2006.
- 9 Sasaki H, Asanuma H, Fujita M, Takahama H, Wakeno M, Ito S, Ogai A, Asakura M, Kim J, Minamino T, Takashima S, Sanada S, Sugimachi M, Komamura K, Mochizuki N and Kitakaze M: Metformin prevents progression of heart failure in dogs: role of AMP-activated protein kinase *Circulation* 119: 2568-2577, 2009.
- 10 Cardinale D, Colombo A, Lamantia G, Colombo N, Civelli M, De Giacomo G, Rubino M, Veglia F, Fiorentini C and Cipolla CM: Anthracycline-induced cardiomyopathy: Clinical relevance and response to pharmacologic therapy. *J Am Coll Cardiol* 55: 213-220, 2010.
- 11 Vaynblat M, Shah HR, Bhaskaran D, Ramdev G, Davis WJ III, Cunningham JN Jr. and Chiavarelli M: Simultaneous angiotensin-converting enzyme inhibition moderates ventricular dysfunction caused by doxorubicin. *Eur J Heart Fail* 4: 583-586, 2002.
- 12 Kushwaha S, Vikram A and Jena GB: Protective effects of enalapril in streptozotocin-induced diabetic rat: studies of DNA damage, apoptosis and expression of CCN2 in the heart, kidney and liver. *J Appl Toxicol* 32: 662-672, 2012.
- 13 Haskova P, Koubkova L, Vavrova A, Mackova E, Hruskova K, Kovarikova P, Vavrova K and Simunek T: Comparison of various iron chelators used in clinical practice as protecting agents against catecholamine-induced oxidative injury and cardiotoxicity. *Toxicol* 289: 122-131, 2011.
- 14 Kim IM, Tilley DG, Chen J, Salazar NC, Whalen EJ, Violin JD and Rockman RA: Beta-blockers alprenolol and carvedilol stimulate beta-arrestin-mediated EGFR transactivation. *Proc Natl Acad Sci USA* 105: 14555-14560, 2008.
- 15 Hjalmarson A, Goldstein S, Fagerberg B, Wedel H, Waagstein F, Kjekshus J, Wikstrand J, El Allaf D, Vitovec J, Aldershvile J, Halinen M, Dietz R, Neuhaus KL, Janosi A, Thorgeirsson G, Dunselman PHJM, Gullestad L, Kuch J, Herlitz J, Rickenbacher P, Ball S, Gottlieb S and Deedwania P: Effects of controlled-release metoprolol on total mortality, hospitalizations, and well-being in patients with heart failure: the metoprolol CR/XL Randomised intervention trial in congestive heart failure (MERIT-HF). *JAMA* 283: 1295-302, 2000.
- 16 Garg R and Yusuf S: Overview of randomised trials of angiotensin converting enzyme inhibitors on mortality and morbidity in patients with heart failure. *JAMA* 273: 1450-1456, 1995.
- 17 Spallarossa P, Garibaldi S, Altiera P, Fabbi P, Manca V, Nasti S, Rossettin P, Ghigliotti G, Ballestrero A, Patrone F, Barsotti A and Brunelli C: Carvedilol prevents doxorubicin-induced free-radical release and apoptosis in cardiomyocytes *in vitro*. *J Mol Cell Cardiol* 37: 837-844, 2004.
- 18 Habon T, Szabados E, Kezmarky G, Halmosi L, Past T, Sumegi B and Toth K: The effect of carvedilol on enhanced adp-ribosylation and red blood cell membrane damage caused by free radicals. *Cardiovasc Res* 52: 153-160, 2001.
- 19 Asensio-Lopez MC, Lax A, Pascual-Figal DA, Valdes M and Sanchez-Mas J: Metformin protects against doxorubicin-induced cardiotoxicity: Involvement of the adiponectin cardiac system. *Free Radic Biol Med* 51: 1861-1871, 2011.
- 20 Lyu YL, Kerrigan JE, Lin CP, Azarova AM, Tsai YC, Ban Y, Tsai, YC and Liu LF: Topoisomerase IIbeta mediated DNA double-strand breaks: implications in doxorubicin cardiotoxicity and prevention by dexrazoxane. *Cancer Res* 67: 8839-8846, 2007.
- 21 Shin D, Kim TE, Yoon SH, Cho JY, Shin, SG, Jang IJ and Yu KS: Assessment of the pharmacokinetics of co-administered metformin and lobeglitazone, a thiazolidinedione antihyperglycemic agent, in healthy subjects. *Curr Med Res Opin* 28: 1213-1220, 2012.
- 22 Kitakaze M, Sarai N, Ando H, Sakamoto T and Nakajima H: Safety and tolerability of once-daily controlled-release carvedilol 10-80 mg in Japanese patients with chronic heart failure. *Circulation J* 76: 668-674, 2012.
- 23 Najiba NM, Idkaideka N, Adela A, Admoura I, Astigarragab REB, De Nuccib G, Alamec SM, Dhamec R and Qumaruzamanc: Bioequivalence evaluation of two brands of enalapril 20mg tablets (Narapril and Renitec) in healthy human volunteers. *Biopharm Drug Disposal* 24: 315-320, 2003.
- 24 Chow WA, Synold TW, Tetef ML, Longmate J, Frankel P, Lawrence J, Al-Khadimi Z, Leong L, Lim D, Margolin K, Morgan RJ, Raschko J, Shibata S, Somlo G, Twardowski P, Yen Y and Doroshow JH: Feasibility and pharmacokinetic study of infusional dexrazoxane and dose-intensive doxorubicin administered concurrently over 96 h for the treatment of advanced malignancies. *Cancer Chemother Pharmacol* 54: 241-249, 2004.
- 25 Chou TC and Talalay P: Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Reg* 22: 27-55, 1984.
- 26 Cheyne RW, Trembleau L, McLaughlin AC and Smith TAD: Changes in 2-Fluoro-2-deoxy-D-glucose incorporation, hexokinase activity and lactate production by breast cancer cells responding to treatment with the anti-HER-2 antibody trastuzumab. *Nucl Med Biol* 38: 339-346, 2011.
- 27 Cooper AC, Fleming IN, Phyu SM and Smith TAD: Changes in ¹⁸F-Fluoro-2-deoxy-D-glucose incorporation induced by doxorubicin and anti-HER antibodies by breast cancer cells modulated by co-treatment with metformin and its effects on intracellular signalling. *J Canc Res Clin Oncol* 141: 1523-1532, 2015.
- 28 Yan T, Deng S, Metzger A, Gödtel-Armbrust U, Porter ACG and Wojnowski L: Topoisomerase IIα-dependent and -independent apoptotic effects of dexrazoxane and doxorubicin. *Mol Cancer Ther* 8: 1075-1085, 2009.

- 29 Arriola E, Marchio C, Tan DS, Drury SC, Lambros MB, Natrajan R, Rodriguez-Pinilla SM, Mackay A, Tamber N, Fenwick K, Jones C, Dowsett M, Ashworth A and Reis-Filho JS: Genomic analysis of the *HER2/TOP2A* amplicon in breast cancer and breast cancer cell lines. *Lab Invest* 88: 491-503 2008.
- 30 Rapoport B, Demetriou GS, Moodley SD and Benn CA: When and how do i use neoadjuvant chemotherapy for breast cancer? *Curr Treat Options Oncol* 15: 86-98, 2014.
- 31 Fleming IN, Andriu A and Smith TAD: Early changes in ¹⁸F-FDG incorporation by breast cancer cells treated with trastuzumab in normoxic conditions: Role of the AKT-pathway, glucose transport and HIF-1 α . *Breast Cancer Res Treat* 144: 241-248, 2014.
- 32 Yamashita-Kashima Y, Shu S, Harada N and Fujimoto-Ouchi K: Enhanced antitumor activity of trastuzumab emtansine (T-DM1) in combination with pertuzumab in a HER2-positive gastric cancer model. *Oncol Rep* 30: 1087-1093, 2013.
- 33 Li XQ, Lu Y, Liang K, Liu BL and Fan Z: Differential responses to doxorubicin-induced phosphorylation and activation of AKT in human breast cancer cells. *Breast cancer Res* 7: R589-R597, 2005.
- 34 McDermott GM, Welch A, Staff RT, Gilbert FJ, Schweiger L, Semple SIK, Smith TAD, Hutcheon AW, Miller ID, Smith IC and Heys SD: Monitoring primary breast cancer throughout chemotherapy using FDG-PET. *Breast Cancer Res Treat* 102: 75-84, 2007.
- 35 Torizuka T, Zasadny KR, Recker B and Wahl RL: Untreated primary lung and breast cancers: Correlation between F-18 FDG kinetic rate constants and findings of *in vitro* studies. *Radiol* 207: 767-774, 1998.

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