available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

The α_1-adrenergic receptor antagonist doxazosin inhibits EGFR and NF-κB signalling to induce breast cancer cell apoptosis

Hongxiang Hui[a], *Manory A. Fernando*[a], *Anthony P. Heaney*[*]

Division of Endocrinology, Suite 24-130, David Geffen School of Medicine at University of California at Los Angeles, 900 Veteran Avenue, Los Angeles, CA 90024, United States

ARTICLE INFO

Article history:
Received 2 July 2007
Received in revised form
28 September 2007
Accepted 9 October 2007
Available online 26 November 2007

Keywords:
Doxazosin
Breast cancer
Apoptosis
EGFR
NF-κB

ABSTRACT

The selective α_1-adrenergic receptor antagonist doxazosin (dox) has been reported to inhibit prostate cancer proliferation. We now demonstrate that dox-treatment inhibits proliferation and induces apoptosis in breast cancer cells *in vitro* by mechanisms that do not wholly involve the α1-adrenergic receptor. Intriguingly, dox-treatment reduced phosphorylated EGFR expression, decreased pERK1/2 levels and decreased NF-κB, AP-1, SRE, E2F and CRE-mediated transcriptional activity. EGF- and TNFα treatment alone failed to block dox-mediated breast cancer apoptotic effects, but combination of EGF and TNFα treatments completely abrogated dox-induced breast cancer cell apoptosis, indicating doxazosin inhibits both EGFR and NF-κB signalling pathways to induce breast cancer cell apoptosis. Doxazosin is proposed as a possible novel medical therapy for breast cancer.

1. Introduction

Current treatment of breast cancer by early surgery, systemic hormone therapy and/or chemotherapy reduces the risk of death by 25–50%,[1–3] and although anti-oestrogens and more recently aromatase inhibitors inhibit oestrogen receptor (ER)-positive breast cancer recurrence,[4] ER-negative tumours are largely unresponsive to hormonal therapy.[5]

Novel promising agents to prevent ER-negative breast cancer recurrence are the growth factor receptor tyrosine kinase inhibitors,[6,7] including epidermal growth factor receptor (EGFR) inhibitors, which although having good tolerability and potential synergy with other treatments have largely demonstrated disease stabilisation or minor responses only in clinical trials in unselected breast cancer patients.[8–10]

The G-protein-coupled alpha$_1$ (α_1) adrenergic receptors (AR) bind the catecholamines epinephrine and norepinephrine to regulate vascular tone and cardiac output,[11] and α_1-adrenergic receptor antagonists, including prazosin, doxazosin and terazosin have been used as anti-hypertensive therapies. Additionally, extensive *in vitro* and *in vivo* studies have demonstrated that doxazosin and terazosin induce prostate cancer apoptosis,[12,13] and the proposed mechanisms have included actions via TGF-β-signalling, Iκ-B induction and reduced focal adhesion kinase.[14,15] Based on these prior studies in prostate cancer and our own studies of doxazosin-mediated inhibition of *in vitro* pituitary tumour proliferation, we sought to test the effects and further elucidate the mechanism of doxazosin actions in breast cancer cells *in vitro*.[12–16]

[*] *Corresponding author:* Tel.: +1 310 825 4980; fax: +1 310 794 7654.
E-mail address: aheaney@mednet.ucla.edu (A.P. Heaney).
[a] Both authors contributed equally to this paper.
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.10.002

In this study, we demonstrate that doxazosin (dox) treatment inhibits proliferation, and induces apoptosis in both ER-positive and ER-negative breast cancer cells. These dox-mediated actions were largely independent of α_1-adrenergic receptor expression, and resulted in reduced phosphorylated-EGFR, phosphorylated-ERK and inhibition of NF-κB signalling. Additionally, co-treatment with doxazosin, and the EGFR TKI, Iressa, synergistically induced increased apoptosis in breast cancer cells.

2. Materials and methods

2.1. Cell culture

Human breast cancer MDA-MB-231 and MCF-7 cells, and normal breast (MCF12A) cells were cultured according to the American Type Culture Collection (Rockville, MD, USA) recommendations.

2.2. PCR for α_1-adrenergic receptors, cell proliferation assay and cell cycle analysis

PCR for the α_{1A}, and α_{1B} receptor was performed as previously described.[16] Cell proliferation was measured using the MTS Assay (Promega, Madison, WI, USA) and the cell cycle analysis was carried out by FACScan (Becton Dickinson, NJ, USA) after the cells were treated with either doxazosin (dox) or vehicle (0.01% DMSO) for 48–72 h.

2.3. Transfections and apoptosis assay

For EGFR and NF-κB-mediated signalling experiments, separate aliquots of MDA-MB231 cells were first transfected[16] with dominant negative-EGFR- (gifted from Dr. Jeffery Kudlow) and/or -NF-κB (gifted from Dr. Eduardo Arzt) constructs.[17,18] Wild-type MDA-MB 231 and transfectant cells were then treated with vehicle, or 10 μg/ml EGF, or 50 ng/ml TNFα alone, or in combination with 25 μM dox for 48 h. For oestrogen experiments, ER-positive MCF-7 cells were cultured in DMEM supplemented with 10% Charcoal stripped serum (CSS) for 72 h, then cell aliquots were treated with dox or vehicle for 48 h either in CSS medium or CSS plus 10 nM estradiol. Cells were then washed, incubated with propidium iodide and FITC-labelled annexin antibody (Pharmingen, San Diego, CA, USA) prior to flow cytometric analysis to quantify apoptotic cells.[16]

2.4. Western blot analysis

Treated cells were lysed in RIPA buffer and proteins (50 μg) electrophoresed and immunoblotted by standard techniques using antibodies to cleaved and total caspase-3 (1:500); ERK1/2, phospho-ERK1/2, β-actin, EGFR, phospho-EGFR (all 1:1000) (all from Santa Cruz Biotechnology Inc., CA, USA) and phosphorylated IκB-α (1:1000) (Cell Signalling, Beverly, MA). For EGFR phosphorylation studies, EGFR was immunoprecipitated with anti-EGFR antibody (Transduction Laboratories, Lexington, KY), bound to protein A agarose, prior to application to gels.

2.5. In vitro AP-1, SRE, E2F and CRE transcriptional activity

MDA–MB231 breast cancer cells were transiently transfected with 1 μg of AP-1, CRE, SRE or E2F luciferase plasmids (Clontech Laboratories, Inc, CA); pTAL-luc was used as negative control, and treated with either dox (25 μM) or vehicle for 6 or 12 h, after which fold-change in luciferase activities was determined.

2.6. Statistical analysis

Experiments were performed in triplicate, in three separate experiments, and analysed by ANOVA (Kruskal–Wallis) with Dunn's multiple comparison tests or non-parametric *t* test. *P* values <0.05 were considered significant.

3. Results

3.1. The α_1-adrenergic receptor antagonist, doxazosin inhibits human breast cancer proliferation

Our first set of experiments investigated MCF-7, and MDA-MB231 breast cancer cell proliferation following doxazosin treatment. As depicted in Fig. 1, dox-treatment inhibited ER-positive, and ER-negative breast cancer proliferation in a dose- and time-dependent manner (Figs. 1a and b). After 48 h dox-treatment, MCF-7 proliferation was inhibited between 30% and 70% (dox 20–30 μM), and proliferative rates were further inhibited 30–90% at 72 h, $P < 0.01$. Likewise in ER-negative MDA-MB231 cells, dox-treatment (20 μM to 30 μM) for 48 h inhibited proliferation 20–60%, increasing to 30–75% at 72 h, $P < 0.01$ (Figs. 1a and b).

3.2. Doxazosin-treatment leads to G0–G1 cell cycle arrest

To gain insight into the mechanism of dox-mediated inhibition of proliferation, we next performed cell cycle analysis in the dox-treated breast cancer cells. Dox-treatment for 72 h resulted in a 2.3-fold increase in G_0/G_1-phase cells at doxazosin doses higher than 25 μM (data not shown, $P < 0.05$), along with a dose-dependent 40–80% decrease ($P < 0.05$) in S-phase cell population (Fig. 1c). Western blot analysis of dox-treated MCF-7 cells revealed decreased phosphorylated retinoblastoma (pRb) protein expression (Fig. 1d), providing a potential mechanism for the dox-mediated G_0/G_1 cell cycle arrest.

3.3. Doxazosin induces breast cancer apoptosis in vitro

We next investigated the effect of dox-treatment on breast cancer cell apoptosis. Following dox-treatment (5–30 μM × 48 h), FACS demonstrated a dose-dependant 10–15-fold increase in Annexin-FITC positive apoptotic MCF-7 cells. In contrast, no significant increase in dox-mediated apoptosis was evident in normal breast epithelial MCF12A cells (Fig. 2a). As previous studies reported differential effects of dox in androgen-receptor positive or negative prostate cancer cells, we examined dox-mediated apoptosis in oestrogen-receptor expressing MCF-7 cells incubated in either charcoal stripped

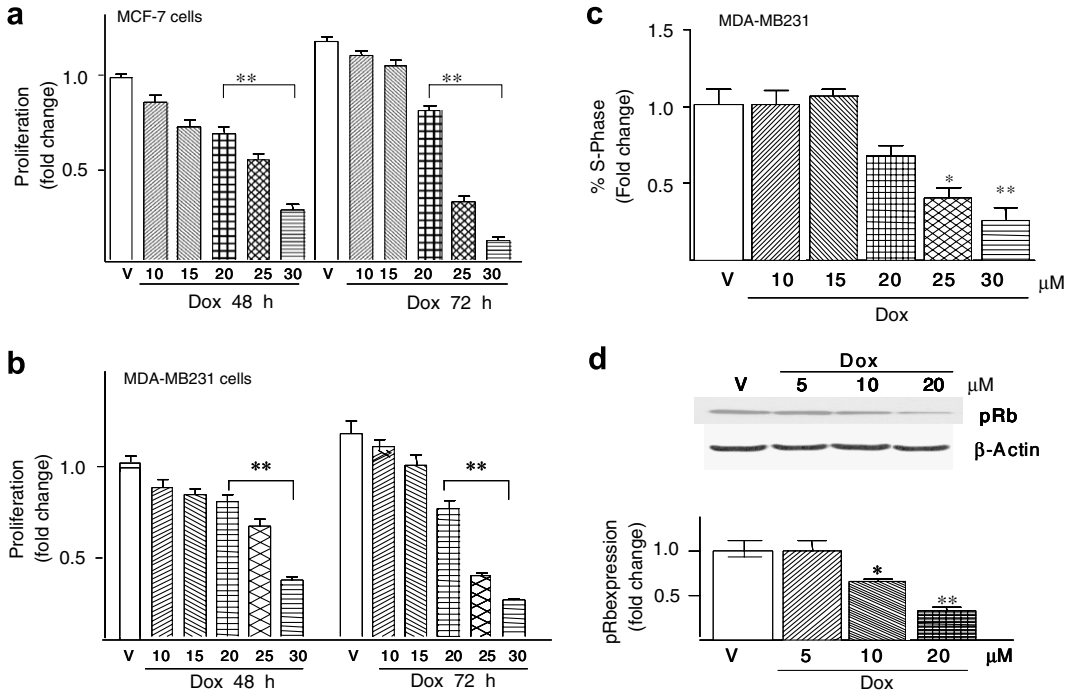

Fig. 1 – Doxazosin (dox) treatment of MCF-7 and MDA-MB231 breast cancer cells inhibited breast cancer cell proliferation (a and b), led to a dose-dependent decrease in S-phase cell population (c), and decreased pRb expression (d). (*, $P < 0.05$; **, $P < 0.01$) V, vehicle; dox, doxazosin, pRb, phosphorylated retinoblastoma protein.

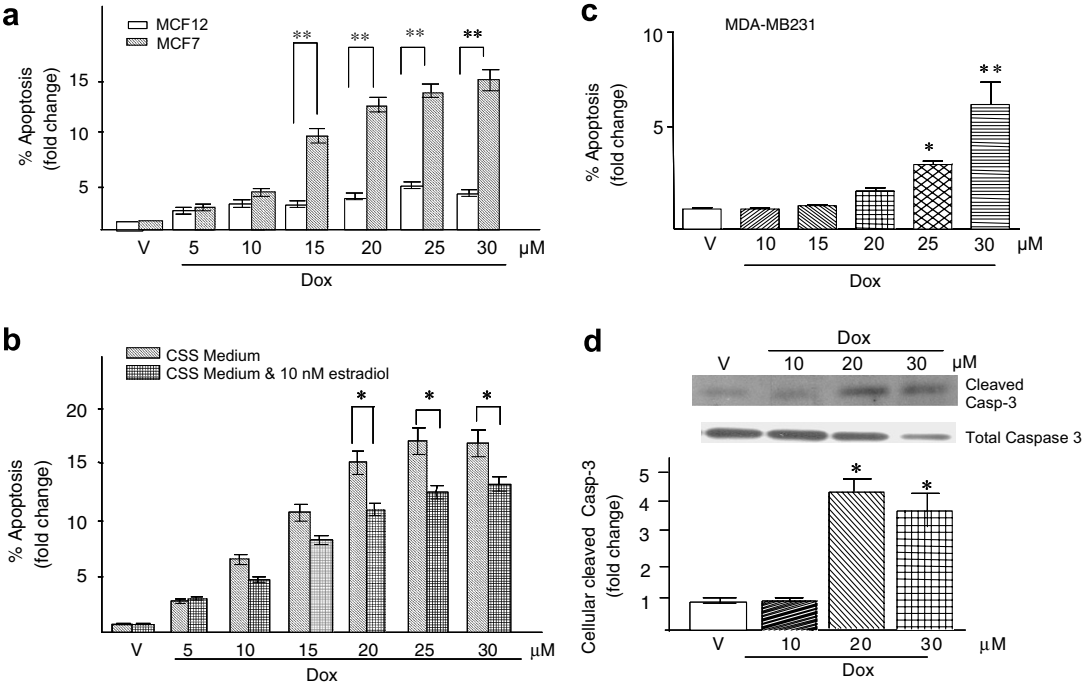

Fig. 2 – Dox-treatment dose-dependently increased breast cancer MCF-7 cell apoptotic rates in comparison to normal breast MCF12A cells (a). Dox-mediated apoptosis was more marked in oestrogen-deprived ER expressing MCF-7 cells (b), but was also evident in ER negative MDA-MB231 cells (c), and associated with increased cleaved caspase-3 expression (d). *, $P < 0.05$; **, $P < 0.001$; V, vehicle; Casp-3, caspase-3; dox, doxazosin.

serum (CSS) alone or CSS plus 10 nM estradiol (E_2) (Figs. 2a and b). Higher apoptotic rates were demonstrated in dox-treated MCF-7 cells in CSS medium alone compared to CSS and E_2 ($P < 0.05$ for 20–30 µM dox). In ER–ve MDA-MB231 cells, dox-treatment also resulted in a 3–7-fold increase in apoptosis (Fig. 2c). Additionally, Western blot analysis revealed

a ~10-fold increase in MDA–MB231 cleaved caspase-3 expression (Fig. 2d), implicating Caspase-3 in dox-mediated breast cancer cell apoptosis.

3.4. *Doxazosin-mediated anti-proliferative and pro-apoptotic actions are independent of the α_1-adrenergic receptor*

Doxazosin's anti-hypertensive action is due to it's ability to bind the α_1-adrenergic receptor, but the mechanism(s) of its anti-proliferative and pro-apoptotic actions in cancer cells are not well understood. To elucidate this issue, α_1-adrenergic (α_{1A} and α_{1B}) receptor mRNA expression was examined in the breast cancer cell lines by RT-PCR. α_{1A} and α_{1B} mRNA expression were demonstrated in MDA–MB-231 cells but were not detectable in MCF-7 cells (Fig. 3a), suggesting doxazosin induced anti-proliferative and pro-apoptotic effects were not entirely dependent on α_1-adrenergic receptor expression. Additionally, co-treatment of α_{1A}, and α_{1B} expressing MDA–MB231 cells with doxazosin, and blocking doses of the irreversible α_{1-} receptor antagonist phenoxybenzamine did not abrogate the pro-apoptotic actions of doxazosin (Fig. 3b).

3.5. *Doxazosin reduces EGFR phosphorylation to inhibit MAP kinase signalling*

As doxazosin has a quinazoline ring structure, similar to the EGFR tyrosine kinase inhibitors, Iressa and Tarceva, we hypothesised a potential interaction of doxazosin via the EGFR in the breast cancer cells. Iressa-treatment alone (1 μM for 48 h) did not significantly alter apoptotic rates, in contrast to doxazosin treatment alone (30 μM for 48 h), which resulted in a 7.2-fold increase in apoptosis ($P < 0.05$) (Fig. 4a). Combination dox- (30 μM) and Iressa- (1 μM) treatment for 48 h resulted in a 14-fold increase in apoptosis ($P < 0.01$), compared to apoptotic rates in vehicle-treated MDA–MB231 cells suggesting synergistic pro-apoptotic actions of these drugs (Fig. 4a). We next examined total ERK1/2 and phosphorylated ERK1/2 (pERK) expression by Western blot in dox-treated MDA-MB231 cells before and after epidermal growth factor (EGF) treatment. As expected EGF treatment (10 ng/ml) for 30 min up-regulated pERK1/2 expression (mean ± SEM, 7.9 ± 0.1-fold, $P < 0.01$) (Fig. 4b, lane 2), but intriguingly, dox-treatment (5–30 μM × 30 min) dose-dependently inhibited EGF-induced breast cancer pERK 1/2 expression (Fig. 4b, lanes 3–8). Iressa-treatment (1 μM × 30 min) also potently inhibited EGF-induced pERK1/2 expression (Fig. 4c, lane 3), and unexpectedly, co-treatment with doxazosin (5–30 μM × 30 min) plus Iressa (1 μM × 30 min) resulted in a further decrease in EGF-induced p-ERK1/2 levels in the breast cancer cells (Fig. 4c, lanes 4–7).

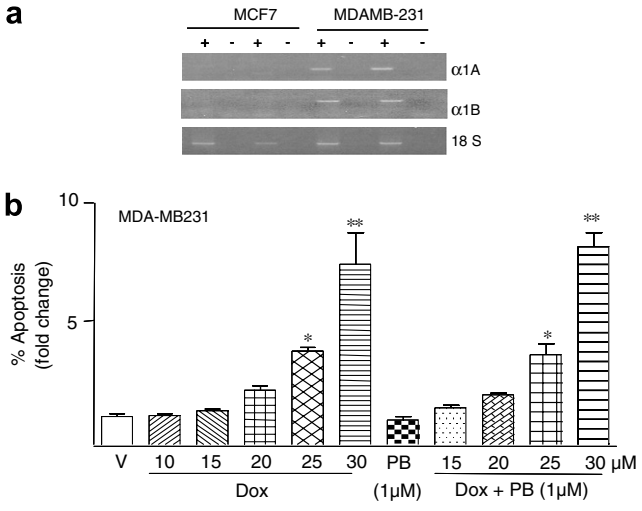

Fig. 3 – RT-PCR demonstrated α_{1A} (α1A)- and α_{1B}-adrenergic receptor (AR) expression in MDA–MB231 cell lines, but α_1 AR expression was absent in MCF-7 cells (a), and dox-mediated apoptosis was not abrogated by co-treatment of breast cancer cells with the α1-AR antagonist phenoxybenzamine. *, $P < 0.05$; **, $P < 0.001$; V, vehicle; PB, phenoxybenzamine; dox, doxazosin. 18S ribosomal RNA served as an internal control.

Given these findings, we next examined activated EGFR expression, and as predicted, EGF-treatment (20 ng/ml × 30 min) led to a 18 fold increase in breast cancer phospho-EGFR levels (Fig. 4d, lane 2) compared to vehicle treatment, which was completely inhibited by Iressa treatment (1 μM × 30 min) (Fig. 4d, lane 3). However, doxazosin treatment alone (30 μM × 30 min) also decreased EGF induced phosphorylated EGFR levels in the MDA–MB231 cells (Fig. 4d, lane 4, $P < 0.05$), suggesting that, like Iressa, doxazosin acts to inhibit breast cancer EGFR activation.

3.6. *Doxazosin inhibits AP-1-, SRE-, E2F- and CRE-mediated transcription activity*

To further characterise dox-mediated actions on MAPK-mediated signal transduction, we transiently transfected activator protein 1(AP-1), serum-response element (SRE), E2F and cAMP-response element (CRE) luciferase reporter plasmids (pTAl-luc) into MDA-MB231 breast cancer cells, and treated transfectants with dox (25 μM) or vehicle for 6 or 12 h, and then determined luciferase activities. Dox-treatment led to a 2.8-fold, 2.8-fold, 1.7-fold and 3.9-fold decrease in AP-1, SRE, E2F and CRE promoter luciferase activities, respectively, in the MDA–MB231 breast cancer cell transfectants compared to the vehicle treatment (Fig. 5a, $P < 0.001$), demonstrating that dox-treatment inhibits EGF-signalling.

3.7. *Doxazosin inhibits NF-κB-mediated transcription*

As dox-treatment has been shown to reduce phosphorylated inhibitor of kappa kinase (IKK) levels, and as dox inhibited the NF-κB transcriptional targets, AP-1, SRE, E2F and CRE, in breast cancer cells, we examined baseline, and TNF-α stimulated phosphorylated IκB-α kinase levels after dox treatment. As expected, TNFα (50 ng/ml × 30 min) induced phosphorylated IκB-α (pIκB-α) expression ~3.9-fold (Fig. 5b, lanes 1 and 2), whereas dox-treatment (20 and 30 μM × 30 min) decreased constitutive MCF-7 breast cancer pIκB-α expression (lanes 3 + 4), and abrogated TNFα induced pIKB-α kinase levels (Fig. 5b, lanes 5 and 6) suggesting NF-κB is also a transcriptional target of doxazosin.

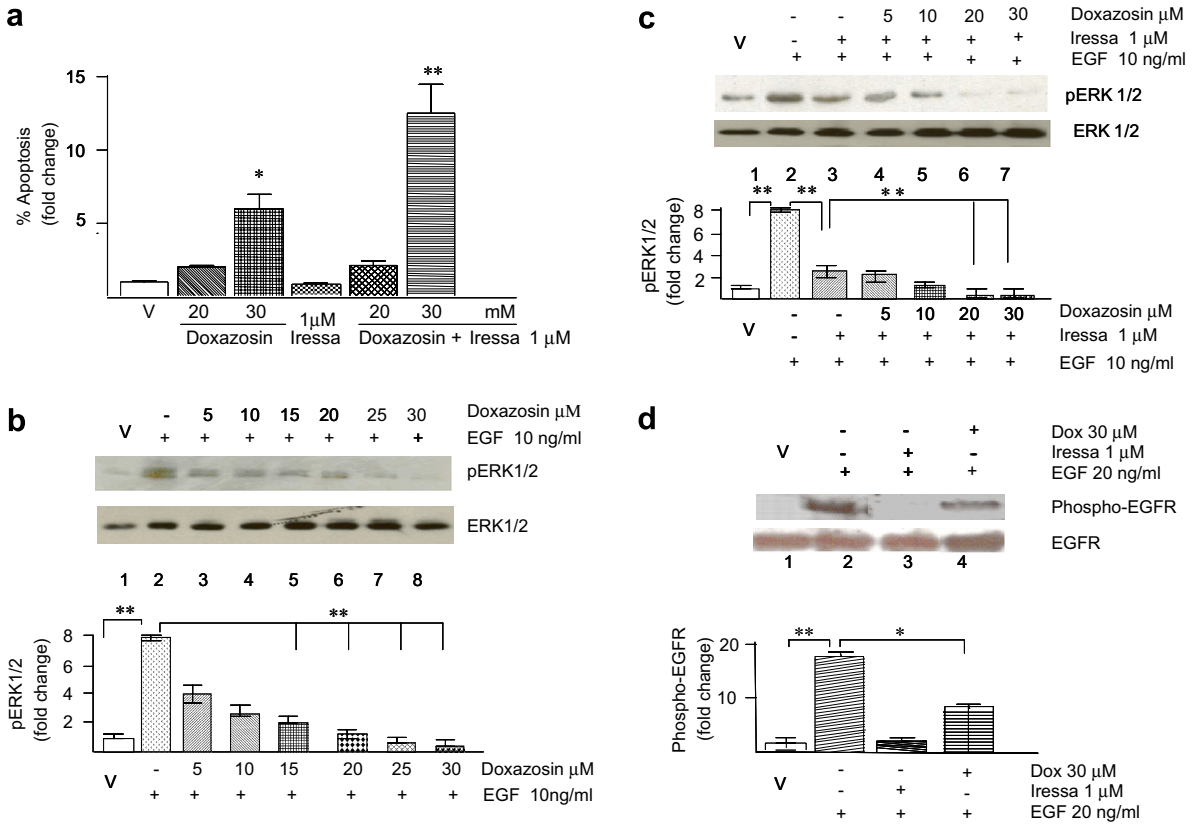

Fig. 4 – (a) Doxazosin-treatment induced MDA-MB231 breast cancer cell apoptosis, whereas Iressa alone did not alter apoptotic rates. Iressa and dox-co-treatment further increased apoptosis compared to doxazosin alone. (b) Doxazosin dose-dependently inhibited (lanes 3–8) EGF-induced breast cancer pERK1/2 expression compared to EGF alone (lane 2). (c) Iressa-treatment partially inhibited EGF-induced pERK1/2 (lane 3), combination dox- and Iressa-treatment further decreased EGF-induced pERK levels (lanes 4–7). (d) EGF-treatment induced phosphorylated EGFR expression (lane 2), which was inhibited by Iressa (lane 3). Dox treatment (30 μm) also inhibited EGF-induced pEGFR expression (lane 4). *, $P < 0.05$; **, $P < 0.01$; V, vehicle.

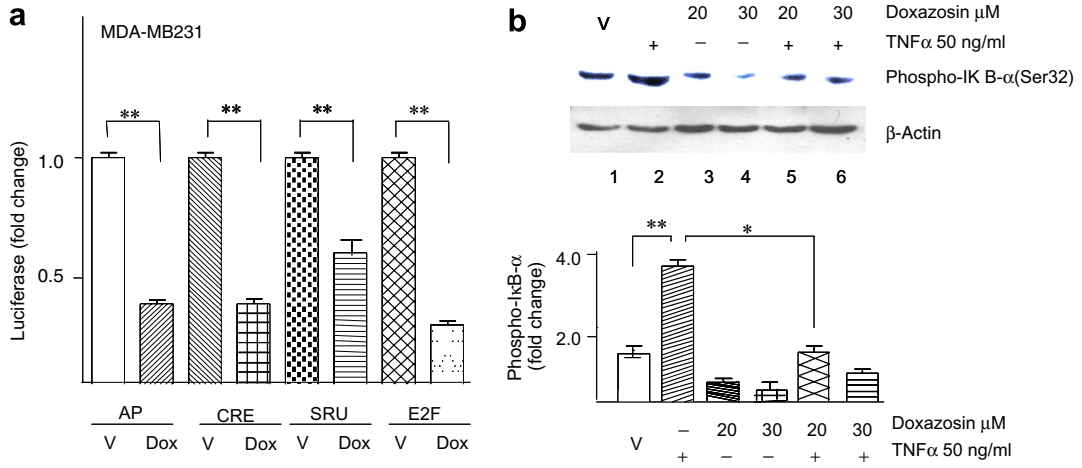

Fig. 5 – Changes in MAPK, and NF-κB-activation in breast cancer cells following dox-treatment of MDA-MB231 cells transiently transfected with AP-1, CRE, SRE and E2F promoter reporter constructs (a), or phosphorylated IκB-α expression following TNFα-treatment (b). *, $P < 0.05$; **, $P < 0.001$; V, vehicle; dox, doxazosin.

3.8. *Combination EGF and TNFα are required to block dox-induced breast cancer apoptosis*

To characterise the contribution of EGFR- and/or NF-κB-mediated signalling to doxazosin-mediated apoptosis, we transiently transfected mutant dominant negative EGFR,[17] and NF-κB[18] constructs into MDA-MB231 breast cancer cells, and then examined doxazosin-mediated apoptotic rates at baseline following vehicle treatment, and following EGF (10 ng/ml) or TNFα (50 ng/ml) mediated EGFR- or NF-κB-activation, respectively. As depicted in Fig. 6, transfection of EGFR- or NF-κB genes into MDA–MB231 cells did not alter base line apoptotic rates of wild-type breast cancer cells (Fig. 6, group 1), although DN-EGFR and DN-NFκB expression increased breast cancer cell sensitivity to dox-mediated apoptosis (Fig. 6, group 2). TNF-α (50 ng/ml) (Fig. 6, group 3) or EGF (10 ng/ml) (Fig. 6, group 5) treatment alone, or combination TNF-α (50 ng/ml) and EGF (10 ng/ml) (group 7) did not alter baseline apoptotic rates in wild-type, DN-EGFR- or DN-NFκB-expressing breast cancer cells. Furthermore, co-treatment of wild-type, DN-EGFR or DN-NFκB expressing breast cancer cells with TNF-α or EGF alone, plus doxazosin (25 μM for 72 h) did not totally abrogate dox-mediated breast cancer apoptotic rates, although apoptotic rates in TNF-α–treated DN-NFκB expressing cells or EGF-treated DN-EGFR-expressing cells were lower than in vehicle treated DN-transfectants (Fig. 6, groups 4 and 6). Only co-treatment with TNF-α and EGF blocked dox-mediated apoptosis in wild-type, DN-EGFR and DN-NFκB expressing cells (Fig. 6 groups 7 and 8), indicating that dox-mediated breast cancer apoptosis involves both EGFR- and DN-NFκB-mediated signalling. Similar results were seen in parallel experiments in MCF-7 cells (data not shown).

4. Discussion

Anti-oestrogens effectively reduce oestrogen receptor (ER)-positive breast cancer recurrence, but additional chemopreventative agents to prevent ER-negative breast cancers are urgently needed.[2,3,19] Several previous studies have reported that clinically approved doses of the quinazoline-based α1-adrenoceptor antagonists doxazosin and terazosin inhibit proliferation and induce apoptosis in prostate cancer cells and it is not clear why this concept has not been further pursued in human clinical trials.[12–15] We extend these findings by demonstrating here that doxazosin inhibits human breast cancer cell proliferation and potently increases apoptosis *in vitro*, in both ER-expressing MCF-7 and ER-negative MDA-MB231 breast cancer cells via multiple mechanisms, which are not entirely mediated via the alpha adrenoreceptor.

In some prostate cancer studies, doxazosin and terazosin treatment appeared primarily to induce apoptosis with a lesser effect on prostate cancer cell proliferation, and different responses have been described in androgen receptor (AR) expression compared to AR-negative prostate cancer cells.[13] Based on our observed reduced S-phase population and decreased pRb expression, doxazosin-treatment significantly inhibits breast cancer cell proliferation, in addition to potently inducing apoptosis, an effect which was more pronounced in breast cancer cells compared to normal breast epithelial cells, and more marked in the absence of oestrogen in ER-expressing MCF-7 cells.

Unexpectedly, doxazosin-mediated anti-proliferative and pro-apoptotic effects were observed in breast cancer cells which did not exhibit significant α_1-AR expression, and α_1-AR blockade did not abrogate doxazosin-mediated pro-apoptotic effects, suggesting dox-mediated apoptotic actions were not entirely mediated via the α-adrenergic receptor. We demonstrate that doxazosin-treatment reduces phosphorylated EGFR and phosphorylated ERK levels to inhibit EGF-stimulated signal transduction and that doxazosin and Iressa actions overlap to increase breast cancer apoptotic rates compared to either drug alone. However, inactivation of EGFR-signalling did not totally abrogate dox-mediated breast cancer apoptosis and our additional studies demonstrate that dox-treatment also reduces constitutive and TNFα-induced breast cancer pIKK levels. However, as for EGFR-inactivation, blockade of NFκB signalling alone did not totally abrogate dox-mediated breast cancer apoptosis, and EGF- and

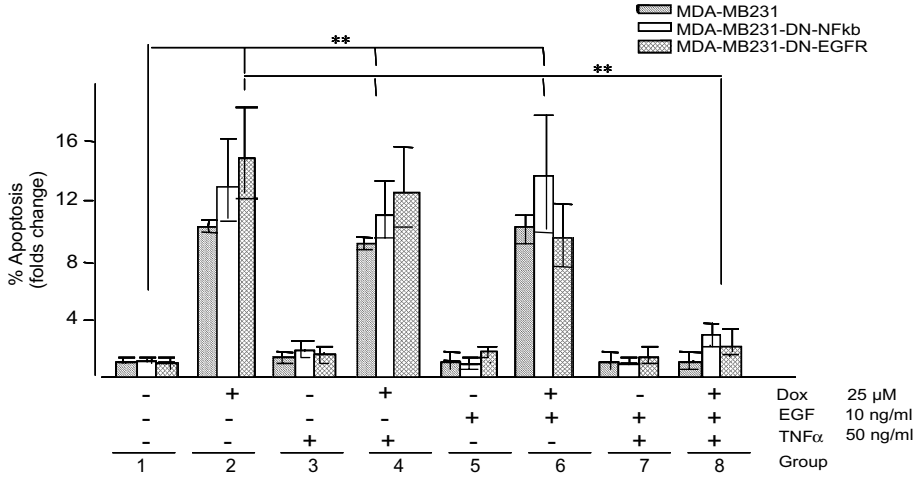

Fig. 6 – Changes in dox-mediated breast cancer apoptosis following transient expression of DN-EGFR or DN-NF-κB constructs in the presence or absence of EGF- and/ or TNFα-treatment. *, $P < 0.05$; **, $P < 0.01$;. DN-EGFR: dominant negative EGFR; DN-NF- κB: dominant negative NF-κB; V, vehicle; dox, doxazosin.

TNFα- co-treatment was required to inhibit the pro-apoptotic effects of doxazosin. We speculate that the different apoptotic response in cancer versus normal cells may be due to greater activation of EGFR- and NF-κB-mediated signalling pathways in breast cancer versus normal breast cells.

Given its multiple mechanisms of action to inhibit both EGFR- and NF-κB-mediated actions, it is a well established safety profile, and comparatively low cost, clinically approved doxazosin doses may ultimately offer a novel therapeutic option for both ER positive and ER negative breast cancer patients. However, further *in vivo* breast cancer studies are now necessary to pave the way to clinical trials of doxazosin therapy in patients with breast and other cancers.

Conflict of interest statement

None declared.

Acknowledgements

We thank Dr. Jeffery Kudlow and Dr. Eduardo Arzt for kind donation of the mutant EGFR and NF-κB plasmids, respectively. This work was supported by a Borden Foundation seed grant, the Margaret Early Foundation and the Cedars-Sinai Research Institute.

REFERENCES

1. Boring CC, Squires TS, Tong T, Montgomery S. Cancer statistics 1994. *CA Cancer J Clin* 1994;**44**:7–26.
2. Hortobagyi GN, de la Garza Salazar J, Pritchard K, et al. The global breast cancer burden: variations in epidemiology and survival. *Clin Breast Cancer* 2005;**6**:391–401.
3. Hortobagyi GN. Treatment of breast cancer. *N Engl J Med* 1999;**339**:974–84.
4. Berry DA, Cronin KA, Plevritis SK, et al. Cancer Intervention and Surveillance Modeling Network (CISNET) Collaborators. Effect of screening and adjuvant therapy on mortality from breast cancer. *N Engl J Med* 2005;**353**:1784–92.
5. Buchanan G, Birrell SN, Peters AA, et al. Decreased androgen receptor levels and receptor function in breast cancer contribute to the failure of response to medroxyprogesterone acetate. *Cancer Res* 2005;**65**:8487–96.
6. Takahashi T, Ohmichi M, Kawagoe J, et al. Growth factors change nuclear distribution of estrogen receptor-alpha via mitogen-activated protein kinase or phosphatidylinositol 3-kinase cascade in a human breast cancer cell line. *Endocrinology* 2005;**146**:4082–9.
7. Xia W, Gerard CM, Liu L, Baudson NM, Ory TL, Spector NL. Combining lapatinib (GW572016), a small molecule inhibitor of ErbB1 and ErbB2 tyrosine kinases, with therapeutic anti-ErbB2 antibodies enhances apoptosis of ErbB2-overexpressing breast cancer cells. *Oncogene* 2005;**24**:6213–21.
8. Hart S, Fischer OM, Prenzel N, et al. GPCR-induced migration of breast carcinoma cells depends on both EGFR signal transactivation and EGFR-independent pathways. *Biol Chem* 2005;**386**:845–55.
9. Pianetti S, Arsura M, Romieu-Mourez R, Coffey RJ, Sonenshein GE. Her-2/neu overexpression induces NF-kappaB via a PI3-kinase/Akt pathway involving calpain-mediated degradation of IkappaB-alpha that can be inhibited by the tumor suppressor PTEN. *Oncogene* 2001;**20**:1287–99.
10. Kaklamani V, O'Regan RM. New targeted therapies in breast cancer. *Semin Oncol* 2004;**31**:20–5.
11. Steinberg SF. The molecular basis for distinct beta-adrenergic receptor subtype actions in cardiomyocytes. *Circ Res* 1999;**85**:1101–11.
12. Kyprianou N, Benning CM. Suppression of human prostate cancer cell growth by alpha 1 adrenoceptor antagonists doxazosin, and terazosin via induction of apoptosis. *Cancer Res* 2000;**60**:4550–5.
13. Alberti C. Apoptosis induction by quinazoline-derived alpha 1-blockers in prostate cancer cells: biomolecular implications and clinical relevance. *Eur Rev Med Pharmacol Sci* 2007;**11**:59–64.
14. Partin JV, Anglin IE, Kyprianou N. Quinazoline-based alpha-1 adrenoceptor antagonists induce prostate cancer cell apoptosis via TGF-beta signalling and I kappa B induction. *Br J Cancer* 2003;**88**:1615–21.
15. Walden PD, Globina Y, Nieder A. Induction of anoikis by doxazosin in prostate cancer cells is associated with activation of caspase-3 and a reduction of focal adhesion kinase. *Urol Res* 2004;**32**:261–5.
16. Fernando MA, Heaney AP. Alpha1-adrenergic receptor antagonists: novel therapy for pituitary adenomas. *Mol Endocrinol* 2005;**19**:3085–96.
17. Xie W, Paterson AJ, Chin E, Nabell LM, Kudlow JE. Targeted expression of a dominant negative epidermal growth factor receptor in the mammary gland of transgenic mice inhibits pubertal mammary duct development. *Mol Endocrinol* 1997;**11**:1766–81.
18. Costas MA, Muller Igaz L, Holsboer F, Arzt E. Transexpression of NF-kappaB is not required for glucocorticoid-mediated protection of TNF-alpha-induced apoptosis on fibroblasts. *Biochim Biophys Acta* 2000;**1499**(1-2):122–9.
19. Goldberg JI, Borgen PI. Breast cancer susceptibility testing: past, present and future. *Expert Rev Anticancer Ther* 2006;**6**:1205–14.