Sdox, a H2S releasing anthracycline, with a safer profile than doxorubicin toward vasculature

Sdox, a H_2S releasing anthracycline, with a safer profile than doxorubicin toward vasculature

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Abstract

Sdox is a synthetic H_2S-releasing doxorubicin (Dox) less cardiotoxic and more effective than Dox in pre-clinical, Dox-resistant tumour models. The well-known anthracycline vascular toxicity, however, might limit Sdox clinical use. This study aimed at evaluating Sdox vascular toxicity *in vitro*, using Dox as reference compound.

Both vascular smooth muscle A7r5 and endothelial EA.hy926 cells were more sensitive to Dox than Sdox, although both drugs equally increased intracellular free radical levels. Sdox released H_2S in both cell lines. The H_2S scavenger hydroxocobalamin partially reverted Sdox-induced cytotoxicity in A7r5, but not in EA.hy926 cells, suggesting a role for H_2S in smooth muscle cell death. Markers of Sdox-induced apoptosis were significantly lower than, in A7r5 cells, and comparable to those of Dox in EA.hy926 cells. In A7r5 cells, Dox increased the activity of caspase 3, 8, and 9, Sdox affecting only that of caspase 3. Moreover, both drugs induced comparable DNA damage in A7r5 cells, while Sdox was less toxic than Dox in Ea.hy926 cells.

In fresh aorta rings, only Dox weakly increased phenylephrine-induced contraction when endothelium was present. In rings cultured with both drugs for 7 days, Sdox blunted phenylephrine- and high K^+-induced contractions though at a concentration 10-fold higher than that of Dox.

In conclusion, Sdox may represent the prototype of an innovative anthracycline, effective against Dox-resistant tumours, displaying a more favourable vascular toxicity profile compared to the parent compound.

Keywords: A7r5 cells, doxorubicin, EA.hy926 cells, rat aorta ring, vascular toxicity

1. Introduction

Doxorubicin (Dox) is still a frontline treatment option for several types of cancer, despite significant advances in target therapy. However, serious adverse reactions, including Dox-induced cardiovascular toxicity [1] and multidrug resistance (MDR), due to increased expression of the efflux ATP binding cassette (ABC) transporters (e.g., P-glycoprotein; P-gp) in tumour cells, represent primary hindrances towards successful chemotherapy [2].

To overcome these limitations, a series of H_2S-releasing Dox (e.g., Sdox), characterised by reduced cardiotoxicity and active against Dox-resistant/P-gp expressing tumour cells, have been obtained by combining the anthracycline with appropriate H_2S donor moieties [3].

Sdox is more effective than Dox against human osteosarcoma cells with increasing degrees of Dox-resistance. Furthermore, Sdox preferentially accumulates within the endoplasmic reticulum (ER), where it activates several ER stress-dependent apoptotic pathways and sulfhydrated ER-associated proteins, including P-gp, thus promoting their subsequent ubiquitination [3;4]. Moreover, Sdox overcome Dox resistance, both *in vitro* and *in vivo*, in an androgen-independent prostate cancer model [5].

Sdox and its liposomal formulation conjugated with hyaluronic acid (HA-LSdox), are effective against Pgp-overexpressing/Dox-resistant osteosarcoma cells and xenograft, compared with Dox and also Caelyx®, the pegylated liposomal Dox, currently used in clinical setting [6]. Noticeably, Sdox is not cytotoxic as Dox in H9C2 cardiomyocytes [3] and displays the same cardiotoxic profile of Caelyx® in osteosarcoma xenografts [6].

Although Sdox may represent a promising drug for the treatment of chemoresistant tumours, its clinical development might be limited by the same severe vascular toxicity characterizing the parent Dox [7-9]. Therefore, the aim of this study was the *in vitro* evaluation of Sdox vascular toxicity, along with the reference compound Dox.

2. Materials and methods

2.1 Cell culture

Rat aorta vascular smooth muscle A7r5 cells (ATCC® CRL–1444™) and human macrovascular endothelial EA.hy926 cells (ATCC® CRL-2922™) were cultured as previously described [10].

2.1.1 Drug treatments and Cell viability assay

To assess the effects of Dox and Sdox on cell viability, A7r5 or EA.hy926 cells were treated with drugs for 24, 48 or 72 h, renewing the solution every 24 h. At each time points MTT (3-[4,5-

dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed as already reported [10].

2.1.2 Apoptosis assays

Flow cytometry assays such as cell cycle and sub-G0/G1 population analysis and annexin V/propidium iodide (AV/PI) staining were used to check for apoptotic-mediated cell death in A7r5 and EA.hy926 cells treated for 24 h with 1 μM Dox and 3 μM Sdox [11].

2.1.3 ROS detection

2′,7′-Dichlorofluorescin diacetate (DCFDA) was employed to evaluate intracellular ROS contents in A7r5 and EA.hy926 treated with drugs for 24 h [12].

2.1.4 DNA damage assessment

A7R5 and EA.hy926 cells treated with either Dox or Sdox for 24 h were incubated with the mouse monoclonal IgG anti-phospho (Ser139)-H2AX primary antibody as previously described [9]. Appearance of phospho(Ser139)-H2AX foci, defined as γH2AX foci, in the cell nucleus was taken as index of DNA damage [13].

2.1.5 Caspase Activity

Proteins of either Dox- or Sdox-treated cells lysates were incubated with specific caspase-3, -8, -9 fluorescent substrates as previously described [14].

2.1.6 Intracellular H_2S-release measurement

A7r5 and EA.hy926 cells were loaded with 100 μM WSP-1 (1,3′-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9′-xanthen]-6′-yl-2(pyridin-2-yldisulfanyl benzoate) fluorescent dye for 30 min; then the supernatant was removed and replaced with medium containing drugs. WSP-1 releases a fluorophore detectable at 465 (excitation) and 515 nm (emission) after reacting with H_2S [15] and the $AUC_{(0\text{-}24h)}$ of the curve describing changes in fluorescence monitored at time 0, 1 h, 4 h and 24 h (ThermoLabsystems Synergy HTX reader, BioTek, Winooski, USA), was calculated.

2.2 Animals

The investigation conformed to the European Union Guidelines for the Care and the Use of Laboratory Animals (Directive 2010/63/EU) and was approved by the Italian Ministry for Health (666/2015-PR).

Male Wistar rats (300-350 g; Charles River Italia, Calco, Italy), anaesthetized with a gas mixture of 4% isoflurane and 96% O_2 by using Fluovac (Harvard Apparatus, Holliston, USA), were decapitated and exsanguinated and aortas quickly removed.

2.2.1 Rings preparation

Aorta rings (2 mm width), either endothelium-intact or –denuded, were prepared as previously described [16]. Their functional integrity was assessed as reported by Fusi et al. [17]. Endothelium removal was validated by adding 10 μM acetylcholine at the plateau of phenylephrine-induced contraction: a relaxation lower than 10% denoted the absence of a functional endothelium [16].

2.2.*2 Effect of Dox and Sdox on phenylephrine-induced and K^+-induced contraction*

The potential vasorelaxant activity of both Dox and Sdox was assessed in rings, either endothelium-intact or -denuded, contracted by 0.3 μM phenylephrine.

To study the antispasmodic activity of both anthracyclines, a cumulative concentration-response curve to K^+ was constructed in endothelium-denuded rings exposed to vehicle or varying concentrations of either Dox or Sdox for 30 min before, and throughout the duration of the concentration–response curve [18].

2.2.3 *Cultured rings*

To assess the potential long-term effects of Dox and Sdox on the mechanical function of vascular smooth muscle, rat aortic rings were cultured as previously described [9]. Preparations were incubated with either Dox or Sdox or vehicle for 7 days at 37 °C in an atmosphere of 95% air and 5% CO_2, renewing the medium every 2 days. Cultured rings, along with a fresh preparation, were mounted in an organ bath and stimulated by either 0.3 μM phenylephrine or 60 mM K^+ [9].

2.3 Whole-cell patch clamp recordings

Whole-cell $Ca_V1.2$ channel currents ($I_{Ca1.2}$) were recorded in A7r5 cells upon application of 250-ms clamp pulses to 10 mV from a Vh of -50 mV, low-pass filtered at 1 kHz and digitized at 3 kHz. Current amplitudes were corrected off-line for leakage and residual outward currents using nifedipine (10 μM) which blocked $Ca_V1.2$ [19].

2.4 Materials

Sdox (2-((2S,4S)-4-(((2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-2-yl)-2-oxoethyl 4-(4-phenyl-3-thioxo-3H-1,2-dithiol-5-yl)benzoate) was synthetized by the reaction of 14-bromodaunomicin with corresponding carboxylic acid in dry dimethylformamide in the presence of potassium fluoride as a weak base, as previously reported (Chegaev et al., 2016).
Phenylephrine was dissolved in 0.1 M HCl, Dox in distilled water. Sdox, in DMSO, was diluted at least 1000 times before use.

2.5 Data analysis

All data are shown as mean ± SEM and analysed by using Student's *t* test for unpaired samples, one sample *t* test or ANOVA followed by Bonferroni post-test as appropriate (GraphPad Prism version 5.04, GraphPad Software Inc., San Diego, CA, USA). In all comparisons, $P < 0.05$ was considered significant. Fluorescence arising from compounds tested was taken into account when operating in the same range of emission wavelengths of fluorescent dyes (see Supplementary Fig 1).

3. Results

Effects of Dox and Sdox on A7r5 and EA.hy926 cells

Dox and Sdox caused a concentration-dependent reduction in cell viability. Both A7r5 and EA.hy926 cells, however, were more sensitive to Dox than to Sdox (Table 1). This was particularly evident after 24 h, IC_{50} values differing by ~6 and ~10 fold in smooth muscle vascular- and endothelial-cells, respectively.

Table 1. Effects of Dox and Sdox on A7r5 and EA.hy926 cell viability

	A7r5 IC_{50} (µM)			**EA.hy926 IC_{50} (µM)**		
	24 h	48 h	72 h	24 h	48 h	72 h
Sdox	2.96±0.58	1.54±1.23	1.53±1.00	3.66±0.86	1.32±0.24	0.56±0.17
Dox	0.51±0.33***	0.48±0.23**	0.29±0.17***	0.36±0.39***	0.71±1.03	0.27±0.33*

IC_{50} values are the mean ± S.D. of at least three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs Sdox matched time point, Student's t-test for unpaired samples

Cytotoxicity was accompanied by changes in cell morphology as assessed by contrast light microscopy: after 24 h treatment with concentrations close to drug IC_{50} values, both A7r5 and EA.hy926 cells clearly showed apoptotic-like features (see Supplementary Fig 2). Thus, to examine

in more detail these cytotoxic effects, the following assays were performed after 24 h treatment with 1 μM and 3 μM Dox and Sdox, respectively.

Being Sdox an anthracycline with a H_2S donor moiety, its ability to release H_2S was assessed. In both cell lines, intracellular H_2S levels significantly increased following a 24-h treatment with Sdox (Fig 1A). To verify the possible role of H_2S in the Sdox-mediated cytotoxic effects, the viability of cells in the presence of the H_2S scavenger hydroxocobalamin (OHCob), was investigated. Interestingly, OHCob (100 μM for 24 h) added along with Sdox (3 μM for 24 h) reverted its cytotoxic effects in A7r5 cells, while it was ineffective in the endothelial cell line (Fig 1B).

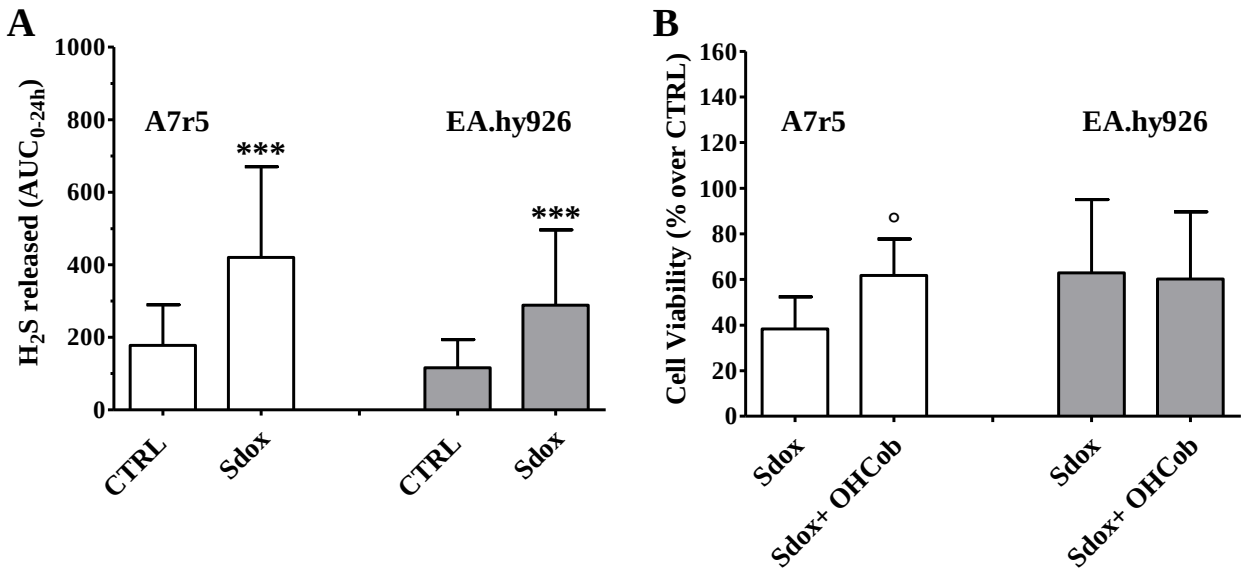

Figure 1. Effects of Sdox on intracellular H_2S levels and cell viability of A7r5 and EA.hy926 cells. (A) Total amount of H_2S released by vehicle (CTRL) and 3 μM Sdox during 24 h, expressed as $AUC_{(0\text{-}24h)}$. (B) Cell viability assessed in presence of Sdox (3 μM for 24 h) and Sdox + OHCob (100 μM for 24 h). Data are reported as mean ± S.D. of at least four independent experiments. ***P < 0.001 *vs* CTRL, ° P< 0.05 vs Sdox same cell line, Student *t* test for unpaired samples.

To study in more detail the mechanisms causing cell death observed after 24 h of treatment with both anthracyclines, flow cytometry-mediated cell cycle analysis was performed. Results showed that sub-diploid cells were significantly increased by Dox in both cell lines, while Sdox raised, although to a lesser extent than Dox, only those of endothelial cells (Fig 2A). Interestingly, Sdox caused an arrest in both S and G2/M phase (A7r5 cells) or only in G2/M (EA.hy926 cells), which was accompanied by a decrease in the number of cells in G0/G1 phase (Fig 2).

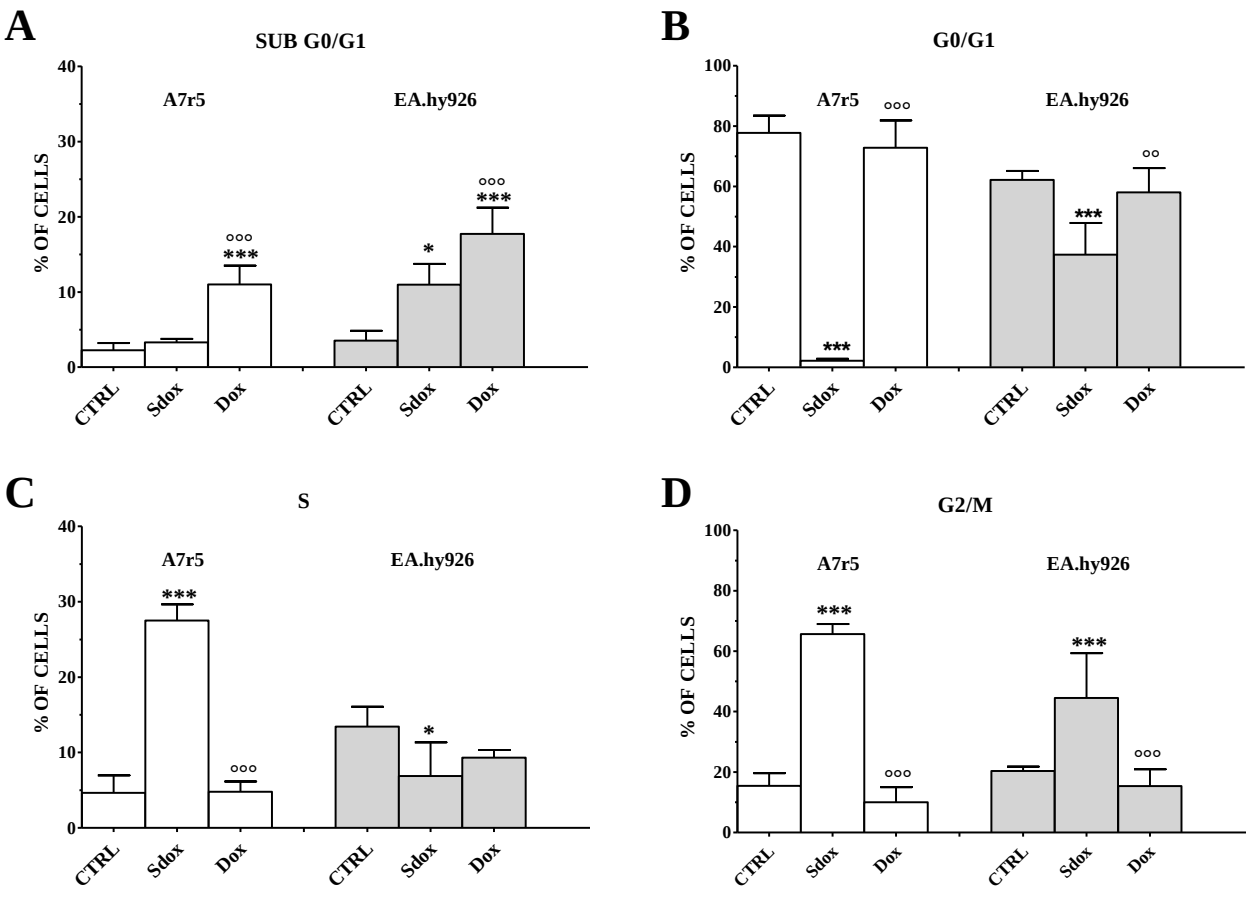

Figure 2. Doxorubicin- (1 μM Dox, 24 h) and Sdox- (3 μM, 24 h) induced changes in A7r5 and EA.hy926 cell cycle. Percentage of cells in the (A) sub G0/G1 (apoptotic), (B) G0/G1, (C) S, and (D) G2/M phases. Data are reported as mean ±S.D. of at least four independent experiments. *P < 0.05, ***P < 0.001 vs control (CTRL); °°P < 0.01, °°°P < 0.001 vs Sdox in the same cell line (ANOVA followed by Bonferroni post-test).

Apoptotic death was also highlighted by AV/PI assay. As shown in Fig 3, Dox increased the percentage of early (AV+, PI-, in both cell lines) or late (AV+, PI+, in EA.hy926) apoptotic cells, proportionally decreasing healthy (AV-, PI-) cells without affecting that of necrotic (AV-, PI+). Sdox increased the percentage of early apoptotic cells, mainly in EA.hy926. This effect was accompanied by an increase in necrotic A7r5 cells along with a proportional decrease in healthy cells. Moreover, Dox and Sdox significantly increased DCF fluorescence, consistent with higher levels of intracellular reactive oxygen species (Supplementary Fig 3) in both cell lines.

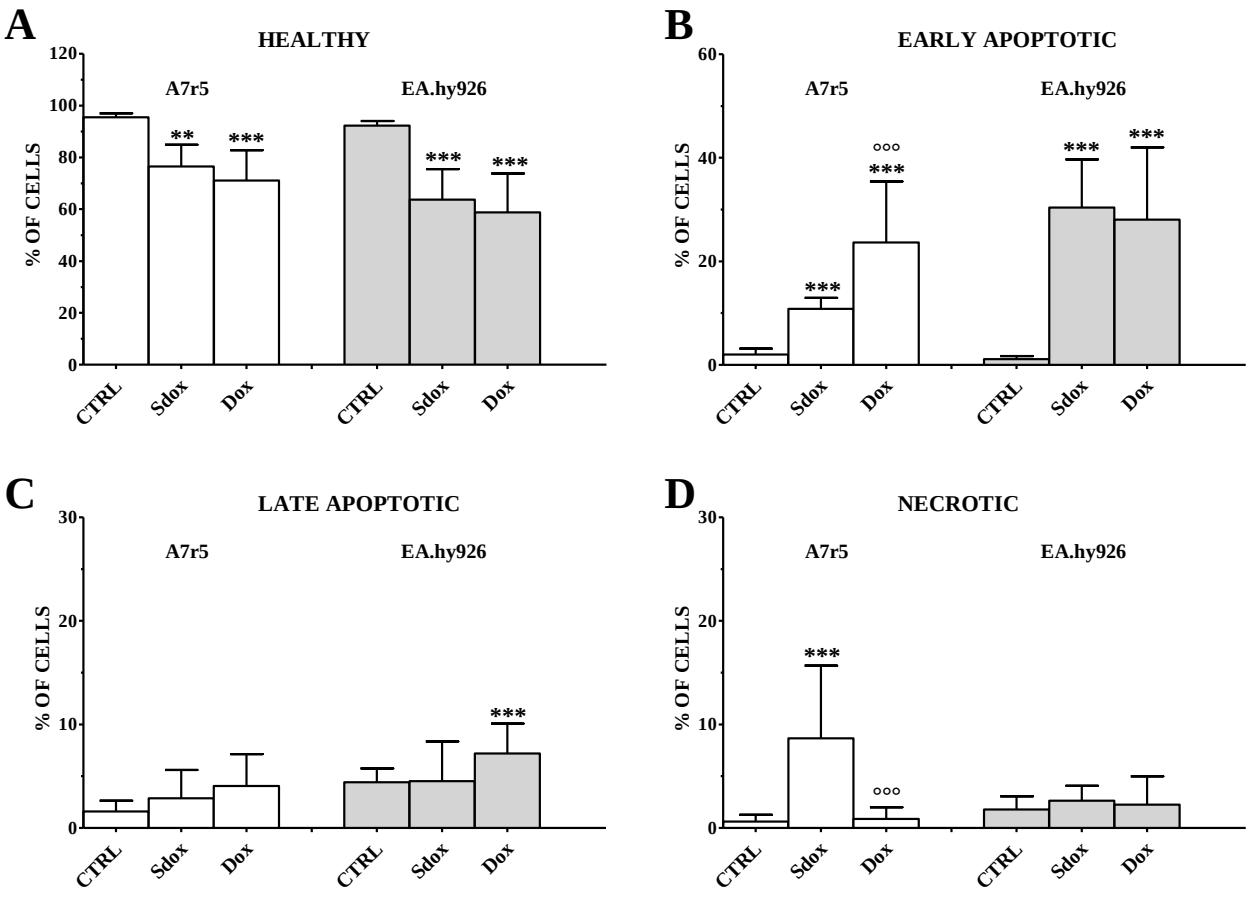

Figure 3. Doxorubicin- (1 μM Dox, 24 h) and Sdox- (3 μM, 24 h) induced changes in the percentage of A7r5 and EA.hy926 healthy (AV-, PI-), early (AV+, PI-) and late apoptotic (AV+, PI+), as well as necrotic (AV-, PI+) cells. Data are reported as mean ± S.D. of at least four independent experiments. **P < 0.01, ***P < 0.001 vs control (CTRL); °°P < 0.01, °°°P < 0.001 vs Sdox in the same cell line (ANOVA followed by Bonferroni post-test).

Among the different mechanisms of apoptosis underpinning cell death, mitochondria- and extrinsic receptor-mediated pathways, involving caspase 9 and 8, respectively, play a fundamental role. These, in turn, activate caspase 3, thus causing DNA fragmentation. Thus, the involvement of mitochondria- rather than extrinsic-pathways in Dox- and Sdox-mediated cytotoxic effects was investigated. Caspase 3 activity was significantly increased by Dox in both cell lines and by Sdox only in A7r5 cells (Fig 4). Dox boosted also caspase 8 and 9 activities in A7r5 cells while decreasing them in EA.hy926 cells. Similarly, a modest decrease in caspase 8 activity was observed in endothelial, but not in vascular smooth cells, upon Sdox treatment, while that of caspase 9 was not affected in both cell lines (Fig 4).

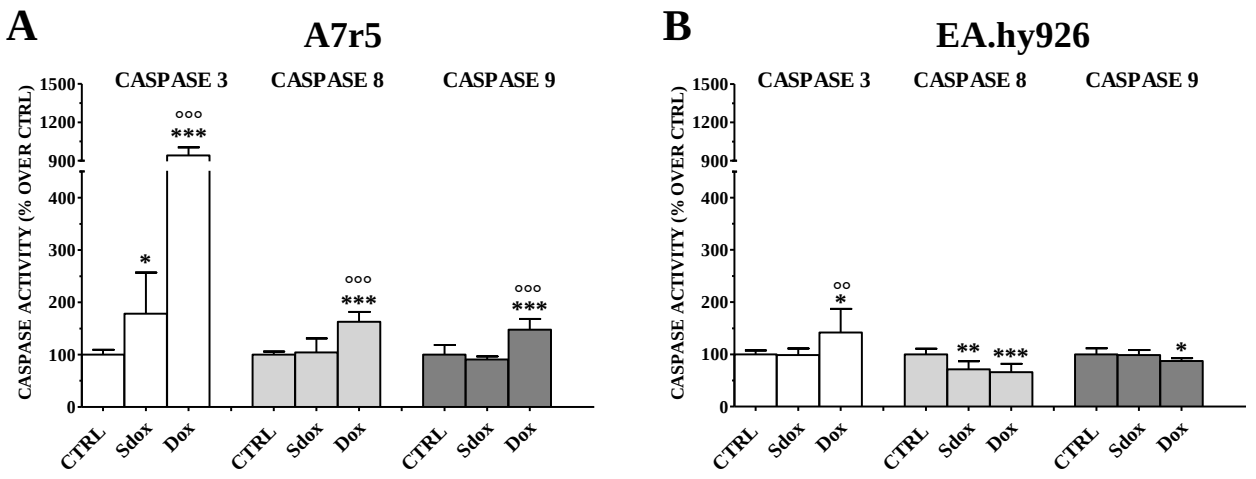

Figure 4. Doxorubicin- (1 μM Dox for 24 h) and Sdox- (3 μM for 24 h) induced changes in caspase 3, 8, and 9 activity in (A) A7r5 and (B) EA.hy926 cells. The specific fluorogenic substrates DEVD-AMC (caspase 3), IETD-AMC (caspase 8), and LEHD-AMC (caspase 9) were used, and the fluorescence of AMC-fragment released by active caspases measured at 380 nm and 460 nm excitation and emission wavelengths, respectively. Data are reported as mean ± S.D. of at least three independent experiments. *P<0.05, **P<0.01. ***P<0.001 vs control (CTRL); °°P<0.01, °°°P<0.001 vs Dox (ANOVA followed by Bonferroni post-test).

To investigate in depth the mechanisms involved in anthracycline-induced cytotoxicity, DNA double strand breaks (DSBs) were quantified as the percentage of cells presenting nuclei with γH2AX foci. After 24 h treatment, Dox (1 μM) and Sdox (3 μM) induced a similar percentage of foci in the nucleus of A7r5 cells, whereas Sdox was significantly less toxic than Dox in EA.hy926 cells (Fig 5).

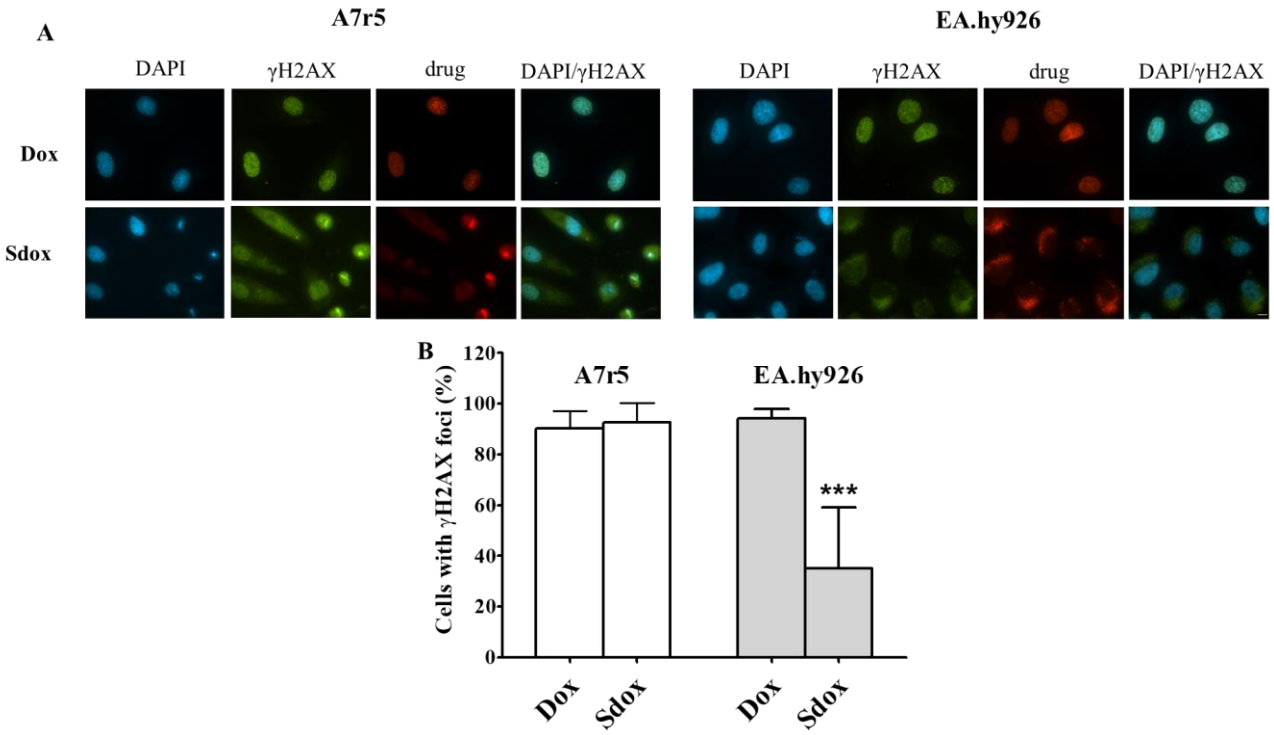

Figure 5. Immunofluorescence analysis of γH2AX foci in A7r5 and EA.hy926 cells treated with either 1 μM doxorubicin (Dox) or 3 μM Sdox for 24 h. (A) Representative micrographs showing the effect of both drugs on (left) A7r5 and (right) EA.hy926 cell nuclear DNA. Magnification: 60X, objective lens (1.49 numerical aperture). Bar: 10 μm. (B) Percentage of visualized cells containing nucleus with foci. Data are reported as mean ±S.D. of three independent experiments. ***P < 0.001 vs Dox (Student's t test for unpaired samples).

Effects of Sdox and Dox on rat aorta rings

These experiments were undertaken to assess the effects of Dox and Sdox on rat aorta rings. Sdox was ineffective on both phenylephrine- and K^+-induced contractions (Fig 6A, C), whereas Dox induced a modest, though significant, increase of phenylephrine-induced tone in endothelium-intact rings (Fig 6B), without modifying the K^+ concentration-response curve (Fig 6D).

Long-term effects of Sdox on vascular smooth muscle function were investigated in cultured rat aorta rings, assessing the contractile response of preparations to either 0.3 μM phenylephrine or 60 mM K^+. In rings pre-treated for 7 days with 10 μM Sdox, both phenylephrine- and K^+-induced contractions were abolished (Fig 6E, F), whereas Dox exhibited the same activity, though at a concentration 10 times lower (Fig 6 E, F).

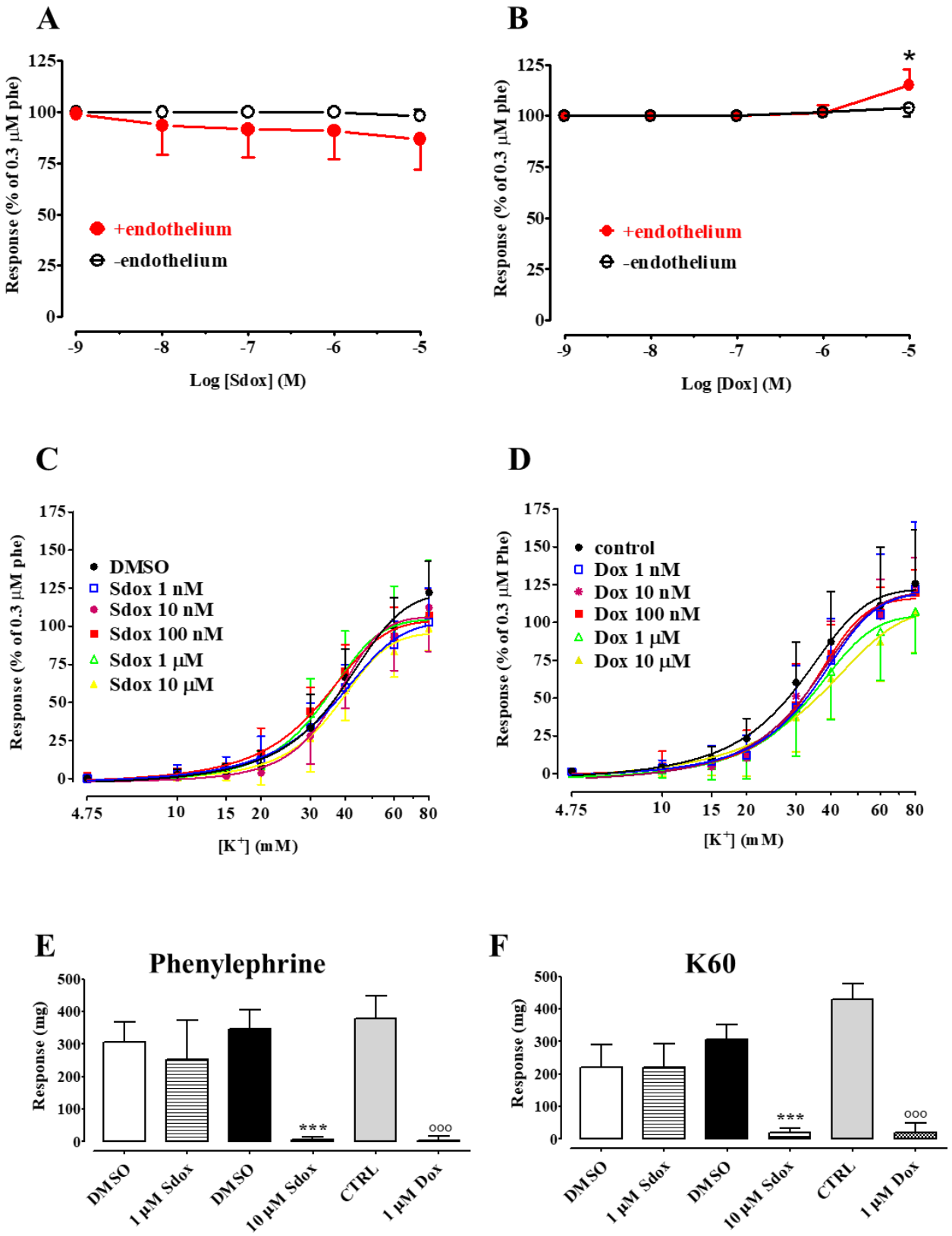

Figure 6. Effects of Sdox and doxorubicin (Dox) on rat aorta rings. (A, B) Sdox and Dox concentration-response curves constructed either in endothelium-denuded (−endothelium) or

endothelium-intact (+endothelium) fresh aorta rings pre-contracted by 0.3 μM phenylephrine (Phe). In the ordinate scale, relaxation is reported as a percentage of the initial tension induced by 0.3 μM Phe. Data points are mean ± S.D. (n = 4-5) of at least three independent experiments. *P < 0.05, vs 100%, one sample t-test. (C, D) Effects of Sdox and Dox on the contraction induced by high K^+. K^+ concentration-response curves were constructed in presence of various concentrations of compounds or vehicle. Data points are mean ± S.D. (n=4-8) of at least three independent experiments and represent the percentage of the maximum response to 0.3 μM Phe. (E, F) Contractile responses to (E) 0.3 μM phenylephrine or (F) 60 mM K^+ (K60) in rings cultured for 7 days with either vehicle or compound. Each treatment is paired with the corresponding control column. Response is reported in mg of contractile isometric tension developed by the rings. Columns represent mean ± S.D. (n = 3–12) of at least three independent experiments ***P < 0.001 vs DMSO, °°°P < 0.001 vs control (CTRL), Student's t-test for unpaired samples.

3.3. Effects of Sdox and Dox on $I_{Ca1.2}$ in A7r5 cells

The effect of both anthracyclines on $I_{Ca1.2}$ was investigated in A7r5 cells to provide evidence of any potential Ca^{2+} antagonist activity. Sdox reduced current intensity in a concentration-dependent manner by 29.4% and 46.7% at 1 μM and 10 μM, respectively, whereas Dox was ineffective (Supplementary Fig 4).

4. Discussion

The present results suggest that Sdox possesses a more favourable vascular toxicity profile than the parent compound Dox, thus raising hopes for its future development as a drug.

In A7r5 and EA.hy926 cells, Sdox, an anthracycline carrying a H_2S donor moiety, was capable to release H_2S. This gasotransmitter mediated, at least in part, its cytotoxic activity in A7r5 cells. H_2S can promote or inhibit vascular smooth cell apoptosis as a function of its concentration and of exposure duration [20]. Moreover, exogenous H_2S can induce apoptosis of human aorta myocytes *via* increased ERK activity. This transduces the apoptotic signal to its downstream enzyme cascades, ultimately activating caspase-3 [21]. The present results indicate that Sdox released enough H_2S to trigger cell death and this conceivably occurred through the ERK-mediated pathway(s), as suggested by the increase of caspase 3 activity observed in A7r5 cells.

Arterial smooth muscle cells are normally quiescent. In fact, more than 80% of A7r5 cells were found in the G0/G1 phase of the cell cycle. However, the majority of cells treated with Sdox were in S (A7r5 cells) and G2/M (A7r5 and EA.hy926 cells) phases, indicating that this agent targeted the regulation of G2-M transition controlling cell growth, thereby facilitating apoptosis death, probably via H_2S.

The fact that in non-transformed rat intestinal epithelial cells [22] and in many tumour cell lines [23;24] sodium hydrogen sulphide causes the same effect support this hypothesis.

Dox and Sdox caused apoptosis-mediated cell death, as demonstrated by sub G0/G1 cell cycle analysis and AV/PI assay. In A7r5 cells, Dox induced the activation of caspase 3, 8, and 9 as previously observed in fibroblast [25], Jurkat cells [26], and human urinary bladder cancer cells [27], whereas in neonatal rat cardiomyocytes [28] only caspase 3 and 9 were activated. Sdox apoptotic activity was significantly lower in A7r5 cells and only caspase 3 was activated, thus indicating a more encouraging profile for this agent as compared to the parent compound. Further experiments are needed to clarify why Sdox activated only caspase 3, though inducing apoptosis.

In endothelial cells, Dox increased ROS production, caspase 3 activity, and induced apoptosis, as previously observed in HUVEC [29] and in cultured bovine aortic endothelial cells [30], poorly affecting caspase 8 and 9 activity. On the other hand, Sdox did not affect the activity of caspase 3 and 9 and weakly reduced that of caspase 8, thus suggesting that it might cause apoptosis-like programmed cell death characterized by less compact chromatin condensation, phosphatidylserine exposure, and absence of executioner caspase activation [31]. Though questionable, the modest reduction (~ 10-30%) in the activity of caspase 8 induced by both anthracyclines, and caspase 9 induced by Dox in EA.hy926, never reported so far, warrants further examination to appreciate its biological meaning, if any.

Dox is a cytotoxic drug capable of inducing DNA damage, thus leading to apoptosis [32]. In A7r5 cells, both anthracyclines gave rise to a comparable level of γH2AX foci, while in EA.hy926 cells, Sdox appeared less toxic. The different compartmentalization of the two agents may account for this difference. Indeed, in both cell lines, Dox accumulated in the nucleus, while Sdox localized both in the nucleus and in the cytosol in A7r5 cells, and mostly outside the nucleus in EA.hy926 cells, as previously reported in cancer cells [4]. Therein, several factors – including the different intracellular localization – may explain the different toxicity profile between cancer and non-transformed cells, but also between different non-transformed components of cardiovascular system.

Finally, in EA.hy926 cells Dox and Sdox might trigger ER-mediated apoptosis, as already observed in heart tissue [33], in human Dox-sensitive osteosarcoma U-2OS, and Saos-2 cell lines [4].

Functional experiments demonstrated that Sdox was devoid of acute vascular activity. In fact, it did not affect smooth muscle contraction evoked by either electro-mechanical or pharmaco-mechanical coupling. However, Dox exhibited a modest contractile effect in rings stimulated by phenylephrine, as previously reported [9;34]. Surprisingly, the electrophysiology data recorded in A7r5 cells showed that, unlike Dox, Sdox caused a concentration-dependent $Ca_V1.2$ channel blockade that, however, did not manifest in depolarized aorta rings, where contraction is elicited by extracellular Ca^{2+} influx

through the open $Ca_V1.2$ channels. These apparently conflicting observations are likely the consequence of the different experimental conditions that characterised functional and patch-clamp experiments. In fact, as previously observed by Dai et al. [35] and Orlov et al. [36] inhibition of $Ca_V1.2$ channels observed in A7r5 cells, where the patch-clamp technique in the whole-cell configuration allows the study of the current in isolation from all the other transporters, channels, second messengers, etc., can be masked in the whole tissue (i.e. in intact aorta rings) by the concomitant effect of H_2S released from Sdox on other targets, namely the $Na^+/K^+/2\ Cl^-$ cotransporter [36].

When both anthracyclines were assessed for their long-term vascular toxicity, Dox blunting of both phenylephrine- and high K^+-induced stimulation substantiated previous observations [9;37]. Similar results were obtained in rings cultured with Sdox for 7 days, though at a concentration 10-fold higher than that of Dox, thus highlighting a noteworthy decrease in long-term vascular toxicity.

5. Conclusion

In conclusion, Sdox exhibited a more favourable vascular toxicity profile than Dox. Taking into account its greater preclinical efficacy in resistant tumours, Sdox is worth of further investigations in preclinical and clinical settings.

CRediT authorship contribution statement

Miriam Durante: Methodology, Investigation.

Maria Frosini: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing.

Elda Chiaino: Methodology, Investigation

Fabio Fusi: Formal analysis, Writing - review & editing.

Alessandra Gamberucci: Investigation.

Beatrice Gorelli: Investigation

Konstantin Chegaev: Resources.

Chiara Riganti: Writing - review & editing.

Simona Saponara: Conceptualization, Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

None

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