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Streptozotocin-induced hyperglycemia unmasks cardiotoxicity induced by doxorubicin

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Late-onset cardiotoxicity induced by anthracyclines occurs years to decades after completion of anti-cancer therapy and is associated with increased morbi-mortality of cancer survivors. Chemotherapy at the time of treatment probably causes cardiac damages for which the juvenile heart compensate. Co-morbidities happening in the adulthood such as type 1 diabetes (DT1), affect the heart and thus can unmask chemotherapy induced cardiotoxicity. To prove our hypothesis, we induced hyperglycemia [Streptozotocin treatment (STZ), 50 mg/kg/day for 5 days] in 11 weeks old mice who previously received doxorubicin treatment (Dox, 3 mg/kg) when they were six-weeks old. Interestingly, streptozotocin-induced hyperglycemia in Dox-pretreated mice (Dox-STZ) induced a higher mortality ($p < 0.05$) and more severe cardiac dysfunction ($p < 0.0001$) when compared with mice receiving Dox or STZ alone. Apoptosis evaluated by caspase 3 protein expression and *Bax/Bcl2* genes expression was higher in Dox-STZ mice compared to STZ or Dox alone. While Dox and STZ independently induced capillary rarefaction, cardiomyocytes atrophy was only induced by STZ. Furthermore, Sirius-red staining of cardiac sections showed higher fibrosis levels ($p < 0.0001$) in Dox-STZ compared to Dox or STZ alone. All together, these results demonstrate that STZ precipitates and unmask cardiac dysfunction in previously treated Dox animals.

Anthracycline-based chemotherapy is one of the most commonly used anti-neoplastic regimens[1,2]. Although highly effective, anthracyclines are cardio-toxic which may affect up to 39% of cancer patients[3]. Late-onset anthracycline cardiotoxicity occurs years to decades after anthracycline treatment has been completed[4,5] which is a lifelong threat especially in cancer survivors who have a long life-expectancy after successful antineoplastic treatment. Late-onset cardiac failure may be the result of the inability of the reduced number of healthy myocytes to fulfill after a long-term asymptomatic period the demands of additional cardiovascular strain such as diabetic cardiomyopathy.

Moreover, diabetes has been associated with poor outcomes and overall survival in patients with cancer who were treated with anthracyclines-based chemotherapy[6,7]. It is reasonable to expect that a cancer patient with diabetic cardiomyopathy would be more susceptible to the anthracyclines-induced cardiac damage due to the pre-existing cardiac dysfunction[8]. However, the role of diabetes in the initiation or unmasking sub-clinical cardiotoxicity and the exact mechanisms on how the additional stress from diabetes, remain largely unexplored.

Few experimental studies showed that doxorubicin pharmacokinetics are impaired in diabetic heart leading to increased fat accumulation and decreased renal clearance[9–12]. Other mechanisms involve decrease of antioxidant defense, defective β-oxidation of fatty acids followed by energy depletion[13,14]. However, the role of diabetes-induced cardiac atrophy and capillary rarefaction in anthracyclines-induced cardiotoxicity has never been explored yet. The pathophysiology of cardiomyocyte injuries by diabetes and anthracyclines-induced cardiotoxicity may be commonly linked to oxidative stress, apoptosis, fibrosis, inflammation and metabolic remodeling[15–18]. Doxorubicin undergoes redox cycling by mitochondrial complex I NADH dehydrogenase, leading to oxidative stress, membrane lipid peroxidation, mitochondrial dysfunction, and

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altered cardiac gene expression collectively leading to cardiomyocyte death[19]. Diabetic cardiomyopathy involves coronary microcirculation defects, hormonal dysregulation, all leading to a diastolic and then a systolic cardiac dysfunction[20–22].

We hypothesize that anthracyclines could induce aberrant cell signaling and oxidative stress overlapping with diabetes and leading to a vicious cycle liable to expedite and to exacerbate the process of heart failure. Using an in vivo juvenile mice model of doxorubicin induced cardiotoxicity and streptozotocin induced hyperglycemia together with an in vitro model of cardiomyocytes subjected to doxorubicin and hyperglycemia, we aim to demonstrate that streptozotocin inducing hyperglycemia thus mimicking a type 1 diabetes will unmask doxorubicin induced cardiotoxicity.

Material and methods
Animal experimental procedures
Animal handling practice during experimentation was followed and approved by the Animal Ethics Committee Lariboisière–Villemin according to the guidelines of the Laboratory Animal Ethic Regulation (APAFIS#12111-2017110817428853V3). The ARRIVE rules and guidelines were taken into consideration by the authors.

Mice were housed with a 12/12 h light–dark cycle and with ad libidum access to food and water. All experimental procedures were performed in accordance with the Directive 2010/63/eu of the European Union.

Six-week old male SWISS mice (Janvier) were injected intraperitonealy, four times every 5 days, with either doxorubicin (Sigma-Aldrich, Saint Quentin Fallavier, France; Dox; n = 34) or saline (n = 24). The first 2 doses of doxorubicin were at a concentration of 1 mg/kg, and the last 2 injections were at 0.5 mg/kg[23]. Control animals were injected with an equivalent volume of saline. Animals were observed daily and weighed weekly. Two weeks after the last injection, streptozotocin-induced hyperglycemia was initiated.

It was induced as previously described[21]. Saline or Dox treated mice received daily intra-peritoneal injections for 5 days with streptozotocin (STZ) (40 mg/kg/d) dissolved in sodium citrate buffer (Sigma-Aldrich, Saint Quentin Fallavier, France). Control animals received sodium citrate buffer injections. Three days after the last injection, whole-blood glucose was monitored using the Euroflashmonitor (LifeScan, Milpitas, CA, USA) in fasted mice. STZ-treated mice with blood glucose concentration higher than 15 mM were considered diabetic. DT1 was induced for a period of 6 weeks.

At the end of the protocol, mice were sacrificed with an i.p. overdose of pentobarbital (50 mg/kg) injection. The hearts were arrested in diastole by an intravenous injection of saturated KCl, they were quickly removed, weighed and cut transversely at the ventricles equator. The upper parts of the heart were mounted, frozen in isopentane precooled with liquid nitrogen, and kept at -80 °C until use, as previously described[24].

Echocardiography
Echocardiography was performed on lightly sedated mice with ketamine (80 mg/kg) as previously described[25]. The thickness of the left ventricle LV and fractional shortening were measured using a GE Vivid-7 machine (General Electric Medical Systems, Paris, France) equipped with an 8–14 MHz linear transducer. Echocardiography was performed 5 times, before and after treatments (Dox or STZ) and at the end of the protocol (Fig. 1A).

In vitro studies
Rat cardiomyocytes H9C2 cells (Sigma-Aldrich, 88092904) were cultured in DMEM (Sigma-Aldrich, Saint Quentin Fallavier, France) supplemented with 10% of fetal bovine serum (Fisher Scientific, Illkirch, France) and 1% of penicillin–streptomycin antibiotics (Sigma-Aldrich, Saint Quentin Fallavier, France), at 37 °C and 5% Co2. Plated cardiomyocytes were starved (2% serum) for 10–12 h before treatment with Dox (1 μM) and/or glucose (33 mM) for 24 h. Cell survival was assessed by counting alive cells using Malassez counting-chamber. The cells were then centrifuged (300 g, 5 min) and the pellet used for protein and RNA preparation. All the treatments were done in triplicates and the experiments were repeated three times.

For cell morphology assessment, cells were grown on coverslips which were fixed at the end of the protocol with 4% paraformaldehyde for 10 min following a thorough wash with 1% sterile phosphate buffered saline (PBS). This was followed by immunofluorescence staining.

RNA preparation and real-time quantitative RT-PCR
RNA were prepared from mice heart tissue using QIAGEN RNeasy Mini Kit (Qiagen, Courtaboeuf, France) according to manufacturer instructions and as previously described[26]. RNA concentration was determined using the ND-100 spectrophotometer (Nanodrop, Thermo Fischer Scientific Waltham, USA). The cDNA synthesis was performed using the QuantiTect® Reverse Transcription Handbook Kit (Qiagen, Courtaboeuf, France). Real-time quantitative RT-PCR was performed using 1.25 ng cDNA and 10 nmol of specific primers (Supplemental Table 1) for amplification of the genes of interest using the LightCycler 96 thermal cycler (Roche Diagnostics France SAS, Meylan, France).

Protein preparation and western blot
Proteins were prepared from mice heart tissue and H9C2 cells in RIPA lysis buffer (0.02M Tris HCl pH7.5, 0.1M KCl, 1mM EDTA, 1mM EGTA, 1mM DTT, 0.04M B-glycérophosphate, 2mM sodium Ortho-Vanadate, 1mM PMSF, 0.01Mm NaF) in the presence of anti-proteases and anti-phosphatases (Sigma-Aldrich, Saint Quentin Fallavier, France). Protein yield was measured by spectrophotometry using the Pierce BCA Protein Assay kit (ThermoFischer Scientific, France). Proteins (20μg) were heated at 99 °C for 7 min before being separated by SDS-page electrophoresis using on a 4–20% polyacrylamide gel (Biorad, Hercules, USA) and transferred to 0.2-μm nitrocellulose membrane. Nitrocellulose blots were blocked and incubated with primary and secondary
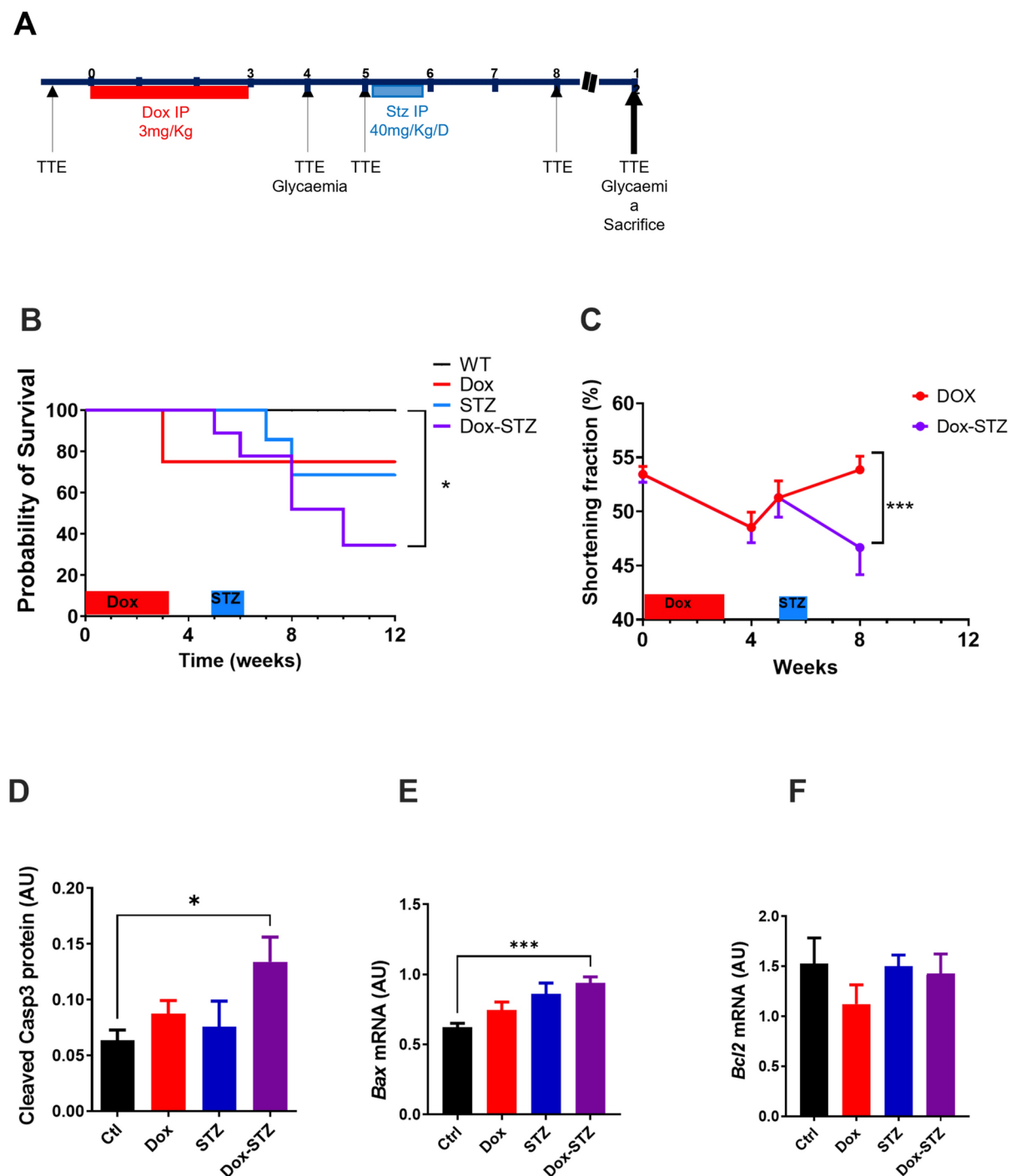
Fig. 1. Streptozotocin-induced hyperglycemia aggravates cardiac dysfunction and mortality induced by doxorubicin. (**A**) Schematic representation of the protocol used in vivo. (**B**) Kaplan-Meyer plot of mice surviving during the in vivo experimental protocol in the 4 groups of mice treated with saline (Ctl), doxorubicin (Dox), streptozotocin (STZ) or both (Dox-STZ). (**C**) Cardiac function evaluated by fractional shortening measured by transthoracic echocardiography (TTE) of mice after doxorubicin treatment (represented arrow *Dox* on the x-axis) and type 1 diabetes (represented by *Stz* on the x-axis). (**D**) Cleaved caspase 3 protein expression in the cardiac tissue evaluated by western blot. (**E, F**) mRNA expression of *Bax* and *Bcl2*, respectively measured in the cardiac tissue par RT-qPCR. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

antibodies (Supplemental Table 2). Chemiluminescence was measured using AZURE (Azure Biosystems) and measured using MultiGauge V2.02 software (Fuji).

Immunostaining
Double immunostaining was performed on cryostat heart tissue sections using vinculin and caveolin-1 antibodies. Phalloidin antibody was used for H9C2 immunostaining. All antibodies are listed in Supplemental Table 2.

Cardiac fibrosis
Cardiac cryosections (7 μm) were stained with Sirius red (0.5% in saturated picric acid). Fibrosis was semi-quantified, as previously described[24] with a severity scale ranging from slight fibrosis (1) to high collagen accumulation (5). Fibrosis quantification was double-blinded.

Statistical analyses
All values are expressed as mean ± SEM. Statistical comparisons were performed using GraphPad Prism software (version 6.00 for Windows, GraphPad Software, La Jolla California, USA www.graphpad.com). Variables comparison was done using one-way ANOVA and Dunn's correction or two-way ANOVA with Bonferroni correction. Survival rate was analyzed by the Kaplan–Meier method with the log-rank test. Statistical significance was considered when $p < 0.05$.

Results
Doxorubicin induces acute cardiac dysfunction and hyperglycemia
One week after the last injection of doxorubicin, mice had an echocardiography to detect early cardiotoxicity, defined as minus 10% in fractional shortening (FS) when compared to the echocardiography done before the onset of doxorubicin treatment. Ten mice (29%) presented with an acute doxorubicin induced cardiotoxicity (Supplemental Figure 1A). Only mice presenting with no cardiac dysfunction were included in the experimental DT1 like induction.

Interestingly, doxorubicin induced an increase in glycaemia ($p < 0.05$) when compared to saline treated mice (Supplemental Figure 1B). A concomitant decrease of GLUT4 protein expression in the cardiac tissue of doxorubicin treated mice was observed ($p < 0.05$), with no change in GLUT1 protein expression (Supplemental Figure 1C). However, this hyperglycemia induced by doxorubicin wasn't exacerbated post STZ treatment (Supplemental Figure 2).

STZ-induced hyperglycemia aggravates cardiac dysfunction and mortality induced by doxorubicin
Doxorubicin and STZ-induced hyperglycemia independently induced mice mortality (Fig. 1B). Interestingly, STZ-induced hyperglycemia induction in doxorubicin pretreated mice induced a higher mortality in mice. STZ-induced hyperglycemia, two weeks after induction, induced a decrease in FS in doxorubicin treated mice (Fig. 1C) probably involved in the highest percentage of death observed in the Dox-STZ experimental group. Diabetes induced a decrease in body weight, heart and liver weight with no added effect due to doxorubicin treatment (Table 1). At the end of the protocol, no significant difference in systolic cardiac function was observed between the four experimental groups (Table 1), probably associated with a bias induced by death of diseased

Groups	Ctl	Dox	STZ	Dox-STZ	2-Way ANOVA		
					Dox	STZ	Interaction
N	10	9	10	7			
Body weight (g)	45.6 ± 1.5	40.8 ± 1.3	35.8 ± 1.0**	34.0 ± 1.53**	NS	$p < 0.01$	NS
Echocardiography							
IVSd (mm)	1.2 ± 0.05	1.15 ± 0.04	0.97 ± 0.05	0.89 ± 0.06	< 0.0001	< 0.0001	< 0.0001
LVd (mm)	3.8 ± 0.10	4.3 ± 0.08	3.6 ± 0.20	3.7 ± 0.14	NS	0.03	NS
LVPWd (mm)	1.1 ± 0.04	1.0 ± 0.04	1.1 ± 0.05	1.1 ± 0.10	NS	NS	NS
SF (%)	52.1 ± 3.1	51.1 ± 2.2	51.9 ± 1.7	49.7 ± 1.7	NS	NS	NS
Anatomical data							
Heart weight (mg)	177.3 ± 6.0	175.5 ± 5.9	166.6 ± 5.6	104.3 ± 11.6	NS	< 0.001	NS
Heart weight/TL	9.4 ± 0.3	9.7 ± 0.4	6.0 ± 0.5	5.4 ± 0.7	NS	< 0.001	NS
Lungs weight/TL (mg/mm)	10.7 ± 0.5	9.4 ± 0.8	9.3 ± 0.6	8.7 ± 0.5	NS	NS	NS
Liver weight/TL (mg/mm)	105.7 ± 6.64	97.1 ± 2.72	85.7 ± 8.28	73.0 ± 14.4	NS	0.0265	NS

Table 1. Echocardiography and anatomical data of control, doxorubicin and/or type 1 diabetes treated mice, at the end of the protocol. Ctl: control mice; Dox: doxorubicin treated mice; STZ: Streptozotocin-induced hyperglycemia mice; Dox-STZ: Streptozotocin-induced hyperglycemia in doxorubicin treated mice; IVS: intraventricular septum; LV: left ventricular diameter; LVPW: left ventricular posterior wall; SF: shortening fraction; TL: tibia length.

mice. Evolution of echocardiographic parameters from baseline to the end of the protocol are illustrated in Supplemental Table 3 & 4.

We observed an increase in active caspase 3 protein level and the pro-apoptotic *Bax* mRNA level in the Dox-STZ group compared to control group ($p < 0.05$, Fig. 1D, E) whereas the anti-apoptotic *Bcl2* mRNA levels remained stable (Fig. 1F). All together, these results indicated an increased apoptosis in the cardiac tissue of the Dox-STZ mice group.

Furthermore, in vitro experiments showed that doxorubicin or hyperglycemia treatments induced cardiomyocytes mortality which was further higher when the cells were incubated with both doxorubicin and high glucose concentration (Fig. 2A).

STZ-induced hyperglycemia and not doxorubicin induced cardiomyocytes atrophy

Cardiac cachexia assessed by echocardiography showed that doxorubicin and type 1 diabetes individually decreased intraventricular septum diameter when compared to control mice (Table 1). This decrease was aggravated when doxorubicin treatment was followed by STZ-induced hyperglycemia ($p < 0.001$) (Table 1).

Interestingly, cardiomyocytes diameter measured on cardiac sections stained with vinculin antibody showed that only diabetes induced cardiomyocytes atrophy (Fig. 3A, B). Whereas no change in Atrogin 1 protein levels was observed between the experimental mice groups (*data not shown*), an increase of MurF1 protein levels was observed in the cardiac tissue of STZ treated mice (Fig. 3C). These effects of diabetes on cardiomyocyte atrophy is blunted when the mice were pretreated by doxorubicin (Fig. 3A, B).

High glucose concentration had the same effect in vitro by inhibiting the trophicity of cultured H9C2 (Fig. 2B, C). However, doxorubicin induced cardiomyocytes atrophy in vitro which was not observed in vivo (Fig. 2B, C).

Doxorubicin and STZ-induced hyperglycemia independently induced capillary rarefaction

Each stress independently induced a decrease in the number of capillaries per cardiomyocyte (Fig. 3A, D) without an aggravation of this effect when the mice were affected with both stresses (Fig. 3D). In addition, a decrease in cardiac VEGF-A protein level was observed in response to doxorubicin and STZ (Fig. 3E).

STZ-induced hyperglycemia aggravates cardiac fibrosis in doxorubicin treated mice

Cardiac fibrosis quantified on Sirius red stained cardiac sections showed that doxorubicin or STZ-induced hyperglycemia induced increased collagen deposition (Fig. 4A). Interestingly, fibrosis was aggravated in STZ-induced hyperglycemia in doxorubicin pretreated mice (Fig. 4B). We also observed an increase in fibronectin
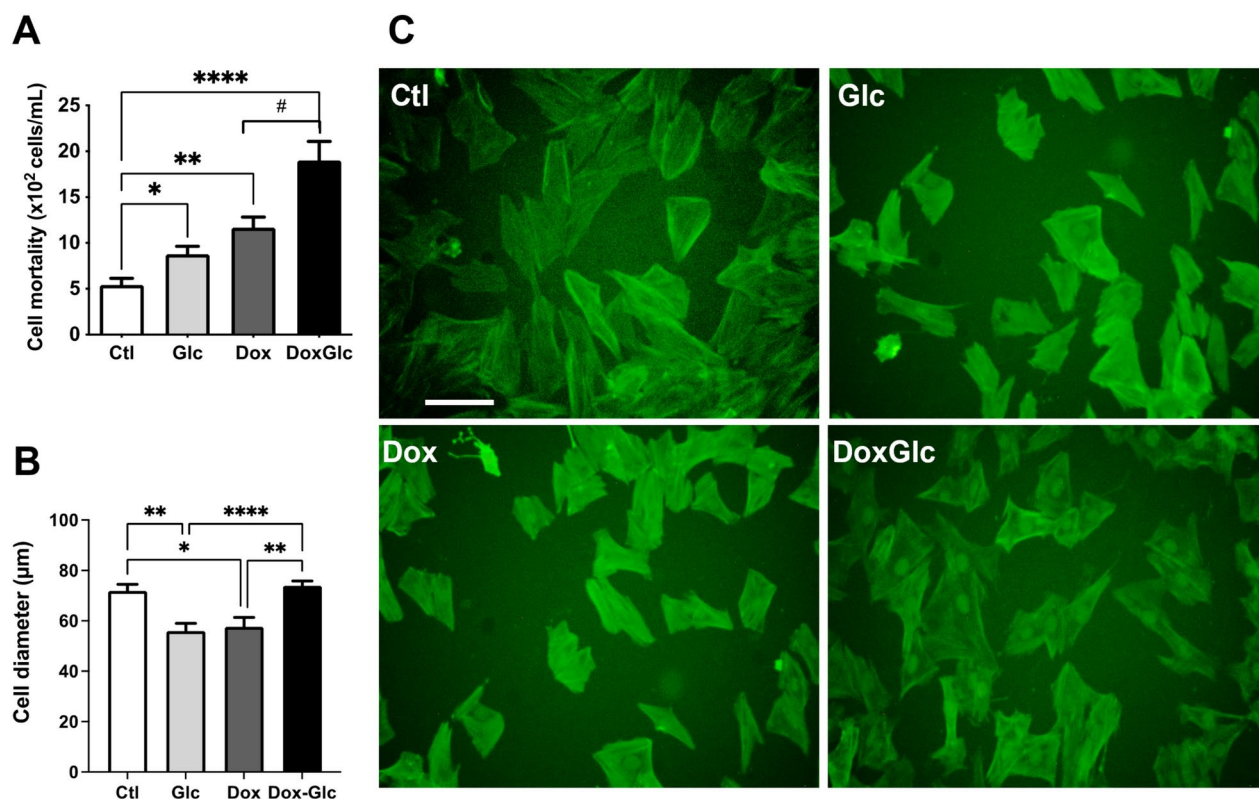

Fig. 2. Effect of doxorubicin and glucose on cardiomyocytes survival and size. (**A**) H9C2 Cardiomyocytes mortality 24h after doxorubicin (Dox), Glucose (Glc) or both (DoxGlc) treatment. (**B, C**) cross section of cultured H9C2 labeled with phalloidin (green). Ctl represent the non-treated condition. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared to the "Ctl" condition. #$p < 0.05$ compared to the "Dox" condition.
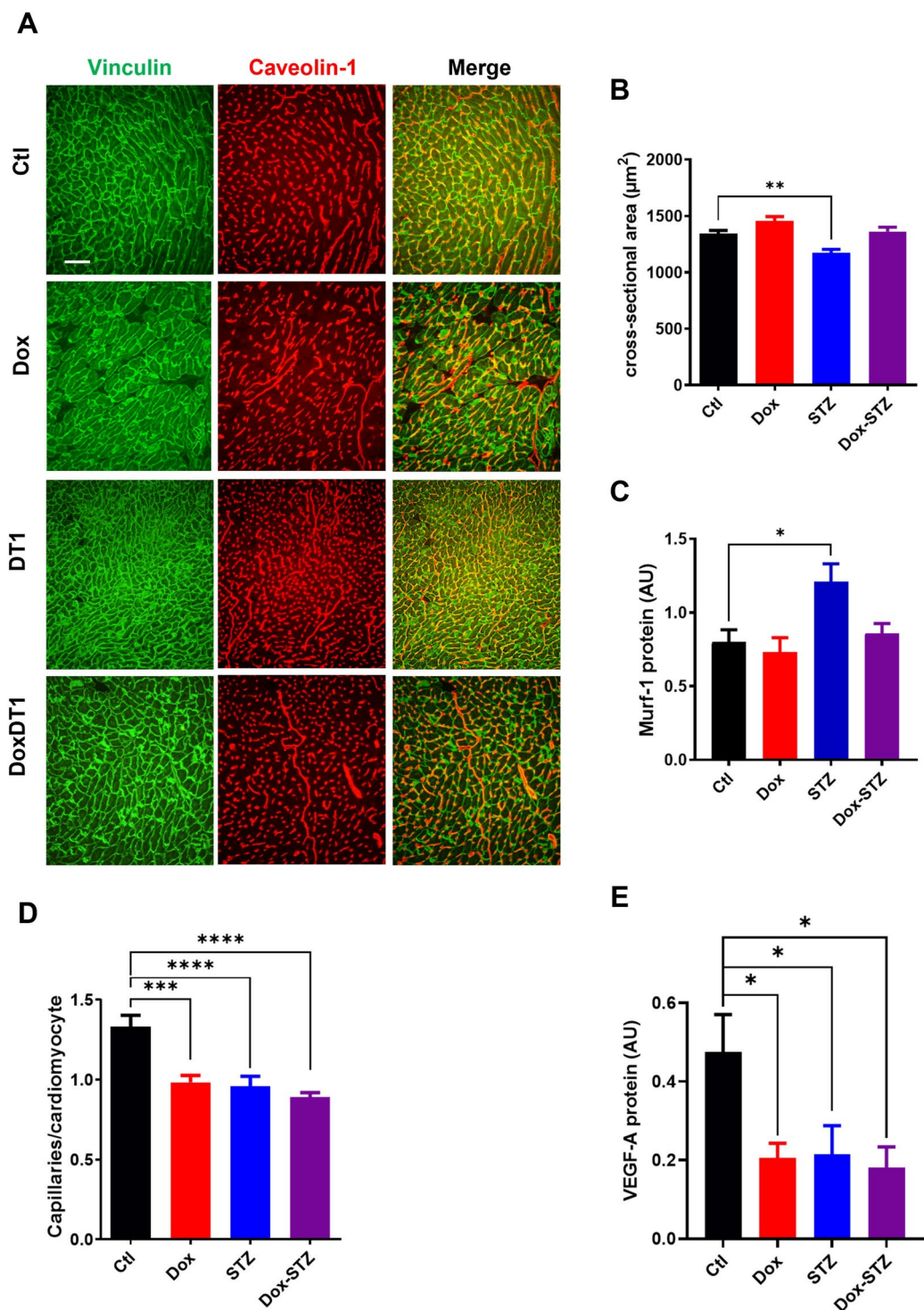
Fig. 3. Effect of streptozotocin-induced hyperglycemia and doxorubicin on cardiomyocytes atrophy and capillary rarefaction. (**A**) Double immunostaining of vinculin (green) and caveolin-1 (red) proteins on cardiac sections of saline (Ctl), doxorubicin (Dox), streptozotocin (STZ) and both doxorubicin and diabetes (Dox-STZ) treated mice. Histograms indicate the cross-sectional area of myocytes (**B**) and the capillary/cardiomyocyte ratio (**D**) measured in the 4 groups. (**C,E**) protein expression of MuRF-1 and VEGF-A, respectively, measured in the cardiac tissue par western blot. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ compared to the "Ctl" condition or otherwise specified. Scale, 100 µm.
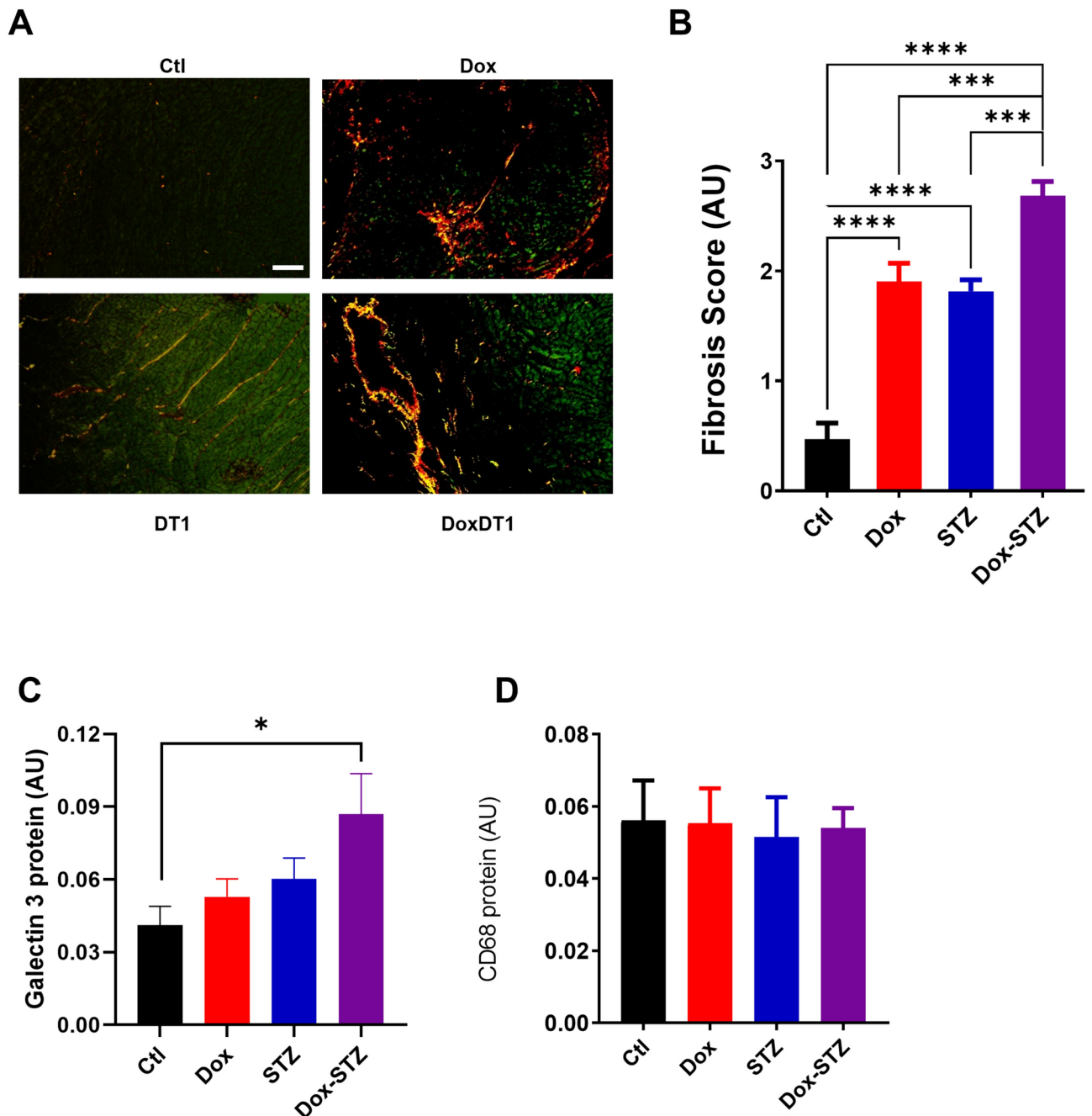
Fig. 4. Streptozotocin-induced hyperglycemia aggravates cardiac fibrosis in doxorubicin treated mice. (**A**) Distribution of fibrillar collagens in the left ventricle of a mouse examined by polarized light microscopy after Sirius Red staining. (**B**) Semi-quantitative analysis of cardiac fibrosis. (**C,D**) protein expression of Galectin 3 and CD68, respectively, in the cardiac tissue. $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the "Ctl" condition. Scale, 100 μm.

protein expression and a decreased laminin protein expression in Dox-STZ mice group (Supplemental Figure 3A & B). Fibronectin upregulation was driven by doxorubicin but not by hyperglycemia as no significant change was observed in STZ group not receiving doxorubicin. Furthermore, we observed an increase in the pro-fibrotic galectin-3 protein levels in the Dox-STZ mice group (Fig. 4C) and no change in CD68 protein level whatever the experimental group (Fig. 4D).

Discussion

Our study demonstrated that type 1 diabetes unmasks infra-clinical cardiotoxicity induced by doxorubicin treatment. The mechanisms by which type 1 diabetes precipitates doxorubicin induced cardiotoxicity involved to
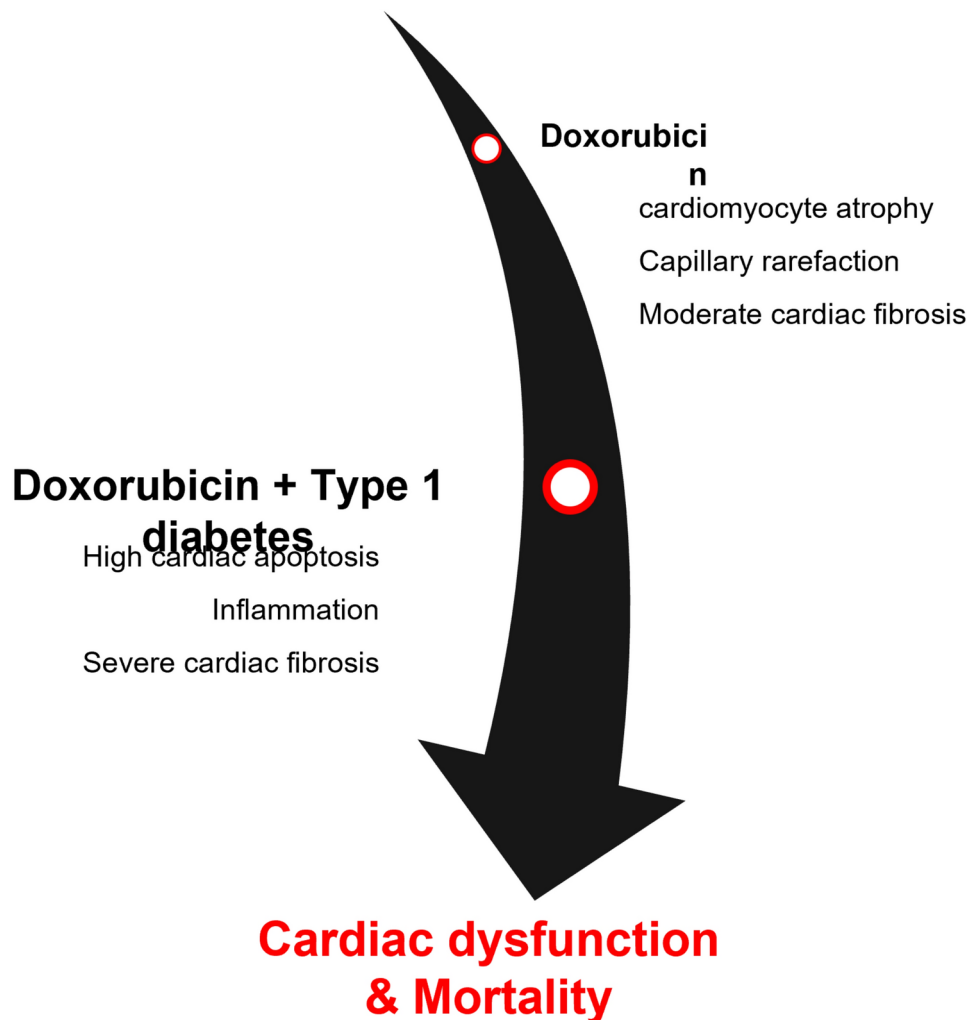
Fig. 5. Scheme illustrating how type 1 diabetes aggravates the doxorubicin-induced cardiac dysfunction leading to mortality.

an increased cardiomyocyte death and consequently extracellular matrix remodeling thus inducing contractile cardiac dysfunction and ultimately death (Fig. 5).

Cancer and diabetes mellitus are two leading causes of death in the world representing health and economical concerns (WHO;[27]). Epidemiological studies have demonstrated a strong link between certain cancers and diabetes mellitus[28,29], with cancer increasing diabetes incidence[30] and vice versa[31,32].

The lifelong risk of cardiotoxicity is complex and multifaceted. Key roles have been attributed to mitochondrial dysfunction[33], anthracyclines metabolism[34], inflammation and fibrosis[35] together with myofibril disorganization[36], cardiomyocyte senescence and apoptosis[37]. Late-onset cardiotoxicity mechanisms are difficult to understand. One can speculate that infra-clinical cardiotoxicity is already present in the heart of patients after the termination of their treatment with anthracyclines as recently confirmed by meta-analysis showing that clinically overt cardiotoxicity occurred in 6% of patients, whereas subclinical cardiotoxicity developed in 18% of patients[6]. Our study emphasizes the potential association of anthracycline cardiotoxicity with another stressor, here represented by type 1 diabetes, which unmask the infra-clinical toxicity.

Relationship between cancer treatment induced cardiotoxicity and diabetes has been previously investigated. Studies showed that preexisting diabetes in cancer patients who will undergo a chemotherapy treatment will have higher incidence of cardiovascular events[38]. This was confirmed by an experimental study demonstrating an increased sensitivity of the diabetic heart to doxorubicin treatment[7,11].

Our study is novel and shows for the first time the consequence of type 1 diabetes on a pretreated heart with anthracyclines. Importantly, it has been shown that cancer and its treatment can induce later on diabetes mellitus[39,40]. Furthermore, it has been shown that -Pancreatic cancer increases diabetes incidence[41]. The relation between cancer, anthracyclines and diabetes is even more complex. Indeed, Type 1 and type 2 Diabetes were shown to be associated with increased risk of cancer incidence[42,43] and can worsen mortality induced by cancer[44].

In our study, one week after doxorubicin treatment completion, twenty percent of the mice presented with acute cardiotoxicity (decrease of more than 10% of FS). This result is in accordance with what has been published concerning juvenile model of cardiotoxicity[23]. In addition, doxorubicin acutely induced hyperglycemia which

wasn't aggravated later on when type 1 diabetes was induced, which confirms the acute and transient state of this increase in blood glucose. It has been described that cytotoxic chemotherapy is associated with transient and acute hyperglycemia in patients[45–47]. This increase in blood glucose was assigned to acute beta-cell damage and a drop in protein synthesis causing an increase demand in gluconeogenic precursors[45,46].

In accordance with our results, an experimental study showed that doxorubicin induces hyperglycemia and insulin resistance in rats[48]. Downregulation of the AMPK signaling leading to a decrease in GLUT-4 expression, in the skeletal muscle of doxorubicin treated rats was suggested by the authors to be responsible of doxorubicin induced glucose intolerance. One week after doxorubicin treatment, hyperglycemia was associated with a decreased expression of GLUT4 in mice heart (Supplemental Figure 1) with no change in AKT and insulin receptors expression (data not shown), as previously described[48]. Together with cardiac atrophy and cachexia, decreased GLUT-4 expression in the heart and probably in skeletal muscles explain doxorubicin induced hyperglycemia even if we cannot exclude any doxorubicin toxic effect on pancreatic cells.

Hyperglycemia itself contributes to cardiac toxicity as demonstrated by increased cardiomyocytes mortality when incubated in vitro with high glucose concentration. High glucose induced cardiotoxicity has previously been described in vitro[49] and in vivo[50]. Furthermore, it has been shown that hyperglycemia increases doxorubicin accumulation in the heart tissue[11,51]. Even if we didn't observe a higher glycaemia when type 1 diabetes was induced in doxorubicin pretreated mice compared to doxorubicin treated mice without type 1 diabetes, our findings showed, in vitro and in vivo, that combination of doxorubicin treatment and hyperglycemia aggravated cardiomyocytes apoptosis and cardiac dysfunction.

Furthermore, it has been shown that metformin, an oral antihyperglycemic drug used for type 2 diabetes, reduces the risk of cancer in patients with diabetes mellitus[52]. Very recently, Metformin combination with neoadjuvant chemotherapy was shown to be safe, tolerable, and to improves non-significantly the clinical and pathological tumor response of breast cancer patients[53]. Metformin is cardio-protective in a context of myocardial ischemia[54] and recently against doxorubicin-induced cardiotoxicity[55]. The mechanism by which metformin prevent cardiotoxicity induced by doxorubicin seems to be partially linked to decreased inflammation and apoptosis in the heart[56–58]. Unfortunately, none of these studies were done in a context of diabetes which might dysregulates the balance favoring cardio-protection induced by metformin.

Another very promising drugs in this context are the Sodium-glucose cotransporter 2 (SGLT2) inhibitors (e.g., gliflozin, empagliflozin, dapagliflozin), the novel oral glucose-lowering medication, which were shown to be cardioprotective against doxorubicin treatment[59–61]. This cardioprotective effect was also demonstrated in a doxorubicin and diabetic mouse model[62]. Furthermore, two recent retrospective studies showed a beneficial association between SGLT2 inhibitors use and decreased mortality, cardiovascular events and rehospitalization for cancer patients treated with anthracyclines[63,64].

Moreover, preclinical studies evaluating concomitant use of SGLT-2 inhibitors with doxorubicin in cancer models demonstrated a beneficial and synergic effect of SGLT-2 inhibitors on tumor growth[65,66]. These studies further demonstrated that the use of SGLT2-inhibitors allows for the use of lower doses of doxorubicin thus preventing its side effects.

Another factor precipitating cardiac dysfunction and mortality in mice receiving both doxorubicin and streptozotocine in our study, is cardiac fibrosis. Cardiac fibrosis induced cardiac dysfunction has been largely investigated in the past[67]. Doxorubicin and streptozotocin induce cardiac fibrosis associated with cardiac dysfunction[21,59]. Interestingly, cardiac fibrosis induced cardiac dysfunction is a late-onset effect of doxorubicin which can appear during the recovery phase, weeks after treatment termination[35]. Extracellular matrix proteins profiling evaluated in cardiac tissues was similar to what was previously described with doxorubicin inducing collagen 1, 3 and fibronectin expression but decreasing laminin expression thus disrupting the structural and functional maintenance of cardiac cells[68,69].

Interestingly, cardiac dysfunction and mortality in hyperglycemic and doxorubicin treated mice wasn't associated with cardiomyocytes atrophy. This is surprising as doxorubicin and diabetes alone induce cardiomyocytes atrophy as shown by other studies[21,70]. We only observed cardiomyocytes atrophy in diabetic mice not receiving doxorubicin. This was associated with increased MuRF1 expression[71,72]. One can speculate that maintained cardiomyocytes size in diabetic mice receiving doxorubicin is an adaptive response of the resting cardiomyocytes following cardiac apoptosis, and should be further investigated in the future.

Although our study is novel and translational with clinical relevance for cancer survivors presenting higher risk of developing later on cardiovascular comorbidities such as diabetes mellitus which can unmask a subclinical cardiotoxicity induced by chemotherapy treatment, it presents with some limitations. First of all, only type 1 diabetes was mimicked by STZ-induced hyperglycemia despite the fact that it represents less than 10% of diabetes mellitus. Second, only cardiomyocyte cell lines, and not primary cells, were used. Third, our model doesn't include cancer which itself can induce cardiac changes leading to cardiac dysfunction[73]. Fourth, insulin resistance and glucose tolerance tests were not performed in this study limiting the evaluation of doxorubicin metabolic effect.

Data availability
Data is provided within the manuscript or supplementary information files.

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Declarations

Competing interests

The authors declare no competing interests.

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