ORIGINAL ARTICLE

Treatment with 17β-estradiol protects donor heart against brain death effects in female rat

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ABSTRACT

The viability of donor organs is reduced by hemodynamic and immunologic alterations caused by brain death (BD). Female rats show higher heart inflammation associated with the reduction in female sex hormones after BD. This study investigated the effect of 17β-estradiol (E2) on BD-induced cardiac damage in female rats. Groups of female Wistar rats were assigned: Sham-operation (Sham), brain death (BD), treatment with E2 (50 μg/ml, 2 ml/h) 3 h after BD (E2-T3), or immediately after BD confirmation (E2-T0). White blood cell (WBC) count was analyzed; cytokines and troponin-I were quantified. Heart histopathological changes and expression of endothelial nitric oxide synthase, endothelin-1, intercellular adhesion molecule-1, BCL-2, and caspase-3 were evaluated. Cardiac function was continuously assessed for 6 h by left ventricular pressure-volume loop analysis. E2 decreased the BD-induced median serum concentration of troponin-I (BD:864.2 vs. E2-T0:401.4; $P = 0.009$), increased BCL-2 (BD:0.086 vs. E2-T0:0.158; $P = 0.0278$) and eNOS median expression in the cardiac tissue (BD:0.001 vs. E2-T0:0.03 and E2-T3:0.0175; $P < 0.0001$), and decreased caspase-3 (BD:0.025 vs. E2-T0:0.006 and E2-T3:0.019; $P = 0.006$), WBC counts, leukocyte infiltration, and hemorrhage. 17β-estradiol treatment was effective in reducing cardiac tissue damage in brain-dead female rats owing to its ability to reduce leukocyte infiltration and prevent cardiomyocyte apoptosis.

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Key words
brain death, estradiol, female Wistar rats, heart, inflammation

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Introduction

Various clinical and experimental studies have demonstrated the negative impact of brain death (BD) on the viability of the organ to be transplanted. The reduced number of suitable organs, mainly heart and lungs, has been a limiting factor to attend the growing number of patients on the waiting lists [1]. Therefore, an understanding of the alterations generated by BD in the donor organs will help in optimization of the clinical management of these patients and assist in obtaining more suitable organs for transplantation. It has been reported that the induction of BD due to increase in the intracranial pressure leads to a hyperdynamic reaction in the heart, causing ventricular function changes, hemodynamic instability, decrease in myocardial isoenzymes, and histological injury [2].

According to the International Society for Heart and Lung Transplantation, donor-recipient gender mismatch is an important factor that impacts the survival of heart

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transplant patients, especially after the first year of transplantation [3]. Other studies have also suggested donor gender as a factor that influences the outcome of heart transplantation [4,5]. In this context, experimental studies have shown severe inflammatory response in brain-dead female rats, owing to BD-induced estradiol reduction [6,7]. In another study, estradiol deficiency was found to be correlated to kidney transplantation failure in female rats [8]. Since the reduction of estradiol concentration by BD is a potential modulator of inflammation, we aimed to investigate whether treatment with 17β-estradiol could be beneficial in female heart donors.

Materials and methods

Animals

Female Wistar rats (7–8 weeks old; $n = 32$) were obtained from our animal facilities. The rats were allowed free access to water and food before the experimental procedure and housed at 23°C ± 2°C in a 12 h light-dark cycle. All rats received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996). The Animal Subject Committee of Sao Paulo University Medical School approved the experimental protocol (SDC No. 4350/16/016).

Groups and treatment

Female rats from maximal estradiol secretion to heat period were assigned to four groups ($n = 8$): (i) Sham-operated animals (Sham)—rats subjected to trepanation without BD induction, (ii) brain death (BD)—rats subjected to BD, (iii) E2 3 h (E2-T3)—rats administered 17β-estradiol (Sigma-Aldrich, USA) in saline (50 μg/ml to 2 ml/h); 3 h after BD induction, and (iv) E2 6 h (E2-T0)—rats administered 17β-estradiol (Sigma-Aldrich, USA) in saline (50 μg/ml to 2 ml/h) immediately after BD induction. Sham and BD groups received fluid replacement (saline solution, 2 ml/h).

Estrous cycle identification and hormone quantification

Fluid obtained after vaginal lavage with phosphate-buffered saline (PBS) was placed on slides and stained with crystal violet solution (0.2%). Estrus and proestrus cycle were identified using an optical microscope.

The quantification of estradiol and corticosterone was performed by ELISA kit (Cayman Chemical Company, USA), following the manufacturer's protocols.

Induction of brain death.

Anesthesia was performed in a chamber with 5% isoflurane, followed by intubation and ventilation with a rodent ventilator (Harvard Apparatus, model 683, USA), at a frequency of 70 breaths/min (tidal volume 10 ml/kg) and 2% isoflurane. The carotid artery was cannulated for blood sampling under continuous blood pressure monitoring. Saline solution (2 ml/h), with or without treatment, was infused through cannulation of the jugular vein.

BD model was based on the method described by Breithaupt-Faloppa et al. (2016) [6]. Briefly, a catheter Fogarty 4F (Baxter Healthcare Co., USA) was inserted intracranially through a drilled parietal burr hole. BD was induced by rapid balloon inflation (400 μL—saline solution), confirmed by mydriasis, apnea, and absence of reflexes; isoflurane inhalation was interrupted. All animals were monitored for 6 h.

White blood cell count (WBC)

A 20 μL volume of blood was withdrawn, and WBC was analyzed using an automatic hematologic counter (Mindray BC 28000 Vet, China).

Quantification of cytokines and troponin-I

The levels of granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory proteins (MIP)-1α, MIP-2, interleukin (IL)-1β, IL-10, monocyte chemoattractant protein-1 (MCP-1), cytokine-induced neutrophil chemoattractant-1 (CINC-1), vascular endothelial growth factor (VEGF), and tumor necrosis factor-α (TNF-α) were quantified in serum samples collected at the end of the experimental protocol, using commercial kit Milliplex® (Merck Millipore, USA). Quantification of the serum levels of troponin-I made by ELISA (Elabscience®, USA).

Histopathologic heart examination

At the end of the experiment, heart samples were obtained and fixed in 10% formaldehyde for 24 h, embedded in paraffin, sectioned (4 μm), and stained with hematoxylin and eosin. Leukocyte infiltration,

edema, and hemorrhage were evaluated by two observers blinded for intervention, and the mean values from each were analyzed by correlation tests to determine interobserver variability. Intercellular myocardial edema and hemorrhage were reported as units per square millimeter, whereas leukocytes infiltration was reported in cells per square millimeter of cardiac tissue.

Immunohistochemistry

Frozen serial sections of the heart tissue (8 μm) were fixed in acetone (10 min), washed in TRIS-buffered saline Tween-20 (TBST), permeabilized with TBST and Triton X-100, followed by blockade of nonspecific sites with TBST, containing 2% bovine serum albumin (BSA) and then peroxidase with H_2O_2. Sections were incubated in TBST (complemented with 2% BSA and rabbit antibodies to BCL-2 [1:100; Abcam, Cambridge, MA] and caspase-3 [1:200; Abcam, Cambridge, MA]) for 12 h at 4°C. After washing with TBST, all sections were incubated for 1 h at 37°C with anti-rabbit secondary antibodies (1:200) conjugated to peroxidase (HRP; Millipore). Next, the sections were washed with TBST and stained with peroxidase substrate 3-amino-9-ethylcarbazole (AEC; Vector Laboratories, USA) (5-10 min) and counterstained with hematoxylin. Negative control sections were incubated in absence of primary antibodies.

To evaluate the expression of intercellular adhesion molecule-1 (ICAM-1), paraffin-embedded tissues were sectioned (4μm), rehydrated, and incubated with citrate buffer (pH 6.0) for 20 min at 100°C to retrieve antigens. Next, the sections were submitted to blocking of nonspecific sites and endogenous peroxidase, and further incubated with mouse antibodies to rat ICAM-1 (1:50 – 12h at 4°C) (Cedarlane, Canada). After washing with TBST, the sections were incubated for 2 h at room temperature with anti-mouse secondary antibodies attached to HRP (1:400, Novus Biologicals, USA), rinsed, stained with AEC (5-10 min) (Vector Laboratories, USA), and counterstained with hematoxylin. Negative control sections were incubated in the absence of primary antibodies.

The analysis was performed through image acquisition system with a digital camera DS-Ri1 (Nikon, Japan) coupled to a Nikon microscope and analyzed using the NIS-Elements BR software (Nikon, Japan).

Hemodynamic measurements: left ventricular pressure-volume loop (LVPV)

The right carotid artery was dissected and a 2F microtip pressure-conductance catheter (SPR-838; Millar Instruments, USA) was inserted and placed in the left ventricle (LV). To record signals for 6 h, a pressure-volume conductance system (MPVS-Ultra, Millar Instruments, USA) was connected to a data acquisition system (PowerLab, AD Instruments, USA). After stabilization, data related to ejection fraction (EF), stroke volume (SV), end-diastolic volume (EDV), systolic pressure (SP), stroke work (SW), maximum rate of rise of left pressure (dP/dT max), and time constant of left pressure decay (Tau) were obtained.

Statistical analysis

All data were presented as mean ± standard error of the mean (SEM) or as median and 95% percentile interval. Normally distributed data were analyzed by ANOVA followed by Dunnett or Sidak's tests for multiple comparisons, with *P*-values adjusted to account for multiple comparisons. Abnormally distributed data were analyzed using a Kruskal–Wallis test followed by a post hoc Dunn multiple comparison test also, with *P*-values adjusted to account for multiple comparisons. Statistical analysis was performed using GraphPad Prism software v.8.3.

Results

Estradiol and corticosterone concentration

The quantification of serum estradiol levels showed that BD group had reduction in estradiol, while the Sham group had significant increase in estradiol after 3 h and 6 h after the beginning of experiment. Compared to the BD group, both the estradiol-treated groups showed an increase in estradiol concentration after the beginning of treatment; the E2-T0 group had changes after 3 h and 6 h, while the E2-T3 group showed an increase in estradiol after 6 h (Fig. 1a). The estradiol infusion was able to maintain the hormone concentration stable during the experimental period.

In addition, the serum concentration of corticosterone was evaluated. Compared to the BD group, the Sham group had higher concentration of corticosterone at 3 h and 6 h after the beginning of the experiment. However, no difference was observed in corticosterone levels among BD, E2-T3, and E2-T0 groups (Fig. 1b).

WBC count

At 3 h and 6 h after the beginning of the experiment, BD group had lower number of lymphocytes,

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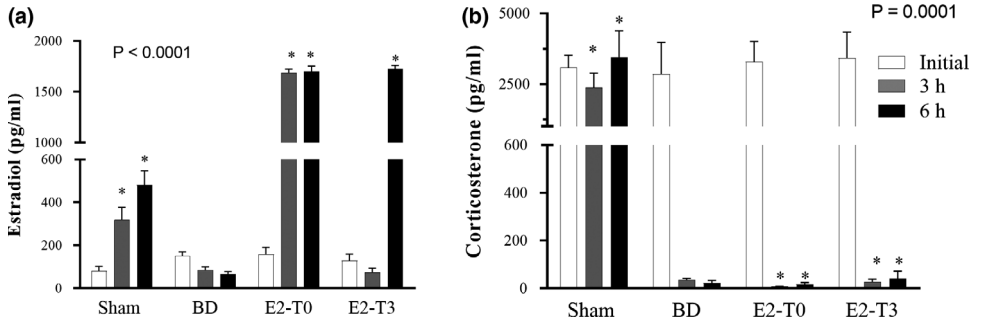

Figure 1 Serum (a) estradiol and (b) corticosterone concentrations. Sham, false-operated rats; BD, rats submitted to encephalic death; E2-T0, 17β-estradiol (E2) treated rats after confirmation of BD and E2-T3, 17β-estradiol (E2)-treated rats after 3 h of confirmation of BD. Data expressed as mean standard error of the mean (SEM; $n = 8$) *$P < 0.0001$ in comparison with BD group.

granulocytes, and monocytes than those in the Sham group. However, BD group showed leukocytosis after 3 h, which was maintained until 6 h. In contrast, 17β-estradiol-treated female rats did not develop leukocytosis until the end of the follow-up time (Table 1).

Serum cytokines

BD caused an increase in the serum levels of CINC-1, TNF-α, and MIP-1α compared with the Sham group. However, no statistically significant differences were detected in the estradiol-treated groups (Table 2).

Histopathological analysis

In Fig. 2, 17β-estradiol was able to reduce leukocyte infiltration to the heart and the hemorrhage.

Endothelial function

BD group had expression of eNOS protein decreased while the expression of ICAM-1 increased. In contrast, treatment with 17β-estradiol increased the eNOS expression without altering the expression of ICAM-1. There were no differences in the protein expression of endothelin-1 (Fig. 3).

Table 1. Total and differential white blood cell counts

		Leukocytes (cells/mm^3) Total	Lymphocytes	Neutrophils	Monocytes
Sham	0h	10670 ± 1111	6470 ± 388	3860 ± 778	340 ± 50
	3h	28270 ± 2908*,**	5630 ± 788**	21560 ± 2349*,**	1100 ± 117*,**
	6h	18120 ± 3562	3750 ± 577*	13700 ± 2915*	670 ± 144*
BD	0h	10110 ± 964	6440 ± 523	3230 ± 443	440 ± 76
	3h	14790 ± 2122*	3510 ± 333*	10870 ± 1850*	390 ± 77
	6h	13060 ± 1304	3870 ± 431*	11270 ± 2050*	440 ± 58
E2-T0	0h	8900 ± 1175	4930 ± 699	4000 ± 485	370 ± 67
	3h	9250 ± 1033	2550 ± 174*	6720 ± 692*	390 ± 80
	6h	8460 ± 1052β	2530 ± 332*	5430 ± 664	380 ± 81
E2-T3	0h	10500 ± 737	6110 ± 440	4000 ± 485	390 ± 38
	3h	10060 ± 902	2970 ± 270*	6720 ± 692*	340 ± 70
	6h	8770 ± 830	3010 ± 312*	5430 ± 664	330 ± 56
	P ANOVA (group)	<0.0001	0.0051	<0.0001	0.0004
	P ANOVA (time)	<0.0001	<0.0001	<0.0001	0.0046

Data expressed as mean ± SEM ($n = 8$). Statistical testing consisted of ANOVA followed by Dunnett test for multiple comparisons. SHAM, false-operated rats; BD, brain death; E2-T3, 17β-estradiol (E2)-treated rats after 3h of confirmation of BD; E2-T0, 17β-estradiol (E2)-treated rats after confirmation of BD.

*$P < 0.05$ in comparison with Initial.

**$P < 0.05$ in comparison with BD.

Table 2. Measurement of inflammatory parameters

(pg/ml)		Sham	BD	E2-T0	E2-T3	*P* value
Cytokines	CINC-1	24.3 (0.2–1504)	1687 (0.3–2999)	1164 (0.3–1897)	986 (0.1–2614)	0.069
	TNF-α	7.78 (2.38–32.79)	28.1 (6.15–37.5)	8.92 (3.28–30.87)	20.4 (4.2–38.79)	0.212
Interleukines	IL-1 β	52.9 (9.3–108.2)	71.5 (31.2–267.9)	63.1 (5.2–265.9)	55.7 (3.7–244.1)	0.600
	IL-10	242 (75–878)	285 (77–689)	290 (30–706)	228 (122–363)	0.953
Chemokines	MIP-1α	555 (330–751)	855 (70–2058)	668 (305–1080)	682 (66–884)	0.776
	MCP-1	2638 (953–5599)	7960 (1927–12890)	9969 (3534–18633)	8923 (2486–17536)	0.008
	MIP-2	201 (14–787)	180 (25–380)	67 (26–430)	162 (14–732)	0.870
Growth factors	G-CSF	4.63 (0.3–10.4)	1.58 (0.3–3.2)	5.56 (0.25 –63.3)	1.66 (0.68–15)	0.350
	GM-CSF	0.87 (0.13–3.58)	0.74 (0.13–1.49)	1.28 (0.14–6.04)	0.27 (0.13–1.21)	0.447
	VEGF	109 (35–1134)	113 (33–262)	158 (62–280)	200 (41–312)	0.748

Data (pg/ml) are expressed as median and 95% percentile interval (n = 8). Statistical testing consisted of Kruskal–Wallis test followed by Dunn's test for multiple comparisons. SHAM, false-operated rats; BD, brain death; E2-T3, 17β-estradiol (E2)-treated rats after 3h of confirmation of BD; E2-T0, 17β-estradiol (E2)-treated rats after confirmation of BD. G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; MIP, macrophage inflammatory protein; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; CINC-1, cytokine-induced neutrophil chemoattractant-1; VEGF, vascular endothelial growth factor; TNF-α, tumor necrosis factor-α.

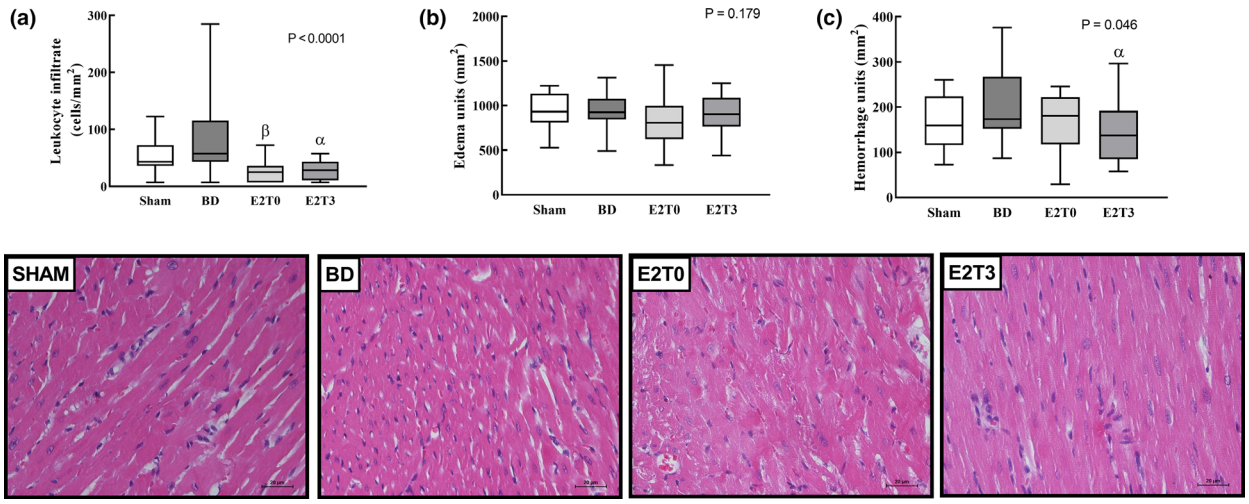

Figure 2 Histopathological analysis of heart tissue. (a) leukocyte infiltration, (b) edema units, and (c) hemorrhage units. Sham, false-operated rats; BD, rats submitted to encephalic death; E2-T0, 17β-estradiol (E2)-treated rats after confirmation of BD and E2-T3, 17β-estradiol (E2)-treated rats after 3 h of confirmation of BD. Formaldehyde-fixed sections of heart tissue were stained with hematoxylin-eosin. Original magnification 40 × for all images. Data expressed as median and 95% percentile interval (n = 8 per group; 1 tissue section per animal, 5 fields) $^{\alpha,}$ $^{\beta}p < 0.05$ in comparison with BD group.

Quantification of troponin-I serum concentration

A significant increase in the level of serum troponin-I was observed in the BD group compared with Sham group (Fig. 4). In contrast, treatment with 17β-estradiol, immediately after BD, reduced the troponin-I levels.

BCL-2 and caspase-3 expression

In Fig. 5a, there was a significant upregulation in the expression of the apoptotic protein, caspase-3 in BD group, which was reversed by 17β-estradiol treatment. In addition, there was upregulation in the expression of anti-apoptotic protein, BCL-2 in E2-T0 group (Fig. 5b).

LVPV analyses

All parameters were analyzed continuously during the 6 h of experiment (Table 3). All groups submitted to BD presented with a decreased EF after 6 h compared to the initial value. At the end of follow-up time, BD group showed reduced EF values compared with the

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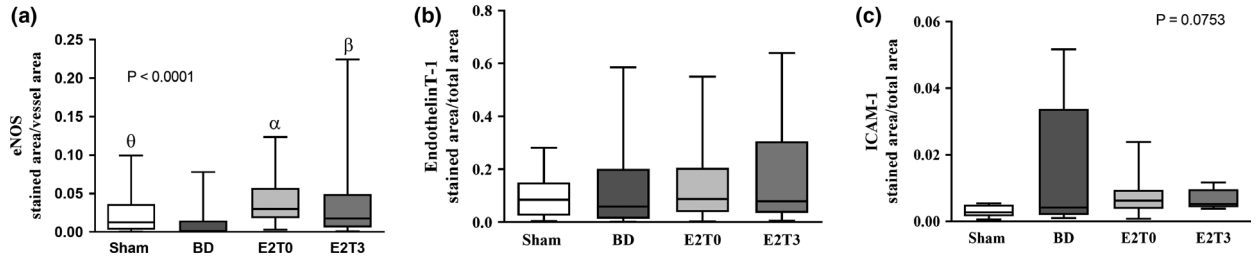

Figure 3 Quantification of protein expression of eNOS (a), endothelin-1 (b), and ICAM-1 in cardiac tissue. Sham, false-operated rats; BD, rats submitted to encephalic death; E2-T0, 17β-estradiol (E2) treated rats after confirmation of BD and E2-T3, 17β-estradiol (E2)-treated rats after 3 h of confirmation of BD. Data expressed as median and 95% percentile interval (n = 5 per group; 1 tissue section per animal, 5 fields). $^{\alpha,\beta,\theta}p < 0.05$ in comparison with BD group.

Sham group. In both the estradiol-treated groups, significant increases of EDV were observed after 6 h of treatment when compared to the initial values. Furthermore, compared to the initial values, SW was reduced in BD, E2-T3, and E2-T0 groups after the 6 h of experiment. Other hemodynamic measurements (SVSP, dP/dT max, and Tau) did not differ among the groups during the follow-up time.

Discussion

Results from previous studies by our group have demonstrated a worst inflammatory female donor profile in comparison with male donor profile, which is related to acute estradiol reduction after BD [6,7]. Findings from this study suggest that 17β-estradiol treatment was efficient in reducing heart injury in brain-dead female rats due to its ability to reduce myocardial tissue compromise, modulation of the expression of apoptosis mediators, reduction in the troponin-I levels, and increased eNOS expression.

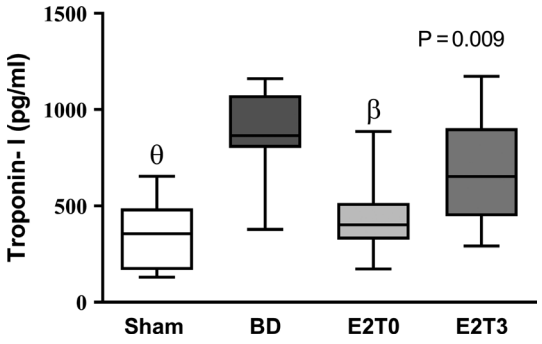

Figure 4 Serum troponin-I concentration. Sham, false-operated rats; BD, rats submitted to encephalic death; E2-T0, 17β-estradiol (E2)-treated rats after confirmation of BD and E2-T3, 17β-estradiol (E2)-treated rats after 3 h of confirmation of BD. Data expressed as median and 95% percentile interval (n = 8). $^{\beta,\theta}p < 0.05$ in comparison with BD group.

These results reiterate the findings from previous studies, thus suggesting the cardiovascular protective effects of 17β-estradiol as anti-apoptotic, anti-inflammatory, and vasodilator [9,10]. Despite the documented inflammatory modulation, 17β-estradiol treatment did not affect the heart function decrease observed after BD induction.

With respect to systemic BD alterations, the analysis of systemic release of inflammatory mediators showed an increase in the levels of TNF-α after BD and increase in the serum levels of CINC-1, a neutrophil chemoattractant cytokine which is associated with the increase in circulating leukocytes, specifically neutrophils. MIP-1α was also increased in the serum of brain-dead rats and similar to CINC-1, it also led to increase in the neutrophil count [11]. Unlike in the BD group, 17β-estradiol-treated rats did not develop leukocytosis after 6 h of BD induction. However, estradiol treatment did not result in significant changes in the levels of systemic cytokines, despite the observation of this influence in male animals [12].

The insult produced by BD results in morphophysiological derangements in the heart and the lungs. In the rodent model, female rats tend to develop more edema, hemorrhagic foci, and recruitment of inflammatory cells in these organs when compared to male rats [13]. An increased expression of the adhesion molecule protein is responsible for the attachment and influx of leukocytes into peripheral tissue and is associated with the reduction in serum corticosterone levels after BD [14]. Infiltrated leukocytes secrete a plethora of proinflammatory substances such as chemokines, cytokines, complement components, proteases, growth factors, and reactive oxygen metabolites; thereby inducing tissue damage and resulting in cell death [15]. The acute reduction in estradiol after BD leads to increase in leukocyte influx into female cardiac tissue. In this study, BD female rats and 17β-estradiol-treated rats showed decreased

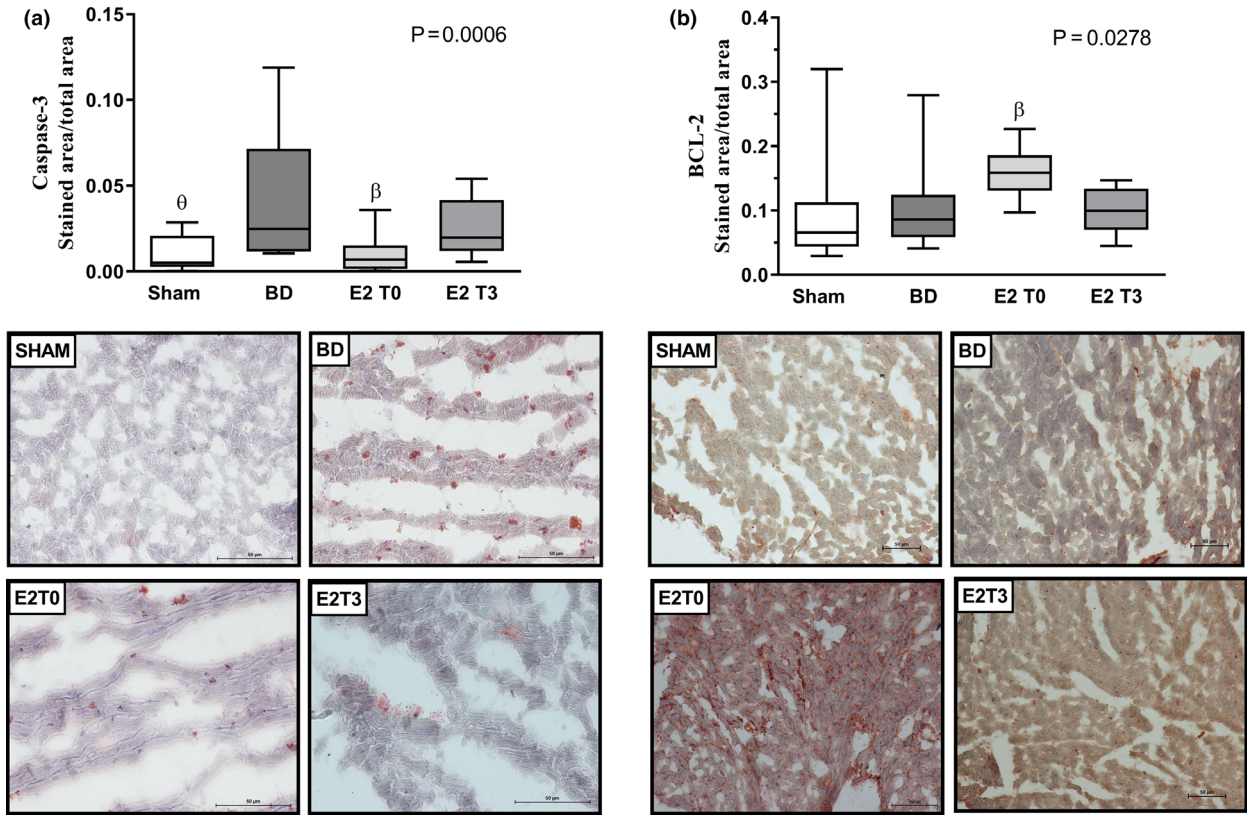

Figure 5 Immunostaining for apoptotic protein in cardiac tissue. Apoptotic protein, caspase-3 (a) and anti-apoptotic protein, BCL-2 (b) Sham, false-operated rats; BD, rats submitted to encephalic death; E2-T0, 17β-estradiol (E2)-treated rats after confirmation of BD and E2-T3, 17β-estradiol (E2)-treated rats after 3 h of confirmation of BD. Original magnification images: 40 × for caspase-3 and 20 × for BCL-2. Data expressed as median and 95% percentile interval (n = 5 per group; 1 tissue section per animal, 5 fields). $^{\beta,\theta}P < 0.05$ in comparison with BD group.

leukocyte infiltration into the heart. Once the reduced number of circulating leukocytes was confirmed in estradiol-treated animals, it was inferred that this low cell count might result in diminished infiltration to the tissue. Our results showed that BD was able to increase ICAM-1 expression in the heart. However, no significant reduction in ICAM-1 levels was observed in the estradiol-treated groups. In contrast, data not published pointing to the expression of these adhesion molecules were significantly reduced by 17β-estradiol treatment in the lung tissue.

Endothelial dysfunction after BD is characterized by impaired vasodilation and a proinflammatory status [12]. It has been well documented that BD induces hypoperfusion in the mesenteric microcirculation, which is correlated to the reduction in the level of serum corticosterone in male rats [14,16]. Correia et al (2019) [16] described that microcirculatory hypoperfusion after BD is reduced by hypertonic saline infusion, by increase in the levels of eNOS and endothelin-1 expression, which play key roles in the inflammatory process in vascular wall and vessel tone regulator. Estrogens exert increased bioavailability of nitrous oxide (NO) in the vascular system through nongenomic pathway, once estradiol binds to estrogen receptor-α, leading to eNOS (endothelial isozyme) phosphorylation/activation [17]. In our study, eNOS expression was reduced in cardiac tissue after BD. In contrast, estradiol treatment significantly increased the expression of this enzyme in the female heart. Additionally, maintenance of the levels of endothelin-1 protein expression immediately after BD in the treated groups, confirm the anti-inflammatory action of 17β-estradiol in the female heart, once endothelin-1 has been involved in activation of transcription factors, such as NF-kB and expression of TNF-α and IL-1β [18].

Apoptosis is associated with organ dysfunction and graft deterioration in heart transplantation [19]. Cell death pathway, signaled by an increase in the expression of pro-apoptotic protein, caspase-3 expression and decrease in the expression of anti-apoptotic protein, BCL-2 was observed in the brain-dead female heart

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Table 3. Left ventricular pressure-volume analysis

		Sham	BD	E2-T0	E2-T3	*P* ANOVA
EF (%)	0h	60 ± 5	63 ± 3	68 ± 2	70 ± 2	<0.0001
	3h	55 ± 4	45 ± 7*	50 ± 4*	52 ± 4*	
	6h	50 ± 5**	28 ± 3*	35 ± 6*	31 ± 4*	
SV (μL)	0h	131 ± 18	132 ± 15	165 ± 20	154 ± 15	0.9169
	3h	123 ± 11	122 ± 22	135 ± 17	145 ± 22	
	6h	133 ± 7	109 ± 21	135 ± 19	118 ± 19	
EDV (μL)	0h	185 ± 19	194 ± 21	159 ± 23	188 ± 26	0.0124
	3h	228 ± 22	195 ± 12	231 ± 35	200 ± 45	
	6h	255 ± 22	269 ± 16	266 ± 25*	328 ± 48*	
SP (mmHg)	0h	101 ± 7	108 ± 6	108 ± 4	90 ± 6	0.0733
	3h	96 ± 8	95 ± 6	96 ± 9	100 ± 5	
	6h	83 ± 4	96 ± 6	85 ± 7	93 ± 3	
SW (mmHg/μL)	0h	12847 ± 3806	10961 ± 649	13490 ± 1444	11630 ± 2018	<0.0001
	3h	11125 ± 1954	7936 ± 1848	9447 ± 1982*	7662 ± 2085*	
	6h	10853 ± 2136	7669 ± 1774	8734 ± 1397*	6068 ± 1578*	
dP/dT max (mmHg/s)	0h	5461 ± 629	6179 ± 715	6092 ± 304	5754 ± 345	<0.0001
	3h	5170 ± 723	4842 ± 449	5081 ± 592	5643 ± 425	
	6h	4427 ± 625	4201 ± 467	4217 ± 531	3763 ± 382	
Tau (ms)	0h	12 ± 2	11 ± 1	14 ± 3	10 ± 1	0.0007
	3h	14 ± 3	16 ± 3	18 ± 3	17 ± 2	
	6h	10 ± 1	14 ± 1	21 ± 3	17 ± 2	

Data are expressed as mean ± SEM (6 rats per group). Statistical testing consisted of ANOVA followed by Sidak's test for multiple comparisons. Ejection fraction (EF), stroke volume (SV), end-diastolic volume (EDV), systolic pressure (SP), stroke work (SW), maximum rate of rise of left pressure (dP/dT max) and time constant of left pressure decay (Tau).

*$P < 0.05$ in comparison with Initial.

**$P < 0.05$ in comparison with BD.

tissue. One important cardioprotective effect of estrogen is the myocyte death inhibition. Previous studies have shown that the activation of phosphoinositide-3 kinase/Akt pathway (PI3K-PKB/AKt) by estrogen modulates a wide range of physiological responses, including metabolism, genic transcription factors, and cell survival [20]. In this regard, Wang et al. (2009) [20] showed that estrogen receptor-β mediates increased activation of Akt signaling cascade, leading to myocardial protection by decreasing pro-apoptotic protein, caspase-3 and increasing anti-apoptotic protein, BCL-2 in female hearts followed to acute ischemia. Similar results were observed in this study, once 17β-estradiol treatment was able to increase BCL-2 expression and decrease caspase-3 expression in the female heart tissue after BD. Troponin-I is a sensitive and specific marker of slight myocardial injury. Elevated troponin-I serum levels is related to worse prognosis after heart transplantation [21]. In line with cell death data, the level of troponin-I was significantly increased in BD female, suggesting cardiac tissue degradation. However, treatment with 17β-estradiol during 6 h after BD decreased serum troponin-I, which indicated a reduction of cardiac injury.

According to previous studies [22,23], BD donors have cardiac dysfunction (with an EF < 50%) or regional wall motion abnormalities on echocardiography. In our study, despite the anti-inflammatory action, treatment with 17β-estradiol did not change cardiac function during the 6 h period after BD. This can be attributed to the absence of important hemodynamic changes in our model, as we found a significant reduction in the EF in the BD group when compared to the Sham group. In a similar rodent model, Magalhães et al (2019) [24] highlighted the beneficial action of hypertonic solution in the heart function of brain-dead male rats, (partially attributable to its characteristic of being a volume expander) used to reestablish hemodynamics in hypotensive events as hemorrhagic shock. Recently, studies have claimed that donor hearts with low EF (40%–50%) showed notable functional recovery after transplant [25], thus minimizing the relevance of this event.

This study has some limitations. We used an investigation time of 6 h, which is usually the standard in this type of experimental model. However, the observation of the benefits of 17β-estradiol in cardiac inflammation and myocardial tissue compromise in BD female rats was shortened to early histopathologic and inflammatory findings in the donor organ, limiting to point out possible mechanisms.

In conclusion, 17β-estradiol treatment was effective in preventing heart injury caused by BD in female rats. Estradiol treatment immediately after BD was found to be better in reducing the levels of key damage markers, such as troponin-I and caspase-3, and upregulating the levels of protective mediators, such as eNOS and BCL2. Based on these findings, 17β-estradiol therapy might be an alternative to mitigate BD-induced systemic inflammatory profile and improve female heart viability for transplantation. Considering female brain death donors, when there is a reduction in the concentration of female sex hormones, it can be suggested that treatment with estradiol may contribute to maintaining the quality of the organs.

Authorship

RAJr and FYRS designed and performed the study, analyzed the data, and wrote the manuscript. CJC and MVS contributed to the collection and analysis of data. RSCS and LFA contributed to data collection. HGDL and LFPM contributed to data analysis and wrote the manuscript. ACBF designed the study, analyzed data, and wrote the manuscript.

Conflict of interest

The authors have no conflicts of interest to disclose.

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