The role of estradiol in the maintenance of brain-dead organ donors

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CHAPTER 7

Summary, general discussion of the thesis and future perspectives

SUMMARY AND GENERAL DISCUSSION

Studies confirm that donor gender influences the success of organ transplantation and shows a worse prognosis in kidney, heart, and lung transplantation with grafts from women (1–3). Female sex hormones participate in modulating the magnitude of the inflammatory response, and their acute reductions may cause changes in several systems, such as the immune system (4,5). Moreover, females also preserve hemodynamic parameters after hemorrhagic shock (6,7). Experimentally, the literature suggest that the repercussions of trauma in females are less intense than in males, and that those under treatment with estradiol also develop less severe injuries (8–11).

Based on the idea that there is sexual dimorphism in the immune system response, the purpose of this thesis was to discuss the mechanisms of sex-dependent differences in the evolution of organ inflammation in brain death (BD) induction models. Furthermore, to increase the pool of viable organs for transplantation, 17β-estradiol was discussed as a strategy to improve the quality of BD-female donor organs.

Chapter 1 was a brief introduction of BD and sex influences on the inflammatory processes following it. Previous studies indicate that female lungs are generally worse in comparison to male lungs, due to a higher inflammatory frame in BD-female donors, which associated with acute estradiol reduction after BD (12). In parallel, **Chapter 2** discussed that male and female animal differed in terms of microvascular perfusion following BD. BD led to hypoperfusion in males, but not in females. This was associated with greater endothelial nitric oxide synthase (eNOS) expression in females with high estradiol concentration BD (13). eNOS exerts its protective role by maintaining vascular tone and integrity, favoring the preservation of microvascular perfusion/flow in female rats than in male rats.

However, once brain death is induced resulting into the acute depletion of female sex hormones and higher ICAM-1 expression and leukocyte infiltration favor a proinflammatory organ status.

Based on previous studies that associated an exacerbated inflammatory response in female rats with acute reduction of female sex hormone concentration following BD, therapy with 17β-estradiol (E2) was suggested to control organ injury in the BD-female donor. The following chapters aimed to investigate whether treatment with E2 may be beneficial in female organ donors.

As a consequence of cerebral injury and BD, increased levels of circulating cytokines lead to a local inflammatory response (14). The systemic and local inflammatory response consists of complement and endothelial activation, cytokine and chemokine release – such as IL-6, CINC-1, IL-10, and monocyte chemoattractant protein (MCP-1) – and the influx of leukocyte into the organs. In **Chapter 3**, we described that rat treated with E2 did not develop leukocytosis after 6 h of BD induction. Nevertheless, E2 treatment did not cause a significant change in the levels of systemic cytokines.

Endothelial injury following BD is characterized by impaired vasodilatation and proinflammatory status (15,16). At the site of an endothelial injury, the influx of inflammatory cells and synthesis of proinflammatory mediators promote and magnify both local and systemic inflammation (17). One pivotal mechanism underlying endothelial activation/dysfunction is the depletion of eNOS bioavailability (17). In vivo and in vitro studies have shown that treatment with E2 leads to a rapid increase in vascular NO release through induction of eNOS (18,19). In **chapter 3** we showed that E2 treatment reverted the reduced eNOS expression in cardiac tissue after BD, possibly through a non-genomic pathway, activating the phosphatidylinositol 3-kinase/Akt pathway (20).

Another pathway modulated by estrogen is the phosphoinositide-3 kinase/Akt. This pathway is associated with a wide range of physiological responses, gene transcription, and cell survival (21). Wang et al. (21) demonstrated that ER-β mediates increased activation of the Akt signaling cascade, resulting in myocardial protection by reducing pro-apoptotic protein and caspase-3, and increasing anti-apoptotic protein and BCL-2 in female hearts after acute ischemia. Similar results were observed in our study, in which E2 treatment increased BCL-2 expression and reduced caspase-3 expression in female heart tissue following BD. In consonance with cell death data, serum troponin-I, a sensitive and specific marker of cardiac injury, was significantly increased in BD-female rats. In contrast, E2 treatment decreased serum troponin-I levels after 6 h of BD induction, reiterating the cardioprotective effect of E2 in females.

The lungs are also affected by BD (22). Breithaupt-Faloppa et al. (12) demonstrated that lungs from female rats subjected submitted to BD presented a greater inflammatory response associated with the acute reduction of estrogen, a well-established protector against lung inflammation (23). In fact, we described in **Chapter 4** that E2 reduced pulmonary inflammation caused by BD. In classical pulmonary inflammation, alveolar macrophages synthesize cytokines, such as TNF-α and IL-1, which induce endothelial cells to express adhesion molecules and release chemokines (24). In parallel, during inflammatory responses, when leucocyte recruitment is accelerated by chemokines (e.g., MIP-1α, MIP-2, and CINC-1), a supplementary enzyme capable of synthesizing NO is induced, called inducible nitric oxide synthase (iNOS) (25–27). iNOS presents proinflammatory characteristics, impacting the synthesis of cytokines, endothelial expression of P-selectin and ICAM-1, and consequently neutrophil infiltration (28). In our study, BD triggered an inflammatory response, as evidenced by the expression of MIP-1α, MIP-2, CINC-1, IL-1β, iNOS, and VCAM, and remarkably high leukocyte infiltration.

Besides cytokines and chemokines released during the course of lung inflammation, metalloproteinase (MMP), a proteinase responsible for degrading the extracellular matrix, also disrupts resident cells and amplifies the inflammatory process (29). In our study, following BD induction, the MMP-9 gene and protein expression and MMP activity were increased. Like other inflammatory mediators, MMP-9 is also released by the trigger of TNF-α, IL-1β, and IL-8 largely on inflammatory cells, such as monocytes, macrophages, neutrophils, and endothelial cells (30–32). In this study, the lung MMP activity was diminished by E2 treatment. Previous studies have highlighted the role of E2 in downregulating MMP-9 gene expression, and correlated it to a reduction in hemorrhage, permeability, and leukocyte infiltration (33,34).

Cellular damage and its related molecular products are recognized as crucial triggers of inflammation following acute tissue injury (35). Acute kidney injury (AKI) is correlated with systemic and renal inflammation (36). In C**hapter 5**, we tested the effect of E2 on renal injury in BD-female rats. In BD-organ donors, the complement system is activated at a systemic and local level and is a relevant mediator of inflammation and renal injury (37,38). Complement activation results in the generation of mediators, such as opsonins (C3b, iC3b, C3dg, C3d, C4b, and C4d) and anaphylatoxins (C3a and C5a), which interact with their respective receptors (C3aR, C5aR1, and C5aR2) and recruit leukocytes to renal tissue (38,39). Activated renal parenchymal and dendritic cells synthesize chemokines that develop acute neutrophil- and monocyte/macrophage–dependent proinflammatory responses in AKI (40,41). Although there was no reduction in C3 formation, E2 treatment downregulated C3aR and C5aR expression in BD-female kidneys, which may be correlated to reduced expression of IL-6, MMP2, and MMP9 in renal tissue. In parallel, the attenuation of the complement system by E2 treatment was observed by a reduced formation of the membrane attack complex (MAC/C5b-9), a multimeric product of complement system activation that generates a trans-plasma membrane channel on the surface of the target tissue membrane, resulting in cell lysis and death (39).

In line with inflammatory mediators related to tissue injury, BD-female rats presented higher expression of KIM-1, a transmembrane glycoprotein that plays a role in the elimination of apoptotic cells and necrotic fragments (42–44). Furthermore, high KIM-1 expression is correlated with a reduction in glomerular function (45). Similar to El-Lateef et al.'s (46) results, which suggest a protective effect of E2 in gentamicin-induced AKI, BD-female rats treated with E2 reduced protein expression of KIM-1 and had improved renal function.

The maintenance of serum estradiol levels, due to treatment immediately after BD induction, resulted in non-progression of the inflammatory process in the heart, lung, and kidney. Furthermore, once the inflammatory process was established, late treatment was effective in reducing inflammation and tissue injury caused by BD in the organs that were studied.

Finally, in **Chapter 6**, we focused on a procedure that has gained importance in renal transplant research. The so-called isolated perfused kidney (IPK) has been used to assess function before transplantation, helping make clinical decisions on questionable kidneys. It also enables potential treatment and repair of injured kidneys (47,48). Together with the knowledge that male and female renal inflammation is caused by BD, especially the role of female sex hormones in the microvascular circulation and inflammatory response. We designed a study to compare renal inflammation in male and female patients with BD followed by IPK. Previous data indicated an equal inflammatory response in both sexes; however, in our study, kidneys from BD-males presented a reduced flow in the IPK.

We suggest that the reduction in renal perfusion in BD-males is correlated with intravascular microthrombi formation. Experimental evidence has shown that BD stimulates clot formation and triggers hypercoagulation in BD-male pigs (49). Such alterations were in the early phase of the BD experiment, and normal values were restored 180 min after BD. Correia et al. (50) described decreased clot lysis capability and hypercoagulation in BD-male rats, as demonstrated by the diminished time required for clot formation following BD. This reduction

may provide the intravascular concentration of microthrombi in BD-male rats, compromising microcirculatory perfusion. In female rats to BD, the depletion of clot firmness and clot lysis capability did not affect the final perfusion, which was preserved owing to the lesser clot formation.

In conclusion, female sex hormones, especially estradiol, exert an important influence on the repercussions caused by BD in female donors. Acute reduction of estradiol leads to an exacerbated inflammatory response. However, E2 treatment was effective in preventing and attenuating organ injury caused by BD in female rats. Thus, treatment with estradiol may contribute to maintaining or improving the quality of transplantation organs.

Future perspectives

As mentioned previously, preserving graft viability and optimizing donor management is of paramount importance. In the present study, we found sex differences in BD. For optimal organ preservation and an increased pool of viable grafts for transplantation, sex-specific care must be considered (Figure 1).

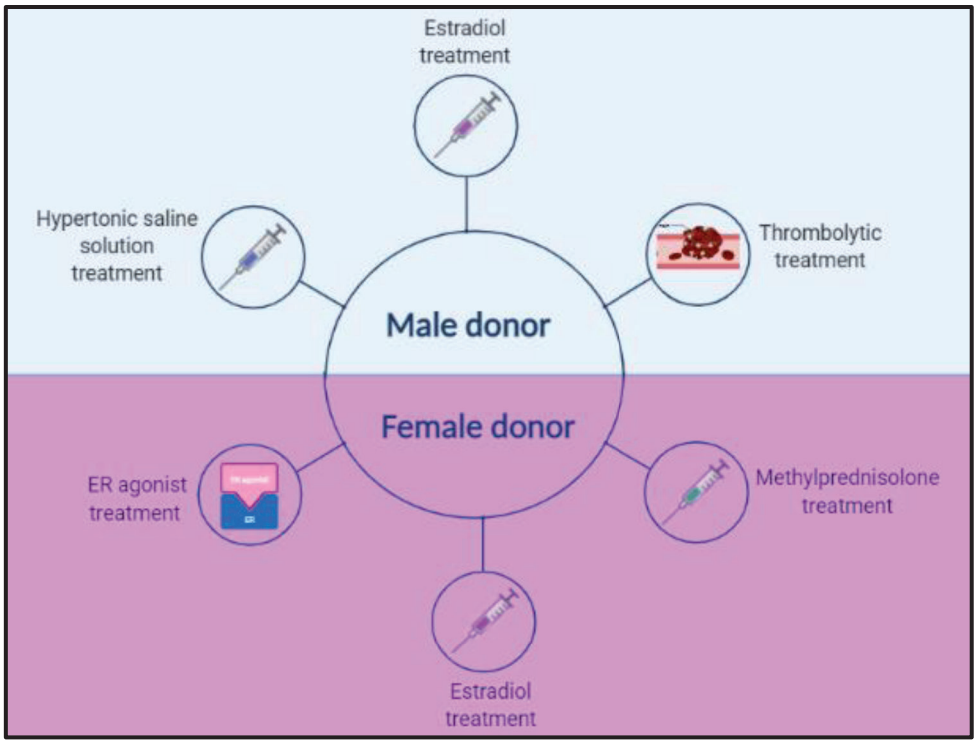

Figure 1. Schematic representation of gender-specific care.

- Brain dead-female donors

There was a greater inflammatory response in brain-dead female donors than in brain-dead male donors, characterized by activation of the complement system cascade, pronounced leukocyte infiltration into organ tissue, increased permeability, local release of inflammatory mediators (such as cytokines and chemokines), and cell death activation. Thus, we suggest a beneficial effect of anti-inflammatory treatment in brain-dead female organ donors.

Administration of estradiol and/or estrogen receptor agonist

In previous chapters, we showed that 17β-estradiol therapy efficiently reduced heart, lung, and kidney injury triggered by brain death. A greater understanding of the molecular and cellular responses to estrogen may offer further insights into the findings of experimental and clinical studies. Differences in expression and cellular/intracellular distribution of estrogen

receptor-α (ER-α) and estrogen receptor-β (ER-β) are considered to be responsible for the specificity and differences in the effects of estrogen.

In addition, the use of ER-α and ER-β agonists showed beneficial effects in several models (51–53). Brinckmann et al. (54) showed that ER-α stimulation by the propylpyrazole trio, an ER-α selective agonist, reduced apoptosis and increased the survival of myocytes co-cultured with post-infarct cardiac c-kit+ cells.

Administration of methylprednisolone

In the context of brain death in females, it is important to consider that the reduction in glucocorticoid and female sex hormone concentrations have an important influence on the systemic inflammatory process and the functional state of various organs.

Both estrogen and corticoid receptors are found in several cells, both immune and non-immune. In immune cells, these hormones act by modulating cellular development and function, whether in a convergent manner, although there are no further studies on how the respective receptors are related to this system (55). However, studies with several other cell types have an interaction between ERs and GRs, showing mechanisms related to the respective receptors (56–59). Genes involved in the inflammatory process are targets of both estradiol and glucocorticoid actions (60,61). Estradiol and corticosteroids interact with important transcription factors related to the inflammatory process, such as AP-1, Sp1, Stat1, and NF-κB (62–65). Edgar et al. (66) showed that there is synergism between hormones in inflammatory response modulation within the microvasculature, which form a complex that binds to NF-κB and inhibits the transcription of several inflammatory mediators.

- Brain dead-male donors

Regarding brain-dead male donors, experimental evidence demonstrated a compromise in microcirculatory perfusion following brain death in male rats, and indicated hemodynamic instability, which impacts the perfusion of organs and affects their viability for transplantation (50,67–69). Therefore, the implementation of therapeutic strategies to improve hemodynamic performance should be considered.

Administration of hypertonic saline solution

Hypertonic saline solution has been extensively studied as a fluid replacement in patients with septic and hemorrhagic shock, and has been shown to ameliorate hemodynamic performance by causing rapid intravascular volume expansion. The known mechanisms include reduction of endothelial cell edema and blood viscosity and improvement of cardiac contractility (70–72). Correia et al. (69) demonstrated that the infusion of hypertonic saline solution to BD-male rats improves microvascular perfusion owing to its effects on eNOS and the expression of the endothelin-1 protein. Thus, the use of hypertonic saline solution in brain-dead donors may ameliorate organ viability and transplant outcomes.

Thrombolytic Therapy

Thrombolytic therapy involves the use of drugs to dissolve thrombi. Examples include streptokinase, heparin, and alteplase. In lung transplants from cardiac arrest donors, thrombolytic therapy has been used (74) to reduce thrombi and enable perfusion in ex vivo models seeking organ reconditioning (75). In the treatment of pulmonary embolism, thrombolytic use has effectively improved pulmonary perfusion and right ventricular function (76). In a clinical study involving more than 100 liver donors with cardiac arrest, donors who underwent thrombolytic therapy had improved organ functionality (77).

Alteplase improves angiographic and hemodynamic changes faster than heparin does (78). Compared to streptokinase, alteplase effectively and rapidly improves hemodynamic changes

in patients with pulmonary embolism (79). In cases of cerebrovascular accident, treatment with intravenous alteplase 4 h after confirmation improves hemodynamic and neural function levels (80).

Administration of estradiol

Although less expressive than in brain-dead female donors, the inflammatory response is also present in male rats, resulting in tissue injury (12,16,73,81). Experimental evidence shows that 17β-estradiol effectively attenuates pulmonary and intestinal injury in brain-dead male rats, predominantly because of its capacity to modulate nitric oxide synthases (16,73).

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