CHAPTER 1

General Introduction and Outline of the Thesis

INTRODUCTION

Organ transplantation is the treatment of choice for patients with end-stage failure. In the past few decades, the success rate of transplants has progressively increased due to a better understanding of organ dysfunction, the rejection process, and the development of more appropriate immunosuppressive treatments (1–3).

Brain-dead donors are still the main source of transplantation organs. However, different situations compromise the viability of these organs (1,4), which highlights the importance of donor clinical conditions in the success rate of transplantation (5).

Brain death and organ transplantation

Most of the organs used for transplantation come from donors who have been confirmed to be brain dead. Currently, the biggest challenge in transplantation surgery is the low number of viable organs. Technical advances in donor management and organ preservation have provided improved graft results with clear benefits to the recipient.

In addition to saving lives, heart, liver, and lung transplantations promote significant physical and social rehabilitation of patients, reintegrating them into society with a better quality of life. Kidney transplantations also promote resource savings, as alternative treatments for maintaining life, such as dialysis, are more expensive in the long run (1–3).

Clinical and experimental evidence highlight the influence of BD on the viability of the organs to be transplanted (6–8), pointing to the importance of the donor's physical status in the success rate of transplantation (5). Therefore, an understanding of the alterations caused by BD allows for the optimization of clinical conduct and the acquisition of organs considered viable for transplantation.

BD caused by trauma, infarction, or intracranial hemorrhage occurs due to increased intracranial pressure, and short, intense sympathetic activity. This period is marked by high hemodynamic instability in response to an increase in serum catecholamines (autonomic storm). Catecholamines are released from the adrenal glands and nerve endings, resulting in hypertension, tachycardia, and arrhythmia. However, after the release of catecholamines, there is an imbalance in the consumption and availability of oxygen in the heart, leading to ischemic damage (9,10).

In the lungs, the consequences of a large sympathetic stimulus are edema, hemorrhage, and increased capillary permeability (11).

After a catecholamine storm, an episode of compensatory arterial hypotension occurs. This is accompanied by intense sympathetic stimulation causing severe vasoconstriction, leading to an increase in systemic vascular resistance and tachycardia (12,13). In this phase, the perfusion of peripheral organs decreases, and an inflammatory and immune response develops as a consequence of ischemia, hypoperfusion, and edema. The result is a greater expression of inflammatory mediators, cytokines, chemokines, and adhesion molecules, accompanied by the infiltration of cells in all organs, such as the heart, lung, liver, and kidney (2,4,14).

Significant hormonal and metabolic disturbances occur following pituitary failure in experimental models of BD (15). Metabolic disorders are closely linked to the inflammatory process; therefore, these changes are associated with events that precede BD, such as trauma/hemorrhage, and add to the inflammatory response in the organ donor. The development of diabetes insipidus due to antidiuretic hormone reduction occurs in approximately 80% of organ donors. In addition, the significant reduction in cortisol concentration, associated with the interruption of adrenocorticotrophic hormone release by the anterior hypophysis, compromises the donor's endogenous response to the stress generated by BD (16).

BD should not be considered as a static situation, but as a dynamic process with a striking influence on the quality and viability of organs intended for transplantation (2,17). BD leads to gradual organ dysfunction, which is worsened by hemodynamic dysfunction. Additionally, BD triggers the inflammatory status of the potential donor organ, irrespective of the presence of hypotension. The disorders observed may induce the graft to supplementary damage from ischemia/reperfusion in the transplant procedure (2,18). Therefore, organs from donors with BD may possibly be immunologically active before transplantation, which may interfere with the result when comparing them to organs from living donors (1,10).

In the heart, BD induction through increased intracranial pressure leads to a hyperdynamic reaction, culminating in echocardiographic alterations and hemodynamic instability, decreasing myocardial isoenzymes and resulting in histological injury. These consequences of BD are associated with alterations in the expression of myocardial genes, in particular the activation of the α-adrenergic receptor (19,20). The reduction in cardiac function in brain-dead donors may be caused by an imbalance between oxygen demand and supply to the myocardial tissue or even by direct injury caused by the massive release of catecholamines (21). One of the central causes of death following heart transplantation is primary graft dysfunction, which may be influenced by the donor's characteristics.

The records of the International Society for Heart and Lung Transplantation demonstrated that a sex mismatch between donor and recipient affects heart and lung transplant survival, especially after the first year (22). Other studies confirmed that the donor's gender influences the success of organ transplantation and demonstrated the differences in short-and long-term prognosis in various organ transplantations between sexes (23–27). Some surveys showed worse prognosis in heart and lung transplantation with grafts from women (27,28).

Experimental studies highlighted that acute reduction of female sex hormones caused by BD result in a negative effect on lung inflammation (29,30). Additionally, Simão et al. (31)

demonstrated inflammatory histological changes in the heart and lungs of female rats subjected to BD, with a remarkable increase in the number of polymorphonuclear and lymphomononuclear cells when compared to males. These data suggest that the inflammation generated by BD may be exacerbated in female animals and that female sex hormones, as modulators of the immune response, may be considered potential modulators of events triggered by BD.

Estradiol, immune system, and inflammation

Previous studies have revealed that the repercussions of trauma are less intense in females than in males, who, under treatment with estradiol, also develop less intense injuries (32). In this context, females also have better preservation of hemodynamic parameters after hemorrhagic shock (32,33). Clinical studies show that women, when compared to men, have fewer cases of sepsis, pneumonia, and insufficiency of multiple organs and systems (34–37). Even in cases of cardiovascular disorders, sexually active women are more protected, with the most critical cases being described in postmenopausal women (38).

There is a growing number of investigations on the contribution of female sex hormones to inflammatory processes (39–41). Studies have demonstrated the participation of female sex hormones in modulating the magnitude of the inflammatory response, revealing the complex role of sexual steroids. The gonadal steroid 17β-estradiol protects the cardiovascular system against ischemic, inflammatory, and metabolic injuries (39,40). In addition, prior experimental evidence clearly highlights that estrogen and estrogen receptor (ER) agonists are beneficial therapeutic adjuncts in preserving organ function and improving outcomes after trauma-hemorrhage (42).

The beneficial effects of estrogen in preserving organ function are due to a genomic action modulated by the interaction between intracellular receptors ERα and ERβ, as well as through a non-genomic action mediated by cell-surface estrogen-binding receptors like GPR30 (39) (Fig 1).

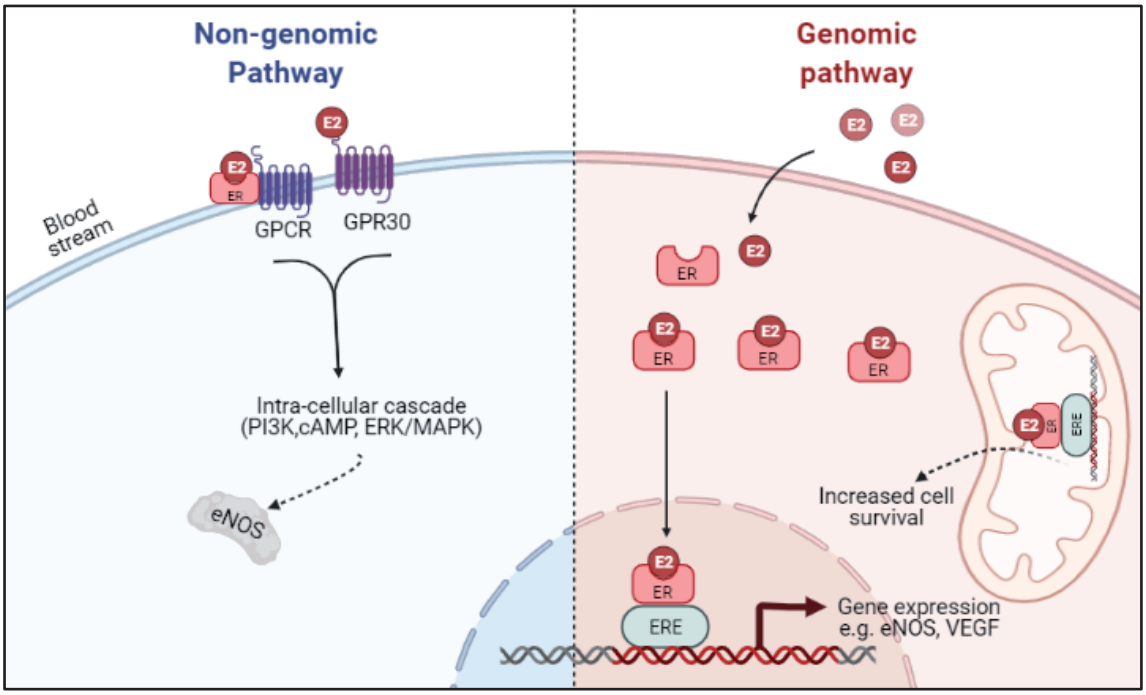

Figure 1. Overview of genomic and non-genomic effects of estrogen (E2). E2 can modulate the gene expression and activity of signaling molecules by binding to estrogen receptors (ER) through genomic and/or non-genomic pathways.

Estrogen protects endothelial cells against the prevention of atherosclerosis; consequently, it has been used to prevent cardiovascular diseases in postmenopausal women. Estrogen reduces the expression of endothelin-1 (a pro-myogenic and vasoconstriction factor), reduces the expression of adhesion molecules in response to cytokines, and stimulates the synthesis of nitric oxide (NO) by endothelial cells through phosphorylation and activation of endothelial nitric oxide synthase (eNOS) (acute or immediate effect), as well as by regulating the increase in eNOS gene expression (chronic effect) (43,44). Considering the effects of NO on the tone of vascular smooth muscle, its increase may have additional relevance for the maintenance of homeostasis of the cardiovascular system (45). Moreover, there is evidence that the influence of estradiol in

reducing the expression of transcription factors, such as nuclear factor kappa B (NF-κB) and activating protein-1 (AP-1), in the presence of experimental hemorrhagic shock (46).

Acute reductions in the concentration of female sex hormones may cause changes in several systems, including the immune system. Previous studies have shown that the removal of estradiol by ovariectomy has an important impact on gene expression in myocardial tissue, as it reduces the expression of genes that mediate vasodilation and increases the expression of genes relevant to the induction of inflammation, apoptosis, and proteolysis (47).

SCOPE OF THE THESIS

In this context, the aim of this thesis is to describe the effects of female sex hormone modulation on systemic inflammation and organ injury in brain-dead female donors. In **Chapter 2** we analyze the differences in microcirculatory changes caused by BD and inflammatory mediators between sexes.

Considering female sex hormones as immunomodulators and the association between severe organ inflammation in female animals following BD with acute estradiol reduction, we investigated the potential therapeutic effects of 17β-estradiol treatment to mitigate the deleterious process in the heart (**Chapter 3**), lung (**Chapter 4**), and kidney (**Chapter 5**) from brain-dead female rats.

In parallel, preservation solutions and perfusion techniques are important for the maintenance and improvement of the donor organ, which is directly related to morbidity and survival after transplantation. In **Chapter 6**, we compare renal inflammation in male and female patients with BD followed by isolated kidney perfusion.

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