Brain death: from inflammation to metabolic changes

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INTRODUCTION

THEORETICAL FRAMEWORK

Organ transplantation remains the best therapeutical option for the majority of patient with end-stage organ failure. Many improvements in terms of organ donation, immunosuppressive therapies, post-operative and intensive care have been made over the last twenty years. However the disparity between organ donation and waiting list is still a major problem for the medical community. Besides the total number of organs, also the decreasing quality of available donor organs is cause for concern.

This thesis focuses on organ care and improvement for transplantation, with special attention for liver and kidney.

ORGAN DONATION

In the last 10 years deceased donation has expanded. New donor sources like deceased circulatory dead donors (DCD) formerly named, non heart-beating donors and older donors are being used. It is important to realize that these changes have a substantial impact on the donated organ quality [7, 16, 18, 33, 50]. Therefore, understanding and improvement of donor physiology are two decisive research fields that potentially can contribute to a better transplant outcome.

LIVING-RELATED AND DECEASED CIRCULATORY DEAD DONORS

These groups of donors are the opposite edges of the same road. A living related donor is the best opportunity for the recipient [52]. It is a highly selected donor, usually young healthy people, with optimal organ quality associated with shorter ischemic periods than cadaveric donation. However, this option is not always available and is more difficult for liver donation, just 30% of candidates for hepatic living donation, finally will donate [17]. Nowadays, some concern has been raised in regards of an increased risk of end-stage renal failure associated with living kidney donation, although small, this represents a potential problem for living kidney donation [45].

On the other hand, a DCD donor represents the most complex kind of donation. Due to many injurious periods like hypotension and prolonged ischemia, organs derived from DCD donors are associated with a higher rate of early and late complications. They usually have a warm ischemic period longer than deceased brain death donors. Despite utility of living and DCD donors, brain dead donors remain as the major source of organs. [20, 60].

BRAIN DEAD DONORS

It was in 1959 when two French neurologists Mollaret and Goulon described a state of irreversible coma without reflexes (*coma dépassés*) after massive cerebral injury that required mechanical ventilation. The natural history after this event is cardiorespiratory

arrest days or even weeks later. This situation introduced the dilemma of a deceased patient with intact circulation. In 1968, at Harvard

Medical School, a committee was established to deal with this problem [1]. They suggested specific criteria for diagnosis of brain death, which are still used nowadays in most countries.

A permanently non-function brain state was defined when: unreceptivity and unresponsitivity plus no movements or breathing plus no reflexes were present. A flat electroencephalogram can be included as a fourth condition. These test should be repeated at least 24 hrs with no change. Other confusing conditions should be excluded: hypothermia or central nervous system depressant [1]. This definition made donation, after declaration of brain death, possible. These donors were called heart-beating donors referring to their autonomous cardiac output that remains functional after brain died. Despite functional circulation, organs derived from BD donors are of less quality compared to living donors [52].

Since the concept of brain death was introduced, many efforts have been made to understand the impact of this condition on donation and transplantation. In the 90's a number of brain death animal models were developed. These models made it possible to study and understand the hemodynamic changes, hormonal impairment and inflammatory response related with brain death [4, 25, 42].

Brain Death (BD) represents the culmination of progressive rostral-to-caudal ischemia of the central nervous system [59]. During the onset of BD, as a consequence of cerebral ischemia and intracranial hypertension, there is a strong parasympathetic activity followed by a severe vasoconstriction (sympathetic response) due to endogenous catecholamines release. This hemodynamic response is called the Cushing's reflex and it aims to maintain the cerebral perfusion pressure. Secondly, a progressive paralysis of the spinal cord takes place. This paralysis terminates the sympathetic tone and triggers peripheral vasodilatation. Hemodynamic instability characterizes this period [10].

In addition, hormonal deregulations are described. Pituitary function is affected following BD as adrenocorticotrophic hormone (ACTH) secretion is altered resulting in a transient rise in cortisol levels, which then progressively diminishes below baseline levels. In addition other hormones including T_4/T_3, anti-diuretic hormone (ADH) and insulin are reduced as a consequence of BD [41].

BD results in a systemic inflammatory state as illustrated by the influx of polymorphonuclear neut-rophils (PMNs) into kidney and liver tissues [21]. Systemic production of circulating cytokines including interleukin-6 (IL-6), interleukin-10 (IL-10), Tumor Necrosis Factor-alpha (TNF-α), Trans-forming Grown Factor-beta (TGF-β) and Monocyte chemotactic protein 1 (MCP-1) are thought to orchestrate this response [29, 36, 51, 54]. The trigger for this inflammatory process is not well understood, however recent evidence suggests there could be a role for cerebral cytokines that cross

the blood-brain barrier, in addition to complement activation [12, 13] and intestinal bacterial translocation [27, 46].

Brain death could be described as an inflammatory status, comparable to SIRS (systemic inflammatory response syndrome) without neuro-endrocine control, but under irreversible conditions.

ORGAN RESPONSE TO BRAIN DEATH

Interestingly, the impact of brain death has some organ-specific features. How the hemodynamic impairment, the neuroendocrine failure and the systemic inflammatory state affects each organ has not been deeply studied. Here, some characteristics will be summarized, focusing on liver and kidney since they are the most transplanted organs.

Liver

It is known that the hemodynamic state directly affects hepatocyte function. Nagareda et al. described in a study using sequential liver biopsies in humans as long as 48 days after the on-set of brain death the occurance of central venous congestion during the first days, probably caused by brain death-induced circulatory failure [38]. No significant central fibrosis, fatty change, piecemeal necrosis, or periportal fibrosis were observed. Weiss et all. showed that liver grafts from brain dead donors had a poor performance in response to ischemia reperfusion injury compared with living donors [58]. Using the brain dead rat model in Groningen, Van der Hoeven, working as PhD student in the Surgical Research Laboratory in the UMCG, reported increased levels of AST (aspartate transaminase) and GST (glutation s-transferase) in brain dead rats, which could be explained through liver cell dysfunction induced by brain death. Accordingly, the authors report increased cell adhesion molecule (VCAM-1 and ICAM-1) expression on liver parenchyma in the brain death group, as well leukocyte infiltration. In a second experiment the detrimental effect of brain death status on the liver graft after trans-plantation was reported. [53–55].

Kidney

Also kidneys suffer from BD induced hemodynamic instability and inflammation. Several studies in rat models and humans report increased inflammatory cytokines, infiltration of mac-rophages and T cells and up-regulation of adhesion molecules (selectins and Ig superfamily members). Several studies show increased plasma creatinine levels in brain dead animals and humans samples, as well as leukocyte influx, complement activation, adhesion molecules like VCAM-1 and ICAM-1, and expression of early gene FOS in response to cellular damage, as HO-1 and HSP-70 as well. [12, 40, 56].

BRAIN DEAD DONOR MANAGEMENT

Currently the management of brain dead donors is focused on three key aspects, hemodynamics, ventilation and hormonal replacement therapy.

Hemodynamics

As pointed out above, the hemodynamic impairment is a key factor in the pathophysiology of brain death. Because of that, the main goal is to maintain adequate circulating blood volume, proper cardiac output and perfusion pressure in order to sustain optimal tissue oxygen delivery.

In order to achieve this goal, a proper monitoring is needed. The most utilize device is the Central Venous Catheter (CVC). Central Venous Pressure (CVP) is a rough measure of the hemodynamic status. Thus, to assess this condition the gold standard in most Intensive Care Units (ICUs) is the Pulmonary artery Catheter (PAC), which gives an estimation of the left ventricular pre-load and the cardiac output through pulmonary artery occlusion pressure (PAOP). There are other costly possib-ilities as well, like trans-pulmonary thermo-dilution or trans-oesophageal ultrasound. By using this technique the continuous cardiac output could be determined, as well as the intrathoracic blood volume (ITBV) and extravascular lung water (EVLW). The ITBV could be a better determination of left ventricular pre-load. The EVLW reflects pulmonary edema, which is important for lung donors [16, 59, 61].

Despite the possible monitoring process, the most widely objectives are main arterial pressure (MAP) above 70 mm Hg, a CVP of 4-10 mm Hg, a PAOP of 8-12 mm Hg, an adequate urine output (≥ 1,5ml/kg) on dopamine infusion if is needed, and haematocrit ≥ 30% or haemoglobin level ≥ 10 g/dL [8, 14]. In order to achieve these goals the first strategy is to maintain or restore a normal intravascular volume. Initially, colloids or crystalloids could be used. There are not enough data to choose one of them and, in fact, they are often used in combination in brain death donor management. It is reasonable to think that crystalloid-based fluid therapy is related with neurogenic pulmonary oedema [14, 43]. On the other hand, the use of colloids, like HAES, is related with kidney toxicity. A recent review by Myburgh JA et al.[37] pointed out that the CHEST study found a significant increase in renal replacement therapy rate using colloids as resucitation fluids. Although the third generation of this products could be less deleterious [5]. In the same line, administration of packed red cells it is recommended to restore a physiological haemoglobin level, but there are no studies that test the administration of red cells in this setting [32, 57].

In almost all BD donors, vasoactive drugs are required, considering the negative effects on myocar-dium because of sympathetic storm [44], it seems reasonably, not to use high doses of catecholamines. Norepinephrine in donors has been correlated to primary non-function of heart grafts, how-ever the same report showed an improvement of kidney graft survival and no change in liver graft survival [48]. Dopamine is an inotropic drug that could improve the cardiac output and it could play an important role on inflammatory response. The role as inotropic drug is inconclusive but dopamine could augment oxygen consumption and tachycardia, which are both deleterious for myocardial muscle. There is some experience using dopamine in combination with norepineph-rine with better

outcomes in graft function [9]. The role of dopamine to dampen the inflammatory response is encouraging, Koetting et al. report on improvement of liver graft response to cold ischemia, using dopamine in the preservation solution [24]. Liu et al. report lower tubulitis in kidneys pretreated with dopamine [31]. This effect is apparently related with a preconditioning effect, activating adenylate cyclase and subsequently increasing cAMP that via protein-kinasa A (PKA) in-hibit translocation of nuclear factor-κB (NF-κB) modulating the innate inflammatory response [3]. Lazou et al. showed a protective effect on heart muscle using dopamine. This effect is quite similar to ischemic preconditioning by activating adrenergic receptors [30]. A maximum dopamine dose of 10 μg/kg/min has been suggested, but there is not enough evidence to support this assumption [19, 59].

Pulmonary management

As mentioned, neurogenic pulmonary oedema is a common problem in the brain dead organ donor. In addition pneumonia and the inflammatory response make lung donation difficult. The pulmonary care has to focus on preserving the perfusion pressure without increasing extravascular water. In addition, not to increase barotrauma. This requires an aggressive approach including the use of positive end expiratory pressure (PEEP), chest physiotherapy, attention to fluids balance, bronchial toilette, antibiotics and bronchoscopy [49].

Alveolar recruitment can be undertaken when the initial blood analysis show a PaO_2/FiO_2 ratio (PAFI) of less than 300 mm Hg, or in case of presence of pulmonary infiltrates/pulmonary oedema, and/or atelectasis [7]. To promote recruitment, it is important to avoid depressurization, in that sense the apnoea test could be made within CPAP mode with a PEEP at 10 cm H_20. After that, a protective ventilator strategy, with a Current Volume at 6-7 ml/kg (bwt), FiO_2 enough to keep the PaO_2 near 100 mm Hg or oxygen saturation $\geq$ 95%. The PEEP value has to be kept as low as possible to keep PaO_2 near 100 mm Hg, but it is necessary to use at least 8 cm H_20 to avoid atelectasis [14]. Recruitment manoeuvres include increased PEEP to 15 cm H_2O, Tidal volume of 4-8 ml/kg and a plateau pressure of $\leq$ 30 cm H_2O, Ventilatory Rate < 35 b/min, inspiratory:expiratory ratio 1:1 - 1:3 [7, 34].

Hormonal Replacement Therapies

The neuroendocrine impairment potentiates the inflammatory environment. In management of severe sepsis patients, steroids utility it is well know [15]. The same concept can be apply to brain dead donors but is not standard care. The use of an hormonal cocktail containing thyroid hormones, vasopressin, steroids and insulin permitted to improve the donor condition in case of inappropriate hemodynamic response [41, 59].

Vasopressin is utilized to control Diabetes Insipidus, but it also has a function as vasopressor, reducing the hemodynamic need of catecholamines in BD donors. The use

of arginine-vasopressin at doses greater than 0.04 U/min may cause coronary, renal and splanchnic vasoconstriction jeopardizing donor organ function [16].

Insulin therapy is part of the ordinary attention in critical cares units [23], as well as vasopressin in hemodynamic control. But for thyroid hormones the evidence is controversial.

Steroids seem to improve graft function and donor stability. Some authors observed an anti-inflammatory role of steroids and decreased organ damage in brain dead donors treated with Methylprednisolone [35]. This effect can be assessed in each organ and by cytokines plasma levels measurement [41]. The recommended dose is a single bolus of 15 mg/kg bwt, but some authors, like Pratschke et al. propose 250 mg in bolus follow by 100mg/hr until organ harvest [26, 28].

For decades thyroid supplementation was a fundamental part in heart donor management, since it improves graft function, however some evidence in liver and kidney donors shows the opposite [2, 11, 22].

Recent data shows a potential benefit for the administration of other hormones, like erythropoietin that could improve the outcomes in kidney and liver transplant, but there is not enough evidence available. It seems clear that it is necessary to advance in basic research and clinical trials to improve our knowledge about this condition and the best way to prevent or decrease organ injury secondary to brain death state [6, 39, 47].

AIM

The aim of this thesis was to study the response of livers and kidneys to brain death and identify key elements in brain dead donor management.

Since the inflammatory response is a major feature of brain death, we investigated the effect of the anti-inflammatory drug prednisolone on kidney and liver grafts from BD rats (chapter two and three) focusing on functional and cellular-injury markers. A second step was to test a non-anti-inflammatory therapy; in this case we studied the preconditioning action of thyroid hormone (T_3) on liver graft of BD rats. (chapter four).

As hemodynamic changes are also a key element in brain death, we performed an experiment comparing two different hemodynamic profiles of brain death induction in rats (chapter five). The slow induction model previously described by Kolkert et al. in Groningen [25] and a fast induction model, these two models develop opposite hemodynamic profiles. Slow induction model has a short hypotensive period just before the onset of brain death while fast induction model has a hypertensive peak during induction phase without hypotensive period. This determined a differential pattern of injury on kidney and liver related with hemodynamic response.

The second part of this thesis follows the thought that key elements in brain death as hemodynamic, hormonal changes and inflammation response are influencing cell metabolism and cell survival. Using the slow induction model we studied metabolic changes in brain death (chapter six). We assessed changes in carbohydrates metabolism as well as

fatty acid oxidation. Since metabolism is closely related with organ perfusion and oxygen delivery we developed a non-invasive MR technique to assess organ perfusion and oxygen consumption in our animal model (chapter seven). We investigated differences in liver and kidney perfusion during brain death as differences in oxygen consumption. Because the final energy producer in the cell is the mitochondria, we studied their function in kidney and liver tissue of brain dead animals (chapter eight). As a final conclusion results were summarized and discussed in perspective in chapter nine.

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