UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

ATIVIDADE INFLAMATÓRIA INDUZIDA PELA MORTE ENCEFÁLICA NO TECIDO PANCREÁTICO HUMANO

TESE DE DOUTORADO

TATIANA HELENA RECH

Porto Alegre, dezembro de 2012

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Orientadores: Profa. Dra. Cristiane Bauermann Leitão

Dra. Daisy Crispim Moreira

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul (UFRGS) como requisito parcial para a obtenção do título de Doutor em Endocrinologia.

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LISTA DE ABREVIATURAS

ATP	Adenosina trifosfato
BD	*Brain death*
BMI	*Body mass index*
cDNA	*Complementary deoxyribonucleic acid*
CI	*Confidence interval*
CIHDOTT	Comissão Intra-Hospitalar de Doação de Órgãos e Tecidos para Transplante
DM	Diabetes melito ou *diabetes mellitus*
ELISA	*Enzyme-linked immunosorbent assay*
ERK	Cinase extracelular sinal-regulada
FIPE	Fundo de Incentivo à Pesquisa e Ensino
FT	Fator tecidual
GLP-1	*Glucagon-like peptide 1*
GRADE	*Grading of Recommendations Assessment, Development and Evaluation*
HbA1c	*Glycated hemoglobin*
HCPA	Hospital de Clínicas de Porto Alegre
HLA	*Human leukocyte antigen*
IBMIR	*Instant blood-mediated inflammatory reaction*
IFN-γ	*Interferon-γ*

IL-1β	*Interleukin-1β*
IL-10	Interleucina-10
IL-6	*Interleukin-6* e interleucina-6
IκB	Inibidor κB
ME	Morte encefálica
MeSH	*Medical Subject Headings*
mRNA	*Messenger ribonucleic acid*
NF-κB	*Nuclear factor-κB* e fator nuclear-κB
PRISMA	*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*
RCT	*Randomized clinical trial*
RNA	*Ribonucleic acid*
RR	*Relative risk*
RT-qPCR	*Reverse transcription quantitative polymerase chain reaction*
SD	*Standard deviation*
TF	*Tissue factor*
TNF-α	*Tumor necrosis factor-α* e fator de necrose tumoral-α
UCP-2	Proteína desacopladora 2
UFRGS	Universidade Federal do Rio Grande do Sul
WMD	*Weighted mean difference*

Esta tese de doutorado será apresentada no formato exigido pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia. Ela será constituída de uma introdução em português e de dois artigos em inglês, estes formatados conforme as exigências das respectivas revistas médicas às quais serão submetidos para avaliação e posterior publicação. Os artigos em inglês desta tese são um artigo do tipo Revisão Sistemática e Meta-Análise e outro do tipo Artigo Original.

RESUMO

O transplante de ilhotas pancreáticas restabelece a secreção de insulina em pacientes diabéticos tipo 1 lábil. A despeito dos avanços da técnica de isolamento de ilhotas, a inabilidade de se obter um número suficiente de células de um único doador persiste como um obstáculo para o sucesso desse transplante. A identificação dos fatores relacionados com o dano das ilhotas pancreáticas durante todo o procedimento do transplante tem sido buscada na tentativa de desenvolver terapias capazes de minimizar a perda de células e otimizar a enxertia das ilhotas transplantadas, reduzindo, assim, a necessidade de múltiplos doadores para o alcance da independência de insulina. A morte encefálica (ME) está associada a uma inflamação sistêmica que produz profundas alterações fisiológicas na condição hemodinâmica do doador, antes mesmo do início do processo de retirada dos órgãos. Essa característica única do doador cadavérico influencia negativamente a função dos órgãos pós-transplante, o que torna os cuidados com o doador uma peça chave no cenário dos transplantes de órgãos. Contudo, o uso de protocolos com terapias específicas, como a reposição de hormônios tireoidiano e suprarrenal, tem demonstrado eficácia muito limitada em melhorar os desfechos de órgãos transplantados. Os resultados desta pesquisa demonstram que marcadores inflamatórios estão aumentados no estado de ME. A precipitação da ME é seguida de um aumento das concentrações de fator de necrose tumoral-α (TNF-α) e interleucina-6 (IL-6) no sangue e de TNF-α no tecido pancreático, mas não de fator tecidual (FT), cuja expressão já conhecida em ilhotas isoladas provavelmente se deva a fatores de estresse

relacionados ao isolamento das células. Esse aumento de TNF-α pode explicar, pelo menos em parte, os melhores desfechos alcançados por protocolos de transplante de ilhotas que incluem o uso de receptores solúveis do TNF-α. Em conclusão, a ME está associada a um aumento da expressão de TNF-α no sangue e no tecido pancreático humano. Portanto, o uso de terapias anti-inflamatórias dirigidas ao doador de múltiplos órgãos pode tornar-se uma estratégia promissora para melhorar os resultados dos transplantes de ilhotas.

CAPÍTULO 1

Introdução

O diabetes melito (DM) é uma síndrome caracterizada por alterações metabólicas associadas a elevada morbidade e mortalidade e que atinge milhões de pessoas em todo o mundo. O DM tipo 1, responsável por 5 a 10% dos casos de diabetes, é uma doença autoimune resultante da destruição das células β pancreáticas, o que determina a deficiência total da produção de insulina e a necessidade de administração de insulina exógena para a sobrevivência (1).

O transplante de pâncreas é, no momento, a maneira mais eficaz de se restabelecer a homeostase glicêmica em pacientes diabéticos tipo 1 com controle metabólico instável (2). O transplante de pâncreas como órgão inteiro promove controle glicêmico adequado e reduz as complicações crônicas do diabetes. Além disso, os pacientes submetidos ao transplante apresentam uma boa sobrevida a longo prazo (3). No entanto, esse procedimento está associado à morbidade de uma cirurgia de grande porte. Nesse cenário, a ideia da substituição do pâncreas endócrino deficiente por células produtoras de insulina através do transplante de ilhotas pancreáticas foi introduzida por Lacy em 1960. Seus relatos pioneiros demonstraram que ratos diabéticos ficavam normoglicêmicos após serem submetidos a transplante de ilhotas pancreáticas (4). Ao longo das últimas décadas, o aperfeiçoamento das técnicas de isolamento de ilhotas avançou muito (5), e, no ano 2000, o transplante de ilhotas se consolidou como uma opção de tratamento para pacientes com diabetes tipo 1 com

controle instável, com base nos estudos de Shapiro *et al.*, que propuseram um protocolo de imunossupressão livre de corticosteroides (6). A qualidade de vida é afetada positivamente pelo transplante (7), e a percepção da hipoglicemia é restaurada (8), promovendo a estabilização do controle glicêmico e a prevenção de hipoglicemias graves.

Em relação ao transplante de órgão inteiro, o transplante de ilhotas tem a grande vantagem de ser menos invasivo, uma vez que a injeção das células é feita através da canulação percutânea da veia porta (9). Por outro lado, um controle glicêmico adequado pós-transplante exige que um grande número de ilhotas seja transplantado. Frequentemente, são necessários transplantes sequenciais de dois ou mais pâncreas para se atingir a independência de insulina (10, 11). A escassez de órgãos para transplante acaba, então, sendo um forte limitador dessa terapia (12, 13). Em razão disso, vêm-se estudando exaustivamente maneiras de atingir o máximo aproveitamento de ilhotas por pâncreas doado (14, 15). É preciso ainda muito desenvolvimento nesse campo para que o transplante de ilhotas se torne a terapia padrão para o tratamento do diabetes tipo 1. O grande objetivo dessa terapia é o alcance da independência de insulina com apenas um doador (3).

Durante o processo de retirada e estocagem do pâncreas, as ilhotas são submetidas a múltiplos fatores deletérios celulares, entre eles a isquemia fria, as mudanças súbitas de temperatura, o estresse oxidativo, as forças de cisalhamento que agem sobre o órgão, além do processo de digestão necessário ao isolamento das células (16, 17). A esses agravos soma-se o intenso estresse inflamatório produzido pela morte encefálica (ME), resultando em lesão tecidual e na redução da função e da sobrevida dos enxertos (18).

A maior fonte de órgãos para transplante e a única fonte substancial de pâncreas é o doador cadavérico em ME (19). O doador ideal de ilhotas pancreáticas é um homem jovem, vítima de trauma, com índice de massa corporal ≥ 25 kg/m^2, controle glicêmico adequado, curto período de internação em unidade de tratamento intensivo e sem instabilidade hemodinâmica prolongada (20-23).

A ME é uma síndrome inflamatória com efeitos adversos graves bem definidos sobre os desfechos dos transplantes. A influência não imunológica da ME sobre os órgãos captados foi inicialmente estudada no rim. Enxertos renais de ratos receptores de rins cadavéricos apresentaram um curso mais acelerado de rejeição crônica do que os daqueles de doadores vivos (24). Mesmo rins de doadores vivos sem compatibilidade HLA (*human leukocyte antigen*) apresentaram uma sobrevida maior do que rins de doadores em ME em estudo bem desenhado com 368 transplantes entre cônjuges (25).

Órgãos provenientes de qualquer doador vivo, relacionado ou não relacionado, demonstram resultados consistentemente superiores quando comparados aos de doadores cadavéricos. Uma lesão cerebral catastrófica leva a ME, e esta desencadeia alterações hemodinâmicas, neuro-humorais e imunológicas que afetam a qualidade dos órgãos (26). A liberação aguda maciça de catecolaminas, conhecida como tempestade autonômica, é consequência da herniação cerebral e é tanto mais intensa quanto maior for a velocidade de instalação da hipertensão intracraniana. Essa liberação explosiva de catecolaminas produz um aumento na expressão de citocinas nos órgãos sólidos, além de mediar a ativação do complemento (27). O gatilho inflamatório que afeta adversamente a função dos órgãos transplantados de uma maneira antígeno-independente foi bem documentado por Kasuka *et al.*, que quantificaram a expressão de fator de necrose tumoral-α (TNF-α), interleucina-1β (IL-1β) e interleucina-6 (IL-6) em cobaias submetidas a ME ou somente ventilação mecânica. Após 5 dias, ocorreu uma

densa infiltração dessas citocinas nos túbulos e glomérulos renais dos ratos em ME (28). Contreras *et al.*, por sua vez, demonstraram que a ativação de citocinas pró-inflamatórias tem um impacto importante e tempo-dependente na função das células β pancreáticas. Em ratos, a precipitação da ME é seguida de um aumento imediato das concentrações de TNF-α, IL-1β e IL-6, que induzem a disfunção e morte da célula β, principalmente por apoptose (29). Há evidências também da implicação do aumento da interleucina-10 (IL-10) e do interferon-γ (INF-γ) na apoptose de ilhotas nesse cenário (30, 31).

Pâncreas originados de doadores vivos estão associados com maiores taxas de pureza, viabilidade e funcionalidade das células pós-isolamento em modelos animais (32) e humanos (33). Os efeitos inflamatórios deletérios da ME sobre o isolamento e a funcionalidade das células β são muito relevantes, na medida em que determinam uma menor recuperação de ilhotas por pâncreas doado. Por outro lado, a independência de insulina depende do transplante de um grande número de células β.

Além da perda de ilhotas secundária ao estresse inflamatório induzido pela ME e pelo estresse oxidativo da estocagem e do isolamento (34), um percentual significativo de ilhotas é destruído imediatamente após a infusão das células na circulação porta. Esse fenômeno, chamado de reação inflamatória instantânea sangue-mediada (do inglês *instant blood-mediated inflammatory reaction*, IBMIR), caracteriza-se por uma intensa atividade pró-coagulante, com eventos deletérios trombótico-inflamatórios responsáveis pela perda precoce de células transplantadas (35). Esse estado pró-coagulante induzido pelo transplante de ilhotas pode culminar, inclusive, com trombose da veia porta. A expressão do fator tecidual (FT) tem sido implicada como o principal gatilho da IBMIR no transplante de ilhotas e de hepatócitos (36, 37).

O FT é uma glicoproteína de 47 kDa responsável por desencadear a formação do coágulo, transformando o fator VII em fator VIIa. O complexo FT-fator VIIa é pró-inflamatório. A expressão de FT pelas ilhotas provoca intensa reação inflamatória no momento em que elas entram em contato direto com sangue ABO-compatível e ocorre a ligação rápida de plaquetas na sua superfície. Existe uma associação entre a intensidade da expressão do FT nas ilhotas e a magnitude com que a IBMIR afeta a função das células transplantadas (38). A perda de ilhotas desencadeada pela IBMIR foi estimada em 50 a 60% do total de células em primatas não humanos (39). Um estudo pequeno sugeriu que o bloqueio do FT através do uso de anticorpos monoclonais específicos anti-FT poderia aumentar a taxa de sucesso do transplante de ilhotas humanas (40).

A escassez de órgãos é o principal fator limitante ao desenvolvimento dos transplantes como terapia viável (41, 42). Desta forma, o manejo do doador de múltiplos órgãos concretiza-se como um capítulo muito importante na medicina intensiva (43). Uma das razões principais para potenciais doadores não se tornarem doadores de fato é o suporte inadequado das funções vitais no período que permeia a ME (44). Sabe-se que o uso sistemático de protocolos de cuidados com o doador aumenta a taxa de captação de órgãos (45-47). Porém, não se sabe, de forma consistente, quais terapias especificamente estão associadas à melhora dos resultados.

Diante do exposto, esta tese tem dois objetivos:

- Determinar quais procedimentos adotados no manejo do doador de múltiplos órgãos em ME alteram a qualidade dos órgãos a serem enxertados, através de uma revisão sistemática e meta-análise da literatura;
- Avaliar se a ME está associada a aumento de marcadores pró-inflamatórios e pró-trombóticos no tecido pancreático humano, por meio de um estudo de casos e controles.

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CAPÍTULO 2

Management of the brain-dead organ donor: a systematic review and meta-analysis

Short title: Management of brain-dead organ donor

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Key words: Brain death; randomized clinical trials; directed tissue donation; tissue and organ procurement.

Abstract

The shortage of organs is a limitation for transplantation, making the care of potential organ donors an important issue. The present systematic review and meta-analysis was carried out to assess the efficacy of interventions to stabilize hemodynamics in brain-dead donors or improve organ function and outcomes of transplantation. Medline, Embase and Cochrane databases were searched. Of 5096 articles retrieved, 39 randomized clinical trials (RCTs) were selected. Twenty were included in a qualitative synthesis, providing data on 1277 patients. The main interventions described were desmopressin use, triiodothyronine and methylprednisolone replacement, fluid management, vasopressor therapy, mechanical ventilation strategies, and surgical techniques. Three meta-analyses were conducted: the first included two studies and showed that desmopressin administered to brain-dead patients was not advantageous with respect to early organ function in kidney recipients (RR 0.97; 95%CI 0.85-1.10; I^2 0%, p=0.809). The second included four studies and showed that triiodothyronine did not add hemodynamic benefits vs. standard management (weighted mean difference 0.15; 95% CI -0.13-0.42; I^2 17.4%, p=0.304). The third meta-analysis (two studies) showed that ischemic liver preconditioning during harvesting procedures did not benefit survival (RR 1.0; 95% CI 0.93-1.08; I^2 0%, p=0.459). The present results suggest limited efficacy of interventions focusing on the management of brain-dead donors.

Introduction

Organ transplantation is the treatment of choice for many end-stage organ diseases. However, it is still strongly limited by organ shortage (1), with increasing disparity between organ supply and demand (2). One promising way to overcome this problem is to optimize brain-dead organ donation.

Brain death is an inflammatory syndrome (3, 4) that causes a massive catecholamine release, with a sudden decrease in cortisol, insulin, thyroid and pituitary hormone levels (5). Hormonal replacement therapy has been reported to stabilize and improve cardiac function in brain-dead donors (6, 7). Rosendale et al. (8) described a retrospective analysis of more than 10 thousand consecutive donors, suggesting that aggressive pharmacologic therapy results in more transplanted organs. However, other studies have failed to confirm these benefits (9, 10).

In addition, hormonal alterations have a profound hemodynamic and metabolic impact on potential donors, inducing a variety of deleterious effects that can threat organ perfusion and result in cardiac arrest (11). Frequently, hemodynamic collapse precludes organ donation (12). Therefore, early donor management is associated with increased organ retrieval (13), and many transplantation centers and critical care societies have developed standardized donor management protocols that focus on hemodynamic and hormonal resuscitation (14-16).

However, the strategy of brain-dead donor management is still controversial; it is time and resource consuming and has potential deleterious effects, such as metabolic acidosis. Therefore, the present systematic review and meta-analysis was carried out to assess the impact of interventions focusing on the care of brain-dead donors on transplantation outcomes.

Methods

Search strategy and study selection

To identify randomized clinical trials (RCTs) comparing any category of intervention on management of brain-dead organ donors, in August 2012 we performed an initial electronic literature search in MEDLINE, EMBASE and Cochrane as well as the Cochrane Controlled Trials Register, without language or date restriction, using the following medical subject headings (MeSH): "Tissue and Organ Procurement"[MeSH] or "Directed Tissue Donation"[MeSH] or "Brain Death"[MeSH]. A high sensitivity strategy for the search of RCTs was used (17). Additionally, we manually searched the references of the selected studies. This systematic review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (18).

Eligibility criteria

We included RCTs comparing interventions aimed to stabilize hemodynamics in brain-dead donors or to improve donated organ function or organ receptor outcomes after transplantation as compared to a control group. The following were excluded: 1) RCTs that did not provide information regarding the associations of the intervention with donor stability, organ function or receptor's outcomes in the experimental group, the control group, or both; 2) duplicate publications or substudies of included trials.

Data extraction

Titles and abstracts of retrieved articles were independently evaluated by two reviewers (T.H.R. and R.B.M.). Disagreements were solved by consensus or by a third reviewer (C.B.L.). The investigators were not blinded to authors, institutions or

journals. Articles whose abstracts did not provide enough information regarding the inclusion and exclusion criteria were retrieved for full text evaluation. To avoid possible double counting of patients included in more than one report by the same authors or working groups, recruitment periods were evaluated. If necessary, the corresponding author was contacted for elucidation. Two reviewers (T.H.R. and R.B.M.) independently conducted data extraction.

Assessment of risk of bias and study quality

Risk of bias was evaluated according to GRADE (The Grading of Recommendations Assessment, Development and Evaluation) recommendations (19). Study quality assessment included adequate sequence generation, allocation concealment, blinding of outcomes assessment, and intention-to-treat analysis. Quality assessment was independently performed by two reviewers (T.H.R and R.B.M) and disagreements solved by consensus or a third reviewer (C.B.L).

Statistical analysis

The clinical outcomes of interest were hemodynamic parameters before transplantation, quantification of organ retrieval, organ function after transplantation, and patient or graft survival after transplantation. Studies reporting a similar intervention and outcome were grouped, and a separate forest plot was constructed whenever possible (similar intervention/outcomes in two or more studies identified during the search). For analysis of single studies (that could not be grouped), only qualitative assessment was performed.

For continuous variable outcomes, means or differences between means and respective dispersion values were extracted, and pooled-effect estimates were obtained

by comparing the least squares mean percentage change from baseline to the end of the study for each group; these results were expressed as the weighted mean difference between groups. For categorical outcomes, the total number of patients included and the number of participants with the outcome were used to calculate the overall relative risk (RR) of the intervention to improve an outcome.

Cochran's Q test was used to evaluate heterogeneity between studies, and a threshold *p* value= 0.1 was considered statistically significant. The I^2 test was also conducted to evaluate the magnitude of the heterogeneity between studies. We used risk estimates obtained with a fixed-effect meta-analysis because no significant heterogeneity was found between the studies. For the triiodothyronine meta-analysis, publication bias was assessed using a contour-enhanced funnel plot of each trial's effect size against the standard error (20). Funnel plot asymmetry was evaluated by Begg and Egger tests, and a significant publication bias was considered if the *p* value was less than 0.1 (21, 22). All statistical analyses were performed by Stata 11.0 software (Stata, College Station, TX, USA).

Results

Literature search results and study characteristics

We identified 5094 potentially relevant citations from electronic database search and two from manual search. After duplicates were removed, 2726 studies were screened on the basis of title and abstracts, resulting in 39 RCTs for further evaluation. Twenty studies fulfilled our inclusion criteria, providing data on 1277 patient (Table 1). Of these, 12 trials could not be grouped (single intervention and/or outcome) (11, 23-

33) and were evaluated qualitatively, and 8 studies (15, 16, 34-39) were included in the meta-analysis. A flowchart of search and selection criteria is shown in Figure 1.

The trials were published from 1977 to 2010. The main interventions included: desmopressin use (n=146 patients), triiodothyronine replacement (n=264 patients), methylprednisolone replacement (n=150 patients), triiodothyronine and methilprednisolone replacement (n=60 patients), fluid management (n=33 patients), vasopressor therapy (n=264 patients), mechanical ventilation strategies (n=118 patients), and surgical techniques (n=242 patients). The outcomes assessed varied substantially between studies, from hemodynamic parameters and organ function to graft and patient survival. There were no major treatment-associated adverse events.

Table 2 shows the risk of bias in each trial. Eight studies were blinded, seven used placebo controlled groups, nine trials described adequate sequence generation and allocation concealment, 15 used the intention-to-treat principle for statistical analysis, two declared to have received grant support from the pharmaceutical industry and three were stopped early, one because of harm (impaired immediate renal function of kidney recipients), one because of benefit, and the other because of termination of funding.

Trials included in qualitative-only analysis

Twelve studies (11, 23-33) reported interventions and/or outcomes not duplicated by other authors. Because it was not possible to group them, they were not therefore included in a meta-analysis. One of them, a well-designed multicenter RCT including 118 patients, demonstrated that more lungs were eligible for transplantation when a lung protective ventilator strategy with low tidal volumes was used as compared with a conventional ventilator strategy (30). Another trial, performed in 60 European centers, allocated brain-dead patients to pretreatment with low-dose dopamine or no

treatment. The results showed that early kidney graft function was improved with the pharmacological approach, but with no impact on patient survival (33). Two underpowered studies tested the role of fluid management of donors. When low molecular weight hydroxyethyl starch was administered to liver donors, no differences in early function were found (31). However, impaired immediate renal function in kidney recipients was reported when hydroxyethyl starch was used as plasma expander in kidney donors, leading to early termination of the trial (24).

The impact of methylprednisolone replacement on brain-dead donors was investigated in three different RCTs that evaluated distinct organ outcomes. Hormone therapy was not effective to either increase lung yield (13) or improve early kidney graft function (23). Only one study demonstrated a protective effect of methylprednisolone on liver grafts, with significant downregulation of inflammation markers and decreased incidence of acute rejection after liver transplantation (29).

Two out of six studies of thyroid hormone replacement therapy described study-specific outcomes and were not included in the meta-analysis. The first one measured the impact of triiodothyronine on liver function tests during the first week post-transplantation, obtaining similar results in both groups and worse metabolic acidosis in the treatment group (32). The other study showed a trend toward less inotrope need in the treatment group (28).

Circulatory deterioration and cardiac arrest usually occur soon after brain death. However, long-term maintenance of circulation of brain-dead donors was reported in a desmopressin-treated group when compared to a control group (27).

No difference regarding graft performance was found in a trial designed to compare two preservation solutions as the initial flush in hepatic allograft procurement (25). Finally, a trial of the impact of donor harvesting technique using a modified

double (aortic and portal) perfusion technique for suboptimal liver grafts was terminated early because of benefit, with improved six-month graft and patient survival rates when compared with a single aortic perfusion technique (26).

Trials included in meta-analyses

We retrieved eight studies that could be meta-analyzed, two evaluating desmopressin (n= 121 patients), four intravenous triiodothyronine (n = 209 patients), and two ischemic liver preconditioning (n = 151 patients).

The two studies on desmopressin use (36, 37) assessed the effects of desmopressin administration to brain-dead donors on early graft function in kidney recipients. As shown in Figure 2A, no benefits of desmopressin on early graft function of kidney transplants were observed (RR = 0.97, CI = 0.85 – 1.10, I^2 = 0% and *p* for heterogeneity = 0.819).

Four trials allocated brain-dead patients to receive intravenous triiodothyronine or placebo and used cardiac index as outcome (9, 10, 38, 39). No differences in cardiac index were found between groups (difference between groups: 0.15, CI = -0.13 – 0.42 L/min/m², I^2 = 17.4% and *p* for heterogeneity = 0.304; Figure 2B). Funnel plot analysis did not show significant publication bias for the triiodothyronine intervention.

Figure 2C depicts the meta-analysis of two RCTs (34, 35) that assessed the effects of ischemic liver preconditioning during the donor harvesting procedures. No differences were observed in patient survival at 24-25 months (RR = 1.00, CI = 0.93 – 1.08, I^2 = 0% and *p* for heterogeneity = 0.459).

Discussion

Aggressive donor management protocols must rely on strong pathophysiological evidence. However, the present systematic review and meta-analyses did not find consistent support for recommending such strategies, especially hormonal replacement.

It has been suggested that the hemodynamic instability associated with brain death is in part a result of diminished levels of circulating thyroxine, leading to a reduction of myocardial energy stores and a shift from aerobic to anaerobic metabolism (7). Experimental studies demonstrated improved cardiac function following thyroid hormonal replacement therapy in brain-dead baboons (40). Many retrospective analyses suggest that thyroid hormonal replacement could improve cardiac function and increase the number of organs transplanted per donor (8, 41, 42). We retrieved six RCTs designed to evaluate the effects of triiodothyronine replacement to organ donors, and all of them had consistent negative results. Moreover, the pooled analysis of four RCTs (n=209 patients) evaluating cardiac index as outcome turned out to be negative. Another recent meta-analysis has evaluated the effect of triiodothyronine replacement on donor heart function, and no benefit was found (43). These inconsistent findings suggest that depletion of triiodothyronine (and the subsequent relative hypothyroid state) is not the major determinant of myocardial dysfunction in these patients, but rather perhaps only an adaptive response to illness. Similarly, the evidence in favor of the administration of triiodothyronine in another setting of adaptive relative hypothyroidism, that is, critical care, is far from compelling, to the point that some authors advise withholding its use in critically ill patients unless there is clear evidence of previous hypothyroidism (44).

The complex hemodynamic dysfunction related to brain death is frequently associated with major complications in the potential donor, and has multiple causes (1). Diabetes insipidus may be present in up to 80% of these patients, with severe

dehydration and hypovolemia (11). The use of desmopressin was not associated with better kidney graft outcomes in the present meta-analysis (36, 37), but it is safe and useful to limit the harmful effects of profuse polyuria, decreasing the need for large volume infusions and preventing hemodynamic collapse (45). Studies have suggested the use of colloid solutions as an option to avoid the infusion of large volumes to treat hypovolemia, since fluid overload could be deleterious to lung grafts (46). The only two RCTs retrieved by us dealing with fluid replacement did not support this notion. On the contrary, one study showed no difference between treatment groups of liver donors when hydroxyethyl starch was used compared to crystalloid solutions. The other study was terminated early because of harm to immediate renal function of kidney recipients (24, 31). To this point, there is no evidence supporting the use of hydroxyethyl starch in brain-dead or other critical care patients (47). Vasodilation and hypotension are almost always present in these patients, but no high level evidence for the choice of one or another vasopressor agent is available. Donor treatment with dopamine resulted in a reduction in dialysis requirement after kidney transplantation, with no clinically significant impact on graft or patient survival (33). This fact, taken together with the results of a recent meta-analysis of septic shock patients, would advise in favor of norepinephrine, because dopamine was associated with greater mortality and higher incidence of arrhythmias when compared to norepinephrine in this study (48).

Marginal livers, which have been used to increase the donor pool, are especially susceptible to ischemia-reperfusion injury. Ischemic preconditioning during harvesting is a strategy to prevent the deleterious effects of ischemia-reperfusion on the liver graft, probably by modulating the inflammatory response (49). Compared with standard orthotopic liver transplant, this strategy is associated with better tolerance to ischemia, but with no significant difference on patient survival (34, 35).

Brain death is associated with a profound pro-inflammatory process. Under these circumstances, a beneficial role of steroids could be expected, as suggested by retrospective studies (42, 50). However, RCTs using methylprednisolone in brain-dead donors contradict this hypothesis. Methylprednisolone neither increased lung yield (13), nor improved kidney function post-transplantation (23). The only positive effect was observed for a surrogate outcome: downregulation of inflammatory and apoptotic markers in liver biopsies (29). As brain-dead donors might have a relative adrenal insufficiency (51), another potential benefit of corticosteroid use is promotion of hemodynamic stability. However, only methylprednisolone, which lacks significant mineralocorticoid activity, has been evaluated in RCTs. The use of hydrocortisone or even fludrocortisones may result in better outcomes and should be evaluated in future RCTs.

The best evidence in the management of organ donor refers to mechanical ventilation. The use of lung protective strategies with low tidal volumes increases the yield of lungs when compared to conventional ventilatory strategies (30). High tidal volumes are known to be detrimental to patients with acute lung injury (52, 53), and prevention of overdistension seems to be beneficial to potential lung donors.

This study has several limitations. First, there are few RCTs dealing with the management of brain-dead donors. Second, the general quality of these studies was considered low according to GRADE guidelines (19), raising the possibility of bias. Third, the end points of various trials differed, and many of them had evaluated only surrogate hemodynamic end points as their primary outcomes, such as hemodynamic parameters and initial organ function. Besides, studies had reported no major-treatment-associated adverse events, raising the question if they were properly evaluated. However, similar results were obtained in a systematic review and meta-analysis

focusing specifically on clinical trials of thyroid hormone administration to brain-dead potential organ donors (43), which concludes that the use of thyroid hormone in marginal donors is based on low-level evidence.

It should be noted that the interventions found in this study to be ineffective to increase patient or organ survival when used alone are nevertheless recommended by international guidelines (1, 5, 14, 16, 54). However, we believe that a multi-intervention strategy protocol conducted by a dedicated senior physician at the bedside could produce more favorable outcomes. An early combined strategy holding the best choice of fluids, vasopressor drugs, mechanical ventilation parameters, surgical techniques and combined hormonal replacement therapy should be tested in a well-designed clinical trial.

In summary, despite the implementation of aggressive donor care protocols focusing on hemodynamic and hormonal resuscitation by many transplantation centers and critical care societies, these recommendations are weakly supported, with most evidence based on surrogate outcomes and retrospective data. We recognize the great importance of brain-dead donor care to improve transplantation outcomes, but this systematic review and meta-analysis did not provide consistent evidence for recommending this strategy. Therefore, further RCTs are required to elucidate to what extent a multi-intervention management strategy of brain-dead donors is helpful for transplant recipients.

Contributors

T.H.R participated in the study conception and design, data acquisition, analysis, and interpretation of data, drafting of the manuscript and revision of the manuscript. R.B.M participated in data acquisition, analysis, and interpretation, drafting of the

manuscript and revision of the manuscript. D.C. and M.A.C. critically reviewed the manuscript for intellectual content. C.B.L participated in the study conception and design, analysis and interpretation of data, revision of the manuscript and statistical analysis.

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Table 1. Summary of Randomized Controlled Trials of Interventions for Care of Brain-Dead Organ Donors

Intervention, source and number of patients	Treatment groups	Control group	Outcome	Specific outcome	Organ function post TX	Survival
ADH						
Iwai et al, 1989 (28) - 25 patients	Group 1: epinephrine for SBP > 100 mmHg Group 2: ADH 0.1-0.4 U/hr Group 3: ADH 1-2 U/hr + epinephrine	Epinephrine	Donor survival	Hours to death	No	No
Pennefather et al, 1995 (31)* - 24 patients	ADH 300 $\mu U.kg^{-1}min^{-1}$	Placebo	Hemodynamic parameters	CI, MAP, pressor dose	Yes	Patient
Guesde et al, 1998 (32)* - 97 patients	ADH 1 μg 2/2h when diuresis > 300 mL/h	No treatment	Renal function	Hemodialysis requirement, creatinine D1 to D15	Yes	Graft
Methylprednisolone						
Chatterjee et al, 1977 (24) - 50 patients	Methylprednisolone 5 g prior to harvesting	No treatment	Renal function	Graft failure at 1 and 3 months	Yes	Graft
Kotsch et al, 2008 (25) - 100 patients	Methylprednisolone 250 mg bolus + 100 mg/h until recovery of organs	No treatment	Liver function	ALT/AST/BB D1 and D10	Yes	Graft
Venkateswaran et al, 2008 (9) - 60 patients	Methylprednisolone 1 g	Placebo	Lung function and suitability	PaO_2/FiO_2 ratio	No	No

Triiodothyronine						
Mariot et al, 1991 (14)* - 40 patients	1/1h or 0.5/0.5h: T_3 2 or 4 µg and hydrocortisone 100 mg	Placebo	Hemodynamic parameters; organ retrieval	CI, MAP, pH pre and post protocol	No	No
Randell et al, 1992 (26) - 25 patients	T_3 2 µg/h started immediately before surgery	No treatment	Hemodynamic parameters; liver function	Maximum ALT/AST/BB to D7, pressor dose	Yes	No
Goarin et al, 1996 (13)* - 37 patients	T_3 0.2 µg/kg bolus	Placebo	Hemodynamic parameters; heart function	CI, LVF, MAP pre and post protocol	No	No
Jeevanandam et al, 1997 (27) - 30 patients	T_3 0.6 µg/kg bolus	Placebo	Hemodynamic parameters; kidney function	Creatinine, pressor dose	Yes	Graft
Perez-Blanco et al, 2005 (33)* - 52 patients	T_3 1 µg/kg bolus + 0.06 µg/kg/h	Placebo	Hemodynamic parameters	CI, CO2 gap and lactate pre and post protocol	No	No
Venkateswaran et al, 2009 (34)* - 80 patients	T_3 0.8 µg/ kg bolus + 0.113 µg/kg/h and/or methylprednisolone 1000 mg bolus	Placebo	Hemodynamic parameters	CI, LVSWI, SVR pre and post protocol	No	No
Fluid management-hydroxyethyl starch						
Randell et al, 1990 (22) - 16 patients	Hydroxyethyl starch according to hemodynamic data	Crystalloid	Hemodynamic parameters; liver function	HR, MAP, electrolyte balance	Yes	No
Cittanova et al, 1996 (23) - 27 patients	Hydroxyethyl starch up to 33 mL/kg	Gelatin	Kidney function	Creatinine at D10, hemodialysis requirement	No	No

Vasopressor- dopamine						
Schnuelle et al, 2009 (21) - 264 patients	Dopamine 4 μg/kg/m	No treatment	Renal function	Hemodialysis requirement	Yes	Graft and Patient
Surgical technique- ischemic preconditioning						
Azoulay et al, 2005 (35)* - 91 patients	10 min of ischemic liver preconditioning before harvesting	Conventional technique	Liver function	ALT/AST D5 and D10, BB D7 and D15, PT D3-D15	Yes	Patient
Amador et al, 2007 (36)* - 60 patients	10 min of ischemic liver preconditioning before harvesting	Conventional technique	Liver function	ALT/AST D1-D10, apoptosis	Yes	Graft and patient
Donor harvesting technique						
D'Amico et al, 2007 (30) - 35 patients	Modified double perfusion technique	Conventional technique	Liver function	ALT/AST/BB/PT D2 and D7	Yes	Graft and patient
Preservation solution (Euro-Collins)						
Cofer et al, 1992 (29) - 56 patients	Euro-Collins solution	University of Wisconsin solution	Liver function	ALT/AST/BB/PT	Yes	Patient
Mechanical ventilation						
Mascia et al, 2010 (20) - 118 patients	Lung protective strategy	Conventional ventilator strategy	Lung function and suitability	PaO_2/FiO_2 ratio, pH, MAP	No	Patient

ADH: desmopressin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BB: bilirubin; CI: cardiac index; HR: heart rate; LVF: left ventricular function; LVSWI: left ventricular stroke work index; MAP: mean arterial pressure; PT: prothrombin time; SVR: systemic vascular resistance; TX: transplantation. * Trials included in meta-analysis.

Table 2. Risk of bias in studies

Source	Adequate sequence generation	Allocation concealment	Blinding	Complete organ function data addressed	Complete survival outcome data addressed
ADH					
Iwai et al, 1989 (28)	Unclear	Unclear	No	NM	NM
Pennefather et al, 1995 (31)*	Unclear	Unclear	No	No	No
Guesde et al, 1998 (32)*	Yes	Yes	Yes	No	No
Methylprednisolone					
Chatterjee et al, 1977 (24)	Yes	Yes	Yes	No	No
Kotsch et al, 2008 (25)	Yes	Yes	Yes	No	Unclear
Venkateswaran et al, 2008 (9)	Yes	Yes	Yes	NM	NM
Triiodothyronine					
Mariot et al, 1991 (14)*	Yes	No	Yes	NM	NM
Randell et al, 1992 (26)	Unclear	Unclear	No	No	NM
Goarin et al, 1996 (13)*	Yes	Unclear	Yes	NM	NM
Jeevanandam et al, 1997 (27)	Unclear	Unclear	Yes	No	No
Perez-Blanco et al, 2005 (33)*	Unclear	Unclear	Yes	NM	NM
Venkateswaran et al, 2009 (34)*	Yes	Yes	Yes	NM	NM
Fluid management-hydroxyethyl starch					
Randell et al, 1990 (22)	Unclear	Unclear	No	No	NM
Cittanova et al, 1996 (23)	Unclear	Unclear	Yes	NM	NM
Vasopressor- dopamine					
Schnuelle et al, 2009 (21)	Yes	Yes	No	No	NM

Surgical technique-ischemic preconditioning					
Azoulay et al, 2005 (35)*	No	No	No	No	No
Amador et al, 2007 (36)*	Yes	Yes	No	No	No
Donor harvesting technique					
D'Amico et al, 2007 (30)	Yes	Yes	No	No	No
Preservation solution (Euro-Collins)					
Cofer et al, 1992 (29)	Unclear	Unclear	No	No	No
Mechanical ventilation					
Mascia et al, 2010 (20)	Yes	Yes	No	NM	No

ADH: desmopressin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BB: bilirubin; CI: cardiac index; HR: heart rate; LVF: left ventricular function; LVSWI: left ventricular stroke work index; MAP: mean arterial pressure; NM: not measured; PT: prothrombin time; SVR: systemic vascular resistance; TX: transplantation.

* Trials included in meta-analyses.

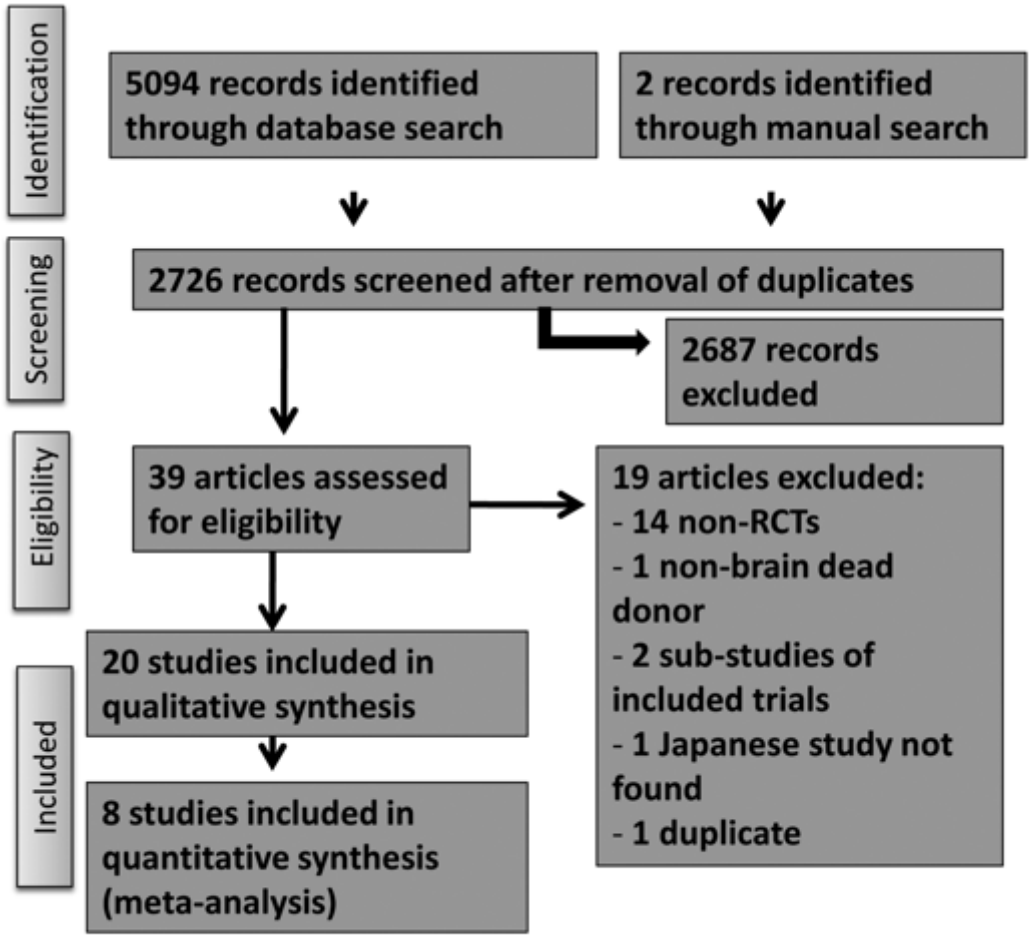

Figure 1. Flowchart summarizing search strategies and selection of trials.

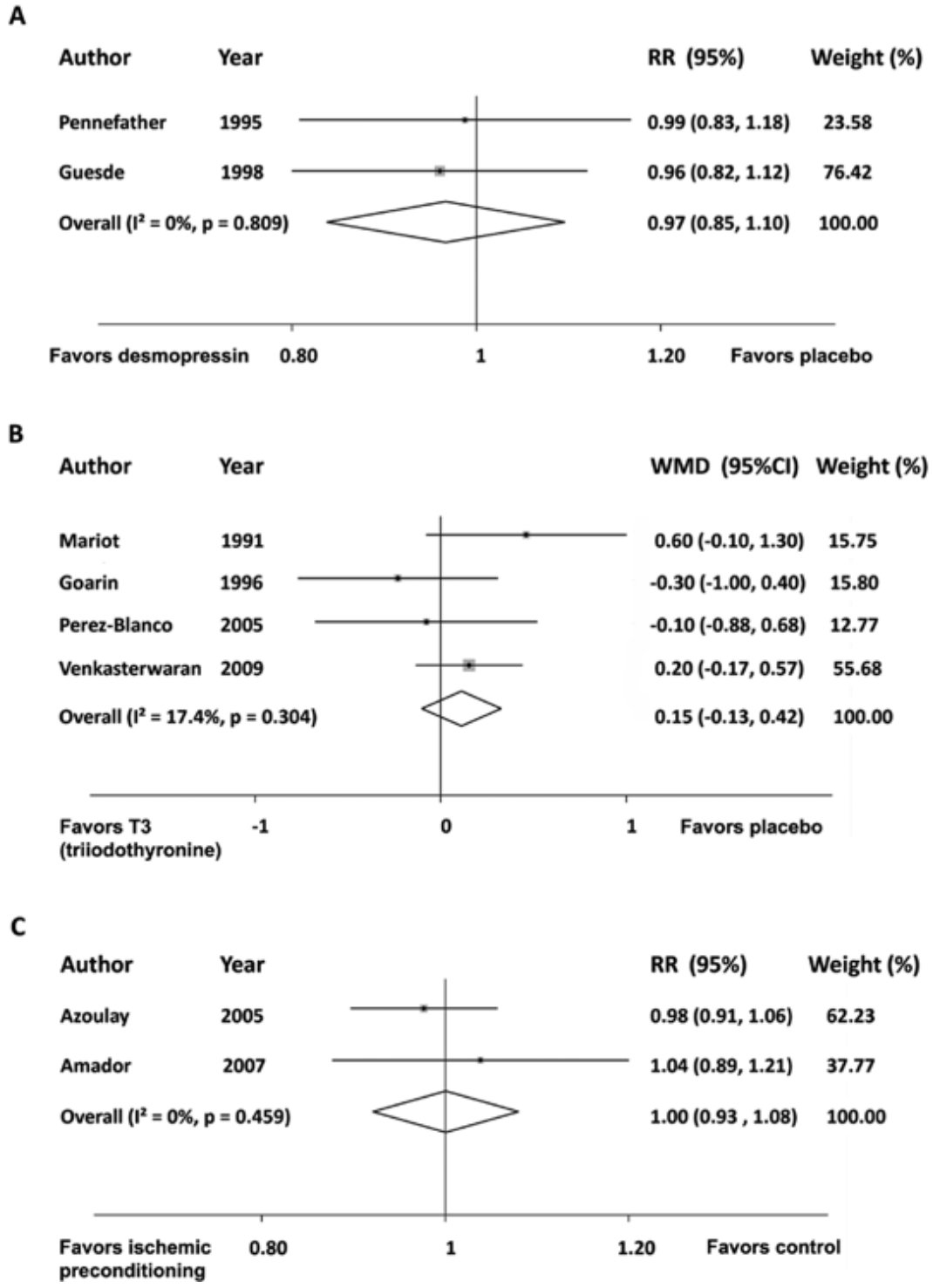

Figure 2. A. Forest plot comparing the effects of desmopressin versus placebo on early graft function in kidney recipients. B. Forest plot comparing the effects of triiodothyronine versus placebo on cardiac index in donor hearts. C. Forest plot comparing the effects of liver preconditioning during harvesting procedures with conventional technique on patient survival. WMD = weighted mean difference.

CAPÍTULO 3

Brain death-induced inflammatory and procoagulant activity in human pancreatic tissue: a case-control study

Short title: Brain death-induced human pancreatic tissue inflammation

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Key words: brain death; cytokines; thromboplastin; islets of Langerhans

Abstract

Long-term insulin independence after islet transplantation depends on engraftment of a large number of islets. However, the yield of islet cells from brain-dead donors is negatively affected by the up-regulation of inflammatory mediators. Brain death (BD) is also believed to increase the expression of tissue factor (TF), which further contributes to a low rate of islet cell engraftment. We conducted a case-control study to assess BD-induced inflammatory and thrombotic effects in human pancreatic tissue.

Seventeen brain-dead patients and 20 control patients undergoing pancreatectomy were included in the study. Serum TNF-α, IL-6, IL-1β, IFN-γ, and TF were measured using commercial ELISA kits. Gene expressions of these cytokines and *TF* were evaluated by RT-qPCR on pancreatic samples. Protein quantification was performed by immunohistochemistry in paraffin-embedded pancreas sections.

Brain-dead patients had higher serum concentrations of TNF-α and IL-6 in comparison to controls. The groups had similar TNF-α, IL-6, IL-1β, and IFN-γ mRNA levels in pancreatic tissue. RT-qPCR revealed significant up-regulation of *TF* mRNA expression in control patients. Immunohistochemical analyses showed that brain-dead patients had increased TNF-α protein levels compared to controls.

BD induces profound inflammatory derangements that are evidenced by the up-regulation of TNF-α in serum and pancreatic tissue. Blocking the expression of key inflammatory mediators in brain-dead donors should be evaluated as a new approach to improve the outcomes of islet transplantation.

Introduction

Type 1 diabetes mellitus is characterized by severe insulin deficiency resulting from progressive destruction of pancreatic beta-cells by the immune system (1). Islet transplantation is an effective therapy for unstable type 1 diabetes mellitus (2, 3). However, restoration of β-cell function after transplantation and the ensuing improvement in glycemic control (4) depend on a high rate of islet engraftment, which is hindered by the marked destruction of islets during the transplantation process (5) as a consequence of pancreas preservation, isolation procedures (6), and donor and recipient characteristics (7). Even if important benefits are conferred by partial function (8, 9), the ultimate goal of insulin independence after islet transplantation still requires infusion of a large number of islets, and therefore multiple donors (10).

Previous studies have reported that the source of transplants, from either brain dead or living donors, impacts graft survival rate (11, 12). Jung *et al* showed a better yield of islet cell mass from living pancreatic donors compared with brain-dead donors (13). Animal and human studies suggest that BD causes the release of potent pro-inflammatory cytokines (14-17) and procoagulant mediators (18, 19) into the circulation, with potential deleterious effects on transplanted organs (20). Contreras *et al* demonstrated that BD-associated up-regulation of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) significantly reduces isolated islet yield, viability, and function after transplantation in rats (21). Therefore, it has been suggested that cytokine blockade could enhance islet engraftment (3, 22).

Recently, expression of tissue factor (TF), a glycoprotein implicated in extrinsic and intrinsic coagulation pathways, has been reported in the islets of Langerhans (23). Infusion of TF-expressing islets into the portal vein triggered an instant blood-mediated inflammatory reaction (IBMIR) characterized by rapid binding of platelets to islet

surface as the islets come into direct contact with ABO-compatible blood (24, 25). TF up-regulation was demonstrated in a rat model of BD, suggesting that BD could stimulate TF expression (18).

To the best of our knowledge, there are no studies focusing on BD-induced inflammation in human pancreatic tissue. The aim of this case-control study was therefore to assess the inflammatory and procoagulant effects induced by BD in human pancreatic tissue.

Patients and Methods

Brain-dead patients and controls

The study protocol was approved by the research ethics committee at Hospital de Clínicas de Porto Alegre. Informed consent was obtained from control patients or from the next of kin in the case of brain-dead individuals. BD was assessed independently by two physicians, according to Brazilian law (26), and was based on the following criteria: coma with complete unresponsiveness, including absence of all brain stem reflexes, apnea test, and confirmation image with absence of cerebral blood flow. All brain-dead patients (n=17) older than 18 years and not accepted for pancreas donation were prospectively included in the study from November 2010 to December 2011. Pancreas was procured during multiorgan harvest for organ transplantation, stored in histidine-tryptophan-ketoglutarate (HTK, Custodiol®) preservation solution and immediately transferred to the islet isolation laboratory, where a $2cm^2$ biopsy was taken. A $2cm^2$ pancreas biopsy was also obtained from control patients (n=20) during partial or total pancreatectomy for the treatment of underlying lesions (mainly periampullary tumors). Control patients were prospectively enrolled during the same period as cases.

Clinical and biochemical data were recorded at the time of BD diagnosis or on the day before surgery for controls.

Serum TNF-α, IL-6, IL-1β, IFN-γ and TF determinations

A 20-mL whole blood sample was collected in silicone-coated tubes (Vacutainer) at the time of surgical procedures for all patients, and was centrifuged at 2500*g* for 10 min at 4°C. Serum was separated and immediately stored at -20°C until analysis. Circulating levels of TNF-α, IL-6, IL-1β, interferon-γ (IFN-γ), and TF were assessed by enzyme-linked immunosorbent assay (ELISA) using commercially available kits with primary polyclonal antibodies following the manufacturer's recommendations (Biosource Europe S.A., Nivelles, Belgium).

TNF-α, IL-6, IL-1β, IFN-γ, and TF mRNA isolation and quantification by RT-qPCR

Pancreatic tissue biopsies were excised, snap-frozen in liquid nitrogen and stored at -80°C until use. Pancreatic tissue (120mg) was homogenized in phenol-guanidine isothiocyanate (Invitrogen Life Technologies, Carlsbad, CA). RNA was extracted with chloroform and precipitated with isopropanol by centrifugation (12,000g) at 4°C. RNA pellet was washed twice with 75% ethanol and re-suspended in 10-50µl of water treated with diethylpyrocarbonate. The concentration and quality of total RNA samples were assessed using a NANODROP 2000 spectrophotometer (Thermo Scientific Inc., Newark, DE). Only RNA samples with adequate purity ratios (A260/A280=1.9-2.1) were used for subsequent analyses (27). In addition, RNA integrity and purity was also checked on agarose gel containing GelRed™ Nucleic Acid Gel Stain (Biotium Inc., Hayward, CA). The mean RNA concentration isolated was 800µg/120mg pancreatic tissue.

Real-time reverse transcription-PCR was performed in two separate reactions. First, RNA was reverse-transcribed into cDNA. cDNA was then amplified by quantitative real-time PCR (RT-qPCR). Reverse transcription of 1μg of RNA into cDNA was carried out using the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies) following the manufacturer's protocol for the oligo (dT) method (28-31).

RT-qPCR experiments were performed in a Vii™ 7 Fast Real-Time PCR System Thermal Cycler with Vii™ 7 Ruo Software (Life Technologies, Foster City, CA). Experiments were performed by real-time monitoring of the increase in fluorescence of SYBER® Green dye (Life Technologies) (32). Primer sequences for *TNF-α*, *IL-6*, *IL-1β*, *IFN-γ*, *TF*, and *β-actin* genes were designed using Primer Express 3.0 Software (Life Technologies) and are depicted in Table 1. PCR reactions were performed using 5μl of 2x Fast SYBER® Green Master Mix (Life Technologies), 0.5μl (1ng/μl) of forward and reverse primers for *TNF-α*, *IL-6*, *IL-1β*, *IFN-γ*, *TF*, or *β-actin* and 1μl of cDNA template (5μg/μl), in a total volume of 10μl. Each sample was assayed in triplicate and a negative control was included in each experiment. Thermocycling conditions for these genes were as follows: initial cycle of 95^{o}C for 5s and 60^{o}C for 1min10s. RT-qPCR specificity was determined using melting curve analyses. All primers generated amplicons that produced a single sharp peak during the analyses.

Quantification of *TNF-α* mRNA was performed using the relative standard curve method (Life Technologies) (27) and the *β-actin* gene as reference. Relative standard curves were generated for both target and reference genes by preparing serial dilutions of a pool of cDNA samples with a known relative quantity. Then, relative amounts of each *TNF-α* mRNA sample were obtained by normalizing their signal by those of *β-actin*. Results are represented as arbitrary units (AU). Relative quantification of *IL-6*,

IL-1β, IFN-γ and *TF* cDNA was performed using the comparative ΔΔCq method (27, 33). Quantities were expressed relative to the reference gene (*β-actin*). Validation assays were done by separate amplification of the target (*IL-6*, *IL-1β*, *IFN-γ* and *TF*) and reference (*β-actin*) genes, using serial dilutions of a pool of cDNA samples. As a requirement of this method, both target and reference genes exhibited equal amplification efficiencies (E=95% to 105%) in all experiments. The ΔΔCq method calculates changes in gene expression as relative fold difference (n-fold changes) between an experimental and external calibrator sample (27, 33).

Immunohistochemistry for TNF-α, IL-6, IL-1β, IFN-γ, and TF proteins in human pancreatic tissue

TNF-α, IL-6, IL-1β, IFN-γ and TF protein distributions and intensities were determined by immunohistochemistry in formalin-fixed, paraffin-embedded pancreas sections. Anti-TNF-α, anti-IL-6, anti-IL-1β, anti-IFN-γ, and anti-TF rabbit polyclonal antibodies (Santa Cruz Biotechnology, Inc.) were used to detect TNF-α, IL-6, IL-1β, IFN-γ, and TF protein expression in human pancreatic tissue, with intestine and placenta used as positive controls. Immunohistochemical analyses were performed on 4-μm pancreas sections. Routine immunohistochemical techniques comprised deparaffination and rehydration, antigenic recovery, inactivation of endogenous peroxidase, and blocking of non-specific reactions. Slides were incubated with primary antibody and then with a biotinylated secondary antibody, streptavidin horseradish peroxidase conjugate (LSAB; Dako Cytomation Inc., Carpinteria, CA), and diaminobenzidine tetrahydrochloride (kit DAB; Dako Cytomation Inc., Carpinteria, CA). Quantifications of TNF-α, IL-6, IL-1β, IFN-γ, and TF proteins were performed by digital image analyses using Image Pro Plus software, version 4.5 (Media Cybernetics,

Bethesda, MD). Images were visualized through a Zeiss microscope (model AXIOSKOP-40; Carl Zeiss, Oberkochen, Germany) and captured using the Cool Snap-Pro CS (Media Cybernetics, Bethesda, MD, USA) camera in a blinded fashion. Two independent blinded investigators (T.H.R and S.S.B) analyzed the intensity of brownish-colored immunostaining in pixels in 10 fields from each slide. A Pearson correlation of r^2=0.902 (P< 0.001) was obtained for the two analyses. The mean number of pixels identified by the two investigators was used to quantify TNF-α, IL-6, IL-1β, IFN-γ, and TF protein expression in each sample. Paraffin-embedded pancreas sections from 10 adult pancreatic necropsies performed during the study period were used as additional controls for TF protein expression, since pancreatic cancer may induce TF expression (34), which would make our controls unsuitable for this marker.

Statistical Analysis

Variables with normal distribution are presented as mean ± SD. Variables with skewed distribution were log-transformed before analysis and are presented as median and interquartile intervals. Categorical variables are presented as percentages. Serum TNF-α, IL-6, IL-1β, IFN-γ, and TF levels and mRNA and protein expressions were compared between the groups using Student's *t* test. Statistical significance of differences in TF protein expression in brain-dead individuals, controls, and necropsies was determined by one-way ANOVA with Bonferroni-Dunn post hoc test. Pearson's test was used to assess correlations between different quantitative variables. A multiple linear regression was performed in order to adjust TNF-α expression for possible confounding factors. Values were considered statistically significant if P<0.05. All statistical analyses were performed using SPSS 18.0 (Chicago, IL).

Results

The characteristics of 17 brain-dead patients and 20 control patients included in the study are summarized in Table 2. Periampullary tumors were the indication for pancreatectomy in the control group, except in one patient with a cystic tumor. Two control patients were excluded after their biopsies revealed severe chronic pancreatitis. Severe spontaneous intracranial hemorrhage was the cause of BD in 13 patients and cardiac arrest resulting in severe anoxic encephalopathy in 4 patients. Fifty-six organs were retrieved for transplantation (2 hearts, 4 lungs, 16 livers, and 34 kidneys). Age, body mass index (BMI), plasma glucose or glycated hemoglobin (HbA_{1C}) did not differ significantly between the groups. Brain-dead patients were mostly men, while controls were mainly women. As expected, brain-dead patients spent more hours on mechanical ventilation, had more episodes of cardiac arrest and persistent hypotension, and more frequently developed hypernatremia and low platelet levels than controls. Desmopressin and steroids were used as part of the care of potential organ donors in 10 patients.

Serum TNF-α, IL-6, IL-1β, IFN-γ, and TF quantifications by ELISA

Blood samples and pancreas biopsies were obtained at different time points in brain-dead patients and controls (median 12 hours [10-18] vs. 3.5 hours [2.6-4], respectively; P<0.001). As shown in Fig. 1, brain-dead patients had higher concentrations of TNF-α (12.03 pg/mL [6.2-23.6] vs. 3.8 pg/mL [3.4-6.7]; P=0.005) and IL-6 (1127.1 pg/mL [335.7-4571.6] vs. 77.4 pg/mL [48.1-186.6]; P<0.00001) in comparison to controls. Serum IL-1β, IFN-γ and TF were similar in brain-dead patients and controls (IL-1β 0.1 pg/mL [0.1-92.2] vs. 0.1 pg/mL [0.1-0.1], P=0.516; IFN-γ 0.03 pg/mL [0.02-0.05] vs. 0.03 pg/mL [0.02-0.03], P=0.128; TF 126.8 pg/mL [80.6-291.4] vs. 76.9 pg/mL [63.6-102.5], P=0.170).

TNF-α, IL-6, IL-1β, IFN-γ, and TF mRNA quantification in human pancreatic tissue by RT-qPCR

Real-time qPCR analysis was performed in 35 pancreatic biopsies (17 brain-dead patients and 18 controls), as shown in Fig. 2. *TNF-α, IL-6*, *IL-1β* and *IFN-γ* mRNA levels in pancreatic tissue were similar in the two groups (*TNF-α* brain-dead 4.65 A.U [1.4-8.7] vs. controls 2.53 A.U [2.0-9.1], P=0.875; *IL-6* brain-dead 0.47-fold [0.06-1.2] vs. controls 0.58-fold [0.03-1.5], P=0.887; *IL-1β* brain-dead 0.16-fold [0.03-1.2] vs. controls 0.06-fold [0.001-3.1], P=0.148 and *IFN-γ* brain-dead 0.54-fold [0.04-2.4] vs. controls 0.94-fold [0.3-1.6], P=0.330). For *TF* analysis, we excluded three control patients considered to have advanced malignant disease. Unexpectedly, RT-qPCR revealed significant up-regulation of *TF* mRNA expression in control patients (brain-dead 0.39-fold [0.1-1.2] vs. control 1.38-fold [0.7-2.0], P=0.049).

TNF-α, IL-6, IL-1β, IFN-γ, and TF protein immunohistochemistry quantifications in human pancreatic tissue

Immunohistochemical studies were conducted to quantify protein expression of BD-induced inflammatory cytokines and procoagulant activity in human pancreatic tissue (Table 3).

Immunohistochemical analysis showed that brain-dead patients had increased TNF-α protein expression compared to controls (16.81 ± 5.2 pixels vs. 11.57 ± 4.93 pixels; P<0.005), in agreement with the results obtained for serum TNF-α levels (r=0.451; P=0.014). Moreover, TNF-α protein was widely distributed in all pancreatic tissue, including islets (Figure 3). After controlling for possible confounding factors identified in the univariate analysis (sex, ventilation support, and vasopressor support),

BD remained independently associated with TNF-α protein up-regulation (beta= 7.64 [CI 0.81-14.48]; P=0.030).

IL-6 and IL-1β proteins were identified in both ductal cells and islet cells, but no significant differences were observed between cases and controls (IL-6 15.65 ± 6.3 pixels vs. 19.89 ± 9.25 pixels, P=0.132; IL-1β 12.89 ± 6.2 pixels vs. 11.87 ± 8.02 pixels, P=0.683). This indicates that IL-6 and IL-1β expression in human pancreatic tissue was not affected by BD, as also demonstrated by mRNA quantification.

Staining of human pancreas sections with polyclonal antibodies against IFN-γ and TF showed that IFN-γ and TF were minimally present in pancreatic tissue. The mean ± SD TF protein concentration for the entire pancreatic tissue was 15.71 ± 9.53 pixels. Moreover, islets of Langerhans were not stained, indicating no expression of TF in the endocrine pancreas. Because an increase in TF mRNA expression by RT-qPCR was observed in control patients compared to brain-dead patients, we also used 10 pancreas sections from necropsies as a second set of controls, and no significant differences were found among groups (brain-dead 15.63 ± 9.6 vs. control 16.39 ± 8.32 vs. necropsies 14.7 ± 12.01, P= 0.909).

Discussion

In this study, BD was associated with systemic inflammation, as demonstrated by an increase in TNF-α and IL-6 serum levels, as well as pancreatic inflammation, reflected by the up-regulation of TNF-α protein expression in pancreatic tissue of brain-dead subjects as compared to controls. BD-associated increases in IL-1β, IFN-γ or TF were not detected.

It is well known that BD has a negative non-immunological effect on organ function (11, 35). Understanding the mechanisms by which BD affects pancreatic

function is of great interest to islet transplantation. To the best of our knowledge, the present case-control study was the first to show that damage to human pancreatic tissue is caused by BD before other stress factors related to isolation and transplantation procedures are triggered. The increase in TNF-α in the pancreatic tissue of brain-dead patients prior to islet isolation and transplantation suggests that BD itself determines the onset of the inflammatory response detected in human pancreas. These results are in accordance with the results of Birks *et al*, who compared the expression of TNF-α in myocardial biopsies of brain-dead and living donors immediately before transplantation, showing that the increase in TNF-α in cadaveric donors was a consequence of BD (36). Moreover, the outcomes of islet transplantation seem to have improved since the soluble TNF receptor antagonist etanercept was introduced as part of immunosuppression protocols (3, 22), suggesting that TNF-α may be a relevant factor damaging the islets. In fact, TNF-α induces nuclear factor-κB (NF-κB) activation, a pro-apoptotic mechanism contributing to pancreatic beta cell death in type 1 diabetes (37, 38), while blockage of NF-κB activation protects beta cells against TNF-α-induced apoptosis (39). In islet transplantation, TNF-α may act at different points in the process, leading to pathological signs of islet cell death: at the tissue level, before pancreas recovery, as demonstrated by us, during isolation procedures, as a consequence of cytokine production by islets (40), and at the graft site, as part of recipient inflammatory response (41).

IL-1β induces endothelial activation, promoting leukocyte interactions with adhesion molecules expressed on the cell surface (42). Moreover, IL-1β and IFN-γ induce beta cell apoptosis via activation of beta cell gene networks under the control of NF-κB and STAT-1 (43). In our study, IL-1β and IFN-γ were equally distributed in blood and pancreas tissue from brain-dead and control patients, suggesting that BD was not linked to the release of these cytokines. These results differ from those obtained

with an animal model of BD, which demonstrated an intense IL-1β response in serum and myocardium of rats (42). Because our study was the first study to evaluate the behavior of IL-1β after BD in humans, we believe this might be reflecting a difference between species. Weiss *et al* showed that IFN-γ mRNA expression was consistently higher in liver biopsies from brain-dead donors compared to living donors at the time of donor laparotomy (44), a finding not observed by us.

Contreras *et al* also reported the presence of a pro-inflammatory state shortly after the induction of BD in rats, as demonstrated by the up-regulation of cytokines, including TNF-α, IL-1β, and IL-6, in serum and pancreatic tissue (21). Our study corroborates the up-regulation of TNF-α in human serum and pancreatic tissue. However, although IL-6 was increased in serum, IL-6, IL-1β, and IFN-γ were not expressed in pancreatic tissue. A possible explanation for these differences lies on the fact that, in comparison to that previous study, our samples were collected later after the diagnosis of BD. However, it could also be that the difference is merely reflecting the unique characteristics of different species.

Also, differently from experimental models (18, 19), we did not find an increase in TF in human pancreas from brain-dead patients. Furthermore, our results demonstrated an increase in *TF* mRNA in controls, but this increase was not confirmed at the protein level, suggesting that *TF* gene expression undergoes an important post-transcriptional regulation. To investigate these unexpected findings, pancreas sections from necropsies were also studied, and no significant differences in TF expression related to BD were found. We believe that this could be explained by the specific conditions of our controls, since most had malignant tumors, and TF is suspected of being implicated with thromboembolic events in pancreatic cancer (34).

TF expression has been reported in islets in different experimental models (24, 25, 45) as well as in human islet isolates (46). It is also identified as the main trigger of IBMIR, which is one of the possible explanations for the poor engraftment following islet transplantation. So, we focused on the influence of BD on TF expression in human pancreas. However, the present results do not confirm this hypothesis, as BD was not associated with TF increase in pancreatic tissue. Saito *et al* reported similar findings in a rat model of BD in which TF was observed to increase in isolated islets, but not in pancreatic tissue before digestion procedures (18). That suggests that TF expression is triggered by cold ischemia and isolation procedures, rather than BD. We therefore believe that attempts to block TF expression should target the isolated islets and not the brain-dead donor.

This study has some limitations. First, the case-control design prevented us from establishing an ideal control group. Patients diagnosed with severe pancreatitis and advanced malignant tumors were excluded, because they have the potential to up-regulate pro-inflammatory and procoagulant mediators (34, 47). Additionally, a second control group was used in TF analyses. Second, the median time to collection of blood and pancreas samples was different between groups, since the duration of a pancreatectomy is shorter than that needed to complete a multiorgan donation protocol. Nevertheless, this difference might weaken and not strengthen our findings, because cytokines peak early after BD (21). Third, protein quantification by immunohistochemistry was not specifically determined in the islets, but rather in the total slide. However, knowing the exocrine cell-staining pattern is important because islet suspensions prepared for clinical transplantation contain up to 40% of nonendocrine duct cells (48).

In conclusion, our data suggest that brain death itself impacts IL-6 and TNF-α cytokine content before any transplantation procedures. This association of cytokine content and BD can explain, at least in part, the more favorable outcomes of living donation. Randomized controlled trials to test the performance of TNF-α blockers administered to brain-dead donors prior to organ harvesting procedures are therefore warranted.

Contributors

T.H.R participated in the study conception and design, data acquisition, analysis, and interpretation of data, drafting of the manuscript and revision of the manuscript. D.C and C.B.L participated in the study conception and design, analysis and interpretation of data, revision of the manuscript and statistical analysis. J.R, S.S.B, A.B.O, T.J.M.G.F and C.R.P.K participated in data acquisition and revision of the manuscript. J.L.G critically reviewed the manuscript for intellectual content.

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Table 1. Primer sequences used for *TNF-α, IL-6, IL-1β, IFN-γ* and *TF* gene expression analyses.

Sequences	
TNF-α gene [a]	F 5'- CCCAGGGACCTCTCTCTAATCA -3'
	R 5'- GGTTTGCTACAACATGGGCTACA -3'
IL-6 gene [a]	F 5'- AGCCCTGAGAAAGGAGACATGTA -3'
	R 5'- TCTGCCAGTGCCTCTTTGCT -3'
IL-1β gene [a]	F 5'- TGATGTCTGGTCCATATGAACTGAA -3'
	R 5'- GGACATGGAGAACACCACTTGTT -3'
IFN-γ gene [a]	F 5'- CCAACGCAAAGCAATACATGA -3'
	R 5'- TCCTTTTTCGCTTCCCTGTTT -3'
TF gene [a]	F 5'- TGTTCAAATAAGCACTAAGTCAGGAGAT -3'
	R 5'- TCGTCGGTGAGGTCACACTCT -3'
β-actin gene [a]	F 5'- GCGCGGCTACAGCTTCA -3'
	R 5'- CTTAATGTCACGCACGATTTCC -3'

F= forward primer; R= reverse primer.

[a] = Primers were designed using published human gene sequences and the Primers Express 3.0 Software (Life Technologies). They were projected to target two consecutive exons of a gene in order to prevent the amplification of any contaminating genomic DNA.

Table 2. Baseline characteristics of brain-dead patients and controls.

	Brain-dead (n=17)	Controls (n=20)	p value
Cause of brain death or pancreatectomy	13 strokes and 4 anoxic injuries	6 benign tumors and 14 malignant tumors	-
Age (years)	54 ± 11	58 ± 13	0.34
Men (n, %)	12 (70.6)	7 (35)	0.03
BMI [+]	25.9 ± 3.6	24.1 ± 3.6	0.13
Hypothermia (n, %)	2 (11.8)	0	0.115
Ventilation support (hours)	72 (48-114)	6 (5-6.7)	<0.001
Time from BD to pancreas harvest or time to biopsy (hours)	12 (10-18)	3.5 (2.6-4)	<0.001
Episode of cardiac arrest (n, %)	5 (29.4)	0	0.009
Vasopressor support (n, %)	17 (100)	6 (30)	<0.001
Persistent hypotension (n, %)	4 (23.5)	0	0.022
Use of desmopressin (n, %)	10 (58.8)	0	<0.001
Use of steroids (n, %)	10 (58.8)	0	<0.001
Plasma sodium (mEq/L)	158 ± 10	141 ± 3	<0.001
Plasma glucose (mg/dL)	174 ± 51	156 ± 67	0.40
HbA_{1C} (%)	6.2 ± 2.03	5.5 ± 1.42	0,2
Platelet count ($x10^9$/L)	185 ± 66	250 ± 56	0.002
Serum amylase (UI/L)	42 (30-199)	90 (49-114)	0.42
Serum lipase (UI/L)	22 (17-26)	73 (32-171)	0.01

All values were recorded at the time of brain death diagnosis or on the day before surgery for controls.

BMI: body mass index; HbA_{1C}: glycosylated hemoglobin.

+: calculated as weight in kilograms divided by the square of height in meters.

Table 3. TNF-α, IL-6, IL-1β, IFN-γ and TF protein immunohistochemistry quantifications* in human pancreatic tissue.

	Brain-dead (n=17)	Controls (n=18)	Necropsies (n=10)	p value
TNF-α	16.81 ± 5.2	11.57 ± 4.93	-	p<0.005
IL-6	15.65 ± 6.3	19.89 ± 9.25	-	p=0.132
IL-1β	12.89 ± 6.2	11.87 ± 8.02	-	p=0.683
IFN-γ	5.72 ± 3.74	3.99 ± 2.11	-	p=0.107
TF	15.63 ± 9.6	16.39 ± 8.32	14.7 ± 12.01	p=0.909

* Values presented in pixels.

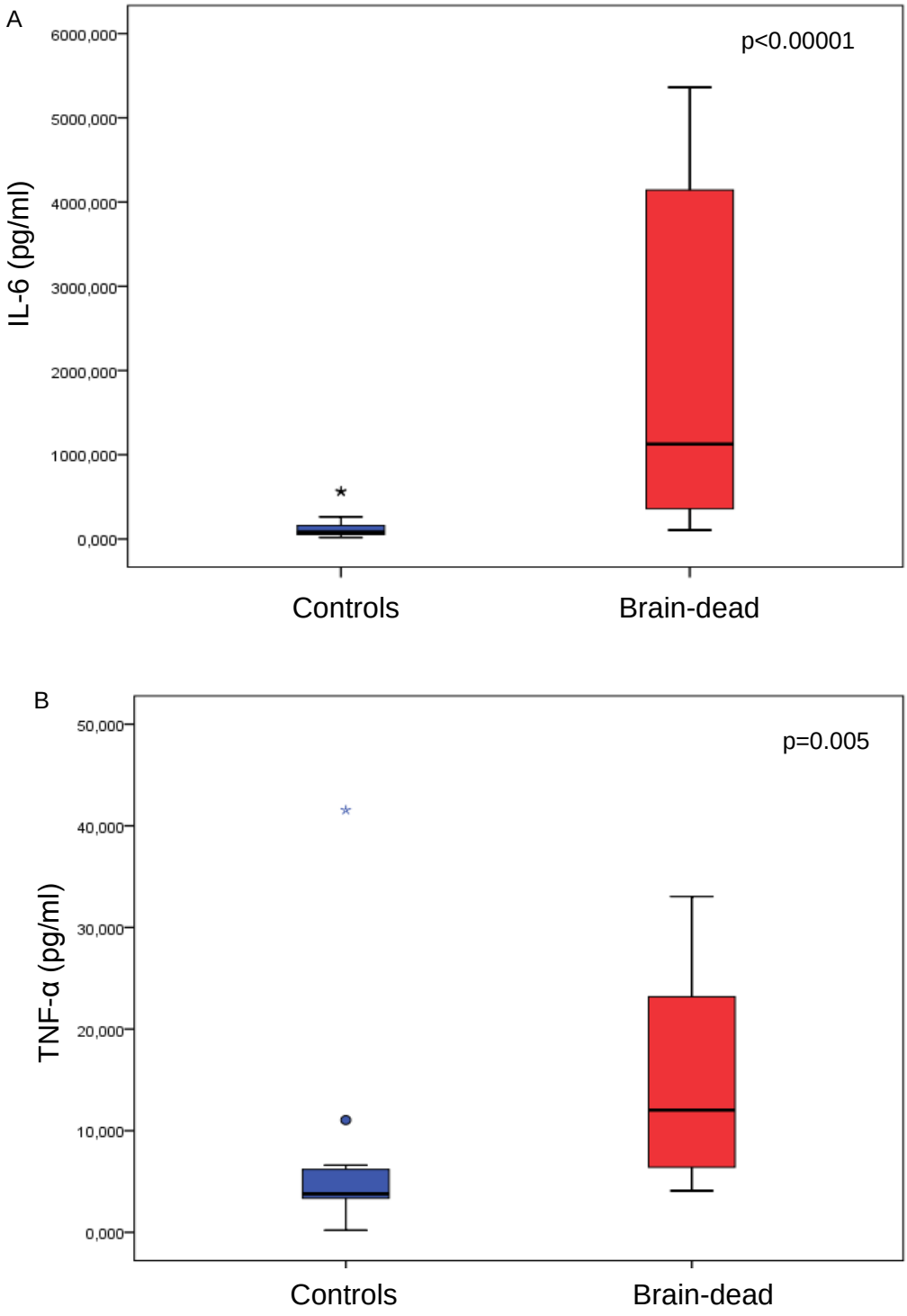

Figure 1. A. Serum IL-6 levels in brain-dead and controls. B. Serum TNF-α in brain-dead and controls.

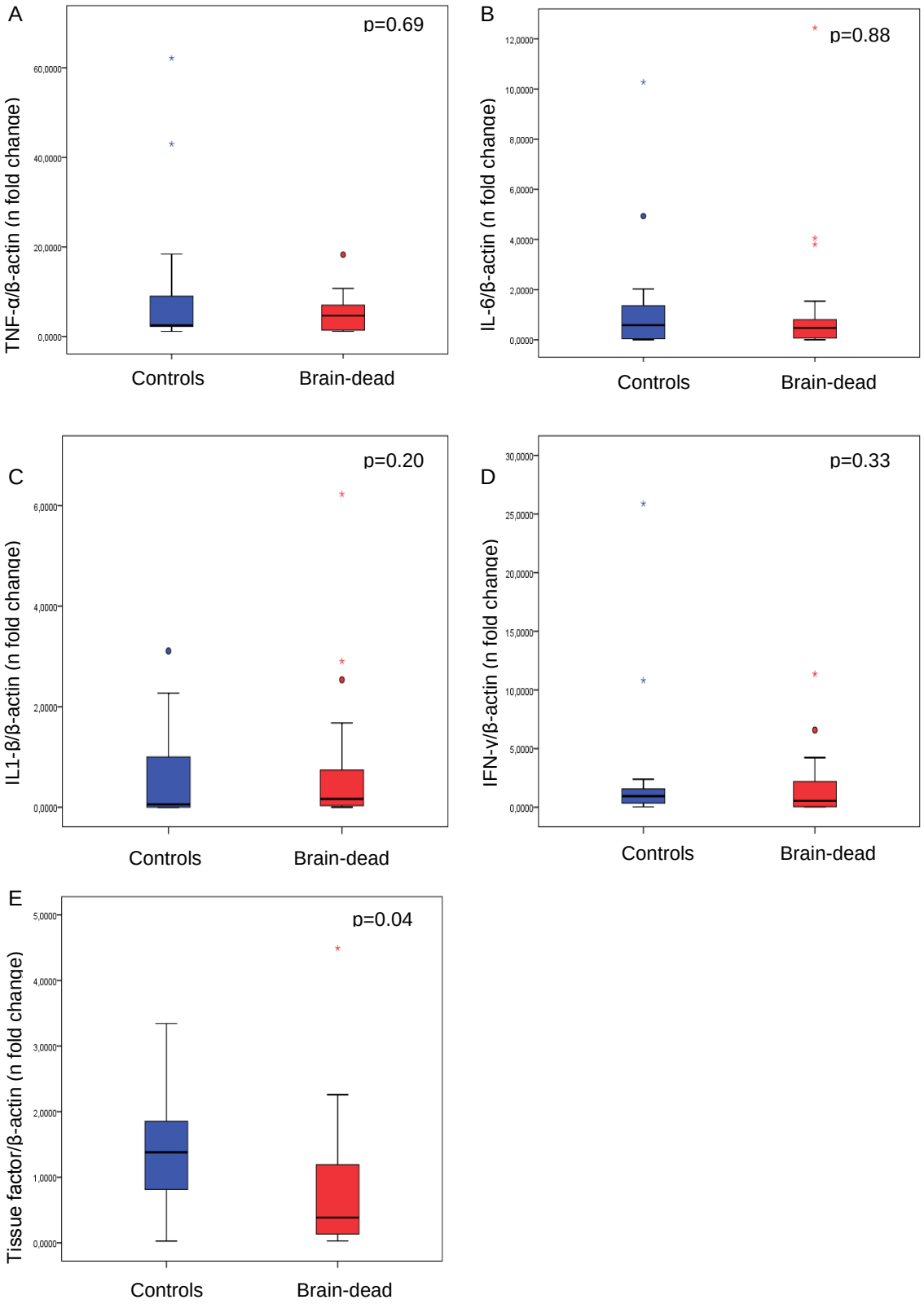

Figure 2. Cytokines quantifications by RT-qPCR in human pancreatic tissue, expressed in A.U (A. TNF-α) and in n-fold change related to calibrator sample (B. IL-6. C. IL-1β. D. INF-γ. E. Tissue factor).

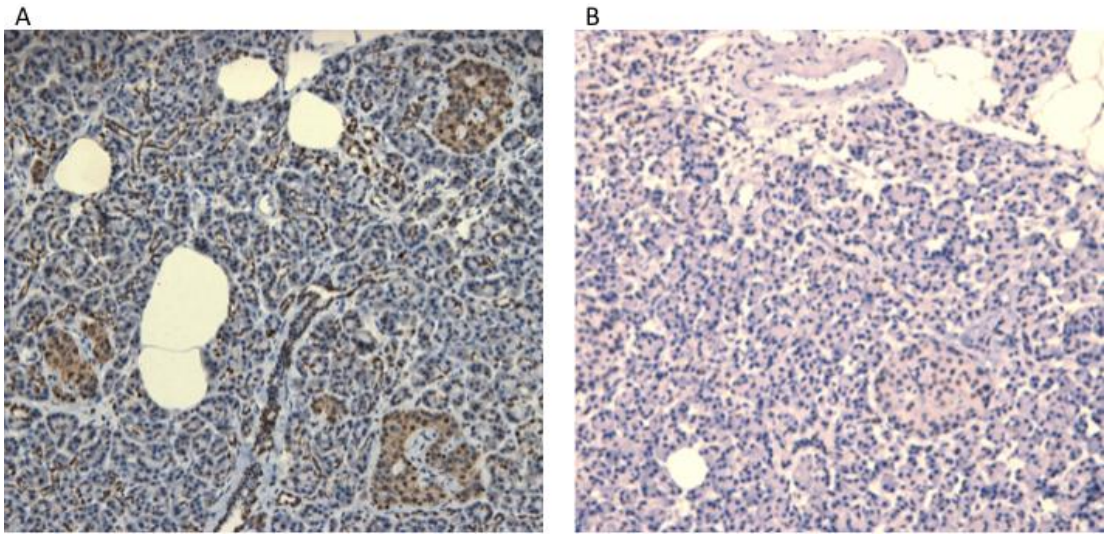

Figure 3. Staining of human pancreas with anti-TNF-α polyclonal antibody showing islets of Langerhans in brain-dead patients (A) and controls (B).

CAPÍTULO 4

Perspectivas futuras

Desde a década de 1990, quando o primeiro transplante de ilhotas em humanos foi realizado com sucesso (1), o transplante de ilhotas pancreáticas tornou-se uma terapia promissora para pacientes com DM tipo 1 com controle metabólico instável. Contudo, nos anos subsequentes, quando esse tipo de transplante passou a ser realizado em diversos centros ao redor do mundo, as taxas de sucesso relatadas não atingiram níveis satisfatórios (2, 3).

O desfecho de um transplante é resultado de uma complexa interação entre todos os eventos que acontecem desde o insulto que culmina com a ME até o período após o implante de órgão no receptor. O doador de múltiplos órgãos é uma peça fundamental dessa intrincada relação.

Há muito o que estudar no que concerne aos cuidados com o doador de órgãos. Este grupo de pesquisa tem focado seus esforços no estudo da ME e de suas consequências inflamatórias no pâncreas, como forma de aperfeiçoar o transplante de células β pancreáticas.

Recentemente, um alvo de pesquisas em DM tem sido o *glucagon-like peptide-1* (GLP-1). O GLP-1 é um hormônio peptídico liberado pelas células L do intestino delgado, cuja secreção é dependente da presença de nutrientes no lúmen do intestino. Sua liberação na corrente sanguínea estimula a secreção de insulina dependente de glicose e inibe a secreção de glucagon (4, 5). As propriedades antidiabetogênicas desse

hormônio levaram à pesquisa e ao desenvolvimento de um análogo do GLP-1, chamado exenatida. A exenatida, aprovada para uso clínico no tratamento do DM tipo 2 no Brasil, funciona como um forte agonista do receptor de GLP-1 (6-8).

Além do seu uso terapêutico no tratamento de pacientes com DM, a exenatida demonstra propriedades citoprotetoras para ilhotas pancreáticas (9, 10). A estimulação do receptor de GLP-1 produz efeitos diretos em células β, estimulando a regeneração e proliferação celular e reduzindo a apoptose, tanto em modelos animais como em ilhotas humanas isoladas (11, 12). Assim, seu potencial como agente citoprotetor no transplante de ilhotas foi testado clinicamente. Os resultados positivos desses estudos clínicos sugerem que, em breve, a exenatida poderá ser incluída como parte do tratamento dado a pacientes transplantados de ilhotas (13, 14).

A exenatida também possui atividade anti-inflamatória em linhagens de células β e ilhotas pancreáticas isoladas. Alguns estudos mostram a ação protetora da exenatida contra a atividade pró-inflamatória de citocinas como a IL-1β (15, 16). Por fim, um estudo publicado recentemente por Cechin *et al.* demonstrou que o tratamento com exenatida *in vitro* reduz a produção de citocinas e FT, além de ativar cinases, tais como Akt e ERK 1/2, em ilhotas humanas isoladas (17). Tais cinases fazem parte de diferentes vias de sinalização intracelular com funções pró-proliferativas e antiapoptóticas.

Desta forma, acreditamos que as propriedades anti-inflamatórias e pró-proliferativas da exenatida possam ser utilizadas para amenizar os danos causados pela ME nas ilhotas pancreáticas, melhorando a qualidade das células a serem transplantadas e reduzindo a perda da massa de ilhotas. Estamos testando essa hipótese através de um modelo experimental de ME em ratos Wistar, projeto atualmente em desenvolvimento

no Centro de Pesquisa Experimental do HCPA. Em um futuro próximo, planejamos reproduzir esse mesmo projeto com o uso de bloqueadores de TNF-α.

Além disso, consideramos que a ME possa estar envolvida na perda precoce de ilhotas causada por apoptose das células β, através da ativação da via do fator de transcrição NF-κB.

O fator de transcrição NF-κB é um heterodímero composto de duas subunidades, uma de 50 kDa e outra de 65 kDa. É encontrado no citoplasma da célula, associado a uma proteína inibitória, a IκB. O estímulo de citocinas inflamatórias resulta na fosforilação da IκB mediada pelo complexo da cinase da IκB, levando à degradação da IκB e consequente translocação do NF-κB para o núcleo (18). O NF-κB tem sido implicado como uma molécula sinalizadora do controle do balanço entre o ciclo celular normal e a apoptose (19, 20).

Em células β pancreáticas, o NF-κB controla diversas redes de genes que contribuem para a apoptose por ativação do estresse do retículo endoplasmático (21). Sabe-se que a exposição das células β a IL-1β *in vitro* induz a disfunção celular e, em combinação com IFN-γ ou TNF-α, provoca a morte celular, principalmente por apoptose. A inibição *in vitro* do NF-κB nessas células leva à diminuição da morte celular induzida por citocinas (22).

Chen *et al.* demonstraram, em ilhotas de porcos, que a atividade do NF-κB induzida por citocinas e sob condições de hipóxia desempenha um papel negativo sobre as ilhotas pancreáticas, levando à apoptose (23). Um estudo realizado em camundongos transgênicos que expressavam um super-repressor do NF-κB relatou uma diminuição do desenvolvimento de diabetes em resposta a múltiplas doses de estreptozotocina (24).

Os estudos que relacionam o fator de transcrição NF-κB com a apoptose das células β são direcionados à compreensão da fisiopatologia do DM tipo 1 (25, 26).

Entretanto, nenhum estudo avaliou o papel da ME na ativação do NF-κB no tecido pancreático humano e seu potencial efeito na apoptose das ilhotas. Desta forma, a amostra de pacientes do estudo caso-controle objeto desta tese está sendo ampliada, com o objetivo de comparar os efeitos da ME na apoptose pela ativação da via do NF-κB no tecido pancreático.

Por fim, a ME está implicada no desenvolvimento de disfunção primária dos enxertos (DPE) transplantados (27, 28), de maneira não completamente compreendida e provavelmente subestimada. A injúria de isquemia-reperfusão é uma causa reconhecida de DPE (29, 30). A atividade inflamatória induzida pela ME parece ter um papel intensificador da lesão de isquemia-reperfusão, por aumento de espécies reativas de oxigênio. Especula-se que os agravos da ME possam tornar os órgãos mais suscetíveis aos efeitos deletérios da isquemia-reperfusão (31).

A proteína desacopladora 2 (do inglês *uncoupling protein 2*, UCP-2) é uma proteína inserida na membrana mitocondrial interna e faz parte de uma superfamília de proteínas transportadoras. A UCP-2 tem uma distribuição tecidual ampla e atua desacoplando a oxidação dos substratos da síntese de ATP, dissipando a energia do potencial de membrana e, consequentemente, diminuindo a produção de ATP pela mitocôndria. Esse desacoplamento está associado à regulação do metabolismo de ácidos graxos livres e à diminuição da formação de espécies reativas de oxigênio pela mitocôndria (32, 33).

O aumento da expressão da UCP-2 parece ter um efeito antiapoptótico na maioria dos tipos celulares, uma vez que reduz a produção de espécies reativas de oxigênio. Entretanto, acredita-se que o papel da UCP-2 possa ser tanto pró-apoptótico quanto antiapoptótico, dependendo da regulação transcricional, do tipo celular e dos diferentes estímulos bioquímicos (34). De fato, o papel da UCP-2 na apoptose das

células β pancreáticas é ainda bastante controverso. Alguns estudos indicam que a expressão aumentada da UCP-2 teria uma função antiapoptótica, por proteger as células do estresse oxidativo (35-37). Outros estudos relatam justamente o contrário: que o bloqueio da UCP-2 teria um efeito pró-apoptótico (38, 39).

Considerando-se a indefinição do papel da UCP-2 e de sua resposta aos estímulos inflamatórios da ME nas células β, decidimos avaliar o efeito do bloqueio e da superexpressão da UCP-2 na viabilidade, na função e na expressão de genes pró e antiapoptóticos de células INS-1E e de ilhotas pancreáticas humanas submetidas a diferentes condições pró-inflamatórias e pró-apoptóticas.

Frente a todo o exposto, concluímos que ainda há muito a estudar sobre a ME e seus efeitos no tecido pancreático, especialmente nas células β.

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