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ORIGINAL ARTICLE

Does liraglutide alleviate inflammation in brain-dead donors? A randomized clinical trial

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Abstract

Brain death triggers an inflammatory cascade that damages organs before procurement, adversely affecting the quality of grafts. This randomized clinical trial aimed to compare the efficacy of liraglutide compared to placebo in attenuating brain death-induced inflammation, endoplasmic reticulum stress, and oxidative stress. We conducted a double-blinded, placebo-controlled, randomized clinical trial with brain-dead donors. Fifty brain-dead donors were randomized to receive subcutaneous liraglutide or placebo. The primary outcome was the reduction in IL-6 plasma levels. Secondary outcomes were changes in other plasma pro-inflammatory (IL-1β, interferon-γ, TNF) and anti-inflammatory cytokines (IL-10), expression of antiapoptotic (*BCL2*), endoplasmic reticulum stress markers (*DDIT3/CHOP*, *HSPA5/BIP*), and antioxidant (*superoxide dismutase 2*, *uncoupling protein 2*) genes, and expression TNF, DDIT3, and superoxide dismutase 2 proteins in liver biopsies. The liraglutide group showed lower cytokine levels compared to the placebo group during follow-up: Δ IL-6 (−28 [−182, 135] vs. 32 [−10.6, 70.7] pg/mL; $p = 0.041$) and Δ IL-10 (−0.01 [−2.2, 1.5] vs. 1.9 [−0.2, 6.1] pg/mL; $p = 0.042$), respectively. The administration of liraglutide did not significantly alter the expression of inflammatory, antiapoptotic, endoplasmic reticulum stress, or antioxidant genes in the liver tissue. Similar to gene expression, expressions of proteins in the liver were not affected by the administration of liraglutide. Treatment with liraglutide did not increase the organ recovery rate [OR = 1.2 (95% CI: 0.2–8.6), $p = 0.82$]. Liraglutide administration reduced IL-6 and prevented the increase of IL-10 plasma levels in brain-dead donors without

Abbreviations: *BCL2*, antiapoptotic B cell lymphoma 2; BD, brain death; cDNA, complementary DNA; *DDIT3/CHOP*, DNA damage inducible transcript 3; GLP-1, glucagon-like peptide-1; *HSPA5/BIP*, heat shock protein family A; IFN-γ: interferon-γ; qPCR, quantitative polymerase chain reaction; SAPS 3, Simplified Acute Physiology score 3; *SOD2*, superoxide dismutase 2; T1, time point 1; *UCP2*, uncoupling protein 2; Δ, delta value

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affecting the expression of genes and proteins related to inflammation, apoptosis, endoplasmic reticulum stress, or oxidative stress.

INTRODUCTION

Experimental studies have reported an upregulation of cytokines after brain death (BD),[1–3] resulting in harmful effects on organs suitable for transplantation.[1,2,4,5] Our previous research has demonstrated that BD induces inflammation at levels similar to sepsis, which is known to cause significant organ damage.[6]

Glucagon-like peptide-1 (GLP-1) is a hormone secreted by the intestinal epithelium L-cells in response to food.[7] Besides its main effect of stimulating glucose-dependent insulin release, it has anti-inflammatory, antiapoptotic, and cytoprotective properties,[7,8] and the administration of the GLP-1 agonist exenatide has been shown to increase pancreatic islet viability[9] and reduce liver damage[10] in rats. Additionally, its use has been shown to protect renal tissue from ischemia-reperfusion damage, an effect mediated by changes in the expression of genes related to oxidative stress, endoplasmic reticulum stress, and inflammation.[11,12]

Although liraglutide, another GLP-1 analogue, is an FDA-approved drug for treating type 2 diabetes and obesity,[13–15] its anti-inflammatory and antiapoptotic properties have not been studied in reducing inflammation, endoplasmic reticulum stress, and oxidative stress of organs from brain-dead donors. Thus, our randomized clinical trial aimed to investigate the potential of liraglutide in attenuating BD-induced inflammation, endoplasmic reticulum stress, and oxidative stress in brain-dead donors compared to placebo.

METHODS

Trial design and oversight

A double-blinded, placebo-controlled, single-center, randomized clinical trial was conducted at Santa Isabel Hospital in Blumenau, SC, Brazil. The study protocol was previously registered at ClinicalTrials.gov ID NCT03672812. Ethical approval to conduct the trial was obtained by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (the reference Ethics Committee in Research, project No. 2018-0170) and by the State Transplant Center of Santa Catarina. Informed consent was obtained from the closest relative, simultaneously with the written consent for organ donation. The study adheres to the principles set forth in the Declaration of Helsinki and Istambul, as well as local standards and Brazilian legislation.[16] Given the minimal risk associated with the study for the organ recipient, since liraglutide is a medication with a very safe profile and longstanding clinical use, the ethics committee waived the consent form for the recipient. It is worth noting, however, that the transplant teams were duly informed of the donor's participation in the study at the time of organ allocation.

Study population

Patients were deemed eligible for the study after undergoing BD protocol and if they were aged over 18 years. Patients who had received potent anti-inflammatory drugs, such as anti-TNF agents, were excluded from the study. Treatment with corticosteroids was not an exclusion criterion, and the type and dose of corticosteroids were recorded. Two physicians not affiliated with the study independently diagnosed BD in accordance with Brazilian legislation.[17] The study adhered to the following exclusion criteria: pregnancy, hemodialysis-dependent renal failure, advanced hepatic insufficiency, known allergy to liraglutide, and family refusal to participate. Following the determination of complete eligibility, randomization and initiation of study protocol ensued.

Randomization and trial interventions

Randomization was computer-generated, and brain-dead donors were randomized in a 1:1 ratio (using the website www.randomization.com) to receive either liraglutide (intervention group) or placebo (placebo group). The dosage of liraglutide administered in our study was equivalent to the highest clinically recommended dose used for treating patients with obesity.[18] The intervention group received 3 mg of liraglutide subcutaneously, corresponding to 0.5 mL immediately after randomization and every 6 hours until organ recovery. We selected a 6-hour timeframe to provide sufficient intervals for the administration of repeated doses, considering that a BD protocol typically does not extend beyond 24 hours. The placebo group received 0.5 mL saline solution immediately after randomization and every 6 hours subcutaneously until organ recovery. Liraglutide (Victoza®) and placebo were administered subcutaneously. The study pharmacist prepared identical syringes containing either the medication or the placebo, while the research team remained unaware of their respective content.

Liraglutide is an FDA-approved medication with a well-established clinical experience and safety profile. The main potential adverse effects of the medication are not applicable to brain-dead donors (constipation, nausea). The choice of dosage and dosing interval underwent thorough deliberation within the Ethics Committee to ensure no harm would result from the intervention on organs to be donated or organ recipients. Due to the nature of liraglutide, which does not cause hypoglycemia, no adverse events related to glycemic control were anticipated during treatment.

Data collection

The management of brain-dead donors was discrete and conducted by the critical care team in accordance with local standards.[19–21] They were unaware of study group allocation. The research personnel collected clinical and laboratory data from the electronic medical records.

Plasma IL-6, IL-1β, IL-10, IFN-γ, TNF, and BCL-2 determinations

A 20 mL whole blood sample was collected at the time of BD diagnosis, immediately before the first dose of either liraglutide or placebo was administered (time point 1, T1), and again before organ procurement (time point 2). Blood samples were centrifuged at 2500 g for 10 minutes at 4°C. Plasma was separated and immediately stored at 80°C until analysis. Circulating levels of IL-6, L-1β, IL-10, interferon-γ (IFN-γ), and TNF were assessed by the multiplex ELISA method (magnetic bead assay) using the Human Magnetic Custom Luminex® Kit (Invitrogen Life Technologies, Carlsbad, USA) and the Luminex® 200™ magnetic card reader (Luminex, Austin, USA), following the manufacturer's recommendations. Results are expressed as pg/mL. BCL2 was assessed by ELISA using a commercially available kit and following the manufacturer's recommendations (detection levels: BCL-2 $<$ 0.5 ng/mL) (Invitrogen Life Technologies, Carlsbad, USA). The time difference (in hours) between time point 2 and T1 is the delta value (Δ).

Liver RNA extraction and quantification of BCL2, HSPA5, DDIT3, SOD2, UCP2, and TNF genes qPCR

Liver tissue biopsies were obtained during hepatectomy before the liver was flushed with preservation fluid, snap frozen in liquid nitrogen, and stored at −80°C until use. Total RNA was extracted from liver tissue (100 mg) using the Purelink RNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA). The concentration and quality of total RNA samples were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Only RNA samples with adequate purity ratios were used for subsequent analyses.[22]

Real-time reverse transcription-quantitative PCR (qPCR) was performed in 2 separate reactions. Firstly, RNA was reverse-transcribed into complementary DNA (cDNA). cDNA was then amplified by qPCR. Reverse transcription of 200 ng of RNA into cDNA was carried out using the SuperScript IV Vilo Master Mix (Thermo Fisher Scientific) following the manufacturer's guidelines. qPCR experiments were performed in a Vii7 Fast Real-Time PCR System Thermal Cycler (Thermo Fisher Scientific), following thermal conditions suggested by Thermo Fisher Scientific for the specific qPCR buffer.

For antiapoptotic B cell lymphoma 2 (*BCL2)*, heat shock protein family A (*HSPA5)*, DNA damage inducible transcript 3 (*DDIT3)*, superoxide dismutase 2 (*SOD2)*, and uncoupling protein 2 *(UCP2)* genes, qPCR experiments were performed by real-time monitoring of the increase in fluorescence of the SYBER Green dye (47). Primer sequences for the target and the reference gene *YWHAZ* (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta) were designed using the Primer Express 3.0 Software (Thermo Fisher Scientific) and are depicted in Supplemental Table 1, http://links.lww.com/LVT/A527. qPCR reactions were performed using 5 µL of 2X PowerUp SYBER Green Master Mix, 0.5 µL (1 ng/µL) of forward and reverse primers for target and reference genes, and 1 µL of cDNA template (2.5–10 ng/µL depending on the target), in a total volume of 10 µL. The specificity of the qPCR experiments was verified through melting curve analyses, which showed that all primers generated amplicons that produced a single sharp peak.

For the *TNF* gene, qPCR reactions were performed using 5 µL of 2X TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific), 0,5 µL of 20X TaqMan Gene Expression Assay [for *TNF* (assay ID Hs00174128_m1) or tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (assay ID: Hs01122445_g1), Thermo Fisher Scientific], and 0,5 µL of cDNA template (200 ng/µL), in a total volume of 10 µL.

For all genes, each sample was analyzed in triplicate, and a negative control was included in every experiment. The comparative ΔΔCq method was employed for the relative quantification of genes. The ΔΔCq method estimates changes in gene expression as n-fold changes relative to the calibrator sample (pool of cDNAs).[22,23] A blinded researcher conducted the experiments.

Immunohistochemistry for DDIT3, SOD2, and TNF proteins in human liver tissue

DDIT3, SOD2, and TNF protein levels were determined by immunohistochemistry in formalin-fixed, paraffin-embedded liver sections. Anti-DDIT3/CHOP mouse monoclonal antibodies (Thermo Fisher Scientific), anti-SOD-2 rabbit

monoclonal antibodies (Cell Signaling Technology), and anti-TNF rabbit polyclonal antibodies (Thermo Fisher Scientific) were used to detect DDIT3 (1:50), SOD2 (1:200), and TNF (1:50) protein expression in human liver tissue, respectively. Positive controls for the experiments were brain, spleen, and intestine samples. Immunohistochemical analyses were conducted on 4 μm liver sections using routine immunohistochemical techniques, which included deparaffination and rehydration, antigenic recovery, inactivation of endogenous peroxidase, and blocking of nonspecific reactions. Slides were incubated with a primary antibody and then with a biotinylated secondary antibody, streptavidin-horseradish peroxidase conjugate anti-mouse (Santa Cruz Biotechnology, SC-516102) or anti-rabbit (EMD Millipore, code AP132P). The reaction visualization was obtained with Liquid Dab (Dako, K3468), according to the manufacturer's recommendations. For each slide, a blinded researcher captured images of 5 random fields at × 400 magnification. The selection of these fields was determined by the absence of artifacts and the amount of tissue. Images were visualized with a Zeiss microscope (model AXIOSKOP-40; Carl Zeiss, Oberkochen, Germany) and captured using the Cool Snap-Pro CS camera (Media Cybernetics). The staining was done using diaminobenzidine chromogen, and the quantification of DDIT3, SOD2, and TNF proteins was performed using the Image J software with the color deconvolution plugin (National Institutes of Health, NIH). Results are presented in pixels.

Outcomes

The primary outcome was the reduction in IL-6 plasma levels. The secondary outcomes were changes in other pro-inflammatory (IL-1β, INF-γ, TNF) and anti-inflammatory plasma cytokines (IL-10), expression of *BCL2*, endoplasmic reticulum stress markers (*DDIT3*, *HSPA5*), and antioxidant (*SOD2*, *UCP2*) genes, and expression of TNF, DDIT3, and SOD2 proteins in liver biopsies.

Sample size estimation

A sample size of 46 patients was estimated to detect a difference of 1 log in IL-6 levels between treatment groups, considering an 80% power and a 5% α-error rate.[4] To account for possible losses to follow-up, a sample of 50 participants was planned (25 for each group).

Statistical analysis

Categorical variables were expressed as percentages. Continuous data were expressed as mean and SD if normally distributed or as median and interquartile range otherwise. Data normality was assessed by visual inspection of the distribution. Groups were compared using Student's *t*-test, the Mann-Whitney *U* test, or the chi-square test. Correlations between variables were estimated using Spearman's test. Two-way ANOVA was performed to verify the difference between means according to the treatment group in the expression of genes considering the median of IL-6 at the first time point (131 pg/mL). To assess the OR and 95% CI of the IL-6 and number of donated organs, logistic regression models were built, and the model was adjusted for SAPS 3 score, time from BD to biopsy, and length of hospital stay before BD. To account for potential logistic issues with the study protocol, all analyses were conducted based on the intention-to-treat principle. This approach ensures that participants are analyzed according to their assigned treatment groups, regardless of any protocol deviations. Besides, an exploratory analysis per protocol was performed on the main outcome. Two-sided *p* values under 0.05 were considered statistically significant. Statistical analyses were conducted in SPSS 21.0 (Chicago, IL, USA). Graphs were created using GraphPad Prism version 9.5 (San Diego, CA, USA).

RESULTS

Patient characteristics

Between September 2018 and December 2020, 93 patients were evaluated for eligibility after the critical care team, who were not involved in the study, completed the BD protocol. In total, 50 brain-dead donors were included in this study (Figure 1). The main baseline characteristics were, in general, well balanced between groups, except for the BD cause (Table 1). Briefly, 62% were male, the mean age was 54 ± 16 y, and SAPS 3 score was 66 ± 14. The most common causes leading to BD were subarachnoid hemorrhage (n = 14, 28%), followed by stroke (n = 13, 26%), traumatic brain injury (n = 12, 24%), and cardiorespiratory arrest (n = 4, 8%). The presence of sepsis and the use of corticosteroids, both of which can potentially influence inflammation status, were similar between the groups. The median time to the first dose of the study medication after randomization was 20 (10–30) minutes. Patients received a mean of 1.9 ± 0.6 doses of the study medication: 11 patients (22%) received 1 dose, 31 (62%) received 2 doses, and 8 (16%) received 3 doses. The median time from randomization to organ biopsies was 11 (7.5–12) hours. The time interval between the last dose of the study medication and organ retrieval ranged from 2 to 5 hours.

Primary Outcome

The administration of liraglutide resulted in a decrease in IL-6 levels, while the placebo group showed an

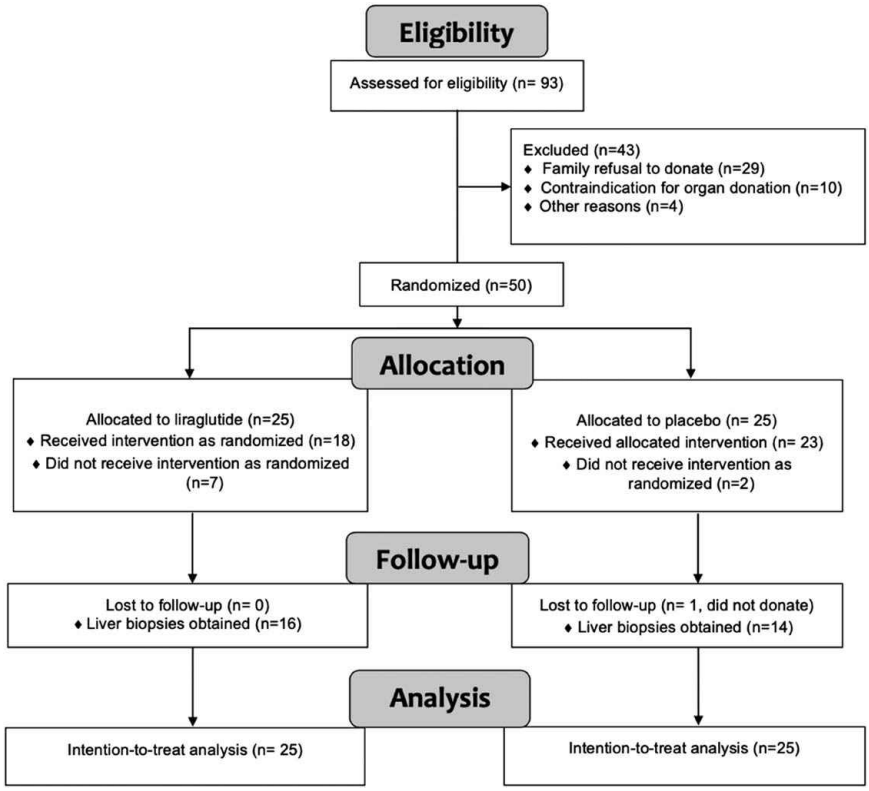

FIGURE 1 Consort flow diagram.

increase [−28)−182, 135) vs. 32 (−11, 71) pg/mL, $p = 0.041$] (Figure 2, Table 2). These results were also confirmed in the exploratory analysis conducted per protocol, which showed a similar difference in Δ IL-6 between the liraglutide and placebo groups [−31 (−200, 27) vs. 43 (−6, 198) pg/mL, $p = 0.005$].

Secondary Outcomes

Plasma IL-1β, IL-10, IFN-γ, TNF, and BCL-2 determinations

Table 2 shows the plasma cytokine profile during BD. The levels of IL-10 slightly increased from baseline in both groups, but the Δ IL-10 was significantly lower in the liraglutide than in the placebo group [0 (−2, 6.1) vs. 1.9 (−0.3, 6.1) pg/mL, $p = 0.036$]. However, the per-protocol analysis did not show any significant difference in Δ IL-10 levels between the two groups [0.2 (−2, 3.4) vs. 0.9 (−1.6, 5.5) pg/mL, $p = 0.511$]. Other plasma cytokines and BCL-2 levels did not differ significantly between groups (Table 2).

BCL2, HSPA5, DDIT3, SOD2, UCP2, and TNF gene expression in human liver tissue

The administration of liraglutide did not significantly alter the expression of inflammatory, antiapoptotic, endoplasmic reticulum stress, or antioxidant genes in the liver tissue obtained from brain-dead donors. The gene expression levels did not differ significantly between the liraglutide and placebo groups. Table 3 provides a summary of these findings.

Immunohistochemistry for DDIT3, SOD2, and TNF proteins in human liver tissue

Similar to gene expression, expressions of proteins in liver tissue were not affected by the administration of liraglutide. Quantifications of SOD2 [41 (14, 183) vs. 111 (19, 209) × 10^5 pixels, p = 0.546] and TNF [201 (12–250) vs. 192 (40–241) × 10^5 pixels, $p = 0.51$] did not differ between the liraglutide and placebo groups. The quantification of DDIT3 showed a trend to be higher in the liraglutide group compared to placebo [49 (48–49) vs. 45 (44–48) × 10^5 pixels, $p = 0.053$].

Exploratory outcomes

From the 50 donors included in the study, 30 donors underwent liver biopsies, with 16 in the liraglutide group and 14 in the placebo group (Figure 1). Not all biopsied organs were procured due to several reasons, including the absence of suitable recipient matches, the availability of transplant surgical teams, and complications arising

TABLE 1 Baseline characteristics of the brain-dead donors

	Liraglutide group (n = 25)	Placebo group (n = 25)
Demographics		
Age (y ± SD)	52 ± 18	57 ± 15
Men (n, %)	14 (56)	17 (68)
BMI (kg/m^2)	22 ± 4.5	22 ± 3.7
Cause of brain death (n, %)		
Subarachnoid hemorrhage	6 (24)	8 (32)
Traumatic brain injury	7 (28)	5 (20)
Stroke	8 (28)	5 (20)
Cardiac arrest	4 (16)	0 (0)
Others	0 (0)	7 (28)
Management site		
ICU (n, %)	21 (84)	18 (72)
Emergency room (n, %)	4 (16)	7 (28)
Disease severity		
SAPS 3 score	66 ± 14	66 ± 13
Presence of sepsis (n, %)	10 (40)	12 (48)
Pulmonary (n, %)	5 (62.5)	9 (90)
Other (n, %)	3 (37.5)	1 (10)
Need for vasopressors (n, %)	24 (96)	25 (100)
Cardiac arrest (n, %)	4 (16)	3 (12)
Use of corticosteroids (n, %)	18 (72)	18 (72)
Nutrition		
None (n, %)	16 (64)	15 (60)
Oral or enteral (n, %)	9 (36)	10 (40)
Biochemical measurements		
Hematocrit (%)	34 ± 8	31 ± 7
Hemoglobin (g/dL)	11.2 ± 2.3	10.2 ± 2.3
Leukocytes (10^3/mm^3)	17.2 ± 5.5	14.7 ± 5.6
Platelets (10^3/mm^3)	163 (104, 255)	158 (107, 229)
ALT (U/L)	34 (20, 70)	44 (21, 68)
AST (U/L)	40 (33, 80)	40 (25, 74)
Bilirubin (mg/dL)	0.8 (0.4, 1.2)	0.8 (0.4, 1)
PTTa (seconds)	30 ± 4	31 ± 5
Creatinine (mg/dL)	1.1 (0.7, 1.6)	0.8 (0.7, 1.3)
Urea (mg/dL)	59 (37, 87)	51 (32, 71)
Sodium (mEq/L)	147 ± 10	148 ± 13
Amylase (IU/L)	58 (37, 135)	51 (24, 133)
Lipase (IU/L)	41 (22, 66)	27 (20, 55)
Blood glucose (mg/dL)	255 ± 83	243 ± 91

Note: Values are mean ± SD or median and interquartile range.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; ICU, intensive care unit; PTTa, partial thromboplastin time activated; SAPS 3 score, Simplified Acute Physiology 3 score.

from the COVID-19 pandemic. Finally, 29 livers were transplanted, 12 in our Liver Transplant Center and 17 elsewhere. Early allograft dysfunction developed in 3 patients (25%), 2 in the liraglutide group and 1 in the placebo group. The follow-up biochemical data from the 12 recipients from our center is presented in Table 4. No adverse event was attributed to the study medication in these 12 patients.

Liraglutide treatment did not affect the number of livers retrieved from brain-dead donors [OR = 0.3 (95% CI: 0.05–2.5), $p = 0.29$] or the overall number of organs recovered [OR = 0.6 (95% CI: 0.04–9.83), $p = 0.74$].

To investigate the impact of liraglutide on gene and protein expression in brain-dead donors with higher levels of inflammation, a post hoc prognostic enhancement strategy was employed. Patients were divided based on the median of IL-6 at T1 into two groups, either < or > 131 pg/mL. Analysis of patients with IL-6 levels above the median did not reveal any significant differences between the liraglutide and placebo groups in terms of gene expression of *BCL2* [3.3 (1.2, 6.6) vs. 2.3 (2.0, 4.2), $p = 0.60$], *HSPA5* [1.3 (0.2, 14.0) vs. 1.1 (0.8, 2.1), $p = 0.99$], *DDIT3* [0.2 (0.1, 12.5) vs. 3.3 (2.3, 3.7), $p = 0.13$], *SOD2* [0.8 (0.2, 1.3) vs. 1.7 (0.9, 2.6), $p = 0.13$], *UCP2*[1.1 (0.6, 1.8) vs. 0.7 (0.5, 0.9), $p = 0.18$], and TNF [0.7 (0.4, 1.4) vs. 0.4 (0.7, 0.6), $p = 0.08$], protein expression of DDIT3 [49 (37, 49) vs. 48 (48, 48) × 10^5 pixels, $p = 0.8$], SOD2 [93 (7, 227) vs. 84 (20, 190) × 10^5 pixels, $p = 0.63$], and TNF [225 (195, 268) vs. 107 (20, 220) × 10^5 pixels, $p = 0.33$].

Our analysis did not reveal any significant correlation between IL-6 levels at T1 and markers of inflammation and disease severity (Supplemental Table 2, http://links.lww.com/LVT/A528). Similarly, there was no significant correlation between Δ IL-6 and gene and protein expressions (Supplemental Table 3, http://links.lww.com/LVT/A529).

DISCUSSION

In this double-blinded, placebo-controlled, and randomized clinical trial of brain-dead donors, liraglutide treatment reduced IL-6 and prevented the increase of IL-10 levels compared to placebo. Gene expression and protein content related to inflammation, endoplasmic reticulum stress, and oxidative stress were not affected by liraglutide treatment in liver tissue.

IL-6 is a pro-inflammatory cytokine that plays a key role in regulating immune responses. It induces the expression of transcription factors responsible for the acute inflammatory response, mainly through the signal transducers and activators of transcription and the NF-κB pathways.[24] Elevated plasma levels of IL-6 in brain-dead donors before transplantation have been associated with increased postoperative complications in liver transplantation and the development of bronchiolitis obliterans in lung transplantation.[25,26] Moreover, higher plasma IL-6 levels in brain-dead donors have been associated with prolonged hospitalization in organ

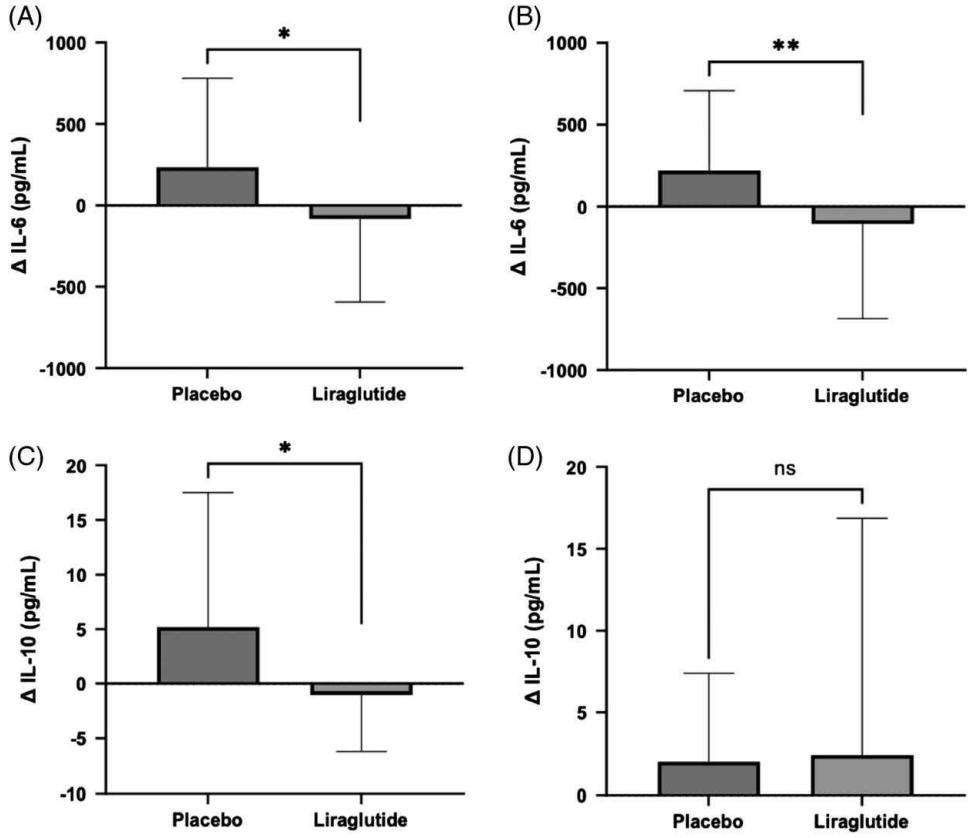

FIGURE 2 IL-6 and IL-10 levels in liraglutide and placebo groups. (A) The difference in IL-6 levels between the second minus the first time point (Δ IL-6) using intention-to-treat analysis. (B) The difference in IL-6 levels between the second minus the first time point (Δ IL-6) using the per protocol analysis. (C) The difference in IL-10 levels between the second minus the first time point (Δ IL-10) using intention-to-treat analysis. (D) The difference in IL-10 levels between the second minus the first time point (Δ IL-10) using the per protocol analysis.

recipients and delayed graft function.[27,28] Our results revealed that liraglutide treatment reduced IL-6 plasma levels compared to placebo. However, IL-10 is an anti-inflammatory cytokine that can suppress several inflammatory events, including the production of IFN-γ, IL-1β, IL-6, and TNF in macrophages and monocytes.[29] In addition to its recognized anti-inflammatory role, IL-10 has also been reported to enhance various immune processes that are crucial to organ transplantation. These events include immunoglobulin production by B cells, increased cytotoxicity of natural killer cells and CD8+ T cells, and the proliferation of thymocytes.[30] Both liraglutide and placebo groups showed a slight increase in IL-10 plasma levels over time, but liraglutide resulted in a smaller increase in IL-10 plasma levels than placebo, suggesting that liraglutide interferes with both inflammatory and anti-inflammatory pathways. Studies have shown that substantial trauma-induced release of IL-6 and IL-10 contributes to immune dysfunction, as the balance between pro and anti-inflammatory cytokines seems to be important for both the activation and subsequent downregulation of the immune system.[29,31] This study provides preliminary evidence that liraglutide may influence key immune responses involved in organ transplantation.

We hypothesized that the use of liraglutide would reduce inflammation and oxidative stress, leading to the downregulation of transcription factors of genes related to these processes. However, our results did not support this hypothesis, as there were no significant changes in gene or protein expressions in brain-dead donors treated with liraglutide. Possibly, mechanisms other than those affected by liraglutide treatment, such as coagulation, vascular morphogenesis, and extracellular restructuring,[32] are more important determinants of gene expression. Besides, the short duration of exposure to liraglutide, with most patients receiving only 2 doses before organ procurement, may have precluded the full effects of liraglutide. Therefore, initiating liraglutide treatment earlier in the process, before completion of BD protocol, may be necessary to fully obtain its anti-inflammatory effects.

Experimental studies suggested that BD is associated with increased apoptosis in different tissues.[10,33,34] Carlessi et al reported that exenatide decreased BD-induced apoptosis in liver tissue in rats.[10] The lack of effect of liraglutide on markers of apoptosis in our study may be due to differences in the type of GLP-1 agonist used, the duration of treatment, the timing of treatment initiation, or the higher

TABLE 2 Time course of plasma cytokine levels in brain-dead donors from randomization to organ recovery

Variables	Liraglutide (n = 25)	Placebo (n = 25)	*p*
IL-6 at T1	220 (76, 509)	78 (46, 297)	0.15
IL-6 at T2	179 (62, 392)	110 (58, 295)	0.71
Δ IL-6	−28 (−182, 135)	32 (−11, 71)	**0.04**
IL-1β at T1	3.4 (1.5, 4.3)	2.7 (2, 3.9)	0.70
IL-1β at T2	2.5 (1.7, 3.5)	2.7 (1.5, 4.9)	0.86
Δ IL-1β	−0.5 (−0.8, 0)	0 (−1.2, 2.9)	0.44
IL-10 at T1	1.9 (0.7, 5.4)	3.2 (1.4, 7.3)	0.16
IL-10 at T2	2.6 (0.8, 5.4)	6 (3.0, 9.4)	**0.02**
Δ IL-10	0 (−2.1, 1.4)	1.9 (−0.3, 6.1)	**0.04**
IFN-δ at T1	12 (4.5, 12.6)	12 (7.8, 12.6)	0.46
IFN-δ at T2	10 (7.7, 12.6)	12 (7.8, 12.7)	0.32
Δ IFN-δ	0 (0, 3.9)	0 (−0.1, 1.2)	0.42
TNF at T1	17 (8.4, 48)	13 (6.1, 31.5)	0.18
TNF at T2	25 (6.6, 81)	19 (6.1, 72)	0.93
Δ TNF	−2 (−10, 15)	3 (−7.6, 13)	0.46
BCL2 at T1	11 (2, 14.7)	10 (2, 16)	0.65
BCL2 at T2	10.2 (2.5, 13.5)	15 (2.5, 21.4)	0.42
Δ BCL2	0 (−4.5, 0.7)	1.1 (0, 9.6)	0.12

Notes: Citokine values are expressed in pg/mL, except for BCL-2, which is expressed in ng/mL.
Mann-Whitney *U* test for independent samples.
Analyses are intention-to-treat.
Abbreviation: BCL2, B cell lymphoma 2; IFN-δ, interferon-delta.

complexity of BD in humans. Some studies reported *BCL2* downregulation in tissues from BD donors,[35,36] whereas others did not,[33,37,38] which may reflect differences between species. Here, *BCL2, HSPA5,* and *DDIT3* expressions were not significantly affected by liraglutide treatment. However, despite not being statistically significant, protein expression of DDIT3 was higher in the liraglutide group compared to the placebo group.

Oxidative stress has a role in BD-induced organ damage, contributing to the development of complications following organ transplantation, mainly ischemia-reperfusion injury, delayed graft function, and primary graft dysfunction.[34,39] Our findings indicated that liraglutide treatment did not affect the expression of *SOD2* and *UCP2* in liver tissue, which is consistent with previous experimental studies demonstrating that exedin-4 reduced *UCP2* and *SOD2* expression in pancreatic tissue but not in liver tissue,[9,10] suggesting a tissue-specific pattern of GLP-1 effects on its receptor.[40–42]

TABLE 3 Gene expressions in liver tissue recovered from brain-dead donors

Genes	Liraglutide (n = 16)	Placebo (n = 14)	*p*
BCL2	3.2 (1.6, 4.1)	2.5 (1.7, 3.3)	0.29
HSPA5	0.8 (0.4, 1.4)	0.9 (0.51, 1.4)	0.89
DDIT3	0.6 (0.8, 1.9)	1.7 (0.3, 3.4)	0.17
SOD2	0.8 (0.4, 1.5)	0.8 (0.4, 1.7)	0.82
UCP2	0.9 (0.7, 1.5)	0.7 (0.6, 1.2)	0.18
TNF	0.9 (0.7, 1.4)	0.7 (0.4, 1.3)	0.31

Gene expressions are expressed in n-folds in relation to the calibrator sample.
Analyses are intention-to-treat.
Abbreviations: BCL2, B cell lymphoma 2; DDIT3 (CHOP), DNA damage inducible transcript 3; HSPA5 (BIP), Heat Shock Protein Family A (Hsp70) Member 5; SOD2, Superoxide dismutase 2; TNF, Tumor necrosis factor; UCP2, uncoupling protein 2.

Donor-based research is relatively rare. There are a limited number of clinical articles focused specifically on donor conditions and treatment, as conducting studies on organ donors is inherently challenging due to a myriad of ethical issues involved in the donation and transplantation processes. Nevertheless, we successfully conducted the first double-blinded, placebo-controlled, randomized clinical trial to test a drug with anti-inflammatory, antioxidant, and antiapoptotic properties in human brain-dead donors. However, some limitations require attention. Firstly, the study was conducted at a single center, had a slow recruitment rate, and had some protocol deviations, mainly incorrect liraglutide administration to 2 donors randomized to the placebo group due to a pharmacist error and the lack of liraglutide administration in seven cases of the experimental group. To maintain randomization, we performed an intention-to-treat analysis, as

TABLE 4 Peak levels of transaminases, bilirubin, and international normalized ratio of liver recipients on ICU admission, on days 1, 2, and 7 of ICU stay

ICU admission	Liraglutide (n = 5)	Placebo (n = 6)	*p*
AST	1047 (688, 3498)	1015 (449, 2879)	0.93
ALT	617 (430, 1880)	841 (210, 1850)	0.93
INR	2.6 (2.3, 4.8)	1.9 (1.7, 5.5)	0.25
BT	4.2 (3.2, 21.8)	1.7 (1.3, 8.9)	0.12
ICU day 1	Liraglutide (n = 4)	Placebo (n = 5)	*p*
AST	521 (425, 9366)	547 (266, 4586)	0.73
ALT	484 (400, 3112)	682 (247, 3206)	0.99
INR	1.9 (1.3, 2.4)	1.6 (1.4, 4.2)	0.73
BT	4.7 (2.8, 8.9)	1.14 (0.6, 10.4)	0.41
ICU day 2	Liraglutide (n = 4)	Placebo (n = 4)	*p*
AST	268 (236, 2914)	569 (202, 7559)	0.73
ALT	357 (335, 1803)	975 (254, 16255)	0.99
INR	1.5 (1.2, 1.8)	1.4 (1.2, 3.5)	0.99
BT	5.7 (2.3, 6.6)	1.2 (0.3, 13.8)	0.29
ICU day 7	Liraglutide (n = 4)	Placebo (n = 4)	*p*
AST	64 (38, 76)	62 (50, 94)	0.99
ALT	146 (100, 251)	194 (106, 287)	0.69
INR	1.1 (1.1, 1.4)	1.1 (1.0, 1.1)	0.49
BT	3.3 (1.5, 8.7)	0.4 (0.3, 0.7)	0.03

Abbreviations: ALT, alanine aminotransferase (in U/L); AST, aspartate aminotransferase (in U/L); BT, bilirubin (in mg/dL); ICU, intensive care unit; INR, international normalized ratio.

planned and recommended. Secondly, due to the complex logistics of the study, we could not obtain liver tissue from all brain-dead patients, potentially limiting the power to detect differences in gene and protein expressions. Thirdly, the exposure of brain-dead donors to liraglutide treatment may have been insufficient to significantly modify inflammation at higher levels, as liraglutide exhibits its maximum concentration after 8 hours.[18] Perhaps, early liraglutide administration to comatose patients with severe brain injuries at the imminence of BD may better uncover its full anti-inflammatory effects. Also, this is a proof-of-concept study, and the lack of benefit on organ retrieval or impact on the development of early allograft dysfunction has to be considered with caution, as the study was designed to look at inflammatory markers, not to look at clinical outcomes related to this inflammatory markers. Besides, donation outcomes are influenced by behavioral factors rather than solely biological factors, and there may be several unaccounted variables that could have influenced these results. Finally, the liver possesses a unique anatomical structure, including a dual inflow vascular system and a diverse population of resident immune cells. It is in a constant state of stimulation, which has a key role in maintaining a balance between tolerance and inflammation in healthy livers.[43] These specific characteristics may contribute to the relatively lower levels of inflammation observed and the limited treatment effects of an anti-inflammatory agent. It is possible that the effects of liraglutide might be more pronounced in organs other than the liver, given their different immunological profiles and responses.

In summary, we present here preliminary evidence that treatment of brain-dead donors with liraglutide reduces IL-6 and prevents the increase of IL-10 levels compared to placebo, 2 critical cytokines implicated in BD-induced inflammatory cascade. This hypothesis-generating study suggests that alleviating inflammation may reduce BD-induced organ damage. Investigating the effects of liraglutide or other more potent GLP-1 analogue agents on outcomes of organ transplantation, as well as exploring different doses and durations of exposure, warrants further assessment through a multicentric clinical trial.

AUTHOR CONTRIBUTIONS

Geisiane Custódio participated in the study design, data collection, and data interpretation and drafted the manuscript. Andrew Maykon Massutti and Mauro Rafael da Igreja obtained organ biopsies. Daisy Crispim, Natália Emerim Lemos, Fernanda Visioli, and Victor de Mello Palma performed the experiments. Cristiane Bauermann Leitão participated in the study conception and design, data interpretation, and statistical analysis. Tatiana Helena Rech participated in the

study conception and design, data interpretation, and statistical analysis and drafted the manuscript. All authors revised the manuscript.

Tatiana Helena Rech is the guarantor of this study and, as such, has complete access to data and takes full responsibility for the integrity of data and accuracy of analysis.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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