Experimental study to assess the impact of vasopressors administered during maintenance of the brain-dead donation in the quality of the intestinal graft

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BACKGROUND: The hemodynamic maintenance of brain-dead donors will influence the quality of the organs procured for transplantation, including the intestine. Although norepinephrine (NE) and dopamine (DA) are commonly used to sustain mean arterial pressure in humans, there are no standardized protocols for their use during maintenance of brain-dead donors. Our aim was to compare the effects of each drug, in the intestinal graft quality using a rat brain-dead donation model.

METHODS: Wistar rats (N = 17) underwent brain death (BD) for 2 hours with NE (NE group) or with DA (DA group) administration; the control group was mechanically ventilated for 2 hours without BD. Jejunum biopsies were obtained at the end of the maintenance period. Histological damage was evaluated using Park-Chiu scale. Villi/crypt ratio, mucosal thickness, Goblet cell count, and villi density were evaluated using ImageJ software (US National Institutes of Health, Bethesda, MD). Barrier damage was assessed by bacterial translocation culture counting on liver samples. The inflammatory status of the intestine was evaluated by $CD3^+$ counting by immunohistochemistry and gene expression analysis of interleukin (IL)-6, IL-22, and CXCL10.

RESULTS: Norepinephrine-treated donors had higher focal ischemic injury in the intestinal mucosa without a substantial modification of morphometrical parameters compared with DA-treated donors. $CD3^+$ mucosal infiltration was greater in intestines procured from brain-dead donors, being highest in NE ($p < 0.001$). Local inflammatory mediators were affected in BD: DA and NE groups showed a trend to lower expression of IL-22, whereas CXCL10 expression was higher in NE versus control group. Brain death promoted intestinal bacterial translocation, but the use of NE resulted in the highest bacterial counting in the liver ($p < 0.01$).

CONCLUSION: Our results favor the use of DA instead of NE as main vasoactive drug to manage BD-associated hemodynamic instability. Dopamine may contribute to improve the quality of the intestinal graft, by better preserving barrier function and lowering immune cell infiltration. (*J Trauma Acute Care Surg.* 2022;92: 380–387. Copyright © 2021 Wolters Kluwer Health, Inc. All rights reserved.)

KEY WORDS: Brain dead; intestinal transplantation; graft quality; hemodynamic maintenance; rat.

Intestinal transplantation remains as the main therapeutic alternative for patients with intestinal failure and complications associated to the chronic use of total parenteral nutrition.[1,2] The vast majority of intestines procured for transplantation are obtained for donation after brain death (BD),[3] with a limited worldwide experience using living donation and donors after cardiac death. Organs from BD are known for being of inferior quality than those from living donation[4,5] because of hormonal, metabolic, and circulatory events related to BD, which are also concomitant to inflammatory events that results in changes at the tissue and cellular levels that may require specific interventions for optimal outcomes.[6]

Brain death, itself, triggers different biological processes related to dysregulated homeostatic circuits. Among them, the release of catecholamines might originate a transitory rise of arterial pressure that is usually followed by hypotension due to changes in hormone levels related to water and electrolyte management (insipidus diabetes) and drop in endogenous catecholamine levels.[7,8] Usually, in this phase, most patients required different vasopressor agents to maintain mean arterial pressure (MAP) within physiologic limits. The hemodynamic management required as part of the BD maintenance will condition the quality of the different organs for transplantation, including the intestinal graft.

Intestine has been shown as one of the most labile organs for ischemia-reperfusion injury.[9] Important efforts have been taken to improve the quality of organ preservation during procurement.[10] However, improving the quality of organs by donor management will also contribute to better performance of the different interventions performed downstream along the procedure, being intensive care unit (ICU) donor maintenance a critical part of the whole procedure.

There are different reports on the use of both norepinephrine (NE) and dopamine (DA) to improve MAP; however, ICUs mostly do not adopt a standardized protocol for using vasopressors during the donor maintenance or procurement.[11,12] Although there are some randomized-controlled studies indicating that low-dose DA administration may be useful in heart and kidney transplantation, there is no generalized consensus in the selection of vasoactive drug for brain-dead donor management.[13,14] Models to reproduce BD in rodents are complex, not only because of the procedure required to cause occlusive BD but also because of the complexity required to sustain ventilatory support and reproduce vasopressor support. We have previously published our experience of using a brain-dead rat model generated by intracranial balloon inflation at controlled rate; adding to the described procedure different refinements, such as regulating the speed of inflation to simulate different types of situations such as traumatic versus cerebrovascular cause of death, has been proposed.[15,16] In most of the experimental BD protocols in rats, the management of hemodynamic instability is achieved by the alternative use of NE or DA as a vasopressor drugs; however, the differential effects of these drugs on the quality of intestinal graft, which has been shown as

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one of the most reactive organs after BD,[5,17] has not been assessed yet. Given that increased intestinal mucosal damage associated with adrenergic receptor activation has been reported,[18,19] we hypothesize that NE use may induce a differential effect compared with DA use to sustain MAP.

With the aim of analyzing the differential effect of DA versus NA in the management of BD on the intestinal graft quality, an experimental protocol was designed to further study the effects of BD and the associated vasopressor support with NE or DA in the morphological, physiological, and mechanistic aspects of the intestinal graft.

MATERIALS AND METHODS

Animal Use and Care

Adult male Wistar rats (mean ± SD, 300 ± 20 g) were housed in a climate-controlled room (21°C ± 2°C and relative humidity of 45% ± 15%) on a 12-hour light-dark cycle at our institution's animal facilities. The protocol was approved by the Animal Welfare Ethics Committee of The Veterinary Sciences School of the National University of La Plata, Argentina (54-1-15 T).

Surgical Procedure

All animals were anesthetized with isofluorane, in a saturated chamber for induction, then with a mask, and, finally and for the whole procedure, using the endotracheal intubation.

Under surgical microscope, lines were placed in the carotid artery and jugular vein to control MAP and deliver solutions. Using the same surgical approach, a tracheostomy is performed, and an endotracheal tube is placed to initiate the assisted ventilatory support.

All animals were maintained under mechanical ventilation with 70 breaths per minute, 2.5 mL/kg and positive end expiratory pressure 1 to 4 cmH2O (683 Small Animal Ventilator; Harvard Apparatus, Holliston, MA), controlling MAP (70–120 mm Hg), SpO_2, heart rate, and temperature (37–38°C). Animals with MAP below 60 mm Hg for more than 10 minutes were discarded.

After 2 hours of maintenance, a midline celiotomy was done, and the small bowel was dissected according the technique previously described;[20] the aorta was clamped proximal to the superior mesenteric artery and flushed with 5 mL of cold lactate Ringer's solution; and then, samples of small intestine and liver were taken.

BD Model

The BD model used was the gradual onset of procedure described by Pratschke et al.[21] Through a drilled Bregma's frontlateral burr hole, a catheter Fogarty 4F (Edwards LifeSciences Co., Irvine, CA) was inserted in the subdural space. Brain death was induced by a progressive inflation of the balloon with 500 µL of saline solution using a syringe infusion pump (PC11UBT; Apema S.R.L., Buenos Aires, Argentina), at 1 mL/h rate. After 30 ± 5 minutes, BD was confirmed by apnea, Cushing response, and maximal pupil dilatation.

Experimental Design

Three groups were established (Fig. 1).

Live donor group (control group [CG]) (n = 6), used as CG: rodent under assisted ventilation for 2 hours, under anesthesia without BD procedure or use of vasopressor drug support.

Brain death norepinephrine group (n = 6): 2 hours of ventilatory support after BD combined with the use of NE as vasopressor agent was used with a maximum dose of 1 µg/kg per minute, to keep MAP between 70 and 120 mm Hg, a dose higher than the proposed one, which was considered as exclusion criteria.

Brain death dopamine group (n = 5): 2 hours of ventilatory support after BD combined with the use of DA as vasopressor agent; 5 µg/kg per hour was considered as a limit for exclusion criteria (Fig. 2).[23,24]

A total of six additional rats were discarded during the surgical procedures. Three of them were because of hypotension during maintenance that could not be controlled with vasopressors, two because of bleeding at the arterial cannulation site, and one because of acute pulmonary edema.

Sampling

Intestinal samples from jejunum were collected for histopathological injury assessment and gene expression analysis. Liver samples from the distal part of the left lateral lobe were aseptically collected to analyze bacterial translocation.

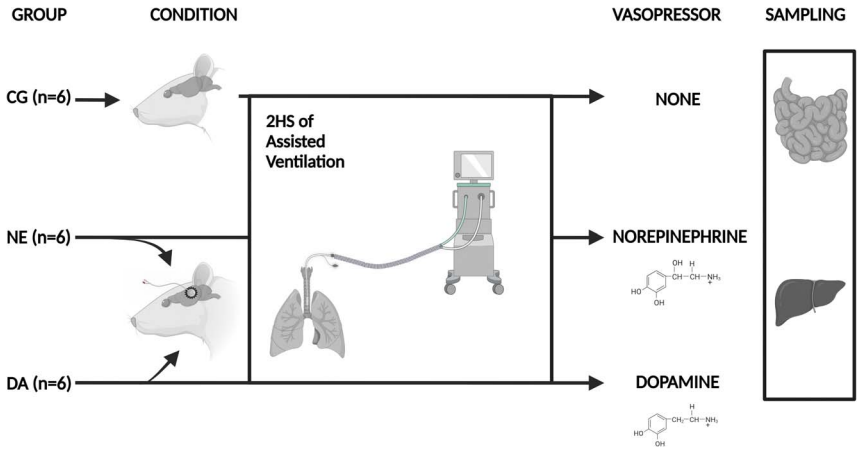

Figure 1. Representation of the experimental design. Three groups were performed, two of them with BD during 2 hours. In each group, the correction of the MAP during the BD period was done using either NE or DA. The CG included animals mechanically ventilated for 2 hours. Once the stipulated time was over, intestinal and liver samples were taken for subsequent analysis. Created with BioRender.com.

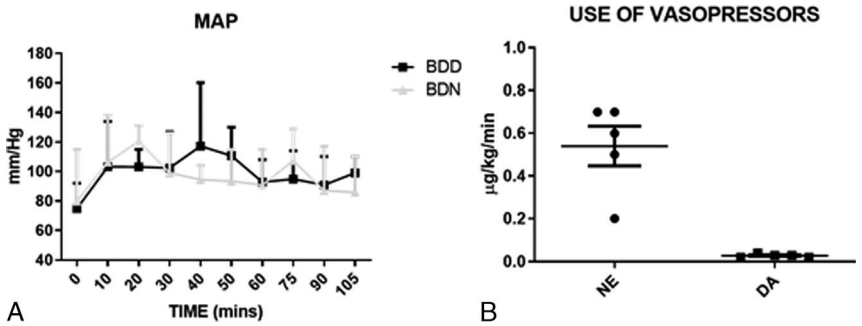

Figure 2. Arterial pressure and dose of vasopressors used during the 2 hours of BD period. (*A*) Variation in MAP during the BD experimental phase. No significant differences between groups were detected at any time point. (*B*) Norepinephrine and DA use of vasopressors during the experiment. For each animal, the total dose of vasopressors infused along the BD period is represented in NE equivalence using a 83.3:1 ratio taken from De Bakker et al.[22]

Intestinal Histological Evaluation

Sections of the animals' small bowel were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin for histological evaluation.

Intestinal ischemic histological damage was scored according to Park-Chiu classification scale (PC scale) as previously described[25,26] (0, normal mucosa; 1, subepithelial space at villus tip; 2, more extended subepithelial space; 3, epithelial lifting along villus side; 4, denuded villi; 5, loss of villus tissue; 6, crypt layer infarction; 7, mucosal infarction; 8, transmural infarction).

To perform a more descriptive analysis that includes both global and focal injury, ischemic damage was analyzed on 9 randomized fields at 10 times magnification for each sample, which was assigned a score within the PC scale. For each individual, the median of the nine randomized fields was taken as representative, and the results are shown as mean and SD within each group, representing the global damage. In the case of focal damage analysis, fields with values greater than or equal to 4 on the PC scale are counted and assigned a percentage value for each individual, and then, the results are displayed as mean and SD within each group. Using ImageJ software(US National Institutes of Health, Bethesda, MD),[27,28] intestinal villus height and crypt depth were measured to calculate villus/crypt ratio and mucosal thickness.

At least 20 individual villi and crypt were measured in each rat to determine this parameter. We also quantified the total mucosal area and counted the number of fully functional villi (≤3 on PC scale) with the aim to calculate effective villi density. Goblet cell count was performed on Alcian Blue/neutral red coloration,[29] counting 25 randomly selected villi per animal. The immunohistochemistry technique was performed using an anti-CD3 antibody (DakoCytomation, Santa Clara, CA) and the LSAB2 kit (DakoCytomation) as detection method using 3,3′-diaminobenzidine as substrate. Antigen retrieval was performed with the steam method.[30] Hematoxylin was used as contrast colorant, and synthetic balm was used as montage media. $CD3^+$ lymphocytes were counted in 150 randomly selected fields at 40 times magnification per individual.

Bacterial Culture

A portion of the liver (approximately 1 g) was aseptically collected and placed in sterile tubes containing 3 mL of sterile phosphate saline buffer. The organ was homogenized, and the suspension was diluted in series 1:10 and 1:100; then, 100 µL of that suspension was seeded on a Luria-Bertani-agar plates. Bacterial growth was assessed by colony counting after 48 hours at 37°C. The results were expressed as colony-forming units per gram of tissue.

To validate the aseptic sampling, in this case, we add a sham operated group (n = 4), where the individuals where anesthetized, aseptically conditioned, and sampled through a midline laparotomy (not shown).

RNA Isolation and Quantification

Total RNA extraction intestinal graft was performed using the NucleoSpin RNA II kit (GE Healthcare, Aurora, OH). Reverse transcription was performed using random primers and MMLV-Reverse transcriptase (Invitrogen, Carlsbad, CA) as previously described.[20] Real-time quantitative polymerase chain reaction was performed following manufacturer's protocol using the iCycler iQ5 thermal cycler (BioRad, Philadelphia, PA). Primers for rat interleukin (IL)-6, IL-22, CXCL10, and Actin-b were designed by us or adapted from literature: qRat-actB-forward ACAACCTTCTTGCAGCTCCTC 1, qRat-actB-reverse ACA ACCTTCTTGCAGCTCCTC 1, qRat-IL6-forward CTGATT GTATGAACAGCGATG 1, qRat-IL6-reverse GAACTCCAGAAG ACCAGAG 1, qRat-CXCL10-forward CTGCACCTGCATCGAC TTCC, qRat-CXCL10-reverse TTCTTTGGCTCACCGCTT TC, qRat-IL22-forward TGGTGCCTTTCCTGACCAA self-designed, and qRat-IL22-reverse GTTCTGGTCATCACCGCTGAT self-designed. Relative difference calculation using the ΔΔCt method was previously described.[31,32]

Statistical Analysis

Comparisons among groups were performed using an analysis of variance or Kruskal-Wallis, followed by Dunn's test for multiple comparisons, or Student's *t* test or Mann-Whitney test for unpaired or paired data, as appropriate using GraphPad 5.0 software (GraphPad Software Inc, San Diego, CA). Results were expressed as mean ± standard error of the mean. Differences between means were considered statistically significant when $p < 0.05$.

RESULTS

Higher Focal Ischemic Injury in the Intestinal Mucosa Was Observed in NE-Treated Donors Compared With DA-Treated Donors

Brain death has a negative impact on the intestinal mucosa. Both groups under BD condition show a higher score on

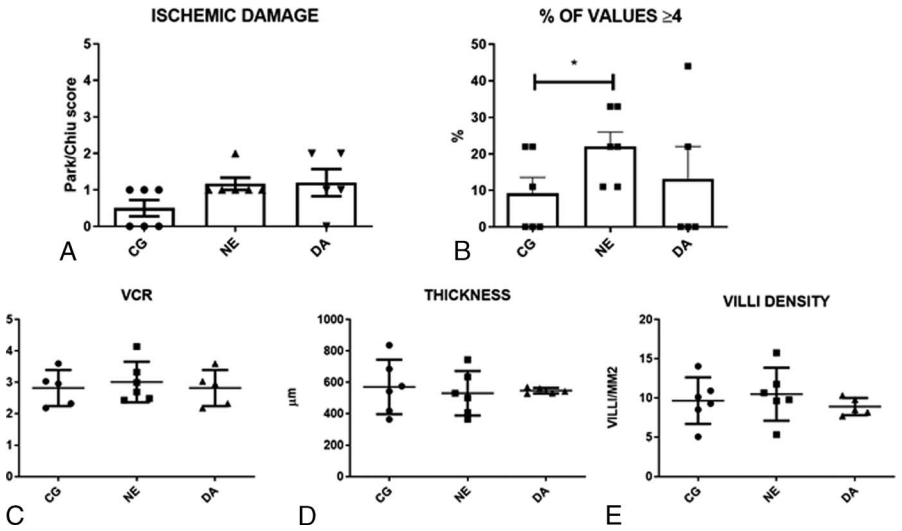

Figure 3. Intestinal histopathological parameters to characterize global ischemic damage. (*A*) Ischemic damage under Park/Chiu score (*9* random images of intestinal tissue of each individual are analyzed). The line is located at the mean value of the distribution. (*B*) Focal damage represented by samples that have a Park-Chiu score of 4 or higher (*t* test CG vs. NE, $*p = <0.05$). (*C–E*) Morphometric analysis of the intestinal mucosa. (*C*) The villus/crypt ratio; (*D*) the mucosal thickness, calculated as the sum of villus length and crypt depth; and (*E*) the effective villi density, estimated by measuring the mucosal area and counting the villi with a maximum of 3 in PC scale (which still maintains epithelial lining); no significant differences were observed between the groups.

the PC scale compared with the CG. Norepinephrine group was the most damaged (1.94 ± 1.57 PC scale) followed by the DA group (1.33 ± 1.24 PC scale), and finally, the CG was the one with the least degree of injury (1.11 ± 1.56) (Fig. 3*A*). To determine the extension of focal damage, the percentage of tissue showing values higher than 4 in the PC scale was calculated for each condition (Fig. 3*B*). The NE group showed the highest focal damage including 22% of values ≥4. The DA group presented 13.20% of values ≥4 on PC scale, whereas, in the CG group, 9.16% showed focal damage. These results indicate that the selection of vasopressor has an impact on mucosal integrity.

Furthermore, villi/crypt ratio was slightly higher in NE group (3.31 ± 1.03) in comparison with CG (2.94 ± 0.96) and DA groups (2.75 ± 0.88) (Fig. 3*C*). Intestinal mucosa was found thicker in treated groups. Dopamine group showed the greater value (546.1 ± 70.95) followed by NE (488.1 ± 136.5) and CG groups (472.4 ± 153.3), respectively (Fig. 3*D*). On the other hand, villi density was not substantially affected with the use

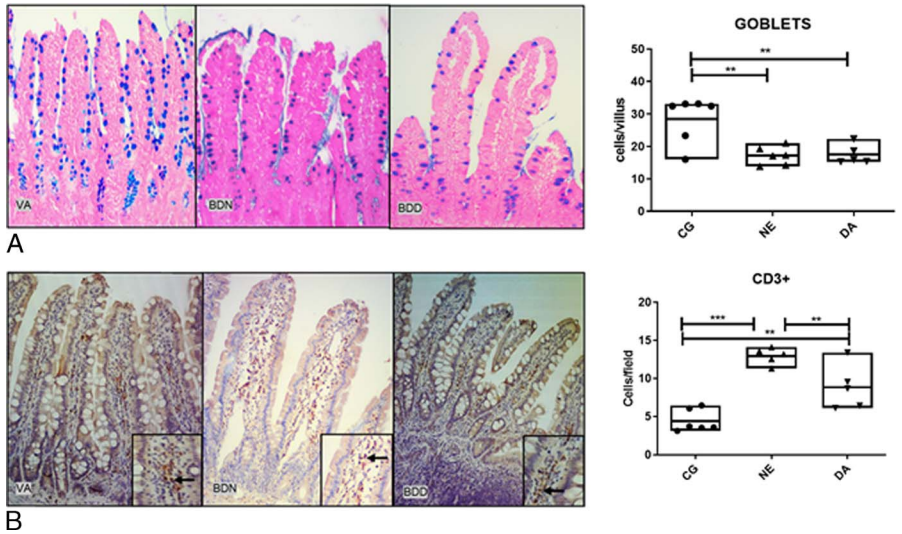

Figure 4. Differential cell population counting. (*A*) Representative microphotographs of Alcian Blue staining that allows the counting of GCs. The GC number per villi was evaluated for each individual by counting at least 25 individual villi. Box plot represents the mean value and superior and inferior quartiles of the distribution of values. Brain death significantly affects GC number, with no differential effect with either NE or DA treatment ($**p < 0.01$). (*B*) $CD3^+$ T lymphocyte immunohistochemical staining. Representative 10 times microphotograph (enlarged) and 40 times insets are depicted; black arrows indicate a positive CD3 T lymphocyte. $CD3^+$ count was significantly higher in NE group, almost triplicating the count for CG ($**p < 0.01$; $***p < 0.001$).

of vasopressors (CG, 10.08 ± 5.52; NE, 11.12 ± 6.17; and DA, 9.051 ± 2.93) (Fig. 3*E*).

BD Affects Goblet Cells Population in the Villi

Goblet cells (GCs) constitute one of the most significant innate defenses barriers against crypt mucosal injury, by preventing direct bacterial contact with enterocytes. Brain death significantly decreases the number of GCs all along the villi (Fig. 4*A*, $p < 0.01$). The CG showed a mean ± SD of 28.45 ± 7.18 GCs per villi; meanwhile, the average number of GCs per villi in the DA and NE groups was 17.54 ± 3.01 and 17.07 ± 2.8, respectively, showing that, regardless of the vasopressor drug used, there is a marked decrease in the GC count. This decrease in GCs is more pronounced in the NE group with respect to CG ($p = 0.0025$, *t* test performed) and a little less noticeable but still significant between CG and DA ($p = 0.0059$, *t* test performed).

$CD3^+$ Mucosal Infiltration Is Greater in Intestines Procured From Brain-Dead Donors Treated With NE

As previously mentioned, the organs from donors with BD are of lower quality compared with living donors; one of the aspects that impact on tissue status is the inflammatory activity in the lamina propria. We analyzed the lymphocyte infiltration on intestinal lamina propria under different situations. A mean ± SD of 4.4 ± 1.45 $CD3^+$ lymphocytes per 40 times field is observed in the CG; these values were duplicated in the case of DA-treated donors (DA, 8.8 ± 2.94) and were even higher in NE-treated donors (NE, 12.95 ± 1.07) (Fig. 4*B*; $p < 0.0001$). These results confirm in intestine what it was already described for other tissues[33,34] and show that NE treatment is associated with highest levels of lymphocyte infiltration in lamina propria.

Local Inflammatory Mediators Are Affected in BD

To investigate the inflammatory gene expression of the intestinal graft, the levels of IL-6, IL-22, and CXCL10 expression were evaluated in the different groups. Interleukin-22 showed a trend to lower expression in the DA and NE groups compared with the control, while, in the case of CXCL10, the NE group showed the highest expression of this cytokine compared with the other groups under study ($p = <0.05$). In the case of IL-6, NE was the group with a trend to show the highest expression of this cytokine, while DA and CG showed similar results (Figs. 5*A–C*).

The Use of NE Affects the Intestinal Barrier Permeability Amplifying Bacterial Translocation

Brain death has a deleterious effect on the intestinal barrier, promoting bacterial migration from the lumen to the bloodstream colonizing different abdominal organs.[35,36] By using NE, we could observe a significant increase in bacterial translocation to the liver, compared with the CG (CG vs. NE, $p = 0.044$; *t* test performed), whereas the use of DA showed intermediate results (Fig. 5*D*).

To perform this experiment, we use a sham control with no BD, showing no bacterial translocation to liver, validating the methodology used (not shown).

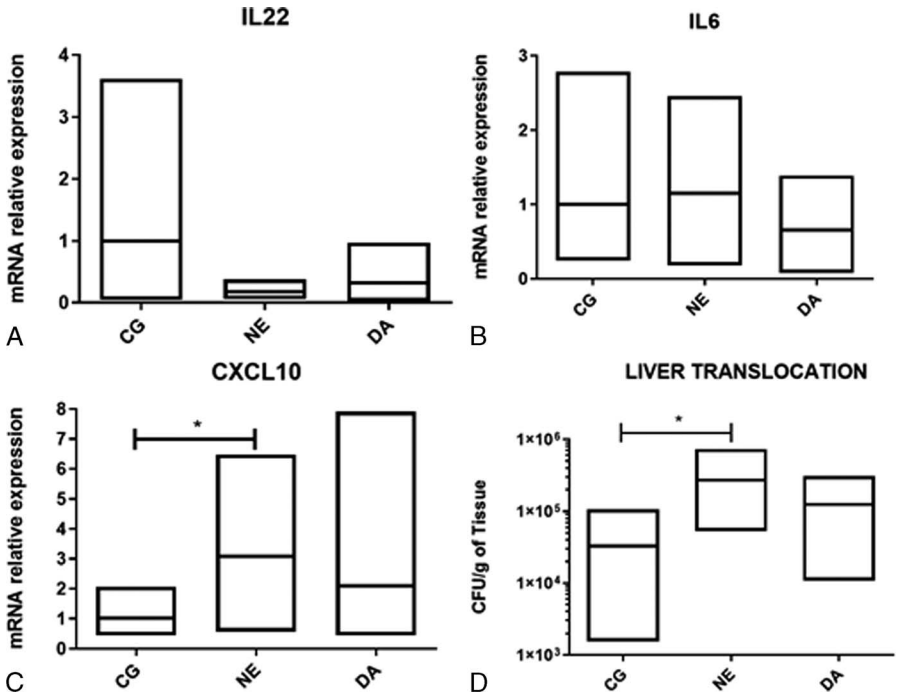

Figure 5. Gene expression evaluation and intestinal barrier function (*$p < 0.05$). (*A–C*) Gene expression in intestinal samples. (*A*) Interleukin-6 expression. No statistical differences between groups were observed. (*B*) Interleukin-22 expression. (*C*) CXCL10 expression. Higher values were observed in NE group compared with CG group (*$p < 0.05$). (*D*) Assessment of intestinal barrier integrity evaluated by total bacterial counting in liver. Norepinephrine group showed two orders of magnitude higher bacterial count than the CG group. Box plot represents the mean value and superior and inferior quartiles of the distribution of values.

DISCUSSION

Previous reports[37] described the direct negative effect of BD and maintenance on the procured grafts, but little has been written on the effect on the intestinal mucosa and intestinal graft.

Our results showed that alterations in hemodynamic parameters can produce alterations of the mucosal architecture; it was observed that changes were worse in animals under NE as vasopressor, compared with the ones receiving DA. Those observations might be based on the fact that DA increases superior mesenteric artery blood flow and is able to sustain appropriate small bowel oxygenation, as it was shown in a brain-dead pig model.[38] On the other hand, NE used in critical patients has been associated with enterocyte damage, which is in line with our observations, supporting our findings in a translational research model with rodents.[33]

Beyond hemodynamic instability, BD effects on the procured organs could be related to the release of proinflammatory mediators to circulatory compartment, which has been linked with changes in endothelial adhesion molecules.[5] This process causes a secondary effect by increasing immune cell infiltration.[37] In concordance, we found a marked increase of $CD3^+$ T lymphocytes in intestines from the brain-dead groups. Remarkably, the DA-treated animals showed significantly lower CD3 infiltrates compared with NE-treated animals. It has been reported that, under stressful clinical conditions, like heat stress; the use of NE produced a deficient expression of tight junction proteins,[39] increasing epithelial permeability inducing bacterial translocation and immune cell migration toward the intestinal mucosa, as observed in our experimental settings. In our experiments, the increased liver bacterial content could be attributed to changes described in mucosal permeability, causing a significant injury of the intestinal barrier function. Remarkably, we observed that the BD by itself produces a rise in bacterial traslocation, which is also in concordance with previous reports of increased systemic endotoxin in brain-dead animal model without using vasopressors.[17,39] Interestingly, we also observed a rise in proinflammatory gene expression in brain-dead animal. The fact that a reduced expression of IL-22 is found in brain-dead animals compared with controls adds that BD might also have an associated impairment of the intestinal epithelial regeneration pathway.[40]

There are several reviews on the superiority of NE versus DA in terms of shock events in ICUs;[41–43] even so, most of the parameters analyzed are focused on the patient under treatment and his/her survival. The situation of organ donation, in which the use of one or another vasopressor is important, could have an impact on the survival of the transplanted organs.[38,44] Although studies reported that the effect of high dose of vasopressor use has not shown differences in 1-year survival,[45] short-term effects that may be clinically relevant such as delayed graft function, ICU stay, length of dependence on ventilator assistance, and time to discharge, among others, were not evaluated. Besides this, there is also a lack of information regarding the number of donors that are not accepted to be used because of the high doses of NE used to sustain shock as result of the BD storm, which also requires the need of a progressive increase of fluid resuscitation, which also has a deleterious effect for the abdominal organs by producing edema and reducing the intestinal motility, as it is observed at the retrieval. An additional problem in most countries is related to the lack of recorded information, since, in general, those problems are registered as "poor organ/donor quality" without additional information, with pancreas and intestine being the most jeopardized organs.[46]

Although there is a lack of controlled studies that may contribute to the selection of vasopressive agent, the knowledge produced in this study might contribute to produce a clinical study aiming to maintain donors with the understanding of using the vasopressor that will benefit to preserve the integrity of the organ or organs to be used.

Our experimental study has the limitations inherent to the use of rodent models that make extrapolations to clinical situations not straightforward. We have selected a single period to analyze the effects of BD, a condition that may have dynamic evolution. We have also measured cell infiltrates without assessment of cell death/migration, which may account for part of the differences observed. Despite these limitations, our results highlight the impact of BD and the required support in the quality of the intestinal graft, generating evidence for further experimental studies on ischemia-reperfusion injury, graft rejection, and graft versus host disease. A better understanding on the underlying pathophysiology will allow improved donor management in the ICU and alternative preconditioning or postconditioning strategies to improve the quality and the availability of adequate organs to perform effective transplant procedures.[47,48]

Overall and in line with studies that suggest a beneficial effect of low-dose DA use to maintain donors,[33] our results indicate that DA should be considered as the main vasoactive drug to manage BD-associated hemodynamic instability by contributing to improve the quality of the intestinal graft, including the preservation of barrier function, and by reducing the risk for bacterial translocation.

AUTHORSHIP

L.E.V.D., P.L.S., and M.R. performed the study conception and design, data collection, analysis and interpretation of data, and manuscript writing. D.E.R., I.M.I.M., J.V., J.C.A.Z., and M.A.M. performed the data collection and analysis. G.E.G. and N.R.L. performed the critical revisions, which are important for the intellectual content.

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DISCLOSURE

The authors declare no conflicts of interest.

REFERENCES

1. Solar H, Ortega M, Gondolesi GE. Quality of life after intestinal transplantation. *Curr Opin Organ Transplant*. 2021;26(2):200–206.
2. Jansson-Knodell CL, Mangus RS, Gilmore AC. Intestinal failure: what all gastroenterologists should know. *Clin Gastroenterol Hepatol*. 2021;19(5):885–888.
3. Tzvetanov IG, Tulla KA, Benedetti E. Living donor intestinal transplantation. *Gastroenterol Clin North Am*. 2018;47(2):369–380.
4. Floerchinger B, Oberhuber R, Tullius SG. Effects of brain death on organ quality and transplant outcome. *Transplant Rev*. 2012;26(2):54–59.
5. Koudstaal LG, 't Hart NA, Ottens PJ, van den Berg A, Ploeg RJ, van Goor H, Leuvenink HG. Brain death induces inflammation in the donor intestine. *Transplantation*. 2008;86(1):148–154.

6. Meyfroidt G, Gunst J, Martin-Loeches I, Smith M, Robba C, Taccone FS, Citerio G. Management of the brain-dead donor in the ICU: general and specific therapy to improve transplantable organ quality. *Intensive Care Med*. 2019;45(3):343–353.
7. Lopau K, Mark J, Schramm L, Heidbreder E, Wanner C. Hormonal changes in brain death and immune activation in the donor. *Transpl Int*. 2000;13(Suppl 1):S282–S285.
8. Al-Khafaji A, Elder M, Lebovitz DJ, Murugan R, Souter M, Stuart S, et al. Protocolized fluid therapy in brain-dead donors: the multicenter randomized MOnIToR trial. *Intensive Care Med*. 2015;41(3):418–426.
9. Grootjans J, Hundscheid IH, Lenaerts K, Boonen B, Renes IB, Verheyen FK, Dejong CH, Von Meyenfeldt MF, Beets GL, Buurman WA. Ischaemia-induced mucus barrier loss and bacterial penetration are rapidly counteracted by increased goblet cell secretory activity in human and rat colon. *Gut*. 2013;62(2):250–258.
10. Oltean M. Intestinal preservation for transplantation: current status and alternatives for the future. *Curr Opin Organ Transplant*. 2015;20(3):308–313.
11. van Erp AC, van Dullemen LF, Ploeg RJ, Leuvenink HGD. Systematic review on the treatment of deceased organ donors. *Transplant Rev*. 2018;32(4):194–206.
12. Frenette AJ, Williamson D, Weiss MJ, Rochwerg B, Ball I, Brindamour D, Charbonney E. Worldwide management of donors after neurological death: a systematic review and narrative synthesis of guidelines. *Can J Anaesth*. 2020;67(12):1839–1857.
13. Benck U, Hoeger S, Brinkkoetter PT, Gottmann U, Doenmez D, Boesebeck D, Lauchart W, Gummert J, Karck M, Lehmkuhl HB, et al. Effects of donor pre-treatment with dopamine on survival after heart transplantation: a cohort study of heart transplant recipients nested in a randomized controlled multicenter trial. *J Am Coll Cardiol*. 2011;58(17):1768–1777.
14. Schnuelle P, Gottmann U, Hoeger S, Boesebeck D, Lauchart W, Weiss C, Fischereder M, Jauch KW, Heemann U, Zeier M, et al. Effects of donor pretreatment with dopamine on graft function after kidney transplantation: a randomized controlled trial. *JAMA*. 2009;302(10):1067–1075.
15. Rebolledo RA, Hoeksma D, Hottenrott CM, Bodar YJ, Ottens PJ, Wiersema-Buist J, Leuvenink HG. Slow induction of brain death leads to decreased renal function and increased hepatic apoptosis in rats. *J Transl Med*. 2016;14(1):141.
16. Vecchio Dezillio L, Romanin D, Ivanoff Marinoff I, Vernengo J, Abate Zárate J, Saves J, Machuca MLN, Stringa P, Rumbo M. *O-36: intestinal graft quality after norepinephrine and dopamine management in an experimental brain dead model. *Transplantation*. 2021;105(7S):S19.
17. Koudstaal LG, Ottens PJ, Uges DR, Ploeg RJ, van Goor H, Leuvenink HG. Increased intestinal permeability in deceased brain dead rats. *Transplantation*. 2009;88(3):444–446.
18. Takenaka MC, Guereschi MG, Basso AS. Neuroimmune interactions: dendritic cell modulation by the sympathetic nervous system. *Semin Immunopathol*. 2017;39(2):165–176.
19. You XY, Zhang HY, Han X, Wang F, Zhuang PW, Zhang YJ. Intestinal mucosal barrier is regulated by intestinal tract neuro-immune interplay. *Front Pharmacol*. 2021;12:659716.
20. Stringa P, Romanin D, Lausada N, Papa Gobbi R, Zanuzzi C, Martín P, Abate JC, Cabanne A, Arnal N, Vecchio L, et al. Gut permeability and glucose absorption are affected at early stages of graft rejection in a small bowel transplant rat model. *Transplant Direct*. 2017;3(11):e220.
21. Pratschke J, Wilhelm MJ, Kusaka M, Laskowski I, Tilney NL. A model of gradual onset brain death for transplant-associated studies in rats. *Transplantation*. 2000;69(3):427–430.
22. De Backer D, Creteur J, Silva E, Vincent JL. Effects of dopamine, norepinephrine, and epinephrine on the splanchnic circulation in septic shock: which is best? *Crit Care Med*. 2003;31(6):1659–1667.
23. De Backer D, Creteur J, Silva E, Vincent JL. Effects of dopamine, norepinephrine, and epinephrine on the splanchnic circulation in septic shock: which is best? *Crit Care Med*. 2003;31(6):1659–1667.
24. Goradia S, Sardaneh AA, Narayan SW, Penm J, Patanwala AE. Vasopressor dose equivalence: a scoping review and suggested formula. *J Crit Care*. 2021;61:233–240.
25. Park PO, Haglund U, Bulkley GB, Falt K. The sequence of development of intestinal tissue injury after strangulation ischemia and reperfusion. *Surgery*. 1990;107(5):574–580.
26. Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg*. 1970;101(4):478–483.
27. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671–675.
28. Abràmoff MD, Magalhães PJ, Ram SJ. Image processing with ImageJ. *Biophotonics Int*. 2004;11(7):36–42.
29. Casselbrant A, Söfteland JM, Hellström M, Malinauskas M, Oltean M. Luminal polyethylene glycol alleviates intestinal preservation injury irrespective of molecular size. *J Pharmacol Exp Ther*. 2018;366(1):29–36.
30. Mason DY, Cordell J, Brown M, Pallesen G, Ralfkiaer E, Rothbard J, Crumpton M, Gatter KC. Detection of T cells in paraffin wax embedded tissue using antibodies against a peptide sequence from the CD3 antigen. *J Clin Pathol*. 1989;42(11):1194–1200.
31. Ramos HC, Rumbo M, Sirard JC. Bacterial flagellins: mediators of pathogenicity and host immune responses in mucosa. *Trends Microbiol*. 2004;12(11):509–517.
32. Anderle P, Sengstag T, Mutch DM, Rumbo M, Praz V, Mansourian R, Delorenzi M, Williamson G, Roberts M-A. Changes in the transcriptional profile of transporters in the intestine along the anterior-posterior and crypt-villus axes. *BMC Genomics*. 2005;6(1):69.
33. Habes QLM, van Ede L, Gerretsen J, Kox M, Pickkers P. Norepinephrine contributes to enterocyte damage in septic shock patients: a prospective cohort study. *Shock*. 2018;49(2):137–143.
34. Gosain A, Jones SB, Shankar R, Gamelli RL, DiPietro LA. Norepinephrine modulates the inflammatory and proliferative phases of wound healing. *J Trauma*. 2006;60(4):736–744.
35. Weaver JL, Matheson PJ, Matheson A, Graham V, Harbrecht BG, Downard CD, Garrison RN, Smith JW. Direct peritoneal resuscitation reduces intestinal permeability after brain death. *J Trauma Acute Care Surg*. 2018;84(2):265–272.
36. Stanley D, Mason LJ, MacKin KE, Srikhanta YN, Lyras D, Prakash MD, Nurgali K, Venegas A, Hill MD, Moore RJ, et al. Translocation and dissemination of commensal bacteria in post-stroke infection. *Nat Med*. 2016;22(11):1277–1284.
37. Koudstaal LG, 't Hart NA, Van Den Berg A, Olinga P, Van Goor H, Ploeg RJ, Leuvenink HGD. Brain death causes structural and inflammatory changes in donor intestine. In: *Transplantation Proceedings* vol. 37. New York, NY: Elsevier; 2005.
38. Martikainen TJ, Kurola J, Kärjä V, Parviainen I, Ruokonen E. Vasopressor agents after experimental brain death: effects of dopamine and vasopressin on vitality of the small gut. *Transplant Proc*. 2010;42(7):2449–2456.
39. Luo Y, Ma H, Niu S, Li X, Nie L, Li G. Effects of norepinephrine on colonic tight junction protein expression during heat stress. *Exp Ther Med*. 2021;21(5):421.
40. Keir M, Yi Y, Lu T, Ghilardi N. The role of IL-22 in intestinal health and disease. *J Exp Med*. 2020;217(3):e20192195.
41. De Backer D, Biston P, Devriendt J, Madl C, Chochrad D, Aldecoa C, Brasseur A, Defrance P, Gottignies P, Vincent JL. Comparison of dopamine and norepinephrine in the treatment of shock. *N Engl J Med*. 2010;362(9):779–789.
42. Vasu TS, Cavallazzi R, Hirani A, Kaplan G, Leiby B, Marik PE. Norepinephrine or dopamine for septic shock: systematic review of randomized clinical trials. *J Intensive Care Med*. 2011;27(3):172–178.
43. Sakr Y, Reinhart K, Vincent JL, Sprung CL, Moreno R, Ranieri VM, De Backer D, Payen D. Does dopamine administration in shock influence outcome? Results of the Sepsis Occurrence in Acutely Ill Patients (SOAP) Study*. *Crit Care Med*. 2006;34(3):589–597.
44. Korte C, Garber JL, Descourouez JL, Richards KR, Hardinger K. Pharmacists' guide to the management of organ donors after brain death. *Am J Health Syst Pharm*. 2016;73(22):1829–1839.
45. Pahari H, Rizzari M, Safwan M, Khan H, Jafri SM, Muszkat Y, Collins K, Yoshida A, Abouljoud M, Nagai S. Effect of donor vasopressor requirements on recipient outcomes for intestinal and multi-visceral grafts — is it overrated? *Transplantation*. 2017;101(6S2):S17.
46. Wiseman AC, Wainright JL, Sleeman E, McBride MA, Baker T, Samana C, Stock P. An analysis of the lack of donor pancreas utilization from younger adult organ donors. *Transplantation*. 2010;90(5):475–480.
47. Malinoski DJ, Daly MC, Patel MS, Oley-Graybill C, Foster CE 3rd, Salim A. Achieving donor management goals before deceased donor procurement is associated with more organs transplanted per donor. *J Trauma*. 2011;71(4):990–995.
48. Salim A, Martin M, Brown C, Rhee P, Demetriades D, Belzberg H. The effect of a protocol of aggressive donor management: implications for the national organ donor shortage. *J Trauma Acute Care Surg*. 2006;61(2):429–435.