Norepinephrine and Arginine Vasopressin Increase Hepatic but Not Renal Inflammatory Activation During Hemodynamic Resuscitation in a Rodent Model of Brain-dead Donors

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Abstract

Objectives: **Hypotension that occurs after brain death causes a deterioration in organ function, which in turn restricts the number of organs that can be retrieved and leads to graft dysfunction. The correction of hypotension by the administration of norepinephrine increases the number of organs suitable for retrieval but is associated with cardiac allograft failure. Arginine vasopressin is relatively less cardiotoxic; however, the effect of that drug on intra-abdominal organs is unknown. We used a rodent model and real-time reverse transcription polymerase chain reaction to assess changes in the expression of inflammatory mediators in livers and kidneys that occurred in response to resuscitation with those drugs.**

Materials and Methods: **Fifty outbred male Wistar rats were anesthetized, and an intracranial balloon was inserted. In 35 rats, the balloon was inflated to induce brain death and subsequent hypotension. In 20 of those rats, hypotension was corrected with either norepinephrine (n = 10) or vasopressin (n = 10), while the remaining 15 rats received no resuscitation. Brain death was not induced in 15 rats that did not become hypotensive or receive resuscitation. Organs were retrieved 30 minutes, 2 hours, and 5 hours after balloon insertion, and inflammatory activation was assessed via real-time reverse transcription polymerase chain reaction.**

Results: **Significant time-dependent up-regulation of CXC motif chemokine ligand 1, interleukin-1ß, and heme oxygenase 1 occurred after brain death. Significantly greater up-regulation of CXC motif chemokine ligand and interleukin-1ß occurred in the livers of rats that received norepinephrine and vasopressin than in those that received no resuscitation. No increase in the expression of those mediators was noted in the kidneys.**

Conclusions: **This study showed that both norepinephrine and vasopressin amplified the inflammatory response that followed brain death in the livers, but not the kidneys, of rats in this model.**

Key words: *Brain death, Resuscitation, Norepinephrine, Vasopressin*

Brain-dead donors remain the major source of organs for transplant. Brain death is associated with profound physiologic and immunologic derangement, both of which lead to inferior recipient outcomes after organ transplant from a brain-dead donor (1). The most obvious changes that occur after brain death are hemodynamic, with severe hypotension observed in almost all such donors (2). Our group has previously shown that the transient hypertensive crisis that occurs at the onset of experimental brain death initiates a systemic inflammatory response (3). The hypertensive crisis is followed by severe hypotension that is caused by sympathetic collapse and compounded by volume depletion resulting from diabetes insipidus. The reduction in perfusion pressure exacerbates the systemic inflammatory response and causes ischemia in potential allografts.

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Ischemia impairs oxidative metabolism, increases anaerobic glycolysis, and causes adenosine triphosphate depletion (4). Those changes lead to a local inflammatory response characterized by the up-regulation of proinflammatory cytokines that include the CXC motif chemokine ligand (CXCL1) (5) and interleukin (IL)-1β (6). The effects of those cytokines may be somewhat mitigated by the increased expression of anti-inflammatory mediators such as heme oxygenase 1 (HO-1). However, the overall effect is to increase organ immunogenicity, which can lead to primary and chronic allograft dysfunction (7).

Early management of brain-dead donors can reduce local and systemic immunogenicity and increase the number of organs that are suitable for retrieval (8). Fluid administration corrects the volume deficit caused by diabetes insipidus and increases mean arterial pressure. This improves renal function but can also increase cardiac preload and impair alveolar gas exchange (9).

Fluid resuscitation alone is rarely sufficient to correct hypotension in marginal organ donors, and exogenous vasopressors may be required. Norepinephrine, which is the most commonly used agent, causes excessive Ca^{2+} influx in cardiomyocytes (10) and is associated with inferior outcomes after cardiac transplant (11).

Arginine vasopressin, a less cardiotoxic alternative to norepinephrine (12), is widely used in low doses to treat diabetes insipidus. The use of vasopressin at higher vasoactive doses is limited by the theoretical concern that it may exacerbate vasoconstriction in the abdominal organs (9). The effect of vasopressin on the liver and kidneys has not been assessed in the setting of the experimental brain-dead organ donor.

We sought to determine the effect of resuscitation with norepinephrine and vasopressin in a rodent model of the unstable brain-dead donor by comparing the regulation of inflammatory markers in rat livers and kidneys.

Materials and Methods

Animal model

Fifty adult male Wistar rats were used in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986 in an established model of the unstable brain-dead donor. All protocols were approved by a local ethics committee. The rats were anesthetized with 5% isoflurane, intubated with a 14-G cannula, and ventilated with an Inspira Advanced Safety Volume Control ventilator (Harvard Apparatus, MA, USA). Anesthesia was maintained with inhaled 2.5% isoflurane. A tail vein and femoral artery were cannulated to enable fluid administration and invasive arterial pressure monitoring. All rats received intravenous fluid replacement with 5 mL/kg/h of gelatin solution. A frontolateral cranial trepanation was made with a dental drill, and a 4F Fogarty catheter was inserted caudally.

The rats were assigned to 1 of 4 experimental groups. In the brain death group (n = 15), the intracranial catheter was inflated with 0.5 mL of water over 30 seconds to induce brain death, which was verified by pupil dilatation and the loss of brainstem auditory evoked potentials. In the norepinephrine group (n = 10) and the vasopressin group (n = 10), brain death was induced in the same manner as that used in the brain death group, but a continuous intravenous infusion of either norepinephrine or arginine vasopressin was initiated after balloon inflation and was titrated to maintain a mean arterial pressure of 60 mm Hg. In the sham group (n = 15), an intracranial catheter was inserted but not inflated. Those rats did not become brain dead or hypotensive and did not receive norepinephrine or vasopressin.

Livers and kidneys were retrieved 30 minutes, 2 hours and 5 hours after balloon insertion in the brain death and sham groups, were snap frozen in liquid nitrogen, and were stored at –80°C. Organs were retrieved from the norepinephrine and vasopressin groups at 2 and 5 hours only and were stored under the same conditions. Thus, 5 animals were used per treatment group per time point.

Ribonucleic acid extraction and real-time reverse transcription polymerase chain reaction

Total ribonucleic acid (RNA) was extracted from the livers and kidneys with the Tri-Reagent method (Sigma-Aldrich UK, Gillingham, UK). The quality of that RNA was proven by spectrophotometry and gel electrophoresis. The RNA samples were treated with deoxyribonuclease-1 (Invitrogen, Breda, The Netherlands) to reduce genomic deoxyribonucleic acid contamination. Reverse transcription was performed

with the Superscript-III reverse transcriptase method (Invitrogen) according to the manufacturer's instructions.

Sequence amplification and detection were performed with Taqman gene expression assays (Applied Biosystems, Foster City, CA, USA) and the Prism 7000 Sequence Detection System (Applied Biosystems). All reactions were run in duplicate. Reaction efficiency was proven for all genes by the amplification of serial dilutions. The absence of genomic deoxyribonucleic acid contamination was proven in all reactions by the amplification of control samples in which RNA was either absent or not reverse transcribed.

The relative expression of markers was assessed with the $2^{-\Delta\Delta CT}$ method, normalized to endogenous control genes, and validated by the method of Vandesompele and colleagues (13). Renal RNA was normalized to an average of β-glucuronidase and phosphoglycerate kinase 1 expression. Hepatic RNA was normalized to β-actin and TATA box binding protein.

Results

Hemodynamic changes

The induction of brain death in this model resulted in highly repeatable hemodynamic changes that have been previously described in detail (4). Intracranial balloon inflation led to brain death in all rats and to a concomitant hypertensive crisis. This was replaced by hypotension, and the mean arterial pressure deteriorated over the following 5 hours in the brain death group. The target mean arterial pressure of 60 mm Hg was achieved 15 minutes after the initiation of infusion in the norepinephrine group and 30 minutes after the initiation of infusion in the vasopressin group. The mean dosage of norepinephrine administered was 82 μg/kg/h (95% CI, 44-121), and the mean dosage of arginine vasopressin was 0.27 U/kg/h (95% CI, 0.22-0.32).

The effect of brain death on biomarker expression

The expression of CXCL1, IL-1β, and HO-1 messenger ribonucleic acid was significantly greater in liver and kidney samples from the brain death group at 2 and 5 hours after intracranial balloon insertion than in the sham group at 30 minutes following insertion (Figure 1). Significant up-regulation in both CXCL1 and HO-1 occurred in kidney samples from the sham group at 2 and 5 hours when compared with samples taken after 30 minutes. The expression of CXCL1 at 2 hours and Il-1β and HO-1 at 5 hours remained significantly greater in the brain death group than in the sham group at the equivalent time point.

In liver samples from the sham group, we noted a significant up-regulation in CXCL1 at 2 hours and in IL-1β and HO-1 at 2 and 5 hours when compared

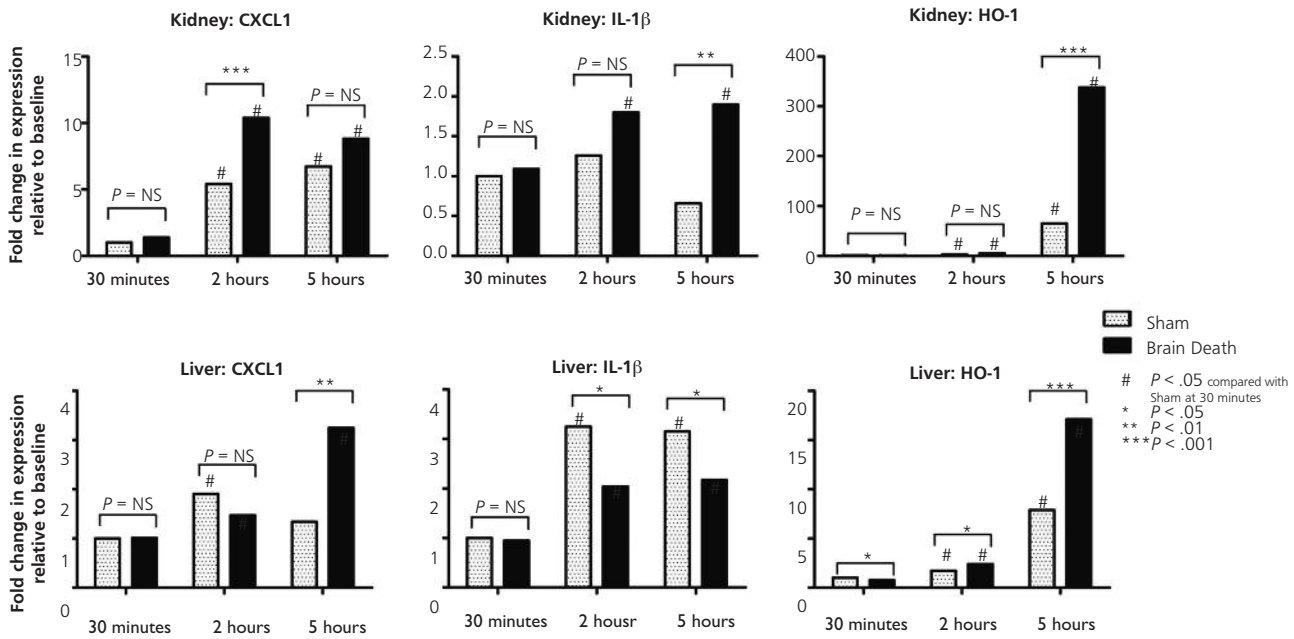

Figure 1. Comparison of the expression of biomarkers in the sham and brain death groups 30 minutes, 2 hours, and 5 hours after intracranial balloon insertion.

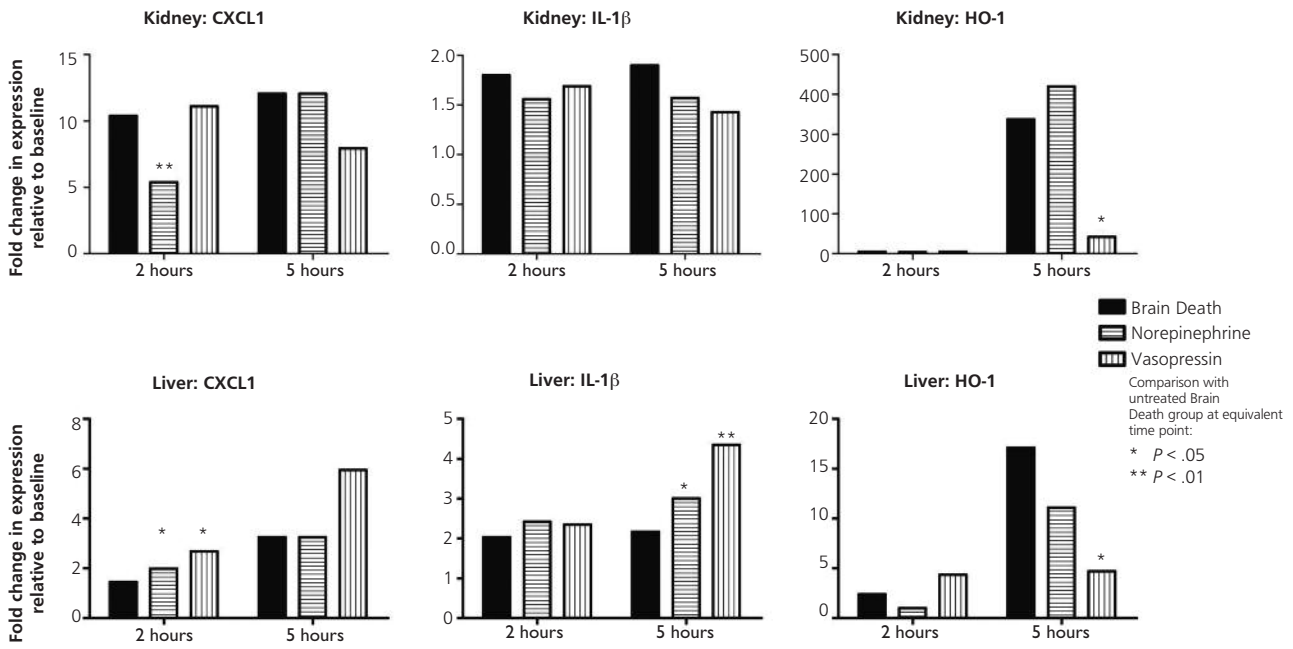

Figure 2. Comparison of the expression of biomarkers in the brain death (no resuscitation), norepinephrine, and vasopressin groups 2 and 5 hours after intracranial balloon insertion.

with samples taken at 30 minutes following balloon insertion.

In the kidney, CXCL1 expression was significantly lower in the norepinephrine group at 2 hours and HO-1 expression was significantly lower in the vasopressin group at 5 hours than in the brain death group at corresponding time points (Figure 2). There was no significant difference in IL-1β expression between the treated and untreated groups.

In liver samples from the norepinephrine and vasopressin groups, CXCL1 expression was significantly greater at 2 hours and Il-1β expression was significantly greater at 5 hours when compared to brain dead animals that did not receive resuscitation. HO-1 expression was significantly lower in the vasopressin group at 5 hours than in the brain death group. Rats in the brain death group exhibited significant metabolic acidosis that was attenuated in the groups treated with norepinephrine or vasopressin, as previously described (4).

Discussion

We have previously shown that hemodynamic resuscitation with norepinephrine can attenuate the progression of the systemic inflammatory response that follows brain death. Furthermore, both norepinephrine and vasopressin can reduce the expression of proinflammatory cytokines in the lungs and limit development of pulmonary edema (14). The results of this study suggest that neither norepinephrine nor vasopressin increases inflammatory activation in the kidneys. Those findings support the results of studies showing that norepinephrine (15) and vasopressin (16) do not tend to impair renal perfusion in other forms of shock and may improve function (17,18). The reduction in CXCL1 expression noted in the norepinephrine (but not the vasopressin) group at 2 hours might also have been due to the recognized anti-inflammatory effect of catecholamines (19).

Both norepinephrine and vasopressin increased the expression of CXCL1 and IL-1β in the liver. The mechanism for this is likely to be hemodynamic rather than immunologic. The stimulation of both V1 (20) and α-adrenoceptors (21) might impair liver function by reducing splanchnic perfusion. In a porcine model, Voelckel and colleagues have shown that vasopressin tends to reduce mesenteric blood flow to a greater degree than does norepinephrine, although those investigators found no macroscopic evidence of liver damage (22). Westphal and colleagues have demonstrated that the administration of vasopressin can decrease mesenteric mucosal perfusion and potentiate IL-6 expression (23). However, the relative effects of

vasopressin and norepinephrine on human mesenteric perfusion during shock remains controversial.

The pronounced reduction in HO-1 expression in vasopressin-treated animals is a novel finding. HO-1 induction is associated with improved recipient outcomes after liver or kidney transplant (24,25). Although vasopressin has been reported to reduce HO-1 expression in vitro (26), that phenomenon has not been described clinically. Further studies are required to determine whether the effect is clinically significant.

To our knowledge, this is the first study that has evaluated the effect of hemodynamic resuscitation with norepinephrine and vasopressin after brain death upon livers and kidneys. The rodent is an established model of brain death; however, a trial of human patients is required to determine the overall clinical relevance of the changes observed.

We concluded that both norepinephrine and arginine vasopressin can be used to resuscitate brain-dead rats and that those agents amplified the hepatic (but not the renal) inflammatory response that follows brain death in that model.

References

1. Terasaki PI, Cecka JM, Gjertson DW, Takemoto S. High survival rates of kidney transplants from spousal and living unrelated donors. N Engl J Med. 1995;333(6):333-336.
2. Salim A, Martin M, Brown C, Belzberg H, Rhee P, Demetriades D. Complications of brain death: frequency and impact on organ retrieval. Am Surg. 2006;72(5):377-381.
3. Avlonitis VS, Wigfield CH, Kirby JA, Dark JH. The hemodynamic mechanisms of lung injury and systemic inflammatory response following brain death in the transplant donor. Am J Transplant. 2005;5(4 Pt 1):684-693.
4. Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. Lancet. 2004;364(9447):1814-1827.
5. Molls RR, Savransky V, Liu M, et al. Keratinocyte-derived chemokine is an early biomarker of ischemic acute kidney injury. Am J Physiol Renal Physiol. 2006;290(5):F1187-F1193.
6. Kato A, Gabay C, Okaya T, Lentsch AB. Specific role of interleukin-1 in hepatic neutrophil recruitment after ischemia/reperfusion. Am J Pathol. 2002;161(5):1797-1803.
7. Pratschke J, Wilhelm MJ, Kusaka M, et al. Brain death and its influence on donor organ quality and outcome after transplantation. Transplantation. 1999;67(3):343-348.
8. Rosendale JD, Chabalewski FL, McBride MA, et al. Increased transplanted organs from the use of a standardized donor management protocol. Am J Transplant. 2002;2(8):761-768.
9. Pennefather SH, Bullock RE, Dark JH. The effect of fluid therapy on alveolar arterial oxygen gradient in brain-dead organ donors. Transplantation. 1993;56(6):1418-1422.
10. Hori M, Sato H, Kitakaze M, et al. Beta-adrenergic stimulation disassembles microtubules in neonatal rat cultured cardiomyocytes through intracellular Ca2+ overload. Circ Res. 1994;75(2):324-334.
11. Schnuelle P, Berger S, de Boer J, Persijn G, van der Woude FJ. Effects of catecholamine application to brain-dead donors on graft survival in solid organ transplantation. Transplantation. 2001;72(3):455-463.
12. Rostron AJ, Avlonitis VS, Kirby JA, Dark JH. Haemodynamic resuscitation of the brain-dead organ donor and the potential role of vasopressin. Transplant Rev. 2007;21(1):34-42.
13. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002;3(7):RESEARCH0034.
14. Rostron AJ, Avlonitis VS, Cork DM, Grenade DS, Kirby JA, Dark JH. Hemodynamic resuscitation with arginine vasopressin reduces lung injury after brain death in the transplant donor. Transplantation. 2008;85(4):597-606.
15. Bellomo R, Kellum JA, Wisniewski SR, Pinsky MR. Effects of norepinephrine on the renal vasculature in normal and endotoxemic dogs. Am J Respir Crit Care Med. 1999;159(4 Pt 1):1186-1192.
16. Kang CH, Kim WG. The effect of vasopressin on organ blood flow in an endotoxin-induced rabbit shock model. J Invest Surg. 2006;19(6):361-369.
17. Holmes CL, Walley KR, Chittock DR, Lehman T, Russell JA. The effects of vasopressin on hemodynamics and renal function in severe septic shock: a case series. Intensive Care Med. 2001;27(8):1416-1421.
18. Redl-Wenzl EM, Armbruster C, Edelmann G, et al. The effects of norepinephrine on hemodynamics and renal function in severe septic shock states. Intensive Care Med. 1993;19(3):151-154.
19. Panina-Bordignon P, Mazzeo D, Lucia PD, et al. Beta2-agonists prevent Th1 development by selective inhibition of interleukin 12. J Clin Invest. 1997;100(6):1513-1519.
20. Woolsey CA, Coopersmith CM. Vasoactive drugs and the gut: is there anything new? Curr Opin Crit Care. 2006;12(2):155-159.
21. Zhang H, De Jongh R, De Backer D, Cherkaoui S, Vray B, Vincent JL. Effects of alpha - and beta -adrenergic stimulation on hepatosplanchnic perfusion and oxygen extraction in endotoxic shock. Crit Care Med. 2001;29(3):581-588.
22. Voelckel WG, Lindner KH, Wenzel V, et al. Effects of vasopressin and epinephrine on splanchnic blood flow and renal function during and after cardiopulmonary resuscitation in pigs. Crit Care Med. 2000;28(4):1083-1088.
23. Westphal M, Freise H, Kehrel BE, Bone HG, Van Aken H, Sielenkämper AW. Arginine vasopressin compromises gut mucosal microcirculation in septic rats. Crit Care Med. 2004;32(1):194-200.
24. Kato H, Amersi F, Buelow R, et al. Heme oxygenase-1 overexpression protects rat livers from ischemia/reperfusion injury with extended cold preservation. Am J Transplant. 2001;1(2):121-128.
25. Exner M, Böhmig GA, Schillinger M, et al. Donor heme oxygenase-1 genotype is associated with renal allograft function. Transplantation. 2004;77(4):538-542.
26. Ishizaka N, Griendling KK. Heme oxygenase-1 is regulated by angiotensin II in rat vascular smooth muscle cells. Hypertension. 1997;29(3):790-795.