Effects of Cyclodextrin Curcumin Formulation on Ischemia-Reperfusion Injury in Porcine DCD Liver Transplantation

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Background. Curcumin is a pleiotropic antioxidant polyphenol, which has proven to be highly protective in various models of liver injury and inflammation. We hypothesized that adding a stable aqueous curcumin formulation which comprises a water-soluble cyclodextrin curcumin formulation (CDC) complex of the water-insoluble curcumin molecule (Novobion, Espoo, Finland) to preservation solution during liver procurement may reduce ischemia-reperfusion injury and improve graft function after liver transplantation using donation after circulatory death (DCD). **Methods.** In a preclinical pig model of DCD-liver transplantation, livers exposed to 15' of warm ischemia were either modulated (N = 6) with a flush of preservation solution (histidine-tryptophan-ketoglutarate) containing CDC (60 μmol/L) through the vena porta and the aorta, or not (controls, N = 6) before 4 h of cold storage. Area under the curve of log serum aspartate aminotransferase, markers of graft function (lactate, glycemia, prothrombin time, and bile production), inflammation (tumor necrosis factor-alpha), and survival were monitored. **Results.** Area under the curve of log serum aspartate aminotransferase were similar between curcumin and control groups (22.12 [20.87–24.88] versus 25.08 [22.1–26.55]; $P = 0.28$). No difference in the liver function markers were observed between groups except a lower serum lactate level 3-h post-reperfusion in the curcumin group (3 [1.95–6.07] versus 8.2 [4.85–13.45] mmol/L; $P = 0.05$). Serum tumor necrosis factor-alpha levels were similar in each group. Recipient survival rates were found similar. **Conclusions.** CDC added to the preservation solution in DCD liver pig model did not improve ischemia-reperfusion injury severity, liver function, or survival. Further efforts are needed to explore this strategy, particularly with dynamic preservation, which finds its way into clinical practice.

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INTRODUCTION

Livers previously considered unsuitable for transplantation or so-called extended criteria donors (donor age >65 y, graft steatosis, prolonged cold ischemia time [CIT], and donation after circulatory death [DCD]) are increasingly used for transplantation to attenuate the imbalance between organ shortage and the unacceptable rate of patient mortality on the waiting list.[1] Such high risk and especially DCD livers are more susceptible for ischemia-reperfusion injury (IRI).[2] During DCD liver transplantation (LT), the cumulative ischemic injury of (1) warm ischemia (WI) injury before organ preservation, (2) cold injury during preservation, and (3) ischemic rewarming injury during surgical implantation and the liver graft reperfusion elicit a complex cascade of events with a self-amplifying loop of inflammation and cellular damage, known as IRI.[3] Although IRI is unavoidable during LT, its degree may vary from minimal to severe and/or full destruction of the graft.[4] IRI may clinically go

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N.M. performed the literature research and collected retrospective data from their previous liver transplantation model to generate the hypothesis. N.M. and D.M. designed the study and drafted the article. N.M., T.W., V.H., and L.C. participated in the performance of the study. N.M., S.F., and D.M. analyzed the data. J.P. and I.J. co-authored the writing of the article and contributed to the study design. All authors read and approved the final article for publication.

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unnoticed (immediate graft function with no or minimal damage), present as dysfunction (10%–30%) or as graft failure (<5%). The biliary tree is regarded especially vulnerable to IRI, and ischemic cholangiopathy[5] are a common cause of IRI-related late graft loss. Graft failure, dysfunction, and ischemic cholangiopathy negatively affect both short- and long-term outcomes in terms of morbidity and mortality.[4,6] Accordingly, understanding and attenuating hepatic IRI is an imperative strategy to improve outcomes of liver grafts. Past decade has seen remarkable advances in the field of organ preservation providing experimental data evidence that tackling IRI with pharmacological agent is a promising strategy.[4] Conditioning preservation solutions with agents with pleiotropic properties have been of particular interest as associated to a reduction of reactive oxygen species (ROS) and ROS-induced effects, blockade of immune activation, or modulation of cytokine response.[7]

Curcumin is a natural polyphenol derived from root of the plant Curcuma longa and is well-known for its antioxidant and anti-inflammatory properties, as shown in diverse experimental rodent models of liver diseases and damages, for example, cirrhosis, steatohepatitis, and cholestasis.[8-10] Protective effects by targeting the liver IRI inflammatory cascade by curcumin have also been described in various rodent models.[11-15] In these models of in situ WI with[11,12] or without reperfusion,[13] ex situ liver perfusion,[14] and LT,[15] a strong anti-inflammatory effect by curcumin is exerted through prevention of ROS formation,[11,13-15] induction of antioxidant enzymes (glutathione transferase, heme oxygenase-1, and catalase),[11,13] and inhibition of nuclear factor kappa B activation and nuclear factor kappa B-induced pro-inflammatory cytokine expression tumor necrosis factor-alpha (TNF-α) and interleukin-1B and -6[12,14,15] resulting in a reduction of liver injury serum markers aspartate transaminase (AST) and alanine transaminase[11,12,14] and apoptosis histological assessment.[11-15]

Despite curcumin's potential to tackle LT-related IRI, use of curcumin is impaired by its hydrophobic properties and low systemic bioavailability after oral dosing[16] that precludes a more widespread clinical applicability. Various strategies have been developed to enhance curcumin tissue delivery as liposomes incorporation[17] and phospholipids encapsulation.[18] Herein, we used a water-soluble cyclodextrin curcumin formulation (CDC), well-known to reduce curcumin insolubility and improve its bioavailability, which can be added to the preservation solutions used in transplantation.[19] Reportedly, flushing and preserving porcine DCD-kidneys with 65 μM CDC in preservation solution resulted in better recovery of kidney function, improved histology (delayed onset of chronic graft fibrosis), reduced inflammation, and was associated with better recipient survival.[20] Supplementation of curcumin dissolved and added to different cold preservation solutions was found feasible and beneficial in an isolated rat liver perfusion model resulting in decreased post-reperfusion AST/alanine transaminase levels and an improvement of liver function recovery as assessed by a decrease of lactate levels and bile flow.[21]

Despite beneficial effects reported in rodent IRI model with 60 μM CDC,[13] and a porcine kidney transplantation model with 65 μM CDC,[20] the effect of water-soluble CDC formulation on hepatic IRI in a large preclinical LT model has not yet been assessed. Therefore, based on the design and using an almost similar efficacy-proven dose of these previous studies (60 μM CDC),[13,20] we aimed to evaluate the effect of a CDC formulation supplemented to the cold flush and preservation solution on IRI severity and inflammation, liver function, and recipient survival in a stringent and well-validated porcine DCD-LT model.[22]

MATERIALS AND METHODS

Study Design

The 2 study groups differed with respect to the post-WI flush solutions. In the control group, flush was performed with Ringer followed by histidine-tryptophan-ketoglutarate (HTK), whereas in the curcumin group it was both Ringer and HTK that were supplemented with CDC (1.5 mL/L to 60 μM), as liver graft conditioning. The dose of 60 μM CDC was deliberately chosen for the design of this study. Indeed, no titration experiment were warranted as obvious efficiency against IRI was described in an in vitro and renal autotransplantation pig DCD model with 65 μM CDC dissolved in the preservation solution.[20] Accordingly, we previously showed protection against oxidative stress when donor livers were conditioned with an intra-portal injection of 60 μM CDC in a rodent model of in situ WI.[13]

CDC Formulation

CDC was manufactured as a sterile solution under good medical practice conditions as described earlier[19] and provided by Novobion (Espoo, Finland). The CDC solution contained 12 mg/mL of curcumin and was stored at 2–8 °C.

Animals

Inbred male pigs (Topigs 20; T. Janssen, Nijmegen, The Netherlands) weighing 25–30 kg were used as donors and recipients and housed in a conventional, closed housing system. They received a maintenance diet (MPig-H; ssniff, Soest, Germany) with unlimited access to tap water. Twelve hours before surgery, they were fasted with unlimited access to water. Experiments were approved by the animal care committee (KU Leuven, P079/2011) and performed in accordance with the European guidelines regarding animal welfare.[23]

Anesthesia and Surgery

A validated IRI pig model of controlled DCD-LT was used as described earlier.[22] In brief, donor pigs were premedicated with intramuscular Xylazine 10 mg/kg (Arendonk, Belgium) and Tiletamine/Zolazepam 20 mg/kg (Virbac, Barneveld, The Netherlands). After endotracheal intubation, general anesthesia was induced and maintained by intravenous (IV) fentanyl (8 μg/kg/h as a continuous infusion), Pavulon (1 mg/kg 1–2 mL in bolus every 30–45 min), and 0.6%–1% isoflurane mixture of 40%–50% O_2 and room air. Catheters were inserted in the left carotid artery and the external jugular vein. Through a midline laparotomy, the liver was freed from its peritoneal attachments and the common bile duct, portal vein, and hepatic artery were dissected. The infrarenal aorta and inferior vena cava were prepared for cannulation. IV heparin (500 U/Kg) was given 5 min before cardiac arrest. To mimic controlled DCD, cardiac arrest was provoked by mechanical ventricular fibrillation after insertion, under direct vision, of a needle directly in the ventricular muscle through the diaphragm and connected

to the pulse generator (amplitude range ±15 volts, currency <300 mAmp at a frequency of 502 Hz). Ventricular fibrillation and cardiac arrest were immediately confirmed on electrocardiogram with subsequent and concomitant drop of the arterial pressure, resulting in an almost absent agonal phase. Concomitantly with ventricular fibrillation, pigs were extubated. After exposing the unperfused liver to 15 min of WI, the liver was flushed by gravity (via portal vein and the hepatic artery simultaneously) with a warm (25 °C) Ringer pre-flush (1 L) and a consecutive cold (4 °C) HTK flush solution (9 L) with or without supplemental CDC (1.5 mL/L to 60 μM). After the hepatectomy, livers were cold stored in 1 L of cold HTK for 4 h (4 °C). WI was defined as the time period from the initiation of cardiac arrest until the start of cold perfusion. CIT was defined as the period from the start of cold perfusion until the liver graft was taken of the preservation solution at the start of the implantation.

The recipient pig was anesthetized as described above and underwent hepatectomy of the native liver. The donor liver graft was first reperfused after completion of the suprahepatic inferior vena cava and the portal vein anastomoses, followed by anastomosing the infrahepatic inferior vena cava and the hepatic artery in continuity with an aortic conduit. During the anhepatic phase, no veno-venous bypass was used, and it was described as well-tolerated if kept short.[22] Mean arterial and central venous pressures were monitored throughout the procedure. Bile was drained externally and readministrated BID through a feeding jejunostomy to prevent interruption of the entero-hepatic biliary circulation.

Postoperatively, animals received standard fluid replacement and analgesia with intramuscular buprenorphine 0.01 mg/12 h (Temgesic). Tacrolimus (0.1 mg/kg) was given orally and BID. To minimize confounding effects caused by rejection, sepsis, and other later-onset phenomena, the observation period was limited to 4 d. On day 4, animals were anesthetized as described previously.[22] Following anesthesia, relaparotomy and graft inspection (anastomoses and macroscopic aspect of the liver) were performed, and animals were euthanized by IV potassium chloride injection. Autopsy was performed in animals who survived <4 d.

Analyses

The primary endpoint of the study was the effect of CDC liver graft conditioning on the degree of IRI as reflected by AST, an accepted and early surrogate parameter of liver IRI,[24] as measured during 4 d post-reperfusion and by the calculated area under the curve (AUC) of the log AST within the first 3-h post-reperfusion. Secondary endpoints were pathophysiological parameters of IRI and included the incidence of primary nonfunction (PNF), markers of liver function, inflammation, and recipient survival at day 4. PNF was defined as post-LT encephalopathy (failure to wake up after LT despite standard cessation of anesthetics at 60 min after reperfusion), irreversible metabolic acidosis (lactate >7 mmol/L, pH <7.1), profound hypoglycemia (<30–50 mg/dL), coagulopathy (massive hemorrhagic ascites and prothrombin time [PT] <20%–30%), and very low (<2 mL) or absent bile production within 3 h after reperfusion.[22] Conversely, liver function was assessed from the recipient's ability to recover from metabolic acidosis, hypoglycemia, coagulopathy, and bile production impairment. Serum AST (IU/L), lactate (mmol/L), and glucose (mg/dL) were determined after donor and recipient induction as baseline, at 5 and 15 min, 1, 2, and 3 h after reperfusion, and on postoperative days (PODs) 1, 2, 3, and 4. PT was measured at recipient induction as baseline, 1, 2, and 3 h, and POD 1. Bile production (mL) was measured within the first 3-h post-reperfusion and collected once a day until POD 4. Serum TNF-α, a parameter of inflammation and activation of liver Kupffer cells, was analyzed from recipient induction to POD 2 (pg/mL).

Statistics

Continuous data were compared between groups using Student's *t* test (normality criteria were met) or using Mann-Whitney *U* test (normality criteria were not met). Recipient survival rate was calculated by Kaplan-Meier analysis. $P < 0.05$ were considered statistically significant.

Sample size was calculated on the basis of the AUC of log AST values measured within the first 3 h after graft reperfusion and obtained in a series of prior experiments with the same DCD-LT model. These values followed a log-normal distribution. We assumed that would allow for a 20% reduction of the mean AUC of log AST. Using a 2-sided 2-sample pooled *t* test of the mean ratio with log-normal data, we determined that 6 animals were needed to obtain a difference between groups of 20%, with 80% power and alpha of 0.05.

RESULTS

Baseline Demographic Data

Baseline demographics, namely weight of the donor and recipient pigs and weight of the donor liver were comparable between the curcumin and control groups (Table 1).

TABLE 1.

Baseline demographics and operative characteristics between the curcumin and control groups

Variables	Control (n = 6)	Curcumin (n = 6)	*P*
Weight of pig (kg)			
Donor	34.8 (30.3–37.4)	41 (35.1–50.7)	0.06
Recipient	35.7 (33.1–36.6)	41.77 (34.5–48.3)	0.18
Weight of liver (g)	927 (855–1029.5)	1115 (938.5–1203.5)	0.18
Cold ischemic time (min)	256.5 (245–269.5)	263 (258.5–266.5)	0.48
Warm ischemic time (min)	16 (15–16.7)	15.5 (15–17.2)	0.82
Anhepatic phase (min)	21.5 (21–23)	24 (21.7–25)	0.09

Data are given as median (interquartile range).

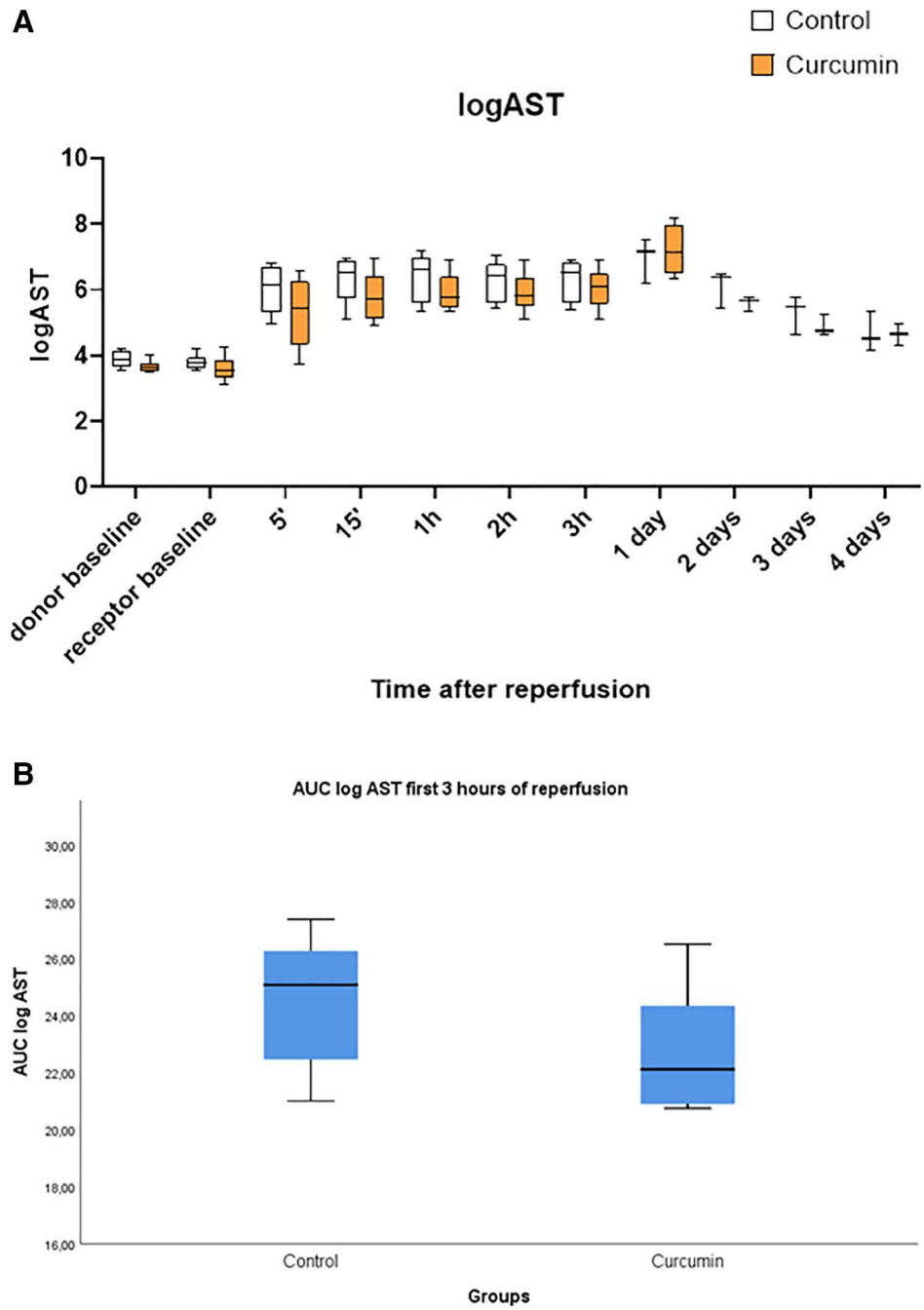

FIGURE 1. Effect of CDC on the degree of IRI. Box plots of serum log AST levels before (baseline) and after liver graft reperfusion until postoperative day 4 (POD 4) (A) and AUC log AST within the first 3 h after liver reperfusion in control and curcumin groups (B). Median values, interquartile ranges, and ranges are represented by horizontal bars, boxes, and error bars, respectively. Differences between control and curcumin groups were not significant. AUC, area under the curve; CDC, cyclodextrin curcumin formulation; IRI, ischemia-reperfusion injury; log AST, log aspartate transaminase.

Operative characteristics such as CIT, WI time, and anhepatic phase duration were not different between both groups.

Effect of CDC on the Degree and Pathophysiological Parameters of IRI and Liver Function

After reperfusion, serum AST levels and log AST were not statistically different between the curcumin and the control groups. The peaks of AST were observed on POD1 in each group and were similar between the curcumin and control groups: (1271 [810–2214] versus 1285 [885–1540] IU/L; $P = 0.55$) (Figure 1A). The AUC of log AST within 3-h post-reperfusion was similar between the curcumin and control groups: (22.12 [20.87–24.88] versus 25.08 [22.1–26.55]; $P = 0.28$) (Figure 1B).

PNF was observed in none of the 6 pigs in the curcumin group (0%) and 2 of 6 pigs in the control group (33%) ($P = 0.45$). After a similar increase of serum lactate levels in both groups during the first 15 min post-reperfusion; lactate declined to normal levels more rapidly in the curcumin than the control group (Figure 2A) and was significantly lower at 3-h post-reperfusion (3 [1.95–6.07] versus 8.2 [4.85–13.45] mmol/L; $P = 0.05$]. Lactate returned to normal (≤1

mmol/L) in both groups at POD 3. There were no differences between the curcumin and the control groups in terms of post-reperfusion glycemia levels, PT recovery, and bile production (Figure 2B–D). Serum total bilirubin (0.18 [0.18–0.48] versus 0.46 [0.35–0.84] mg/dL; $P = 0.37$) and alkaline phosphatase (204 [199–210] versus 276 [255–296]

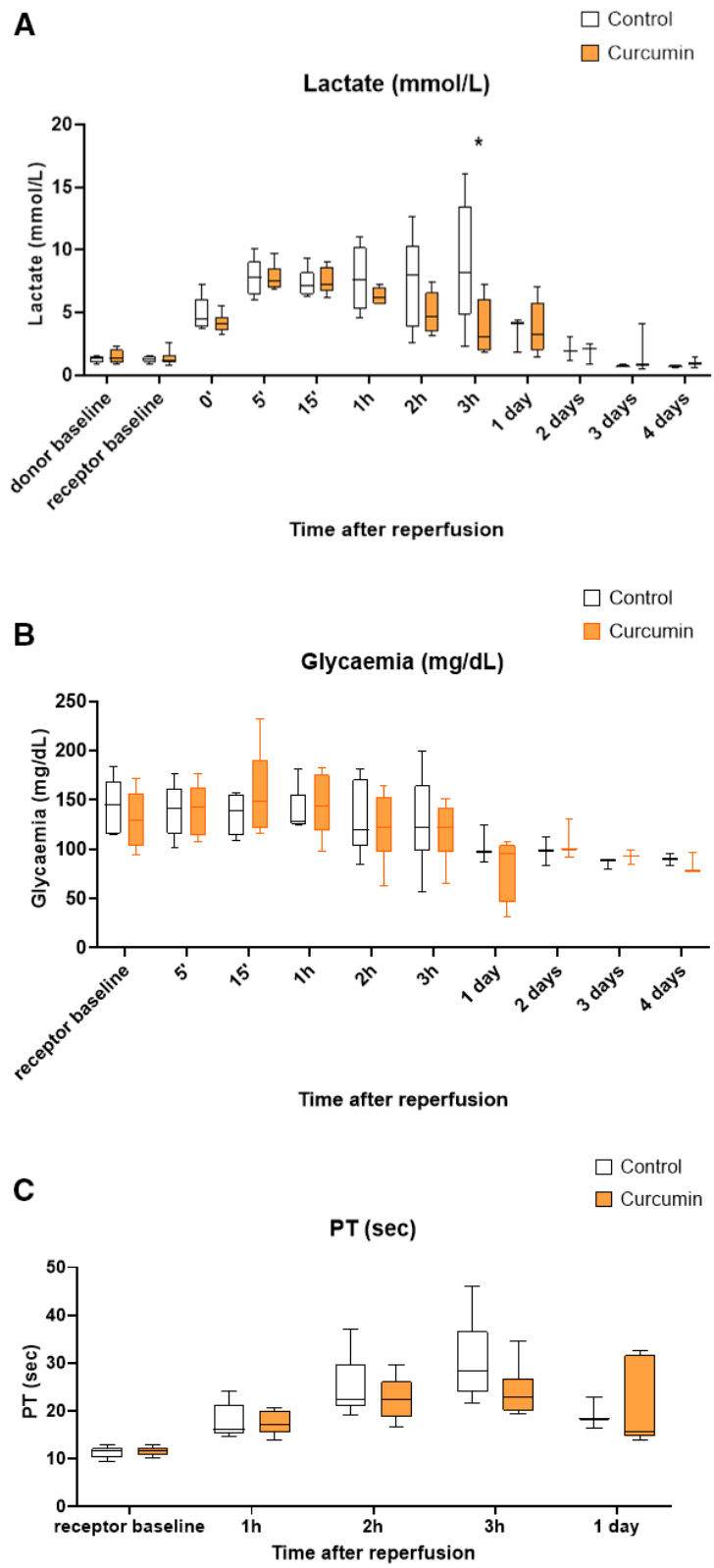

FIGURE 2. Effect of CDC on pathophysiological parameters of IRI and liver function. Box plots of serum lactate levels (A), serum glycemia levels (B), PT (C), and bile production (D) after liver reperfusion in control and curcumin groups. There was no significant difference between groups except for serum lactate levels at 3-h post-reperfusion that were found significantly lower in the curcumin group (*$P = 0.05$). CDC, cyclodextrin curcumin formulation; IRI, ischemia-reperfusion injury; PT, prothrombin time. (*Continued*)

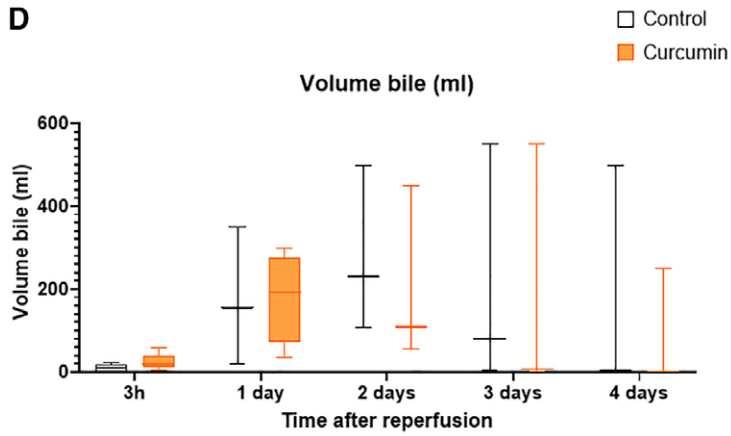

FIGURE 2. Continued.

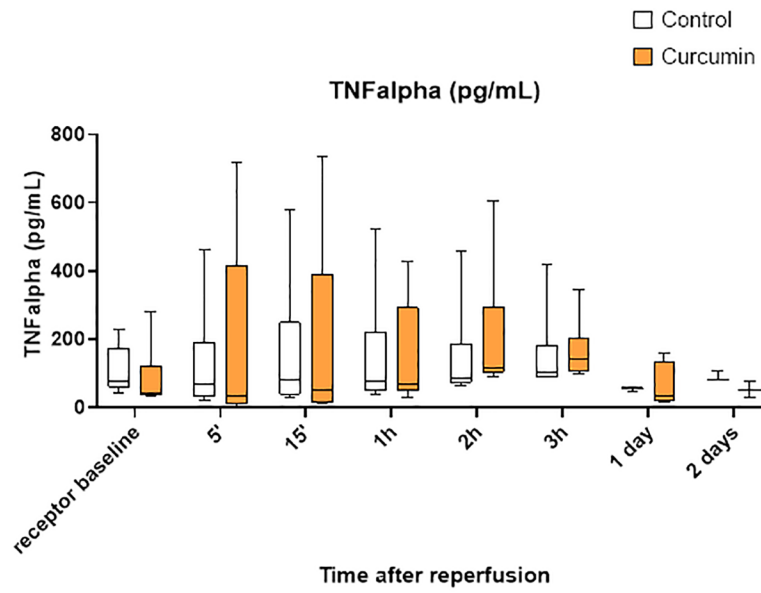

FIGURE 3. Box plots of serum TNF-α concentration in control and curcumin groups before (baseline) and after liver graft reperfusion. No difference was observed between groups. TNF-α, tumor necrosis factor-alpha.

U/L; $P = 0.25$) at POD 1 were similar between curcumin and control groups, respectively. In both groups, serum TNF-α levels similarly increased immediately after graft reperfusion and declined thereafter (Figure 3).

Effect of CDC on Recipient Survival

In both groups, 3 of 6 (50%) pigs were alive at POD 4. In the control group, 2 recipients developed PNF (could not be weaned from the ventilator) and died within 3 h after graft reperfusion. Another recipient in the control group could be weaned from the ventilator but died after 6 h because of severe liver dysfunction (irreversible hypoglycemia and acidosis) and hemorrhagic ascites was found at autopsy. In the curcumin group, 1 pig died at 6 h and 2 pigs on POD 1 after reperfusion because of severe graft dysfunction (Figure 4).

DISCUSSION

In this study, flushing and preserving liver grafts with a water-soluble curcumin supplemented preservation solution resulted in a lower immediate graft failure but not in less IRI severity, improved liver function, decreased inflammation, or superior recipient survival after porcine DCD-LT.

Our findings could not confirm any of the protective effects exerted by the antioxidant and anti-inflammatory properties of curcumin, as observed in various rodent models of IRI (lower post-reperfusion transaminase, better survival, and lower TNF-α concentration)[11-15] and in a renal porcine autotransplantation model (better function and recipient survival).[20]

One possible explanation for this nonbeneficial effect may relate to the moment of curcumin administration. Reportedly, rodent models of LT in which donors were pretreated by intraperitoneally injection up to 7 d (100 mg/kg)[15] before the ischemic injury, result in a reduction of liver injury while improving liver function and overall post-LT survival.[15] Such a prolonged pretreatment seems necessary since oral administration just before the ischemic injury does not mitigate the IRI while ensuring adequate protective levels before the ischemic injury as shown in this rat model of in situ WI induced by Pringle maneuver,[25] even

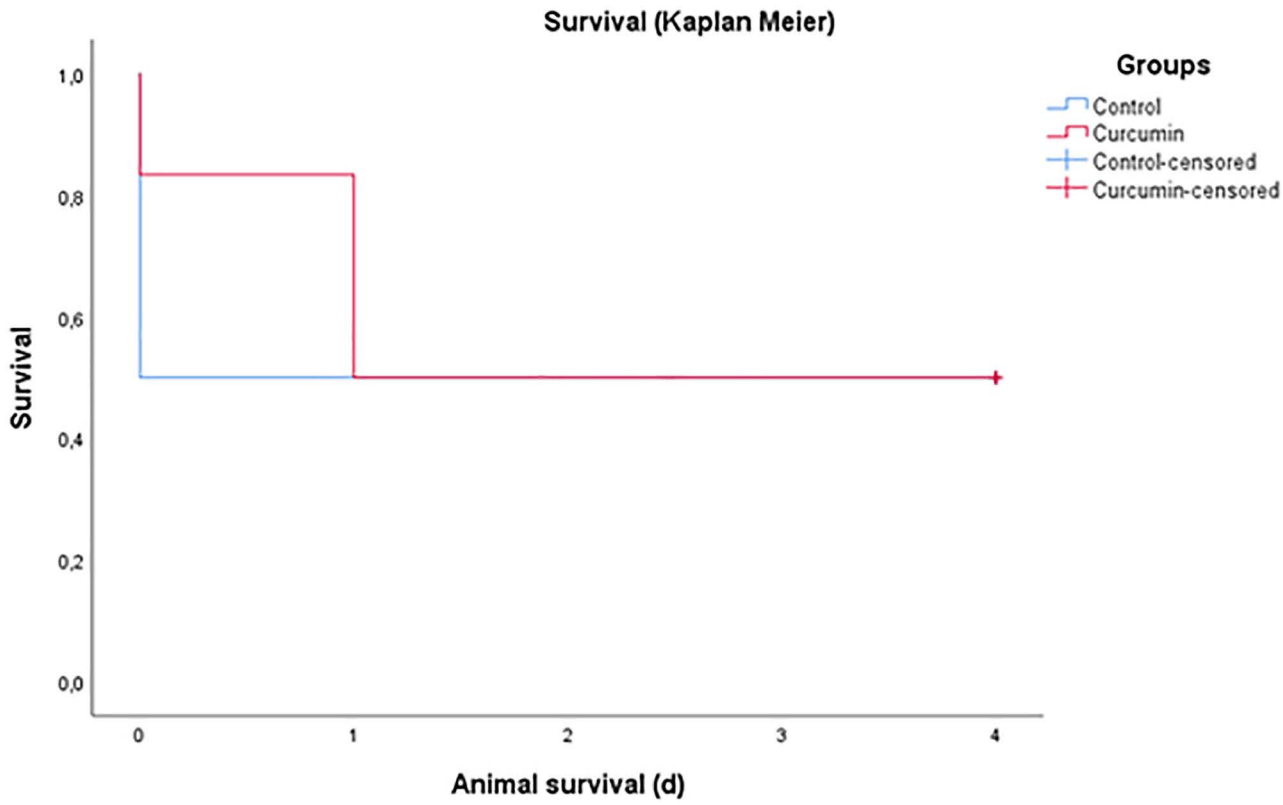

FIGURE 4. Kaplan-Meier survival curves of the recipients in the control and curcumin groups. No difference between groups was not observed.

with higher doses (200 mg/kg) but results are conflicting in the literature.[12,14] Translation of this approach from rat to pig has also been demonstrated feasible: in a pig model of inflammatory-induced cardiopulmonary bypass and extracorporeal support[26] in which animals were orally treated for 3 d before surgery with high doses (130 mg/kg), an optimal protective exposure to curcumin could be obtained and associated with significantly reduced serum TNF-α levels. In humans, oral bioavailability is also regarded low because of a low intestinal absorption by small intestine, the extensive reductive and conjugative liver metabolism and the capacity of enterocytes to modify the structure of curcumin after binding.[27,28] In the sequence of deceased organ donation, oral pretreatment of the donor for several days is not conceivable since deceased donation is per definition a fateful and unplanned event. Moreover, pretreatment, especially in DCD donation is still not widely accepted because of ethical reasons.[29]

To closely mimic the current clinical practice of DCD-LT, we added a water-soluble formulation of curcumin to the flush and preservation solution. A similar approach was applied for autotransplantation of porcine kidneys, exposed to 60 min of WI, followed by 24 h of cold ischemia, flushed, and preserved with the University of Wisconsin with CDC, resulting in better function and less histological injury.[20] For this porcine renal autotransplantation model described by Thuillier et al,[20] a concentration of 65 μM CDC—similar to the 60 μM concentration used in our experiments—was applied and administered after exposure to WI. This concentration was found optimal in preceding in vitro IRI experiments using kidney endothelial cells resulting in reduced lactate, improved mitochondrial activity, reduced expression of Toll-like receptor, P-selectin, and TNF-α.[20] In addition, our group demonstrated that donor intra-portal injection of 60 μM CDC in rat livers previously exposed to 60 min of WI reduced oxidative stress and parenchymal histological injury.[13] In this model, a homogenous intrahepatic and intracellular CDC distribution of curcumin in rat livers was observed, regardless of exposure to WI or cold ischemia, and independent of the type (University of Wisconsin versus HTK) and the temperature of the pre-flush preservation solution in which CDC was dissolved.[13] However, curcumin was no longer detectable on liver tissue sampling by fluorescence after 4 h of cold storage suggesting that curcumin was rapidly metabolized during the preservation into the liver.[13] Since the liver is the primary site of curcumin degradation through well-known fast and complex reduction and conjugative mechanisms,[27] we speculate that curcumin may no longer be available in its most active formulation at the end of cold storage and thus just before graft reperfusion and the ensuing reperfusion injury. A possible reason for the discrepancy between the kidney and liver preclinical DCD transplant models may relate that, contrary to the liver, curcumin is not degraded in the kidney and thus available and capable of exerting all its protective properties at the moment of kidney reperfusion.[20] However, this hypothesis cannot be evaluated because of the lack of tissue samples and technology to measure active and degraded curcumin levels in the liver. Besides, testing this hypothesis with additional exposure to curcumin including repeated flush out of CDC just before reperfusion would be interesting for a future study. Considering this,

the perfusion machine would definitely serve as an ideal platform to maintain optimal curcumin concentration in its active form during the whole dynamic perfusion preservation phase and optimize the exposure of curcumin to the liver until the anhepatic phase.

Another reason possibly accounting for the absence of any protective effect of curcumin may be inherent to the DCD-LT preclinical model itself. We preferred to test the CDC in our model with a well-defined severity of ischemia, which is well-tolerated in terms of graft function recovery (very low rate of PNF and/or delayed graft function) but still associated with substantial IRI (ie, increase in AST levels) and previously also shown capable to evaluate novel therapies against IRI.[22] Hence, we exposed livers to 15 min of WI and 4 h of CIT; this results in an increased level of serum AST compared with CIT alone, allowing us to use AST as a primary endpoint while also to calculate the sample size needed.[22] However, given the lack of any protective effect—apart from a lower rate of immediate graft failure—we speculate that our pig DCD-LT model was either not injurious enough to highlight the impact of CDC or, on the contrary, even too harmful to observe protective effects of curcumin. The degree of IRI and CDC exposure could be addressed in a distinct but future study by balancing the titration of CDC doses along with a titration of the IRI damage model. Finally, although the study was powered to detect differences in AST, the small sample size did not allow a claim on the absence of an effect in this technically challenging model.

In conclusion, conditioning DCD liver grafts with CDC added to the preservation solution in a DCD liver pig model did not improve IRI or survival at a dosage reported to be effective in a pig kidney transplantation model. To unravel the potential beneficial effect of curcumin during preservation on outcome of high-risk grafts and curcumin metabolism liver properties, further studies are warranted to assess curcumin bioavailability, particularly with dynamic preservation, which has now found its way into clinical practice.

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REFERENCES

1. Wall S, Plunkett C, Caplan A. A potential solution to the shortage of solid organs for transplantation. *JAMA*. 2015;313:2321–2322.
2. Briceno J, Marchal T, Padillo J, et al. Influence of marginal donors on liver preservation injury. *Transplantation*. 2002;74:522–526.
3. Eltzschig H, Eckle T. Ischemia and reperfusion—from mechanism to translation. *Nat Med*. 2011;17:1391–1401.
4. Zhai Y, Petrowsky H, Hong J, et al. Ischemia-reperfusion injury in liver transplantation-from bench to bedside. *Nat Rev Gastroenterol Hepatol*. 2013;10:79–89.
5. De Vries Y, von Meijenfeldt FA, Porte RJ. Post-transplant cholangiopathy: classification, pathogenesis, and preventive strategies. *Biochim Biophys Acta Mol Basis Dis*. 2018;1864:1507–1515.
6. Zhai Y, Busuttil RW, Kupiec-Weglinski JW. Liver ischemia and reperfusion injury: new insights into mechanisms of innate-adaptative immune-mediated tissue inflammation. *Am J Transplant*. 2011;11:2279–2296.
7. Soares RO, Losada DM, Jordani MC, et al. Ischemia/reperfusion injury revisited: an overview of the latest pharmacological strategies. *Int J Mol Sci*. 2019;20:5034–5079.
8. Bavarsad K, Riahi MM, Saasat S, et al. Protective effects of curcumin against ischemia-reperfusion injury in the liver. *Pharmacol Res*. 2019;141:53–62.
9. Rivera-Espinoza Y, Muriel P. Pharmacological actions of curcumin in liver disease or damage. *Liver Int*. 2009;29:1457–1466.
10. Strimpakos AS, Sharma RA. Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. *Antioxid Redox Signal*. 2008;10:511–545.
11. Shen SQ, Zhang Y, Xiang JJ, et al. Protective effect of curcumin against liver warm ischemia/reperfusion injury in rat model is associated with regulation of heat shock protein and antioxidant enzymes. *World J Gastroenterol*. 2007;13:1953–1961.
12. Wang L, Li ND, Zang Y, et al. Curcumin protects against hepatic ischemia/reperfusion induced injury through inhibiting TLR4/NF-KB pathway. *Oncotarget*. 2017;8:65414–65420.
13. Saidi SA, Meurisse N, Jochmans I, et al. Hepatocellular uptake of cyclodextrin-complexed curcumin during liver preservation: a feasibility study. *Biopharm Drug Dispos*. 2018;39:18–29.
14. Moussavian MR, Scheuer C, Schmidt M, et al. Multidrug donor preconditioning prevents cold liver preservation and reperfusion injury. *Langenbecks Arch Surg*. 2011;396:231–241.
15. Liu Y, Zhang W, Cheng Y, et al. Activation of PPARγ by curcumin protects mice from ischemia/reperfusion injury induced by orthotopic liver transplantation via modulating polarization of Kupffer cells. *Int Immunopharmacol*. 2018;62:270–276.
16. Prasad S, Tyagi A, Aggarwal B. Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: the golden pigment from golden spice. *Cancer Res Treat*. 2014;46:2–18.
17. Matloob A, Mourtas S, Klepetsanis P, et al. Increasing the stability of curcumin in serum with liposomes or hybrid drug-in-cyclodextrin-in-liposome systems: a comparative study. *Int J Pharm*. 2014;476:108–115.
18. Sou K, Inenaga S, Takeoka S, et al. Loading of curcumin into macrophages using lipid-based nanoparticles. *Int J Pharm*. 2008;352:287–293.
19. Yadav VR, Prasad S, Kannappan R, et al. Cyclodextrin-complexed curcumin exhibits anti-inflammatory and antiproliferative activities superior to those of curcumin through higher cellular uptake. *Biochem Pharmacol*. 2010;80:1021–1032.
20. Thuillier R, Allain G, Giraud S, et al. Cyclodextrin curcumin formulation improves outcome in a preclinical pig model of marginal kidney transplantation. *Am J Transplant*. 2014;14:1073–1083.
21. Chen C, Johnston TD, Wu G, et al. Curcumin has potent liver preservation properties in an isolated perfusion model. *Transplantation*. 2006;82:931–937.
22. Monbaliu D, Crabbe T, Roskams T, et al. Livers from non-heart-beating donors tolerate short periods of warm ischemia. *Transplantation*. 2005;79:1226–1230.
23. EUR-Lex. Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. 2010. Available at http://eur-lex.europa.eu/. Accessed September 22, 2022.
24. Jochmans I, Monbaliu D, Pirenne J. The beginning of an end point: peak AST in liver transplantation. *J Hepatol*. 2014;61:1186–1187.
25. Oguz A, Kapan M, Onder A, et al. The effect of curcumin on the liver and remote organs after hepatic ischemia reperfusion injury formed with Pringle manoeuvre in rats. *Eur Rev Med Pharmacol Sci*. 2013;17:457–466.
26. Ma P, Tumin D, Cismowski M, et al. Effects of preoperative curcumin on the inflammatory response during mechanical circulatory support: a porcine model. *Cardiol Res*. 2018;9:7–10.
27. Dei Cas M, Ghidoni R. Dietary curcumin: correlation between bioavailability and health potential. *Nutrients*. 2019;11:2147–2161.
28. Anand P, Kunnumakkara AB, Newman RA, et al. Bioavailability of curcumin: problems and promises. *Mol Pharm*. 2007;4:807–818.
29. Meurisse N, Monbaliu D, Berlakovich G, et al. Heterogeneity of bile duct management in the development of ischemic cholangiopathy after liver transplantation: results of a European liver and intestine transplant association survey. *Transplant Proc*. 2019;51:1926–1933.