Title

The Protective Effects of Carbon Monoxide Against Hepatic Warm Ischemia-Reperfusion Injury in MHC-inbred Miniature Swine

Short title:

Carbon Monoxide Protects Livers from Ischemia-Reperfusion Injury

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Abstract

Background: The development of treatment strategies to protect against ischemia-reperfusion injury (IRI) to livers is important not only for liver surgeries but also in regard to increasing the utilization of livers from marginal donors. In this study, we examined whether inhalational carbon monoxide (CO) therapy reduced IRI after a 45-minute (min) warm ischemia (WI) in a miniature swine model.

Materials and methods: Six CLAWN miniature swine underwent a 45-min hepatic WI induced by clamping the portal vein and proper hepatic artery. Three animals were subjected to control conditions while the remaining three were treated with CO inhalation for a total of 345-min, including 120-min after reperfusion to maintain a concentration of CO-Hb under 15% (CO-treated group). IRI of the livers was evaluated by liver function tests, serum pro-inflammatory cytokines and liver biopsies.

Results: All controls had statistically significant increased levels of liver enzymes compared to the CO-treated group ($p<0.05$). In controls, liver biopsies at 2 hours after reperfusion showed marked histological changes including diffuse hemorrhage, congestion, necrosis, vacuolization and neutrophil infiltration with apoptosis. In contrast, the CO-treated group showed less obvious or only minimal histological changes.

Furthermore, increases in HMGB1, TNF-α and IL-6 in sera that were induced by IRI in controls were markedly inhibited by the CO-treatment.

Conclusion: We demonstrated that low-dose CO inhalation reduces hepatic warm IRI, potentially through downregulation of pro-inflammatory mediators and activation of anti-apoptotic pathways. To our knowledge, this is the first report demonstrating CO-inhalation attenuated hepatic IRI following WI in a large animal model.

Key Words:

Carbon monoxide, Ischemia-reperfusion injury, Liver, Large animal model, Proinflammatory cytokines, HMGB1

Introduction

Due to critical organ shortage the criteria for usable organs has been expanding. In the field of renal transplantation, grafts are now routinely utilized with success from donors after cardiac death (DCD) [1, 2]. However, the use of DCD donors remains a challenge in regard to liver transplantation (LTx) [3]. It has been shown that one of the main drivers of acute graft failure has been reperfusion injury following the extended hepatic ischemia with DCD donors. Hepatic IRI directly leads to the death of hepatocytes and sinusoidal endothelial cells [4], and upon reperfusion indirectly triggers the activation of Kupffer cells, the release of reactive oxygen species, pro-inflammatory cytokines, increased expression of adhesion molecules [5, 6] and infiltration of leukocytes and micro-circulatory disturbance [7]. All of which are contributing factors that lead to liver dysfunction and failure. Although different approaches to address hepatic IRI have been investigated [8, 9], further studies utilizing large animal preclinical models are needed.

Carbon monoxide (CO) is generally known as a toxic gas, however it has been recently investigated as a potent regulatory signaling molecule with anti-inflammatory, anti-apoptotic, anti-oxidative and vasodilative effects [10]. In rodent models, the beneficial

effects of CO under various pathophysiologic conditions such as shock, sepsis and IRI have been investigated to better understand the mechanisms that have been demonstrated with in vitro studies [11]. We have previously reported inhalation of the optimal dose of CO reduced IRI of lungs as well as prolonged survival of lung allografts in using our MHC- inbred CLAWN miniature swine [12, 13]. In this study, we investigated if this same CO therapy would translate to warm hepatic IRI in a large animal model. Here we demonstrate the dramatic reduction in warm hepatic IRI after perioperative inhalation of low-dose CO using our established, clinically relevant CLAWN miniature swine model.

Materials and Methods

Animals

CLAWN miniature swine weighing between 18 and 28 kg were obtained from the Kagoshima Miniature Swine Research Center (Isa, Kagoshima, Japan). The study protocol was approved by the Ethical Committee of the Faculty of Medicine at Kagoshima University and all animal care and procedures were performed in accordance with the guidelines of Kagoshima University Institutional Animal Care and Use Committee.

Warm Hepatic Ischemia-Reperfusion Injury (IRI) Model

After general anesthesia, peripheral venous access was obtained from the ear and central venous access was obtained by catheter insertion via the left external jugular vein. The left carotid artery was used for continuous arterial blood pressure monitoring during the experiment. A midline laparotomy was performed, and the portal trunk and the proper hepatic artery were isolated from the hepaticoduodenal ligament. Catheters (14-16 Fr) were inserted into the splenic vein and the right external jugular vein for portal bypass, which avoided congestion and thrombosis of the mesenteric venous return during portal clamping. After the intravenous administration of 300 IU/kg heparin, warm ischemia (WI) was induced by clamping the common hepatic artery and the portal trunk for 45 minutes. Concurrently, the portojuglar bypass system was initiated utilizing an external pump, and bypass flow was maintained corresponding to a flow of 10 to 20 mL/kg/min (HAS-CFP-MA, Senko Medical Instrument Mfg. Co. Ltd. Tokyo, Japan). After 45 minutes of WI, the portal trunk was unclamped for reperfusion of the liver, immediately followed by cessation of the portojugular bypass system. The proper hepatic artery was unclamped 30 minutes after reperfusion to mimic conditions during a clinical liver transplantation. Cholecystectomy was performed before abdominal closure to prevent

cholecystitis.

Experimental Design

Six CLAWN miniature swine [14-16] were divided into two groups: a CO-treated group (n=3) and a control group (n=3). In the CO-treated group, the swine inhaled CO continuously for a total of 345 minutes from the beginning of the procedure until 2 hours after reperfusion (Figure 1A).

CO Inhalation

As described previously, 1% CO in oxygen was connected to the isoflurane vaporizer and administered via the endotracheal tube [12, 13]. CO levels in the inhaled gas mixture and arterial carboxyhemoglobin (COHb) levels were monitored [12]. The level of CO was maintained as a COHb concentration under approximately 15%.

Assessment of Liver Injury by Liver Function Tests

Venous blood samples were taken via the central venous catheter for assessment of liver injury. Liver injury was measured by liver function tests including serum aspartate

aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH), which were measured at baseline and compared to measurements taken at 0.5-, 1-, 2-, 4-, 6-, 8-, 24-hour after reperfusion. Measurements were then taken daily until post-operative day 7, and then at day 14, and at one month. Total bilirubin (T-bil) was also measured at the same time points after IRI as markers of bile duct injury.

Analyzing Liver Injury via Histopathologic Evidence

The biopsy samples were analyzed by a blinded pathologist with light microscopy. Open wedge liver biopsies were performed, and the specimens were taken from the peripheral edges of the liver at baseline (before hepatic IRI), 2 hours and 4 days after reperfusion. The liver specimens were prepared in 10% formalin solution, embedded in paraffin and stained with hematoxylin and eosin (HE). Hepatic IRI was assessed by sinusoidal hemorrhage and congestion, degeneration, apoptosis and necrosis of hepatocyte and neutrophil infiltration [17]. Hepatocyte apoptosis was assessed by the terminal deoxynucleotidyl transferase-mediated UTP-biotin nick end labeling (TUNEL) method.

Measurement of Serum Levels of TNF-α, High-Mobility Group Box 1 and IL-6

Serum concentrations of TNF-α, High-Mobility Group Box 1 (HMGB1) and IL-6 were measured by enzyme-linked immunosorbent assay (ELISA) at baseline, at 0.5-, 1-, 2-, 4-, 6-, 8-, 24-, 48-hour after reperfusion according to the manufacture's protocol (HMGB1: Shino-Test Corporation, Sagamihara, Japan; TNF-α, IL-6: R&D Systems, Minneapolis, MN, USA).

Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM). Group comparisons were performed by the Student t test, the Mann–Whitney U test, or analysis of variance as appropriate. Repeated measured two-way ANOVA was used to examine the effects of two categorical variables. Calculations were made with GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA).

Results

Establishment of a Non-Lethal Hepatic Warm IRI Model in CLAWN Miniature Swine

This study successfully established a non-lethal warm hepatic IRI model in CLAWN miniature swine. During the 45-min of WI, the small intestine was found to be free of venous congestion via the portojugular active bypass system in all animals in the study. Although mean blood pressure measured by the arterial catheter transiently dropped after clamping of both the portal vein and proper hepatic artery, the pressure subsequently recovered in the post-reperfusion period without clinical sequelae. Average surgical time was six hours four minutes(min) ±15 min, and all animals recovered from surgery without incident.

COHb Levels during the Experiment

COHb levels at baseline in the CO-treated group were 0.5 % ± 0.2%. In regards to the inhalation of CO in the experimental group, COHb levels increased sharply and then plateaued at 14.0% ± 0.6% (Figure 1B). After withdrawal of CO inhalation at 2 hours after reperfusion, COHb levels decreased precipitously.

Low- dose CO Inhalation had no Adverse Effects during and after the Procedure

No significant difference in mean arterial blood pressure was observed between the

control and CO-treated groups during the hepatic IRI procedure (103 ± 6.4 mm Hg in the control group vs 93 ± 2.6 mmHg in the CO-treated group just before the start of ischemia, 72 ± 0.7 mmHg in the control group vs 67 ± 1.0 mmHg in the CO-treated group 2 hours after reperfusion. p=0.27). All animals in both groups had no evidence of respiratory distress during the procedure and were able to be extubated after the procedure without incident. The low dose inhalation of CO had no apparent side effects on behavior after the procedure in the CO-treated group **for one month.**

CO Improved Liver Function Tests

All measured parameters of hepatic injury were significantly lower in the CO-treated group than the control group (Figure 2). More specifically, the levels of serum AST in the control group increased dramatically and peaked to 2224 ± 326 U/L at 2 hours after reperfusion (Figure 2A). The levels of ALT in the control group also showed a similar progressive increase with a peak at 2 hours, 98 ± 50 U/L (Figure 2B). Serum LDH levels in the control group had a slightly delayed peak at POD 1 with 1915 ± 406 U/L (Figure 2C). The levels of T-bil in the control group also rose higher and demonstrated a delayed recovery as compared to the CO-treated group (Figure 2D).

Light Microscopic Findings Revealed that Animals Treated with CO Possessed Fewer Histopathologic Changes

In the control group, liver biopsies demonstrated acute ischemic injury of the liver with massive congestion and hemorrhage, degeneration, apoptosis and necrosis of hepatocytes with neutrophil infiltration diffusely at 2 hours after reperfusion (Figure 3B, C). Even though the livers demonstrated some histological evidence of recovery, the congestion, necrosis of hepatocytes, and inflammatory cell infiltration remained even at 4 days after reperfusion (Figure 3D). In contrast, the CO-treated group demonstrated only limited areas of injury with evidence of hepatic ischemia, with minimal progression of congestion, degeneration and necrosis of hepatocytes with limited neutrophil infiltration at 2 hours after reperfusion (Figure 3F, G). These changes were completely diminished 4 days after reperfusion in the CO-treated group (Figure 3H).

Inhibition of Apoptosis of Hepatocytes by CO Treatment in the IRI Liver

We assessed whether CO prevented apoptosis induced by the IRI using TUNEL staining. In the control group, numerous TUNEL-positive cells were observed at 2 hours after

reperfusion compared to the CO-treated group (Figure 4A, C). At 4 days after reperfusion, the CO-treatment resulted in only a small number of TUNEL- positive cells, while the control group continued to demonstrate TUNEL-positive cells without recovery at this time point (Figure 4B, D).

CO Inhibited Serum Inflammatory Cytokines; TNF-α, HMGB1 and IL-6

Levels of serum inflammatory cytokines were evaluated by ELISA and a marked elevation were observed in response to IRI in the control group compared to the experimental group (Figure 5). The concentration of TNF-α and HMGB1 in the sera peaked at 0.5-hr and 1hr after reperfusion, respectively (Figure 5A, B). Subsequently, serum levels of IL-6 increased considerably with a peak reached at 2-hr after reperfusion. The level of HMGB1 in the CO-treated group were significantly lower at 1-hr (p=0.0010) and 2-hr (p=0.0007) than those in the control group (Figure 5B). The level of TNF-α in the CO-treated group were also significantly lower than those in the control group at 0.5-hr (p=0.005), 1-hr (p=0.0007) and 2-hr (p=0.0035) (Figure 5A). Although not significant, the level of IL-6 in the CO-treated group were lower than those in the control group (Figure 5C).

Discussion

IRI is one of the initial non-immunologic triggers that activates the innate immune response that causes tissue damage and potential devastating complications including graft failure after transplant [8]. IRI of liver grafts in particular leads to early allograft dysfunction or primary-non function (PNF) [18]. Attenuating IRI is therefore essential to improving both short and long-term outcomes after LTx. This is even more relevant for marginal grafts, such as DCD grafts, steatotic livers and livers from the elderly, known as "extended criteria" liver grafts [19]. The length of inevitable prolonged warm ischemia influences whether the DCD graft can be utilized or discarded. In fact, approximately 60% of the potential DCD livers are discarded because of irreversible ischemia-reperfusion injury [20]. A number of therapeutic approaches including pharmacologic, gene or cell therapy [21], development of new preservation solutions [22] and machine perfusion techniques [23] have been reported to ameliorate hepatic IRI with differing levels of success [9, 21]. However, a universal and practical strategy to protect marginal liver grafts with prolonged warm ischemia have not been established.

In this study, we have established a clinically relevant large animal model of hepatic warm IRI and shown that low-dose CO inhalation can significantly reduce the severity of hepatic warm IRI. We initially established a miniature swine non-lethal warm IRI model in the liver to investigate the function and pathophysiology in vivo during both the acute and chronic periods. We showed that in the control group, although a 45-min WI induced significant hepatocyte damage, the animals were able to survive the ischemic insult and enabled us to observe them longer to assess the safety of CO administration. After 45-min of WI, administration of low-dose gaseous CO by inhalation before and after reperfusion significantly mitigated hepatic IRI with data demonstrating (i) improved levels of serum liver functioning tests, (ii) minimized histologic hepatocyte changes, (iii) remarkably fewer apoptotic cells, and (iv) lower production of serum pro-inflammatory cytokines such as TNF- α and HMGB1. To our knowledge, this is the first report of translational evidence that low-dose CO inhalation showed the cyto-protective effects against hepatic warm IRI in a preclinical large animal model. Although the number of animals in each group was relatively small, the marked difference in results observed between the two groups in this model can provide significant information. The reproducibility of the “all or none” phenomenon in these two protocols using MHC-

inbred CLAWN miniature swine in which the background of MHC is known has provided a unique opportunity to study not only the induction and maintenance of tolerance [13, 16], but also testing IRI [12] in which immunologic responses are involved.

This study was set up after first establishing a hepatic warm IRI model to determine not only the efficacy and safety of the CO therapy, but also the duration of WI that could be tolerated without causing fatal hepatic injury without CO therapy. Less than 20-30 min of true warm ischemia (interval between significant ischemic insult and initiation of perfusion) are recommended to prevent complications [3]. Although experimental models utilizing pigs are clinically-relevant due to their size and physiologic similarities to humans [21], pigs poorly tolerate hepatic ischemic injury compared to humans [24]. In a pig DCD LTx model, ≧30 min WI showed an unacceptably high rate of PNF (50%) [25]. Because LTx includes a multitude of factors including T cell responses as well as the innate responses which induce inflammatory cytokines, we chose a 45-min WI period in this IRI model without LTx. The 45-min WI lead to consistently severe but reversible IRI of livers in controls which allowed us to study the effects of CO with a small number of animals. Adding portojugular bypass during portal clamping was crucial for animal

survival because it prevented lethal intestinal venous congestion and thrombosis in the acute and subsequent chronic phase of the IRI.

The heme oxygenase-1 (HO-1) enzyme system has become an attractive therapeutic target for the development of anti-inflammatory regimens [11]. HO-1 is the rate-determining enzyme that disassociates the central iron molecule from heme proteins, and degrades the protein into biliverdin IXα and CO [26]. HO-1 induction has been shown to have protective effects against cellular damage caused by the generation of free radicals, because of the catalysis of potentially pro-oxidant and cytotoxic heme, as well as the generation of antioxidant and cyto-protective byproducts [27]. It has also been shown that exogenously provided CO via inhalation protects endothelial cells and hepatocytes against cytotoxic agents in vitro and in vivo after IRI in various injury models [11]. **As reviewed in the article by Ozaki et al. [28], it has been reported that gaseous CO can mitigate hepatic cold IRI using rat models. Exogenously provided 300 ppm of CO to the graft for 2 hours after 24-hour cold storage ameliorated hepatic IRI in an ex vivo perfusion model of the isolated rat liver with COHb concentration at 6.8 ± 1.5% in the perfusate [29]. In a rat liver transplantation model, hepatic cold IRI induced by**

18-hour cold preservation was ameliorated with either in vivo treatment of recipients with inhaled 100 ppm CO (serum COHb: 13.5 ± 0.1%) for 1 hour before and for 24 hours after the transplant surgery [30, 31] or ex vivo treatment during cold storage with 5% CO supplementation of UW solution [32]. However, thus far there are very few studies in the literature that have demonstrated the protective effects of exogenously delivered CO against warm IRI of the liver [33-35]. Furthermore, these studies utilized small animal models, which are of limited applicability to humans due to the size and physiologic differences in metabolism and liver anatomy [21]. Also, these studies employed a CO delivery method by utilizing a CO-releasing molecule, which are drugs that are not used in current clinical practice. In fact, low-dose CO inhalation therapy is already used in clinical practice as a treatment option in various clinical trials, and to date no adverse effects have been reported [36]. **In this experiment, in concordance with published articles [30, 12, 31], our results have shown the safety and efficacy of perioperative inhalation of CO adjusted with serum COHb level below 15% and all swine survived for one month in stable condition even after CO inhalation.** Utilizing large animals, we have previously demonstrated the beneficial effects of clinically applicable gaseous CO on lung IRI using a large animal transplant model [12, 13]. To

further study if our results would be applicable to liver transplants, we designed a large animal hepatic IRI model with gaseous CO inhalation. Our results from this study may reveal a new therapy applicable not only for the recovery of the liver from ischemic injury, but useful for the recovery of all organs after an ischemic insult in the critically ill patient.

The role of cytokines has been well documented as either pro-inflammatory or an anti-inflammatory mediator in liver IRI. One notable finding in this regard from our study is the markedly lower expression of the inflammatory cytokines TNFα, HMGB1 and IL-6. This would suggest that the cyto-protective effects exerted by CO are induced partly due to the suppression of the pro-inflammatory cytokine productions. TNF-α is is produced by Kupffer cells and is considered the key aspect of the pro-inflammatory cytokine cascade in liver IRI [37]. In rodent models, CO lead to an anti-inflammatory response after IRI of the liver by blocking the inflammatory signaling of Kupffer cells in the graft liver upon transplantation [31, 38], up-regulating the anti-inflammatory MAPK pathway [39], preserving glycogen synthase kinase 3b phosphorylation status and regulating the microRNA-34a/SIRT1 pathway [40]. HMGB1, one of the damage-associated molecular patterns, is known to behave as a pro-inflammatory stimulator when passively released

from damaged or ischemic cells, or actively secreted by monocytes or macrophages and is a critical mediator of injury and inflammation in the acute phase following IRI of the liver, kidney, brain and heart [39-43]. The release of HMGB1 from damaged or ischemic cells may act as an early inflammatory responder via signaling by TNF-α or IL-6 and activation of the TLR4 system [41]. In our previous renal IRI model using CLAWN miniature swine we also found that neutralization of the HMGB1 antibody has cytoprotective effects [44]. In a mouse model, Tsung et al showed that administration of a neutralizing antibody to extracellular HMGB1 reduces hepatic IRI. More recent data suggested CO may increase SIRT1 expression by decreasing acetylation of HMGB1 and subsequently reduce its translocation and release, as a possible mechanism of the protection against hepatic IRI . Further research aimed at blocking HMGB1 specifically as a therapeutic target in hepatic IRI may be considered.

Our study did not address the beneficial effects that CO inhalation have on a molecular level. In rodent models, CO has been shown to have anti-apoptotic functions due to the upregulation of antiapoptotic signals of the B cell lymphoma-2 family, as well as the down regulation of pro-apoptotic signals like caspase 3 and B cell lymphoma-2 associated X [34,

[31]. Consistent with these data, we demonstrated in this study that CO via direct inhalation has potent anti-apoptotic effects on hepatic warm IRI.

Although our data represent a proof of principle of the effectiveness of CO in an *in vivo* large animal model, we are aware of the limitations of this study, as we investigated only a 45-min period of warm ischemia. Considering that the type (warm or cold) and time of ischemia lead to differences in the mechanisms of hepatic IRI and in the effects of therapeutic strategies [9], further experiments are required to test this strategy and to develop a clinically applicable protocol of CO in a cold ischemia model or at various ischemic times, which would more closely mimic a clinical scenario.

In conclusion, we have demonstrated that low-dose CO administration by inhalation reduces hepatic warm IRI in a pre-clinical large animal model, potentially through downregulation of pro-inflammatory mediators and activation of anti-apoptotic pathways. Our data suggest that inhalation of low-dose CO may be a useful therapeutic option for minimizing warm IRI during human LTx, which could potentially expand the use of

marginal grafts which include DCD grafts. Our next step is to determine if CO therapy is effective in a DCD liver model, as well as the timing of CO therapy. Our preliminary data in CLAWN lung transplant models indicate that donor treatment with CO inhalation was more protective from IRI injury in lungs than post lung transplant treatments (Sahara et al. manuscript in preparation). CO pre-treatment may prove to be a good strategy in protecting the liver grafts of DCD and could potentially be applied to donors after brain death with longer cold ischemic time to ensure better quality of the liver grafts.

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Figure Legends

Figure 1. (A) Scheme of the experimental protocol for evaluating the efficacy of perioperative carbon monoxide (CO) inhalation. Warm ischemia was induced in the control group by clamping the portal trunk and the hepatic artery for 45 min. After 45 min, the portal trunk was unclamped to permit reperfusion. The common hepatic artery was de-clamped 30 min after reperfusion. PV: Portal Vein. PHA: Proper Hepatic Artery. (B) Percent carboxyhemoglobin (COHb) in the blood during and after carbon monoxide

(CO) inhalation in the CO treated group. CO inhalation was initiated at the beginning and continued to maintain a CO-heme concentration of approximately 15% for 180 min. After 180 min, the warm ischemic period was induced. After 45 min of warm ischemia, CO inhalation was continued to maintain this level of CO-heme concentration. 2 hours after reperfusion CO inhalation was ceased.

Figure 2. Analysis of serum markers of hepatic injury (black bars: Control group; white bars: carbon-monoxide (CO) treated group). The CO-treated group showed significantly lower levels of liver enzymes: (A) Serum aspartate transferase (AST) peak AST: 2224 ± 326 U/L vs 456 ± 81 U/L, $p<0.05$, (B) Serum alanine transferase (ALT) peak ALT: 98 ± 50 U/L vs 23 ± 5 U/L, $p<0.05$. (C) Lactate dehydrogenase (LDH), peak LDH: 1915 ± 406 U/L vs 1157 ± 110 U/L, $p<0.05$. (D) Total Bilirubin (T-bil), peak T-bil: 0.7 ± 0.03 mg/dl vs 0.4 ± 0.03 mg/dl. Data are expressed as Mean ± SEM.

Figure 3. Histological analysis of representative liver biopsies by light microscopy of HE-stained liver specimens in both the control group and CO-treated group obtained at baseline (A, E), on 2 hours after reperfusion (B, C, F, G) and on day 4 (D, H) are shown.

At 100 x magnification, the (B) control group showed massive sinusoidal congestion and hemorrhage compared with the (F) CO-treated group. At 600 x magnification, degradation and vacuolization of hepatocytes with neutrophil infiltration were more remarkable in the (C) control group compared to the (G) CO-treated group. Well preserved hepatocytes were also seen around damaged hepatocytes in CO-treated group. These changes were almost completely resolved in the (H) CO-treated group on day 4, whereas the (D) control group still displayed congestion, degeneration and necrosis of hepatocytes with inflammatory cell infiltration.

Figure 4. Representative liver biopsy specimens of TdT-mediated dUTP nick end labeling (TUNEL) staining on 2 hours (A, C) and 4 days (B, D) after reperfusion. TUNEL-positive cells were obviously seen in the control group (A) at 2 hours after reperfusion. In contrast, fewer numbers of TUNEL positive cells were observed in the CO-treated group at 2 hours after reperfusion (C). At 4 days after reperfusion, the CO-treatment resulted in only a small number of TUNEL-positive cells (D), whereas the control group showed a moderate number of remaining TUNEL-positive cells (B).

Figure 5. Serum levels of pro-inflammatory cytokines, TNF-α (A), HMGB1 (B) and IL-6 (C) at early time points after ischemia-reperfusion (black bars: control group; white bars: CO- treated group). HMGB1, TNF-α and IL-6 markedly increased in the control group while these elevations were almost entirely suppressed in the CO-treated group. Notably, levels of TNF-α and HMGB1 were statistically lower in the CO-treated group (A, B). Serum levels of IL-6 were also lower in the CO-treated group, although this did not reach statistical significance (C). *p<0.05. HMGB1, High Mobility Group Box 1.

Permissions: N/A

Fig 1: Schema of the experimental protocol (A) and the concentration of COHb in the CO-treated group (B)

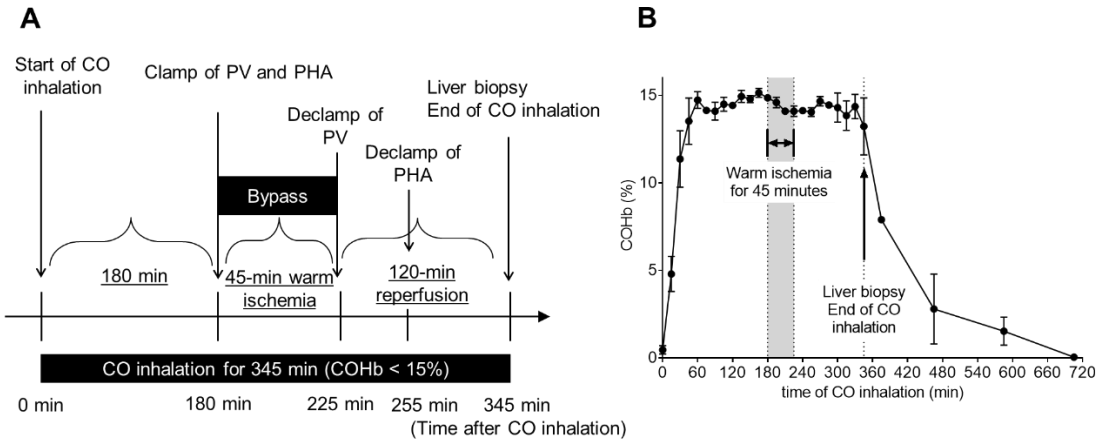


Fig 2: Analysis of serum markers of hepatic injury

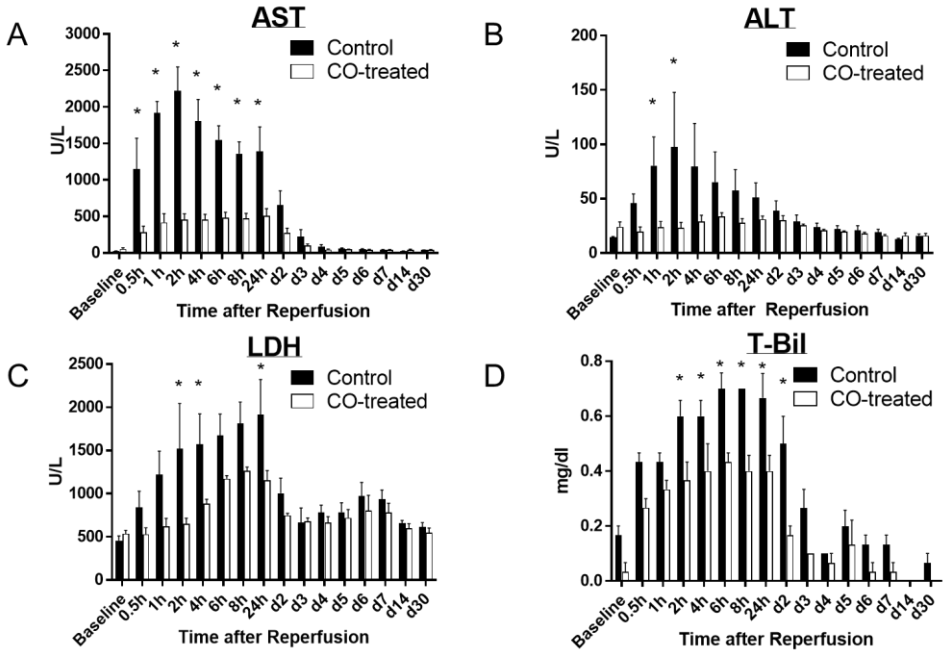


Fig 3: Histological analysis of the livers

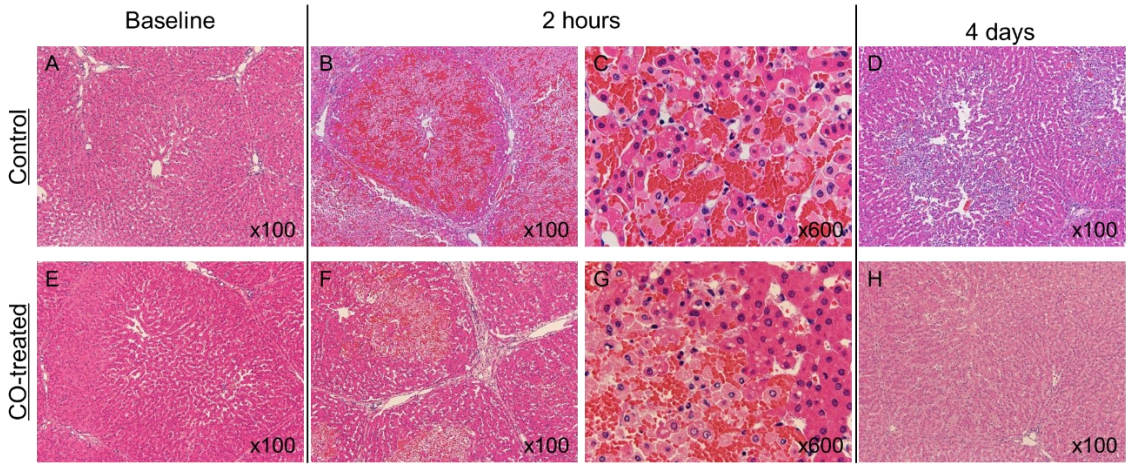
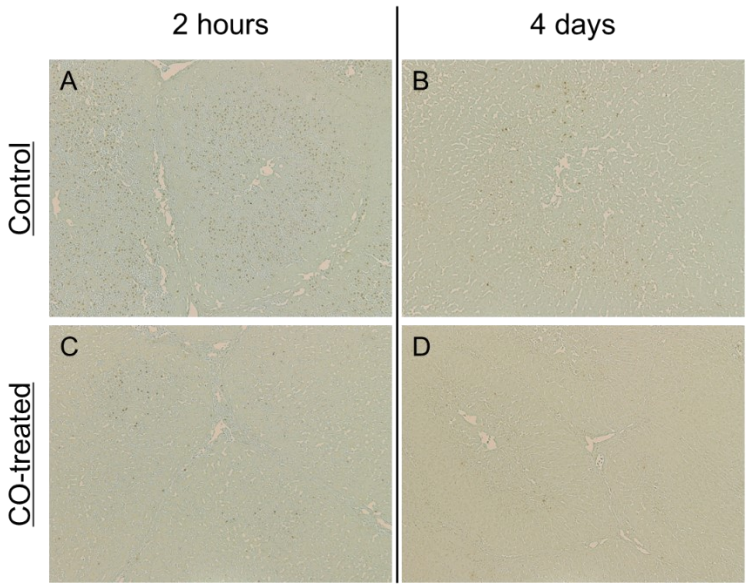


Fig 4: TUNEL staining of the Livers



TUNEL x100

Fig 5: Serum cytokine levels: TNF-α (A), HMGB1(B) and IL-6 (C) at early time points after ischemia-reperfusion