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Ultrafast cooling with total liquid ventilation mitigates early inflammatory response and offers neuroprotection in a porcine model of cardiac arrest

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Non-standard abbreviations and Acronyms

CA: cardiac arrest

IL: interleukin

ROSC: Resumption of spontaneous circulation

TLV: Total liquid ventilation

Abstract

Background: Brain injury is the one of the most serious complications after cardiac arrest (CA). To prevent this phenomenon, rapid cooling with Total Liquid Ventilation (TLV) has been proposed in small animal models of CA (rabbits and piglets). Here, we aimed to determine whether hypothermic TLV can also offer neuroprotection and mitigate cerebral inflammatory response in large animals.

Methods and results: Anesthetized pigs were submitted to 14 min of ventricular fibrillation followed by cardiopulmonary resuscitation. After return of spontaneous circulation (ROSC), animals were randomly subjected to normothermia (Control group, n=8) or ultrafast cooling with TLV (TLV group, n=8). In the latter group, TLV was initiated within a window of 15 min after ROSC and allowed to reduce tympanic, esophageal and bladder temperature to the 32-34°C range within 30 min. After 45 min of TLV, gas ventilation was resumed and hypothermia was maintained externally until the 3rd h after CA, before rewarming using heat pads (0.5-1°C/h). After an additional period of progressive rewarming for 3 h, animals were euthanized for brain withdrawal and histological analysis. At the end of the follow-up (i.e., 6 h after CA), histology showed reduced brain injury as witnessed by the reduced number of Fluororjade C positive cerebral degenerating neurons in TLV vs Control. IL-1ra and IL-8 levels were also significantly reduced in the cerebrospinal fluid in TLV vs Control along with cerebral infiltration by CD3+ cells. Conversely, circulating levels of cytokines were not different among groups, suggesting a discrepancy between local and systemic inflammatory levels.

Conclusions: Ultrafast cooling with TLV mitigates neuroinflammation and attenuates acute brain lesions in the early phase following resuscitation in large animals submitted to CA.

Keywords: Cardiac arrest, Hypothermia, Inflammation, Liquid ventilation

Research perspective

What is new?

- The ultra-rapid hypothermia afforded by total liquid ventilation can prevent brain injury in large animals.
- The neuroprotective effect of total liquid ventilation is associated with brain inflammation reduction despite early rewarming after a short hypothermic episode.

What Question Should be Addressed Next?

- Total liquid ventilation can be an original strategy to evaluate the therapeutic window of hypothermia when induced very rapidly in the clinical setting.
- Potential benefits need to be investigated in a clinical study under preparation.

Introduction

Following cardiac arrest (CA), most surviving patients present multiple complications including cardiac and neurological injuries, leading to a post-CA syndrome^{1,2}. In animal studies, temperature reduction and mild hypothermia (32-34°C) were shown to provide potent benefits against the brain injury occurring after return of spontaneous circulation (ROSC)^{3,4}. This was demonstrated in many experimental conditions and recently emphasized by a meta-analysis of experimental studies in small and large animals^{3,4}. Conversely, therapeutic hypothermia (33°C) did not seem to improve the outcome in patients when compared to sub-normothermia (36°C) or strict normothermia (37°C) after ROSC^{5,6}. Accordingly, the recent ERC-ESICM recommendations suggested that targeted temperature management with active fever prevention could be sufficient in most patients resuscitated after CA⁷. This apparent discrepancy between animal and human studies might be explained by different windows of application of hypothermia with very short time to target temperature in bench studies (< 1 h after ROSC in most studies)⁸ vs delayed hypothermia in most clinical reports (> 6-8 h after ROSC)⁷. The recent ERC-ESICM guidelines also state that “*the therapeutic window within which temperature control with hypothermia may be effective in the clinical setting is unknown*”⁷.

In this context, our team has investigated total liquid ventilation (TLV) as an innovative technique for very rapid cooling induction. This technique consists in lung filling and tidal ventilation with temperature-controlled breathable liquids⁹, enabling body cooling with a core target temperature of 32-34°C within less than 30 min in both small and large animals^{4,10-14}. Importantly, hypothermic TLV was further demonstrated to be potently cardio- and neuroprotective in rabbits and piglet models of CA^{4,11}. For instance, hypothermic TLV instituted 15 min after ROSC decreased interleukin-6 blood levels very rapidly after CA and attenuated

brain injury regarding both clinical outcome and histopathological markers after 72 h of follow-up¹⁰. However, the relationship between systemic and local cerebral inflammation was not properly established and this was only observed in small animals until now¹⁰. Accordingly, the goal of the present study was to evaluate the neuroprotective effect of TLV in a large animal porcine model of CA, as well as its ability to mitigate local inflammatory response in the brain.

Material and Methods

Data availability statement

Data is available upon request to the corresponding author.

Board Statement

All experiments were approved by the French ethical board for animal research n°16 (Comité d’Ethique Anses – ENVA – UPEC; APAFIS #32855-2021090315125530 v2) and fully complied with French and European regulations concerning the use of laboratory animals.

Animal preparation

Female swine (Lebeau, Gambais France) were fasted overnight. Prior to the procedure, animals were sedated using a mixture of tiletamine and zolazepam (10 mg/kg each, i.m.). Anesthesia was induced by propofol (2.5 mg/kg i.v. and 10 mg/kg/h, i.v. for induction and maintenance, respectively). Analgesia was provided by an administration of methadone (0.75 mg/kg, i.m.). After endotracheal intubation, animals were mechanically ventilated ($FiO_2=30\%$, tidal volume=6-8 mL/kg, positive end-expiratory pressure = 5 cmH₂O, respiratory rate=20-25 cycles/min, Monnal T60®, Air Liquide Medical Systems, Antony, France) and the ventilation parameters were adjusted to optimize oxygenation and target normocapnia. Temperature probes were inserted into the bladder, eardrum and esophagus. At baseline, all swine were placed on a heating pad to maintain their body temperature at $38.0\pm 0.5^\circ\text{C}$.

Two sheaths were introduced into the femoral vessels using the Seldinger technique in order to introduce central catheters and monitor arterial and right atrial blood pressures using pressure transducers (Millar®, SPR-524, Houston, TX, USA). In order to compensate fluid loss, animals received a standardized administration of Ringer Lactate (4 mL/kg).

CA and CPR protocol

After a period of stabilization, ventricular fibrillation (VF) was induced by an electrical stimulation (A/C 10 V) with a pacemaker probe introduced into the right ventricle through the venous femoral sheath. Mechanical ventilation was simultaneously interrupted. After 14 min of *no flow*, cardiopulmonary resuscitation (CPR) was initiated with continuous chest compressions using an automated device (100 compressions/min; Lucas®) and resumption of mechanical ventilation (FiO₂=100%; respiratory rate=10 cycles/min; tidal volume=6-8 mL/kg.). Following 3 min of CPR, epinephrine (0.5 mg) was administered and external defibrillations were attempted until ROSC. After ROSC, norepinephrine was administered to maintain mean arterial blood pressure (MAP) at 75±5 mmHg if needed. Ventilatory parameters were set at similar levels as baseline and readjusted, if required, to target normocapnia and prevent hyperoxia. In the absence of ROSC after 20 min, experiments were interrupted. In resuscitated animals, a second administration of methadone was performed 4 hours after ROSC (0.75 mg/kg i.m.).

Experimental groups

As described in Figure 1A, resuscitated pigs were randomly subjected to normothermia or hypothermic TLV after ROSC (Control and TLV groups, respectively; both n=8). In the Control group, animals underwent convention gas ventilation throughout the procedure and body temperature was maintained at ~38°C throughout follow-up. In the TLV group, liquid ventilation was implemented within 15 min after ROSC to achieve a core body temperature of 32 to 34°C. TLV was only used for cooling induction, after which gas ventilation was resumed and hypothermia maintained externally until the end of the 3rd hour following the onset of CPR. Then, a slow rewarming phase was initiated with heating pads during an additional period of 3

hours (+0.5 to 1°C per hour). Animals were euthanized in both groups after a total period of 6 hours of follow-up after CA.

Beyond the animals included in the main experiments and submitted to CA, six additional animals were anesthetized in order to sample cerebrospinal fluid (CSF) with neither CA nor TLV (Sham group). This allowed the evaluation of usual cytokine concentrations in the CSF as comparison with values obtained in Control and TLV groups after CA (see below).

Total liquid ventilation procedure

In the TLV group, animals received rocuronium (3 mg/kg i.v.) after ROSC. Then, liquid ventilation was instituted using a dedicated TLV device (Supplemental Figure 1) with perfluorooctylbromide (PFOB), as breathable liquid. At the onset of the procedure, the endotracheal tube was disconnected from the conventional ventilator to be connected to a remote Y piece of the TLV device. This piece evaluates breathable liquid temperatures and pressures throughout the procedure. Once connected, TLV was started with an initial lung filling with a residual liquid volume of 12 ml/kg. In case of insufficient lung filling, this target could be increased within the 12-18 ml/kg range and further maintained throughout the procedure by an embedded algorithm. Respiratory rate and tidal liquid volume were set at 6 cycles / min and 8 ml/kg, respectively. The fraction of oxygen infused in the disposable kit was also adjusted to target a pulsed oximetry between 94 and 98%. Finally, PFOB temperature was automatically controlled in order to maximize thermal exchange while preventing temperature undershoot. After 45 min of TLV, the procedure was ended by a dedicated mode allowing to collect from the lung as much of the breathable liquid as possible. Then, animals were reconnected to the conventional gas ventilator until the end of the procedure using the same ventilator parameters as previously mentioned for Control animals.

Investigated parameters

During follow-up, heart rate (HR) and mean arterial blood pressure (MAP) were continuously monitored. Blood samples were collected regularly to assess arterial blood pH, O₂ and CO₂ partial pressures (PaO₂ and PaCO₂, respectively), lactate and bicarbonate levels. Additional blood samples were collected at 2 and 6 hours after CA for further analyses, as well as CSF as previously described¹⁵. These additional samples were stored at -80°C before evaluation of inflammatory mediators and/or neurofilament light chain (NFL). The CSF samples of Sham animals (see above) were also analyzed for the determination of usual cytokine levels in the CSF. Briefly, cytokine concentrations were assessed using a dedicated porcine multiplex evaluating of Tumor Necrosis Factor- α (TNF- α), Interleukin (IL)-1ra, IL-6, IL-8, IL-10, IL-18, matrix metalloproteinase-1 (MMP1) and CD31 (8-plex R&D systems # LXSAPM-08, L151732; Bio-Plex 200, Bio-Rad). NFL was also evaluated by a dedicated porcine kit (Neuro 2-Plex B Advantage Kit #103520; Simoa SR-X, Quanterix).

End of protocol

At the end of the follow-up period, animals were euthanized and brains and lung samples were extracted and fixed with 4% paraformaldehyde for histological analyses. Fluorochrome staining was used to spot degenerating neurons in non-overlapping fields within the cortex (Merck Millipore, Burlington, MA). Infiltrated T cells into the brain were detected and counted using a monoclonal anti-CD3 antibody (Anti-CD3 #A0452, Dako). Four lung samples were also prepared and stained with hematoxylin-eosin for histological analyses (two samples from ventral left and right diaphragmatic lobes and two samples from the dorsal parts of the same lobes). Extension and severity of the lung lesions, which consist in cardiogenic edema, congestion, interstitial inflammation or necrosis (pulmonary infarct-like pattern), were blindly assessed on a

0 to 5 scale for each sample (0: normal appearance; 5: extended and severe lesions). The mean value was calculated for each animal for each type of lesion.

Statistical Analysis

Data are presented as mean \pm standard error of the mean (SEM). All statistical analyses were conducted using GraphPad Prism® software (GraphPad® Software, La Jolla, CA, USA). Continuous variables were compared between groups using a two-way analysis of variance for repeated measures (ANOVA), followed by Sidak's multiple comparison test if needed. Post-hoc analysis was only made between groups but not among the different time points, except for cytokines and NFL for which we aimed at investigating variation as compared to baseline. In the CSF, we did not compare values between time-points as we did not have baseline values. Accordingly, we compared values to Sham animals. The number of degenerating neurons in the brain and the score of lung lesion at histology were compared between groups using non-parametric Mann-Whitney test. Statistical significance was set at $p < 0.05$.

Results

General CPR parameters

Thirty animals were submitted to CA, among which 16 were successfully resuscitated and allocated to Control or TLV groups (both n=8). Body weight was not different among groups (34 ± 1 vs 31 ± 1 kg in TLV vs Control, respectively). As shown in Table 1, time to achieve ROSC, epinephrine dose and number of external defibrillations before ROSC were not different among groups in successfully resuscitated animals.

Targeted Temperature Management

As illustrated in Figure 1, tympanic, esophageal and bladder temperatures were similar between groups at baseline. After CA, these temperatures remained within the normal range in the Control group and decreased below 34°C within 15 to 30 min in the TLV group. During the rewarming period, temperatures increased by $+0.5$ to 1°C per hour. Full rewarming was not achieved in the TLV group before the end of the protocol, *i.e.*, 360 min after CA.

Hemodynamics

At baseline, MAP and HR did not differ between TLV and Control groups. Following CA, HR significantly decreased in TLV vs Control group (127 ± 4 vs 168 ± 10 bpm at 30 min after CA, respectively). MAP was significantly increased in TLV vs Control group at 240 min after CA (84 ± 2 vs 67 ± 4 mmHg, respectively), despite reduced doses of norepinephrine (Figure 2).

Blood gases

As shown in Table 2, there was no difference in biochemical parameters between groups at baseline. After CA, we observed a metabolic acidosis characterized by increased lactate levels and decreased bicarbonate levels in both groups. For instance, lactate levels reached 10.55 ± 0.52 vs 11.46 ± 0.95 mmol/L at 30 min after CA in TLV vs Control. At the same time-point, the

arterial blood pH averaged 7.09 ± 0.02 vs 7.16 ± 0.04 in TLV vs Control, respectively. During the hypothermic phase, PaO_2 was not different between groups. However, PaCO_2 was higher at 30 min following CA in TLV vs Control (Table 2).

Inflammatory markers

At baseline, both groups had similar blood cytokine concentrations. As illustrated in Figure 3, blood cytokine concentrations did not significantly differ after CA between Control and TLV groups. Conversely, most cytokine concentrations in animals submitted to CA increased in the CSF (i.e., Control and TLV groups) when compared to Sham animals, as illustrated in Figure 4. IL-1ra and IL-8 CSF levels were significantly reduced in TLV vs Control at 6 h after CA. For instance, IL-8 concentration achieved 147.19 ± 71 vs 735.83 ± 243.28 pg/mL in the CSF in TLV vs Control, respectively. In addition, IL-6 concentrations in the CSF tended to decrease in TLV vs Control without reaching statistical significance (9.7 ± 5.05 vs 1669.61 ± 1093.53 pg/mL at 120 min after CA, respectively).

Neurological outcomes and immunohistochemistry

As illustrated in Figure 5, NFL blood levels significantly increased at the end of the protocol as compared to baseline in Control conditions but not after TLV group. In addition, NFL blood levels was lower in TLV vs Control at the end of the protocol (30.96 ± 5.51 vs 79.93 ± 33.55 pg/mL, respectively), but this difference did not achieve statistical significance when considering the multiple comparison correction ($p=0.040$).

The total number of degenerating neurons was also significantly reduced in the cortex of TLV animals vs Control (10 ± 1 vs 27 ± 4 neurons/field, respectively). The number of CD3+ cells counted at brain immunohistochemistry was also significantly lower in TLV vs Control-group (Figure 5).

Lung histology

As illustrated in Figure 6 (Panel A-C), the morphological appearance of the lung samples at histology revealed alveolar serous cardiogenic edema, congestion and interstitial inflammation. Foci of pulmonary infarct-like lesions were also observed in 5/8 swine in the Control group vs 0/8 in the TLV group (Figure 6D). Overall, histological lesional scores were significantly decreased for cardiogenic edema, congestion and pulmonary infarct-like lesions in TLV vs Control (Figure 6A). Scores of inflammation were not different among groups. In one lung sample from the TLV group, we also observed few macrophages with foamy appearance, suggesting clearance of the breathable liquid residues (Figure 6E).

Discussion

In this study, we investigated the impact of hypothermic TLV on the inflammatory response and brain injury after CA in pigs. Remarkably, TLV attenuated local immune response in the brain through reduced cytokine concentrations in the CSF and cerebral infiltration by CD3+ cells. TLV also attenuated brain injury regarding histopathological markers. This was not associated with a systemic effect on circulatory cytokine blood levels.

This study extends previous results obtained with TLV regarding its ability to induce very rapid hypothermia in large animals, providing a strong translational insight. Target temperature (<34°C) was achieved in all body compartments within 30 min, corresponding to a cooling rate of ~ -8°C/h in body core. This was associated with a significant reduction in the overall number of degenerating neurons in the brain, and a trend toward reduced NFL blood levels. To our knowledge, this is the first demonstration of the neuroprotective effect of TLV in large animals, as it was mostly documented in rabbits ^{4,10,12,14} and piglets ¹³ until now. Importantly, a short hypothermic phase was sufficient to provide neuroprotective effects whereas patients are generally treated with hypothermia for at least 24 hours when instituted in the clinical arena ⁷.

Importantly, the neuroprotective effect of TLV was observed with a procedure that started within 15 min after ROSC, allowing to achieve the target temperature within 45 to 60 min after the onset of CPR. This corresponds to a very early therapeutic window of application when compared to clinical trials on target temperature management requiring more than 6 to 8 hours to achieve target temperature after CA ^{5,6,16}. However, TLV was instituted post-ROSC, which differs from the intra-arrest cooling that provides the most potent protection in small animals ^{8,17}. This also differs from other small animal studies in which hypothermia is achieved virtually

immediately after cooling initiation, due to their low body mass, even when induced after ROSC⁸. In the present study, cooling protocol also differs from most reports performed in large animals, *i.e.*, pigs and dogs. In a recent meta-analysis of experimental studies in the field, Arrich et al. indeed demonstrated that among 17 large animals studies, 11 investigations started cooling at ROSC and 6 studies from 5 to 60 min after ROSC⁸. Among the latter studies, one study achieved target temperature within < 60 min¹⁸, 2 others required 60 min^{19,20} and 3 more than 90 min²¹⁻²³. Interestingly, this meta-analysis demonstrated that cooling rate was an independent predictor of the benefit of therapeutic hypothermia in large animals, along with hypothermia duration and target temperature, which was not the case for the time to target temperature. This suggests that cooling rate could be more important than the moment of cooling induction after ROSC among the included investigations. Importantly, we should again consider that most experimental studies evaluated short period of hypothermia, as we did here with only 3 h of hypothermia before starting rewarming, which is major difference with clinical practice.

This study also highlights the cerebral anti-inflammatory effects of TLV. These anti-inflammatory effects were observed not only during the brief hypothermic period but also throughout the rewarming phase, suggesting that despite the shortness of the hypothermic episode, the effect of hypothermia was sustained beyond this period. These interesting results are in accordance with a previous study that demonstrated that mild therapeutic hypothermia resulted in a decrease in inflammatory cytokine expression in the cortex of swine submitted to CA²⁴. Conversely, we did not observe any difference in blood cytokine levels in TLV vs Control animals. This differs from our previous study in rabbits in which IL-6 blood levels were reduced during the hypothermia episode in TLV animals vs Control¹⁰, suggesting that swine are less prominent to develop systemic than cerebral immune response after CA. Interestingly, our results

are consistent with clinical reports demonstrating no effect of hypothermia in patients on the cytokine blood levels after CA ^{10,25-27}.

Nevertheless, the key finding is that the local effect of TLV on cytokine concentrations in the CSF was ultimately associated with reduced infiltration by CD3+ lymphocytes, as a marker of neuroinflammation. This is consistent with previous experimental reports in animal models of focal cerebral ischemia showing that hypothermia not only decrease cerebral pro-inflammatory cytokine concentrations ²⁸ but also infiltration by circulatory leukocytes ^{27,28}. The mechanism of action of hypothermia on lymphocyte migration has not been fully investigated but could involve, at least in part, the reduction in pro-inflammatory cytokine levels in the CSF, reduced brain-blood barrier opening ¹² and a direct effect of temperature reduction on leukocytes reactivity ¹⁰.

In addition to brain injury evaluation, the present study also investigated the effect of TLV on lung recovery after cardiac arrest. Importantly, we observed a trend toward moderately lower blood oxygenation at the end of the follow-up in TLV vs Control animals, despite reduced lung lesions. We could speculate that TLV actually preserved lung morphology but could temporarily favor atelectasis during the evaporation phase of the breathable liquid from the lung. In the present study, PEEP was indeed limited to 5 cmH₂O after cardiac arrest, which is likely insufficiently to completely prevent some atelectasis. This could explain mildly reduced blood oxygenation despite reduced lung lesions after TLV as compared to Control animals.

This study presents some limitations. First, the hypothermic period was limited to 3 hours which is interesting to evaluate the early action of hypothermia but not comparable to the clinical practice. Second, animals were also not completely rewarmed by the end of the follow-up period. Third, due to a small sample size and gender selection, the results can hardly be extrapolated to a

wider population. In addition, when the sample size is small, p-value is not a great criterion for identifying significant differences. For instance, a power analysis demonstrated that at least 84 animals would be required to evidence a significant group effect for NFL blood levels at the end follow-up, considering an hypothetical group effect similar to that observed in the present study with $\beta=80\%$. It also suggests that histopathological parameters of neurological injury, which were less variable, might more appropriate for experimental studies with small sample size. Finally, the follow-up duration was limited to 6 hours after resuscitation, since we focused on acute brain injury and assessed early local cytokine production in the CSF. Longer period of follow-up would be necessary to assess delayed brain injury.

Conclusion

In conclusion, the rapid induction of hypothermia by TLV attenuates acute brain injury and reduces the local inflammatory response occurring in the early phase following resuscitation in swine submitted to CA. These findings support further evaluation in patients resuscitated after CA to investigate potential neuroprotection.

Declarations

Statements: All authors have read the journal's authorship agreement and policy on disclosure of potential conflicts of interest.

Conflict of interest: M Nadeau, P Micheau, M Kohlhauer and R Tissier are shareholders and experts of the company dedicated to total liquid ventilation that developed the investigational device (Orixha). M Libardi and S Perrotto are employees of Orixha.

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Ethics approval: The protocol was approved by the French national ethical committee on animal research (ComEth Anses/EnvA/UPEC n°16, project #27365-2020092810214926).

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Figure 1: Experimental protocol and body temperature

A- Schematic representation of the experimental protocol.

B- Esophageal, tympanic and bladder temperatures in animals submitted to the Control procedure or to rapid cooling with total liquid ventilation (TLV).

CA, cardiac arrest; VF, ventricular fibrillation; CPR, cardiopulmonary resuscitation; *, $p < 0.05$ between TLV and Control groups throughout the corresponding period.

Figure 2: Hemodynamic parameters

Heart rate, mean blood pressure and doses of norepinephrine administered after resuscitation to prevent hypotension in animals submitted to the Control procedure or to rapid cooling with total liquid ventilation (TLV).

*, $p < 0.05$ between TLV and Control groups.

Figure 3: Blood circulatory inflammatory markers

Blood concentration of cytokines at baseline, 120 min and 360 min after cardiac arrest in animals submitted to the Control procedure or to rapid cooling with total liquid ventilation (TLV).

*, $p < 0.05$ vs Corresponding baseline; †, $p < 0.05$ vs corresponding value at 120 min

Figure 4: Inflammatory markers in the cerebrospinal fluid (CSF)

Cytokine concentrations in the CSF at 120 min and 360 min after cardiac arrest in animals submitted to the Control procedure or to rapid cooling with total liquid ventilation (TLV), as compared to Sham animals neither submitted to cardiac arrest nor TLV

*, $p < 0.05$ vs Control-group; †, $P < 0.05$ vs Sham

Figure 5: Brain injury markers at the end of the protocol

A- Neurofilament light chains (NFL) blood levels at baseline and 360 min after cardiac arrest in animals submitted to the Control procedure or to rapid cooling with total liquid ventilation (TLV)

B- Mean number of degenerating neurons spotted with Fluorojade C staining per field in the cortex.

C- Morphological appearance of the cortex after Fluorojade C staining with very few positive cells in the TLV vs Control groups.

D- Mean number of CD3+ cells per field in the cortex, allowing to identify infiltrated T-cells into the brain.

E- Morphological appearance of the cortex after immunohistochemistry staining showing no or very few CD3+ cells in TLV vs Control.

*, $p < 0.05$ vs Control; †, $p < 0.05$ vs corresponding baseline

Figure 6: Histological appearance of the lung

A- Lesional scores on a 0-5 scale for cardiogenic serous edema, lung congestion, interstitial inflammation and infarct-like lesions of pulmonary parenchyma

B- Morphological appearance of one lung sample from the Control group demonstrating extensive and severe serous edema and congestion

C- Morphological appearance of one lung sample from the TLV group demonstrating moderate congestion without alveolar serous edema

D- Morphological appearance of one lung sample from the Control group demonstrating infarct-like lesions

E- Morphological appearance of one lung sample from the TLV group with one macrophage with a foamy appearance (arrow)

Table 1: Weight and resuscitation parameters in resuscitated animal submitted to the Control procedure or to rapid cooling with total liquid ventilation (TLV)

	Control	TLV
Weight (Kg)	31±1	34±1
Time to resumption of spontaneous circulation (ROSC, min)	8±1	6±1
Total dose of epinephrine before ROSC (mg)	0.6±0.3	0.5±0.2
Number of external defibrillation before ROSC	6±1	5±1

Table 2: Blood pH, O₂ and CO₂ partial pressures (PaO₂ and PaCO₂, respectively), lactate and bicarbonate levels in animal submitted to the Control procedure or to rapid cooling with total liquid ventilation (TLV)

	Baseline	Time after ROSC			
		30 min (During TLV)	120 min	180 min	360 min
Arterial blood pH					
Control	7.43±0.01	7.16±0.04	7.37±0.02	7.39±0.02	7.38±0.03
TLV	7.45±0.01	7.09±0.02	7.36±0.02	7.39±0.01	7.38±0.04
Arterial blood PaCO₂ (mmHg)					
Control	41±1	53±3	41±2	43±1	45±2
TLV	38±1	75±4*	43±1	41±2	50±3
Arterial blood PaO₂ (mmHg)					
Control	158±6	120±4	151±7	146±3	138±4
TLV	173±3	151±30	168±20	115±9	105±19
Bicarbonate arterial levels (mmol/L)					
Control	26.20±0.95	18.16±1.16	23.08±0.80	25.19±0.51	26.00±1.32
TLV	25.84±0.72	22.64±1.00	24.61±0.98	25.38±1.34	28.88±1.11
Lactate arterial levels (mmol/L)					
Control	2.9±0.4	11.5±1.1	6.1±1.2	4.6±0.6	3.5±1.1
TLV	3.1±0.3	10.6±0.5	7.8±0.8	6.0±0.8	3.7±1.2

PaCO₂, partial pressure of carbon dioxide; PaO₂, partial pressure of oxygen; *, $p < 0.05$ vs corresponding Control value (this time point occurs ~10 min after the onset of TLV)

Supplemental material

Supplemental Figure 1: Schematic representation and picture of the liquid ventilator