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Original Article

Liquid extracorporeal carbon dioxide removal: use of THAM (tris-hydroxymethyl aminomethane) coupled to hemofiltration to control hypercapnic acidosis in a porcine model of protective mechanical ventilation

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Abstract: A promising approach to facilitate protective mechanical ventilation is the use of extracorporeal CO_2 removal techniques. Several strategies based on membrane gas exchangers have been developed. However, these techniques are still poorly available. The goal of this study was to assess the efficacy and safety of THAM infusion coupled to hemofiltration for the management of hypercapnic acidosis. A severe respiratory acidosis was induced in seven anesthetized pigs. Five of them were treated with THAM 8-mmol·kg^{-1}·h^{-1} coupled to hemofiltration (THAM+HF group) at 100 mL·kg^{-1}·h^{-1}. After 18-hours of treatment the THAM infusion was stopped but hemofiltration was kept on until 24-hours. The 2 other animals were treated with THAM but without hemofiltration. After 1-hour of treatment in THAM+HF, $PaCO_2$ rapidly decreased from a median of 89.0 (IQR) (80.0, 98.0) to 71.3 (65.8, 82.0) mmHg ($P<0.05$), while pH increased from 7.12 (7.01, 7.15) to 7.29 (7.27, 7.30) ($P<0.05$). Thereafter $PaCO_2$ remained stable between 60-70 mmHg, while pH increased above 7.4. After stopping THAM at 18 hours of treatment a profound rebound effect was observed with severe hypercapnic acidosis. The most important side effect we observed was hyperosmolality, which reached a maximum of 330 (328, 332) mOsm·kg H_2O^{-1} at T18. The animals treated only with THAM developed severe hypercapnia, despite the fact that pH returned to normal values, and died after 12 hours. Control-group had an uneven evolution until the end of the experiment. A combined treatment with THAM coupled to hemofiltration may be an effective treatment to control severe hypercapnic acidosis.

Keywords: Acute respiratory distress syndrome, extracorporeal CO_2 removal, hemofiltration, hypercapnia, Tris hydroxymethyl aminomethane (THAM), ventilator induced lung injury

Introduction

During the last years there has been an increasing interest for introducing extracorporeal CO_2 removal ($ECCO_2R$) techniques in the management of patients with severe respiratory failure [1, 2]. One of its major potential roles appears to be the possibility of providing more protective mechanical ventilation (MV) to patients with moderate to severe acute respiratory distress syndrome (ARDS) to avoid ventilator induced lung injury [3]. Other source of interest derives from patients with decompensate chronic obstructive pulmonary disease, who do not respond to noninvasive ventilation, and in whom there is general reluctance to consider intubation [4].

The first techniques to provide $ECCO_2R$ were tested several decades ago, by using conventional circuits for extracorporeal membrane oxygenation (ECMO), but at lower blood flows.

This technology has remained available for decades [5, 6], but its use has been limited by several factors, including risks of hemorrhage and complex technical requirements similar to classical ECMO. More recently, several alternatives of $ECCO_2R$ have been developed based on smaller double-lumen central venous catheters, and simpler circuits, which resemble those used for continuous renal replacement therapies (CRRT) [4-7]. These novel $ECCO_2R$ systems use lower blood flows in the range of 0.5 to 1 L min^{-1}, and they are able to remove around 50% of CO_2 produced by metabolism. However, the technology is still poorly available, they are rather expensive, they include lung membrane gas exchangers, the catheters required are larger than those usually applied in most intensive care units (ICUs) to provide CRRT, and they still require significant anticoagulation.

Tris hydroxymethyl aminomethane (THAM) is a buffer (121 daltons) used in Europe since 1959 as an alternative to sodium bicarbonate for the treatment of acidosis [8]. It has the ability to bind protons in equimolar proportion, and its pKa is 7.8 at 37°C, which allows a better buffering capacity at physiologic pH. In addition, in contrast to bicarbonate, THAM does not increase CO_2 content. It is eliminated via glomerular filtration [9]. It has been used to treat not only metabolic acidosis, but also hypercapnic acidosis in critically ill patients [10]. However, it has a limited efficacy due to the relatively low dose that can be used due to accumulation. Current maximal dosing recommendations are 2 mmol·kg^{-1}·h^{-1}, and 15 mmol·kg^{-1} per day. Nevertheless, this restriction may be avoided by increasing THAM clearance through hemofiltration, which is frequently used in critically ill patients. Recently, Russ and co-workers tested this concept in a short experimental study (only 1 hour) [11]. Healthy anesthetized animals were hypoventilated up to a $PaCO_2$ around 70 mmHg, and then THAM was infused at 8.8 mmol·kg^{-1}·h^{-1}, combined with hemofiltration. Hypercapnia was partially controlled and arterial pH was normalized with this strategy.

The goal of this study was to assess in an experimental model the efficacy and safety of a high dose THAM infusion coupled to hemofiltration during 18 hours for the management of hypercapnic acidosis induced by hypoventilation.

Material and methods

Animals and instrumentation

The Institutional Review Board for animal care of the Universidad Andres Bello approved the study. *Guide for the Care and Use of Laboratory Animals*, 8th Edition, from the National Academy of Sciences of the United States of America. Pigs (*Sus scrofa*) of 23 to 33 kg weight were used in the study. Animals were fasted for 24 hours before the experiments, but with free access to water. They were premedicated with ketamine (Ketostop, Drag Pharma S.A, Chile) 20 mg·kg^{-1} and xylazine (Ronpum, Bayer Health Care, Germany) 2 mg·kg^{-1} i.m. After inserting a peripheral i.v. line, and injecting fentanyl (Fentanyl, Laboratorio Biosano, Chile) 30 μg·kg^{-1} and atracurium (Tracrium, GlaxoSmithKline, UK) 2 mg·kg^{-1} i.v., pigs were intubated and connected to mechanical ventilation in volume control ventilation mode (Savina® 300, Dräger, Germany) with positive end expiratory pressure (PEEP) of 8 cmH_2O, tidal volume (Vt) 12 ml·kg^{-1}. Respiratory rate was adjusted to keep end tidal CO_2 between 35 and 45 mmHg. Anesthesia was maintained with a continuous infusion of midazolam (Dormonid, Roche, Switzerland) 2 mg·ml^{-1}, fentanyl 20 μg·ml^{-1} and ketamine 20 mg·ml^{-1}, set at 4 ml·kg^{-1}·h^{-1} during invasive procedures, and at 2 ml·kg^{-1}·h^{-1} thereafter until the end of the experiment. Muscle paralysis was maintained with atracurium 0.5-0.25 mg·kg^{-1}·h^{-1} throughout all the experiment. A bolus of 10 ml·kg^{-1} of lactated Ringer's solution (Fresenius Kabi, Germany) was infused after anesthesia induction and a continuous infusion was kept at 4 ml·kg^{-1}·h^{-1} during the instrumentation period. Thereafter, during the rest of the experiment the infusion was kept at 2 ml·kg^{-1}·h^{-1}. In parallel, 10% dextrose solution (Fresenius Kabi, Germany) was infused starting at 1 ml·kg^{-1}·h^{-1}, and adjusted to keep glucose plasma concentration between 80 and 150 mg·dl^{-1}. The left carotid artery and the right and left jugular veins were surgically exposed for catheter insertion. A pulmonary artery catheter (Edwards Lifesciences, USA) was placed through the left jugular vein. In the animals treated with hemofiltration, a double-lumen 12Fr. dialysis catheter (Haemocat® Signo, B.Braun, Germany) was inserted through the right jugular vein. During the experiment, end-tidal CO_2 (Dräger Infinity Mcable-Maistream CO_2, USA), electrocardiogram, heart rate, pulse oxygen saturation, inva-

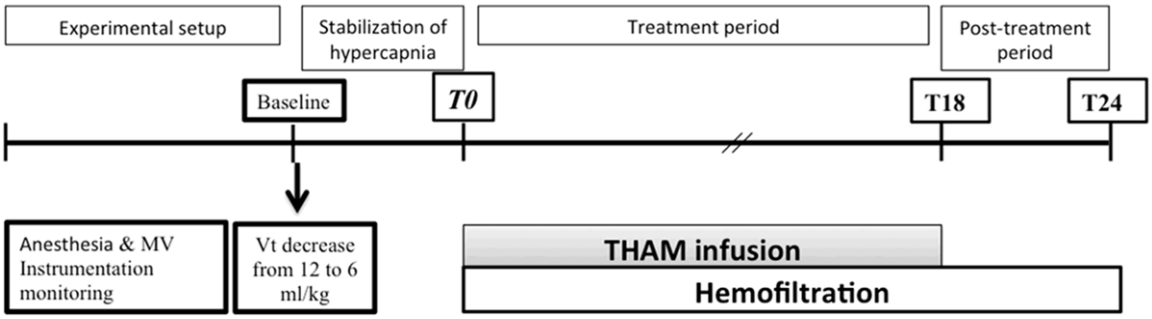

Figure 1. Experimental design for THAM+HF group. The figure describes the four protocol periods, the timeline, and the main events. Experimental setup took 2 to 3 hours, which was followed by baseline measurements. There after tidal volume was decreased from 12 to 6 ml·kg^{-1} and followed by a period of 90 minutes for stabilization of hypercapnia. After taking measurements (T0), THAM infusion and hemofiltration were started and maintained for a treatment period of 18 hours (T18). Then THAM infusion was stopped and hemofiltration was kept on for six additional hours to assess the post-treatment effects. MV, mechanical ventilation; Vt, tidal volume.

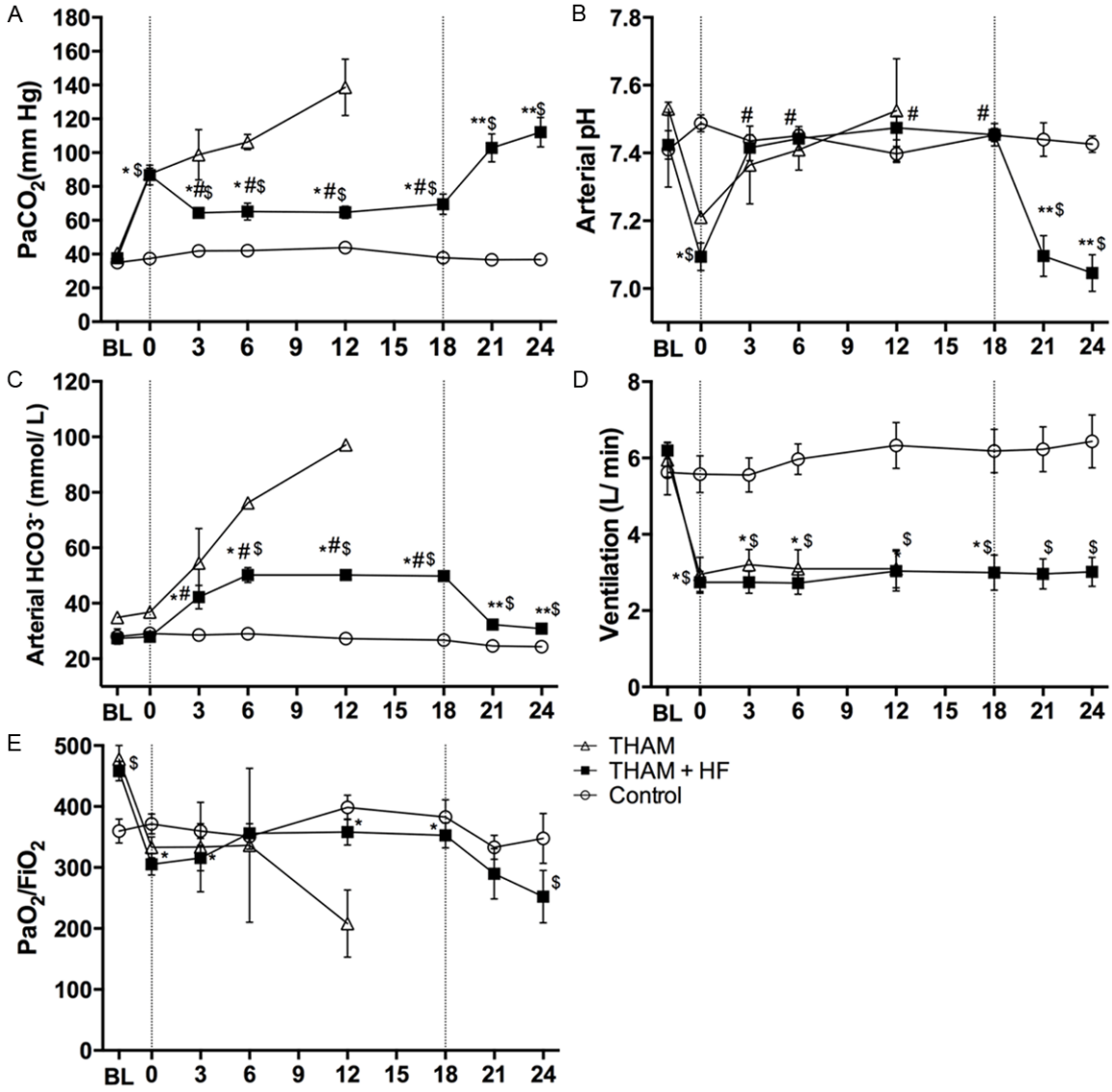

Figure 2. Respiratory and acid-base variables. Data of arterial partial pressure of carbon dioxide (A), arterial pH (B), arterial bicarbonate (C), ventilation (D), and PaO_2/FiO_2 ratio (E) for Control, THAM+HF, and THAM groups. Vertical broken lines at T0 and T18 indicate the start and the end of THAM infusion, respectively. Results are presented as median and interquartile range. *P<0.05 compared to baseline (only up to T18); #P<0.05 compared to T0 (only up to T18); **P<0.05 for T21 and T24 compared to T18; $P<0.05 for THAM+HF compared to control group. BL, baseline (just before decreasing tidal volume from 12 to 6 ml·kg^{-1}). No statistics were performed for THAM group, as only two animals were included.

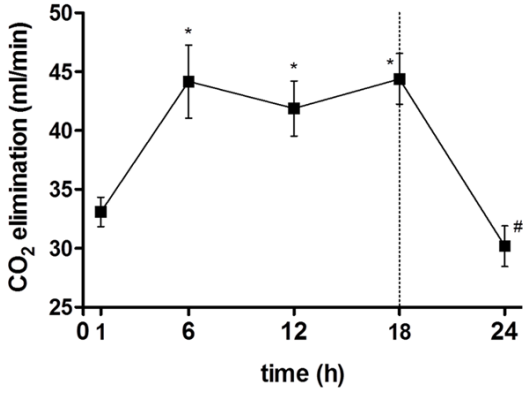

Figure 3. Extracorporeal CO_2 removal by THAM coupled with hemofiltration. Vertical broken line at T18 indicate the end of THAM infusion. Results are presented as median and interquartile range. *P<0.05 compared to T1 (only up to T18); #P<0.05 for T24 compared to T18.

sive arterial blood pressure, central venous pressure, pulmonary artery pressure, pulmonary artery occlusion pressure, cardiac output, core temperature (Infinity® Delta XL, Dräger, USA) and urine output, were monitored every hour.

*Experimental design (**Figure 1**)*

In seven animals we induced hypercapnic acidosis by hypoventilation. Five of them were allocated to be treated with THAM plus hemofiltration (THAM+HF group), and the other 2 to be treated just with THAM, but without hemofiltration (THAM group). In addition to these seven animals, we included five animals as sham (Control group). In these 5 control animals we kept them anesthetized and with controlled ventilation at Vt 12 ml·kg^{-1} and respiratory rate adjusted to maintain $PaCO_2$ between 35 and 45 mmHg during the 24-hours study period. In the seven animals allocated to hypercapnic acidosis, baseline measurements were performed after the instrumentation period, and then Vt was decreased from 12 to 6 ml·kg^{-1}, keeping respiratory rate constant. A 90-min period was allowed for stabilization of hypercapnia. There after (time 0, T0), in the THAM+HF group, hemofiltration was started while THAM 3 Molar (Tris 36.34% B.Braun, Germany) was infused by prefilter at 8 mmol·kg^{-1}·h^{-1} (treatment period). In this group THAM infusion was stopped after 18 hours (T18), but hemofiltration was kept on until T24. The purpose of this final post-treatment period of 6 hours was to assess the relative effect of THAM infusion on hypercapnic acidosis, as well as any potential post-treatment effect. The THAM group received a THAM infusion at 8 mmol·kg^{-1}·h^{-1} through the central venous catheter, but without hemofiltration. Efficacy was evaluated through arterial blood gases (i-STAT, Abbott Point of Care Inc. IL, USA [by cartridge CG4+]) which measures directly pH, pCO_2, pO_2, lactate and calculates bicarbonate [12, 13]; while safety was evaluated by measuring repeatedly blood glucose and lactate levels (i-STAT, Abbott Point of Care Inc. IL, USA), osmolality by freezing point (Fiske® 210, Norwood, Massachusetts, USA), phosphatemia (Hitachi U-5100 UV-Vis Spectrophotometer), free hemoglobin (spectrophotometer assays based on a peroxidase reaction, Hitachi U-5100 UV-Vis Spectrophotometer), as well as systemic hemodynamics, temperature and vital signs. Each experiment ended with euthanasia of the pig.

Setup of hemofiltration

Hemofiltration was performed using a continuous renal replacement therapy machine (Diapac Acute, B.Braun, Germany) with a polysulfone filter (2-m^2 surface area, inner diameter of capillaries 200 µm, wall thickness 40 µm, ultrafiltration coefficient 58 mL·h^{-1}·mmHg^{-1} and priming volume bloodside 121 ml [Diacap HI-PS 20 filter, B.Braun, Germany]). Blood flow was fixed at 200 ml·min^{-1}. Lactated Ringer's solution (Fresenius Kabi, Germany) was infused prefilter (predilution) as replacement fluid at 100 mL·kg^{-1}·h^{-1}. Hemofiltration was always isovolumetric. THAM 3 molar was infused prefilter within the circuit at 8 mmol·kg^{-1}·h^{-1} proximal to the replacement fluid. Unfractioned heparin (Heparin sodium 5000 UI·ml^{-1}, Fresenius Kabi) was infused prefilter and titrated to achieve an

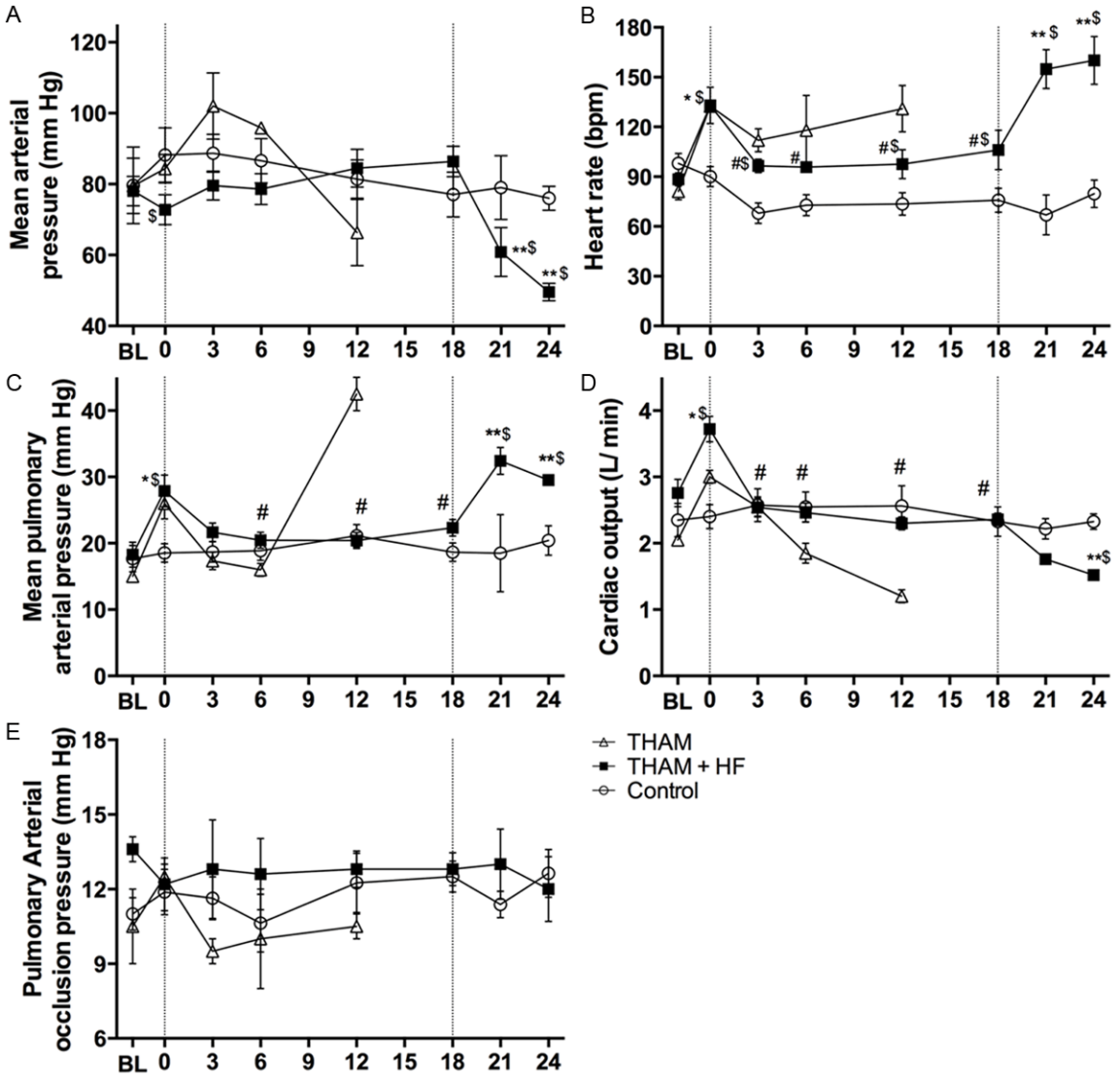

Figure 4. Hemodynamics variables. Data of mean arterial pressure (A), heart rate (B), mean pulmonary arterial pressure (C), cardiac output (D), and pulmonary arterial occlusion pressure (E) for Control, THAM+HF, and THAM groups. Vertical broken lines at T0 and T18 indicate the start and the end of THAM infusion, respectively. Results are presented as median and interquartile range. *P<0.05 compared to baseline (only up to T18); #P<0.05 compared to T0 (only up to T18); **P<0.05 for T21 and T24 compared to T18; $p<0.05 for THAM+HF compared to control group. BL, baseline (just before decreasing tidal volume from 12 to 6 ml·kg^{-1}). No statistics were performed for THAM group, as only two animals were included.

activated partial thromboplastin time in the out of extracorporeal circuit of 60-80 seconds.

Statistical analysis

Data are presented as median and interquartile range (IQR). Comparisons of continuous variables between the two groups were conducted with Mann-Whitney U test. Comparisons of different time points within a single group were performed by analysis of variance or by Friedman test, followed by post-hoc Bonferroni's or Dunn's test, as appropriate. P<0.05 was considered as statistically significant. Data were analyzed with STATA version 12.0 (StataCorp LP, College Station, TX).

Results

Acid-base variables

In the control group, $PaCO_2$, pH and bicarbonate levels remained rather normal and stable throughout the experiment (**Figure 2**). In groups

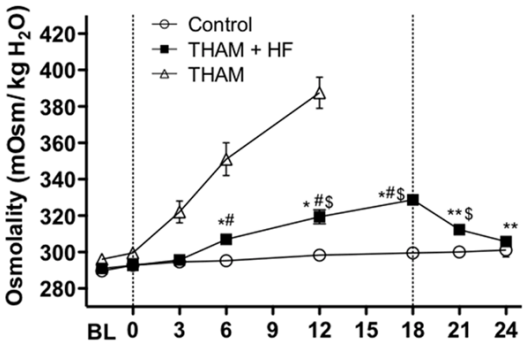

Figure 5. Evolution of the osmolality. Vertical broken lines at T0 and T18 indicate the start and the end of THAM infusion, respectively. Results are presented as median and interquartile range. *P<0.05 compared to baseline (only up to T18); #P<0.05 compared to T0 (only up to T18); **P<0.05 for T21 and T24 compared to T18; $p<0.05 for THAM+HF compared to control group. BL, baseline (just before decreasing tidal volume from 12 to 6 ml·kg^{-1}). No statistics were performed for THAM group, as only two animals were included.

THAM+HF and THAM, after decreasing Vt to 6 ml·kg^{-1}, $PaCO_2$ rapidly and progressively increased while pH decreased during the 90 min stabilization period. At T0 hypercapnic acidosis was still worsening with $PaCO_2$ and pH at 89.0 (80.0, 98.0) mmHg and 7.12 (7.01, 7.15) in the THAM+HF group, and 91.0 (90.4, 91.5) mmHg and 7.20 (7.20, 7.22) in the THAM group, respectively.

In the THAM+HF group a few minutes after starting treatment, end tidal CO_2 stopped increasing, and rapidly decreased towards a lower level. At time T1 $PaCO_2$ had already decreased to 71.3 (65.8, 82.0) mmHg, while pH had increased to 7.29 (7.27, 7.30). In the following hours of treatment $PaCO_2$ remained rather stable but pH continued increasing up to T9, after which it remained stable at rather normal levels, until T18, when THAM infusion was stopped. There after, hypercapnic acidosis reappeared rapidly within the following hours with $PaCO_2$ at 113.4 (93.4, 132.0) mmHg and pH at 7.04 (6.93, 7.16) at the end of the experiment at T24. Bicarbonate concentrations in plasma increased up to 40 to 50 mmol·L^{-1} showing a parallel trend to that of pH (**Figure 2C**). In the THAM group $PaCO_2$ and pH rapidly improved at T1, but thereafter $PaCO_2$ started to increase again rapidly going over 100 mmHg after T3, with both animals dying at T12 (**Figure 2**). Bicarbonate increased progressively since the start of THAM infusion reaching 76.3 (75.6, 76.9) mmol·L^{-1} at T6. In contrast, pH remained within rather normal values until the end.

Extracorporeal CO_2 removal through hemofiltration ranged 35 to 45 ml·min^{-1} (**Figure 3**), which represented 35 to 40% of total CO_2 production.

Systemic hemodynamic variables

There were no relevant hemodynamic changes along time in the control group (**Figure 4**). Induction of hypercapnic acidosis in groups THAM+HF and THAM were associated to tachycardia, pulmonary hypertension, and increased cardiac output. In the THAM group there was a transient decrease in tachycardia and pulmonary hypertension after starting THAM infusion, but cardiac output continued decreasing until both animals died. In the THAM+HF group, heart rate, pulmonary arterial pressure and cardiac output rapidly normalized after starting treatment and kept within normal ranges until THAM infusion was stopped at T18. Thereafter, pulmonary hypertension, severe hypotension, and tachycardia, became evident again (**Figure 4**).

Safety data

The most important side effect in the THAM+HF group was hyperosmolality (**Figure 5**). Osmolality increased significantly after T6 and reached a maximum of 330 (328, 332) mOsm·kg H_2O^{-1} at T18, decreasing towards normal values after stopping THAM infusion. Creatine kinase increased steadily in the 3 groups even after stopping THAM infusion (**Table 1**). In the THAM group, osmolality and creatine kinase rose even more rapidly and steadily reaching a maximum of 411 (398, 425) mOsm·kg H_2O^{-1} and 5343 (1887, 8799) U·L^{-1}, respectively. In addition, there was mild hyponatremia in the THAM+HF group. Phosphatemia was lower in the THAM+HF group compared to the control group, but within normal values. Plasma free hemoglobin increased only in the THAM group. Concerning hematologic variables, the only alteration was a drop in the platelets, however these were similar in the three study groups (Table S1). There was no hypoglycemia and the glucose requirements were similar among the three groups (dextrose infusion was 1.5 (1.4, 1.5), 1.7 (1.5, 1.7) and 1.6 (1.5, 1.8) mg·kg^{-1}·min^{-1} in THAM+HF, Control and THAM groups, respectively; P = 0.2).

Table 1. General blood biochemistry

	Group	T0	T6	T12	T18	T24
Sodium (mmol L^{-1}) [136-146]	THAM+HF	136 (134, 137)	132 (131, 133)$^{\$}$	130 (130, 133)$^{\#,\$}$	131 (128, 134)$^{\#,\$}$	132 (131, 137)$^{\$}$
	Control	140 (139, 142)	139 (135, 141)	141 (139, 141)	140 (140, 141)	141 (139, 143)
	THAM	135 (134, 135)	134 (133, 135)	125 (124, 125)		
Potasium (mmol L^{-1}) [3.5-4.9]	THAM+HF	4.1 (4, 4.2)	3.9 (3.9, 4)	4.3 (4.1, 5.2)	5.1 (4.1, 5.5)$^{\#}$	4.7 (3.8, 5.5)$^{\$}$
	Control	3.7 (3.4, 3.9)	4.2 (4.1, 4.5)$^{\#}$	4.3 (3.9, 4.4)$^{\#}$	4.2 (3.9, 4.9)$^{\#}$	3.9 (3.6, 4.2)
	THAM	4.5 (4.4, 4.6)	4.2 (3.8, 4.6)	5.8 (5.5, 6.1)		
Chloride (mmol L^{-1}) [98-109]	THAM+HF	98 (94, 100)	97 (94, 98)	102 (99, 103)	100 (100, 105)	104 (103, 106)
	Control	96 (95, 97)	98 (98, 99)	98 (97, 103)	101 (98, 103)	100 (98, 102)
	THAM	97 (95, 98)	94 (93, 95)	90 (88, 93)		
Phosphate (mg dL^{-1}) [2.6-4.5]	THAM+HF	7 (6, 7.4)	4.9 (4.9, 5.8)$^{\#,\$}$	3.7 (3.6, 4)$^{\#,\$}$	3.7 (3, 4.2)$^{\#,\$}$	5.4 (4.9, 5.6)$^{**,\$}$
	Control	7.4 (6.9, 8)	6.5 (5.4, 7.6)	8.3 (6.8, 8.9)	7.4 (5.6, 10.7)	8.2 (7.5, 9.4)
	THAM	8 (7.7, 9)	9 (8.5, 9.6)	6.8 (4.9, 8.7)		
Creatinine (mg dL^{-1}) [0.6-1.3]	THAM+HF	0.90 (0.6, 1.1)	0.68 (0.59, 0.86)$^{\#,\$}$	0.9 (0.7, 1)$^{\$}$	0.96 (0.77, 1.07)$^{\$}$	1.1 (1.08, 1.26)
	Control	0.94 (0.7, 1.1)	1.04 (0.8, 1.1)	1.2 (1.1, 1.3)	1.3 (1.2, 1.5)$^{\#}$	1.3 (1, 1.5)
	THAM	1 (0.95, 1.06)	1.5 (1.3, 1.7)	2.1 (1.9, 2.3)		
Blood urea nitrogen (mg dL^{-1}) [8-26]	THAM+HF	8 (5, 8.4)	7 (6.7, 8)$^{\$}$	5.5 (5, 7)$^{\$}$	8 (6, 8)$^{\$}$	8 (7, 9)$^{\$}$
	Control	9 (7, 10)	13 (9, 13)	18 (14, 22)$^{\#}$	16 (12, 26)$^{\#}$	18 (13, 29)
	THAM	9 (8, 10)	13 (8, 15)	16 (13, 18)		
Creatine Kinase ($U\ L^{-1}$) [38-174]	THAM+HF	554 (359, 771)$^{\$}$	1632 (983, 2273)$^{\#}$	2265 (1213, 4405)$^{\#}$	3614 (1912, 6040)$^{\#}$	4903 (2622, 7236)$^{\$}$
	Control	853 (430, 1722)	2371 (1111, 3631)$^{\#}$	2239 (1132, 6548)$^{\#}$	2455 (1178, 10421)$^{\#}$	2391 (1439, 10628)
	THAM	1069 (534, 1604)	3790 (1571, 6009)	5343 (1887, 8799)		
Plasma Free Hemoglobin (mg dL^{-1}) [0-10.6]	THAM+HF	3.6 (3, 7.1)$^{\$}$	3.8 (3, 6)$^{\$}$	5 (4.5, 9)$^{\$}$	3.2 (2.6, 13)$^{\$}$	8.3 (6, 11)**
	Control	8.5 (5.2, 11)	7.8 (5.9, 9.9)	2.3 (2, 2.6)	7.4 (2.8, 11.9)	6.5 (2, 11)
	THAM	2.4 (2, 2.7)	11.4 (2, 20.8)	31.2 (22.5, 40)		
Arterial Lactate (mmol L^{-1}) [0.36-1.25]	THAM+HF	1.9 (1.3, 2.2)	2.7 (1.7, 4.9)$^{\$}$	2.7 (2.3, 5.1)$^{\$}$	4.1 (3.1, 6.3)$^{\#,\$}$	5.2 (2.1, 8.3)$^{**,\$}$
	Control	1.5 (0.9, 2)	1.8 (0.8, 2.8)	1.9 (0.8, 2.8)	1.2 (0.6, 2.1)	1.1 (0.6, 1.8)
	THAM	1.2 (0.3, 2.1)	3.9 (2, 4.1)	10.6 (7.4, 13.8)		

In the first column each parameter is shown with its corresponding units () and normal range []. Results are presented as median and interquartile range. $^{\#}$P<0.05 compared to T0 (only up to T18); **P<0.05 for T24 compared to T18; $^{\$}$P<0.05 for THAM+HF compared to control group. No statistics were performed for THAM group, as only two animals were included.

In the THAM+HF group there was a rise in lactate levels during the treatment period which reached a peak of 4.1 (3.1, 6.3) mmol·L^{-1} at T18, and which further increased in the post-treatment period (**Table 1**). In the THAM group there was an early and severe hyperlactatemia.

Discussion

The main finding of this experimental study is that THAM coupled to hemofiltration appears to be an effective treatment to revert hypercapnic acidosis secondary to prolonged hypoventilation. This novel therapeutic approach appears to be safe in general, and the main side effect was hyperosmolality, which appears to be due to THAM accumulation.

The use of THAM without CRRT in the treatment of acidosis is well established [9]. It has even been used in the treatment of hypercapnic acidosis in patients with acute lung injury, ARDS [10] and severe asthmatic crisis at doses up to 2 mmol·kg^{-1}·h^{-1} [14]. It has also been used to induce short periods of apnea for bronchoscopy procedures. In a recent experimental study six pigs were subjected to complete apnea for up to 4 hours while treated with THAM, but a huge dose of 20 mmol·kg^{-1}·h^{-1} [15]. Four of them survived until the end of the experiment keeping pH at a physiologically acceptable level. In critically ill patients prolonged infusions as long as 5 days of low doses (2 mmol·kg^{-1}·h^{-1}) of THAM have been reported, without evident adverse events [16].

Dialysis and hemofiltration without the use of bicarbonate solutions have the ability to clear CO_2 through bicarbonate removal [17]. However, this effect is limited because of the inability to maintain electrolyte concentrations and pH while removing bicarbonate. Numerous approaches to replace bicarbonate have been attempted using sodium hydroxide, THAM, and other organic anions. However, these attempts have failed when applied for extended use

because of fluid gain, hyperchloremic acidosis, hemolysis, cardiac arrhythmias and acid-base disorders [17-19]. In this study, by combining a high dose infusion of THAM coupled to hemofiltration, we were able to overcome most of these limitations and could treat effectively a severe hypercapnic acidosis for at least 18 hours.

The rationale of this strategy is that THAM captures protons favoring the dissociation of carbonic acid into bicarbonate, decreasing $PaCO_2$ and increasing bicarbonate and base excess. Although protonated THAM and bicarbonate can then be excreted by urine, this occurs at a rather low rate, which limits the maximal efficacy of the strategy and its time extension. However, if hemofiltration is added, the dose of THAM can be increased leading to more excretion of THAM, protons and bicarbonate, besides allowing for a longer treatment.

In a recent experimental study in which severe respiratory acidosis was induced in pigs, Karagiannidis and co-workers [20] observed that when using lung membrane oxygenators, blood flows over 750 ml·min^{-1} were required to restore a normal pH. In the present study pH was effectively corrected with an extracorporeal blood flow of only 200 ml·min^{-1}, which corresponded to 5.2 (4.7, 8.4)% of cardiac output. Although acidosis was fully corrected by THAM+HF, as reflected by pH which increased over 7.4, hypercapnia was only partially reverted. Bicarbonate increased slowly but progressively, in parallel to osmolality, during the 18 hours of THAM infusion. This increase in bicarbonate level had already been described in a previous study using a high dose of THAM [15], but reaching markedly higher levels than those observed in our study in the THAM+HF group.

While protons may be removed bound to THAM, or buffered by increasing concentrations of THAM, CO_2 removal remains dependent on bicarbonate elimination. The increasing concentrations of bicarbonate as well as progressive hyperosmolality, which suggest THAM accumulation, indicate that the dose of THAM relative to hemofiltration rate was perhaps still too high. However, when comparing it to the levels observed in the THAM group (without HF), it becomes evident that hemofiltration had a substantial contribution to the buffering efficacy of THAM (by removing most of the protonated THAM and bicarbonate generated).

Other factors related to hemofiltration may have increased the alkalinizing effect of the treatment. Lactate present in the replacement fluid can be metabolized generating bicarbonate [21]. Removal of unmeasured anions by hemofiltration may have also contributed to this effect [22].

The use of THAM and hemofiltration appears to have several potential advantages compared to conventional $ECCO_2R$ techniques based on lung membranes. First, renal replacement equipment to provide hemofiltration is widely available worldwide [23]. On the other hand, THAM is a commercially available buffer already used in clinical practice [9]. Second, the technique proposed is relatively inexpensive. Third, it can be applied through conventional dialysis catheters. Forth, it requires less anticoagulation. Fifth, hemofiltration has the ability to remove not only CO_2, but also other molecules, and fluids. Besides, because lung edema plays a major role in ARDS, the possibility of inducing negative fluid balance is highly relevant. However, other forms of renal replacement therapy such as dialysis or hemodiafiltration with used of bicarbonate-free solutions, may also potentially work to enhance THAM removal. This alternative should be assessed in the future.

Concerning safety, the main side effects previously reported for THAM were hypoglycemia and hyperkalemia [9]. We observed no hypoglycemia and although glucose at low dose was infused regularly to keep normal glucose levels, there was no difference between groups in the amount of glucose infused. Potassium increased moderately in the THAM group but not in the THAM+HF group. The main side effect that we identified was hyperosmolality, which was progressive and reached a peak level around 330 mOsm·kg H_2O^{-1} at time T18 in the THAM+HF group, but more than 400 mOsm·kg H_2O^{-1} in the THAM group already at T12.

Elevated blood lactate concentrations in THAM+HF group were probably related to the use of a lactate based solution as fluid replacement for hemofiltration, as has been reported previously [24]. In fact, after stopping THAM infusion at T18, the concentration of lactate continued to rise. A different situation may have occurred in the THAM group, where lactate levels at T12 were greater than 10 mmol·L^{-1}, probably reflecting a situation of systemic toxicity, secondary to accumulation of THAM.

We think that hyperosmolality reflects both, bicarbonate and THAM accumulation. Although in this study we used a fix and rather high THAM dose, accumulation could be potentially prevented in the future by increasing the hemofiltration rate. We acknowledge several limitations of our study. First, we included only two animals in the THAM group. Our purpose with this group was just to make evident that hemofiltration played a major role in the efficacy of the strategy. After treating these 2 animals with THAM without hemofiltration, and observing how early they died after severe hemodynamic instability, it was neither ethical nor necessary to include more animals in this group. Second, we tested the strategy in animals without acute lung injury. Our goal was just to validate the concept and feasibility. However, we cannot extrapolate our results obtained in these animals intentionally hypoventilated, to conditions of acute respiratory failure where such a strategy appears as an attractive alternative to avoid ventilator-induced lung injury. Third, the doses of THAM and hemofiltration rate used were probably not optimal, as we did not perform a dose-response curve.

In conclusion, this experimental study shows that a combined treatment with THAM and hemofiltration may be an effective alternative to control severe hypercapnic acidosis for at least 18 hours. The main side effect was serum hyperosmolality.

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Disclosure of conflict of interest

None.

Authors' contribution

PT and AB participated in all aspects of the study and wrote the manuscript. LA organized the experiments, and performed animal experiments. FL performed the surgical procedures. KH took care of THAM infusion and the hemofiltration system. LE and DS contributed to biochemical analysis. FS contributed to data analysis and writing the manuscript. All authors read and approved the final manuscript.

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Table S1. Hematologic, hepatic and biochemical laboratory data

Variable	Group	T0	T6	T12	T18	T24
Hemoglobin ($gr{\cdot}dL^{-1}$) [7.1-12]	THAM+HF	7.4 (7.3, 8.3)	8.3 (7.6, 8.9)	8.1 (7, 8.5)	8.5 (6.8, 8.7)	7.5 (5.7, 9.3)
	Control	7.3 (6.8, 7.7)	8.4 (7.3, 9)	8.6 (7.6, 8.8)	8.2 (7.2, 8.4)	8.1 (7.7, 8.9)
	THAM	8.4 (8.2, 8.5)	9.8 (8.8, 10.7)	12.4 (11.7, 13)		
White blood cells (x $10^3/\mu L$) [4.5-11]	THAM+HF	11.4 (11.1, 11.6)	N/A	11.9 (11.4, 11.8)	11.8 (11.7, 12.2)	12.5 (11.3, 13.8)**
	Control	11.6 (11.5 , 11.8)	N/A	11.8 (11.7, 11.9)	12.7 (12, 13.3)	11.7 (11.4, 11.9)
	THAM	12 (11.9, 12.2)	N/A	11.1 (10.9, 11.3)		
Platelet count (x $10^3/\mu L$) [150-400]	THAM+HF	403 (381, 424)	N/A	202 (149, 257)#	191 (152, 217)#	169 (124, 189)
	Control	367 (340, 390)	N/A	172 (165, 179)#	160 (153, 166)#	159 (144, 174)
	THAM	340 (323, 358)	N/A	222 (186, 259)		
Alanine transaminase ($U{\cdot}L^{-1}$) [10-40]	THAM+HF	37 (27, 42)	N/A	40 (28, 45)	52 (37, 61)	60 (30, 121)$
	Control	45 (42, 66)	N/A	42 (41, 66)	38 (34, 72)	38 (27, 66)
	THAM	45 (39, 51)	N/A	27 (13, 41)		
Aspartate aminotransferase ($U{\cdot}L^{-1}$) [10-55]	THAM+HF	17 (16, 20)$	N/A	68 (66, 121)#	495 (438, 750)#,$	1369 (1312, 4160)**,$
	Control	34.5 (27, 59)	N/A	58 (42, 87)	40 (31, 121)	63.5 (47, 102)
	THAM	32 (29, 34)	N/A	171 (116, 225)		
Alkaline phosphatase ($U{\cdot}L^{-1}$) [45-115]	THAM+HF	115 (96, 132)	N/A	272 (144, 307)#,$	536 (260, 574)#,$	616 (407, 630)$
	Control	81 (67, 95)	N/A	91 (70, 137)	51 (40, 126)	109 (91, 130)
	THAM	100 (88, 112)	N/A	197 (184, 209)		
Total Bilirubin ($mg{\cdot}dL^{-1}$) [0-1]	THAM+HF	0.06 (.04, 0.09)$	N/A	0.08 (0.07, 0.1)$	0.27 (0.1, 0.66)#,$	0.8 (0.57, 1.4)**,$
	Control	0.13 (0.1, 0.15)	N/A	0.15 (0.1, 0.15)	0.1 (0.05, 0.15)	0.15 (0.1, 0.15)
	THAM	0.06 (0.03, 0.08)	N/A	0.08 (0.05, 0.1)		
Indirect Bilirubin ($mg{\cdot}dL^{-1}$) [≤ 0.3]	THAM+HF	0.04 (0.04, 0.05)	N/A	0.08 (0.05, 0.13)	0.17 (0.09, 0.34)#,$	0.68 (0.53, 1.05)**,$
	Control	0.06 (0.04, 0.09)	N/A	0.05 (0.04, 0.08)	0.04 (0.03, 0.09)	0.05 (0.04, 0.09)
	THAM	0.05 (0.02, 0.07)	N/A	0.09 (0.08, 0.09)		
Gamma-glutamyl transferase ($U{\cdot}L^{-1}$) [4-50]	THAM+HF	28 (26, 29)	N/A	22 (21, 28)	27 (24, 32)	44 (31, 52)**
	Control	36 (22, 46)	N/A	38 (20, 41)	35 (32, 51)	33 (21, 40)
	THAM	36 (33, 39)	N/A	42 (38, 46)		
Prothrombin time (sec) [2-8]	THAM+HF	11 (10.7, 11.2)	N/A	12.5 (11.5, 13.7)	11.3 (10.5, 14)	24.3 (22.1, 31)**,$
	Control	10.2 (10, 10.3)	N/A	10.2 (9.3, 11.1)	11 (10.6, 11.3)	11.3 (10.6, 12)
	THAM	10.8 (10.6, 10.9)	N/A	10.5 (10.3, 10.6)		
Lactate dehydrogenase ($U{\cdot}L^{-1}$) [135-225]	THAM+HF	318 (298, 339)$	N/A	397 (348, 788)#	744 (544, 1146)#	1936 (1365, 2722)**,$
	Control	396 (352, 577)	N/A	466 (346, 661)	545 (395, 672)#	876 (450, 970)**
	THAM	455 (423, 487)	N/A	837 (783, 890)		
iCa++($mmeq{\cdot}L^{-1}$) [2.2-2.6]	THAM+HF	2.7 (2.6, 2.7)	2.5 (2.3, 2.5)	2.6 (2.5, 2.6)	2.6 (2.5, 2.6)	2.9 (2.6, 2.9)$
	Control	2.6 (2.5, 2.6)	2.4 (2.3, 2.5)	2.6 (2.5, 2.6)	2.5 (2.3, 2.5)	2.2 (2.1, 2.4)
	THAM	2.6 (2.5, 2.6)	1.8 (1.7, 1.9)	1.3 (1.2, 1.3)		
Proteins ($gr{\cdot}dL^{-1}$) [5.5-8.0]	THAM+HF	4.1 (3.5, 4.2)	N/A	4.2 (3.1, 4.4)	3.9 (3.3, 4.3)	2.8 (2, 3.6)$**
	Control	4.1 (4, 4.1)	N/A	4 (3.8, 4.3)	4.1 (3.8, 4.5)	3.8 (3.5, 4)
	THAM	4.9 (4.8, 5.1)	N/A	5.4 (5.1, 5.7)		
Albumine ($gr{\cdot}dL^{-1}$) [3.5-5.0]	THAM+HF	2.1 (1.9, 2.2)	2 (2, 2.1)	2.1 (2, 2.3)	2.1 (1.9, 2.5)	1.6 (1.5, 2.3)
	Control	2 (1.9, 2.1)	2.2 (1.9, 2.4)	1.9 (1.8, 1.9)	1.8 (1.7, 2)	1.8 (1.7, 1.9)
	THAM	2.4 (2, 2.9)	2.7 (2.5, 2.9)	2.6 (2.2, 2.9)		

In the first column each parameter is shown with its corresponding units () and normal range []. Results are presented as median and interquartile range. #p<0.05 compared to T0 (only up to T18); **p<0.05 for T24 compared to T18; $p<0.05 for THAM+HF compared to control group. No statistics were performed for THAM group, as only two animals were included. N/A, not assessed.