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Physiological Response to a Simplified Veno-Venous Perfusion-Induced Systemic Hyperthermia System

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Abstract

Our original venovenous perfusion-induced systemic hyperthermia (vv-PISH) system appeared to significantly improve lung cancer patient survival, but was too complex with numerous dialysis problems. We tested a simplified vv-PISH circuit that includes the AvalonElite double lumen cannula, a modified heat exchanger, water heater/cooler, and a centrifugal pump. The purpose of this study was to evaluate this simplified vv-PISH system (without hemodialyzer) and to investigate the physiological response to whole body hyperthermia in pigs. We tested our vv-PISH circuit in healthy female adult swine (n= 7, 55-68 kg). The therapeutic core temperature (42°C), calculated as mean of rectal, bladder, and esophageal temperatures, was achieved in six swine. A maximum difference of 0.5°C was observed between the individual temperature sensor readings, indicating homogeneous heat distribution. Heart rate and mean arterial pressure were transiently altered, but were safely managed. A significant elevation in pulmonary artery pressure occurred during the heating phase, resulting in one pig death. In all other pigs, pulmonary artery pressure returned to physiologic values during the therapeutic phase. Arterial blood electrolytes were maintained without the need of a dialyzer. Major organ function was within normal parameters. The simplified vv-PISH circuit reliably delivered the hyperthermic dose with no need of dialysis.

Keywords

systemic hyperthermia; advanced cancer; whole-body hyperthermia

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INTRODUCTION

Hyperthermia selectively kills cancer cells and enhances the cytotoxicity of certain chemotherapy drugs.¹⁻⁴ Numerous clinical trials have reported improved outcomes with local/regional hyperthermia plus chemotherapy for some localized cancers.^{2,3,5,6} The use of whole body hyperthermia for refractory cancer with remote metastasis has been reported but is limited due to difficulties in administering and monitoring the therapeutic thermal dose.^{7,8} Our group has developed a veno-venous perfusion-induced systemic hyperthermia (VV-PISH) system for homogeneous whole body hyperthermia with accurate thermal dosimetry.⁹ We tested this vv-PISH system in a phase I clinical trial in 10 patients with advanced non small cell lung cancer (NSCLC). The retrospectively selected, matched control group median survival was 96 days, while the hyperthermia group median survival was significantly greater at 450 days.¹⁰ Based on these initially promising results we elected to further refine the hyperthermia delivery circuit.

Although our original vv-PISH system functioned adequately, there were two critical issues preventing further application of vv-PISH. First, the complicated circuit required a bulky dialysis unit, multiple pumps and very long tubing connections. Second, the patients were somnolent for up to 48 hrs, although they eventually recovered.¹⁰ The purpose of this study was to evaluate a simple vv-PISH system without a hemodialyzer and to investigate the physiological response to whole body hyperthermia in pigs. In this paper, we show that the pig blood electrolytes and pH can be easily maintained within the normal physiological range without hemodialysis. We also found a significant increase in pulmonary artery pressure which must be addressed for safe vv-PISH application.

METHODS

Animal Preparation and Instrumentation

Seven healthy adult female swine (55-68 kg) were used in this study. This study was approved by the Institutional Animal Care and Use Committee at the University of Kentucky and was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals".

Intravenous telazol (2.0 mg/kg), ketamine (1.0 mg/kg), and xylazine (1.0 mg/kg) were administered for anesthesia induction. After intubation, the swine were transferred to the operating room and general anesthesia was maintained with isoflurane inhalation through the Narkomed 2B anesthesia machine (DRAGER, Telford, PA). Two 16 G catheters (Becton Dickinson, Sandy UT) were placed into the femoral artery and vein via a right groin cut-down for blood sampling/pressure monitoring and fluid administration, respectively. A Swan-Ganz catheter (Edwards Lifesciences, Irvine CA) was placed percutaneously through the left jugular vein to the pulmonary artery (PA) for measurement of cardiac output, PA pressure (PAP), and central venous pressure (CVP). The catheters were connected to Tru-Wave transducers (Edwards Lifesciences Irvine, CA) for the monitoring of arterial blood pressure (ABP), CVP and PAP via a MP-50 monitor (Phillips, Boeblingen, Germany). Temperature probes were placed in the midesophagus, rectum, bladder (Foley catheter), blood in/out of animal, and pulmonary artery. After instrumentation, operative anesthesia

was switched to a fentanyl/diazepam (8 mcg/ml + 0.08mg/ml) continuous infusion to avoid the isoflurane-induced vasodilation during hyperthermia.

Data Acquisition

The data acquisition system we used in this study was the cDAQ9172 (National Instruments, Austin, TX) with a temperature module (NI-9219), a pressure module (NI 9237), and a flow module (NI 9215). The temperature module was connected to the temperature probes placed in rectum, bladder, esophagus, and blood in/out tubing for constant temperature measurement. The core temperature was defined as the average of the rectal, bladder and esophageal temperatures. The pulmonary artery temperature measured via the Swan-Ganz catheter was not included in the core temperature calculation due to its close proximity to the site where the heated blood enters the right atrium via the DLC. The pressure module was connected to the pressure sensors for PAP, ABP and CVP monitoring. The flow module was connected to the flow meter (T110, Transonic Systems Inc.) for circuit blood flow monitoring. Data acquisition software (DAQ, Labview 8.6, National Instruments, Austin, TX) was used to record the temperature, blood pressure, and pump flow rates simultaneously at 5Hz.

The vv-PISH System

Compared to the previous complicated version,^{9,10} the new vv-PISH circuit was significantly simplified (**Figure 1**) by 1) removal of the bulky hemo-dialyzer and two associated pumps, 2) one compact CentriMag® centrifugal pump (Levitronix, Waltham, MA) to replace the bulky roller pump, 3) a compact heat exchanger cut off from an Affinity® gas exchanger (Medtronic, Minneapolis, MN) to replace the long ECMO heat exchanger, and 4) a one-site venous cannulation via a double lumen cannula¹¹ (Avalon Elite™, Avalon Laboratories, LLC, Rancho Dominguez, CA) to replace the two separate cannulations (two individual cannulas) and associated long tubing connection. A water-circulated heater/cooler (Model 8212, Polysciences, Inc, Warrington, PA) connected to the heat exchanger was used for heating and cooling blood.

Establishment and Maintenance of vv-PISH

Systemic anticoagulation was initiated with a bolus of intravenous heparin (150 U/kg) and maintained at an activated clotting time of 180-250 seconds throughout the experiment. The double lumen cannula (DLC) was inserted through a small incision on the jugular vein into the superior vena cava (SVC), traversing the right atrium (RA), with the tip positioned in the inferior vena cava (IVC). Correct positioning of the DLC was assured by anatomical data from previous pig autopsies.

This DLC was connected to the vv-PISH circuit which was previously primed with a heparin/Ringer's solution (3 U heparin/ml). When the pump was started, the venous blood was drained from the DLC drainage lumens (IVC and SVC) and sent to the heat exchanger for heating. The heated blood was pumped back through the DLC infusion lumen into RA-pulmonary circulation. During the heating phase, the blood flow was set at 1-1.5 L/min and the water-to-circuit blood temperature gradient was less than 4°C with a maximum water temperature of 45.5°C until the 42°C core temperature was achieved. This core temperature

was maintained at 42-42.5 °C for 2 hrs for the cancer therapeutic window. Then, the cooling phase was started by circulating cool water through the heat exchanger until the core temperature reached 38 °C. Perfusion was then terminated and the pigs were euthanized with Beuthanasia-D (1 mL/10 lb body weight, Schering-Plough Animal Health, Union, NJ).

Free hemoglobin was measured at baseline and end-cooling time-points to assess hemolysis. Arterial blood chemistries and complete blood counts were measured at the following time-points: 1) baseline, 2) start therapeutic, 3) mid-therapeutic, 4) end-therapeutic, and 5) end-cooling. Arterial blood gases/electrolytes were measured every 15 minutes using a blood gas analyzer (Cobas b221 Roche Diagnostics Indianapolis, IN). Hemodynamic parameters were continuously monitored, and cardiac output was measured every 15 minutes. Urine output was measured hourly. Continuous intravenous 0.9% sodium chloride infusion (100-999 mL/hr) was administered to maintain the blood volume (CVP) for stable hemodynamics. Phenylephrine (0.5 to 2.0 µg/kg/min) was given as needed to maintain the systolic arterial blood pressure above 90 mm Hg. Supplemental intravenous calcium chloride (100 mg/mL) was applied to correct hypocalcemia as necessary. An intravenous bolus of furosemide (5-20 mg) was given if hourly urine output was less than 50 mL.

Data Analysis

All data are expressed as mean ± standard deviation (SD). A p-value of < 0.05 was considered statistically significant. Differences between the baseline and subsequent time-points were evaluated using analysis of variance with a Dunnett post test.

RESULTS

Six of the seven swine survived the experiment and achieved the therapeutic hyperthermia window (42 °C for 2 hrs). One swine developed circulatory collapse from severe pulmonary hypertension and subsequently died during the heating phase. Therefore, data from six swine was included in the physiological analysis. The simplified vv-PISH system maintained blood electrolytes/volume in physiological range without hemodialysis.

vv-PISH performance

In the 6 swine surviving the experiment, 85±34 min of heating time was required to achieve the therapeutic core temperature. After the therapeutic window of 120 minutes was completed, 57±19 min was required to cool the swine to 38°C. The swine temperature did not exceed 42.5°C at any measured site. The temperature difference between the rectal, bladder, and esophageal probe readings was only 0.5 °C (**Figure 2A**). The vv-PISH circuit blood flow rate was 1.3 ± 0.2 L/min. During the heating phase, the circuit blood infusion temperature reached a maximum of 43.5 ± 1.2 °C with the greatest difference between the blood infusion and core temperatures being 2.6 °C (**Figure 2B**). During the cooling phase, there was up to a 3.4 °C difference between the blood infusion and core temperatures.

Hemodynamic Response

Mean arterial pressure (MAP), CVP, and cardiac output were maintained in the physiological range throughout the experimental period (**Table 1**). Heart rate significantly

increased during the therapeutic phase, but returned to baseline during the cooling phase. There were no significant changes in PAP during the therapeutic and cooling phases (**Table 1**). During the heating phase, however, PAP was significantly elevated in 4 out of 7 pigs (**Figure 3**). This temporary increase in PAP caused severe circulatory collapse in one pig in the heating phase.

Fluid and blood electrolyte balance

Normal saline infusion at 100-500 ml/hr was used to maintain the blood volume (CVP) for stable hemodynamics. Urine output was 586 ± 160 mL/hr. Blood sodium, potassium, chloride, bicarbonate and arterial pH were maintained in physiological range (**Table 2**). Ionized calcium levels were significantly reduced, requiring calcium supplementation.

Blood parameters and liver/kidney function

Hemoglobin, hematocrit, and red blood cell counts were unaffected by hyperthermia (**Table 3**). Free hemoglobin was below 7 throughout the study. Total white blood cell counts were unchanged. Granulocytes were significantly elevated and lymphocytes were significantly reduced. Platelet counts were reduced significantly, but were within normal range.

Blood glucose was significantly elevated when the target core temperature was met but returned to baseline by the end-cooling time-point (**Table 3**). Alkaline phosphatase and alanine aminotransferase levels remained stable, suggesting the absence of hepatocellular injury. Urine output was 586 ± 160 mL/hr, blood urea nitrogen, and creatinine was in normal range, indicating normal kidney function. Albumin and total protein were unchanged by hyperthermia.

DISCUSSION

The current study showed that our simplified vv-PISH system can consistently deliver the hyperthermic dose (42° C for 2 hrs) to adult healthy swine. Without a hemodialyzer, the vv-PISH system can still maintain the blood volume-acid/base balance and electrolytes in physiological range. However, vv-PISH caused severe pulmonary hypertension in the heating phase, warranting further investigation.

A hemodialyzer included in our previous vv-PISH circuit was used for balancing blood volume and maintaining acid/base-electrolytes in physiological range. However, the hemodialyzer in the vv-PISH system required two additional pumps to drive and control the dialysate and an additional circuit to connect to the system. The hemodialyzer made the vv-PISH system complicated and expensive, which: 1) compromised vv-PISH performance, requiring higher blood temperature (48 ° C) to compensate for heat loss from the extra long circuit, 2) caused potential blood damage by the higher blood temperature, 3) prolonged the heating time to reach therapeutic window, and 4) increased blood contact foreign surface area and related activation of thrombosis/inflammation.

In the current *in vivo* large animal study, the vv-PISH circuit was significantly simplified by eliminating the hemodialyzer and two related pumps. The blood volume was easily maintained/balanced by fluid therapy and light diuresis. The blood electrolytes were well

balanced except for mild hypocalcemia, which was easily corrected by supplemental intravenous calcium chloride doses. Therefore, based on this experience, the hemodialyzer can be safely removed from the vv-PISH circuit.

Heart rate was significantly increased during the therapeutic phase, but did not affect other hemodynamic parameters. Moreover, this hyperthermia-induced elevation in heart rate has been previously reported.^{8-10,12,13} CVP was stable in our study, demonstrating our ability to adequately maintain/balance blood volume.

In this study, we found a serious complication of pulmonary hypertension during the heating phase. In the previous pig studies with the more complicated vv-PISH circuit, there was a trend for increased pulmonary artery pressure during the heating phase, but only one time-point was used during this phase.⁹ Pulmonary artery pressure was not measured in the previous clinical study.¹⁰ The pulmonary hypertension in the current study caused one pig to experience severe circulatory collapse as evidenced by increased PAP and HR combined with a severe and rapid drop in MAP. The possible reason may be that rapid heating causes less gas (O₂/N₂) solubility, generating micro-bubbles in the blood and resulting in pulmonary embolism. The air bubble can be absorbed with time; therefore, the pulmonary hypertension was temporary. Thus, pulmonary hypertension is a significant problem with vv-PISH and will have a negative impact on vv-PISH clinical application, requiring complete resolution.

The two types of systems that have been used clinically to provide whole-body hyperthermia are radiant heat devices and extracorporeal (vv-PISH) circuits. Radiant heat devices use infrared A radiation to heat the body.^{8,12,14-16} While radiant heat devices have the advantage of being non-invasive, these external heating systems do possess certain disadvantages. Major heat loss from perspiration causes significant fluid loss.^{8,12,16} Prolonged heating times (2-3 hrs) are also needed to reach the desired therapeutic temperature.^{8,12,13} Most importantly, patient complications such as severe skin burns and peripheral neuropathy seriously impact quality of life.^{8,12,14,16} By contrast, vv-PISH extracorporeal circuits are invasive, but provide homogeneous internal heating with no severe skin burns. Also, the vv-PISH circuits have shorter heating times than the radiant heat devices^{9,10} which is advantageous since rapid heating rates are associated with enhanced hyperthermia-induced cancer cell killing.¹⁷ While the original vv-PISH circuit had several advantages over radiant heat devices, this vv-PISH circuit was also complicated, expensive, and likely caused blood trauma. In the current study, we have improved upon the original vv-PISH system by simplifying the circuit through removal of the hemodialyzer component and use of a double lumen cannula. In this paper, we report that a hemodialyzer is not required for maintenance of blood volume/electrolytes/acid-base balance. Furthermore, no blood trauma was evident using our simplified vv-PISH system as evidenced by stable red blood cell counts, total/free hemoglobin, and hematocrit values.

Conclusion

The simplified vv-PISH system achieved target homogeneous heating to 42 °C while maintaining blood volume/electrolytes/acid-base balance, demonstrating that a hemodialyzer

is not required for this systemic hyperthermia circuit. This simplified vv-PISH circuit delivered the therapeutic hyperthermia dose as evidenced by the absence of blood trauma and hepatocellular/kidney injury. However, pulmonary hypertension is a serious complication in vv-PISH application, warranting further investigation.

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Figure 1.

The VV-PISH Circuit. Our new simplified vv-PISH circuit included 1) the Avalon Elite™ double lumen cannula, 2) a heat exchanger, 3) a water heater/cooler, and 4) a centrifugal blood pump.

Figure 2.

Hyperthermia Temperature Profile. **2A:** Rectal, bladder, and esophageal temperatures measured during the heating, therapeutic, and cooling phases. Homogeneous heat distribution was observed with no temperature readings above 42.5°C. **2B:** The relationship between core temperature and circuit infusion (blood in)/drainage (blood out) blood temperatures. Maximal circuit blood infusion temperature was 43°C.

Figure 3.

Pulmonary Artery Pressure During the Heating Phase. A significant elevation in pulmonary artery pressure occurred in four out of seven pigs during the heating phase. Each line represents pulmonary artery pressure data from one pig.

Table 1

Hemodynamics

Parameter	Baseline	Start Therapeutic	Mid Therapeutic	End Therapeutic	End Cooling
MAP	86±9.3	98±11.4	88±8.2	82±5.4	76±9.4
HR	76±20	123±34 *	138±31 *	128±31 *	96±32
CVP	9±1.7	8±3.3	8±3.6	7±2.9	9±4.5
PAP	19±2.1	16±5.1	17±3.6	16±3.9	22±4.7
CO	5.31±0.9	6.89±2.5	7.29±2.3	7.01±2.1	7.14±2.9

Hemodynamics were recorded at Baseline, Start Therapeutic (when 42°C target was met), Mid Therapeutic (1 hr at 42°C), End Therapeutic (2 hrs at 42°C), and End Cooling (baseline temperature restored) time-points. MAP=mean arterial pressure, HR=heart rate CVP=central venous pressure, PAP=pulmonary artery pressure, and CO=cardiac output. N= 6 pigs,

*
p<0.05 vs baseline.

Table 2

Electrolytes

Parameter	Baseline	Start Therapeutic	Mid Therapeutic	End Therapeutic	End Cooling
Sodium (Na ⁺)	142±1.4	143±2.0	142±3.7	143±3.1	144±5.3
Potassium (K ⁺)	3.57±0.19	4.12±0.44	3.89±0.36	3.59±0.48	3.86±0.87
Calcium (Ca ²⁺)	1.29±0.05	1.12±0.13	0.96±0.12 *	0.92±0.13 *	1.16±0.19
Chloride (Cl ⁻)	103±2.4	105±2.9	106±2.1	107±2.3	104±5.4
Bicarbonate (HCO ₃ ⁻)	27±1.4	26±2.2	23±1.8	23±3.9	25±3.2
pH	7.41±0.04	7.42±0.04	7.42±0.07	7.38±0.09	7.35±0.06

Arterial blood electrolytes were measured at Baseline, Start Therapeutic (when 42°C target was met), Mid Therapeutic (1 hr at 42°C), End Therapeutic (2 hrs at 42°C), and End Cooling (baseline temperature restored) time-points. N= 6 pigs,

*
p<0.05 vs baseline.

Table 3**Blood Parameters**

Parameter	Baseline	Start Therapeutic	Mid Therapeutic	End Therapeutic	End Cooling
Hematocrit (%)	28±2.0	30±2.2	29±4.9	28±5.9	28±5.2
Hemoglobin (g/dL)	9.8±0.45	10.9±0.60	10.4±1.5	10.2±1.8	10.0±1.7
RBC (x 10 ⁶ /μl)	5.61±0.28	6.24±0.43	6.02±0.76	5.88±0.96	5.74±0.80
WBC (x 10 ³ /μl)	18±3.3	15±3.6	14±6.6	14±6.0	15±5.7
Granulocytes (%)	44±9	54±9	61±11 *	66±9 *	70±6 *
Lymphocytes (%)	51±7	40±9	32±10 *	28±9 *	24±5 *
Monocytes (%)	7±0.7	7±0.8	7± 1.1	6±0.8	6±0.8
Platelets (x 10 ³ /μl)	310±37	283±27	266±27	240±33 *	333±46 *
Glucose (mg/dL)	114±15	156±25 *	132±24	142±11	115±17
ALP (IU/L)	138±35	145±35	129±39	135±40	130±36
ALT (IU/L)	30±3	29±3	27±4	27±2	26±7
BUN (mg/dL)	7.2±2.1	8.0±2.4	8.5±3.8	9.3±4.5	9.5±3.5
Albumin (g/dL)	3.9±0.8	3.4±0.1	3.1±0.4	3.1±0.2	2.8±0.6
Total Protein (g/dL)	5.1±0.9	5.2 ±0.4	4.8 ±0.8	4.8±0.3	4.4±0.5
Creatinine (mg/dL)	1.3±0.3	1.4±0.2	1.4±0.2	1.5±0.2	1.5±0.2

Complete blood counts and blood chemistry were measured at Baseline, Start Therapeutic (when 42°C target was met), Mid Therapeutic (1 hr at 42°C), End Therapeutic (2 hrs at 42°C) and End Cooling (baseline temperature restored). RBC =red blood cells, WBC=white blood cells, ALP=alkaline phosphatase, ALT=alanine aminotransferase, GGT=gamma-glutamyl transferase, and BUN=blood urea nitrogen. N= 6 pigs,

* p<0.05 vs baseline.