A Novel Approach for Inducing Selective Brain Hypothermia

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ABSTRACT

The effectiveness of hypothermic brain neuroprotection has been linked to how rapidly cooling is initiated, how quickly and uniformly the therapeutic hypothermic zone (THZ) is reached. Body surface or invasive intravascular methods are encumbered by equipment requirements, time delays for implementation, surface injury, and systemic instability. The nasopharyngeal (NP) approach is uniquely suited for selective brain hypothermia due to proximity to the cerebral circulation. However, current methods of NP cooling with gases or liquids are limited by low heat capacity and potential respiratory compromise, respectively. As an alternative, the rapid evaporative characteristics of NP aerosolized perfluorochemical (PFC) increases heat carrying capacity of respired gas. Additionally, high respiratory gas solubility and homogenous surface contact associated with PFC further support its use to rapidly induce selective brain cooling without compromising cardiopulmonary stability. To test this approach, anesthetized and ventilated juvenile sheep (n = 30) were instrumented with multiple temperature probes and vascular catheters then randomized to receive aerosolized PFC introduced via the nasopharyngeal approach (NP-PFC: PFC / O2 spray device; 1 ml/min/kg:1 L/min/kg for ≤ 45 min then studied to 2 hrs post-initiation; BeneChill, Inc.) or whole body surface cooling by circulating water blanket (WBS: 4ºC water blanket for 2 hrs). Regional temperatures, systemic and pulmonary arterial pressure were monitored continuously. Cardiac output and blood chemistry were assessed serially. As a preliminary evaluation of maximum PFC uptake and elimination with this technique, 2 additional animals were exposed to double PFC flow rates during the NP-PFC procedure. PFC was measured serially in blood by headspace gas chromatography. Regional cooling rates were calculated and evaluated (ANKOVA) as a function of method (NP-PFC vs WBS) and time to reach the brain THZ defined as at least -3oC below baseline. Tissue samples along the nasopharyngeal pathway were obtained for histology.

°C/hr; x ± SE	Brain	Vascular	Rectal
NP – PFC*, **	8.95 ± 1	4.96 ± 0.49	2.33 ± 0.31
WBS	1.40 ± 0.12	1.39 ± 0.16	1.59 ± 0.13

Independent of region, cooling was faster (*p < 0.001) during NP-PFC vs WBS. During NP-PFC, brain > vascular > rectal cooling rates (**p < 0.001), brain to systemic temperature gradients were maintained, and the brain THZ was reached in 20.1 ± 1.10 SE min. In addition, with NP-PFC there were no differences in cooling rates within the brain and the nasopharyngeal epithelial surface appeared intact. During WBS, brain vs systemic cooling rates were not significantly different and the brain THZ could not be reached by 2 hrs. Arterial blood pressure and cardiac output remained stable during NP-PFC but decreased during WBS cooling. Gas exchange was stable with both methods. PFC uptake in blood reached a maximum of 23.5-ng/mL and elimination half-life of less than 15 min. These values are orders of magnitude lower than reported with direct intravascular exposure of PFC blood substitutes (mg/mL) or intrapulmonary exposure during PFC liquid ventilation (µg / mL). In conclusion, the NP-PFC procedure more rapidly induces selective and relatively homogenous brain cooling as compared to other reported procedures. Using this non-invasive clinical approach, it is possible to reach a therapeutic hypothermic brain temperature within minutes of treatment using a relatively simple device. As such, this approach may be the most effective means to initiate and achieve hypothermic brain neuroprotection.

INTRODUCTION

Cerebral hypoxic-ischemic events are the leading cause of acute neurological injury at birth, and, cause of long-term disability across age worldwide. A 2-5ºC reduction in brain temperature after this event can improve neuropathological, cerebral energetic, and electrophysiological outcomes. The neuroprotective efficacy of cerebral hypothermia has been linked to how rapidly cooling is initiated, how quickly the brain is cooled, and the extent of tissue that reaches the therapeutic hypothermic zone. Whole body surface (WBS) or intravascular methods for brain cooling are encumbered by equipment, systemic hypothermia, slow response, and concomitant systemic instability. Selective head cooling by circulating cold water cap shows promise, though regional gradients in brain temperature remain. Due to proximity to the cerebral circulation, the nasopharynx is uniquely suited for selective and homogenous brain cooling; however, nasopharyngeal (NP) cooling with oxygen or liquid is limited by low heat capacity and/or respiratory compromise. As an alternative, the favorable distribution and rapid evaporative properties of nasopharyngeal aerosolized perfluorochemical (PFC) increases the heat capacity of respired gas, thus should facilitate rapid induction and maintenance of global brain cooling without substantial compromise in systemic temperature.

Theoretical Basis for PFC Nasopharyngeal Cooling

1. Latent Heat of Vaporization
   - Phase Change of PFC liquid to PFC vapor
   - Endothermic reaction
   - ▲Heat ∝ (Q_{PFC} x CP x Surface Area)
2. Hematogenous Cooling
3. Conductive Cooling

HYPOTHESIS

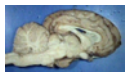
PFC nasopharyngeal cooling will rapidly induce global cerebral hypothermia

OBJECTIVES

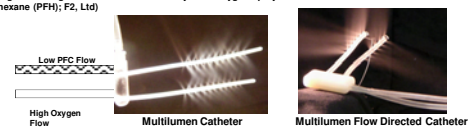
- Characterize brain cooling rates as a function of PFC/Gas flow ratio and catheter design
- Compare brain and systemic cooling rates and temperature gradients during PFC nasopharyngeal cooling to whole body surface cooling.
- Characterize PFC blood uptake and elimination

METHODS

- **Animal Model**
  - Normal Weanling Sheep (n = 30; 21 – 25 kg)
  - Ketamine (10 mg/kg) and butorphanol tartrate (1 mg/kg) pre-anesthesia (IM)
  - Sodium Pentobarbital (12.5 mg/kg) supplemented by continuous infusion (1.25 mg/kg/hr)
- **Instrumentation**
  - Carotid Artery, bilateral external jugular and pedal veins, trachea cannulation
  - Pulmonary Artery (Thermodilution Catheter)
  - ECG leads and Pulse Oximeter Probe
  - Temperature Probes
    - Brain (Inferior Frontal: 5 cm; 3rd Ventricle: 5 cm; Superficial Parietal: 3 cm) placement confirmed by fluoroscopy and Evans Blue
    - Ear (approximating tympanic membrane)
    - Vascular (High: external jugular into thoracic cavity; Low: pedal vein into abdominal cavity)
    - Subcutaneous
    - Intramuscular
    - Rectal



- **Management**
  - Mechanical Ventilation (Time-cycled, volume-controlled) with 100% O_2)
  - Target PaO_2 > 300 mmHg; $PaCO_2$ = 35 - 45 mm Hg; pH = 7.35 – 7.45
  - Skeletal Muscle Paralysis (Pancuronium Bromide: 0.10/ mg/kg/hr)
  - Maintenance Fluids (D_5 @ 7.5 mL/kg/hr)
- **Protocols**
  - Whole Body Surface Cooling induced and maintained by circumferential, cold water (0ºC) circulating blankets
  - Nasopharyngeal Cooling induced and maintained by PFC/ Oxygen spray cannula devices. (Perfluorohexane (PFH); F2, Ltd)



PFC (room temperature) and Oxygen flow: independently regulated by mass flow meters at predetermined rates

Nasal Temperature = 2 – 5 ºC

Induction: Continuous spray to target -3.5 ºC change from baseline
Maintenance: Intermittent spray: restart @ 0.5 ºC above target; terminate when target temperature was restored

External warming blanket applied if rectal temperature <37 ºC

- **Measurements**
  - Temperature, systemic and pulmonary arterial blood pressure, heart rate: continuous recordings
  - Arterial Blood Chemistry and Cardiac Output: Baseline, mid and final induction, serially throughout maintenance; PFC blood concentration by headspace gas chromotography
- **Calculations and Analyses**
  - Brain cooling rates as a function of PFC/Gas Flow, Regional Cooling Rates as a function of method, Time to Therapeutic Brain Hypothermic Zone, histology, PFC blood uptake and elimination

RESULTS

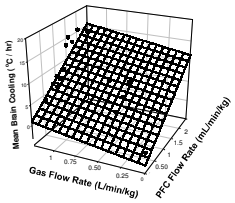


Fig 1: Interrelationship between PFC flow rate, gas flow rate, and mean brain cooling rate. p < 0.0001, r = 0.88, r² = 0.78, r² adjusted = 0.75.

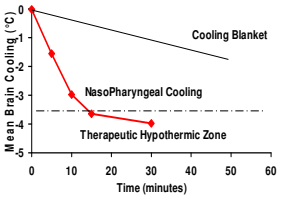


Fig 2: The therapeutic hypothermic zone was reached within 15 mins. with PFC nasopharyngeal cooling but not achieved with the whole body surface cooling blanket during the 2h protocol

RESULTS

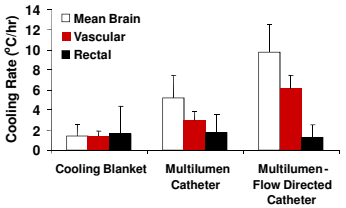


Fig 3: Absolute cooling rates (X ± SE) during blanket and perfluorochemical (PFC) nasopharyngeal (NP) cooling (PFC/O2 Flow: 1 mL/min/kg: 1 L/min/kg) protocols. ANOVA demonstrated significant difference (p < 0.001) in brain and vascular cooling rates between techniques with PFC NP by either catheter > blanket. During PFC NP by either catheter, cooling rates for brain > vascular > rectal (p< 0.001); brain and vascular cooling rates were greatest with flow-directed catheter.

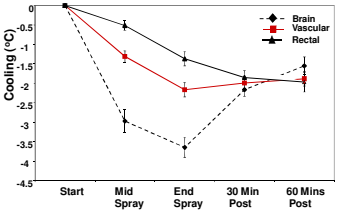


Fig 4: Brain to systemic temperature gradient increased during the nasopharyngeal cooling protocol, diminished with termination, and was no longer observed following 30 min of active cooling.

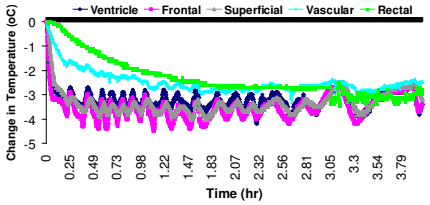


Fig 5: Regional brain and systemic temperature changes during induction and 4 h maintenance protocol with PFC nasopharyngeal cooling. Note consistency between brain regions demonstrating ability to rapidly induce and maintain hypothermia in deep and more superficial brain structures with minimal differences in cooling rates.

RESULTS

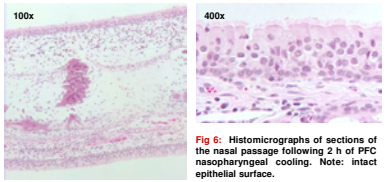


Fig 6: Histomicrographs of sections of the nasal passage following 2 h of PFC nasopharyngeal cooling. Note: intact epithelial surface.

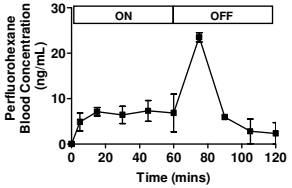


Fig 7: Perfluorohexane (PFH) uptake and elimination shown as blood concentrations (X ± SE) during (ON) and following (OFF) exposure to PFC NP cooling at double PFC flow rate (2 mL/min/kg). As a point of reference and comparison, PFH is the active ingredient in the clinically approved ultrasound imaging agent, Imagent, wherein microbubbles of PFH gas are injected directly into the blood stream. When healthy human volunteers were exposed to 4 mL/kg Imagent containing 20 µg/kg PFH, this technique resulted in mean maximum PFH blood uptake of 28 ng/ml with 75% elimination by 3 h (IMUS-012-USA; NDA21-191; Imagent ADME). With PFC NP cooling at 10'x PFH exposure as compared to IV Imagent (200 gm/kg vs 20 µg/kg) comparable peak blood levels were found and 75% elimination occurred more quickly, by 28 mins. vs 3 h.

CONCLUSIONS

- With PFC nasopharyngeal cooling ,
  - PFC / Gas flow ratio directly influenced brain cooling rates.
  - the therapeutic hypothermic zone in the brain was reached within 15 mins; whole body surface cooling did not obtain this zone within 2 h.
  - absolute compartmental cooling rates and brain to systemic temperature gradients were significantly greater than with whole body surface cooling.
  - brain to systemic temperature gradients demonstrated preferential brain cooling relative to vascular and rectal temperature profiles. Vascular and rectal cooling rates were approximately one-half and one-third of brain cooling rate, respectively.
  - a multilumen flow directed catheter further amplified the brain to systemic temperature gradients.
  - the nasopharyngeal epithelial surface remained intact.
  - PFC uptake and elimination were orders of magnitude lower and faster, respectively, than intravascular or neat intrapulmonary exposure.
  - global cerebral hypothermia was rapidly induced and maintained

SUMMARY

The principle findings of this study are that the therapeutic hypothermic zone targeted for global brain neuroprotection can be rapidly reached and maintained by PFC nasopharyngeal cooling. This approach maintains brain to systemic temperature gradients with preferential brain cooling relative to vascular or rectal temperatures and requires relatively little instrumentation. PFC uptake is minimal and rapidly eliminated. On-going studies are focused at increasing the brain to systemic temperature gradient by modulating the PFC/gas flow ratio and incorporating external warming blankets commensurate with the PFC nasopharyngeal cooling. Studies are planned to evaluate the neuroprotective effect of this approach in models of cerebral compromise.

ACKNOWLEDGMENTS

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