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Combined Neuroprotective Modalities Coupled with Thrombolysis in Acute Ischemic Stroke: A Pilot Study of Caffeinol and Mild Hypothermia

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Abstract

Background—Both caffeinol and hypothermia are neuroprotective in preclinical models of transient middle cerebral artery occlusion. We tested whether combining caffeinol and hypothermia with t-PA in acute stroke patients is safe and feasible.

Methods—20 patients with acute ischemic stroke were treated with caffeinol (caffeine 8-9 mg/kg + ethanol 0.4g/kg IV X 2 hours, started by 4 hrs after symptom onset) and hypothermia (started by 5 hrs and continued for 24 hrs (target temp 33-35°C) followed by 12 hrs of rewarming). IV t-PA was given to eligible patients. Meperidine and buspirone were used to suppress shivering.

Results—All patients received caffeinol, and most reached target blood levels. Cooling was attempted in 18 patients via endovascular (n=8) or surface (n=10) approaches. Two patients were not cooled due to catheter or machine failure. Thirteen patients reached target temperature; average time from symptom onset was 9hrs, 43min. The last 5 hypothermia patients received surface cooling with iced saline induction and larger doses of meperidine; all patients reached target temperature, on average within 2hrs 30min from induction and 6hrs 21min from symptom onset. Three patients died: one from symptomatic hemorrhage, one from malignant cerebral edema, and one from unrelated medical complications. No adverse events were attributed to caffeinol. One patient had reduced respiratory drive due to meperidine, requiring BiPAP.

Discussion—Combining caffeinol with hypothermia in acute stroke patients given IV t-PA is feasible. A prospective placebo-controlled randomized study is needed to further assess safety and to test the efficacy of caffeinol, hypothermia or both.

Introduction

Neuroprotective trials in humans with ischemic stroke have yielded disappointing results^{1, 2}. There are a number of reasons postulated to explain why positive results in animal models of stroke fail to translate to humans with spontaneous ischemic stroke. Among these is failure to initiate treatment in the appropriate timeframe³, doses too low to allow for effective brain penetration of the drug⁴ and targeting a only a single step in the complex ischemic cascade². Most drugs that have shown efficacy in animals used a model of temporary vessel occlusion.

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Recanalization, and more importantly, reperfusion may be essential for drug delivery in concentrations that would be effective and has not been typically addressed in human trials. Lastly, the majority of neuroprotective drugs have greatest benefit on cortical penumbra in animal models³, yet strokes in humans have variable locations and severity of ischemia.

We designed the present study to address each of these concerns. Our neuroprotective strategy was to administer the neuroprotective treatment soon after symptom onset in combination with IV t-PA in order to achieve reperfusion and improve delivery of neuroprotection to target tissue in as many patients as possible. We limited enrollment to patients most likely to have cortical injury; and employed a combination of agents (caffeine and ethanol = caffeinol + hypothermia) that have rapid brain penetration and target multiple aspects of the ischemic cascade.

We chose caffeinol for the following reasons

Caffeinol consists of caffeine and ethanol, and exerts its biological effect through central adenosine, gamma-aminobutyric acid (GABA)_A, and N-methyl-D-aspartate (NMDA) receptors⁵. Caffeine and ethanol are among the most widely consumed substances in most societies. Consequently, there are substantial data and human experience regarding the speed of their effects on the brain, blood levels and side effects in humans that can guide their therapeutic use. As an adenosine receptor antagonist, caffeine speeds up neural activity, stimulates the pituitary-adrenal axis promoting adrenaline release, increases the cerebral metabolic rate, and can actually reduce cerebral blood flow⁵. In rats, the areas of reduced blood flow are the same as those that demonstrate increased metabolic rate. Caffeine may allow a higher metabolic rate for a given rate of cerebral blood flow, which could allow critical metabolic pathways to continue functioning despite reduced perfusion in the setting of ischemic stroke⁵. Caffeine has differential activity at low and high doses. At subtoxic doses, caffeine acts primarily as an antagonist to adenosine receptors, including the A2a subtype of the receptor that has inhibitory effects on synaptic glutamate release and excitotoxicity in context of ischemic stroke⁶. At toxic doses, caffeine inhibits phosphodiesterase, blocks GABA_A receptors, and stimulates the release of intracellular calcium⁵. A survey published in 1996 reported that the estimated mean daily intake of caffeine was in the range of 4mg/kg⁷, and one cup of coffee contains up to 100 mg of caffeine.

Ethanol acts on the brain mainly through GABA_A receptor stimulation and inhibition of NMDA receptors^{8, 9}, thereby helping to promote inhibitory and anti-excitotoxic synaptic pathways. Ethanol has anti-platelet function that may impact post-ischemic cerebral perfusion¹⁰. In low doses, ethanol alone had no effect on infarct volume¹¹. In combination with caffeine, even at very low doses (0.2g/kg), ethanol showed a neuroprotective effect, both on infarct volume, and in neurological outcome¹¹. The mechanism of this neuroprotective effect when in combination with caffeine, but not when given alone, is unknown.

Caffeinol delivered as an intravenous infusion over 2 hours starting 120 minutes after CCA/MCA occlusion in a rat model was effective in reducing the severity of neurological function and the final cortical infarct volume (by 45%) at doses as low as 6 mg/kg of caffeine and 0.2g/kg of ethanol¹¹. Another group confirmed that the same doses of caffeine were effective in reducing infarct volume in a rodent model, and that this effect was restricted primarily to the cerebral cortex¹². One neutral study has been reported with caffeine + t-PA, but used a largely subcortical injury model and different doses of caffeine and t-PA than effective studies¹³. In our animal studies, caffeine did not increase the incidence of hemorrhagic transformation after t-PA¹¹, nor did it affect the thrombolytic effect of t-PA in an ex-vivo bioassay (data on file). The effect of caffeine on stroke volume and neurological outcome in a permanent model of ischemic stroke has not been performed. Because our animal model showing a beneficial effect of caffeine involved reversal of the occlusion, the potential benefit to the ischemic brain in the absence of recanalization is unknown. Pilot clinical trials of caffeine have shown that blood

levels effective in rodents are easily achieved and well tolerated in stroke patients¹⁴. These blood levels (caffeine >8mcg/ml and ethanol 30-50mg/dl) are within the usual limits achieved by “social” consumption of these substances (legal ethanol blood level in most states is 50-80mg/dl). More recently, we found that caffeinol may improve clinical outcome in patients receiving t-PA¹⁵. Finally, our preclinical studies have demonstrated that the robust neuroprotective effect of caffeinol can be augmented by mild hypothermia (35°C)¹¹.

We chose hypothermia for the following reasons

Several studies have shown that patients presenting with ischemic stroke and hyperthermia have worse outcomes and larger infarcts than patients that are normothermic at presentation¹⁶. The ischemic cascade is susceptible to blunting by hypothermia by multiple mechanisms. In addition to reduced cerebral metabolism, reduced blood brain barrier disruption, and blunted inflammatory response, hypothermia also inhibits maladaptive processes in ischemic brain cells which lead to apoptosis. Hypothermia has been effective in reducing ischemic damage in multiple experimental models of stroke¹⁷.

Improved outcomes have been achieved with therapeutic hypothermia in patients with global brain ischemia after cardiac arrest, but these studies were performed on intubated and paralyzed patients^{18, 19}. The COOL AID (Cooling for Acute Ischemic brain Damage) study combined thrombolysis with mild hypothermia. Though few patients were treated (10 in the hypothermia group), adverse events were low and there was a trend towards improved outcome. Small safety and feasibility studies of hypothermia in awake, nonintubated patients with ischemic stroke have shown that shivering is a barrier to effective cooling, and that prevention of shivering can be safely achieved with a combination of buspirone and meperidine²⁰. There are considerable variations in the methods of hypothermia, but to be widely administered in hospitals around the country, the methods need to be not only safe, but feasibly implemented. Intubation and sedation of patients to achieve hypothermia results in a significant demand on health care resources and impairs the ability to follow neurological status.

The present study is the first attempt to combine intravenous thrombolysis with caffeinol and mild hypothermia in awake, nonintubated patients. The specific aim of this study was to conduct a phase I single-center, open label, non-randomized feasibility and safety trial of all three treatments (t-PA, caffeinol and hypothermia).

Methods

Study design

This prespecified first phase of 20 patients had three main objectives: 1. to determine if it was feasible to administer an infusion of caffeinol and hypothermia within strict pre-specified time limits linked to t-PA treatment, 2. to determine if neuroprotective doses of caffeinol were well tolerated, and if target temperatures could be reached in awake stroke patients without excessive shivering or sedation, and 3. to determine if caffeinol and hypothermia could be administered safely together and in patients who had received standard t-PA treatment.

To be eligible, patients had to present with a presumed acute ischemic stroke within 3 hours of symptom onset. Although not required for enrollment, all eligible patients received standard dose intravenous t-PA. The caffeinol infusion and induction of hypothermia had to begin within 4 hrs and 5 hrs of symptom onset, respectively. The intention was to use the same doses of caffeinol previously tested in a first-in-man phase 1 study¹⁴, and to test in sequence various catheter-based and surface cooling systems along with shivering control algorithms.

Inclusion criteria

1. Age 18-85 years (later modified to 18-80 years)
2. Clinical presentation of acute ischemic stroke
 - a. Presumed cortical location of stroke based on cortical findings on neurological examination
 - b. NIHSS ≥ 8 at time of starting caffeineol
3. CT scan of head without evidence of intracerebral hemorrhage or alternate diagnosis
4. Time to caffeineol treatment < 4 hrs from symptom onset and before completion of t-PA infusion (if so treated)
5. Time to hypothermia initiation < 5 hrs from symptom onset
6. Intravenous t-PA treated patients must meet all established criteria for intravenous t-PA

Exclusion criteria

1. Etiology other than ischemic stroke
2. Item 1a on NIHSS > 1
3. Signs/symptoms of purely subcortical, brainstem or cerebellar stroke
4. Symptoms resolving or NIHSS < 8 at time of starting caffeineol
5. NIHSS > 20 if right hemisphere or > 25 if left hemisphere
6. Known alcoholic
7. Clinical or laboratory evidence of alcohol intoxication
8. Historical evidence of exogenous caffeine exposure beyond daily consumption of coffee or soft drinks (i.e. medicinal or herbal caffeine)
9. Known hematologic dyscrasias that affect thrombosis (cryoglobulinemia, Sickle cell disease, serum cold agglutins) or vasospastic disorders (Raynaud's, thromboangiitis obliterans)
10. Comorbid conditions likely to complicate therapy, including but not limited to:
 - a. Severe cardiomyopathy
 - b. Uncompensated or clinically significant arrhythmia
 - c. Myopathy
 - d. Liver disease
 - e. End stage AIDS
 - f. History of significant GI bleeding
 - g. Impaired renal function with creatinine clearance < 50 ml/min (based on $\text{CrCl male} = (140 - \text{age}) \times \text{pt weight in kg} / (72 \times \text{Cr})$ $\text{CrCl female} = 0.85 \times [(140 - \text{age}) \times \text{pt weight in kg} / (72 \times \text{Cr})]$)
11. Intracerebral/intraventricular hemorrhage
12. sBP > 210 or < 100 ; dBP > 110 or < 50 mmHg
13. Severe coagulopathy, (INR > 3 or PTT > 50)

14. Pregnancy
15. Use of monoamine oxidase inhibitor within the preceding 2 weeks
16. Known history of epilepsy

t-PA administration

Patients presenting in the first 3 hours of stroke symptoms were rapidly assessed for eligibility for intravenous t-PA and were treated according to established guidelines²¹. Intra-arterial thrombolytic or mechanical therapy or other experimental therapies were not allowed.

Caffeinol administration

The start of the caffeinol infusion marked the start of the experimental protocol. After informed consent was obtained, caffeine and ethanol levels were sent to the lab and a 2 hour caffeinol infusion (8-9mg/kg caffeine and 0.4gm/kg 10% ethanol) started. Caffeine and ethanol blood levels were drawn at the end of the 2 hour infusion.

Overview of hypothermia phases

The *induction phase* of hypothermia was defined as the interval from initiation of cooling to reaching the target temperature ($\leq 34.5^{\circ}\text{C}$, $> 33^{\circ}\text{C}$). The *maintenance phase* began when the target temperature was reached and continued for 24 hours. If the target temperature was not reached, efforts to cool were stopped 24 hours after the initiation of hypothermia. The *rewarming phase* began after 24 hours of maintenance and consisted of slow, controlled active rewarming to 36.5°C at a rate of $\leq 0.5^{\circ}\text{C}$ per hour, or over 12 hours.

Induction of hypothermia- pharmacological methods

A Foley catheter with a temperature sensor was placed in all patients for continuous temperature monitoring. Buspirone 30mg was given orally, or by nasogastric tube if the patient was unable to swallow. Meperidine 1mg/kg loading dose was given (maximum dose 100mg) prior to initiation, and a maintenance infusion of 30mg/hour was started.

Shivering was assessed every hour during the induction and maintenance phases according to the Shivering Assessment Scale (Table 1). Any degree of shivering was treated to ensure maximal cooling and patient comfort. The meperidine dose was increased by 5mg/hour for shivering, or temporarily held and then the rate reduced for excessive sedation. If target temperature was unable to be reached or tolerated due to shivering, the patient was rewarmed to the lowest tolerated temperature by increasing the target temperature by 0.5°C increments on the cooling system.

After the first 13 patients were enrolled, our methodology was modified to include routine induction of hypothermia with iced normal saline started within the first hour of the caffeinol infusion. Patients were administered one liter of normal saline over one hour and then one additional liter over the next two hours if tolerated and needed to reach the target temperature. Prophylaxis of shivering with a magnesium sulfate infusion (4gm Magnesium SO_4 over 45 minutes followed by 1gm/hour) was also added to the protocol. Serum magnesium levels were measured at baseline, at the end of hypothermia, and at the end of rewarming. In addition, the combination of buspirone and meperidine was initiated earlier and more aggressively, including boluses of 25 mg of meperidine for shivering or before transportation between units. A warming blanket and forced hot air blown onto the face and extremities were provided for patient comfort.

Intravascular cooling methods

Alsius Fortius inferior vena cava catheter™ and CoolGard Temperature Control System®—Endovascular cooling with the Alsius Fortius inferior vena cava “ICY” catheter with CoolGard Temperature Control System was the first cooling method attempted in this study. The CoolGard Temperature Control System consists of a temperature monitor, temperature controller unit, heat exchange unit, and pump to circulate the sterile saline through the tubing and catheter. The Alsius Fortius central venous heat exchange catheter is 9.3 French in shaft diameter with a single central usable lumen. It is inserted via a 12 French sheath with a single, valve locked, side port. The catheter features a spiral balloon. Cold, sterile saline is circulated within the balloon to exchange heat with the central venous blood supply.

The catheter was placed in a controlled, monitored setting (emergency department or intensive care unit per standardized procedure for use of a central venous catheter). The catheter is placed into the femoral vein (inferior vena cava placement of the cooling coils of the catheter is required) over the guidewire following standardized (Seldinger) percutaneous technique. After the catheter placement was radiographically confirmed, the COOLGARD cooling system was started and set at 33°C.

Surface cooling methods

Gaymar hypothermia system®—This external cooling device has two parts: a vest and leg sleeves which connect to a control module that cools and circulates water through the body suit. The control module allowed depth and the rate of cooling to be set.

Medivance Arctic Sun system®—This thermal regulating device consists of a control module and disposable energy transfer pads. The control module recirculates temperature-controlled water to the energy transfer pads. A control algorithm automatically adjusts the water temperature (automatic mode) or the clinician can adjust the water temperature (manual mode) to obtain the desired patient temperature. The energy transfer pads are adhesive; the conductive hydrogel within the pad allows energy transfer by using high flow water to remove the heat from the patient. The pads are applied to the patient’s intact skin on the back, chest, abdomen, and thighs covering about 40% of body surface area, mimicking water immersion.

Cincinnati SubZero (CSZ) system®—This cooling unit has cold water circulating within its pads, and in addition to pads covering the torso, has an additional energy transfer pad that is wrapped around the patient’s neck and head, cooling the superficial venous blood of the head. The control module is very similar to that of the Arctic Sun system, allowing for adjustment in temperature and rate of cooling/rewarming.

Concomitant monitoring and treatments

Enrolled patients were admitted to the same neurological critical care unit for close observation and cared for by the same team of Vascular Neurologists and Intensivists. All patients had continuous cardiac monitoring. Vital signs were recorded every hour until rewarming was complete. A nurse performed an abbreviated NIHSS every hour (LOC, motor). If neurological deterioration occurred, the investigators were notified and emergent brain imaging was obtained. Core body temperature was recorded every 30 minutes during hypothermia induction and every hour during maintenance and rewarming phases using bladder and tympanic measurements.

Upon achieving a temperature of 36.5°C, patients had a noncontrast CT of the head. In cases where the patients had an IVC catheter for cooling, duplex ultrasound was performed to screen for deep venous thrombosis. The Fortius Catheter was removed no more than 48 hours after insertion.

Hypertension greater than a mean arterial pressure of 130 mmHg was treated in patients not treated with thrombolytics, and standard guidelines were followed in patients that received t-PA. Patients received standard of care with regard to antiplatelet use (no antiplatelets for 24 hours after t-PA). All patients received deep venous thrombosis prophylaxis and received standard nursing care and standard femoral catheter care including monitoring for puncture site complications and digital ischemia. Patients were maintained NPO until evaluated by speech pathology or they passed a swallowing screen.

Clinical examinations were assessed using the NIHSS at baseline, before t-PA, before caffeineol, before hypothermia, during hypothermia, after rewarming, and daily until day 5 or discharge (whichever came first), and at 90 days. Functional outcome using the modified Rankin Scale were assessed at day 5 or discharge and at 90 days.

Outcome measures

The primary outcomes were feasibility, tolerability and safety of caffeineol and hypothermia. Feasibility was determined by the ability to start caffeineol and hypothermia within prespecified time intervals linked to t-PA treatment, the ability to reach prespecified therapeutic blood levels of caffeine and ethanol, and ability to reach and maintain prespecified target temperatures of 33-34.5°C within prespecified time intervals, including time required to reach the target core temperature, length of time the temperature could be maintained within this range, and rate of rewarming.

Tolerability was determined by measuring adverse events related to caffeineol, and ability to achieve target temperatures without excessive shivering or sedation. The amount of sedation needed was carefully tracked.

Safety was determined by the incidence of various endpoints including in-hospital mortality, symptomatic intracerebral hemorrhage, symptomatic venous thrombosis, infection, and respiratory, cardiovascular, or other serious adverse events attributed to hypothermia, shivering suppression, or the IVC catheter.

Safety monitoring

An Investigational Device Exemption (IDE) was filed with the Food and Drug Administration (FDA) for use of the IVC and surface cooling systems, and an Investigational New Drug Exemption (IND) filed for use of caffeineol. The study was approved by the University of Texas Committee for the Protection of Human Subjects, and informed consent obtained from the patient or surrogate in all cases before starting either the caffeineol or cooling. An independent Data Safety and Monitoring Board (DSMB) monitored the study in accordance with the provisions of 21CFR.812.46 and related FDA guidelines. After completion of the treatment phase of each enrolled patient, a report summary of treatment including all serious or unanticipated adverse events was submitted to the DSMB. The decision to proceed with further enrollment was made by the DSMB. The study was internally monitored for data integrity. Drs Grotta and Aronowski hold a patent on the use of caffeineol in acute stroke, and a conflict of interest plan was filed and approved with the appropriate UT Medical School oversight committee. This information was clearly noted in the informed consent.

Statistical Issues

We intended to study 20 patients in this first phase. This number was not based on any power analysis. It was chosen because we thought it would be sufficient to 1. Determine if it was feasible to carry out the combinational treatment within 1-2 hours of completing t-PA; 2. Determine if target blood levels and cooling were tolerated; and 3. Determine if rates of hemorrhage, sedation, infection, or DVT were likely to be excessive. It was agreed with the

DSMB that the study would be halted if more than 2 PH-2 hemorrhages occurred in the first 10 patients. There were no pre-specified stopping rules for other safety measures.

Results

20 patients were enrolled (Table 2). 17 of the patients received standard dose t-PA. The other 3 patients had contraindications to thrombolytic therapy. 18 of the patients received caffeine. The other 2 patients were enrolled during a temporary hold on caffeine by our IRB when the protocol was amended for a higher dose of caffeine in the preparation of caffeine. 18 of the patients were assigned to receive hypothermia for 24 hours. One patient was predetermined to receive only induction of hypothermia with an iced-saline infusion. One patient had clinical resolution after t-PA and caffeine and was not cooled. There were technical problems with the catheter in one case and with the cooling console in a second case. Therefore, we induced and attempted to maintain cooling in 16 patients. Overall 14 of the 20 patients (70%) enrolled in the study were treated with t-PA, caffeine, and hypothermia.

Feasibility and tolerability of t-PA followed by caffeine

In the 16 patients that received t-PA and caffeine, all patients received t-PA within 3 hours and all patients received caffeine within 4 hours of symptom onset. Two patients received caffeine after the t-PA infusion was complete, but within the prespecified 4 hours from symptom onset.

Our target caffeine blood level for this study was ≥ 8 mcg/ml based on rodent studies²². The first 11 patients in this study were treated with caffeine containing 8mg/kg of caffeine and the median caffeine level post-infusion in these patients was 8.7mcg/ml (range 6.5-17.4mcg/ml). 6 of the 11 patients (54.5%) reached the target level of >8 mcg/ml. The last 7 patients were treated with 9mg/kg of caffeine. The median caffeine level in these patients was 7.7mcg/ml (range 5-14.3mcg/ml). Only 3 of the 7 patients (42.9%) reached the target level.

Our target blood level for ethanol in this study was ≥ 30 mg/dl based on rodent studies²². The median ethanol levels in our patients were 35.5 mg/dl (range 11-68mg/dl). 12/18 patients reached this target level. These blood levels were to be obtained at the completion of the 2 hr caffeine infusion, but the average was 31.5min afterwards. The $t_{1/2}$ of caffeine in humans is 2-3 hours, so the delay should not have significantly affected caffeine levels, and we found no correlation between the interval from end of caffeine infusion to lab draw and the caffeine level. The half-life of ethanol is highly variable. Our levels inversely correlated with the time from end infusion to measurement ($r=-0.59$, $p=0.011$); patients with target levels had a mean interval of 12 minutes and patients below target levels had a mean interval of 70 minutes.

No adverse events were attributed to caffeine.

Feasibility and tolerability of hypothermia

Antishivering protocol—Some degree of sedation and shivering were experienced in many of our subjects. Sedation was considered significant in 3 patients, required holding or reducing the meperidine infusion, and was reported as adverse events. One patient experienced sedation and reduced respiratory drive requiring treatment with BiPAP. This was reported as a serious adverse event (SAE) and attributed to the effects of meperidine. This patient was 83 years old and as a result of this SAE, we voluntarily reduced our age limit to 80 years of age. The average dose of meperidine administered during the hypothermic period was 570mg. The average shivering score was 2.1. The median serum magnesium level was 4.1 mEq/L; the range was 1.9-5.5 mEq/L. The mean peak CK was 1,540U/L and the median was 607U/L. The highest CK recorded was 8,227U/L with mild and transient worsening of renal function (creatinine

increased to 1.5mg/dl from baseline of 1.3mg/dl). The mean peak CK among our target cooled patients ($<35^{\circ}\text{C}$ for $>12\text{hrs}$) was 2,352U/L and the median was 864U/L. Overall, 7 patients had a CK above 1,000U/L.

Cooling

Endovascular cooling with the Alsius inferior vena cava “ICY” catheter (n=8) and three surface cooling devices (Gaymar n=2, Arctic Sun n=6, Cincinnati SubZero n=2) were used in sequence (Table 3). The average time from symptom onset to induction of cooling was 4 hours and 23 minutes. Of the 18 patients initiated on hypothermia, 4 did not reach the target temperature ($\leq 34.5^{\circ}\text{C}$). 2 reached the target temperature within 1 hour, a total of 4 reached the target temperature within 2 hours, and a total of 8 within 3hrs of induction. Of the 13 patients that ever reached the target temperature during active cooling (72%), the mean and median times to target from symptom onset were 9hrs 43min and 7hrs 55min, respectively. The average duration that subjects who cooled remained in the target temperature range ($33\text{--}35^{\circ}\text{C}$) was 19hrs 48min. Overall, 61.5% (8 of the 13 patients) of the patients that ever reached target temperature and 44.4% (8 of the 18 patients) of the patients that we intended to cool were maintained in the target range for at least 12 hours.

Intravascular cooling—Of the 8 patients in this group, the cooling system malfunctioned in one case, prior to insertion of the catheter. In another case, the saline bag demonstrated an abnormal reduction in volume, suggesting a leak in the tubing or catheter. A leak could not be identified, so hypothermia was aborted for patient safety. Of the 6 remaining patients, 3 patients never reached the target temperature, with a minimum temperature of $35\text{--}35.6^{\circ}\text{C}$. The other 3 patients reached the target temperature in an average time of 380 minutes, and were in the target range for an average of 9.5 of the following 24 hours. The worst shivering scores in these 3 patients were a minimum of 2, and 2 of the 3 patients had uncontrollable shivering at some point during hypothermia despite an average of 519mg of meperidine during the induction and maintenance phases of hypothermia. No patient experienced an adverse event attributed to the catheter or the system.

Surface cooling—We cooled 10 patients with surface cooling (2 with the Gaymar, 6 with the Arctic Sun and 2 with the Cincinnati SubZero devices). All of the patients reached the target temperature, but the average time to do so was 300 minutes from induction. The patients spent an average of 22.9 hours in the target range. The average worst shivering score was a 2, and the average total dose of meperidine given was 700.5mg.

Iced saline induction and modification of anti-shivering regimen

After the first 13 patients were enrolled, we had achieved target cooling within 3 hours of starting the protocol in only 3 patients. Consequently, the protocol was modified to include routine induction of hypothermia with iced saline. In addition, the meperidine antishivering regimen was made more aggressive including boluses and increased rates of infusion for any visible or palpated shivering.

Before these modifications, the average time from symptom onset to induction of hypothermia was 4 hours and 45 minutes. After these modifications, 5 patients were cooled (3 with the Arctic Sun and 2 with the Cincinnati SubZero devices) with the average time from symptom onset to induction of hypothermia one hour shorter (3 hours and 41 minutes). The target temperature was reached in all 5 patients within 2hrs 30min. The median time to target from symptom onset among these 5 patients was 6hrs 35min. The average duration the subjects remained in the target temperature range ($\leq 34.5^{\circ}\text{C}$) was 22 hrs. Compared to patients treated prior to the modifications in protocol, these patients had higher doses of meperidine during the

cooling period (820 vs 525mg), lower shivering scores (1.8 vs 2.3), and were more drowsy (LOC 1a 1.6 vs 1.3).

At the time we began using iced saline, we gave one patient (#16) only 2 liters of iced normal saline over 2 hours without additional cooling methods to see how much temperature reduction would occur from the iced saline alone. Her temperature went from a baseline of 37.0° C to 36.2° C, and then slowly rose to 36.7 ° C over the next 10 hours.

Safety—In-hospital mortality

Three deaths occurred (15%) during hospitalization; one attributed to the single sICH that occurred during the study, one to malignant edema and herniation, and one in a patient with massive stroke, respiratory distress, and an ischemic limb in whom withdrawal of care was requested by the family after the day 5 data was collected.

Safety--Hemorrhage

A total of four parenchymal hemorrhages occurred (three asymptomatic PH1 and one symptomatic PH2), all in patients treated with t-PA. No other serious bleeding episodes occurred, but one elderly patient developed large ecchymoses on her extremities after t-PA. She had no identified coagulopathy and received supportive care.

Safety –Thrombosis

One patient developed thrombotic thrombocytopenia that was attributed to clopidogrel and required treatment with plasmapheresis. Two systemic clotting episodes were identified; one asymptomatic nonocclusive deep venous thrombosis in a patient treated with intravascular cooling through the same limb, and one occlusion of the common femoral artery resulting in an ischemic limb. This occurred in a patient with severe peripheral vascular occlusive disease who received surface cooling.

Safety-- Infectious

A total of 9 infections (45% of patients) were identified and treated during the study period. These included 4 pneumonias (20%), 2 urinary tract infections (10%), 1 case of bacteremia, 1 case of skin infection, and 1 case of ventriculitis in a patient with ICH with intraventricular extension that had an external ventriculostomy placed. All 4 pneumonias occurred in patients who were effectively cooled.

Safety—other serious adverse events

Four patients experienced neurological worsening (increase in NIHSS score ≥ 4 points) during hypothermia. Each of these patients had a large MCA infarct and was effectively cooled for a mean of 20 hours. One patient had bradycardia and then cardiac arrest during hypothermia (temperature 32.6°C). The treating physicians believed the patient might have had a primary cardiac event only “possibly” related to the hypothermia; after he was resuscitated, he was continued on a hypothermia protocol for an additional 24hrs as neuroprotective treatment for witnessed cardiac arrest. This patient improved during his hospitalization and had a 90 day mRS score of 0. One patient returned 9 days after his initial admission with a new brainstem small vessel stroke.

Patient outcomes (Table 4)

The median NIHSS score was 15 at baseline; 9 at 24 hours; 6 by discharge/5d excluding patients that died before day 5. When patients that died were included and scored at the maximal NIHSS of 40, the mean discharge/5d NIHSS score was 10.7 and the median NIHSS score was 7. 75%

(15/20) patients had improvement in the NIHSS by day 5 or discharge. 70% (14/20) patients had improvement of ≥ 4 points on the NIHSS.

Discussion

In this study, we demonstrated the feasibility of combining intravenous t-PA and caffeinol infusions. However, only about half and two-thirds of the patients reached target post-caffeinol caffeine and ethanol levels, respectively. Nevertheless, we think that we have demonstrated the feasibility of achieving therapeutic blood levels of caffeine and ethanol by our dosing regimen because 1. caffeine blood levels in rodents are effective as low as 7-8mcg/ml, and 15/18 (83%) of our patients reached this level, and 2. the delay in blood drawing resulted in lower measured ethanol levels than those actually achieved during the infusion. It is unclear why the increase in the dose of caffeine to 9mg/kg resulted in a lower median caffeine level. This may be due to significant delays in lab draws in the two patients in this group with the lowest levels. We are currently testing blood levels achieved by increasing the caffeine dose to 10mg/kg caffeine in a series of acute stroke patients. In the 6 patients treated with this dose to date, all patients achieved caffeine levels greater than our target of 8mcg/ml.

While we found that it was feasible to begin cooling and link it to prior t-PA and caffeinol infusions within the target time frame (average time from symptom onset to start of cooling was 4 hours and 23 minutes), in general, we found it difficult to achieve rapid and sustained cooling to target levels in our patients. The greatest barrier to effective cooling is the natural shivering response²³. Shivering was very prominent in our patients and essentially prevented effective cooling until adjustments in the protocol that added an initial infusion of iced saline and aggressive antishivering measures. Blocking the shivering response with higher doses of meperidine, magnesium infusion, and warm air blown onto exposed skin, allowed us to reach target temperature quickly in the last 5 patients, and sustain the target range for the majority of the cooling period. Large doses of meperidine were required in many cases to make hypothermia tolerable, however.

We were not successful in cooling patients with the endovascular device, but there are several possible reasons for this other than deficiencies in the device itself. An interventional radiology fellow placed the catheters, and experienced technical difficulty in one case. The median body mass index (BMI) in patients treated with endovascular cooling was 29.6, vs. 25.7 in the patients treated with the surface cooling devices. The BMI in patients who failed to reach and successfully reached target temperature within 4 hours of induction was 31.2 and 25.1, respectively. This indicates that BMI may play an important role in successful cooling of patients. The relatively small ICY catheter (9.3 Fr) was designed for fever control in intubated patients. Achieving hypothermia in large awake patients will probably require a larger catheter. Experience with Innercool's Celsius Control System using a larger endovascular cooling catheter as part of the ICTuS-L²⁰ trial indicates that endovascular cooling can be quite efficient and effective.

The adverse events attributed to t-PA occurred at an expected rate. There was no apparent increase in brain hemorrhage by linking caffeinol and cooling to t-PA. We did not observe cardiac arrhythmias or other side effects attributed to caffeine or ethanol, though it is possible that the anti-shivering regimen for cooling may have masked some side effects of the caffeinol infusion. Taken together with our previous experience with caffeinol, we conclude that the doses of caffeinol used in this study are very well tolerated.

Cooling required ICU care in order to monitor and adhere to the cooling protocol, and because of the risk of respiratory depression resulting from the sedation required to control shivering. There are many potential risks associated with cooling, but in general, except for the discomfort

associated with shivering, we had no serious side effects of cooling other than a possible increased risk of infection. Systemic clotting and bleeding events were minimal with the intravascular catheter. One serious cardiac arrhythmia occurred but this was not felt to be related to the experimental treatment regimen. However, future testing of caffeinol and cooling definitely needs to be linked to careful cardiac monitoring before the cardiovascular safety can be conclusively demonstrated.

Patients treated with hypothermia had a high rate of infection, especially pneumonias, possibly exacerbated by sedation associated with the anti-shivering regimen. It is unclear, and should be tested, whether the risk of pneumonia is due to the sedation or the hypothermia itself. The intention of our protocol was to achieve cooling in awake patients. However, if our antishivering protocol is going to produce pneumonias, then perhaps earlier intubation to achieve airway protection should be considered in subsequent studies. CK becomes elevated in most cooled patients, and should be monitored as well.

In summary, we have demonstrated that it is feasible to start caffeinol and hypothermia within the first hour after t-PA administration in acute stroke patients and have confirmed and that the combination of caffeinol + t-PA is safe. However, achieving hypothermia in awake patients is difficult, labor intensive, and requires careful and aggressive management of shivering. In future studies of hypothermia, the incidence of pneumonia must be closely monitored, and should include careful attention to airway protection and ICU care, and will also need to assess the risks of clotting events and elevations in CK indicative of muscle breakdown.

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Table 1

Shivering Assessment Scale

Score	Finding
0	None
1	Face/jaw muscle fasciculations
2	Peripheral shivering
3	Uncontrolled rigors/shivering

Table 2

Enrollment log.

Patient	Age	Race	Gender	tPA	caffeinol	HT	method	device	Comments
001	61	African American	Female	yes	Yes	yes	Endovascular	ICY/CoolGard	
002	62	African American	Male	yes	Yes	no	Endovascular	ICY/CoolGard	Malfunction of the cooling unit before placement of the catheter
003	68	African American	Female	yes	Yes	yes	Endovascular	ICY/Coolgard	
004	70	Caucasian	Female	yes	Yes	yes	Endovascular	ICY/Coolgard	
005	41	African American	Female	yes	Yes	yes	Endovascular	ICY/Coolgard	
006	45	Caucasian	Male	yes	Yes	yes	Endovascular	ICY/Coolgard	
007	72	African American	Male	yes	Yes	yes	Surface	Gaymar	
008	78	Caucasian	Male	yes	Yes	yes	Surface	Gaymar	
009	67	Caucasian	Female	yes	Yes	yes	Surface	Arctic Sun	
010	48	Hispanic	Male	no INR 1.8	Yes	yes	Surface	Arctic Sun	
011	53	African American	Female	no Presented >3hrs from onset	Yes	yes	Endovascular	ICY/CoolGard	cooled with Arctic Sun after worsened with rewarming
012	59	African American	Female	no History of ICH	No on IRB hold	no	Endovascular	ICY/CoolGard	Malfunction after placement of the catheter
013	46	African American	Male	yes	No on IRB hold	yes	Surface	Arctic Sun	
014	38	Caucasian	Female	yes	Yes	yes	Surface	Arctic Sun	
015	46	African American	Female	yes	Yes	yes	Surface	Arctic Sun	
016	42	Caucasian	Female	yes	Yes	no	Iced NS	Iced NS	Induction hypothermia only
017	39	Hispanic	Male	yes	Yes	yes	Surface	Arctic Sun	
018	58	Caucasian	Male	yes	Yes	yes	Surface	CSZ	
019	44	African American	Female	yes	Yes	no	X	X	NIHSS <8
020	83	Caucasian	Female	yes	Yes	yes	Surface	CSZ	

Table 3

Comparison of hypothermia treatments based on cooling device used.

	Alsius catheter [®]	Gaymar [®]	Arctic Sun [®]	CSZ [®]
Number treated	8	2	6 [*]	2 [*]
Number reached target	3	2	6	2
Median time to target, min	210	390	150	120
Duration in target temp, in hours	9	17	23	28
Lowest temperature	34.2	33.3	34	32.6

* Three patients treated with the Arctic Sun device and two patients treated with the Cincinnati SubZero device also received iced normal saline infusions during induction of hypothermia.

Table 4

Clinical outcomes

Subject #	Discharge mRS	Baseline NIHSS	24hr from onset NIHSS	end of RW NIHSS	Discharge/5d NIHSS*
1	4	15	10	x	5
2	2	19	7	x	5
3	4	24	12	12	12
4	4	14	8	10	10
5	1	14	5	5	3
6	0	16	6	5	0
7	6	25	17	18	patient died
8	6	20	9	21	24**
9	4	11	4	5	10
10	5	14	x	11	10
11	4	20	13	18	8
12	4	9	9	12	12
13	2	10	12	12	6
14	0	13	4	3	1
15	6	19	20	37	patient died
16	1	13	3	x	2
17	4	20	12	5	4
18	1	12	0	2	0
19	0	19	0	x	0
20	5	11	11	18	22

* Data collected at day 5 or discharge, whichever came first.

** Patient died after protracted hospitalization.