Using our novel animal models of severe hemorrhage, focusing on evaluation of outcome to 3-10 days, the following strategies were found superior in terms of intact survival compared to standard resuscitation. Project I on hemorrhagic shock revealed benefit from limited (hypotensive) titrated fluid resuscitation; mild hypothermia during shock and resuscitation; additional use of antioxidant tempol i.v. or intraperitoneal; titrated i.v. administration (instead of bolus) of hypertonic/hyperoncotic solution for preventing cardiac arrest during uncontrolled hemorrhagic shock; and peritoneal oxygenation and medication. Program II on "suspended animation" was in novel animal models of severe hemorrhage to cardiac arrest (no blood flow), the prevalent cause of death in combat. Hypothermic strategies with cold saline flushed into the aorta within the first 5 minutes of cardiac arrest was found to be most effective in preserving the organism for up to 1-2 hours of clinical death. Resuscitation must be with artificial circulation (emergency, portable heart-lung machine). Using small flush volumes plus drugs, the testing of 14 different pharmacologic strategies revealed disappointing results. Special cold preservation solutions tested revealed only minor advantage over cold saline. Industries are being advised for the development of rapid vessel access methods ("smart catheter") and of hypothermia induction with blood cooling in the field.
FINAL REPORT

GRANT NO: N000-97-1-1064, N000-98-2-1064, and N000-14-99-1-0765

PRINCIPAL INVESTIGATOR: Peter Safar, M.D. (e-mail: safarp@anes.upmc.edu)

INSTITUTION: Safar Center for Resuscitation Research, University of Pittsburgh

GRANT TITLE: NOVEL RESUSCITATION FROM LETHAL HEMORRHAGE.
Increasing survival of combat casualties.

Project I: Hemorrhagic shock (HS) in rats. Tisherman et al
Project II: Suspended animation (SA) in dogs. Safar et al


OBJECTIVE: To help maximize the reversibility of presently lethal traumatic hemorrhage. In project I on hemorrhagic shock (HS) (low blood flow) in rats, to extend beyond the “golden hour” of HS tolerance -- the prevalent cause of soldiers having “died of wounds” (DOW) -- by preventing cardiac arrest (CA) during HS and multiple organ failure (MOF) after resuscitation. In project II on rapid exsanguination to CA (no blood flow), in dogs -- the prevalent cause of soldiers killed in action (KIA) -- to develop a totally new approach for this presently unresuscitable condition (and unresuscitable CA in civilian EMS). We term this method “suspended animation (SA) for delayed resuscitation,” i.e., preservation of brain and organism during clinical death of 1-2 h, to buy time for transport, surgical hemostasis, and initiation of resuscitative cardiopulmonary bypass (CPB). Support of projects I and II was initiated by U.S. Navy Commander Lyn Yaffe, project II on SA inspired by U.S. Army surgeon Ronald Bellamy. Our HS and SA projects were designed to systematically explore, in the first 5 years, the most promising pharmacologic and hypothermic strategies to maximally increase the tolerance of these insults in terms of outcome, i.e., survival without brain damage. We specifically explored mild hypothermia (Hth) (33-36°C) or moderate Hth (28-32°C) for HS (spontaneous circulation) and deep Hth (16-27°C) and profound Hth (5-15°C) for CA. Our results should guide future development of devices and techniques for initiation in the field. Both HS and SA studies have been for preservation [treatment during the insult], followed by resuscitation [treatment to reverse the insult]. The efficacy of mild Hth after normothermic CA, first documented by us in dogs, has now also been documented in patients (see NEJM, early 2002 in press, with our editorial).

APPROACH: For project I on HS, we used our established unique outcome models in rats, with targeted modifications in selected studies (2,3). We used uncontrolled HS (tail amputation), pressure-controlled HS, or volume-controlled HS. HS was initiated with bleeding 3 ml/100 g over 15 min. HS phase 1 of 60-180 min with mean arterial pressure (MAP) prevented from dropping below 40 mm Hg; and resuscitation phase 2 of 1-3 h for hemostasis and all-out i.v. fluid resuscitation (FR) with return of initially shed blood plus lactated Ringer’s solution (LR) are followed by observation phase 3 of 72 h (range 24 h - 10 days). Principle endpoints were survival time and survival rate to 24 h or 72 h. We used established outcome measurements of overall, neurologic, and cognitive function, and morphology. We also explored some mechanisms related to treatments studied. Phases 1 and 2 were with standardized light inhalation anesthesia and spontaneous breathing via mask; phase 3 was without anesthesia. During phases 1 and 2 we monitored visceral dysoxia as increased PCO2 on the surface of liver and gut (7-9). During phase 3 we monitored heart rate and activity, and controlled core temperature (T) using a radio transmitter in the abdomen (7-9). We explored therapeutic hypothermia (1,4,10,17,18,45), hyperoxia (increased FiO2) (7-9), novel drugs and fluids (15,16), and peritoneal strategies (13,39,43).

For project II on SA, we used modifications (60) of our unique CA outcome models in custom-bred hunting dogs of 20-25 kg weight (50-64). Normothermic exsanguination over 5 min led to CA no flow for periods ranging between 15 min and 120 min – CA at various temperatures (57,60,61). Preservation was induced within the first 5 min of CA by flushing isotonic NaCl (saline) into the aorta via a balloon catheter, with or without special drugs or fluids added. On drugs, during years 1 and 2, we received input from neuroscience subcontractors Drs. Hayes, Sick, Siesjo, and Bickler. At the end of CA, resuscitation was with closed-chest (emergency) cardiopulmonary bypass (CPB), restoration of spontaneous circulation (ROSC),
benefit does not depend on the ability of Hth to increase MAP (10). In general, non-survivors (irrespective of HS model (5), but when tested in the HS.

Hth supported MAP during in the uncontrolled (Hth) by surface with LR, had survival benefit over normotensive or no FR (2,3). Additional mild hypothermia HS 2 FI0 HS 1.0 during min (lethal with normothermia) can be survived by combining moderate Hth plus HS of 180 report that volume fell of 180 Hg) HS pressure-controlled (MAP 40 mm Hg) HS and FR, increased survival time and 72 h survival rate (5-10). This was a "first.” Even when Hth was delayed until FR (phase 2), did it increase survival time (19). Mild Hth was as effective as moderate Hth. There was no gross shivering when conscious rats were kept under mild Hth (35°C) during phase 3. Hyperoxia with FiO2 1.0 did not significantly increase survival time or rate (9). FiO2 0.5, however, had some survival benefit (NS) when added to mild Hth. FiO2 1.0 during uncontrolled HS and FR supported MAP without increasing bleeding. Hth or hyperoxia transiently mitigated visceral dysoxia during HS. Mild Hth (Tr 34°C) supported MAP during HS in the uncontrolled HS model (5), but when tested in the pressure-controlled (MAP 40 mm Hg) HS model, survival benefit from Hth was retained (10). Therefore, Hth benefit does not depend on the ability of Hth to increase MAP (10). In general, non-survivors (irrespective of temperature) that died on days 2 or 3 had developed gut necrosis. Survivors to 72 h showed grossly normal function and grossly morphologically normal organs.

Mechanism studies were added (10,12,16,17,20). We postulated that the most vulnerable organs after HS are the viscera, while the brain protects itself by vasodilation. Using a pressure-controlled HS outcome model with MAP 30-40 mm Hg for 45-60 min at normothermia, we found normal cognitive function values at 10 days, as well as normal brain histology, normal neuron cell counts, and no neuronal DNA damage (11,21). Rats with poor outcome had apoptotic and necrotic lesions in liver and small intestine (28). Antioxidant reserve in plasma and (more so) in the liver, was reduced to a lesser degree at the end of HS and FR in rats treated with mild Hth compared to normothermic controls (25) (Dr. Kagan). Plasma levels of IL-1ß and IL-6 were lower and those of IL-10 and TNF-α were higher during HS, using mild Hth compared to normothermia (17,20). The increase in survival rate achieved with mild Hth during HS could not be achieved by isolated cooling of the gut – rather it required cooling of the whole organism (18). Recently we found in pigs with liver trauma that mild Hth during HS and FR may not cause coagulopathy (49).

Resuscitation fluids and drugs were explored, i.e., outcome compared to that with use of LR, using mostly the uncontrolled HS model. In our previous studies, colloid or whole blood had proved superior to LR. In our uncontrolled HS model, polynitroxyalted albumin (PNA) plus tempol (a water-soluble antioxidant) increased survival compared to albumin (16). Tempol was effective when given early during HS (38), but not when given late (37). We explored hypertonic/hyperoncotic solution (HHS) we explored with a novel mode of administration, namely titration, instead of the previously studied bolus administration (15); titrating HHS to maintain MAP at ≥ 40 mm Hg during HS of 2 h did not increase survival rate, but did not cause increased bleeding and achieved the same survival with only one-tenth of the fluid volume required with use of LR (15).
Ringer’s ethylpyruvate (an antioxidant) solution did not improve survival time and rate compared to LR (47), nor did amelioride as Na+/H+ exchange inhibitor (48). Oral or enteral IL-6 during HS was not beneficial (12). We explored intra-peritoneal strategies to preserve the viscera during HS. *Intraperitoneal ventilation* with oxygen or air increased survival time and rate (13). Intra-peritoneal (but not enteral) adenosine improved survival (14). In the last study, using lethal uncontrolled HS of 2 h (MAP ≥ 40 mm Hg) an optimized *combination treatment* during HS phase I was most effective (45): compared with control group 1 under normothermia with LR, mild Hth during HS (group 2) increased survival; but mild Hth plus PNA-tempol during HS (group 3) increased survival further. All of the above plus peritoneal adenosine (group 4) gave worse survival data (perhaps because of the added trauma, laparotomy).

**Accomplishments of Project II on SA in Dogs.** In normothermic CA with ≥ 15 min no flow or CA in profound Hth, external CPR is ineffective and CPB is needed to achieve ROSC. In years 1-2, we modified our past SA model (50,51) which used CPB for induction of preservation (SA) at the start of CA with profound Hth. In the modification, the induction of SA was with a single flush of cold saline via an aortic balloon catheter (53). This method is likely to be more feasible than CPB in the field or in field hospitals. For that we are advising industries to work on novel vessel-access methods – insertion of an aortic balloon catheter percutaneously, through cutdown or via thoracotomy (Dr. Klain, Dr. Yaffe, Cardeon Co.); and for a miniaturized cooling/pumping device. In our dog outcome studies (see “approach”) the aortic balloon catheter was placed in the upper thoracic aorta to first rapidly preserve brain and heart. Starting at CA 2 min normothermic no flow, normothermic flush of 500 ml at 37.5°C over 1 min did not improve outcome (53,65); however, the same with saline at 24°C achieved brain (tympanic membrane) temperature (Tty) 36°C, and saline flush at 4°C achieved Tty 34°C, all within 1-3 min flush (53). Flush with 24°C saline at start of CA 15 min no-flow achieved functional normality at 72 h, but with histologic damage (53). Flush with 4°C saline (Tty 34°C) at start of CA 15 min achieved functional and histologic normality (53). Catheter design influenced outcome; with the opening at the tip, the straight flush resulted in better outcome than using a catheter with the tip closed and the flush through multiple lateral openings. For adding drugs with preservation potential, the control protocol had to achieve, at 72 h, survival with brain damage in all dogs. That was CA 20 min no flow and flush at the start of CA with saline at room temperature (24°C) (54).

In years 2-3, we were searching for a breakthrough effect among 14 different drugs added to the saline flush, and in some cases also to the reperfusion fluid. For individual dogs’ and drugs’ results see table in reference (59). The drugs were selected according to one or more of six mechanistic strategies, documented by us and others in the past: 1) delaying energy failure; 2) combating depolarization of membranes; 3) inhibiting proteases; 4) inhibiting apoptosis; 5) inhibiting reoxygenation injury; and 6) protecting mitochondria. The drugs were selected on the basis of published results in rodents and advice from our co-investigators and consultants. Subcontractors supplied their own published and new data. Most drugs tested had not yet been evaluated for outcome after prolonged CA in large animals. We were seeking a breakthrough effect, namely OPC = 1 at 72 h in 2-3 dogs of exploratory mini series of 3-6 experiments each. OPC = 1 was achieved in very few cases (59). Adenosine did not abolish the previously seen histologic damage after CA 15 min (55). In the CA 20 min Tty 36°C model, when thiopental, thiopental plus phenytoin, or insulin plus glucose was added to flush and reperfusion, only 3/20 dogs achieved OPC 1, while 17 achieved only OPC 3 or 4 (severe damage) (56). Even in dogs with OPC 1, none of the brains were histologically normal (total HDS 0-30). HDS were always severe and correlated with NDS. With CA 20 min, flush with the antioxidant tempol resulted in OPC 1 or 2 (good outcome) in all 8 dogs (58). Tempol was also effective with CA 40 min, and 50 ml/kg flush at 4°C, to Tty 28°C (58). In both it improved function, but did not significantly improve brain HDS (58). An explanation for this is speculative. These antioxidant experiments also searched for mechanisms (58). Treatment with any of 11 other drugs resulted only in dogs achieving OPC 3 or 4, severe NDS and severe HDS (55-59). Thus, in this unique systematic exploration in 84 dogs, only tempol looked promising. Either tempol or other brain penetrating antioxidants should be pursued further.

In years 3-4, we used the same model for exploring hypothermic strategies without drugs. We systematically extended the CA (no flow) period from 20 min (54), to 30 min (60), and to 60-120 min (61,62). The lower the Tty after flush the better was preservation for increasing CA duration. Saline at 4°C flushed with a 1L/min flow rate, decreased Tty by 3°C/min. This is more rapid than with any other cooling method except CPB. With CA 20 min no flow (54) aortic arch flush rapidly lowered Tty to 34°C, and achieved survival to 72
h with functionally normal and histologically minimal damage. A delay in flush during normothermic CA to 8 min no flow before start of cold flush negated the preservation achieved with flush starting at CA 2 min or 5 min (84). When we increased CA to 30 min no flow (60), we found that the flush volume of saline at 4°C had to be increased to 100 ml/kg to achieve functionally normal brains, and in some dogs even histologically normal brains, the aortic balloon had to be inflated in the abdominal aorta to also protect the viscera and spinal cord against ischemic damage (60,61,62). For flush preservation in adult humans (70 kg), this translates to 7L of iced saline. Aortic flush toTypography 20°C, 15°C, or 10°C preserved the brain and organism long enough to achieve intact survival (OPC 1) after 60 min, 90 min, and in some dogs even 120 min no flow (61). All 6 dogs with CA 90 min andTypography 10°C were functionally normal. One dog after CA 90 min, one after CA 60 min, and one normal dog received cognitive function tests months later; these were normal. Functional and histologic studies of extracerebral organs after 72 h have been initiated (86). CA up to 60 min was survived with intact viscera, with liver malfunction only transient (75). In the last study, with CA 120 min, when flush was with Normosol, Unisol, and tempol combined, all six dogs achieved good outcome (OPC 1 or 2) (62). We found only minimal outcome benefit from using solutions other than saline for the cold flush, stasis, and reperfusion. Using the CA 30 min model (83), outcome after use of albumin 5% or 20% was not different than with saline. PNA-tempol (Synzyme Co.) gave slightly better NDS than saline. A novel cerebral and cardiac preservation solution designed by our consultant Dr. Taylor (Unisol, extra- and intracellular, Organ Recovery Systems Co.) resulted in easier restoration of stable spontaneous circulation, but no improvement in OPC and NDS (83). We communicated with Dr. Rhee (ONR-USUHS), who was inspired by our work, and who researched SA with asanguinous low flow (not CA), using CPB via thoracotomy in pigs. This adds to our current (year 4) SA trauma studies in preparation for clinical trials.

Other accomplishments. For the rapid induction of mild Hth under spontaneous circulation (after CA, during HS, or other indications), blood cooling proved preferable to surface cooling. We found veno-venous pumping of about 10% cardiac output flow via a cooler to decreaseTypography in dogs to the target level of 34°C in about 6 min (M34). Drs. Safar, Tisherman, Behringer, and alumni of this ONR project contributed to the revisions of Emergency Cardiac Care Guidelines 2000 by the American Heart Association in 2000. The P.I., Co-P.I., and fellows, throughout, presented an average of one lecture per month on a variety of topics, including those related to the HS and SA projects. In 2000, the P.I. presented 10 invited, named lectures outside. In 1999, SCRR Director Dr. Patrick Kochanek, conducted the 20-year anniversary program, which included honoring of and gratitude for the ONR. Subcontractor Dr. Siesjo presented the University of Pittsburgh’s annual Safar Lecture of 1999. We co-initiated and helped the NIH PULSE initiative intended to boost coordinated support of resuscitation research, including traumaology, which was particularly supported by our group. Dr. Tisherman was co-chairman of the CNS panel. Dr. Safar was asked to give input for all NIH panels. Our group led new initiatives at local, national, and international levels for boosting the weakest link in civilian EMS, life-supporting first aid (LSFA) skill acquisition by the public (M8). Three fellows received awards at meetings for our ONR work. ONR held an animal models meeting where our group summarized 15 different models of shock or CA, developed and used since the 1960s. In 1999, we co-conducted in Baltimore, with the Shock Trauma Center, a planning session for clinical trials. On honors and awards, the P.I. gave the invited Wright Memorial Lecture of the American Society of Anesthesiologists on “Resuscitation Medicine in 1000-2000 C.E.” The P.I. received his fourth elected honorary membership of Academies of (Medical) Sciences, i.e., that of Russia. The Co-P.I. gave thanks related to our ONR research for the American Society of Artificial Internal Organs, the Japanese Reanimatology Society 2000, the Shock Society Meeting, and the International Surgical Society (on Fluid Resuscitation). The Co-P.I. received the Shubin-Weil Award of the Society for Critical Care Medicine. The P.I. coached a Ph.D. student on CA-CPCR related research: 1) Earliest possible prediction of futility in comatose survivors of CA (M29); 2) Hypertensive reperfusion from prolonged CA in patients correlates with good cerebral outcome (M28).

CONCLUSIONS: Preservation of the organism during HS (low flow) or CA (no flow) with hypothermic strategies is more effective than with pharmacologic strategies. In clinically relevant rat outcome models of HS, the benefit of mild Hth and of low-volume (hypotensive) FR is reproducible. For life support during uncontrolled HS, titrated hypotensive FR with a hypertonic/hyperoncotic solution and the antioxidant tempol are promising therapies, without risk of rebleeding. Antioxidants may be more effective when given at the beginning of HS or CA than during reperfusion. For severe, prolonged HS, peritoneal strategies should be pursued further for oxygenating, medicating, and cooling. For presently unresuscitable exsanguination CA, buying time beyond the normothermic CA tolerance limit of 5 min is possible with hypothermic strategies:
With Try 10°C (induced with saline 4°C flush starting at CA 2 min) one can count on full preservation of all organs' viability after up to 90 min no flow. On SA, we are on schedule with our initial estimate that it will require about 5 years for pathophysiologic work to clarify limits and potentials (1997-2002) and another 5 years for development of devices and methods and initiation of transfer to patients.

SIGNIFICANCE: Concerning HS (low flow, spontaneous circulation), our documentation in rats of survival benefit from mild hypothermia (34°C), during HS, without causing increased bleeding is a "first." This has considerable clinical potential. How long to sustain Hth during and after FR remains to be determined. The doctrine for first aid of "keep him warm" may have to be changed. Since mild hypothermia is simple to induce and safe, and moderate hypothermia (30°C) as well as hyperthermia are risky, first aid should include temperature control. If trauma more extensive than tail amputation is added, Hth-induced coagulopathy may play an important role. At 34°C, this does not seem to be the case, as in our year-4 study conducted in pigs (also under ONR support). The fact that in rats with healthy lungs, breathing O₂ 100% does not improve outcome, is good news for military strategists concerned with supplies via air. The antioxidant tempol, alone or with colloid (PNA, Synzyme Co.) with outcome benefit in our rat model, should be pursued for FDA approval. The use of small-volume, titrated HHS has significance in the combat situation, in which the volume of available fluid is limited and prevention of CA during very long HS is difficult. The doubling or tripling of the "golden hour" by Hth plus other strategies (antioxidant, minimal novel fluid titrated) could be important. The HS studies are continuing in years 4 and 5 (2001-2003) under ONR support with Dr. Tisherman as P.I. and Dr. Safar as Co-P.I. In preparation for clinical trials, a trauma model in pigs has been added to our rat models.

Concerning CA (SA). Our dog data, so far without trauma, have clarified the limit of tolerance for up to 90 min (perhaps 120 min) CA no-flow at 10°C. This “first” should lead to clinical feasibility trials in trauma hospitals’ emergency rooms, using rapid thoracotomy or femoral cutdown for access to the aorta, with pump and large volumes of cold fluid standby; and emergency (portable) CPB ready for resuscitation. Preservation of viability of brain and organism proved possible within the first 5 min of normothermic no flow. Taking SA into the out-of-hospital arena and the field for initiation by military medics will require a next phase of SA engineering (devices) research. The main scientific significance of our SA results concerns the observation that pharmacologic preservation potentials pale in comparison to hypothermia. After 20 years of frustrating drug-by-drug searches for cerebral resuscitation potentials, by us and others, our systematic exploration of many pharmacologic preservation potentials in the same model was a “first.”

The clinical significance includes SA potentials for the many unresuscitable cases with normovolemic sudden cardiac death — to buy time for bridging to the initiation of prolonged CPB. This normovolemic CA study is planned for year 4. Relevance for that includes military casualties with CA without hemorrhage (e.g., chemical warfare). For all this we are coaching the development of devices and methods for rapid vessel access without thoracotomy (with smart catheters) (Dr. Klain, Dr. Yaffe; Cardeon Co.) and a miniaturized portable blood cooling/pumping device (Biocontrol Co.). With existing tools, a multicenter clinical feasibility trial in trauma centers’ emergency departments, of mild Hth for traumatic HS, and of profound Hth for SA in exsanguination CA (flush via thoracotomy), feasible now, and is being planned now under the leadership of Dr. Tisherman. Subcontractor Dr. Champion helped clarify the number and types of suitable cases, using the National Trauma Database. ONR outcome research in rats and dogs should continue parallel with clinical trials. Continued animal work should pursue many still unanswered questions. These include how tissue injury and coagulopathy would influence our SA results so far without major tissue injury (relevant for exsanguination from a “simple” penetrating injury of a major vessel). For major tissue trauma, we just completed in year 4 the first SA study with major trauma. Military and civilian resuscitation potentials by paramedics vs physician teams, in field vs mobile ICU (or helicopter) vs field hospital, are merging since September 11, 2001. Capabilities and needs of special Armed Forces should make us re-think our research goals.

PATENT INFORMATION: No new patents since start of ONR funding. Earlier, the University of Pittsburgh, under Safar-Klain-Stezoski, obtained a patent for a portable multimodular cardiopulmonary bypass apparatus, and one for a single- or double-balloon aortic catheter. Both patents had been licensed to the Cardeon Corp. of California before start of this ONR project.

AWARD INFORMATION: See “other accomplishments,” above.
PUBLICATIONS
Supported fully or in part by the Office of Naval Research (ONR) grants to the SCRR, University of Pittsburgh 1997-2001 (years 1, 2, and 3)

HEMORRHAGIC SHOCK (HS)

Refereed Publications on HS


Abstracts


SUSPENDED ANIMATION (SA)

Refereed Publications on (SA)


Abstracts on SA


Miscellaneous (books, o.e.) by authors when partially supported by ONR


