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(54) **METHOD AND COMPOSITION FOR RESUSCITATION**

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(57) **ABSTRACT**

The key obstacle for current resuscitation is the acutely limited time window. The major reason for the limited time window is that the brain is extremely vulnerable to hypoxic-ischemic insult. The existence of cerebrospinal fluid (CSF) is the major reason why brain and spinal cord is so vulnerable. A method and a composition for resuscitation of cardiac arrest are provided. The method includes steps of inhibiting CSF production, removing CSF or replacing the CSF with invented composition plus conventional CPR. The composition includes colloidal osmotic agent, insulin, elevated magnesium concentration and ATP in artificial CSF.

METHOD AND COMPOSITION FOR RESUSCITATION

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] This invention is related to a method and a medical composition for resuscitation. In particular, the invention relates to protection of the brain and spinal cord during resuscitation of cardiac arrest. This patent is a continuation of my previous patient application.

[0003] 2. Background Information

[0004] It is estimated that more than 350,000 Americans died of sudden cardiac arrest each year, more than 95 percent of victims die before reaching the hospital. Economic costs for trauma related arrest is over 400 billion dollars each year. Despite numerous scientific advances throughout modern medicine, outcome of resuscitation for arrest victims remains poor, and outcomes in trauma related battlefield statistics have not improved significantly since the Civil War. The cardiopulmonary resuscitation (CPR) practice including ventilation, closed chest compressions or cardiac massage and defibrillation (step ABC, i.e. Airway, Breathing and Circulation) was established in the 1960's. The fundamental strategies for CPR have not changed since these original concepts emerged. The key obstacle for resuscitation is the acutely limited time window. The major reason for the limited time window is that the brain is extremely vulnerable to hypoxic-ischemic insult. Traditionally, it is believed that the maximum tolerant survival time for brain in a cardiac arrest patient is about 5 to 8 minutes. For example, four to five minutes of circulatory arrest causes permanent brain damage in over half of patients, and with ten minutes of cardiac arrest, the likelihood of complete physical and neurological recovery is very low and overall survival is less than ten percent. Therefore, in clinic, the real problem in circulatory arrest is usually not to restore cardiopulmonary function but instead to prevent brain death.

[0005] Shock results in low blood perfusion throughout body. Although the blood perfusion is not completely stopped, shock shares many similar pathological processes with cardiac arrest, and is also a life-threatening condition. Shock can be categorized into anaphylactic, septic, cardiogenic, hypovolemic shock depending on the causes.

[0006] Central nervous system (CNS) consisting of the brain and spinal cord is very vulnerable to hypoxia-ischemia. Currently there are no logical theories to explain its vulnerability. The search for a neuroprotectant has consumed enormous resources from private pharmaceutical companies as well as government supported research. Broad spectrums of compounds with disparate mechanisms of action have been considered. These compounds have ranged from oxygen free radical scavengers, calcium channel blockers, and glutamate receptor antagonists to monoclonal antibodies trying to curtail the inflammation. However, once cerebral ischemic cascade is set into motion by an initiating injury, the resultant damage is still unavoidable, untreatable and permanent. Although several of these agents have shown promise in the laboratory, very few have shown real advantages during in vivo testing or clinical studies. Lacking of an effective approach to protect brain is the ultimate reason why the time window for resuscitation is so limited.

[0007] Current search for a neuroprotective treatment based on various molecular mechanisms has yielded a disappointing result during clinical trial. One possible reason for these failures is because of the negligence of blood perfusion deficit following an initial ischemia. It has been known that after cardiac arrest and global ischemia, the brain suffers a "no-reflow" phenomenon. In the 1960s, Ames and coworkers produced global cerebral ischemia for 6 minutes in rabbits followed by carbon black ink infusion. They found that a large amount of the brain suffered from perfusion deficits. Similar to the "no-reflow" phenomenon, post-ischemic or post-traumatic "hypoperfusion" has also been documented after spinal cord and brain injuries. Therapeutic intervention has seldom targeted this blood perfusion deficit.

[0008] Traditionally, neurons are speculated to be very vulnerable because they have many special characters such as excitability and having synapses, glia cells, similar to other cells outside CNS, are resistant to hypoxia-ischemia. This traditional notion is supported by the fact that compared with other tissues and organs, brain and spinal cord are extremely susceptible to hypoxia-ischemia, because 5-8 minutes of cardiac arrest can result in irreversible brain damage, moreover, among the organs of the body, while comprising just 2% of its mass, the brain requires 20% of the body's blood flow, in addition, it is difficult for neurons to regenerate and it is easy for glia cells to proliferate after brain injuries. However, traditional notion might not be correct based on following facts: 1. Neurons only make up at most 10% of the total cell numbers in brain and spinal cord, gray matter occupies less than 40% of the whole CNS, white matter including glia and fibers is dominant in CNS. In clinic, a cerebral infarct often became liquefied, that means the whole structure in infracted cerebral tissue was destroyed including neurons, glia cells, nerve fibers. 2. Nerve fiber in peripheral nervous system (PNS) can easily survive for 3 hours severe ischemia followed by 7 days reperfusion. 3. Neurons in PNS are also much more tolerate to ischemia than neurons in CNS. We previously showed that neurons in superior cervical ganglion (SCG) could tolerate 2-3 hours of ischemia followed by 7 days reperfusion. Piao and co-workers (Piao D X et al. Arch Histol cytol. 1999 62: 4. 383-392) demonstrated that neurons in enteric nervous system only showed early signs of damage after 3-4 hours complete ischemia followed by 4 weeks reperfusion. Therefore the difference between CNS and PNS may be crucial for understanding why CNS is so vulnerable. Given the fact that the brain and spinal cord are submerged in the CSF, I hypothesize that neurons and glia cells in the CNS, similar to neurons and glia cells in PNS, are resistant to ischemia in nature, the existence of CSF might have altered their resistant nature; the CSF plays an important role in the vulnerability of the brain and spinal cord during ischemia.

[0009] All CNS injuries such as hypoxia-ischemia, trauma, infection, poisoning etc. are invariably associated with cerebral edema which is a common pathway leading to cell death. Our scientific community is familiar with hydrocephalus and cytotoxic, vasogenic cerebral edema, yet the cerebrospinal fluid (CSF), the huge water resource, has seldom been linked with cerebral edema and the vulnerability of the brain and spinal cord to ischemia.

[0010] In a human adult, the CSF occupies about 10 percent of the intra-cranial and intra-spinal volume. The average rate of CSF formation is about 21 to 22 ml/hr, or

approximately 500 ml/day. The CSF formation is related to intracranial pressure (ICP). When the intracranial pressure is below about 70 mm H_2O, the CSF is not absorbed, and production increases. Many agents are known as CSF production inhibitors such as Furosemide and Acetazolamide. It is believed that the CSF serves as a kind of water jacket protecting the spinal cord and brain from potentially injurious blows to the spinal column and skull, it also serves as a buoyancy so that brain and spinal cord virtually float in a CSF jacket with weight greatly reduced. Similar to extracellular fluid or lymphoid fluid, CSF is involved in CNS metabolism. The CSF is secreted from the plasma through the choroid plexuses. Capillaries in the choroid plexus are highly specialized for their function. Unlike those in the rest of the cerebral vessels, capillaries in the choroid plexus are fenestrated, non-continuous and have gaps between the capillary endothelial cells allowing the free-movement of small molecules. Therefore, the CSF consists of micromolecules (i.e. Na^+ 154 mEq/L, K^+ 3.0 mEq/L, Mg^{++} 0.9 mEq/L, Ca^{++} 1.4 mEq/L, Cl^- 136 mEq/L; Glucose 61 mg/dl etc). However, tight junctions linking the adjacent choroidal epithelial and forming what is known as the blood-CSF barrier prevent most macromolecules from effectively passing into the CSF from the blood. It is believed that Blood-CSF barrier together with blood brain barrier (BBB) are for ensuring CNS tissue an "isolated environment" so that harmful substances in the blood such as, circulating drugs, toxins, etc. do not have access to neuron either from blood or from the CSF. Whereas, I believe that CNS might have paid a big price for such an "isolated environment", because all macromolecules such as plasma proteins and other polypeptides can not easily enter the CSF. Many macromolecules are polypeptides and are very important for cell survival. For examples, albumin, the major plasma protein for maintaining colloidal osmotic pressure (COP) is extremely low in the CSF. Insulin, a natural neuroprotectant, has a much lower concentration in CSF compared to plasma. Therefore, the CSF is extremely prone to edema without these macromolecules. The CSF occupies the subarachnoid space, it has free access to neurons and surrounding glia cells through the Vichow-robin space which is also known as the perivascular spaces. Smaller blood vessels, which centripetally penetrate into the brain proper, are accompanied by an extension of the subarachnoid space that forms the Virchow-Robin space and is filled with the CSF. I hypothesize that the CSF might contribute to the vulnerability of CNS tissue through the following cascades: the CSF is readily available for infiltrating cerebral tissues. Cardiac arrest results in complete cerebral ischemia leading to the CSF infiltrating the brain or spinal cord tissue and causing rapid edema, particularly the surrounding tissue of Virchow-Robin space. The swelling tissue reduces the size of the space or causes its complete collapse resulting in constriction of the small blood vessels in Virchow-Robin space. This ultimately leads to long lasting cerebral ischemia even after restoring blood supply, i.e. "no-reflow" phenomenon or "hypoperfusion". This "secondary" blood perfusion deficit induces a feedback loop eventually resulting in irreversible cell death and tissue necrosis and liquefaction.

[0011] Water makes up about 45-65% of total body weight in the human adult. It can enter cells through water channels. For examples, many water channels such as Aquapprin-1 (AQP1), Aquapprin-4 (AQP4), Aquapprin-5 (AQP5), Aquapprin-9 (AQP9) have been identified in CNS and believed to play an important role in the development of cerebral edema. The body fluid must be virtually in "gel form" which only allows small part of fluid to "free flow". It mainly diffuses through the "gel"; that is, it moves molecule by molecule from one place to another by kinetic motion rather than by large numbers of molecules moving together. Excessive "free flow" fluid is one of the important basis of tissue edema. A colloidal osmotic agent is mainly for maintaining colloidal osmotic pressure (COP) and controlling body fluid in human body. Albumin is the major colloidal osmotic agent in plasma, its water holding capacity is so large that it is estimated that one gram of albumin binds 18 ml of water. It measures about 6500 mg/dl in plasma (contributing 26 mm Hg COP), 2% in intercellular fluid and 3-4% in lymphatic fluid. The COP counteracts hydrostatic pressure preventing edema according to Starling's equation. The interstitial fluid and lymphatic fluid pressure at capillary level is believed to be very low or even negative. In CNS however, the CSF contains almost no COP because of the extremely low albumin concentration (25 mg/dl). The molecular weight of albumin is about 60,000 Daltons, it can not easily enter the CSF through choroid plexuses because of the brain-CSF barrier. The ICP which is the hydrostatic pressure of the CSF (normally ranged between 80-180 mm H_2O) is often elevated when cerebral edema occurs.

[0012] Insulin secretion from pancreatic P cells responds very precisely to small changes in glucose concentration in the physiologic range, hereby keeping blood glucose levels within the range of 70-150 mg/dL in normal individuals. The CSF contains about two third of plasma glucose concentration (CSF: 61 mg/dl; Plasma: 92 mg /dl). However it contains about at most one fifteenth of plasma insulin concentration (CSF: 4 μU/ml; fasting plasma: 20-30 μU/ml). If considering fasting insulin in plasma the optimal biological effectiveness to normal blood glucose concentration, the insulin concentration in the CSF is at least three to four times lower than optimal. In addition, evidences have shown that CSF glucose concentration increases easily as plasma glucose concentration escalating, however there is no or very tiny increase of insulin in CSF as plasma insulin and glucose concentration escalating. Insulin is a small protein, with a molecular weight of about 6000 Daltons. It is not easy to enter the CSF through choroid plexuses because of the brain-CSF barrier. Plasma is main source of insulin supply for CNS tissue. Because the amount of insulin does not match the amount of glucose in the CSF, there is a relative insufficient amount of insulin to CNS. The insulin shortage will become apparent when the CNS tissue is damaged and followed by secondary blood perfusion deficit. The effect of glucose during CNS ischemia is controversial. I believe that glucose is neuroprotective only if the original injury is mild, however, glucose is detrimental if CNS ischemia is severe such as cardiac arrest. As a growth factor, insulin has been reportedly neuroprotective independent to its lowing blood glucose effect. Insulin can promote glycolysis through activating phosphofructoskinase-2 (PFK-2). In rats, high concentration of insulin (2500 μU/ml) administered through third ventricle at rate of 5 μU/min does not result in significant plasma glucose reduction. However, direct administering insulin to subarachnoid space for protecting CNS tissue has not been reported.

[0013] Magnesium (Mg^{2+}) is the second highest electrolyte intracellularly (58 mEq/L). ATP (Adenosine 5'-triphosphate) is always present as a magnesium:ATP complex.

Mg^{2+} basically provides stability to ATP. At least more than 260 to 300 enzymes have been found to require Mg^{2+} for activation. Best known among these are the enzymes involved in phosphorylations and dephosphorylations: ATPases, phosphatases, and kinases for glycolytic pathway and krebs cycles. At the level of the cell membrane Mg^{2+} is needed for cytoskeletal integrity, the insertion of protein into membranes, the maintenance of bilayer fluidity, binding of intracellular messengers to the membrane, regulation of intracellular Ca^{2+} release by inositol triphosphate etc. Mg^{2+} also affects the activities of pumps and channels regulating ion traffic across the cell membrane. The potential changes in tissue Mg^{2+} might also affect the tissue ATP levels. In tissue culture and animal models elevated Mg^{2+} concentration has been repeatedly proven to protect cells. The concentration of ATP inside cells is high, whereas the concentration outside cells is very low. Harkness and coworkers showed that the ATP concentrations is about 1 to 20 μmol/l in plasma, however in CSF, ATP could not be detected, and it was estimated to be about less than 0.05 μmol/l. Munoz and coworkers detected that the ATP concentration in CSF is about 16 nM/l. Exogenous ATP provides direct energy to the damaged tissue. Sakama and coworkers showed that continuous application of ATP (100 μM) significantly increased axonal transport of membrane-bound organelles in anterograde and retrograde directions in cultured neurons. Uridine 5'-triphosphate produced an effect similar to ATP. Mg-ATP has been used clinically to protect hepatic and other cells after hypoxia-ischemia.

[0014] Acidosis is a universal response of tissue to ischemia. In the brain, severe acidosis has been linked to worsening of cerebral infarction. Recent evidence however suggests that mild extracellular acidosis protects the brain probably through preventing activation of NMDA receptors and inhibition of Na^+/H^+ exchange. It has been reported mild acidosis provide cell protection down to pH 6.2. The acidosis that accompanies ischemia is an important endogenous protective mechanism. Correction of acidosis seems to trigger the injury. It has also been speculated that mild acidosis might stimulate anaerobic glycolysis that might supplement NADH oxidation and ATP yields.

[0015] U.S. Pat. No. 6,500,809 to Frazer Glenn discloses a hyperoncotic artificial cerebrospinal fluid and method of treating neural tissue edema. A series of patents, U.S. Pat. Nos. 4,981,691, 4,758,431, 4,445,887, 4,445,500, and 4,393,863 to Osterholm disclose an oxygenated fluorocarbon solution for treatment of hypoxic-ischemic neurological tissue.

SUMMARY OF THE INVENTION

[0016] Current cardiopulmonary resuscitation (CPR) practice including ventilation, closed chest compressions or cardiac massage and defibrillation (step ABC, i.e. Airway, Breathing and Circulation) was established in the 1960's. However, CPR procedures lack an important step of protecting brain, therefore the time window for resuscitation is the acutely limited. In clinic, the real problem in resuscitating a patient with circulatory arrest is usually not to restore cardio-pulmonary function but instead to prevent "brain death". The CNS tissue including brain and spinal cord is very vulnerable to hypoxia-ischemia, the reason for this has not been clear, and no effective approaches has been available to protect brain and spinal cord.

[0017] I hypothesize that the existence of the CSF is the major reason why CNS is so vulnerable to hypoxia-ischemia. The CSF is very prone to edema because it lacks macromolecules due to the brain-CSF barrier. Many macromolecules are vital for cell survival. For examples, albumin concentration in CSF is very low resulting in almost zero colloidal osmotic pressure (COP); Insulin, a natural neuroprotectant, its concentration is also very low in CSF. The CSF is readily available to provide endless source of "free flow" water to bath and exert pressure to the CNS tissue. Cardiac arrest results in complete cerebral ischemia, this leads rapid development of cerebral edema. While excessive Na^+ and water molecules inside the cell body is toxic, swelling of the cerebral tissue makes the Virchow-Robin space smaller and may even cause it to collapse, thereby compressing the small blood vessels and resulting in obstruction of the blood flow, such as a "hypoperfusion" or even "no-reflow" phenomenon, which prolongs the original ischemic duration, blocks collateral circulation and induces a feedback loop. These cascade events result in irreversible cell death, tissue necrosis and liquefac-events result in irreversible cell death, tissue necrosis and liquefaction, finally leading to brain death.

[0018] Although the existence of the CSF causes vulnerability of the CNS, it also provides an opportunity for treatment. Completely removing the CSF, or replacing the CSF with modified CSF (increasing the COP, Mg^{2+}, ATP, and insulin concentration) will reduce the cerebral edema herein increasing the cerebral blood flow and protecting the brain and spinal cord tissue. Mild acidosis environment in CSF will enhance glycolytic capacity increasing the tolerant ability of cerebral tissue to ischemic injury.

[0019] This invention provides: 1. A method of resuscitation based on conventional CPR. The method includes injecting CSF production inhibitor and removing the CSF in addition to conventional CPR. This method is especially suitable for out-of-hospital application. 2. A method with a composition for replacing the CSF to resuscitate cardiac arrest patient. The composition contains a mixture of a COP agent, insulin, ATP and increased Mg^{2+} concentration in artificial CSF. The method and the composition according to this invention are useful for resuscitating cardiac arrest and for treating shock for both out-of-hospital and in-hospital application.

[0020] I have found removing the CSF from subarachnoid spaces can effectively prolong the time window for conventional CPR procedure. I have found that replacing the CSF with the invented composition also can effectively prolong the time window for conventional CPR procedure. To use the method to rescue a cardiac arrest patient, first injecting Furosemide or Acetazolamide to stop CSF production, then the CSF will be removed completely from subarachnoid spaces as early as possible, it is preferable that the removal of the CSF start even before conventional CPR. The CSF removal eliminates the major source of water causing cerebral edema, and prevents the onset of the "no-reflow" phenomenon or "hypoperfusion", making CNS tissue resistant to ischemia, and lengthening the therapeutic window for resuscitation. This method is especially suitable for out-of-hospital application before the patient can be transferred to a medical center. The CSF drainage was first used to prevent spinal cord damage during thoracic aorta surgery in 1957 by a group of Japanese surgeons (Miyamoto K, et al, J cardio-

vasc surg. 1960; 16: 188-197). More than 45 years of experiences have proven that CSF drainage is the most effective way to prevent paraplegia in majority of the cases. However, some medical centers also reported failure to prevent paraplegia by CSF drainage (Crawford E S et al, J Vasc Surg. 1991; 13(1): 36-46). I have proven that the CSF is so toxic that a small amount of CSF left in subarachnoid space can damage the local tissue and significantly influence the outcome (I believe the failures reported by Crawford ES et al are simply because of controlling the ICP below the central venous pressure, such pressure control leave large amount of CSF around ischemia damaged spinal cord region). Therefore we must understand that although the CSF removal alone is effective to prolong the time widow for resuscitation, it might not be able to reach the maximum protection. The contour of the CNS is very complex with many sulci, and gyri, therefore complete removal of CSF might be a little bit challenging. Therefore, simple CSF removal might inevitably leave part of the tissue unprotected, this can lead to localized tissue damages despite of successful general resuscitation. Therefore I have invented a composition to replace the CSF. The composition is particularly useful for replacing the CSF that can not be removed from subarachnoid space. To use the composition, after CSF removal, the composition will be injected into subarachnoid space to replace the CSF. The composition can be administered through one infusing catheter and one draining catheter positioned in subarachnoid spaces. The high concentration albumin or gelatin increases COP of the composition limiting "free flow" water. The presence of insulin, ATP, the elevated Mg^{2+} concentration and the mild acidosis in the composition increase glycolytic capacity, yield more energy to ischemia challenged brain. Elimination of cerebral edema prevents the onset of the "no-reflow" phenomenon or "hypoperfusion", making CNS tissue resistant to ischemia, and lengthening the therapeutic window for resuscitation.

[0021] The advantages of the current invention include:

[0022] 1. Removing the CSF has been used to prevent paraplegia during aortic cross-clamping. Furosemide or Acetazolamide has been used in clinic for many years. Therefore, injecting Furosemide or Acetazolamide followed by removing the CSF in addition to conventional CPR can be easily adopted in clinic as a new resuscitation protocol to rescue cardiac arrest. This simple approach can effectively prolong the time window for resuscitation.

[0023] 2. The compositions includes a combination of high concentration COP agent, glucose, insulin, ATP and increased Mg^{2+} concentration in artificial CSF. All components in the composition have been used clinically and proven to be safe.

[0024] 3. In the composition, as a COP agent, albumin is effective. However albumin is also expensive. This invention provides many effective alternative COP agents that are less expensive, such as gelatin.

DETAILED DESCRIPTION OF AN ILLUSTRATIVE EMBODIMENT

[0025] As discussed in the above, the key obstacle for current resuscitation is the acutely limited time window. The major reason for the limited time window is that the brain is extremely vulnerable to hypoxic-ischemic insult. Lacking of an effective approach to protect brain is the ultimate reason why the time window for resuscitation is so limited.

[0026] I have hypothesized that the existence of the CSF is the major reason why brain and spinal cord is so vulnerable to hypoxic-ischemic insult. The CSF drainage has been used to prevent spinal cord damage during thoracic aorta surgery for more than 45 years, and has been proven that it is the most effective way to prevent paraplegia in majority of the cases. The CSF only consists of micromolecules such as water, ions, glucose etc. Blood-CSF barrier prevents most macromolecules from effectively passing into the CSF from the blood. For examples, albumin, the major plasma protein for maintaining COP, is extremely low in the CSF. With almost zero COP, CSF is extremely prone to edema. Insulin concentration is also very low in CSF. Mounting evidences have shown that insulin act as a natural neuroprotectant and growth factor in addition to regulating glucose level. These macromolecules are vital for cell survival. In addition, ATP concentration is also very low in CSF. The CSF occupying the subarachnoid space has free access to neurons and surrounding glia cells through the Vichow-robin space. It is readily available to bath and exert pressure to the CNS tissue. When cardiac arrest, the CSF infiltrates the CNS tissue and causes rapid development of edema, particularly the surrounding tissue of Virchow- Robin space. The swelling tissue reduces the size of the space or causes its complete collapse resulting in constriction of the small blood vessels in Virchow-Robin space. This ultimately leads to long lasting cerebral ischemia even after restoring blood supply, i.e. "no-reflow" phenomenon or "hypoperfusion". This "secondary" blood perfusion deficit blocks collateral circulation and induces a feedback loop eventually resulting in irreversible cell death, tissue necrosis and finally leading to brain death. As the hydrostatic pressure of the CSF, the ICP can deduct the force of colloidal osmotic pressure promoting edema.

[0027] Without the CSF, brain and spinal cord might be as resistant as other organs. Therefore, removing the CSF or replacing the CSF with modified CSF should be able to restore the brain and spinal cord tissue's resistant nature to ischemic events and even may prolong the theraputic window from minutes to hours for cardiac arrest patient. For rescuing cardiac arrest patient, conventional CPR only takes measures trying to restore heart and lung function, but actually not be able to do anything to protect brain. CPR means means cardiopulmonary resuscitation. Since the recognition of CSF toxicity to ischemic brain, I have designed a procedure for resuscitation of cardiac arrest, i.e. N-CPR, meaning neurocardiopulmonary resuscitation.

[0028] The N-CPR procedure comprises of three steps. Step 1. injecting Acetazolamide or Furosemide to reduce CSF production, followed by step 2, completely removing the CSF as soon as possible, and followed by step 3, conventional CPR. Cardiac arrest results in very low or no ICP. Therefore it is a good opportunity to remove the CSF, otherwise once heart beat is resumed, it will make the CSF removal much more difficult. This method is particularly suitable for out-of-hospital application. When a cardiac arrest patient come, injecting Furosemide 40-2000 mg (or Acetazolamide 250-1000 mg) intramuscularly or intravenously, followed by immediately CSF removal, then followed by conventional CPR. The CSF can be removed through a catheter that is placed in the lumbar theca through

lumber puncture. To insure the complete removal of the CSF from whole CNS, when removing the CSF, patient should be kept in a sit position to dump all CSF to the lumber puncture point. Optionally, in addition to lumber puncture, catheters may be placed in cistema magna and lateral cerebral ventricles through puncture, CSF removal can also be performed through these puncture points. Meanwhile a small hole can be drilled on the skull above each cerebral hemisphere, and the dura is punctured, the small holes not only allow the CSF removal, but also overcome the influence of negative ICP, this can make the CSF removal much easier and quicker at lumber puncture point.

[0029] The CSF is so toxic that a small amount of CSF left in subarachnoid space can damage the local tissue and significantly influence the outcome. Although the CSF removal alone is effective to prolong the time widow for resuscitation, it might not be able to reach the maximum protection. The contour of the CNS is very complex with many sulci, and gyri, therefore complete removal of CSF might be a little bit challenging. Therefore, simple CSF removal might inevitably leave part of the tissue unprotected, this can lead to localized tissue damages despite of successful general resuscitation. Therefore I have invented a composition to replace the CSF, particularly the CSF left in subarachnoid space after the CSF removal. The N-CPR procedure will be even more effective if infusing invented composition after CSF removal or simply flushing the invented composition (the purpose of flushing is to replace the CSF which can not be removed with invented composition, and to keep the ICP at very low level to promote blood perfusion). This is suitable for both out-of-hospital and in-hospital application.

[0030] The composition comprises of a mixture of COP agent, insulin, ATP and elevated Mg^{2+} concentration in artificial CSF. There are many COP agents can be chosen, such as, proteins (the protein can be selected from any source, such as animal, vegetable, or microbial, without limitation, the protein can also be modified to increase the ability to absorb water), collagen, fibrin, gelatin. GELOFUSINE® (containing 4-14% gelatin) from Millpledge Ltd can also be chosen. HAEMACCEL® 3.5% colloidal intravenous infusion solution containing gelatin polypeptides being used in clinic in South Africa can also be chosen. Heat shock protein is an inducible protein that have been reportedly neuroprotective can also be chosen. However albumin and gelatin are preferred. The concentration of COP agent should be sufficient to create COP between 1 to 200 mm Hg. It is preferred that COP agent can create a pressure of 20-40 mm Hg. 8% albumin (8 gram in 100 ml solution) creating about 33 mm Hg COP is most preferred. 1-4% gelatin creating about 20-40 mm Hg COP is most preferred too. The insulin concentration should be in a range from 0.01 to 1000 μU/ml. Glucose may or may not be added (0-60 mg/dl). The preferred insulin concentration is between 1 to 60 μU/ml. All growth factors have insulin-like effect and can be chosen to replace insulin. Growth hormones and growth hormone releasing factor have insulin-like effect, can also be chosen to replace insulin. For examples, insulin-like growth factors, nerve growth factor, brain derived neurotrophic factor, neurotrophin, fi-broblast growth factor and glial cell line derived neurotrophic factor, erythroproietin, growth hormone, growth hormone releasing factor etc may be used to replace insulin or may be used in combination with insulin. The ATP concentration should be in a range from 16 nM to 5 mM. The preferred ATP concentration is between 0.001 to 5 mM. The most preferred ATP concentration is between 0.001 to 0.05 mM. Other high energy compound such as Uridine 5'-triphosphate can be used to replace ATP. The artificial CSF used for research usually contains minimums Mg^{2+} of 0.9 meq/L . The elevated Mg^{2+} concentration should be in a range from 0.91 to 8 meq/L. The most preferred Mg^{2+} concentration is between 2.51 to 5.0 meq/L. The pH value of the composition should be in a range between 6.2 to 7.35. The pH value between 6.8-7.0 is preferred. The pH value may be adjusted by phosphate buffer, hydrochloric acid or by bicarbonate. Table 1 shows the components and concentration range of the composition.

TABLE 1

Component	Concentration range
Artificial CSF	
Na	120–155 meq/L
K	0.1–5.0 meq/L
Ca	0.1–3.0 meq/L
P	0.1–10 meq/L
Cl	120–155 meq/L
Mg	0.9–8 meq/L
Albumin	0.1–20 gram/dl
Insulin	1 to 60 μU/ml
ATP	0.001 to 0.05 mM

(Use sterile water for dilution, pH is adjusted to between 6.8–7.3, osmolality ad-justed to between 280–310 mOsm/L, COP between 20–40 mm Hg)

[0031] Optionally the albumin in Table 1 may be replaced by gelatin 0.1-10 gram/dl.

[0032] The mixture of albumin (or gelatin), insulin, ATP, and elevated concentration of Mg^{2+} and mild acidosis have synergic neuroprotective effects. However each individual component dissolving in artificial CSF is also effective and can be used alone.

[0033] To make the composition, albumin (or gelatin), insulin, ATP and elevated concentration of Mg^{2+} in artificial CSF may be manufactured in a ready to use condition. Optionally, artificial CSF with elevated Mg^{2+} concentration may be manufactured in one container, the mixture of albumin (or gelatin), insulin and ATP may be assembled in another container. Since albumin (or gelatin), insulin and ATP are delicate substances, it would be convenient and advantageous to keep their mixture in a cool place and dissolving them in artificial CSF just before use. For example, mixture of albumin (or gelatin), insulin and ATP may be assembled in different quantities in small mapules that is ready for being dissolved in 50 ml, 100 ml and 500 ml of the artificial CSF.

[0034] The composition can also be added any of other nutrients such as, Vitamins (such as, D-Calcium Pantothenate, Choline, Folic acid, i-Inositol, Niacinamide, Pyridoxal, Riboflavin, Thiamine, Vitamin B_{12} etc.), Amino acids (such as, L-Alanine, L-Arginine, L-Asparagine, L-Cysteine, L-glutamine, L-glutamate, Glycine, L-Histidine, L-Isoleucine, L-leucine, L-lysine, L-methionine, L-Phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine etc.), phospholipids, Cholesterol, fat, fatty acid, D,-L-alpha-tocopherol, antioxidant etc. The composition can also be added any of oxygen carriers such as bis-perfluorobutyl ethylene and oxygenated before use. The

composition can also be added any of intermediates of glycolysis (such as fructose-1,6-biphophate, glyceraldehyde-3-phosphate, 1,3 bisphosphoglycerate, 3-phosphoglycerate, 2-phosphoglycerateare, phosphoenolpyruvate, pyruvate, lactate etc.), enzymes for glycolysis (such as hexokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triosephosphate isomerase, glyceraldehydes 3-phosphate dehydrogenase, phosphoglygerate kinase, pyruvate kinase etc.), ketone bodies (such as acetoacetate, β-hydroxybatyrate) and intermediates of krebs cycle.

[0035] The composition herein may also be advantageously combined with any of the agents used to treat stroke or other neurological deficiencies based on other mechanisms including: calcium channel blockers such as Nimodipine, and Flunarizine; calcium chelators, such as DP-b99; potassium channel blockers; Free radical scavengers—Antioxidants such as Ebselen, porphyrin catalytic antioxidant manganese (III) meso-tetrakis (N-ethylpyridinium-2-yl) porphyrin, (MnTE-2-PyP (5+)), disodium 4-[(tert-butylimino) methyl]benzene-1,3-disulfonate N-oxide (NXY-059), N:-t-butyl-phenylnitrone or Tirilazad; GABA agonists including Clomethiazole; GABA receptor antagonists, glutamate antagonists, including AMPA antagonists such as GYKI 52466, NBQX, YM90K, YN872, ZK-200775 MPQX, Kainate antagonist SYM 2081, NMDA antagonists, such as CGS 19755 (Selfotel); NMDA channel blockers including Aptiganel (Cerestat), CP-101,606, Dextrorphan, destromethorphan, magnesium, metamine, MK-801, NPS 1506, and Remacemide; Glycine site antagonists including ACEA 1021, and GV 150026; polyamine site antagonists such as Eliprodil, and Ifenprodil; and adenosine receptor antagonists; Growth factors such as Fibroblast Growth Factor, brain derived neurotrophic factor, insulin like growth factor, neurotrophin. Nitric oxide inhibitors including Lubeluzole, opiod antagonists, such as Naloxone, Nalmefenem, Phosphatidylcholine precursor, Citicoline (CDP-coline); Serotonin agonists including Bay×3072; Sodium channel blockers such as Fosphenytoin, Lubeluzole, and 619C89; Potassium channel openers such as BMS-204352; anti-inflamatory agents; protein kinase inhibitors and other active agents that provide energy to cells, such as co-enzyme A, co-enzyme Q, or cytochrome C. Similarly, agents known to reduce cellular demand for energy, such as phenytoin, barbital, or lithium may also be added. These agents may be added into this invented composition or may be administered orally or intravenously in combination with this invented composition and method.

[0036] To use the composition, removing the CSF as described above, then injecting the invented composition into subarachnoid space to completely replace the whole CSF through the one or all puncture points. The injected composition should be approximately equal or less to the amount of CSF removed to maintain low or zero ICP. Optionally, the composition can be infused continuously from an infusing catheter to a draining catheter in subarachnoid spaces. Lumber puncture point is preferred as the infusing point, and holes on the skull above each cerebral hemisphere as draining points. The infusing rate of the composition can be from 0.001-100 ml/min. Optionally, the composition may be cooled between 4 to 37° C. before use. Alternatively, patient's own CSF may be used to replace artificial CSF in our composition. Usually 50-200 ml of the patient's own CSF can be obtained as a solvent to dissolve the mixture of albumin (or gelatin), insulin and ATP. Elliot B solution is an artificial CSF that has been approved as a solvent since 1996 in USA. Elliot B solution may also be used to replace artificial CSF in our composition. Alternatively, the composition may be served as a flushing fluid and only be kept in subarachnoid space for a few minutes and then may be removed out to keep the ICP at zero level, this will maximize the efficacy of simple CSF removal.

EXAMPLE ONE

Making of the Composition

[0037] Artificial CSF was made according to table 2.

TABLE 2

Component	Amount
NaCl	8.182 gram
KCl	0.224 gram
$CaCl2.2H_2O$	0.206 gram
Na_2HPO_4	0.113 gram
NaH_2PO_4	0.023 gram
$MgSO_4$	0.361 gram
Sterile water for dilution to 1000 ml	
Mixture of Albumin, Insulin and ATP was made according to table 3.	
Table 3.	
Albumin	80 grams
Insulin	3,000 μU
ATP	5.5 milligrams
Mix these substances in one container	

[0038] To make the composition, dissolve the mixture of Albumin, Insulin and ATP in artificial CSF. Final pH of the composition was adjusted to between 6.8 to 7.0.

EXAMPLE TWO

Making of the Composition

[0039] Artificial CSF was made according to table 2 in example one.

[0040] Mixture of Gelatin, Insulin and ATP was made according to table 4.

TABLE 4

Gelatin	10 grams
Insulin	3,000 μU
ATP	5.5 milligrams
Mix these substances in one container	

[0041] To make the composition, dissolve the mixture of Gelatin, Insulin and ATP in artificial CSF. Final pH of the composition was adjusted between to 6.8 to 7.0.

EXAMPLE THREE

N-CPR Procedure for Cardiac Arrest

[0042] 24 rats weighing between 250-300 grams were divided into four groups. 5% Isoflorane was given for anesthetic induction. All animals underwent placement of a saline filled right femoral artery and right femoral vein catheter for monitoring mean blood pressure (MBP) and for drug administration. Following tracheostomy and endotracheal intubation, all animals were mechanically ventilated with 1% isoflorane, 70% nitrous oxide in oxygen at a rate of 50 breaths/minute with tidal volume of 12 ml/kg. A silicone

catheter (0.025 OD, 0.012 ID inch) was surgically implanted in the cisterna magna as a draining route. A hole of 3 mm in diameter was drilled on the skull above each cerebral hemisphere (3 mm lateral to midline and 3 mm in front of the bregma), dura was punctured, an infusing silicone catheter (0.025 OD, 0.012 ID inch) was placed and fixed with glue in the hole into the subarachnoid spaces on the surface of each cerebral hemisphere.

[0043] The cardiac arrest was induced by electrical stimulation (alternating current: 12 V, 50 Hz) via the esophageal electrode and an external electrode covered with electrode gel and placed on the animals chest. Ventilation was stopped. Complete circulatory arrest was indicated by an abrupt decrease in MAP below 15 mm Hg. The cardiac arrest was lasted for 10 minutes.

[0044] For group one (n=6): Conventional CPR. At 10 minutes after cardiac arrest, 0.2 mg/kg epinephrine and 0.5 mmol/kg bicarbonate ($NaHCO_3$) were administered through femoral vein catheter. Simultaneously, animal were ventilated with 100% oxygen at pre-arrest tidal volume and respiration rate. Cardiac massage started at 10 seconds after ventilation to allow the lungs to stretch. Cardiac massage was performed by manual closed chest compression at rate of 200/minute, with two fingers compressing the chest to maximize MAP.

[0045] For group two (n=6): Removing the CSF plus conventional CPR. At 10 minutes after cardiac arrest, first administering 20 mg Furosemide through femoral vein catheter, then the CSF was removed as completely as possible from catheter in cisterna magna and from catheters above each cerebral hemisphere. The CSF removal took about 1 minute. Immediately after the CSF removal, conventional CPR was performed as described in group one.

[0046] For group three (n=6): Removing the CSF and replacing it with invented composition (made according to example one) plus conventional CPR. At 10 minutes after cardiac arrest, first administering 20 mg Furosemide through femoral vein catheter, then the CSF was removed as completely as possible from catheter in cisterna magna and from catheters above each cerebral hemisphere. The CSF removal took about 1 minute. Immediately after the CSF removal, 1 ml of composition (made according to example one) was quickly flushed in from catheters above each cerebral hemisphere and flushed out from catheter in cisterna magna. The flushing took about 1 minute. Immediately after flushing, conventional CPR was performed as described in group one, simultaneously, 3 ml of the composition (made according to example one) was continuously infused from catheters above each cerebral hemisphere and was drained out from the catheter in cisterna magna. The infusion lasted for 3 hours at a rate of 1 ml/hour.

[0047] For group four (n=6): Removing the CSF and replacing it with invented composition (made according to example two) plus conventional CPR. At 10 minutes after cardiac arrest, first administering 20 mg Furosemide through femoral vein catheter, then the CSF was removed as completely as possible from catheter in cisterna magna and from catheters above each cerebral hemisphere. The CSF removal took about 1 minute. Immediately after the CSF removal, 2 ml of composition (made according to example two) was quickly flushed in from catheters above each cerebral hemisphere and flushed out from catheter in cisterna magna. Then the composition was removed from subarachnoid space the as completely as possible. The flushing and removing of composition took about 2 minute. Immediately after flushing and removing, conventional CPR was performed as described in group one.

[0048] At 24 hours, all rats were tested for behavioral deficit by the following criteria: Maximum Score=400 (meaning brain death or death); Minimum Score=0 (meaning normal brain function).

1. Level of Consciousness

[0049] 0=complete awareness of auditory stimuli.

[0050] 30=clouded: apparently conscious but drowsy or intermittently irritable on clapping hands and pinching nailbeds of hindlegs.

[0051] 60=stupor: response with movements to pinching nailbed of hindlimb, open eyes, movements may be either purposeful or reflex.

[0052] 100=coma: no movement on painful stimulation (pinching nailbed of hindlimb; should be confirmed on forelimbs as well).

2. Respiratory Pattern

[0053] 0=normal rate and rhythm.

[0054] 50=abnormal spontaneous breathing (e.g., periodic gasps, irregular rhythm)

[0055] 75=breathing, but not enough to maintain normal arterial blood gases.

[0056] 100=apnea: complete absence of spontaneous respiratory efforts

3. Cranial Nerve Function

[0057] Pupil size: examine in room lighting and record diameters of pupil and iris (R/L)

[0058] 0=normal: 1-2 mm diameter

[0059] 10=abnormal: greater than 3 mm

[0060] 20=severely abnormal: greater than 5 mm, pinpoint, or new anisocoria Papillary response to light: use flashlight (R/L)

[0061] 0=normal

[0062] 10=sluggish

[0063] 20=absent

Eyelid Reflex:

[0064] 0=normal

[0065] 10=sluggish

[0066] 20=absent

Corneal reflex: Test with moist cotton swab, observe for eyelid closure (R/L)

[0067] 0=normal

[0068] 10=sluggish

[0069] 20=absent

Swallow Reflex:

[0070] 0=normal

[0071] 10=sluggish

[0072] 20=absent

4. Motor and Sensory Function

[0073] Motor response to painful stimulus: Pinch each limb, observe for withdrawal response.

[0074] 0=normal

[0075] 25=no response

[0076] 50=coma (no test required)

[0077] Positioning: place rat in left lateral decubitus position and observe position assumed.

[0078] 0=normal

[0079] 25=mildly abnormal or perceptible movement, or intermittent running movements

[0080] 50=markedly abnormal, no any movement.

The Results are as Follow:

[0081] In group one, Four rats were not able to survive for 24 hours. Scores in other two rats are 400 and 360 respectively.

[0082] In group two, the scores are 100, 120, 100, 140, 80 and 150 respectively.

[0083] In group three, the scores are 100, 90, 80, 100, 60 and 60 respectively.

[0084] In group four, the scores are 80, 70, 60, 100, 40 and 60 respectively. It is concluded that N-CPR procedure is effective in resuscitating cardiac arrest.

[0085] While my above description contains many specifics, these should not be construed as limitations on the scope of the invention, but rather as illustrative examples.

1. A method for resuscitating cardiac arrest in a mammal, comprising at least two steps of:

a. Administering a cerebrospinal fluid (CSF) production-suppressing agent in an amount effective to reduce or stop CSF production; wherein said CSF production-suppressing agent is Furosemide or Acetazolamide.

b. Removing the CSF from the subarachnoid space in central nervous system.

c. Conventional cardiopulmonary resuscitation (CPR) procedure including open airway, breathing, and circulation.

2. A method for resuscitating cardiac arrest in a mammal, comprising at least two steps of:

a. Administering a cerebrospinal fluid (CSF) production-suppressing agent in an amount effective to reduce or stop CSF production; wherein said CSF production-suppressing agent is Furosemide or Acetazolamide.

b. Removing the CSF from the subarachnoid space in central nervous system and followed by injecting a volume of composition approximately equal or less to the volume of CSF withdrawn into said subarachnoid spaces; wherein said composition comprising of a mixture of at least one component selected from the group consisting of Albumin 0.1-20 gram/dl, gelatin 0.1-10 gram/dl, insulin 1 to 60 μU/ml and ATP 0.001 to 0.05 mM in an artificial CSF, wherein said artificial CSF comprising of Na 120-155 meq/L, K 0.1-5.0 meq/L, Ca 0.1-3.0 meq/L, P 0.1-10 meq/L, Cl 120-155 meq/L, Mg 0.9-8 meq/L, and water; wherein said composition has a Ph value between 6.8-7.0.

d. Conventional cardiopulmonary resuscitation (CPR) procedure including open airway, breathing, and circulation.

3. A method for resuscitating cardiac arrest in a mammal, comprising at least two steps of:

a. Administering a cerebrospinal fluid (CSF) production-suppressing agent in an amount effective to reduce or stop CSF production; wherein said CSF production-suppressing agent is Furosemide or Acetazolamide.

b. Removing the CSF from the subarachnoid space in central nervous system and followed by injecting a volume of composition into one point of said subarachnoid space and draining out from other point of said subarachnoid spac; wherein said composition comprising of a mixture of at least one component selected from the group consisting of Albumin 0.1-20 gram/dl, gelatin 0.1-10 gram/dl, insulin 1 to 60 μU/ml and ATP 0.001 to 0.05 mM in an artificial CSF, wherein said artificial CSF comprising of Na 120-155 meq/L, K 0.1-5.0 meq/L, Ca 0.1-3.0 meq/L, P 0.1-10 meq/L, Cl 120-155 meq/L, Mg 0.9-8 meq/L, and water; wherein said composition has a Ph value between 6.8-7.0.

d. Conventional cardiopulmonary resuscitation (CPR) procedure including open airway, breathing, and circulation.

4. A method for resuscitating cardiac arrest in a mammal according to step b in claim 1, wherein removing the CSF from the subarachnoid space in central nervous system is performed from at least one point selected from the lumber, cisterna magna, lateral ventricle and skull above each cerebral hemisphere.

5. A method for resuscitating cardiac arrest in a mammal according claim 4, the patient is in a sit position when removing the CSF.

6. A method for resuscitating cardiac arrest in a mammal according to step b in claim 2, wherein removing the CSF from the subarachnoid space and injecting said composition are performed from at least one point selected from the lumber, cisterna magna, lateral ventricle and skull above each cerebral hemisphere.

7. A method for resuscitating cardiac arrest in a mammal according claim 6, the patient is in a sit position when removing the CSF.

8. A method for resuscitating cardiac arrest in a mammal according to step b in claim 3, wherein removing the CSF, injecting and draining said composition is performed from at least one point selected from the lumber, cistema magna, lateral ventricle and skull above each cerebral hemisphere.

9. A method for resuscitating cardiac arrest in a mammal according claim 8, the patient is in a sit position when removing the CSF.

10. A method for resuscitating cardiac arrest in a mammal according claim 6, the ICP is maintained at 0-70 mm H_2O.

11. A method for resuscitating cardiac arrest in a mammal according claim 8, the ICP is maintained at 0-70 mm H_2O.

* * * * *

专利名称(译)	用于复苏的方法和组合物		
公开(公告)号	US20060128797A1	公开(公告)日	2006-06-15
申请号	US11/013167	申请日	2004-12-15
[标]申请(专利权)人(译)	王言明		
申请(专利权)人(译)	王燕鸣		
当前申请(专利权)人(译)	王燕鸣		
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发明人	WANG, YANMING		
IPC分类号	A61K31/34 A61B5/00		
CPC分类号	A61K31/34 A61K31/341 A61K31/433 A61K31/7076 A61K38/17 A61K38/28 A61K38/38 A61K38/39 A61K45/06		
外部链接	Espacenet USPTO		

摘要(译)

当前复苏的主要障碍是时间窗口非常有限。时间窗有限的主要原因是大脑极易受到缺氧缺血性损伤。脑脊液（CSF）的存在是脑和脊髓如此脆弱的主要原因。提供了用于心脏骤停复苏的方法和组合物。该方法包括抑制CSF产生，去除CSF或用本发明的组合物加常规CPR替换CSF的步骤。该组合物包括胶体渗透剂，胰岛素，升高的镁浓度和人工CSF中的ATP。

TABLE 2

Component	Amount
NaCl	8.182 gram
KCl	0.224 gram
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Na_2HPO_4	0.113 gram
NaH_2PO_4	0.023 gram
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Table 3.	
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