Cerebral Resuscitation: State of the Art, Experimental Approaches and Clinical Perspectives

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Every day, up to 1000 persons in the United States and another 1000 in Europe go into cardiocirculatory arrest with subsequent cardiopulmonary resuscitation (CPR), and only about 2% to 12% of them survive. Postarrest brain damage is a key issue. In the western industrialized nations, CPR is attempted in 40 to 90 of 100,000 inhabitants annually, and restoration of spontaneous circulation (ROSC) can be achieved in about 25% to 50% of these patients. The hospital discharge rate, however, is only 2% to 12% (Fig.1) [1]. Therefore, out of up to 300,000 cardiac arrest victims annually in the United States and in Europe, more than 270,000 are not treated successfully, if one uses complete neurologic restoration as the standard.

The major reason for postarrest in-hospital mortality and morbidity is persistent brain damage. Brain damage following cardiocirculatory arrest is related to the short period of tolerance to hypoxic stress and specific reperfusion disorders [2,3]. The individual, social, and economic consequences of brain damage following cardiac arrest are immense [4–6]. One of the most important issues in cardiac arrest and resuscitation (“whole body ischemia and reperfusion”) research, therefore, is cerebral resuscitation and the inhibition of postarrest cerebral damage [3,7]. Current research focuses on pathophysiology and problems during reperfusion [7,8].

The mechanisms of brain damage following global cerebral ischemia and cardiac arrest are complex [2,3,7]. Major issues are hypoxia and subsequent necrosis, reperfusion injury with free radical formation and cellular calcium influx, release of excitatory amino acids, neuronal apoptosis, and cerebral

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microcirculatory reperfusion disorders [2,3,7,9]. Several clinical trials attempted to improve neurologic outcome after cardiac arrest by focusing on brain protection with the therapeutic use of barbiturates and by focusing on reperfusion injury with the use of calcium channel blockers. No positive effects on neurological outcome could be established, however [10,11]. To date, no specific pharmacologic postarrest treatment options are available to improve neurologic outcome following cardiocirculatory arrest in the clinical setting, with cardiocirculatory arrest being the most relevant clinical feature of global cerebral ischemia.

The most important therapeutic options to improve neurologic outcome following cardiac arrest currently under study are focusing on (1) selective neuronal vulnerability and delayed neuronal death [8,12,13], (2) on cerebral microcirculatory reperfusion [14–17] and (3) on therapeutic hypothermia, the topic with the most clinical evidence [18–20].

From experimental concepts to clinical strategies

Selective neuronal vulnerability and delayed neuronal death

Selective neuronal vulnerability and delayed neuronal death contribute to neuronal damage following global cerebral ischemia resulting from cardiac arrest in the clinical and the experimental settings [8]. Short periods of global cerebral ischemia resulting from cardiocirculatory arrest induce neuronal cell damage primarily in so-called “selectively vulnerable” brain areas, such as the hippocampal CA1 sector, the thalamic reticular nucleus, and different layers of the neocortex [8,20]. In a recently developed model of cardiac arrest in rats, strong evidence for neuronal apoptosis in selectively vulnerable areas of the brain was found. Using the TUNEL-technique
(terminal deoxynucleotidyltransferase [TdT]-mediated d-uracil triphosphate [UTP]-biotin nick end-labeling), a characteristic apoptotic morphology with DNA-laddering and apoptotic bodies was detected in the hippocampal CA1 sector and the thalamic reticular nucleus [8,9,13]. Positive TUNEL staining is an important indicator of apoptotic degeneration. Quantitative analysis of TUNEL-positive neurons per tissue section revealed marked differences with regard to the time course between the hippocampal CA1 sector and the thalamic reticular nucleus. TUNEL staining demonstrated early onset degeneration in the thalamic reticular nucleus at 6 hours; it peaked at 3 days. In contrast, degeneration was delayed in the hippocampal CA1 sector, showing an onset at 3 days and a maximum of TUNEL-positive cells at 7 days [8,9,13]. These data suggest that apoptosis contributes to neuronal cell death after cardiac arrest. Moreover, delayed neuronal degeneration reflects a time window in which potential therapeutic interventions can be established after cardiac arrest [7].

Cascades of death

The current concepts of apoptosis suggest that there are several steps between the initial ischemic/hypoxic insult and the final DNA fragmentation leading to cell death (Fig. 2) [21–23]. Within this cell death cascade—consisting of various signals, modulatory proteins, and degradation enzymes—several molecules and proteins facilitating neuronal survival compete with factors contributing to cell death. Ultimately, the balance between survival factors and death factors determines the fate of the cell. Proteins such as

![Diagram of the pathophysiologic concepts of neuronal apoptosis](image)

Fig. 2. A simplified scheme of the pathophysiologic concepts of neuronal apoptosis suggests that there are several steps between the initial ischemic/hypoxic insult and the final DNA fragmentation leading to cell death [9,21,23,24]. Within this cell death cascade, consisting of signals, modulators, and degradation enzymes, there are several molecules and proteins that facilitate neuronal survival and compete with factors that contribute to the cell-death cascade. Ultimately, the balance between survival factors and death factors determines the fate of the cell. Proteins such as Bcl-2 and Bcl-XL promote survival, while Bax, Bid, and Bad promote death. The final step of this cascade is initiated with the activation of caspase 3.
Bcl-2 and Bcl-X\textsubscript{L} promote survival, whereas Bax and Bad promote death. The final step in this cascade is initiated with the activation of caspase 3. Activation of caspase 3 leads to a cleavage of poly(ADP-ribose)polymerase (PARP), which is an important DNA-repair enzyme [21,23,24]. Combined with the cleavage of other important substrates, activation of caspase 3 is believed to be the final trigger of DNA fragmentation and apoptotic cell death, which can be viewed as a form of endogenous “cell suicide.” This final caspase activation step can be blocked by synthetic caspase inhibitors and different viral antiapoptotic proteins, such as the baculovirus protein p35 and the cowpox virus protein crmA [23]. An important step in confirming that delayed neuronal death after cardiac arrest is based on apoptosis was the demonstration of upregulated caspase 3-like protease activity in different brain regions 24 hours after global cerebral ischemia induced by cardiac arrest [25]. In particular, a significant increase in caspase 3 mRNA and caspase 3-like proteolytic activity in the hippocampus was observed. Preincubation of hippocampal extracts with a specific caspase 3 inhibitor completely blocked protease activity. Thus, one of the final steps in an apoptotic cascade, activation of caspase 3, occurs in the brain after cardiac arrest at the transcriptional and the posttranscriptional level [25]. This finding gives further support to the hypothesis that apoptotic degeneration contributes to neuronal death after cardiocirculatory arrest and suggests that antiapoptotic treatment may play an important role in promoting neuronal survival after cardiac arrest in the future.

**Inhibiting cerebral apoptosis**

In an attempt to inhibit neuronal apoptosis, a transgenic rat line was created that expressed the antiapoptotic baculovirus protein p35 in neurons postnatally [26]. Those animals and their nontransgenic littermates underwent a period of 6 minutes of cardiac arrest induced by ventricular fibrillation of the heart. There was a marked difference with regard to the rate of ROSC; and the rate of 7-day survival was significantly higher in p35 transgenic animals [26]. Therefore, antiapoptotic strategies may be indicated to improve outcome following global cerebral ischemia and cardiocirculatory arrest [7,26]. However, additional studies using the intracerebroventricular application of artificial caspase inhibitors, such as the specific synthetic caspase inhibitor z-DEVD-FMK, did not show a significant positive effect on caspase-3 activation, neuronal degeneration, and neurologic outcome following 6 minutes of cardiocirculatory arrest in rats [27].

Recent data from focal cerebral ischemia demonstrate the possibility of inhibiting the apoptotic cascade further upstream with the use of neurotrophins and growth factors. Brain-derived neurotrophic factor (BDNF) is one of the best characterized neurotrophic factors of the nerve growth factor family. BDNF acts on a set of high-affinity receptor kinases (mainly tyrosine kinase B [TrkB]) to promote survival, differentiation, and neurite
extension in many types of neurons in the mammalian central nervous system [28–32]. In vivo, BDNF rescues motoneurons and substantia nigra dopaminergic cells from traumatic and toxic brain injury [33,34]. Intracerebroventricular BDNF administered after focal cerebral ischemia significantly reduced infarct volume, primarily in the cortex [35,36]. Another mechanism of neuroprotection achieved by growth factors after hypoxic/ischemic events is probably prevention of excitotoxicity [31]. Glutamate-triggered excitotoxicity with subsequent Ca2+ overload of cells is thought to be one of the major causes of cellular death after ischemia [37]. BDNF protects neuronal cells in vitro against glutamate-induced neurotoxicity and the subsequently high intracellular calcium levels [31,32]. By inducing an antioxidant defense system, BDNF suppresses the glutamate-triggered accumulation of peroxide, which contributes to the loss of Ca2+ homeostasis [38]. Also, BDNF induces the activation of the IP3 kinase, phospholipase C, and Ras/MAPKinase (MAPK) pathways via the TrkB receptor, exhibiting further neuroprotective cellular stimulation. Those pathways could also be activated effectively by the insulin-like growths factor 1 (IGF-1) and erythropoietin (EPO) [39]. Those growth factors/neurotrophins have been evaluated in models of cardiac arrest with rats. Neither BDNF, nor IGF-1 nor EPO, however, could demonstrate beneficial effects on cerebral recovery and neuronal survival when administered intracerebroventricularly or intraperitoneally after 6 minutes of cardiac arrest [13,40].

**Cerebral microcirculatory reperfusion**

A major area of experimental resuscitation research has focused on cerebral microcirculatory reperfusion and associated disorders following cardiac arrest, including endothelial cell swelling, increased leukocyte-endothelial interactions, and a disseminated intravascular activation of blood coagulation [7,14–17].

A most relevant cause of cerebral dysfunction after cardiac arrest is reflected by the cerebral “no reflow” phenomenon, which describes the regional microcirculatory reperfusion deficits that occur despite adequate systemic hemodynamics. Some years ago, Fischer and Hossmann [16] treated cats after 15 minutes of cardiac arrest and 4 minutes of CPR with hypertonic-hyperoncotic solutions during a 30-minute reperfusion period. Such solutions decrease endothelial cell swelling resulting from the high intravascular osmotic pressure that is generated by this kind of intervention. In these studies, early cerebral microcirculatory reperfusion disorders (cerebral “no reflow”) were reduced with the administration of these solutions, suggesting that therapeutic interventions that focus on a decrease in endothelial cell swelling have positive effects on cerebral microcirculatory reperfusion after cardiac arrest [16]. As in several other experimental studies, cerebral perfusion pressure immediately at the start of reperfusion is correlated negatively with the extent of cerebral microcirculatory
reperfusion disorders—the higher the early reperfusion pressure, the lower the amount of cerebral “no reflow” [14,16,41,42].

**Leukocytes in trouble**

Another important mechanism of reperfusion injury and reperfusion failure in the microcirculation may be leukocyte adherence and leukocyte sticking and, therefore, blocking of microvessels [43,44]. Following 10 minutes of cardiac arrest and 6 hours of reperfusion, an increase in the number of polymorphonuclear leukocytes in the brain suggests that leukocytes may play a role in early cerebral microcirculatory reperfusion failure after cardiac arrest, as they play a role in reperfusion injury in other organs and different models [8]. This finding has been supported by clinical studies demonstrating that cardiocirculatory arrest and successful CPR are associated with a marked increase in the serum levels of polymorphonuclear neutrophil leukocyte (PMN) elastase, complement split products, terminal complement complex (sC5b-9), and soluble intercellular adhesion molecules [45–47].

**Coagulation without fibrinolysis**

The most important pathophysiologic mechanism responsible for cerebral microcirculatory reperfusion disorders seems to be the activation of blood coagulation without adequate activation of endogenous fibrinolysis [48–52]. Intravascular fibrin formation and microthromboses are distributed throughout the entire microcirculation after cardiocirculatory arrest, and interventions that focus on hemostasis may be indicated during reperfusion. In the 1950s, Crowell and coworkers [53] demonstrated beneficial effects of anticoagulatory interventions for the first time in animals. Following 10 minutes of cardiac arrest, only a few dogs survived without heparin pretreatment, while the survival rate was 16% and 67% when doses of heparin, 2 mg/kg and 5 mg/kg of body weight respectively, were given before cardiac arrest [53]. In a later study, Crowell demonstrated beneficial effects of pretreatment with thrombolytic agents before cardiac arrest. In the control group, 14 of 15 animals died after 15 minutes of cardiac arrest [54]. The surviving animal suffered from severe neurologic damage. In contrast, only 2 of 14 animals died if streptokinase had been administered before cardiac arrest. Almost all neurologic deficits after cardiac arrest in this group disappeared within 2 months after stabilization [54]. Lin and coworkers [55] demonstrated that the administration of streptokinase combined with dextran reduces the duration of a flat line electroencephalogram (EEG) and improves cerebral blood flow after cardiac arrest in dogs. Safar and coworkers [56] observed an improvement in neurologic outcome in dogs receiving heparin, dextran, and hypertensive reperfusion as a combined therapeutic approach following 12 minutes of cardiac arrest.

Based on clinical experience [57,58], the effect of thrombolysis during CPR on the extent of the cerebral “no reflow” phenomenon was
investigated in cats [16]. Following 15 minutes of cardiac arrest, animals were allowed to reperfuse spontaneously for 30 minutes. Treated animals received a bolus injection of recombinant tissue-type plasminogen activator (rt-PA), 1 mg/kg of body weight, combined with heparin, 100 U/kg of body weight, during CPR, followed by another 1 mg/kg dose of rt-PA during reperfusion [16]. The administration of rt-PA and heparin led to a significant reduction (8% versus 29%) in the cerebral “no reflow” phenomenon in the entire forebrain. This positive effect was particularly relevant in basal ganglia and brainstem, and bleeding complications did not occur either [16]. Therefore, there is profound experimental evidence suggesting that hemostatic disorders may affect overall outcome, and particularly cerebral outcome, after cardiac arrest.

Clinical investigations

Based on this promising data, a prospective pilot intervention trial in patients undergoing CPR after out-of-hospital cardiac arrest was performed [59]. Overall, 90 patients were included. Heparin and rt-PA were given in 40 patients. In the rt-PA group, ROSC was achieved in 68%; and 58% of patients were able to be admitted to a cardiac intensive care unit, as compared with 44% and 30% of controls. These differences were significant. At 24 hours after cardiac arrest, 35% of patients who were treated with rt-PA (versus 22% of controls) were still alive, and 15% of rt-PA-treated patients (versus 8% of controls) were discharged from the hospital. There were no bleeding complications related to the CPR procedures. These data were supported by a retrospective case-control study of 108 patients who were treated with rt-PA [60]. A randomized and controlled multicenter clinical trial on thrombolysis during CPR is currently underway. Overall, more than 1000 patients will be enrolled in more than 40 centers. The results of this large-scale, randomized, controlled clinical trial to improve microcirculatory reperfusion following cardiac arrest (Thrombolysis in Cardiac Arrest [TROICA] trial) will be available in 2006 [61–63].

Therapeutic mild hypothermia

The use of therapeutic hypothermia following different hypoxic-ischemic insults has played an important role in various concepts of nonspecific protection of cells for a long time [64]. Within several cell and animal experimental models, hypothermia was shown to inhibit a wide range of intracellular death cascades. A large body of evidence suggests that the protective effects of cooling are much greater than can be explained by the reduction in oxygen and glucose metabolism of about 5% to 7% per °C alone [65–72]. Mild therapeutic hypothermia has shown to foster neuroprotection via inhibition of apoptosis [66], reduction of free radicals [67,68] and excitatory neurotransmitters [69–71], and stabilization of membranes [72].
Several case reports of accidental hypothermia and good neurologic outcome even after long periods of ischemia [73] led to clinical attempts to use deep hypothermia (<28°C) in the setting of severe head injury [74] and perioperatively during cardiac surgery and neurosurgery [75–77]. Although the use of deep therapeutic hypothermia after cardiac arrest in the last century did not lead to an improved outcome [78], recent data show positive effects of mild therapeutic hypothermia [18,79–81] after cardiac arrest and other life-threatening events [82]. The data from the European multicenter trial (“hypothermia after cardiac arrest,” HACA trial [19]), as well as those from Australia [18], clearly demonstrate a decrease in mortality (Fig. 3) and a better neurologic outcome for patients who are cooled to 32°C to 34°C for 12 or 24 hours. The European trial included 275 patients with witnessed cardiac arrest and ventricular fibrillation and who were comatose at hospital admission. One hundred thirty-eight of them were cooled to a bladder temperature of 32°C to 34°C for 24 hours (Fig. 4), whereas 137 remained normothermic. The hypothermia group showed a good neurologic outcome (able to live independently and work at least part-time; 55% versus 39%) significantly more often with a number needed to treat (NNT) of 6 (relative risk 1.40; 95% confidence interval 1.08 to 1.81) and higher 6 months survival rate (59% versus 49%; relative risk 0.74; 95% confidence interval 0.58-0.95; NNT = 7). Even when the duration of hypothermia is shortened to 12 hours (target temperature 33°C), as done in the Australian study [18], survival rate with good neurologic outcome is improved (hypothermia 49% versus normothermia 26%; n = 43 versus 34). In 2003, such findings led to the implementation of mild therapeutic hypothermia (32°C–34°C) in the International Liaison Committee on Resuscitation (ILCOR) recommendations and

Fig. 3. Kaplan-Meier blot of the cumulative survival in the normothermia and hypothermia groups of the European trial on hypothermia after cardiac arrest [19]. (From The Hypothermia After Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. N Engl J Med 2002;346:549–56; with permission.)
guidelines for the treatment of unconscious patients after prehospital cardiac arrest [20]:

On the basis of the published evidence to date, the ILCOR ALS Task Force has made the following recommendations:

- Unconscious adult patients with spontaneous circulation after out-of-hospital cardiac arrest should be cooled to 32-34.8°C for 12-/24 h when the initial rhythm was VF.
- Such cooling may also be beneficial for other rhythms or in-hospital cardiac arrest.

Therefore, therapeutic hypothermia should be implemented in clinical practice. It is now the first clinically relevant therapeutic approach to improve cerebral and overall outcome after cardiac arrest.

Glucose control

Hyperglycemia is a common problem in the postarrest period [83,84]. Experimental and clinical data demonstrate that postischemic blood glucose concentration plays an important role in modulating ischemic cerebral infarction and selective neuronal death [85]. Data from several experimental animal studies suggest the use of insulin infusion to overcome those devastating effects of high levels of glucose after ischemic brain damage [86,87]. Recently a large study of critically ill patients admitted to surgical ICUs demonstrated a significant mortality benefit when glucose levels were set to a range between 80 and 110 mg/dL using insulin infusion [88]. Up to now there is no prospective, controlled human trial after cardiac arrest to
support this practice directly. The biologic plausibility of the benefit from controlling hyperglycemia with insulin infusion is strong, however; and therefore there is a ‘low grade’ recommendation to adjust blood glucose levels to between 90 and 145 mg/dL [89].

Future perspectives

More experimental approaches are focusing on the use of a hibernating state after cardiac arrest. Various animals use hibernation during times of extreme environmental conditions like low temperature or food and water deprivation. Hibernation in mammals is characterized by a physiologic state with significant reduction in body-core temperature and metabolic rate [90] resulting from a reduced set-point of thermoregulation. Those physiologic changes provide strong resistance to cerebral ischemia [91]. Reducing body-core temperature by way of modulation of the set-point, as done within hibernation, might be a superior concept to forced hypothermia because of the avoidance of the homeostatic mechanisms counteracting reductions in body temperature. Physiologic stress accompanied by a myriad of responses like shivering and increased catecholamine and cortisol levels during forced hypothermia might decrease cooling speed and efficacy of the hypothermic treatment [92]. Therefore the evaluation of controlled hypothermia- or hibernation-inducing drugs in future studies seems mandatory. Several substances have been tested for their efficiency in inducing regulated hypothermia/hibernation. The hibernation-induction trigger, an 88 kd peptide found in the serum of hibernating ground squirrels, can increase the survival time in a multiorgan preparation model with dogs [93]. Within the same model, it was shown that a delta opioide receptor agonist like D-Ala2, D-Leu5-enkephalin (DADLE) extends hypothermic preservation time of the lung [94]. A modified neotensin 77 given intravenously is able to induce hibernation for several hours in rats [95] and improves neurologic outcome after hypoxic-ischemia [96]. Recently an article in Science demonstrated that H2S induces a suspended–animation-like state in mice by way of inhibiting oxidative phosphorylation [97]. Taken together, there are several at least theoretical possibilities for inducing regulated hypothermia by way of modulation of the thermoregulatory set-point.

Clinical practice

Neuronal injury following global cerebral ischemia continues to be a central problem of patients in the postresuscitation phase. Particular attention must be paid to measures that serve to preserve neurologic function. Besides all measures focusing on rapid restoration of spontaneous circulation, such as the use of defibrillators to treat ventricular fibrillation and the choice of a suitable vasopressor, several postarrest treatment options have been explored in recent years. Probably the most effective treatment after cardiac arrest, as shown by large randomized trials, is the use of therapeutic mild
hypothermia. Current guidelines of ILCOR recommend the use of therapeutic mild hypothermia for all unconscious patients after cardiac arrest.

To date, there is no specific neuroprotective treatment available. However, good practice of critical care, such as the control of blood glucose, blood pressure, and oxygenation, should be provided. Promising animal experimental data concerning the use of thrombolytic agents during cardiopulmonary resuscitation has led to a large European multicenter trial (TROICA trial) that will provide its data in 2006.

References


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