Inflammatory mechanisms involved in brain injury following cardiac arrest and cardiopulmonary resuscitation (Review)

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Abstract. Cardiac arrest (CA) is a leading cause of fatality and long-term disability worldwide. Recent advances in cardiopulmonary resuscitation (CPR) have improved survival rates; however, the survivors are prone to severe neurological injury subsequent to successful CPR following CA. Effective therapeutic options to protect the brain from CA remain limited, due to the complexities of the injury cascades caused by global cerebral ischemia/reperfusion (I/R). Although the precise mechanisms of neurological impairment following CA-initiated I/R injury require further clarification, evidence supports that one of the key cellular pathways of cerebral injury is inflammation. The inflammatory response is orchestrated by activated glial cells in response to I/R injury. Increased release of danger-associated molecular pattern molecules and cellular dysfunction in activated microglia and astrocytes contribute to ischemia-induced cytotoxic and pro-inflammatory cytokines generation, and ultimately to delayed death of neurons. Furthermore, cytokines and adhesion molecules generated within activated microglia, as well as astrocytes, are involved in the innate immune response; modulate influx of peripheral immune and inflammatory cells into the brain, resulting in neurological injury. The present review discusses the molecular aspects of immune and inflammatory mechanisms in global cerebral I/R injury following CA and CPR, and the potential therapeutic strategies that target neuroinflammation and the innate immune system.

Contents

1. Introduction

Cardiac arrest (CA) remains a leading cause of fatality and permanent disability worldwide. Patient care guidelines have been continuously developed and modified so as to increase the proportion of individuals who survive CA (1,2). The recommended treatment is to start cardiopulmonary resuscitation (CPR), including chest compressions and external defibrillation, immediately to achieve return of spontaneous circulation (ROSC) thereby restoring organ perfusion (3,4). Due to the profound impact of advances in CPR, the survival rates to hospital discharge from in hospital CA has improved significantly over the last decade (5). However, nearly 50% of the CA victims who do survive and undergo hospital discharge suffer from moderate to severe long-term neurological deficits that significantly affect their quality of life (6,7). Despite advances in CPR, the persistent neurological deficits, such as neurocognitive impairment, learning and memory difficulties, and other neurological disorders were identified, influencing the American Heart Association (AHA)

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to emphasize cerebral injury associated with CA and CPR by proposing 'cardiopulmonary-cerebral resuscitation' in its 2000 Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care (8). Over decades, however, no specific drug therapy has been shown to improve the neurological outcome following CA and CPR (9).

CA directly causes global cerebral ischemia, which in turn triggers selective, delayed, neuronal cell death. The first aim of CPR is to reestablish sufficient circulation to supply the brain and heart with oxygen. However, emerging evidence supports that initial successful CPR could cause extensive ischemia/reperfusion (I/R) injury to the brain and other vital organs that is closely correlated with poor outcome (7). Although the principal pathophysiology regarding cerebral I/R injury following CA remains to be elucidated, it is well accepted that one of the definitive, but understudied, mechanisms of cerebral I/R injury is inflammation (10). It is characterized by activation of glial cells, influx of peripheral immune and inflammatory cells, high concentrations of reactive oxygen species (ROS) and release of proinflammatory mediators, including cytokines and adhesion molecules (11-13). The inflammatory process collectively inflicts lethal damage to neurons, exacerbates endothelial dysfunction and vasomotor dysregulation and disrupts the blood-brain barrier (BBB), induces edema, leading to tissue-level hypoxia and subsequent neurological damage (14). Despite a more comprehensive understanding regarding the mechanisms of cerebral injury, currently no clinically proven pharmacological therapy data against cerebral I/R damage during CA and CPR are available (15). Increasing evidence reveals that suppressing the inflammatory process facilitates neuroprotection and has potential for use in the clinical treatment of cerebral I/R damage regarding CA (16).

The aim of the present review is to evaluate the specific aspects of the immune and inflammatory mechanisms underlying cerebral I/R injury regarding CA and CPR, and furthermore, this study reviews the potential anti-inflammatory targets in brain injury during CPR and the post-resuscitation phase. Overall, the prospects for a secure clinical strategy to improve neurological outcome following CA remain promising.

2. Cerebral I/R damage following CA and resuscitation

Brain injury from CA and post-resuscitation comes in stages. Within seconds of global cerebral ischemia and hypoxia, cerebral activities are compromised, and within minutes, the ischemic cascade is rapidly initiated, which consists of a series of biochemical events, including depletion of adenosine triphosphate and glucose, Na^+/K^+ pump failure and loss of cell structural integrity (17,18). These events, which subsequently lead to mitochondrial damage and intracellular calcium overload, further exacerbate immediate cellular necrosis or apoptosis when coupled with increased levels of arachidonic acid, glutamate and other toxic excitatory neurotransmitters (19). Restoration of oxygenation, corresponding to reperfusion during CA, limits ongoing hypoxic damage, which is crucial for restoring normal function. However, it can paradoxically cause continued cellular damage and death, which occurs over the subsequent hours and days after successful resuscitation. During reperfusion injury, reoxygenation promotes the generation of ROS and nitrogen metabolites within the active microglia and astrocytes; migration of peripheral macrophages, monocytes and neutrophils; release of cytokines and adhesion molecules by the inflammatory cells, which eventually precipitate lethal damage to neurons, oligodendrocytes and the cerebrovascular endothelium, and disruption of BBB (20).

Furthermore, the innate immune system may have a critical role in cerebral reperfusion injury (21). It has been demonstrated that the brain and innate immune system are engaged in bidirectional crosstalk. In a manner similar to the response defending against pathogen invaders, the cerebral inflammatory cascade comprises an increase of neutrophil recruitment and peripheral macrophages infiltration, activation and migration of microglia, also known as 'brain macrophages', and release of pro-inflammatory stimuli within the brain (20,22). Additionally, microglia and astrocytes express a wide variety of receptors of innate immunity, such as toll-like receptors (TLRs) (23). When an ischemic event occurs, the normally immune-privileged brain environment collapse, danger-associated molecular pattern molecules released by cellular injury can be recognized as invaders by immune cells in terms to induce the activation of TLRs and triggers the activation process of nuclear factor-κB and signal transducer and activator of transcription 3 signaling pathways, which are linked to the transcription of numerous proinflammatory genes (24,25). As a result, encoded cytokines, chemokines and proteins of the complement cascade are upregulated in the cerebral tissue, and consequently, the generation of adhesion molecules on the endothelial cell surface is induced. The described neuroinflammatory changes ultimately lead to neurological damage following CA and CPR.

3. Role of inflammatory cells in cerebral injury following CA and resuscitation

Microglia-innate immune cells of the brain. Microglial cells, the resident macrophages of the brain, are activated immediately following brain injury and have numerous immunological characteristics with blood-derived monocyte/macrophages (26-28). Following ischemia, microglia undergo phenotypic transformation to an 'activated' phenotype which can release various substances, many of which are cytoprotective or cytotoxic (29). Microglia also have a functional role in the phagocytosis of cell debris and in the release of neurotrophic factors, such as brain-derived neurotrophic factor, insulin-like growth factor I and several other growth factors. Acutely activated microglial cells in response to ischemia produce several pro-inflammatory cytokines, such as interleukin-6 (IL-6), IL-1β and tumor necrosis factor-α (TNF-α), as well as other potential cytotoxic molecules including ROS, nitric oxide (NO) and prostanoids (11,30). This initial innate immune response will be evoked rapidly in almost any disruption or loss of brain homeostasis, and it is perceived as a first-line defense against an infection or to blunt further damage following an injury. In cerebral injury following CA and resuscitation, however, this initial response may be maladaptive (31).

Resident microglia are activated within minutes of hypoxia onset and require hours to days to fully develop. Following the resuscitation, reoxygenation promotes a continuous release of signals, including ROS and NO, from microglia (30). These signals also trigger infiltration of peripheral immune cells, such as neutrophils, macrophages and T lymphocytes, to the injured area, all these cell types can in turn affect microglial activation and contribute to ongoing secondary neuronal injury (26,27,30).

The special characteristic of microglia is in its striking ability to multiply and migrate in response to cerebral injury (32,33). Microglial proliferation has been implicated in the onset and progression of cerebral I/R damage (34). Active microglia express a variety of biologically active substances, which induce microglia proliferation (35). A rapid increase in the number of microglia at an injured area is associated with the influx of peripheral immune cells and migration of resident microglia from other sections of the brain (36).

Astrocytes. It is well documented that astrocytes interact with and support neighboring neurons (37). Under hypoxic stress, astrocytes not only release neuroprotective molecules, including metabolic substrates, antioxidants and neurotrophic factors, but also produce a myriad of cytokines, a number of which have dual proinflammatory and anti-inflammatory effects (12,38). In response to I/R injury, astrocytes were activated early (2 h) after reperfusion but confined to the area where neurons started to show degeneration and death hours to days later. This finding demonstrated the importance of astrocytes in delayed neurological damage following I/R injury. Consideration of the role of astrocytes may be an ideal factor for potential neuroprotective strategies (39,40).

Experimental data indicates that astrocyte mitochondria are an important target of I/R insults regarding CA (41,42). Within a few hours of reperfusion, increased ROS production from mitochondrial respiratory chain results in mitochondrial dysfunction in astrocytes, contributes to the loss of glutamate transporter 1 and ultimately to delayed death of neurons (43,44).

Neutrophils. Within a few hours after ischemia onset, peripheral leukocytes adhere to vascular endothelial cell and migrate into damaged brain tissue with the subsequent release of pro-inflammatory molecules and secondary injury in the post-ischemic area. Neutrophils are the earliest leukocyte subtype to show substantial upregulation and to infiltrate areas of brain ischemia (45,46). Similar to in peripheral tissues, the trans-endothelial migration of neutrophils into the brain also appear to demand cellular adhesion molecules (CAMs), including intercellular adhesion molecule 1 (ICAM-1) and P-selectin (47,48). Deficiency of ICAM-1 and P-selectin has been demonstrated to reduce the infarct size and formation of brain edema in mouse models of ischemic stroke (49,50). Infiltrating neutrophils, as well as microglia and macrophages, are also able to produce additional ROS, pro-inflammatory cytokines, including IL-6, IL-1β and TNF-α, and chemokines, such as monocyte chemoattractant protein-1 (MCP-1) and IL-8. By these molecules, infiltrating neutrophils amplify a cerebral inflammatory response that may exacerbate post-ischemic brain damage (49,51,52). Furthermore, infiltrating neutrophils are the primary source of enhanced matrix metalloproteinase 9 activity in the ischemic brain, which is a critical mechanism underlying the breakdown of the BBB and the exacerbation of neurological injury (53).

T lymphocytes. Accumulating evidence supports that the infiltration of T lymphocytes significantly contributes to the pathogenesis of cerebral I/R injury, and different classes of T lymphocytes have differential roles in response to I/R-mediated adaptive immunity (54,55). It is generally known that there are two subtypes of T lymphocytes: Cluster of differentiation 4^+ ($CD4^+$) T-helper (Th) cells and $CD8^+$ cytotoxic T cells. The Th cell subset comprises Th1, Th2, Th17 and Th40 and regulatory T cells (56,57).

Studies using a mouse model of CA/CPR show that CA and resuscitation stimulates rapid infiltrations of $CD4^+$ and $CD8^+$ T cells to the ischemic brain area, which were observed to contribute to the inflammatory and thrombogenic responses, brain infarction and neurological deficit (58). During the I/R injury process, $CD8^+$ cytotoxic T cells directly serve to promote the immune and inflammatory responses through the release of cytokines, including IFN-γ and TNF-α (59). $CD4^+$ Th cells have no cytotoxic activity themselves, but instead aid in the activation of other immune cells, including $CD8^+$ cytotoxic T cells (60). Experimental data also indicates that $CD4^+$ T $cell^{(-/-)}$ and $CD8^+$ T $cell^{(-/-)}$ mice are protected from hippocampal CA1 neuronal cell death, compared with wild-type mice following CPR-induced ischemic brain damage (58).

4. Role of cytokines in cerebral injury following CA and resuscitation

Cerebral injury is correlated to the abundant synthesis of inflammatory cytokines during CA and resuscitation (61,62). In the brain, cytokines are not only produced by the cells of the immune system, but also expressed in resident brain cells, including neurons and glia (63). In addition, cytokines released by peripheral immune cells are involved in neuroinflammation and their inhibition or deficiency is associated with reduced injury (64-66).

The majority of investigations regarding cytokines associated with inflammation in ischemic cerebral injury mostly focuses on IL-6, IL-1β, IL-10, IL-20, TNF-α and transforming growth factor-β (TGF-β). Among these cytokines, IL-1β and TNF-α appear to exacerbate cerebral damage; however, TGF-β and IL-10 may be neuroprotective (67,68). Increased synthesis of pro-inflammatory cytokines and decreased production of the anti-inflammatory IL-10 are correlated with larger infarct volume and poorer neurological outcome (69).

In the reperfusion period, TNF-α levels in the ischemic area elevate markedly and persist at a high level following reperfusion (70). TNF-α expression occurred initially in neurons, and subsequently in glia cells, including microglia and astrocytes as well as in the peripheral immune cells (71-73). Clinical studies have shown that TNF-α-positive cells localize in all ischemic brains of patients who experienced a severe stroke 3 days post-stroke and remain <15 months post-stroke, and the majority of the increased TNF-α are derived from microglia and macrophages (74,75). TNF-α levels in the serum of stroke patents are elevated within 6 h and stay increased for 10 days post-stroke compared to the controls (76,77).

IL-1β levels increased significantly in the reperfusion period and remained at high levels for days after reperfusion (78,79). IL-1β-positive staining was observed in the ischemic regions of the cortex within 16-24 h after stroke (80). An elevated level of IL-1β can potentiate inflammation by activating microglia and induce the infiltration of peripheral leukocytes by increasing the expression of adhesion molecules on endothelial cells; these events are associated with worsening of the infarct severity and progressive neurodegeneration (81-83). In addition to its role as a pro-inflammatory mediator, IL-1β can also elevate the expression of other pro-inflammatory cytokines, such as IL-6 and TNF-α, which can be upregulated following cerebral ischemia (84).

IL-10 exerts potential anti-inflammatory effects by inhibiting IL-1 and TNF-α, and by suppressing cytokine receptor expression and receptor activation as well (85). As a consequence, IL-10 could confer a neuroprotective effect in acute ischemic stroke. In a rat model of cerebral I/R injury, administration of IL-10 appeared to decrease the infarct volume significantly from 30 min to 3 h post-ischemia compared to the control animals (67).

5. Role of chemokines in cerebral injury following CA and resuscitation

In the brain, chemokines, such as IL-8 and MCP-1, are primarily expressed on the neurons and glial cells (63). Circulation chemokines are mainly derived from the immune system, such as neutrophils and macrophages (64). Chemokines are involved in conveying pro-inflammatory signals and inducing immune cells to recruit to the injured tissues. Elevated levels of chemokines, such as MCP-1 (or CCL2) and inflammatory protein-1α (MIP-1α or CCL3), following ischemic injury have been correlated with an increase of peripheral neutrophils infiltration. CCL2 and CCL3 upregulation has been found in the early hours after ischemic stroke (86,87). Intracerebroventricular injection of CCL3 enlarged the infarct territory following transient focal ischemia in rats, CCL2-deficient mice had a smaller infarct size and decreased infiltration of inflammatory cells (86,88). Mice without the chemokine receptor CCR2 are protected against ischemia-reperfusion injury (89).

6. Role of cellular adhesion molecules in cerebral injury following CA and resuscitation

Clinical investigations and animal studies have shown that CAMs are critical participants in the cerebral injury following CA/CPR (49,90). In the ischemic brain, the high level of pro-inflammatory cytokines induces the cerebral endothelial cells to express CAMs that mediate the recruitment of leukocytes to the injured region. Three well-known CAMs, ICAM-1, and P- and E-selectin, are highly expressed at ischemic areas and promote the firm adhesion and migration of neutrophils into damage brain tissues, thereby contributing to neuroinflammation-mediated neurological injury (91-93). In response to I/R injury, P-selectin can be detected as early as 15 min after reperfusion, while E-selectin expression is observed from 2 h after ischemia. Increased expression of ICAM-1 can be observed within hours after ischemia onset (94,95). Compared to wild-type mice, ICAM-1-knockout mice exhibit a reduction in neutrophil infiltration, smaller infarct size, improved cerebral blood flow and lower mortality following I/R injury (47,96). In a model of cerebral I/R, P-selectin-knockout mice exhibit less infarct volume, better functional outcome and an improved return of cerebral blood flow following ischemia (48). E-selectin blockade with specific antibodies 90 min after ischemia onset reduce neutrophil infiltration and infarct volume (93).

7. Development of neuroprotective interventions following CA and resuscitation

General. CA/CPR-induced neurological damage is a progressive process that can develop over a long time following the initial injury (15). As aforementioned, strong evidence now exists to suggest that neuroinflammation continues for days and months, and contributes to the neurological damage that ultimately determines the impaired recovery following CA and CPR (16). This suggests that the neuroprotective anti-inflammatory treatments discussed in the following are promising strategies for CA and resuscitation intervention.

Therapeutic hypothermia (TH). Several recent multicenter, randomized trials report that TH confers significant neuroprotective effects and reduces mortality by >25% when applied for 12-24 h after successfully resuscitated ventricular fibrillation (VF) arrest in adults (97). On the basis of these studies, the AHA published guidelines and awarded the highest level of recommendation for the use of hypothermia in the treatment of patients successfully resuscitated from VF-induced CA. Currently, at 6 months after CA, 55 and 39% of the patients treated with or without TH, respectively, have a favorable neurological outcome. The proposed mechanism underlying the protective effect of TH has generally been attributed to its preservation of metabolic substrates, alteration of cerebral blood flow and prevention of excitatory amino acid accumulation (98). A previous study suggested that TH following CA did not alter serum inflammatory cytokines, including TNF-α, IL-2, IL-10 and MCP-1, demonstrating that the beneficial effects of TH do not arise from alleviation of the inflammatory response (99). Therefore, adjunct treatment targeted to inflammation and immune dysregulation are urgently required to improve the overall efficacy of TH (100).

Minocycline. Minocycline, a semisynthetic tetracycline derivative, has been reported to be neuroprotective against post-arrest global cerebral I/R injury by exerting anti-inflammatory effects, including inhibition of the activation and proliferation of microglia, migration of neutrophils, release of proinflammatory cytokines and chemokines (101). Minocycline administration diminishes infarct volume and improves functional recovery following experimental stroke, and even provides clear protection when the treatment is started 4 h after the onset of stroke (102,103). Furthermore, the outcomes for stroke patients treated with minocycline within 6 to 24 h after ischemia are significantly better compared to the placebo (104).

Molecular hydrogen (H_2). H_2 is a new popular therapeutic agent for cerebral I/R injury treatment (105). In two animal

models of CA/CPR-induced global I/R injury, the beneficial effects of H_2 treatment were associated with the antioxidant and anti-inflammation effects (106,107).

Statins. Statins are the 3-hydroxy-3-methylglutaryl coenzyme, which are reductase inhibitors that have been shown to reduce infarct size in animal models of stroke. Statins may provide pleiotropic neuroprotective effects following cerebral ischemia that are independent of cholesterol lowering, including attenuation of inflammatory responses, amelioration of oxidant stress and improvement of endothelial function (108).

8. Conclusion

CA is a devastating disease process with neurological injury accounting for poor outcome following ROSC. Accordingly, a collaborative effort to resolve the mechanisms underlying neurological injury could potentially enhance our understanding of the pathobiology of brain resuscitation following CA. Emerging preclinical, as well as recent human clinical evidence, suggests that activation of inflammatory cascade and the immune system have critical roles in the pathogenesis of neurological damage following CA/CPR. An increasing number of anti-inflammatory and immunomodulatory compounds have shown feasible potential for the neuroprotective effects in preclinical settings. However, a more efficient development of treatments targeting the elements of these injury cascades is required for an improved outcome and quality of life for CA patients.

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