INTRODUCTION

Restoration of blood supply, referred to as “reperfusion,” is a desired goal for acute stroke treatment. Spontaneous reperfusion occurs commonly after stroke, in about 50–70% of patients with ischemic stroke. Reperfusion can also be achieved either by thrombolytic therapy using tissue plasminogen activator (tPA) or endovascular therapy, including embolectomy surgery using retrieval devices and thrombus disruption using stents. Since the publication of the first edition of Primer on Cerebrovascular Diseases in 1997, there has been considerable progress in the mechanisms study and treatment development for reperfusion injury in stroke. The time window of thrombolysis using tPA has been extended up to 4.5 h after stroke onset; meanwhile the embolectomy surgery using stent retrievers has undergone multiple clinical trials in the United States in the past 10 years with significantly beneficial outcomes and is expected to be widely practiced in the near future. However, despite the beneficial effect of oxygen supply brought by reperfusion, rapid reperfusion also has detrimental impact on brain function, the so-called reperfusion injury. This has been documented by both experimental studies using animal stroke models and clinical evidence, such as rat stroke models showing significantly increased infarct volume after reperfusion compared with permanent occlusion. In addition, MRI studies using fluid-attenuated inversion recovery and perfusion-weighted images for human stroke showed that reperfusion could be linked to an early opening of the blood–brain barrier (BBB) and consequently to secondary reperfusion injury and poor outcome [1]. In this chapter, we briefly discuss the pathophysiology and cellular and molecular mechanisms of reperfusion injury and hemorrhagic transformation (HT), and potential therapeutic strategies against these injuries.

CELLULAR AND MOLECULAR MECHANISMS OF ISCHEMIA-REPERFUSION INJURY AND HEMORRHAGIC TRANSFORMATION

To date the deleterious effect of reperfusion in brain function after stroke has been widely recognized and the underlying cellular and molecular mechanisms are being clarified, part of which are learnt from ischemia-reperfusion injuries in other organs such as heart and liver. The mechanisms of reperfusion injury include oxidative stress, leukocyte infiltration, platelet activation, complement activation, and disruption of the BBB, which ultimately lead to edema or HT. HT significantly contributes to the neurological dysfunction and mortality after acute ischemic stroke, and is further worsened by reperfusion caused by either tPA recanalization or embolectomy surgery. In the past few years, the concept of neurovascular unit (NVU) has been widely accepted, in which multiple cell types including endothelial cells, astrocytes, pericytes, oligodendrocytes, microglia, and neurons functionally interact with each other to maintain brain function. As a consequence, in addition to vascular damage, ischemia-reperfusion also causes deleterious effects on these NVU components. Due to the space limit of this chapter, we mainly focus on endovascular damage caused by ischemia-reperfusion.

Oxidative Stress

Oxidative stress results from an imbalance in which the manifestation of reactive oxygen species overwhelms
the antioxidant capacity of the cells. The overproduction of reactive oxygen species, mainly peroxides and free radicals, causes damages to all components of the cells, including proteins, DNA, and lipids. Oxidative stress has emerged as one of the mechanisms implicated in the pathogenesis and disease progression of many diseases including stroke. Increased ROS production has been demonstrated in ischemic stroke, both during ischemia and reperfusion [2]. The cerebral ischemia-reperfusion model revealed that oxidative stress mediates BBB dysfunction in mice with superoxide dismutase deficiency. Furthermore, free radical generation and oxidative damage in BBB are main triggers of HT after transient focal cerebral ischemia, which is supported by experimental evidence that free radical scavenger can significantly decrease tPA-induced HT in embolic focal ischemia.

Although a large array of experimental studies have established that oxidative stress is an important mechanism of reperfusion-injury and HT, supportive clinical evidence is scant. A clinical investigation for patients with stroke after tPA thrombolysis showed that the oxidative stress markers including malondialdehyde and myeloperoxidase were already increased at baseline of stroke, whereas no further increases were found for these markers after tPA recanalization, suggesting no relationship between free radical–induced oxidative damage to lipids/proteins and reperfusion injury. However, there exists much limitation in clinical studies; for instance, the peak of oxidative stress might have been missed. The contribution of oxidative stress in reperfusion injury cannot be denied, which warrants further investigation in the future.

**Leukocyte Infiltration**

Leukocytes play important roles in cerebral reperfusion injury. During reperfusion, activated leukocytes attach to endothelial cells through chemotactic signals, and matrix metalloproteinase and neutrophil-derived oxidants are subsequently produced to break down the BBB. The leukocytes then extravasate from capillaries and infiltrate into brain tissue, releasing proinflammatory cytokines, which eventually result in deterioration of the penumbra [3].

The destructive effect caused by leucocyte infiltration has been validated by numerous animal studies. It was revealed that in rat stroke models neutrophil accumulation at the neuronal injury site occurred earlier and to a greater extent in reperfusion tissue than in tissue with permanent occlusion. In addition, the contribution of leukocyte infiltration in reperfusion injury is also supported by the beneficial effects of neutrophil depletion, in which the animals after transient ischemia showed smaller infarct size when administered with either antineutrophil antiserum or monoclonal antibodies. Furthermore, leukocyte infiltration is also involved in HT, supported by increased white blood cell count in patients with HT than in those without. The subsequent enhanced leukocyte infiltration may damage microvascular endothelial cells, causing BBB dysfunction and HT.

**Platelet Activation**

Increasing evidence supports a role of platelets in the pathogenesis of ischemic-reperfusion injury. Platelets are activated by ischemia-reperfusion and accumulate in vascular beds early after reperfusion. Upon activation, platelets generate reactive oxygen radicals and release proinflammatory factors such as platelet-derived growth factor, arachidonic acid metabolites, thromboxane A2, and platelet factor 4. In addition, activated platelets adhere to microvascular endothelial cells, causing the latter to release mediators for leukocyte chemotaxis and migration, subsequently exacerbating the inflammatory responses [4].

Furthermore, platelets have the potential to modulate leukocyte functional responses, potentially through the interaction between platelet-released fibrinogen and CD11C/CD18. Conversely, activated leukocytes can also alter platelet function. Indeed, accumulating evidence has supported that platelets and neutrophils act synergistically in the pathogenesis of reperfusion injury, largely through cell–cell interactions mediated by P-selectin.

**Complement Activation**

The complement system as part of the innate immune system comprises a big group of plasma proteins that can be activated by pathogens or other stimuli and subsequently induce inflammatory responses. Previous studies have indicated that the activation of complement system is one of the important mechanisms of
reperfusion injury. During reperfusion the complement system can be activated through different pathways, including the antibody-dependent classical pathway, the alternative pathway, or the mannann-binding lectin/mannann-binding lectin-associated serine proteases pathway [5]. As a result, multiple inflammatory mediators will be released, including anaphylatoxins C5a, and the distal complement component C5b-9 [membrane attack complex (MAC)]. C5a can stimulate leucocyte infiltration into damaged tissue, and may also induce the release of proinflammatory factors such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α. MAC forms transmembrane channels that can increase cell membrane permeability, and ultimately disrupt the phospholipid bilayers of cellular membrane, leading to cell lysis and death. Furthermore, MAC plays an essential role in mediating the recruitment of leukocytes to the reperfused tissue, potentially via local induction of IL-8.

**Blood–Brain-Barrier Disruption**

BBB disruption is a consequence of aforementioned reperfusion-injury mechanisms including oxidative stress, leucocyte infiltration, platelet activation, and complement activation. It has been documented by both animal studies and clinical investigation that BBB disruption occurs during cerebral reperfusion and can lead to vascular edema and HT. Previous studies have reported that BBB disruption occurs after 3 h of reperfusion following transient occlusion, whereas BBB remained intact after 6-h occlusion in a permanent occlusion group. A clinical study involving 144 patients with acute stroke showed that BBB disruption was more common in patients with reperfusion than in those without reperfusion. In addition, in the reperfused group, patients with BBB disruption were more likely to have a poorer clinical outcome than those without disruption [6]. This study established the association of early BBB disruption with HT and poor clinical outcome in humans.

**Inhibition of Leukocyte Infiltration**

Therapeutic strategies targeting leucocyte-mediated reperfusion injury include inhibition of leucocyte adhesion molecule synthesis, inflammatory factor release, and receptor-mediated leucocyte adhesion to endothelial cells. There exists experimental evidence that this strategy is effective in protecting against reperfusion injury. For example, the inhibitors of leucocyte adhesion molecule synthesis, such as glucocorticoids, gold salts, and d-penicillamine, have been shown to have therapeutic effects against leucocyte-mediated reperfusion injury [8]. In addition, lipoxins are potent inhibitors of leucocyte chemotaxis, adhesion, and transmigration induced by inflammatory factors, suggesting that they are part of innate protective pathways dampening the host inflammatory response. Furthermore, administration of biostable lipoxin analogues attenuated PMN (polymorphonuclear leukocytes)-mediated vascular barrier dysfunction and second-organ injury in several models of ischemia-reperfusion.

**Inhibition of Platelet Activation**

Platelets can also be a target for therapeutics against ischemia-reperfusion injury. Accumulating studies have shown beneficial effect of platelet depletion in ischemia-reperfusion injury. It has been demonstrated that platelet depletion using filter improved both hepatic and pancreatic function after ischemia-reperfusion injury, probably through reducing lipid peroxidation in cell membrane and the ratio of thromboxane A2 to prostaglandin I2. In addition, antiplatelet agents including dipyridamole and cilostazol improved myocardial function when combined with statin after ischemia-reperfusion [9]. These findings suggest that inhibition of platelet activation might be a potential therapeutic strategy for ischemia-reperfusion injury in the brain as well. However, the potential risk of bleeding caused by antiplatelet agents should be considered for further development of antiplatelet therapy.
Inhibition of Complement Activation

Inhibition of complement activation is also a potential strategy to prevent ischemia-reperfusion injury as demonstrated in multiple experimental models [10]. As an example, C3 convertase is a member of the serine protease family in the complement system. It has been found that an inhibitor of C3 convertase, the soluble complement receptor 1 (CR1), significantly decreased myocardial infarct size and improved myocardial function in a rat model of ischemia-reperfusion. More interestingly, the CR1 short consensus repeats have been shown to protect against cerebral ischemia-reperfusion injury in rats, decreasing cerebral infarct size and improving neurological function. In addition, C5 is another important component of the complement system, which after cleavage can be converted into C5a and C5b-9, two potent inflammatory mediators that increase vascular permeability, leukocyte adhesion and activation, and endothelial activation. Administration of a recombinant antibody against human C5, namely, pexelizumab (Alexion Pharmaceuticals, Inc., Cheshire, CT), has been shown to significantly attenuate complement activation, leukocyte activation, myocardial injury, and acute postoperative mortality in patients undergoing coronary artery bypass grafting surgery. However, to date not many complement inhibitory reagents have been potent enough to enter human clinical trials. More potent complement inhibitory reagents targeting ischemia-reperfusion are yet to be developed in the future.

CONCLUSION

In summary, over the past decade there have been significant advancements in our understanding of molecular and cellular mechanisms of ischemia-reperfusion injury for brain. The major mechanisms of reperfusion injury include oxidative stress, leukocyte infiltration, platelet activation, complement activation, and breakdown of BBB, which ultimately lead to edema or HT. A number of therapeutics studies are ongoing targeting these injury mechanisms, which, however, are still far from achieving clinical success. Further investigations on the mechanisms of reperfusion injury are warranted, which will be helpful for developing effective therapeutics against reperfusion injury in the brain.

References

学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具