EXPERT REVIEWS

Brain 11: what's new in stroke research?

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The International Society for Cerebral Blood Flow and Metabolism promotes research centered on furthering the understanding of neurological conditions that result from ischemia and related injuries. This previous meeting (Brain 11) focused on state-of-the-art research, which shed light on the pathophysiology of these conditions as they pertained to the cerebral vasculature – including the BBB – and angiogenesis, immune mechanisms, regeneration and repair following such insults. The meeting also covered a variety of potential therapeutic strategies ranging from cell-based therapy, induced tolerance and the targeting of specific immune or cell death pathways. In addition, there was a focus on how the peripheral circulation contributes to such types of brain injury.

Keywords: cerebral ischemia • inflammation • neurogenesis • neuroprotection • neurovascular unit • stem cells

Brain 11 was the 25th International Symposium on Cerebral Blood Flow, Metabolism and Function, and was organized by Anna Planas of Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and colleagues on behalf of the International Society for Cerebral Blood Flow and Metabolism (ISCBFM). ISCBFM is an organization of scientists and physicians interested in the study of cerebral blood flow and metabolism, whose members come from all over the world. This meeting is held biennially, and attracts over 1000 scientists and physicians from 40 different countries. The program included state-of-the-art topics, such as neurovascular biology, cell-based therapy, neuroimmunology and neuroimaging.

Neuron–blood vessel interactions

The meeting began with a plenary lecture given by Berislav V Zlokovic of the University of Rochester (NY, USA) who presented work demonstrating a previously unrecognized role of the cerebral vasculature and neurodegeneration, with an emphasis on pericytes. The role of pericytes in the brain vasculature has only recently been shown to contribute significantly to neurovascular functions. By studying mice deficient in pericytes, Zlokovic and colleagues found that this led to disruption of the BBB, with subsequent leakage of blood-derived neurotoxins [1]. Entry of neurotoxins then led to neurodegeneration. These findings have important clinical implications because they suggest the importance of targeting the brain vasculature in the prevention of neurodegenerative disease.

The link between Alzhiemer's disease and inflammation is often controversial, but lipopolysaccharide (LPS)-induced inflammation appears to prevent the normal efflux of β-amyloid out of the brain, potentially leading to abnormal retention and accumulation.

Inflammation is known to negatively affect BBB integrity. Thus, anti-inflammatory strategies could be neuroprotective by virtue of their salutary effects on the brain vasculature. Cannabinoids are known to be neuroprotective, and one mechanism of protection appears to be through anti-inflammatory properties [2]. LPS treatment increased cannabinoid receptor (CB2) expression, and CB2 agonists increased tight junction protein (TJP) expression and strengthened the BBB while preventing leukocyte adhesion. This would suggest that a potential protective mechanism of cannabinoids is through an anti-inflammatory, BBB strengthening property.

Factors leading to BBB disruption were also discussed in other presentations. While past work has demonstrated the importance of matrix

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metalloproteinase (MMP) activation in BBB disruption, repair mechanisms were also explored [3]. While TJPs disappear early after ischemic stroke, they reappear at 3 weeks in newborn vessels and even surrounding pericytes and astrocytes, and this was correlated to increased MMP-2 (astrocytes) and MMP-3 (pericytes). These observations suggest that MMPs, while acutely damaging to the BBB, may also be important in angiogenesis and BBB remodeling.

Restorative therapies

With the recognition that most patients do not present for medical attention until hours or sometimes days following stroke, efforts to understand how the brain recovers from cerebral ischemic insults and to identify potential therapeutic strategies have been the focus of this field for nearly a decade. With recent interest in the potential of stem cells to cure a variety of diseases, their application in brain ischemia is no exception.

Cell-based therapy

The current status of cell-based therapy in stroke and related conditions was reviewed. Both neuron-derived and bone marrow-derived mesenchymal cells (MSCs) could be administered to laboratory animals subjected to various types of CNS insults [4,5] and new techniques that include antioxidant gene transfer to stem cells proved efficient for the survival of stem cells [6]. These cells integrate into the brain and lead to improvement in long-term neurological function. Furthermore, cotreatment with trophic factors could improve the ability of stem cells to repair injury to the spinal cord. Advanced imaging techniques allow noninvasive nanoparticle labeling of the transplanted cells, and these studies were able to compare the efficiency of transplantation of bone marrow-derived MSCs when given peripherally or intracerebrally, with more efficient uptake occurring when given intracerebrally.

Human studies of cell-based therapies were also reviewed [7]. While the collective clinical experience is still in its infancy, a few anecdotal cases of remarkable functional improvement were shown. Most clinical studies are currently examining the role of MSCs, and the studies are at the Phase II level, where safety, rather than efficiacy, are being evaluated. While it was emphasized that cell-based therapy is still in the early stages of investigation and its precise role in the treatment of patients is unclear, many speakers seemed optimistic.

Endogenous CNS repair mechanisms & neurogenesis

CNS injury frequently leads to an inevitable and irreversible loss of function owing to the widely held notion that neurons, particularly in the adult brain, do not regenerate. However, recent work has shown that neurons are indeed capable of regenerating ('neurogenesis') in certain brain regions and under specific settings. Thus, the possibility that brain cells can be restored through endogenous repair mechanisms opens up a host of therapeutic strategies to optimize this recently recognized property [8].

In one session, neuroscientists discussed novel applications of neurogenesis through the activation of endogenous neural stem cells (NSCs) and/or pharmacological pretreatment of NSCs prior to transplantation. Attempts to maximize the spread of neurogenesis after injury have yielded broad insights into the identification of specific molecules with neuroprotective properties. Several researchers presented new strategies for improving CNS repair during various insults. NSCs pretreated with drugs, such as minocycline, LPS and agmatine (an endogenous inhibitor of nitric oxide synthase), all attenuated infarct size and enhanced neurological recovery when transplanted into the ischemic brains of rodents. Minocycline-treated NSCs subjected to ischemia-like insults *in vitro* followed by reperfusion deregulated several antioxidant genes and trophic factors, such as Nrf2, NADH quinone oxidoreductase 1, HO, BDNF, NGF, GDNF and VEGF. A few investigators also suggested that PI3k/Akt or Akt/JNK signaling pathways were associated with this neuroprotection. Preconditioning and other approaches aimed at enhancing NSC protection, such as transfection of transgenes, were also presented. Some studies explored the role of miRNAs that could regulate the expression of various genes. In one study, inhibiting miRNA (mir1), which binds to the 3′ UTR of the *IGF-1* gene, could promote protection of NSCs, and lead to improved NSCs for transplantation.

Inflammation in stroke

The role of inflammation in brain ischemia has been increasingly studied, and it is generally recognized that the acute inflammatory response negatively affects stroke outcome [9]. Several presentations characterized specific immune targets that could be manipulated to improve outcome from experimental models. Studies of sphingosine kinase antagonists, the P2Y12 nucleotide receptor (and also the site at which the popular antiplatelet drug clopidogrel acts), and agonists of cannabinoids/cannabinoid receptors, all showed salutary effects on brain cell survival and neurological outcome. A few presentations demonstrated that ischemic tolerance, or the phenomenon that sublethal stress leads to protection from a subsequent lethal insult, can be conferred by manipulating certain inflammatory pathways. These included the stimulation of toll-like receptor 3 (TLR3) or the sphingosine kinase-2. Ischemic preconditioning was also correlated to increases in endogenous anti-inflammatory factors, including agmatine.

The role of microglia in brain ischemia was also explored in a symposium that challenged the notion that microglia possess damaging functions in both acute and chronic neurological conditions. While some microglial properties certainly appear to worsen outcome from brain ischemia, ablation of brain microglia in the adult brain led to worsening of ischemic outcome and alteration of the ischemic inflammatory response [10]. This worsening seemed to be related to the depletion of microglia-derived prosurvival factors, such as IGF-1 and galectin-3. However, the deletion of microglia from neonatal brains had no effect on stroke outcome and emphasizes clear differences in responses to ischemic insults in the developing versus adult brain. Other microglial functions appear vital to effective recovery. A newly characterized phagocytic receptor, triggering receptor expressed on myeloid cells (TREM-2), may be important in the removal of injured neurons, and its deficiency could potentially negatively impact stroke outcome. The examination of microglia in aged human brain samples of subjects carrying a diagnosis of Alzheimer's disease and related conditions

also revealed that during senescence, microglia show signs of degeneration and abnormal responses [11]. Furthermore, microglia show signs of damage prior to the development of tau pathology, and would suggest that treatments targeting microglia are unlikely to affect neurodegeneration in conditions such as Alzheimer's disease.

Importance of the peripheral circulation

The peripheral circulation has been understudied in this field, but recent work presented at this meeting has emphasized its importance. The study of bone marrow cells in different stroke models revealed early immune activation in these cells. However, the stress of surgery and anesthesia appeared to contribute to some of these responses and these factors need to be considered when interpreting animal stroke model results. Nevertheless, by utilizing a bone marrow chimeric approach, CD36 (an inflammatory mediator that contributes to ischemic worsening owing to elevated plasma cholesterol [12]) derived from circulating immune cells was shown to contribute to stroke worsening, rather than CD36 derived from the brain. At the clinical level, T-cell responses in acute stroke patients demonstrated a TH1 bias, while proteomic analysis of plasma samples from acute ischemic stroke patients revealed the presence of many proteins involved in inflammation and clotting.

Summary

Current research in the area of stroke and neurodegeneration were presented. Several meeting themes challenged prior concepts showing that vascular stress may precede neurodegeneration, and that microglia possess important prosurvival and protective functions. The peripheral circulation and the status of cell-based and regenerative therapies were also reviewed.

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