

Review Article

Monoclonal antibody as an emerging therapy for acute ischemic stroke

Demi Woods, Qian Jiang, Xiang-Ping Chu

Department of Biomedical Sciences, School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, USA

Received July 13, 2020; Accepted August 20, 2020; Epub August 25, 2020; Published August 30, 2020

Abstract: Acute ischemic stroke (AIS) is the 5th leading cause of death and the leading cause of neurological disability in the United States. The oxygen and glucose deprivation associated with AIS not only leads to neuronal cell death, but also increases the inflammatory response, therefore decreasing the functional outcome of the brain. The only pharmacological intervention approved by the US Federal Food and Drug Administration for treatment of AIS is tissue plasminogen activator (t-PA), however, such treatment can only be given within 4.5 hours of the onset of stroke-like symptoms. This narrow time-range limits its therapeutic application. Administering t-PA outside of the therapeutic window may induce detrimental rather than beneficial effects to stroke patients. In order to reduce the infarct volume of an AIS while increasing the time period for treatment, new treatments are essential. Emerging monoclonal antibody (mAb) therapies reveal great potential by targeting signaling pathways activated after an AIS. With successful application of mAb in the treatment of cancer, other therapeutic uses for mAb are currently being evaluated. In this review, we will focus on recent advances on AIS therapy by using mAb that targets the signaling cascades and endogenous molecules such as inflammation, growth factors, acid-sensing ion channels, and N-methyl-D-aspartate receptors. Therefore, developing specific mAb to target the signaling pathways of ischemic brain injury will benefit patients being treated for an AIS.

Keywords: Acute ischemic stroke, antibody therapy, monoclonal antibody, inflammation, growth factors, acid-sensing ion channel, N-methyl-D-aspartate receptors

Introduction

With more than 15 million people in the world suffering from strokes each year, strokes are an important cause of morbidity and mortality [1]. Strokes can be classified into ischemic or hemorrhagic, in which 85% of strokes are ischemic [1]. Acute ischemic stroke (AIS) is defined as a rapid decrease in blood flow to the brain immediately depriving the brain from oxygen and glucose. The lack in blood supply leads to neuronal and glial cell death with subsequent loss of cerebral function. As the 5th leading cause of death and the leading cause of neurological disability in the United States, novel and effective therapies are critical and warranted [1-3].

Currently, the only pharmacological intervention for AIS treatment is intra-arterial (IA) or intra-venous (IV) tissue plasminogen activator

(t-PA) which acts to dissolve the clot and improve blood flow [1]. IA or IV t-PA was approved by the US Federal Food and Drug Administration (FDA) for AIS therapy. However, t-PA must be given less than 4.5 hours from the onset of symptoms limiting the efficacy of the drug [4, 5]. Aside from the narrow therapeutic window of t-PA, over 50% of patients that receive t-PA acutely post-stroke have significant long-term disability [5]. While t-PA has a narrow therapeutic window, it also has a fatal consequence if administered outside of 4.5 hours [6]. IA or IV t-PA, when not administered at the proper time in the proper dose, can lead to intracerebral hemorrhages which can ultimately lead to death. Therefore, new treatments are necessary in order to improve stroke outcomes in patients that fall outside of t-PA's therapeutic window of 4.5 hours [6]. Here, we reviewed recent advances on emerg-

ing therapies for AIS, with an emphasis on the outcomes associated with using monoclonal antibody (mAb) to combat the neuronal damage associated with AIS.

Inflammation after AIS

Inflammation is one of the major contributing factors worsening the clinical outcome of strokes [7, 8]. Pro-inflammatory cytokines contribute to brain cell death by activating a variety of cells: neurons, astrocytes, microglia, and endothelial cells. This activation leads to neuronal and glial cell death and contributes to the progression of brain injury following an ischemic stroke [7, 8]. After an AIS, the pro-inflammatory cytokines and anti-inflammatory cytokines are activated [8]. For example, tumor necrosis factor- α (TNF- α), interleukin-1, 8 (IL-1, IL-8) and monocyte chemoattraction protein-1 (MCP-1) are pro-inflammatory stroke cytokines that contribute to neuronal cell death, while interleukin-10, 20 (IL-10, IL-20), transforming growth factor- β (TGF- β), and interferon-inducible protein-10 (IP-10) are anti-inflammatory cytokines that are neuroprotective [9-11].

MAB can be administered to block the deleterious pathways associated with inflammation by inhibiting pro-inflammatory cytokines as well as their receptors. For instance, ion channels as well as neurotransmitter receptors are contributing factors to neuronal cell injury after an acute stroke [12]. Generating specific monoclonal antibodies (mAbs) to target specific inflammatory signaling, cell death pathways, ion channels, and neurotransmitter receptors is a promising therapy toward managing the outcome of an acute stroke [12].

Emerging therapies based on neuronal regeneration also provide benefits to patients that are outside of the 4.5-hour time window that t-PA administration requires. For instance, mAb repair therapies can be accessible to a larger patient population because they have a time window of days to weeks compared to hours as seen with t-PA [5, 6, 12]. MAB promotes neuronal repair by binding to receptors and surface markers and function to block or neutralize inhibitors of neuronal cell growth [12]. Three major inhibitors of neuronal cell growth are myelin-associated glycoprotein (MAG), oligo-myelin glycoprotein, and Nogo-A. Following an

acute ischemic stroke, MAG, oligo-myelin glycoprotein, and Nogo-A are upregulated [13, 14]. MAB can be used to block these inhibitors and thus will promote neuronal repair and axonal growth following an acute ischemic stroke [12].

Recently, mAbs have been successfully applied to treat cancer/tumor patients [15-17]. Therefore, it is anticipated that mAbs might be potential therapeutic agents for stroke patients [18, 19]. Most mAbs evaluated for stroke treatment prove effective in reducing infarct volume and improving neuronal performance in animal models of middle cerebral artery occlusion (MCAO) [12]. Although most clinical trials for mAbs on AIS failed right now, however, they are warranted for further studies.

MAB on inflammatory pathways after AIS

Inflammation following damage to tissues begins as an acute process [7, 17]. Inflammatory molecules are upregulated in conditions such as cellular damage, ischemia, hemorrhage, and infections. Inflammatory mediators are responsible for local and systemic effects induced by inflammation [8]. Many molecules are involved in this pathological process. The main molecules responsible for inflammation are cytokines such as ILs and TNF- α , chemokines such as MCP-1, and cellular adhesion molecules [18]. In regards to stroke, inflammatory mediators contribute significantly to the downfall of patient's post-stroke [8]. Following damage to brain parenchyma, cytokines, chemokines, and cellular adhesion molecules released in response to injury create a neurotoxic environment [8].

There are evolving treatments in regards to decreasing inflammation proceeding a stroke. For instance, immunotherapy is not only an effective treatment for targeting cancer cells, but is also under evaluation for blocking the inflammatory response of post-stroke [19, 20]. The results of inflammation as well as the cytokines produced post stroke are the main contributors in the damage that ensues after a stroke [12]. MABs target inflammation and decrease cerebral damage after a stroke [19]. Therefore, the endogenous inflammatory molecules (chemokines, cytokines, and cellular adhesion molecules) are targets of mAb therapy. MABs not only inhibit inflammatory path-

ways and cascades, but also increase the timespan for which reperfusion therapy can be given to treat an AIS [18]. IA or IV t-PA dissolves the fibrin clot responsible for causing an ischemic stroke. However, if given outside of 4.5 hours from the onset of stroke-like symptoms, t-PA has detrimental effects as a result of the accumulation of reactive oxygen species (ROS). Outside of its therapeutic window, t-PA has a significant risk of causing brain hemorrhage and angioedema [4]. However, immunotherapy is an emerging therapy that can widen the therapeutic window of t-PA, allowing t-PA to be administered to a wider spectrum of ischemic stroke patients. Along with increasing the timespan in which t-PA can be administered, immunotherapy is also beneficial in inhibiting inflammation following an acute ischemic stroke and decreasing the infarct volume [19]. Clinical trials utilizing anti-inflammatory agents such as immunotherapy in AIS have only been successful in animal models and have subsequently failed in human stroke models [18, 19]. Human trials may have failed for a variety of reasons. One vital aspect in the lack of reproducibility from animal to human studies is that the stroke was controlled for in animal models. For example, each mouse or rat underwent the exact same procedure to produce a model of middle cerebral artery occlusion (MCAO) and induce an AIS. However, human models uncontrollably have varying causes of strokes to varying degrees of severity. Therefore, a future study is critical to explore the detailed mechanism of strokes in humans. While the outcome of immunotherapy in ischemic strokes has not yet been effective, the combination of multiple therapeutic agents would be desired as an effective treatment for stroke patients [21-23].

In order to reduce inflammation, antibodies are effective molecules to be administered against receptors such as toll-like receptor 4 (TLR4) and adhesion molecules such as α -4 integrin to inhibit the transduction signaling cascades [12]. For example, inhibition of TLR4 via mAb in MCAO mice had beneficial effects in decreasing inflammation. Inflammation was measured based on total infarct volume and brain swelling, both of which decreased after 48 hours in MCAO mice treated with a mAb against TLR4 compared to mice did not get treated with mAb [24].

MAbs against leukocyte adhesion molecules were also evaluated. Specifically, α -4 integrin antibodies were administered against α -4 integrin, a specific leukocyte adhesion molecule [25]. In an experimental model of MCAO mice, mAb administered against α -4 integrin reduced the volume of infarction, however, the study was not successful in human trials for natalizumab. Natalizumab is an antibody formed against leukocyte adhesion molecules, specifically against α -4 integrin. When natalizumab, which was approved by the FDA for the treatment of multiple sclerosis and Crohn's disease, was used in patients with stroke, it did not show a significant reduction of infarct volume which is in contrast to the animal study [25]. Another study using an embolic model of stroke followed by thrombolysis with t-PA was conducted in rabbits [26]. A mAb was directed against a leukocyte adhesion molecule (ICAM-1). Results showed a decrease in inflammatory infiltration, leading to a decrease in neurological damage [26]. Enlimomab is an anti-ICAM-1 mAb, however, the results from clinical trials suggest that enlimomab enhances neutrophil activation and inflammation rather than inhibiting it. Therefore, enlimomab worsens the outcome of AIS [27]. Similar to the lack of reproducibility from animal studies to human studies seen with mAb therapy, further clinical trials are warranted to evaluate mAb as an effective therapeutic treatment of human strokes.

The difference between outcomes of animal models and humans suffering from AIS might be explained by humans and mice/rats having different post-ischemic inflammatory responses. For example, there are potential differences in α -4 integrin expression between species [25]. Another possibility for the lack of reduction of infarct volume with application of mAb in human stroke patients could be attributed to the dose administered. Initially, the study administered 300 mg of natalizumab based on the dosage that is approved as a treatment of multiple sclerosis [25]. The study suggests doses of natalizumab ranging from 450-600 mg might have better clinical outcomes in regards to reduction of infarct volume compared to the original trial of 300 mg [25]. Further clinical trials in which natalizumab is increased from its original 300 mg dosage to 450-600 mg dosage is critical to evaluate this emerging stroke therapy more completely.

Neuronal repair after AIS

Neuronal repair is an important process for restoring the structure and function of neurons in the central nervous system (CNS) following a brain injury [28, 29]. Without neuronal repair and regeneration, stroke patients would not have an opportunity to recover [29]. In comparison to the current therapy of t-PA, neuronal repair therapies are measured in days to weeks and have the potential of benefiting a variety of stroke patients, especially those that fall outside of the narrow therapeutic window of 4.5 hours established by t-PA [12, 30]. In particular, studies examined and evaluated the neuronal repair process after an AIS. Following an AIS, neuronal repair occurs spontaneously and can occur for many years afterwards [29, 30]. Consistent with this idea, animal studies have provided further insight into neuronal repair following an MCAO of AIS. This has led researchers to conclude that the majority of therapies used to promote neuronal repair focus on a single intervention and are thus termed monotherapies [30]. State-of-the-art new techniques such as optogenetics and magnetogenetics are also critical for neuroplasticity after stroke [31-33]. Optogenetics utilizes light to stimulate neuronal activity. In experimental animal models, optogenetic recovery was successful in cloned channelrhodopsin-2 (ChR2) mice [32]. Furthermore, optogenetics stimulated growth-promoting genes in the ChR2 mice. However, the optogenetic light source must be in direct contact with the neuronal tissue. Thus, in order to stimulate deep brain tissue, optogenetics is a rather invasive procedure [32]. Therefore, the use of optogenetics in human models requires further investigation.

A less invasive procedure to stimulate deep brain tissues relies on thermal relaxation techniques. Magnetogenetics utilizes magnetic fields to heat up nanoparticles [33]. Magnetogenetics has been successful in activating a heat-sensitive transient receptor potential vanilloid family member 1 (TRPV1) channel of human embryonic kidney cells. In this study, manganese oxide nanoparticles were stimulated by thermal relaxation, leading to the influx of calcium through the channel [34]. Another example where magnetogenetics was studied was in regards to neuronal stimulation. A neuronal population in the ventral tegmental area of

mice was stimulated, also showing that magnetogenetics can be utilized for deep brain stimulation [35]. Therefore, magnetogenetics is a promising emerging therapy with the potential to stimulate neuronal tissue to allow for regeneration after strokes.

MAB on growth factors for neuronal regeneration after AIS

Growth factors are essential for CNS development and are vital in neuronal repair [30]. Growth factors stimulate angiogenesis, cell proliferation, cell differentiation, cell migration, cell survival, and cellular synaptic plasticity [36-39]. Without the activation of growth factors following an AIS or any CNS injury, neurons and associated blood vessels would not regenerate [40, 41]. A preclinical stroke study suggests that the administration of exogenous growth factors 24 hours prior to the induction of an MCAO in an experimental stroke model has long-term benefit on the behavioral outcome [30]. Specific growth factors utilized in this preclinical study were brain-derived neurotrophic factor, epidermal growth factor, human chorionic gonadotropin, and erythropoietin (Epo) [30, 40, 41]. However, similar to the sparsity of human trials in examining the inflammatory process of a stroke, human trials are also sparse when discussing neuronal regeneration via administering growth factors following a stroke [42].

Preclinical studies that examined the efficacy of Epo in promoting neuronal repair suggest that Epo is beneficial when administered as a monotherapy within 24-48 hours after the onset of the ischemic stroke [43]. Epo was discovered to not only be beneficial but also safe for patients [44]. In a randomized, placebo-controlled study, 167 patients received two doses of Epo and no adverse side effects were reported when comparing Epo versus placebo [43, 44]. However, high dose Epo administered along with t-PA had an increased mortality rate in comparison to the placebo group. As a result of the fatal interaction between Epo and t-PA, further studies are needed to determine if the interaction is dose-dependent or if Epo and t-PA must never be co-administered [43, 44].

Growth factors have been applied for neuronal regeneration in the majority of human trials for patients suffering from an AIS [40, 41]. For

example, hematopoietic growth factors have been used to trigger the neuronal regeneration in an ischemic stroke. In particular, granulocyte-colony stimulating factor (G-CSF) was evaluated in the AX200 for Ischemic Stroke Study (AXIS) [45, 46]. The AXIS study concluded that G-CSF is tolerated when given within 12 hours of an ischemic stroke, however, a meta-analysis conducted after the AXIS study discovered that administration of G-CSF at any time span, ranging from days to years, post-stroke had unfavorable effects [47, 48].

Along with growth factors, mAbs can also be used to promote axonal growth. Specifically, there are three major inhibitors of neuronal regeneration that are targets of such mAbs: myelin-associated glycoprotein (MAG), oligo-myelin glycoprotein, and Nogo-A [12, 48]. Following an ischemic stroke, MAG, oligo-myelin glycoprotein, and Nogo-A are upregulated contributing to the inhibition of neuronal growth and regeneration [49-51].

To examine the importance of neutralizing the inhibitors of neuronal growth mAb GSK-249320 was evaluated. GSK249320 is an IgG1 mAb with a disabled Fc region directed against MAG [52-56]. GSK249320 was tested in experimental stroke models of rats and monkeys. GSK249320 reached the lesion site and improved the functional outcomes when injected 24 hours post-stroke [54, 55]. The results further support the translational potential of this mAb as a restorative therapy for patients with strokes [53, 56]. Not only did this mAb contribute to restoring function in animal studies, but more importantly, mAb GSK249320 is also an emerging therapy for stroke patients. Initially, this mAb was evaluated in healthy volunteers. In this initial study, the GSK249320 did not lead to any clinically significant abnormalities in neurological examinations, nerve conduction tests, quantitative sensory tests, clinical laboratory tests, or electrocardiograms [52]. In regards to side effects in human models, GSK249320 was well tolerated, and no major safety concerns were reported [52]. Recently, one study randomized 42 patients into two groups: placebo group versus the GSK249320 experimental group [56]. To measure an increase in neuronal regeneration, gait velocity was evaluated. Initially, gait velocity showed greater improvement in those that received GSK249320 in comparison to placebo. How-

ever, in a phase IIb double-blind study, it was discovered that two doses of GSK249320 did not improve gait velocity in comparison to placebo [56]. Such results show that although mAb GSK249320 has been proven to increase gait velocity in patients, doubling the dose will actually decrease the efficacy rather than improve outcomes. In addition to improving gait velocity, anti-MAG mAb was also shown to reduce the volume of the lesion when administered at 0.05 µg/mL 1 hour after MCAO in animal models. Animals treated with GSK249320 showed significant improvements in total neurological score at 24 hours, 48 hours, and 7 days after treatment when compared to those of the placebo group. Overall, anti-MAG mAb GSK249320 has been effective in improving gait velocity in humans; however, complete neurological function has yet to be evaluated in human models.

Nogo-A is produced by oligodendrocytes and is another contributing factor to the inhibition of neuronal growth after CNS injury along with MAG [57, 58]. However, the best time to inhibit Nogo-A following an ischemic stroke has not been identified. Nogo-A cannot be inhibited immediately following an ischemic stroke. It has been proven that Nogo-A is initially involved in healing after a stroke. Although it is initially needed for healing, Nogo-A eventually becomes an inhibitor of CNS regrowth. However, the timespan of such events is uncertain [51, 57]. To expand, both Nogo-A knockout mice as well as mice administered anti-Nogo-A antibody following a stroke had increased mortality compared to placebo. MCAO mice that were depleted of Nogo-A had increased mortality after a stroke compared to placebo. This exemplifies the importance of Nogo-A in healing immediately following a stroke. However, administering anti-Nogo-A antibody 7-9 days after MCAO stroke proved beneficial [57]. The administration of anti-Nogo-A a week after MCAO in mice showed increased sprouting, increased neuroplasticity, increased midline crossing of corticorubral axons to the red nucleus of the cerebellum, and new efferent cortical projections [57]. In correlation to the neuronal regeneration in the above study, a study conducted by Wiessner et al. concluded that forepaw function in MCAO mice also improved [58]. In this experiment, purified monoclonal anti-Nogo-A antibody (7B12) was

administered to rats exactly 24 hours after MCAO induction. Forepaw function of rats that had MCAO without 7B12 improved to 40-50% of prelesion levels from weeks 4 to 12 after MCAO. In contrast, the 7B12 experimental group had improved to 40% of prelesion levels at week 4 with 60-70% when evaluated at weeks 7-12 [58]. While administering anti-Nogo-A antibody immediately after MCAO was associated with an increase in mortality, administering it 7-9 days after MCAO increased neuronal regeneration and growth, but administering anti-Nogo-A antibody 24 hours after MCAO improved forepaw function. The different time of administration is associated with drastically different outcomes in animal studies. Thus, more studies are needed in order to experimentally determine the ideal time to administer this antibody as well as to evaluate the outcomes associated with humans.

MAB on acid-sensing ion channels after AIS

Protons have been identified as neurotransmitters in the brain [59]. One of the candidates for sensing protons is acid-sensing ion channels (ASICs). ASICs are highly expressed in both peripheral and central sensory neurons and belong to the degenerin/epithelia Na⁺ channel superfamily [60-66]. ASICs are activated by a decrease in extracellular pH and play a vital role in neuronal cell death after a stroke [67]. Four genes (*ACCN1-4*) encoding at least six ASIC subunits have been cloned. Each subunit has two transmembrane domains with a large extracellular loop and a short intracellular N- and C-termini [67-69]. Structurally, ASICs are trimeric complexes of either identical or different subunits forming into homomeric and/or heteromeric channels respectively [63, 65]. The functional subunits of ASICs reveal distinct electrophysiological and pharmacological properties [68, 69]. For example, homomeric ASIC1a channels have the highest sensitivity with a threshold pH for channel activation at ~7.0 and pH₅₀ around 6.2 to 6.6 [60, 68]. Similar to homomeric ASIC1a, heteromeric ASIC1a/2a channels are activated with a pH drop to slightly below 7.0 with pH₅₀ at ~6.0. In contrast, homomeric ASIC2a channels have the lowest sensitivity to H⁺ with a threshold for channel activation at a pH of 5.5 and pH₅₀ at 4.4 [68]. However, due to the presence of various endogenous modulators, the sensitivity

of ASICs to H⁺ *in vivo* could be dramatically increased [65, 66]. Brain ASICs exist primarily as homomeric ASIC1a and heteromeric ASIC1a/2 channels. However, in a minority of neurons, homomeric ASIC2a channels also exist [68, 70]. All ASICs are inhibited by amiloride, a non-selective ASIC blocker as well as a more selective ASIC blocker A-317567 [70]. In contrast, homomeric ASIC1a and heteromeric ASIC1a/2b channels are inhibited by tarantula toxin *psalmotoxin 1* (PcTx1) [70-73].

Brain ASIC1a contributes to synaptic plasticity, learning/memory, pain and fear conditioning [74, 75]. Both homomeric ASIC1a and heteromeric ASIC1a/2b channels are involved in damage of the brain parenchyma in ischemic strokes [76-86]. In addition to ischemic brain injury, ASICs also play critical roles in neurodegenerative diseases such as multiple sclerosis, Parkinson's disease, Huntington's diseases, seizures, depression and cocaine addiction [67]. Although most ASICs desensitize in the continuous presence of acidosis, the properties of ASICs are subjected to change in pathological conditions [67, 79]. For example, the expression and/or activity of ASICs are dramatically enhanced after peripheral inflammation and global ischemia [67, 79, 82]. Also, ischemic-related signaling molecules such as arachidonic acid and lactate dramatically enhance the activation of ASICs [67, 79, 82]. Thus, ASICs appear to have more important roles in pathological conditions in contrast to their limited physiological functions [67]. Targeting these channels likely has limited side effects [67].

Recently, ASICs have been identified as a potential therapeutic target for ischemic strokes [67, 75, 80, 82-86]. Studies have demonstrated that ASICs contribute to ischemic brain injury [79-82]. During an ischemic stroke, oxygen and glucose deprivation results in anaerobic metabolism. Anaerobic metabolism leads to an accumulation of lactic acids, leading to a decrease in pH. This decrease in neuronal pH subsequently activates neuronal ASICs [79-82]. The activation of ASICs leads to an influx of calcium. Unfortunately, this influx in calcium is a major contributing factor to cell death [67, 79, 80]. Specifically, ASIC1a is the predominant subunit with calcium permeability

in the brain and thus, ASIC1a is a critical therapeutic target for ischemic stroke [67, 79].

A mAb called ASC06-IgG1 has been discovered that specifically blocks ASIC1a [81]. An MCAO stroke was induced in mice, and three hours after ischemia, 4 μ L of phosphate-buffered saline (PBS, vehicle solution) plus 3 μ g/ μ L ASC06-IgG1 were injected intracerebroventricularly. Compared to the control, the group treated with PBS plus ASC06-IgG1 showed a 23% decrease in infarct volume [81]. Such results show that ASICs contribute to neuronal cell death in the brain. Antibodies against ASICs are neuroprotective and might have therapeutic potential in stroke patients [81, 87].

MAb on N-methyl-D-aspartate (NMDA) receptors after AIS

Another vital neurotransmitter in the brain is glutamate. Glutamate is an excitatory neurotransmitter in the brain and plays a critical role in normal physiological functions. For example, glutamate is an essential component in learning/memory and neuronal plasticity [88]. When glutamate levels are off-balance, glutamate can contribute to neurological and psychological diseases [89, 90]. For instance, when glutamate levels are elevated, this can lead to a process known as excitotoxicity and will contribute to cell death [88]. In order to exert its physiological effects, glutamate binds to glutamate receptors. One of the important glutamate receptors in the brain is N-methyl-D-aspartate receptors (NMDARs) [91]. NMDARs are activated during normal physiological processes as well as ischemic stroke conditions. During an ischemic stroke, damaged neuronal tissue releases glutamate into the plasma and cerebrospinal fluid. The rapid increase in glutamate will lead rapid activation of NMDARs [92-96]. Once glutamate binds to NMDARs, there is a subsequent influx of calcium. Similar to how activation ASICs lead to an influx in calcium, the overload of intracellular calcium due to NMDARs activation also contributes to neuronal cell death during an ischemic stroke [92-96].

Because high concentration of glutamate with subsequent activation of NMDARs is a key factor in neuronal cell death, an anti-GluN1 antibody was developed in animal models. Anti-GluN1 antibodies interfere with activated

NMDARs. This mAb decreased the infarct volume after an ischemic stroke was induced in rats. Following an ischemic stroke, disruption of the blood brain barrier (BBB) allows for anti-GluN1 mAb to penetrate the brain via this destructed BBB [97].

Not only do anti-GluN1 antibodies prevent NMDAR-associated calcium influx, but it is also hypothesized that anti-GluN1 antibodies inhibit platelet aggregation [97]. Rats were vaccinated with GluN1 and were evaluated based on the antibodies formed. Various rat anti-GluN1 antibodies were cloned and evaluated based on human platelet aggregation. The study has shown that rats vaccinated with GluN1 have prolonged bleeding while anti-GluN1-S2 antibodies were shown to inhibit platelet activation [97]. By inhibiting platelet aggregation, anti-GluN1-S2 antibodies subsequently decrease the infarct volume in rat studies. However, anti-GluN1-S2 antibodies have not been directly tested in human stroke models [97].

MAb on intracerebral hemorrhage

While a lot of research has been related to mAb in terms of effective therapies for ischemic strokes, they have also been evaluated in intracerebral hemorrhages (ICH) as well. ICH is life-threatening and account for 10-15% of strokes worldwide [98]. When evaluating pro-inflammatory proteins released during ICH, it was discovered that High Mobility Group Box-1 (HMGB1) is a nonhistone proinflammatory DNA-binding protein that is released into the extracellular space following an ICH [98]. Anti-HMGB1 is a mAb directed against HMGB1. This mAb proves promising in decreasing secondary outcomes resulting from ICH [99-102]. For instance, a study conducted in Japan evaluated anti-HMGB1 mAb and its therapeutic benefits for ICH [99]. In this experiment, 0.03U bacterial type IV collagenase was injected into the striatum of rats to induce an ICH. The size of the hematoma was controlled for based on the measure of hemoglobin after 24 hours after ICH. ICH rats were randomly divided into 3 groups. 6 hours after ICH was induced, group 1 was treated with an IV injection of anti-HMGB1 mAb, group 2 received a class-matched control mAb, and the control group of rats received saline instead of type IV collagenase and were injected with saline 6

hours after the initial injection. As a result, group 1 (anti-HMGB1 mAb group) not only had a decrease in the disruption of the BBB, but also had a decrease in the release of inflammatory molecules (TNF- α , iNOS, IL-1, IL-8, COX2, MMP2, MMP9, VEGF) compared to the other groups. The integrity of the BBB was evaluated with Evans blue dye leakage assay 6 hours as well as 3 days after ICH. Anti-HMGB1 mAb group had a lower concentration of dye in the ipsilateral cerebellum, concluding that anti-HMGB1 mAb decreased the damage of BBB in ICH rats. The strength of the BBB is vital to decreasing cerebral edema. If the BBB is severely attenuated, this will lead to the influx of fluids and electrolytes into the brain. Along with evaluating the disruption of the BBB, the extent of oxidative damage was also examined. Group 1 also showed a decrease in oxidative stress compared to the other groups. Oxidative stress was evaluated based on serum bioantioxidant potency. Anti-HMGB1 mAb rats had a decreased serum bioantioxidant potency and thus a decrease in reactive oxygen species (ROS).

To evaluate neurological function, grip strength was tested at baseline and at 6, 24, and 48 hours after ICH. Group 1 had greater improvement in grip strength when compared to initial deficits after ICH. To understand the mechanisms of anti-HMGB1 mAb, Chen et al. have shown that astrocytic HMGB1/IL-6 signaling pathway enhances environment-mediated angiogenesis and functional recovery after ischemic stroke [100]. Overall, anti-HMGB1 mAb has not yet been evaluated in human studies but proves promising as an effective treatment in decreasing inflammation, protecting the BBB, and improving neurological outcomes following an ICH [99-102].

Concluding remarks

While the current pharmacological intervention for patients suffering from ischemic strokes is IA or IV t-PA, the narrow therapeutic window of less than 4.5 hours from the onset of symptoms limits its benefit as a universal stroke treatment [2, 3]. However, recent studies have examined emerging therapies to treat beyond the narrow timeframe of t-PA such as activating growth factors, inhibiting pro-inflammatory cytokines, enhancing anti-inflammatory cytokines, and blocking ion channels and neu-

rotransmitter receptors through mAbs. By increasing the time window allowed for treatment from hours as provided by t-PA to days as allowed with such experimental therapies, a larger patient population will benefit from treatment. The broadened time-span can potentially result in a decrease in the lifelong side effects of acute strokes [3]. For instance, mAbs have the potential to increase neuronal repair and regeneration, ultimately allowing the brain to recover from the stroke more completely. Therefore, developing specific mAb targeting the signaling pathway or cascades of stroke such as inflammation signaling, growth factors, ion channels, and neurotransmitter receptors will reveal a great potential in stroke therapy. Although the mAbs have been effective on experimental stroke models, it is necessary to conduct further clinical research to evaluate the efficacy of such therapies on human stroke patients as well.

Acknowledgements

DW received Sarah Morrison Research Award from the University of Missouri-Kansas City School of Medicine, Missouri, USA. X-PC received the research support from American Heart Association (19AIREA34470007).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiang-Ping Chu, Department of Biomedical Sciences, School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, USA. Tel: +1-816-235-2248; Fax: +1-816-235-6517; E-mail: chux@umkc.edu

References

- [1] Zhou Z, Lu J, Liu WW, Manaenko A, Hou X, Mei Q, Huang JL, Tang J, Zhang JH, Yao H and Hu Q. Advances in stroke pharmacology. *Pharmacol Ther* 2018; 191: 23-42.
- [2] Muñoz Venturelli P, Li X, Middleton S, Watkins C, Lavados PM, Olavarría VV, Brunser A, Pontes-Neto O, Santos TEG, Arima H, Billot L, Hackett ML, Song L, Robinson T and Anderson CS; HEADPOST (Head Positioning in Acute Stroke Trial) Investigators. Impact of evidence-based stroke care on patient outcomes: a multilevel analysis of an international study. *J Am Heart Assoc* 2019; 8: e012640.
- [3] Gomez CR. Time is brain: the stroke theory of relativity. *J Stroke Cerebrovasc Dis* 2018; 27: 2214-2227.

Acute ischemic stroke and monoclonal antibody

- [4] Siniscalchi A, Iannacchero R, Anticoli S, Romana Pezzella F, De Sarro G and Gallelli L. Anti-inflammatory strategies in stroke: a potential therapeutic target. *Curr Vasc Pharmacol* 2016; 14: 98-105.
- [5] Zafar M, Memon RS, Mussa M, Merchant R, Khurshid A and Khosa F. Does the administration of sonothrombolysis along with tissue plasminogen activator improve outcomes in acute ischemic stroke? A systematic review and meta-analysis. *J Thromb Thrombolysis* 2019; 48: 203-208.
- [6] Urdaneta AE and Bhalla P. Cutting edge acute ischemic stroke management. *Emerg Med Clin North Am* 2019; 37: 365-379.
- [7] Shi K, Tian DC, Li ZG, Ducruet AF, Lawton MT and Shi FD. Global brain inflammation in stroke. *Lancet Neurol* 2019; 18: 1058-1066.
- [8] Jayaraj RL, Azimullah S, Beiram R, Jalal FY and Rosenberg GA. Neuroinflammation: friend and foe for ischemic stroke. *J Neuroinflammation* 2019; 16: 142.
- [9] McCann SK, Cramond F, Macleod MR and Sena ES. Systematic review and meta-analysis of the efficacy of interleukin-1 receptor antagonist in animal models of stroke: an update. *Transl Stroke Res* 2016; 7: 395-406.
- [10] Sobowale OA, Parry-Jones AR, Smith CJ, Tyrrell PJ, Rothwell NJ and Allan SM. Interleukin-1 in stroke: from bench to bedside. *Stroke* 2016; 47: 2160-2167.
- [11] Van der Spuy WJ and Pretorius E. Interrelation between inflammation, thrombosis, and neuroprotection in cerebral ischemia. *Rev Neurosci* 2012; 23: 269-278.
- [12] Yu CY, Ng G and Liao P. Therapeutic antibodies in stroke. *Transl Stroke Res* 2013; 4: 477-483.
- [13] Li S and Carmichael ST. Growth-associated gene and protein expression in the region of axonal sprouting in the aged brain after stroke. *Neurobiol Dis* 2006; 23: 362-373.
- [14] Cheatwood JL, Emerick AJ, Schwab ME and Kartje GL. Nogo-A expression after focal ischemic stroke in the adult rat. *Stroke* 2008; 39: 2091-2098.
- [15] Barbier L, Declerck P, Simoens S, Neven P, Vulto AG and Huys I. The arrival of biosimilar monoclonal antibodies in oncology: clinical studies for trastuzumab biosimilars. *Br J Cancer* 2019; 121: 199-210.
- [16] Mahat U, Matar RB and Rotz SJ. Use of complement monoclonal antibody eculizumab in shiga toxin producing escherichia coli associated hemolytic uremic syndrome: a review of current evidence. *Pediatr Blood Cancer* 2019; 66: e27913.
- [17] Hong H, Wang Q, Li J, Liu H, Meng X and Zhang H. Aging, cancer and immunity. *J Cancer* 2019; 10: 3021-3027.
- [18] Nilupul Perera M, Ma HK, Arakawa S, Howells DW, Markus R, Rowe CC and Donnan GA. Inflammation following stroke. *J Clin Neurosci* 2006; 13: 1-8.
- [19] Malone K, Amu S, Moore AC and Waeber C. Immunomodulatory therapeutic strategies in stroke. *Front Pharmacol* 2019; 10: 630.
- [20] Planes-Laine G, Rochigneux P, Bertucci F, Chrétien AS, Viens P, Sabatier R and Goncalves A. PD-1/PD-L1 targeting in breast cancer: the first clinical evidences are emerging. a literature review. *Cancers (Basel)* 2019; 11: 1033.
- [21] Posada-Duque RA, Barreto GE and Cardona-Gomez GP. Protection after stroke: cellular effectors of neurovascular unit integrity. *Front Cell Neurosci* 2014; 8: 231.
- [22] Stoll G and Nieswandt B. Thrombo-inflammation in acute ischaemic stroke - implications for treatment. *Nat Rev Neurol* 2019; 15: 473-481.
- [23] Dillen Y, Kemps H, Gervois P, Wolfs E and Bronckaers A. Adult neurogenesis in the subventricular zone and its regulation after ischemic stroke: implications for therapeutic approaches. *Transl Stroke Res* 2020; 11: 60-79.
- [24] Andresen L, Theodorou K, Grünewald S, Czech-Zechmeister B, Könnecke B, Lühder F and Trendelenburg G. Evaluation of the therapeutic potential of anti-TLR4-antibody MTS510 in experimental stroke and significance of different routes of application. *PLoS One* 2016; 11: e0148428.
- [25] Elkins J, Veltkamp R, Montaner J, Johnston SC, Singhal AB, Becker K, Lansberg MG, Tang W, Chang I, Muralidharan K, Gheuens S, Mehta L and Elkind MSV. Safety and efficacy of natalizumab in patients with acute ischaemic stroke (ACTION): a randomised, placebo-controlled, double-blind phase 2 trial. *Lancet Neurol* 2017; 16: 217-226.
- [26] Bowes MP, Zivin JA and Rothlein R. Monoclonal antibody to the ICAM-1 adhesion site reduces neurological damage in a rabbit cerebral embolism stroke model. *Exp Neurol* 1993; 119: 215-219.
- [27] Becker BJ. Anti-leukocyte antibodies: Leuk-Arrest (Hu23F2G) and Enlimomab (R6.5) in acute stroke. *Curr Med Res Opin* 2002; 18 Suppl 2: s18-22.
- [28] Jia W, Kamen Y, Pivonkova H and Káradóttir RT. Neuronal activity-dependent myelin repair after stroke. *Neurosci Lett* 2019; 703: 139-144.
- [29] Sakai S and Shichita T. Inflammation and neural repair after ischemic brain injury. *Neurochem Int* 2018; 130: 104316.
- [30] Belayev L, Khoutorova L, Zhao KL, Davidoff AW, Moore AF and Cramer SC. A novel neurotrophic

Acute ischemic stroke and monoclonal antibody

- therapeutic strategy for experimental stroke. *Brain Res* 2009; 1280: 117-123.
- [31] Wahl AS. State-of-the-Art techniques to causally link neural plasticity to functional recovery in experimental stroke research. *Neural Plast* 2018; 2018: 3846593.
- [32] Cheng MY, Wang EH, Woodson WJ, Wang S, Sun G, Lee AG, Arac A, Fenno LE, Deisseroth K and Steinberg GK. Optogenetic neuronal stimulation promotes functional recovery after stroke. *Proc Natl Acad Sci U S A* 2014; 111: 12913-12918.
- [33] Nimpf S and Keays DA. Is magnetogenetics the new optogenetics? *EMBO J* 2017; 36: 1643-1646.
- [34] Huang H, Delikanli S, Zeng H, Ferkey DM and Pralle A. Remote control of ion channels and neurons through magnetic-field heating of nanoparticles. *Nat Nanotechnol* 2010; 5: 602-606.
- [35] Chen R, Romero G, Christiansen MG, Mohr A and Anikeeva P. Wireless magnetothermal deep brain stimulation. *Science* 2015; 347: 1477-1480.
- [36] Rust R, Grönnert L, Weber RZ, Mulders G and Schwab ME. Refueling the ischemic CNS: guidance molecules for vascular repair. *Trends Neurosci* 2019; 42: 644-656.
- [37] Kumar P, Kumar A, Srivastava MK, Misra S, Pandit AK and Prasad K. Association of transforming growth factor Beta-1-509C/T gene polymorphism with ischemic stroke: a meta analysis. *Basic Clin Neurosci* 2016; 7: 91-96.
- [38] Lanfranconi S, Locatelli F, Corti S, Candelise L, Comi GP, Baron PL, Strazzer S, Bresolin N and Bersano A. Growth factors in ischemic stroke. *J Cell Mol Med* 2011; 15:1645-1687.
- [39] Oliveira SL, Pillat MM, Cheffer A, Lameu C, Schwindt TT and Ulrich H. Functions of neurotrophins and growth factors in neurogenesis and brain repair. *Cytometry A* 2013; 83: 76-89.
- [40] Ren JM and Finklestein SP. Growth factor treatment of stroke. *Curr Drug Targets CNS Neurol Disord* 2005; 4: 121-125.
- [41] Kolb B, Morshead C, Gonzalez C, Kim M, Gregg C, Shingo T and Weiss S. Growth factor-stimulated generation of new cortical tissue and functional recovery after stroke damage to the motor cortex of rats. *J Cereb Blood Flow Metab* 2007; 27: 983-987.
- [42] Spiliopoulos S, Festas G, Reppas L and Brontzos E. Intra-arterial administration of cell-based biological agents for ischemic stroke therapy. *Expert Opin Biol Ther* 2019; 19: 249-259.
- [43] Jerndal M, Forsberg K, Sena ES, Macleod MR, O'Collins VE, Linden T, Nilsson M and Howells DW. A systematic review and meta-analysis of erythropoietin in experimental stroke. *J Cereb Blood Flow Metab* 2010; 30: 961-968.
- [44] Yip HK, Tsai TH, Lin HS, Chen SF, Sun CK, Leu S, Yuen CM, Tan TY, Lan MY, Liou CW, Lu CH and Chang WN. Effect of erythropoietin on level of circulating endothelial progenitor cells and outcome in patients after acute ischemic stroke. *Crit Care* 2011; 15: R40.
- [45] Schäbitz WR, Laage R, Vogt G, Koch W, Kollmar R, Schwab S, Schneider D, Hamann GF, Rosenkranz M, Veltkamp R, Fiebach JB, Hacke W, Grotta JC, Fisher M and Schneider A. AXIS: a trial of intravenous granulocyte colony stimulating factor in acute ischemic stroke. *Stroke* 2010; 41: 2545-2551.
- [46] England TJ, Abaei M, Auer DP, Lowe J, Jones DR, Sare G, Walker M and Bath PM. Granulocyte-colony stimulating factor for mobilizing bone marrow stem cells in subacute stroke: the stem cell trial of recovery enhancement after stroke 2 randomized controlled trial. *Stroke* 2012; 43: 405-411.
- [47] Ringelstein EB, Thijs V, Norrving B, Chamorro A, Aichner F, Grond M, Saver J, Laage R, Schneider A, Rathgeb F, Vogt G, Charissé G, Fiebach JB, Schwab S, Schäbitz WR, Kollmar R, Fisher M, Brozman M, Skoloudik D, Gruber F, Serena Leal J, Veltkamp R, Köhrmann M and Berrouschot J; AXIS 2 Investigators. Granulocyte colony-stimulating factor in patients with acute ischemic stroke: results of the AX200 for ischemic stroke trial. *Stroke* 2013; 44: 2681-2687.
- [48] Domeniconi M and Filbin MT. Overcoming inhibitors in myelin to promote axonal regeneration. *J Neurol Sci* 2005; 233: 43-47.
- [49] England TJ, Sprigg N, Alasheev AM, Belkin AA, Kumar A, Prasad K and Bath PM. Granulocyte-colony stimulating factor (G-CSF) for stroke: an individual patient data meta-analysis. *Sci Rep* 2016; 6: 36567.
- [50] Irving EA, Vinson M, Rosin C, Roberts JC, Chapman DM, Facci L, Virley DJ, Skaper SD, Burbidge SA, Walsh FS, Hunter AJ and Parsons AA. Identification of neuroprotective properties of anti-MAG antibody: a novel approach for the treatment of stroke? *J Cereb Blood Flow Metab* 2005; 25: 98-107.
- [51] Buchli AD and Schwab ME. Inhibition of Nogo: a key strategy to increase regeneration, plasticity and functional recovery of the lesioned central nervous system. *Ann Med* 2005; 37: 556-567.
- [52] Abila B, Cunningham E and Simeoni M. First-time-in-human study with GSK249320, a myelin-associated glycoprotein inhibitor, in healthy volunteers. *Clin Pharmacol Ther* 2013; 93: 163-169.
- [53] Cramer SC, Abila B, Scott NE, Simeoni M and Enney LA. MAG111539 study investigators. Safety, pharmacokinetics, and pharmacodynamics of escalating repeat doses of

Acute ischemic stroke and monoclonal antibody

- GSK249320 in patients with stroke. *Stroke* 2013; 44: 1337-1342.
- [54] Barbay S, Plautz EJ, Zoubina E, Frost SB, Cramer SC and Nudo RJ. Effects of postinfarct myelin-associated glycoprotein antibody treatment on motor recovery and motor map plasticity in squirrel monkeys. *Stroke* 2015; 46: 1620-1625.
- [55] Cash D, Easton AC, Mesquita M, Beech J, Williams S, Lloyd A, Irving E and Cramer SC. GSK249320, a monoclonal antibody against the axon outgrowth inhibition molecule myelin-associated glycoprotein, improves outcome of rodents with experimental stroke. *J Neurol Exp Neurosci* 2016; 2: 28-33.
- [56] Cramer SC, Enney LA, Russell CK, Simeoni M and Thompson TR. Proof-of-concept randomized trial of the monoclonal antibody GSK-249320 versus placebo in stroke patients. *Stroke* 2017; 48: 692-698.
- [57] Sui YP, Zhang XX, Lu JL and Sui F. New insights into the roles of Nogo-A in CNS biology and diseases. *Neurochem Res* 2015; 40: 1767-1785.
- [58] Wiessner C, Bareyre FM, Allegrini PR, Mir AK, Frenzel S, Zurini M, Schnell L, Oertle T and Schwab ME. Anti-Nogo-A antibody infusion 24 hours after experimental stroke improved behavioral outcome and corticospinal plasticity in normotensive and spontaneously hypertensive rats. *J Cereb Blood Flow Metab* 2003; 23: 154-165.
- [59] Du J, Reznikov LR, Price MP, Zha XM, Lu Y, Moninger TO, Wemmie JA and Welsh MJ. Protons are a neurotransmitter that regulates synaptic plasticity in the lateral amygdala. *Proc Natl Acad Sci U S A* 2014; 111: 8961-8966.
- [60] Waldmann R, Champigny G, Bassilana F, Heurteaux C and Lazdunski M. A protogated cation channel involved in acid-sensing. *Nature* 1997; 386: 173-177.
- [61] Krishtal O. Receptor for protons: first observations on acid sensing ion channels. *Neuropharmacology* 2015; 94: 4-8.
- [62] Uchitel OD, González Inchauspe C and Weissmann C. Synaptic signals mediated by protons and acid sensing ion channels. *Synapse* 2019; 73: e22120.
- [63] Boscardin E, Alijevic O, Hummler E, Frateschi S and Kellenberger S. The function and regulation of acid-sensing ion channels (ASICs) and the epithelial Na⁽⁺⁾ channel (ENaC): IUPHAR review 19. *Br J Pharmacol* 2016; 173: 2671-2701.
- [64] Kellenberger S and Schild L. International union of basic and clinical pharmacology. XCI. Structure, function, and pharmacology. *Pharmacol Rev* 2015; 67: 1-35.
- [65] Vullo S and Kellenberger S. A molecular view of the function and pharmacology of acid-sensing ion channels. *Pharmacol Res* 2020; 154: 104166.
- [66] Soto E, Ortega-Ramírez A and Vega R. Protons as messengers of intercellular communication in the nervous system. *Front Cell Neurosci* 2018; 12: 342.
- [67] Chu XP, Grasing KA and Wang JQ. Acid-sensing ion channels contribute to neurotoxicity. *Transl Stroke Res* 2014; 5: 69-78.
- [68] Gründer S and Pusch M. Biophysical properties of acid-sensing ion channels (ASICs). *Neuropharmacology* 2015; 94: 9-18.
- [69] Tikhonov DB, Magazanik LG and Nagaeva EI. Ligands of acid-sensing ion channel 1a: mechanisms of action and binding sites. *Acta Naturae* 2019; 11: 4-13.
- [70] Baron A and Lingueglia E. Pharmacology of acid-sensing ion channels - physiological and therapeutic perspectives. *Neuropharmacology* 2015; 94: 19-35.
- [71] Rash LD. Acid-sensing ion channel pharmacology, past, present, and future. *Adv Pharmacol* 2017; 79: 35-66.
- [72] Cristofori-Armstrong B and Rash LD. Acid-sensing ion channel (ASIC) structure and function: insights from spider, snake and sea anemone venoms. *Neuropharmacology* 2017; 127: 173-184.
- [73] Vick JS and Askwith CC. ASICs and neuropeptides. *Neuropharmacology* 2015; 94: 36-41.
- [74] Li MH, Inoue K, Si HF and Xiong ZG. Calcium-permeable ion channels involved in glutamate receptor-independent ischemic brain injury. *Acta Pharmacol Sin* 2011; 32: 734-740.
- [75] Huang Y, Jiang N, Li J, Ji YH, Xiong ZG and Zha XM. Two aspects of ASIC function: synaptic plasticity and neuronal injury. *Neuropharmacology* 2015; 94: 42-48.
- [76] Wemmie JA, Taugher RJ and Kreple CJ. Acid-sensing ion channels in pain and disease. *Nat Rev Neurosci* 2013; 14: 461-471.
- [77] Deval E and Lingueglia E. Acid-sensing ion channels and nociception in the peripheral and central nervous systems. *Neuropharmacology* 2015; 94: 49-57.
- [78] Santos PL, Guimarães AG, Barreto RS, Serafini MR, Quintans JS and Quintans-Júnior LJ. A review of recent patents on the ASICs as a key drug target. *Recent Pat Biotechnol* 2015; 9: 30-41.
- [79] Xiong ZG, Zhu XM, Chu XP, Minami M, Hey J, Wei WL, MacDonald JF, Wemmie JA, Price MP, Welsh MJ and Simon RP. Neuroprotection in ischemia: blocking calcium-permeable acid-sensing ion channels. *Cell* 2004; 118: 687-698.
- [80] Wang YZ, Wang JJ, Huang Y, Liu F, Zeng WZ, Li Y, Xiong ZG, Zhu MX and Xu TL. Tissue acidosis induces neuronal necroptosis via ASIC1a

Acute ischemic stroke and monoclonal antibody

- channel independent of its ionic conduction. *Elife* 2015; 4: e05682.
- [81] Qiang M, Dong X, Zha Z, Zuo XK, Song XL, Zhao L, Yuan C, Huang C, Tao P, Hu Q, Li WG, Hu W, Li J, Nie Y, Buratto D, Zonta F, Ma P, Yu Z, Liu L, Zhang Y, Yang B, Xie J, Xu TL, Qu Z, Yang G and Lerner RA. Selection of an ASIC1a-blocking combinatorial antibody that protects cells from ischemic death. *Proc Natl Acad Sci U S A* 2018; 115: E7469-E7477.
- [82] Jiang N, Wu J, Leng T, Yang T, Zhou Y, Jiang Q, Wang B, Hu Y, Ji YH, Simon RP, Chu XP, Xiong ZG and Zha XM. Region specific contribution of ASIC2 to acidosis-and ischemia-induced neuronal injury. *J Cereb Blood Flow Metab* 2017; 37: 528-540.
- [83] Liu S, Cheng XY, Wang F and Liu CF. Acid-sensing ion channels: potential therapeutic targets for neurodegenerative diseases. *Transl Neurodegener* 2015; 4: 10.
- [84] Leng T, Shi Y, Xiong ZG and Sun D. Proton-sensitive cation channels and ion exchangers in ischemic brain injury: new therapeutic targets for stroke? *Prog Neurobiol* 2014; 115: 189-209.
- [85] Wang JJ and Xu TL. Acid-sensing ion channels as a target for neuroprotection: acidotoxicity revisited. *Sheng Li Xue Bao* 2016; 68: 403-413.
- [86] Dibas J, Al-Saad H and Dibas A. Basics on the use of acid-sensing ion channels' inhibitors as therapeutics. *Neural Regen Res* 2019; 14: 395-398.
- [87] Peterson A, Jiang Q and Chu XP. Commentary: potential therapeutic consequences of an acid-sensing ion channel 1a-blocking antibody. *Front Pharmacol* 2019; 10: 954.
- [88] Zhou Y and Danbolt NC. Glutamate as a neurotransmitter in the healthy brain. *J Neural Transm* 2014; 121: 799-817.
- [89] Dong X, Wang Y and Qin Z. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacol Sin* 2009; 30: 379-387.
- [90] Grueter BA, Rothwell PE and Malenka RC. Integrating synaptic plasticity and striatal circuit function in addiction. *Curr Opin Neurobiol* 2012; 22: 545-551.
- [91] Dingledine R, Borges K, Bowie D and Traynelis SF. The glutamate receptor ion channels. *Pharmacol Rev* 1999; 51: 7-61.
- [92] Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ and Dingledine R. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev* 2010; 62: 405-496.
- [93] Paoletti P, Bellone C and Zhou Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nat Rev Neurosci* 2013; 14: 383-400.
- [94] Szydłowska K and Tymianski M. Calcium, ischemia and excitotoxicity. *Cell Calcium* 2010; 47: 122-129.
- [95] Forder JP and Tymianski M. Postsynaptic mechanisms of excitotoxicity: involvement of postsynaptic density proteins, radicals, and oxidant molecules. *Neuroscience* 2009; 158: 293-300.
- [96] During MJ, Symes CW, Lawlor PA, Lin J, Dunning J, Fitzsimons HL, Poulsen D, Leone P, Xu R, Dicker BL, Lipski J and Young D. An oral vaccine against NMDAR1 with efficacy in experimental stroke and epilepsy. *Science* 2000; 287: 1453-1460.
- [97] Green TN, Hamilton JR, Morel-Kopp MC, Zheng Z, Chen TT, Hearn JI, Sun PP, Flanagan JU, Young D, Barber PA, During MJ, Ward CM and Kaley-Zylinska ML. Inhibition of NMDA receptor function with an anti-GluN1-S2 antibody impairs human platelet function and thrombosis. *Platelets* 2017; 28: 799-811.
- [98] Qureshi AI, Mendelow AD and Hanley DF. Intracerebral haemorrhage. *Lancet* 2009; 373: 1632-1644.
- [99] Wang D, Liu K, Wake H, Teshigawara K, Mori S and Nishibori M. Anti-high mobility group box-1 (HMGB1) antibody inhibits hemorrhage-induced brain injury and improved neurological deficits in rats. *Sci Rep* 2017; 7: 46243.
- [100] Halder SK and Ueda H. Amlexanox inhibits cerebral ischemia-induced delayed astrocytic high-mobility group box 1 release and subsequent brain damage. *Pharmacol Exp Ther* 2018; 365: 27-36.
- [101] Chen JY, Yu Y, Yuan Y, Zhang YJ, Fan XP, Yuan SY, Zhang JC and Yao SL. Enriched housing promotes post-stroke functional recovery through astrocytic HMGB1-IL-6-mediated angiogenesis. *Cell Death Discov* 2017; 3: 17054.
- [102] Nishibori M, Mori S and Takahashi HK. Anti-HMGB1 monoclonal antibody therapy for a wide range of CNS and PNS diseases. *J Pharmacol Sci* 2019; 140: 94-101.