We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Pharmacological Treatment of Acute Ischemic Stroke

Humberto Mestre, Yael Cohen-Minian,
Daniel Zajarias-Fainsod and Antonio Ibarra

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/53774>

1. Introduction

Cerebral infarction, generally referred to as stroke for practical purposes henceforth, is a medical emergency that generates severe neurological deficits while compromising cardiovascular and respiratory function. Each year approximately 795,000 people experience a new or recurrent stroke. This disease can be differentiated into two subcategories: hemorrhagic and ischemic. The ischemic subgroup is responsible for up to 87% of all strokes [1]. This pathology is of critical importance to healthcare professionals due to the fact that every 40 seconds someone in the United States suffers a stroke [2]. Moreover, it is the fourth leading cause of death in the US where 1 in every 18 deaths are stroke-related [2]. However, the mortality rate of stroke is relatively low at 8.1% according to the most recent accepted statistics [2]. In consequence, stroke is the leading cause of disability in the United States. Besides the direct effect that stroke has on the economy (US \$18.8 billion), it will indirectly generate an expenditure of US \$2.21 trillion from now until 2050, on account of loss of earnings resulting from the 26% of patients who suffer from a stroke that require assistance with activities in daily living or institutionalization in nursing homes [2]. The epidemiological and economic impact that stroke has on society demands the development of an effective treatment strategy during the acute ischemic phase. Current therapies have been primarily aimed at the four cornerstones of acute ischemic stroke (AIS): (1) the prevention and treatment of secondary complications; (2) reperfusion strategies directed at arterial recanalization; (3) neuroprotective strategies aimed at cellular and metabolic targets; and (4) the inhibition or modulation of the inflammatory response. To date, the mainstay of treatment is arterial recanalization with recombinant tissue plasminogen activator (rtPA), in conjunction with the early onset of an aspirin regimen. It must be noted, however, that the great majority of stroke patients are not eligible for thrombolysis with only around 5% receiving rtPA [3-5]; mainly because the use of intravenous rtPA has many contraindications, a limited time window, and a moderate success rate. Most

patients presenting to hospital stroke units have either a contraindication to rtPA therapy (e.g. a bleeding diathesis, recent surgery, etc.) or, more commonly, are no longer within the time frame for thrombolytic therapy. Although many initiatives to find therapies that will target the other facets of AIS have been undertaken, most have failed. One area of particular interest is that of neuroprotection. Several attempts to generate a neuroprotective drug that will reduce ischemia-associated destruction of neuronal tissue improving the general outcome after AIS have had dismal results. These drugs display a formidable benefit during the animal model phase of research but have been unable to reproduce this effect in human clinical trials. These interventions are aimed at treating stroke in its acute phases and preventing sequels that will result in permanent disability. The ideal treatment of AIS begets a multistep approach: necessary due to the fact that the pathophysiology of stroke is multi-mechanistic. This work will present the current status of drug therapy in AIS and analyze the direction in which the field is moving. The aim of this review is to guide the reader through a general panorama of interventional pharmacological treatment of AIS.

Figure 1. Schematic visualization of the ideal therapeutic approach towards AIS. Due to the notorious failure of interventional stroke research throughout the years it is essential for the field to reboot its attempts and abandon the search for dubiously named “wonder drugs”. The optimal treatment for AIS will lie on effective prevention and if the pathology cannot be prevented it will depend on an integral management. This therapeutic strategy must incorporate and address all of the cornerstones of AIS.

2. Etiology

Stroke is not only a multifactorial disease but also a gamma of different pathologies with markedly varied etiology that manifest themselves in a clinically similar way. For this reason, the accurate diagnosis of the stroke patient involves not only differentiating a stroke from other diseases with comparable clinical features, but also determining the type of stroke and its etiology.

Stroke can be classified as ischemic or hemorrhagic. The latter implies the rupture of intracranial vessels leading, in a very generalized sense, to mass effect, compression, and inflammation leading to neuronal death. The present chapter will be devoted entirely to the pathology that is an acute ischemic stroke (AIS) and the treatment guidelines currently in use as well as novel science in this field. In an effort to appropriately describe the etiology of AIS it is first necessary to explain the different origins of the ischemia, namely: cardioembolic, atheroembolic, atherothrombotic and miscellaneous.

Cardioembolic stroke is the most common and is characterized by the formation of a clot within the cardiac chambers that is ejected and travels peripherally where it finally encounters and lodges in a vessel of sufficiently small caliber obstructing blood flow distally. These emboli are due to numerous pathologies however, the great majority, approximately 75%, are due to atrial fibrillation (AF) [6]. Patients with AF have increased blood residence time in the left atrium; in those who are not adequately anticoagulated, platelet aggregation and coagulation may occur within the atrium. Typically, when a patient with AF is cardioverted to sinus rhythm the ejection fraction from the atria improves substantially increasing the probability that an existing latent thrombus may be expelled into the aorta. Since the common carotid arteries—and consequently the internal carotid arteries—are the most direct path, these emboli usually travel into the cerebral vasculature where, upon obstructing irrigation to brain tissue, cause an acute ischemic stroke. Other, less typical, causes of cardioembolic stroke include emboli originating from thrombi forming on prosthetic or diseased heart valves, cardiac myxomas, vegetations secondary to infectious endocarditis, among others, as well as the direct shunting of venous thrombi to the systemic arterial vasculature by means of a patent foramen ovale.

Although, atheroembolic stroke has a clinical picture akin to that of cardioembolic stroke, the etiology is substantially different. Patients with atheromatous plaques in the ascending aorta, the arteries of the head and neck, or its tributaries, have damaged and reactive endothelial cells in these vessels with exposed tissue factor, etc. This predisposes to the formation of unstable thrombi in these regions. In certain circumstances, particularly during a valsalva maneuver—usually associated with exertion or straining—the friable thrombus fractures releasing an embolus which travels upstream and becomes embedded in the cerebral vasculature. Likewise, atheromatous plaques may rupture releasing a gelatinous cholesterol based substance, which can also cause the embolization of the smaller arteries supplying the brain. Atheroembolic stroke is particularly common in patients with dyslipidemias and is associated with low levels of high-density lipoprotein (HDL) and high levels of low-density lipoproteins (LDL).

In contrast, atherothrombotic stroke predominates in those with dyslipidemia and comorbid pathologies including systemic arterial hypertension and diabetes mellitus. In both diseases

affliction of the smaller cerebral vessels beyond the first bifurcations after the circle of Willis is more common rather than before the anastomosis at the same level as is typical of dyslipidemias. Arterial hypertension causes damage to the endothelium and also hypertrophy of the medial muscular layer of the vessels leading to marked stenosis. Conversely, diabetes leads to angiopathy of both the macrovasculature and microvasculature [7]. Although the microvascular disease associated with hyperglycemia is a recognized factor in the development of generalized brain ischemia it is a chronic degenerative disease rather than a precipitant of acute ischemia. The macrovascular pathology associated with diabetes is less well understood; notwithstanding, the correlation between increased stroke risk and diabetes mellitus is quite established. The mechanism is believed to be multifactorial, probably due to the associated metabolic syndrome, which involves the triad of dyslipidemia, hyperglycemia and hypertension leading to endothelial dysfunction, hypercoagulability and atheroma: all significant stroke factors [8]. The clinical picture of atherothrombotic stroke is gradual in stark contrast to embolic-type strokes and is characterized by repeated transient ischemic attacks (TIAs). The pathogenesis involves the gradual development of atheromatous plaques in the medium caliber arteries of the cerebral vasculature, namely the anterior cerebral, the middle cerebral and the posterior cerebral arteries. Thrombus formation takes place in these dysfunctional vessel walls and the lumen becomes reduced. It is unlikely that the lumen will become completely obliterated through this process, however the unstable thrombus often shifts, briefly obstructing the irrigation upstream leading to a TIA or a stroke in progress. Eventually the obstruction is longer lasting leading to widespread or more permanent damage characteristic of a completed stroke.

It is worth mentioning a fourth miscellaneous category, which groups all other causes of ischemic stroke. Among the notable causes are dissections of neck or thoracic arteries leading to a loss of perfusion to the brain. Moreover, pulmonary thrombosis similarly obstructs blood return to the left heart and leads to brain ischemia. Non-thrombotic emboli, such as air, fat or of tumoral origin can likewise lead to an AIS. These causes are relatively rare and together account for less than 5% of AIS; nevertheless, the clinician should always consider investigating these as plausible causes when determining the origin of an atypical case [2]. The present review shall, nonetheless omit further reference to miscellaneous causes of stroke.

Due to the marked difference in etiology and pathogenesis of these first three types of AIS, it is wise to emphasize the necessity of an accurate diagnosis in order to specifically target therapy to the cause of the stroke in an effort to minimize tissue damage and reduce the risk of further strokes.

3. Risk factors

As previously mentioned AIS is a multifactorial and polycasative pathology and as such, various factors interact to increase the risk of a stroke. However, research in this field has led to the statistical determination of the influence of some of the most common risk factors in an effort to prevent this all-too-common disease. Systemic arterial hypertension (HTN)

is a well-recognized factor leading to stroke and is associated with a two-fold lifetime risk of stroke. However, clarification of this statistic is necessary as HTN, although a strong risk factor for ischemic atherothrombotic stroke as mentioned above, is more often linked to hemorrhagic type strokes. Heart arrhythmias such as AF in particular, have a relative risk for stroke of 5 and thus account for the great majority of cardioembolic stroke with nearly 25% of all strokes in 80-year olds and above being attributable to AF [2]. Smoking is perhaps the most important modifiable risk factor. Smokers are two to four times more likely to suffer a stroke, not to mention have a higher morbidity and mortality rate than non-smokers after a stroke [2]. Moreover, the risk is dose dependent and upon cessation of smoking the risk rapidly falls and after 20 years the risk is nearly that of a person who has never smoked [2]. Additionally, a sedentary lifestyle is associated with a relative risk for stroke of around 3 in contrast to a relative risk of less than 1 for persons who regularly exercise [2]. Other somewhat modifiable risk factors include metabolic disorders: dyslipidemias being a major contributor to stroke risk as they promote the formation of atheroma and cause hematologic disturbances. Diabetes mellitus—with its associated complications—obviously contributes and is a major risk factor; sleep apnea has a two-fold to four-fold risk of stroke depending on the severity of the apnea [2].

Clearly, having knowledge of these risk factors is important when making an effort directed at eliminating risk factors or reducing their impact as a means of prevention

4. Pathophysiology

The core of AIS pathophysiology is the complete interruption of cerebral blood flow (CBF) leading to energy depletion and oxygen starvation with necrotic neuronal death within the first couple of minutes. Modern day therapeutic strategies are aimed at arterial recanalization in order to reestablish CBF. This rapid return to normal CBF is the turning point in salvaging the surrounding tissue of the focal necrotic core. This area lies above the threshold of cell death and below functional levels of CBF, and is commonly known as the penumbra. The penumbra is the principal target of all pharmacological treatments in AIS. The goal of AIS therapeutics is a strategy that will encompass the four cornerstones previously mentioned. The first step depends on the prompt diagnosis of AIS and the treatment aimed at preventing and treating secondary complications of the disease. The second is a fast and effective recanalization of the occluded vessel (i.e. thrombolysis) in order to ameliorate the hypoperfusion of the penumbra. Drugs that target arterial recanalization have the goal of quickly reestablishing CBF and alleviating this area of ischemia allowing the tissue to return to homeostasis. Third, are specific neuroprotective strategies that will intervene in the apoptotic cascade, excitotoxicity, reactive oxygen species (ROS) production and lipid peroxidation further protecting the ischemic tissue or reversing its damage. This branch of interventional AIS research is aimed at discovering compounds that will allow the neural tissue to better survive this period of limited oxygen and nutrients. The fourth and final step is to modulate the inflammatory response to abolish the deleterious effects of unrestrained inflammation. This section will briefly delineate the most

characteristic mechanisms of AIS pathophysiology in order to allow the reader to integrate the mechanisms of action and therapeutic targets of each drug.

Immediately after CBF is interrupted, cells continue to need a constant supply of energy in the form of adenosine triphosphate (ATP). The lack of perfusion decimates the concentration of molecular oxygen forcing the cell to divert energy production from classic aerobic cellular respiration towards anaerobic ATP synthesis (Figure 2A). This alternate metabolic route poses several detriments when compared to homeostatic energy production: firstly, this pathway results in a decrease in the yield of ATP per glucose molecule and, secondly, it creates lactic acid as byproduct. The changes brought about by the energetic deficit and the shift in pH cause energy-dependent ion channels to dysfunction [9]. The loss of ionic interchange alters the cells polarity and inherently affects voltage-dependent mechanisms [10]. One such voltage-dependent process is neurotransmitter release, particularly glutamate. This excitatory neurotransmitter is highly abundant in the central nervous system and is the ligand for the *N*-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid (AMPA) and kainate receptors [10]. After AIS the loss in ionic regulation causes the excessive release of glutamate and impairs its reuptake: a process known as excitotoxicity [11]. The pathway through which glutamate mediates this cytotoxic effect is mediated by calcium ions. The binding of glutamate to its receptors activates the influx of calcium ions into the neuron [12]. The increasing concentration of calcium ions, act as second messengers and overloads the cell by activating intracellular phospholipases, nucleases and proteases. This battery of enzymes degrades essential structures including the cell membrane, DNA, and intracellular proteins [13]. Additionally, the disruption of the ionic gradient of extracellular sodium to intracellular potassium, which relies on ATP-dependent channels, causes changes in the osmotic potential of the cell. The influx of water causes lysis and cytotoxic edema; this reduces the size of the extracellular space and accounts for some of the edema seen after stroke [13]. This state of stress results in the overactive production of ROS which overloads the cell's antioxidant enzymes such as superoxide dismutase and the antioxidant vitamins A and E [14]. The inability to cope with the increased concentration of free radicals causes lipid peroxidation of the cell membrane [15]. The ROS-mediated destruction of the cell membrane further damages the cell and releases phospholipids into the microenvironment that act as precursors in the production of arachidonic acid, which is further transformed into a variety of signaling molecules [16]. Most notably, prostaglandins and leukotrienes are produced, which are responsible for initiating the inflammatory response. The presence of ROS within the cell also opens the mitochondrial permeability transition pore (MPTP), which allows the escape of cytochrome C, a powerful trigger of apoptosis [17]. Other clinically relevant mechanisms of degeneration are the activation of poly-ADP-ribose polymerase (PARP) [18] and cortical spreading depression (CSD) [19]. When talking about AIS, there exists a secondary cascade of degenerative effects known as reperfusion injury (Figure 2D). We recommend referring to the provided source for a complete understanding of this phenomenon as it relates to arterial recanalization [20]. The compound effect of this degenerative cascade that take place after AIS results in the necrosis or apoptosis of the neuronal population within the penumbra in addition to the already irreversibly damaged necrotic core.

Figure 2. A) The pathophysiology of stroke; B) The formation of the thrombus/embolus; C) The mechanism of action of tPA; D) Reperfusion injury.

5. Methodology

The goal of this review is threefold: the first is to clarify for the modern day clinician the accepted stroke treatment guidelines currently in effect; the second is to analyze all clinical trials that have concluded or are still underway; and the third is to analyze animal model studies that have promising results using novel agents that have not been evaluated in a clinical setting.

The systematic review had rigorous search criteria. Firstly, a review of all the accepted guidelines was used to determine the clinical management of AIS. The latest guidelines were published in 2007-2008 and remain in effect today due to the lack of an updated revision. The guidelines that dictate the integral treatment of patients with AIS are those compiled by the American Heart Association (AHA) in 2007, the European Stroke Organisation (ESO) Executive Committee and the National Institute of Health and Clinical Excellence (NICE) of the UK completed in 2008. The latest systematic review on the treatment of AIS were published in by the American College of Chest Physicians and is limited to antiplatelet and antithrombotic management. To facilitate the readers' understanding of current drug therapy in AIS, only recommendations with a level of evidence A-B and a class of I-II were included. In no way does this study substitute the necessity to review the guidelines for the management of AIS in a clinical setting, nor should these recommendations be used in contradiction to nationally accepted practices or hospital protocol.

In order to compile all clinical trials underway, a thorough search of the U.S. National Institutes of Health clinical trial database (www.clinicaltrials.gov), the Internet Stroke Center Trial Registry (www.strokecenter.org/trials/) and the World Health Organization's International Clinical Trials Registry Platform Search Portal (www.apps.who.int/trialsearch/) was undertaken. Due to the nature of the review, only pharmacological interventional randomized controlled trials (RCT) were considered; this meant that all cell-based and physical (this includes cryotherapy and electrical stimulation) therapies were excluded. Other criteria used to refine the search were: trials that had not published preliminary results or had been prematurely terminated, studies that did not evaluate functional outcome, the lack of statistical significance against a placebo or control group, interventions outside the acute setting or those that treated complications of AIS. To aid in the usefulness of this review, the studies excluded by the previous criteria will be briefly mentioned in table format.

The third step consisted of searching for basic experimental research used in animal models of AIS. A computerized search of the National Library of Medicine and the National Institutes of Health MEDLINE database was performed using PubMed. Only published literature in English from 2008 to 2012 was taken into consideration seeing as it was deemed chronologically relevant. Since the objective of this literature revision decided to include only the most promising therapies a strict exclusion criteria was drafted. Parameters of exclusion were studies not performed in *in vivo* models, that had no functional outcome analysis, that did not achieve a $P < 0.05$, that used pre-AIS treatment strategies and that used an invasive administration route that would deem it clinically unfeasible.

The organizational presentation of all existing and potential AIS treatments will help the field by allowing the researcher to see what has and what hasn't been done while introducing the most cutting edge therapies already being studied.

6. Current treatment guidelines

In order to clarify this for the scientific community a systematic review of the existing literature on the pharmacological treatment of AIS is presented below and, in an effort to facilitate the application of the current guidelines in the clinical setting, a treatment algorithm summarizing these recommendations is provided (Figure 3).

Currently the medical treatment approach of AIS focuses on the treatment of the immediate acute phase in an effort to reduce the progression of the ischemia, followed simultaneously by an attempt at revascularization and reperfusion of the brain parenchyma. Further treatment includes the reduction of the damage and neuronal cell death caused by the ischemia and subsequent metabolic cascade brought about by the abrupt reperfusion. This involves the use of neuroprotective strategies and a pharmacological approach to reducing the inflammatory response. Finally, treatment focuses on rehabilitation and retarding the progression of the vascular disease as well as prevention of further strokes. To understand the medical treatment strategies described above, following is provided a detailed description of the pharmacological agents that are used in the treatment of AIS and the science behind these choices of medications.

7. Thrombolysis

The pinnacle of stroke therapy is without doubt thrombolysis and is rapidly becoming the gold-standard treatment in AIS. The NINDS rtPA Stroke Study compared the use of intravenous rtPA given within three hours after stroke onset versus placebo [21]. The rtPA-treated group showed a significant neurological improvement when compared to the untreated group. Despite a greater incidence of intracranial hemorrhage in the treatment group, both treatments exhibited similar survival at three months. This expedited the approval of this therapy by the US Food and Drug administration in 1996.

Recombinant tPA (rtPA) is a genetically synthesized tPA molecule that works in precisely the same way as endogenous tPA. It catalyzes the cleavage of the zymogen plasminogen to yield the active enzyme plasmin. Plasmin in turn is responsible for the degradation of the interlinked fibrin monomers that make up the fibrin clot into soluble products. Endogenous tPA is usually present in relatively small amounts and regulates the breakdown of fibrin plugs in vessels and keeps coagulation in check. In turn, plasminogen activator inhibitor 1 (PAI-1) regulates the activation of tPA, thus hindering the degradation of the fibrin clot. However, when rtPA is administered by infusion, there is insufficient PAI-1 to control the action of tPA, hence activated plasmin is produced in sufficient quantities to breakdown existing fibrin clots (Figure 2C). Perhaps paradoxically, rtPA has been shown to induce fibrinogen binding of platelets and

platelet aggregation. Although thromboxanes were not shown to increase significantly in one study, it is logical to assume that adjunctive therapy with antiplatelet agents such as aspirin or some of the more novel drugs is a sensible approach to preventing rethrombosis after rtPA therapy [22]. Another study showed that rtPA does in fact activate platelets but then in turn is also responsible for inhibiting aggregation [23]. A more recent review on the subject concludes that therapies should consider protecting from extensive activation of platelets after tPA therapy [24].

According to all the recently published major guidelines, intravenous (IV) rtPA thrombolysis is highly recommended in all eligible patients. The criteria for eligibility are however long and strict which accounts for less than 10% of patients being eligible for IV thrombolysis with rtPA. These criteria are summarized in Table 1 in Figure 3. Treatment should be started less than 3 hours from the onset of stroke symptoms, however the guidelines provided by the American College of Chest Physicians recommend against the use of IV rtPA when infusion cannot be started before 4.5 hours have transpired since symptom onset. This left a gray zone between 3 and 4.5 hours in which the benefit of using IV rtPA may, in most cases, outweigh the risks; nevertheless, the evidence for this was not sufficiently strong for a fervent recommendation to be made. Most recently, a study by a science advisory from the American Stroke Association has declared that after reviewing the data from the ECASS-3 trial, sufficient evidence had mounted to make a full recommendation for the use of IV rtPA if therapy was started within 4.5 hours of symptom onset [25].

Therapy with rtPA is given at a dose of 0.9 mg/kg IV without exceeding a maximum dose of 90 mg with 10% given as a loading bolus over 1 minute and the remainder as an infusion over 60 minutes. During the infusion and for one hour after concluding the infusion, the patient's vital signs should be monitored and neurological assessment done every 15 minutes. Thereafter, observations should be carried out every 30 min for the next 6 hours and hourly afterward until 24 hours have transpired since treatment.

Additionally, fibrinolytic therapy can be administered by the intra-arterial (IA) route directly to the artery occluded by the thrombus. This however requires that the center have cerebral angiography equipment and highly trained interventional neuroradiologist to carry out this procedure. The use of IA rtPA therapy is recommended for patients who are no longer eligible for IV infusion of rtPA due to the time-window restraints but are still within the 6-hour cutoff time for IA treatment. Also, patients who are excluded from IV rtPA due to contraindications such as recent surgery may be eligible for IA treatment instead in the case of occlusion of the middle cerebral artery (MCA) or another proximal cerebral artery. Nevertheless, IA therapy should not be considered an alternative to IV infusion when patients are eligible for the latter [26,27]. The combination of IV/IA rtPA therapy is not recommended [25].

8. Antiplatelet therapy

Due to the thrombotic origin of AIS and the involvement of platelet aggregation in the development of said thrombus, antiplatelet drugs play an obvious and pivotal role in the

medical treatment. Perhaps the most widely used antiplatelet agent is non-steroidal anti-inflammatory drugs (NSAID) acetylsalicylic acid, commonly referred to as aspirin, and its many derivatives. Although both historically and currently used as an anti-inflammatory drug, aspirin at low doses is an avid inhibitor of platelet aggregation. The mechanism of action of this medication is as dependent on its pharmacokinetics as its pharmacodynamics. As an anti-inflammatory, aspirin must become distributed within the tissues and inside intracellular compartment in order to effectively block the cyclooxygenases (COX) and thus the synthesis of prostaglandins. This necessitates higher dosages in order to achieve a sufficiently high concentration that falls within the therapeutic window. Conversely, in order to function as a platelet anti-aggregant, aspirin requires significantly lower doses as it must only become distributed within the intravascular compartment—in fact only in the portal circulation thus being independent of systemic bioavailability. Needless to say, aspirin at anti-inflammatory doses achieves a therapeutic effect on platelet binding, however, at antiplatelet doses aspirin has a minimal effect on tissue cyclooxygenase and in consequence the adverse effects of NSAID therapy on the gastric mucosa.

Aspirin binds and inhibits the platelet COX-1 irreversibly and consequently impairs the production of prostaglandins and thromboxanes, noting thromboxane A₂ (TXA₂) in particular. The absence of TXA₂ leads to the reduction in the TXA₂-mediated amplification of platelet activation and thus hinders the platelet aggregation phenotype that includes morphological changes and expression of the fibrinogen receptor necessary for platelet aggregation. Nevertheless, numerous other pathways for platelet activation exist, such as those mediated by thrombin and epinephrine that can sufficiently promote the active phenotype and lead to platelet plug formation in the vessel wall when subendothelial collagen and von Willebrand factor (vWf) are exposed (Figure 2C).

Low-dose (50 – 100 mg daily) aspirin is prescribed typically as a prophylactic in the prevention of cardiovascular and cerebrovascular disease. Taken daily, it effectively reduces platelet efficiency despite adequate platelet concentration in the full blood count. Due to the irreversible inactivation of platelet enzymes, adequate platelet function is only restored upon production of new platelets after halting aspirin treatment. Since platelets have an average lifespan of 10 days it is estimated that 10% of platelets are replaced every day; moreover for proper hematological function it is required that approximately 20% of platelets be functional. Thus, normal blood clotting is achieved two days after discontinuing a low-dose aspirin regimen. Although high-dose aspirin (above 300 mg daily) provides a similar inhibition time window of platelet function and recovery after cessation of treatment; nonetheless, the incidence of gastrointestinal adverse effects (i.e. gastritis) is much higher than on low-dose aspirin. However, if the formulation of high-dose aspirin includes an enteric coating, the therapeutic time window and recovery are significantly prolonged. This effect however is not seen with enteric-coated low-dose aspirin.

The role of aspirin in the prophylaxis of ischemic cerebrovascular events and stroke is well accepted. Numerous studies and systematic reviews have shown a highly significant risk reduction (13%) in the incidence of AIS when daily low-dose aspirin is taken without a greatly significant increase in the incidence of hemorrhagic complications including stroke [28].

Despite the well-recognized use of aspirin in prevention, its use in the initial treatment of AIS is somewhat contested. According to the AHA Guidelines for the early management of adults with ischemic stroke (2007), although aspirin therapy immediately after an AIS is not standard, starting aspirin within 48 hours of the onset of symptoms is routine in many centers and according to studies poses “a modest but statistically significant benefit” [26]. Most recently, in 2012, the American College of Chest Physician published a revised set of guidelines in which starting aspirin at doses of between 160 and 325 mg daily within 48 hours of the onset of symptoms is recommended [27]. The general consensus is that an initial 325 mg dose of aspirin should be given to most patients suffering from a stroke or TIA within 24 hours of the onset of stroke or as early as possible, but not before 24 hours have transpired since thrombolytic therapy, except when contraindicated by evidence of intracranial hemorrhage, bleeding diathesis, recent surgery and sensitivity to aspirin, among others. After the initial loading dose, subsequent daily low-dose aspirin might be more adequate than the higher dose as there is no evidence suggesting that the higher dose provides better protection from further strokes while there is an associated higher risk of intracranial bleeding with the chronic use of high-dose aspirin therapy in comparison with low-dose therapy.

The use of other antiplatelet medication such as clopidogrel, ticlopidine and dipyridamole has not been as formally evaluated in trials, as has aspirin. The routine use of these drugs is not recommended, however it is reasonable to suggest the use of, for example, clopidogrel at an initial dose of 300 mg, as it will efficiently inhibit platelet aggregation, when aspirin is not tolerated by the patient [29]. Likewise, a subsequent daily dose of 75 mg of clopidogrel will maintain platelet aggregation at bay. Furthermore, the guidelines provided by the American College of Chest Physicians recommend the use of aspirin in combination with dipyridamole or clopidogrel over aspirin therapy alone [27].

9. Anticoagulant therapy

Anticoagulants are a heterogeneous group of pharmacological agents that by interacting with the coagulation cascade disrupt the formation of the fibrin mesh that forms the scaffold of the clot. When in homeostasis, the blood elements that participate in this process are kept at check thus preventing the formation of a blood clot *in situ*, or thrombus, inside the blood vessels. Although a comprehensive review of the coagulation cascade would be beyond the scope of this text some knowledge is prerequisite in order to adequately comprehend the pharmacology of these drugs. In simplistic terms, coagulation is activated by two somewhat distinct processes that ultimately lead to a common pathway that results in the activation of prothrombin to thrombin, which in turn converts fibrinogen to fibrin and the formation of the clot thereof. The extrinsic pathway involves the rapid activation of the cascade when clotting factors are exposed to tissue factor (TF) after damage of the vessel endothelium. Alternatively, the intrinsic, or contact activation pathway is triggered by the formation of cascade complexes on collagen after tissue damage. This leads to the eventual activation of the common pathway, although experts now believe that the action of TF is required for the adequate amplification and eventual formation of the thrombus [30]. Anticoagulants interfere with the cascade in

distinct ways: the coumarinic anticoagulants like warfarin inhibit phyloquinone (Vitamin K) epoxide reductase and as a consequence render useless the clotting factors II, VII, IX and X that depend on Vitamin K as a cofactor. This action can be assessed by measuring the action of the extrinsic pathway by means of the time required for coagulation after addition of TF *in vitro* a test known as the prothrombin time (PT) or its normalized equivalent to normal values, the international normalized ratio (INR). Conversely, heparin, another common anticoagulant with numerous variants, activates antithrombin and hence inactivates thrombin and halts coagulation at its final stages. Similarly, this is measured *in vitro* by the activated partial thromboplastin time test, which evaluates the efficiency of the contact activation pathway. Prolongation of the normal times in both instances is interpreted as impaired coagulation.

As with antiplatelet therapy, a distinction between the use of anticoagulants for the prevention of AIS or TIA and that of anticoagulation as a means of treatment in the initial stages post-AIS must be made. Likewise, the incidence of an early recurrence of stroke is considered a complication of AIS and although technically speaking anticoagulants are prescribed prophylactically for this reason, this is considered a standard treatment in the acute phase of stroke and not actual preventive therapy. The use of anticoagulants in the prevention of AIS is beyond the scope of this review, however it must be noted that their use is widespread and is generally accepted. The use of oral coumarinics, such as warfarin, in the prevention of complications of atrial fibrillation such as AIS is common practice.

On the other hand, the use of anticoagulants in the first stages of AIS has been tried with little success. Both the International Stroke Trial and the consensus panel assembled by the National Institute of Neurological Disorders and Stroke (NINDS) recommend against the use of anticoagulants such as heparin within 24 hours of treatment with rtPA [26,27]. This is due to the marked increase in symptomatic intracranial hemorrhage seen in the trials testing anticoagulants for AIS. Additional trials testing the benefits of other anticoagulants yielded less than acceptable results for the low-molecular weight (LMW) heparins dalteparin (compared to aspirin), certoparin, nadroparin and danaparoid [31]. The outcome was particularly dire for those with moderate to severe strokes (National Institutes of Health Stroke Scale (NIHSS) scores of ≥ 15). One trial did however show that heparin administered within the first 3 hours after the onset of stroke improved the outcome significantly. However, since the time-window for the treatment is similar to that of rtPA it is necessary to compare these treatment options, as the concomitant use is not an option. Currently no anticoagulant is recommended in the treatment of the acute stages of AIS nevertheless, there is an interest in the development of a safe anticoagulant that can be co-administered with thrombolytics in order to reduce the risk of re-thrombosis. Additionally, coumarinics have not been tested for use in the acute treatment of stroke as these are mainly oral agents and are therefore reserved for long-term treatment such as in prophylaxis of first or subsequent events. Patients with cardioembolic stroke need to receive oral prophylactic anticoagulation particularly when associated with AF. Initiation must be delayed to avoid the risk of intracranial bleeding: patients with mild stroke or TIA (NIHSS scores of ≤ 10) may be started on warfarin, or newer agents such as dabigatran, titrating dose to an INR between 2.0 and 3.0 after 48 hours if there is no contraindication. Patients

with moderate to severe strokes should not receive anticoagulants after 2 to 4 weeks have elapsed [32].

10. Neuroprotective therapy

Despite widespread interest in this field and the amount of published studies, none have passed clinical trials with the same observable effect seen in animal models. The AHA guideline published in 2007 deemed that at present there are no neuroprotective agents that have shown to reduce tissue damage and improve the neurological outcome of AIS. The committee determined the inexistence of a potential neuroprotective drug with a Class III, Level of Evidence A [26]. Up till now, the recommendations have not changed. Clinical and/or federal authorities have not approved the use of any neuroprotective compound in the management of AIS. The main reason for the inability of these drugs to produce a marked benefit in clinical trials when they are so successful in animal models remains elusive; however in the coming sections several limitations will be described.

11. The state of current science

Drug discovery in the area of interventional AIS research is one of the largest fields in science. Everyday thousands of papers are published trying new or old compounds with a variety of different analysis techniques. The search criteria for this review returned 20,416 papers that commented on possible therapeutic pharmacological interventions in AIS. The exclusion criterion that was employed drastically reduced the database of studies; however the result was still 213 different treatments that are currently being investigated. In order to make this review of greater value only the treatments that have been evaluated by several groups and are closer to the clinic have been included. The following are the most remarkable pharmacological agents currently being considered as treatment for AIS. However, so that the review does not lose the general panorama, several tables have been elaborated in order for the reader to have easy access to supplementary information if necessary.

12. Thrombolytics

The elevated risk of complications after administration of tPA such as hemorrhagic transformation has triggered the search for safer fibrinolytics. Desmoteplase is a plasminogen activator isolated from the saliva of bats. It is currently undergoing clinical trials because it has proven to be more fibrin-selective than recombinant tPA. The secondary effects of tPA such as neurotoxicity and inducing the dysfunction of the blood-brain barrier (BBB) are also bypassed with desmoteplase. The Desmoteplase in Acute Ischemic Stroke (DIAS) trials are now in their third and fourth session and they are ongoing (DIAS 3 and 4). Preliminary results suggest that

create and share your own diagrams at gliffy.com

Figure 3. Treatment algorithm for AIS.

desmoteplase was associated with higher percentages of recanalization and neurological outcomes against a placebo. However, the therapeutic window being evaluated is 3-9 hours, highly similar to tPA [33]. Tenecteplase is a recombinant protein designed from the alteplase molecule, but it is modified at three sites. These modifications make it more fibrin-specific as opposed to tPA and targets the plasminogen within the thrombus allowing for more localized fibrinolysis. The clinical trials for this compound have been slow and mostly terminated, only arriving to phase II. Preliminary results did not yield convincing data compared to controls [34]. There are many limitations to thrombolytic therapy and these mainly reside in the hemorrhagic complications they may cause. The purpose of these is to reestablish CBF by means of arterial recanalization with the goal of saving the penumbra. An interesting alternative to this is mechanical thrombectomy but pharmacological thrombolytics should further be sought because they do not require a highly specialized team and may be administered in the emergency ward. These characteristics have made and continue making thrombolytics a promising area of research.

13. Combination therapies with thrombolytics

Thrombus formation depends on the function of several platelet surface proteins and blood-borne proteins (Figure 2B). A particularly important glycoprotein is IIb/IIIa, which aids in platelet aggregation. To optimize the function of fibrinolysis with tPA and further prevent thrombus formation, a combination scheme with GP IIb/IIIa inhibitors after administration of tPA was started. The Combined Approach to Lysis Utilizing Eptifibatide and rTPA (CLEAR) trial evaluated the use of eptifibatide within the therapeutic window of tPA. The preliminary data suggested that rtPA alone further improved the functional outcome as compared to the combination. The trial was restructured and is now in phase II, known as CLEAR-ER [35]. Other compounds that display synergistic effects with rtPA are matrix metalloproteinase inhibitors, free radical scavengers, NMDA receptor antagonists, AMPA receptor antagonists, antioxidants, anti-inflammatory agents, and antiplatelet drugs [93].

14. Antithrombotic therapies

Drugs that are aimed at preventing the further formation of thrombi are called antithrombotics. Ancrod is isolated from the venom of a pit viper [36]. This molecule degrades fibrinogen instead of fibrin as opposed to thrombolytics. Another example of a fibrinogen-depleting agent is an enzyme found also in snake venom known as batroxobin. Both of these agents have been taken to clinical trials with dismal results. In the preliminary results of ancrod in the Stroke Treatment with Ancrod Trial (STAT) it was suggested that there was a marked reduction in the frequency of symptomatic intracerebral hemorrhage in the group treated with ancrod. A revision of this trial was held by the European STAT database and they concluded that the study showed a lack of efficacy as it did not improve the outcome when administered 6 hours after symptom onset. Batroxobin trials concluded that it could effectively reduce the risk of

recurrent stroke in patients with hyperfibrinogenemia [37]. The true potential of fibrinogen-depleting agents has yet to be seen, but for now they are not considered a promising therapy.

15. Neuroprotection

15.1. Metal chelators

The prolyl hydroxylase inhibitor, deferoxamine mesylate is also an iron ion chelator. This molecule prevents the formation of hydroxyl radicals by neutralizing the reactive iron ions. It also upregulates and stabilizes the transcriptional activator hypoxia-inducible factor-1 (HIF-1) which is responsible for the transcription of several survival genes. The chelator is being studied in the Membrane-Activated Chelator Stroke Intervention (MACSI) trial and has had positive preliminary results [38]. Another metal chelator is DP-b99, which chelates zinc ions. Increased concentrations of zinc are known to be neurotoxic and it is also a cofactor in many degenerative processes. It is currently being studied alongside deferoxamine in the MACSI phase III trial with an intravenous administration route [39].

15.2. Growth factors

Erythropoietin (EPO), a 30-kDa glycoprotein that is in charge of erythropoiesis by means of proliferation, maturation and survival of erythroid progenitor cells [40], was able to reduce infarct volumes and improve motor and memory functions, in rodent models of focal cerebral ischemia. A meta-analysis on the subject suggests that higher doses of EPO are linked to smaller infarct volumes and significant improvement of limb function; however, the effects are time-dependant, having almost no effect on infarct volume and limb function when administered 6 hours after stroke onset [41]. In addition, EPO was able to increase actively proliferating oligodendrocyte progenitor cells in the peripheral white matter zones and in the subventricular zone 7 days after stroke onset, but was unable to prevent the loss of myelinating oligodendrocytes. Nonetheless, a significant rise in myelinating oligodendrocytes and myelinated axons in the peripheral white matter area was observed 28 and 42 days after stroke onset and an improved recovery was also seen in a 6 week long time period, therefore increasing EPO's potential as a therapeutic agent in stroke [42]. On the other hand, Neuro-EPO, a nonerythropoietic variant of EPO, emerges as a potential therapeutic agent for stroke. Contrary to EPO, Neuro-EPO has the advantage of being available in an intranasal absorption route, has a short plasma half-life due to its low sialic acid content, and lacks erythropoietic activity [43]. Rodriguez-Cruz and colleagues, achieved a higher neuroprotective effect with intranasal Neuro-EPO, than the one obtained after intraperitoneal injection of EPO in gerbil models of stroke; this was evidenced by a better neurological state and functional cognitive improvement, as well as a protection of the temporal cortex, thalamus and the CA3 region of the hippocampus [44]. Immunoglobulin G-EPO (IgG-EPO) fusion protein is another re-engineered form of EPO that is fused to a heavy chain of a chimeric monoclonal antibody that is directed against the mouse transferring receptor. This form of EPO is able to easily penetrate the BBB

when compared to original EPO and showed to reduce the hemispheric stroke volume 81% and the neural deficit 78% when administered in high doses (1.0 mg/kg) [45].

Granulocyte colony-stimulating factor was able to enhance not only leptomeningeal collateral growth in an ischemic stroke model, but also circulating blood monocytes and effectively reduced the infarct volume [46]. Granulocyte macrophage colony-stimulating factor (GM-CSF), has obtained similar results as the above when evaluated in adult mice models of cerebral ischemia [47]. A 6-week treatment with GM-CSF accomplished a complete recovery of cerebral blood flow and cerebrovascular capacity together with integrity of hippocampal hypoxia-vulnerable neurons in rat models of ischemia; a significantly higher number of arterioles in parenchymal and leptomeningeal regions were also observed [48].

15.3. Immunomodulators

Copolymer-1 (Cop-1) is a synthetic copolymer that suppresses encephalitogenic processes through an immunological cross-reactivity with myelin basic protein (MBP) [49]. Cop-1, also known as glatiramer acetate or its brand name Copaxone, has been FDA-approved for its use in multiple sclerosis and has shown neuroprotective effects in immune-based neurological pathologies [50] such as stroke. Cop-1 has been able to induce an environment with an adequate balance of Th1 and Th2 that tend to protect the brain tissue. The modulation of innate immunity, blockage of antigen presentation by MHC molecules and T cell receptor antagonism have also been proposed as probable neuroprotective mechanisms [51, 52]. Rina Aharoni and co-workers demonstrated infiltrating Th 2/3 cells' ability to induce an important expression of both neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), and the potent anti-inflammatory cytokines, interleukin-10 (IL-10) and transforming growth factor β (TGF β), by Cop-1-specific T cells *in situ*. All of these molecules play an important role on the protective and regenerative effects of Cop-1 [49]. Cop-1's action in cerebral ischemia was evaluated by our group in a transient MCAO model. Results showed a significant reduction in percentage of infarct volume, significant improvement on neurological recovery and higher tissue preservation when compared to control groups [53]. Cop-1's ability of acting on various mechanisms that present themselves after ischemic insult makes it a strong therapy to be used in stroke [54, 55]. The supporting evidence that has been obtained with the use of Cop-1 calls for more investigation in order to evaluate its overall potential. Poly-YE is a high molecular weight copolymer that has shown to have the ability of downregulating regulatory T cell activity and stimulating $\gamma\delta$ T cells *in vitro* [56]. Poly-YE was used in an ischemic stroke model to enhance a spontaneous response of effector T cells recognizing antigens. In this study poly-YE not only generated a better clinical and behavioral outcome, but also induced neuroprotection and increased neurogenesis in the hippocampus and cerebral cortex. The beneficial effects in this study were observed even with administration of poly-YE up to 24 hours after ischemic stroke [57]. The long therapeutic window makes poly-YE a potential candidate for clinical use, however further research is needed. T cell-based therapeutic vaccination with MBP-derived peptides with attenuated pathogenic properties has also been proven effective in spinal cord injury in rats [58]. Recently; the neuroprotective effect of agents that stimulate toll-like receptor 9 (TLR9), such as K-type cytosine-guanine-rich DNA oligonucleotides was

Drug	Target	Phase	Name
Albumin	Hemodilution	III	Albumin in Acute Stroke (ALIAS)
Ancrod	Fibrinolytic	III	Stroke Treatment with Ancrod Trial (STAT)
Citicoline	Stabilizes membrane	III	ICTUS Study: International Citicoline Trial on Acute Stroke
Deferoxamine mesylate	Chelates iron molecules	II	-
Desmoteplase	Fibrinolytic	II	The Desmoteplase in Acute Ischemic Stroke Trial (DIAS)
DP-b99	Chelates metal ions	II	The Membrane-Activated Chelator Stroke Intervention (MACSI)
Ebselen	Free radical scavenger	III	-
Edaravone (MCI-186)	Free radical scavenger	III	-
Eptifibatide	Gp IIb/IIIa inhibitor	I/II	Study of the Combination Therapy of rt-PA and Eptifibatide to Treat Acute Ischemic Stroke (CLEAR-ER)
GM-CSF (Filgrastim)	Growth factor	II	AXIS 2: AX200 for the treatment of ischemic stroke
hCG (NTx-265)	Growth factor	II	-
Insulin	Glucose-lowering Hormone	III	-
Lovastatin	HMG CoA reductase inhibitor (statin)	II	Neuroprotection with Statin Therapy for Acute Recovery Trial (NeuSTART) I and II
Magnesium sulfate	NMDA channel antagonist	III	The Field Administration of Stroke Therapy-Magnesium (FAST-MAG)
Minocycline	Antibiotic and antiapoptotic properties	III	Neuroprotection With Minocycline Therapy for Acute Stroke Recovery Trial (NeuMAST) and Minocycline to Improve Neurologic Outcome in Stroke (MINOS)
MLC601/901 (NeuroAid™)	Nine herbal and five animal components	III	CHinese Medicine NeuroAid Efficacy on Stroke Recovery (CHIMES)
NXY-059 (Cervive™)	Free radical scavenger	IIb/III	Stroke Acute Ischemic NXY-059 Treatment (SAINT) III.
Simvastatin	HMG CoA reductase inhibitor (statin)	III	-
Tenecteplase	Fibrinolytic	II	-

Table 1. Active Neuroprotection Clinical Trials

reported. These compounds induce tolerance (precondition) to ischemic brain injury. The beneficial effects of this therapy have been shown in both mice and nonhuman primate models of stroke [59]. Although further evaluation is needed, TLR9 agonists can be a possible strategy for stroke.

Tirilazad	Neurotrophins	Calpain inhibitors
Glutamate antagonists	Barbiturates	Gangliosides
Beta adrenergic receptor blockers	Aminophylline	Vasopressors
Anti-ICAM antibodies	Lubeluzole	Fosphenytoin
Basic Fibroblast Growth Factor	Enlimomab	Glycine antagonists
Naftidrofuryl	Nimodipine	Prostacyclins
Neutrophil inhibiting factor	Flunarizine	Opioid antagonists

Table 2. Neuroprotective drugs that have been clinically tested and have failed to improve AIS outcome

15.4. Free radical scavengers

Antioxidant nitrone-derived free radical trapping agents have lately received attention due to their therapeutic benefit [60]. The Stroke-Acute Ischemic NXY Treatment (SAINT-I) study, used NXY-059, in a phase 3 clinical trial and found this agent to reduce disability at 90 days when administered within 6 hours of stroke onset, but failed to markedly improve neurological functioning [61]. Nonetheless, the SAINT-II trial, a larger trial that sought to support SAINT-I trial results, concluded that NXY-059 is ineffective for acute ischemic stroke when administered 6 hours after onset [62]. Despite the stated, studies have shown that NXY-059, when administered 4 hours after stroke onset, was able to reduce BBB permeability, which is damaged by the ischemic insult. Reestablishing the BBB helps restore the brain endothelium and ameliorate endothelium-induced damage [63]. Moreover, NXY-059 was shown to be neuroprotective and safe in acute stroke patients at higher concentrations than the used in experimental models when administered 4 hours after insult [64]. NXY-059 is a potential stroke therapy agent but needs to go through further investigations that will help define its therapeutic window and dose regimens. NOX4 is a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase type 4 that plays an important role in oxidative stress generation in cerebral ischemia; such action was supported when a significant improvement of long-term neurological outcome and reduced mortality was achieved when a NOX4 inhibitor, VAS2870, was applied several hours after ischemia induction. Effects were as protective as NOX4 deficient mice, further supporting their protective potential [65].

Agent	Mechanism of action	Reference
Glutamate oxaloacetate transaminase	Intravascular catabolic enzyme of glutamate	[66]
Prostaglandin E1	Antiapoptotic properties	[67]
Lithium	Antiapoptotic properties	[68]
Sigma-1 receptor agonists	Inhibition of inducible nitric oxide synthase	[69]
Fingolimod (FTY720)	Sphingosine-1-phosphate receptor agonist	[70, 71]
Opioid receptor agonists (Biphalin)	Inhibits postsynaptic potentials by lowering presynaptic Ca ²⁺	[72]
Cinnamophillin	Thromboxane A ₂ receptor antagonist	[73]
Hawthorn extract	Antioxidant properties by incrementing glutathione levels	[74, 75]
Dichlorobenzamil	Sodium and/or calcium exchanger inhibitor	[76]
Cilostazol	Inhibitor of type III phosphodiesterase, antiplatelet agent	[77]
Magnesium sulfate	Inhibits the release of excitatory neurotransmitters	[78, 79]
Arundic acid (ONO-2506)	Astrocyte-modulating agent, inhibits S-100b protein synthesis	[80]
Repinotan	Serotonin or 5-Hydroxytryptophan (5-HT) 1A receptor agonist	[81]
Pioglitazone	Peroxisome proliferator-activated receptor (PPAR)- γ agonist	[82]
NADPH oxidase type 4 (NOX4) inhibitor	Inhibits enzyme that is major source of oxidative stress	[65]
C-Phycocyanin	Antioxidant	[83]
Ginsenoside Rd (<i>Panax ginseng</i>)	Antioxidant and anti-inflammatory	[84]
MFG-E8	Lactadherin glycoprotein that exerts tissue protection	[85]
YC-1	Hypoxia-inducible factor (HIF-1) inhibitor	[86]
Gelsolin	Actin- and calcium-binding protein	[87]

Table 3. Promising agents that are currently searching for clinical trial approval

16. Conclusion

16.1. Failure of clinical trials

Current science has very little to offer in the treatment of AIS. The guidelines available to the practicing clinician are limited and largely antiquated. The reason why these have not changed in the last 30 years is primarily because the field has made very little progress. This is not to say that the scientific community has stopped the search for an integral therapy for ischemic stroke, but there seems to be an error in translation. The main mistakes lie in the experimental model of stroke and the study design of both the preclinical and clinical phases of research.

16.2. Comorbidities

Adapting animal models to fit the human paradigm is an essential part of methodological design. However, these always seem to have limitations. Studies are designed to use young healthy animals from a homogenous population. However, in the clinical setting this is exactly the opposite. The population of individuals who suffer stroke are much older and almost all have comorbidities that either triggered the stroke (e.g. AF), or worsen the outcome (e.g. diabetes mellitus).

16.3. Stroke types

The research model of stroke that is most commonly used is middle cerebral artery occlusion (MCAo) with a filament; this model better represents ischemia-reperfusion after thrombolysis. The onset of stroke is carefully monitored and the duration of ischemia is also controlled. The reproducibility of a MCA ischemic stroke of the same duration across the study population provides for an incredibly standard sample size. The onset of AIS is highly variable with occlusion occurring in any vessel of both anterior and posterior circulation. Added to the variability in the type of stroke there are also differences in the anatomical conformation of the brain in rodents to humans. Humans have about 50% white matter and rodents have 10% [3, 88]. The majority of neuroprotective drugs are aimed at saving the neuronal soma, which constitutes the grey matter. In human studies many patients have a high frequency of sub-cortical damage and diffuse white matter ischemic lesions. This may suggest that grey matter-targeted neuroprotection benefits rodent brains more than it would a human. Attempt to neutralize this have been made by recent publications. The study used gyrencephalic non-human primates, which have the most similar cerebral structure to humans [89].

16.4. Reperfusion

As mentioned above, the model that is predominately used is MCAo. This model includes reperfusion after a time of ischemia; better emulating arterial recanalization after treatment with tPA. However, only about 2 to 5 % of patients with AIS receive this therapy [3]; and if patients do receive thrombolytics only a 30 % recanalization is observed after 6 hours of tPA infusion [90]. This model of reperfusion allows for better post-stroke CBF and allowing the adequate distribution of the drug. Also reperfusion injury causes BBB dysfunction allowing molecules that normally do not penetrate the BBB to enter into the brain.

16.5. Time window

The many syndromes seen secondary to vessel occlusion make the diagnosis of AIS difficult for the untrained eye. This heterogeneous presentation makes the arrival of emergency medical services volatile and the time that it takes for these patients to get to a stroke unit hospital is normally greater than 3 hours. In animal models, the administration of the agent is controlled and normally takes place immediately after the onset of stroke. The study of clinically unrealistic time windows for drugs is a major limitation when these are taken into human trials.

16.6. Neurological outcome measures

The criteria used to evaluate stroke varies greatly among basic and clinical research. In animal models the beneficial effect of an agent is measured by the change in size of the infarct zone through image analysis of histological slices. This morphohistological analysis of stroke is a very objective way of measuring a subjective parameter. The size of infarction can have a milieu of functional effects that varies greatly from subject to subject. In this case, the best way to evaluate stroke recovery is a functional outcome measure. This is the case for AIS in the clinic, the scales of the NIHSS, Barthel index, and Modified Rankin Scale all evaluate changes in the function, and not form, of the ischemic zone.

16.7. Study design

Most studies seen in the field are surrounded by heterogeneity and publication bias. Most preclinical studies do not perform randomized, double-blinded designs as opposed to clinical trials that do. In an attempt to standardize this, the Stroke Therapy Academic Industry Roundtable (STAIR) criterion drafted a set of recommendations [91]. The STAIR documents have the goal of smoothing the transition from the bench to the clinic and only the NXY-059 trial has rigorously adhered to them. The beneficial results observed in that trial suggest that the adherence to the STAIR criterion provide better translation into human studies.

17. Direction of future therapies

Increasingly many drugs are currently being tested as potential therapies in AIS. Most of these have demonstrated promising results in the preclinical phases of research and will probably never see the bedside. With every failed attempt at discovering an effective drug compound to treat stroke, the regulations to monitor which ones make it to the clinical setting will become stricter. A step in this direction is the STAIR criteria; these will unify the way in which science is conducted. The adherence to these recommendations allows for better drugs to reach patients but may also limit potentially beneficial drugs from ever passing the preclinical phase. Most neuroprotectants are designed to target one pathway of the multimechanistic pathophysiology of AIS. This reductionist approach to treatment yields modest results. A recent systematic review and meta-analysis by O'Collins and collaborators analyzed combination therapy in comparison to single treatments [92]. The study included 126 different treatments used in the management of animal models of AIS. Single treatments improved neurological score by 12 % in comparison to controls; when used in combination with a second therapy it improved that efficacy by an additional 25 %. In a separate analysis, combining thrombolysis with another compound extended the therapeutic window from 4.4 to 8 hours in animal models. This incredibly useful review suggests that the best approach to AIS therapy is in fact a combination scheme. A treatment strategy that will target most of the damaging mechanisms of stroke will perhaps allow the field to overcome the bench-to-bedside gap.

Strategies for Intracellular Delivery of Therapeutics

Figure 4. Neuron-specific strategies of drug delivery. Several molecules have been created or modified to be able to carry therapeutic compounds and home in on the target tissue. These strategies reduce secondary adverse side effects by decreasing the systemic concentration and increasing it at the site where it is needed.

One of the main problems of drug design in AIS is making the molecules small enough to diffuse across the BBB and reach the target tissue. In the case of NXY-059, the molecule disufenton sodium had very little BBB penetration and was limited to exerting its effect on the endothelium and neurovascular unit [93]. This particular limitation could be the culprit of why only modest beneficial effects were observed. In an attempt to increase the concentration of drugs that reach the target tissue, researchers have designed nanoparticles that will home in on the stressed neurons in the penumbra. These myriad molecules such as: virus, liposomes, nanospheres, and cell-penetrating peptides will target specific cell populations and spare secondary systemic effects. A very promising therapeutic strategy is cell-penetrating peptides (CPP). These use cell-specific homing proteins (cargo) such as viral surface proteins like transactivator of transcription (Tat) and they are conjugated with proteins that will block intracellular protein-protein interactions (effector) (see Figure 5). Cook and collaborators recently published an example of this. They sidestepped several model limitations by using a gyrencephalic non-human primate that has a brain that shows genetic, anatomical and behavioral similarities to human brains [88]. In this study, they tested the neuroprotective effect of postsynaptic density protein-95 (PSD-95) inhibitors. These compounds uncouple PSD-95 from neurotoxic signaling pathways and results in increased neuroprotection. However, these inhibitors have limited transport into the cell, so in order to improve the effect they used a CPP. The following CPP was used: Tat-NR2B9c, comprising the nine carboxy-terminal amino

acids of the N-methyl-D-aspartate receptor (NMDAR) NR2B subunit fused to the 11-mer HIV-1 Tat protein transduction domain. The results demonstrated that PSD-95 inhibitor CPP exert neuroprotection and improve the functional outcome seen after AIS. Another positive detail of this study is that it adhered completely to the STAIR criteria. The promising results seen with CPP suggest that they will soon be introduced into the clinical testing phase. Please refer to Figure 5 for more examples of CPPs that exert neuroprotective effects in AIS.

Figure 5. Cell-penetrating peptides and their role in AIS. The basic structure of a CPP includes a cell-specific targeting peptide coupled with an effector protein that once inside the cell will exert a protein-protein interaction. This interaction will either activate or inactivate certain metabolic processes and will confer neuroprotection to the ischemic penumbral neuron. Adapted from [94].

This review had the goal of jumpstarting drug discovery in AIS by providing a panorama of the field at present. Enormous strides have been made and shall continue to be made, but in

order to focus our efforts and produce a revolutionary novel therapy in the foreseeable future several steps should be taken. This work urges the researchers of the field to become familiarized with the STAIR criteria and design all experiments in interventional stroke research around it. This will allow all publications to become more homogenous and if a truly promising compound or combination is discovered the distance from the bench-to-bedside will be shortened. The finality of this is to benefit as many people as possible in the shortest time available. The authors suggest that targeted molecules will result in better treatments by limiting adverse side effects at non-target sites. With the literature provided it should be considered that combination therapies hold greater promise than single therapies. The adherence to the STAIR criteria recommends that multiple types of stroke models be used and larger animals be sought. Also, strict analysis of pharmacodynamics and pharmacokinetic parameters shall be done on all experimental agents. The authors hope that with these steps being followed throughout the scientific community the cure for AIS is close at hand. However, until that moment comes the cornerstone of treatment is prevention. Possible therapies aimed at preventing the initial AIS will yield the highest benefits in neurological outcome; more research in this area is required.

Author details

Humberto Mestre¹, Yael Cohen-Minian¹, Daniel Zajarias-Fainsod^{1,2} and Antonio Ibarra¹

¹ Faculty of Health Sciences, Universidad Anáhuac México Norte, Mexico

² Institute of Biomedical Engineering, University of Oxford, UK

References

- [1] Zhang, Y, Galloway, J. M, Welty, T. K, Wiebers, D. O, Whisnant, J. P, Devereux, R. B, et al. Incidence and risk factors for stroke in American Indians: the Strong Heart Study. *Circulation*. (2008). , 118(15), 1577-84.
- [2] Roger, V. L, Go, A. S, Lloyd-jones, D. M, Benjamin, E. J, Berry, J. D, Borden, W. B, et al. Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation*. (2012). , 125(1), 188-97.
- [3] Alberts, M. J. Cerebral hemorrhage, warfarin, and intravenous tPA: the real risk is not treating. *JAMA*. (2012). , 307(24), 2637-9.
- [4] Kleindorfer, D, Lindsell, C. J, Brass, L, Koroshetz, W, & Broderick, J. P. National US estimates of recombinant tissue plasminogen activator use: ICD-9 codes substantially underestimate. *Stroke*. (2008). , 39(3), 924-8.

- [5] Reeves, M. J, Arora, S, Broderick, J. P, Frankel, M, Heinrich, J. P, Hickenbottom, S, et al. Acute stroke care in the US: results from 4 pilot prototypes of the Paul Coverdell National Acute Stroke Registry. *Stroke*. (2005). , 36(6), 1232-40.
- [6] Balami, J. S, Chen, R-L, Grunwald, I. Q, & Buchan, A. M. Neurological complications of acute ischaemic stroke. *The Lancet Neurology*. (2011). , 10(4), 357-71.
- [7] Tiehuis, A. M, Visseren, F. L, Vincken, K. L, Biessels, G. J, et al. Diabetes increases atrophy and vascular lesions on brain MRI in patients with symptomatic arterial disease. *Stroke*. (2008).
- [8] Quinn, T. J, & Dawson, J. Sugar and stroke: cerebrovascular disease and blood glucose control. (2011).
- [9] Candelario-jalil, E. Injury and repair mechanisms in ischemic stroke: Considerations for the development of novel neurotherapeutics. *Curr Opin Investig Drugs*. (2009). , 10(7), 644-54.
- [10] Dirnagl, U. U, & Iadecola, C. C. Moskowitz MAM. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci*. (1999). , 22(9), 391-7.
- [11] Xing, C, Arai, K, Lo, E. H, & Hommel, M. Pathophysiologic cascades in ischemic stroke. *Int J Stroke*. (2012). , 7(5), 378-85.
- [12] Kostandy, B. B. The role of glutamate in neuronal ischemic injury: the role of spark in fire. *Neurol. Sci*. (2012). , 33(2), 223-37.
- [13] Lipton, P. Ischemic cell death in brain neurons. *Physiol Rev*. (1999). , 79(4), 1431-568.
- [14] Lo, E. H, Dalkara, T, & Moskowitz, M. A. Mechanisms, challenges and opportunities in stroke. *Nat Rev Neurosci*. (2003). , 4(5), 399-415.
- [15] Ibarra, A, García, E, Flores, N, Martiñón, S, Reyes, R, Campos, M. G, et al. Immunization with neural-derived antigens inhibits lipid peroxidation after spinal cord injury. *CORD Conference Proceedings*. (2010). , 476(2), 62-5.
- [16] Pallast, S, Arai, K, Wang, X, Lo, E. H, & Van Leyen, K. Lipoxygenase targets neuronal mitochondria under oxidative stress. *J Neurochem*. (2009). , 111(3), 882-9.
- [17] Waldmeier PCP Prospects for antiapoptotic drug therapy of neurodegenerative diseases. *Prog Neuropsychopharmacol Biol Psychiatry*. (2003). , 27(2), 303-21.
- [18] Kuzhandaivel, A, Nistri, A, & Mladinic, M. Kainate-Mediated Excitotoxicity Induces Neuronal Death in the Rat Spinal Cord In Vitro via a PARP-1 Dependent Cell Death Pathway (Parthanatos). *Cell Mol Neurobiol*. (2010). , 30(7), 1001-12.
- [19] Dreier, J. P. The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. *Nature Medicine*. (2011). , 17(4), 439-447.
- [20] Strbian, D, Meretoja, A, Putaala, J, Kaste, M, et al. Cerebral edema in acute ischemic stroke patients treated with intravenous thrombolysis. *Int J Stroke*. (2012).

- [21] Tissue plasminogen activator for acute ischemic stroke The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. *N. Engl. J. Med.* (1995). , 333(24), 1581-7.
- [22] Parise, P, Hauert, J, Iorio, A, et al. Streptokinase and rt-PA activate platelets by a different way: implications on the rethrombosis rate after their administration in myocardial infarction. *J. Lab. Clin. Med.* (1995). , 125(2), 212-21.
- [23] Penny, W. F, & Ware, J. A. Platelet activation and subsequent inhibition by plasmin and recombinant tissue-type plasminogen activator. *Blood.* (1992). , 79(1), 91-98.
- [24] Callahan, K. P, Malinin, A. I, et al. Platelet Function and Fibrinolytic Agents: Two Sides of a Coin? *Cardiology.* (2001).
- [25] del Zoppo GJSaver JL, Jauch EC, Adams HP. Expansion of the Time Window for Treatment of Acute Ischemic Stroke With Intravenous Tissue Plasminogen Activator. *Stroke.* (2009).
- [26] Adams, H. P. del Zoppo G, Alberts MJ, Bhatt DL, Brass L, Furlan A, et al. Guidelines for the Early Management of Adults With Ischemic Stroke. *Circulation.* (2007). ee534., 478.
- [27] Lansberg, M. G, Donnell, O, Khatri, M. J, Lang, P, Nguyen-huynh, E. S, & Schwartz, M. N. NE, et al. Antithrombotic and Thrombolytic Therapy for Ischemic Stroke: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* (2012). suppl) e601S-e636S.
- [28] Tijssen JGPLow-dose and high-dose acetylsalicyclic acid, with and without dipyridamole: A review of clinical trial results. *Neurology.* (1998). Suppl 3) SS16., 15.
- [29] Broussalis, E, Killer, M, McCoy, M, Harrer, A, Trinkka, E, & Kraus, J. Current therapies in ischemic stroke. Part A. Recent developments in acute stroke treatment and in stroke prevention. *Drug Discov. Today.* (2012).
- [30] Woodruff, R. S, Sullenger, B, & Becker, R. The many faces of the contact pathway and their role in thrombosis. *J. Thromb. Thrombolysis.* (2011). , 32(1), 9-20.
- [31] Shahpour, M, Mousavi, S, Khorvash, F, et al. Anticoagulant therapy for ischemic stroke: A review of literature. *Journal of Research in Medical Sciences.* (2012). , 17(6), 396-401.
- [32] Miller, C. S, Grandi, S. M, Shimony, A, Filion, K. B, & Eisenberg, M. J. Meta-analysis of efficacy and safety of new oral anticoagulants (dabigatran, rivaroxaban, apixaban) versus warfarin in patients with atrial fibrillation. *Am. J. Cardiol.* (2012). , 110(3), 453-60.
- [33] Hacke, W, Albers, G, Al-rawi, Y, Bogousslavsky, J, Davalos, A, Eliasziw, M, et al. The Desmoteplase in Acute Ischemic Stroke Trial (DIAS): a phase II MRI-based 9-hour

window acute stroke thrombolysis trial with intravenous desmoteplase. *Stroke*. (2005). , 36(1), 66-73.

- [34] Haley EC JrThompson JL, Grotta JC, Lyden PD, et al. Phase IIB/III trial of tenecteplase in acute ischemic stroke: results of a prematurely terminated randomized clinical trial. *Stroke*. (2010). , 41(4), 707-711.
- [35] Pancioli, A. M, Broderick, J, Brott, T, Tomsick, T, Khoury, J, Bean, J, et al. The combined approach to lysis utilizing eptifibatide and rt-PA in acute ischemic stroke: the CLEAR stroke trial. *Stroke*. (2008). , 39(12), 3268-76.
- [36] Levy, D. E, Trammel, J, & Wasiewski, W. W. Ancrod Stroke Program (ASP) Study Team. Ancrod for acute ischemic stroke: a new dosing regimen derived from analysis of prior ancrod stroke studies. *J Stroke Cerebrovasc Dis*. (2009). , 18(1), 23-7.
- [37] Xu, G, Liu, X, Zhu, W, Yin, Q, Zhang, R, & Fan, X. Feasibility of treating hyperfibrinogenemia with intermittently administered batroxobin in patients with ischemic stroke/transient ischemic attack for secondary prevention. *Blood Coagul. Fibrinolysis*. (2007). , 18(2), 193-7.
- [38] Zhao, Y, & Rempe, D. A. Prophylactic neuroprotection against stroke: low-dose, prolonged treatment with deferoxamine or deferasirox establishes prolonged neuroprotection independent of HIF-1 function. *J Cereb Blood Flow Metab*. (2011). , 31(6), 1412-23.
- [39] Rosenberg, G, Bornstein, N, Diener, H-C, Gorelick, P. B, Shuaib, A, Lees, K, et al. The Membrane-Activated Chelator Stroke Intervention (MACSI) Trial of DP-b99 in acute ischemic stroke: a randomized, double blind, placebo-controlled, multinational pivotal phase III study. *Int J Stroke*. (2011). , 6(4), 362-7.
- [40] Fisher, J. W. Erythropoietin: physiology and pharmacology update. *Exp. Biol. Med*. (2003).
- [41] Minnerup, J, Heidrich, J, Rogalewski, A, Schabitz, W. R, & Wellmann, J. The efficacy of erythropoietin and its analogues in animal stroke models: a meta-analysis. *Stroke*. (2009).
- [42] Zhang, L, Chopp, M, Zhang, R. L, Wang, L, Zhang, J, Wang, Y, Toh, Y, Santra, M, Lu, M, & Zhang, Z. G. Erythropoietin amplifies stroke-induced oligodendrogenesis in the rat. *PLoS One*. (2010). e11016.
- [43] Hermann, D. M. Nonhematopoietic variants of erythropoietin in ischemic stroke: need for step-wise proof-of-concept studies. *Scientific World Journal*. (2010).
- [44] Rodriguez, C. Y, Mengana, T. Y, Munoz, C. A, Subiros, M. N, Gonzalez-quevedo, A, & Sosa, T. I. Garcia Rodriguez JC. Treatment with nasal neuro-EPO improves the neurological, cognitive, and histological state in a gerbil model of focal ischemia. *Scientific World Journal*. (2010).

- [45] Fu, A, Hui, E. K, Lu, J. Z, Boado, R. J, & Pardridge, W. M. Neuroprotection in stroke in the mouse with intravenous erythropoietin-Trojan horse fusion protein. *Brain Res.* (2011).
- [46] Sugiyama, Y, Yagita, Y, Oyama, N, Terasaki, Y, Omura-matsuoka, E, Sasaki, T, & Kitagawa, K. Granulocyte colony-stimulating factor enhances arteriogenesis and ameliorates cerebral damage in a mouse model of ischemic stroke. *Stroke.* (2011).
- [47] Todo, K, Kitagawa, K, Sasaki, T, Omura-matsuoka, E, Terasaki, Y, Oyama, N, Yagita, Y, & Hori, M. Granulocyte-macrophage colony-stimulating factor enhances leptomeningeal collateral growth induced by common carotid artery occlusion. *Stroke.* (2008).
- [48] Schneider, U. C, Schilling, L, Schroeck, H, Nebe, C. T, Vajkoczy, P, & Woitzik, J. Granulocyte-macrophage colony-stimulating factor-induced vessel growth restores cerebral blood supply after bilateral carotid artery occlusion. *Stroke.* (2007).
- [49] Aharoni, R, Kayhan, B, Eilam, R, Sela, M, & Arnon, R. Glatiramer acetate-specific T cells in the brain express T helper 2/3 cytokines and brain-derived neurotrophic factor in situ. *Proc. Natl. Acad. Sci. U. S. A.* (2003).
- [50] Angelov, D. N, Waibel, S, Guntinas-lichius, O, Lenzen, M, Neiss, W. F, Tomov, T. L, Yoles, E, Kipnis, J, Schori, H, Reuter, A, Ludolph, A, & Schwartz, M. Therapeutic vaccine for acute and chronic motor neuron diseases: implications for amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* (2003).
- [51] Arnon, R, & Aharoni, R. Mechanism of action of glatiramer acetate in multiple sclerosis and its potential for the development of new applications. *Proc. Natl. Acad. Sci. U. S. A.* (2004). Suppl 2) 14593-14598.
- [52] Gorantla, S, Liu, J, Sneller, H, Dou, H, Holguin, A, Smith, L, Ikezu, T, Volsky, D. J, Poluektova, L, & Gendelman, H. E. Copolymer-1 induces adaptive immune anti-inflammatory glial and neuroprotective responses in a murine model of HIV-1 encephalitis. *J. Immunol.* (2007).
- [53] Ibarra, A, Avendano, H, & Cruz, Y. Copolymer-1 (Cop-1) improves neurological recovery after middle cerebral artery occlusion in rats. *Neurosci. Lett.* (2007).
- [54] Aharoni, R, Arnon, R, & Eilam, R. Neurogenesis and neuroprotection induced by peripheral immunomodulatory treatment of experimental autoimmune encephalomyelitis. *J. Neurosci.* (2005).
- [55] Yuan, T. F. BDNF signaling during olfactory bulb neurogenesis. *J. Neurosci.* (2008).
- [56] Cady, C. T, Lahn, M, Vollmer, M, Tsuji, M, Seo, S. J, Reardon, C. L, Brien, O, & Born, R. L. WK. Response of murine gamma delta T cells to the synthetic polypeptide poly-Glu50Tyr50. *J. Immunol.* (2000).
- [57] Ziv, Y, Finkelstein, A, Geffen, Y, Kipnis, J, Smirnov, I, Shpilman, S, Vertkin, I, Kimron, M, Lange, A, Hecht, T, Reyman, K. G, Marder, J. B, Schwartz, M, & Yoles, E. A

novel immune-based therapy for stroke induces neuroprotection and supports neurogenesis. *Stroke*. (2007).

- [58] Schwartz, M, & Hauben, E. T cell-based therapeutic vaccination for spinal cord injury. *Prog. Brain Res.* (2002).
- [59] Bahjat, F. R, Williams-karnesky, R. L, Kohama, S. G, West, G. A, Doyle, K. P, Spector, M. D, Hobbs, T. R, & Stenzel-poore, M. P. Proof of concept: pharmacological preconditioning with a Toll-like receptor agonist protects against cerebrovascular injury in a primate model of stroke. *J. Cereb. Blood Flow Metab.* (2011).
- [60] Green, A. R, Ashwood, T, Odergren, T, & Jackson, D. M. Nitrones as neuroprotective agents in cerebral ischemia, with particular reference to NXY-059. *Pharmacol. Ther.* (2003). , 100, 195-214.
- [61] Lees, K. R, Zivin, J. A, Ashwood, T, Davalos, A, Davis, S. M, Diener, H. C, Grotta, J, Lyden, P, Shuaib, A, Hardemark, H. G, & Wasiewski, W. W. NXY-059 for acute ischemic stroke. *N. Engl. J. Med.* (2006).
- [62] Diener, H. C, Lees, K. R, Lyden, P, Grotta, J, Davalos, A, Davis, S. M, Shuaib, A, Ashwood, T, Wasiewski, W, Alderfer, V, Hardemark, H. G, & Rodichok, L. for the SAINT NXY-059 for the Treatment of Acute Stroke: Pooled Analysis of the SAINT I and II Trials. *Stroke*. (2008).
- [63] Culot, M, Mysiorek, C, Renftel, M, Roussel, B. D, Hommet, Y, Vivien, D, Cecchelli, R, Fenart, L, Berezowski, V, Dehouck, M. P, & Lundquist, S. Cerebrovascular protection as a possible mechanism for the protective effects of NXY-059 in preclinical models: an in vitro study. *Brain Res.* (2009).
- [64] Lees, K. R, Barer, D, Ford, G. A, Hacke, W, Kostulas, V, Sharma, A. K, & Odergren, T. Tolerability of NXY-059 at higher target concentrations in patients with acute stroke. *Stroke*, (2003).
- [65] Kleinschnitz, C, Grund, H, Wingler, K, Armitage, M. E, Jones, E, Mittal, M, Barit, D, Schwarz, T, Geis, C, Kraft, P, Barthel, K, Schuhmann, M. K, Herrmann, A. M, Meuth, S. G, Stoll, G, Meurer, S, Schrewe, A, Becker, L, Gailus-durner, V, Fuchs, H, Klopstock, T, De Angelis, M. H, Jandeleit-dahm, K, Shah, A. M, Weissmann, N, & Schmidt, H. H. Post-stroke inhibition of induced NADPH oxidase type 4 prevents oxidative stress and neurodegeneration. *PLoS Biol.* (2010). e1000479.
- [66] Campos, F, Sobrino, T, Ramos-cabrer, P, Argibay, B, Agulla, J, Pérez-mato, M, et al. Neuroprotection by glutamate oxaloacetate transaminase in ischemic stroke: an experimental study. *J Cereb Blood Flow Metab.* (2011). , 31(6), 1378-86.
- [67] Kawamura, T, Horie, S, Maruyama, T, Akira, T, Imagawa, T, Nakamura, N, & Prostaglandin, E. transported into cells blocks the apoptotic signals induced by nerve growth factor deprivation. *J Neurochem.* (1999). , 72(5), 1907-14.

- [68] Gold, A. B, Herrmann, N, & Lanctot, K. L. Lithium and its neuroprotective and neurotrophic effects: potential treatment for post-ischemic stroke sequelae. *Curr. Drug Targets.* (2011). , 12(2), 243-255.
- [69] Vagnerova, K, Hurn, P. D, Bhardwaj, A, & Kirsch, J. R. Sigma 1 receptor agonists act as neuroprotective drugs through inhibition of inducible nitric oxide synthase. *Anesth. Analg.* (2006). , 103(2), 430-4.
- [70] Wei, Y, Yemisci, M, Kim, H-H, Yung, L. M, Shin, H. K, Hwang, S-K, et al. Fingolimod provides long-term protection in rodent models of cerebral ischemia. *Ann. Neurol.* (2011). , 69(1), 119-29.
- [71] Liesz, A, Sun, L, Zhou, W, Schwarting, S, Mracsko, E, Zorn, M, et al. FTY720 reduces post-ischemic brain lymphocyte influx but does not improve outcome in permanent murine cerebral ischemia. *PLoS ONE.* (2011). e21312.
- [72] Zhang, Z, Chen, T-Y, & Kirsch, J. R. Toung TJK, Traystman RJ, Koehler RC, et al. Kappa-opioid receptor selectivity for ischemic neuroprotection with BRL 52537 in rats. *Anesth. Analg.* (2003). , 97(6), 1776-83.
- [73] Chen, T-Y, Tai, S-H, Lee, E-J, Huang, C-C, Lee, A-C, Huang, S-Y, et al. Cinnamophilin offers prolonged neuroprotection against gray and white matter damage and improves functional and electrophysiological outcomes after transient focal cerebral ischemia. *Crit. Care Med.* (2011). , 39(5), 1130-7.
- [74] Elango, C, & Jayachandaran, K. S. Niranjali Devaraj S. Hawthorn extract reduces infarct volume and improves neurological score by reducing oxidative stress in rat brain following middle cerebral artery occlusion. *Int. J. Dev. Neurosci.* (2009). , 27(8), 799-803.
- [75] Elango, C, & Devaraj, S. N. Immunomodulatory effect of Hawthorn extract in an experimental stroke model. *J Neuroinflammation.* (2010).
- [76] Kaur, H, Jaggi, A. S, & Singh, N. Modulation of neuroprotective effect of ischemic post-conditioning by dichlorobenzamil a Na (+)/Ca (2+) exchanger inhibitor in mice. *Biol. Pharm. Bull.* (2010). , 33(4), 585-91.
- [77] Nonaka, Y, Tsuruma, K, Shimazawa, M, Yoshimura, S, Iwama, T, & Hara, H. Cilostazol protects against hemorrhagic transformation in mice transient focal cerebral ischemia-induced brain damage. *Neuroscience Letters.* (2009). , 452(2), 156-61.
- [78] Saver, J. L. Target brain: neuroprotection and neurorestoration in ischemic stroke. *Rev Neurol Dis.* (2010). Suppl 1) S, 14-21.
- [79] Saver, J. L, Kidwell, C, Eckstein, M, & Starkman, S. FAST-MAG Pilot Trial Investigators. Prehospital neuroprotective therapy for acute stroke: results of the Field Administration of Stroke Therapy-Magnesium (FAST-MAG) pilot trial. *Stroke.* (2004). e, 106-8.

- [80] Pettigrew, L. C, Kasner, S. E, Albers, G. W, Gorman, M, Grotta, J. C, Sherman, D. G, et al. Safety and tolerability of arundic acid in acute ischemic stroke. *J. Neurol. Sci.* (2006).
- [81] Green, A. R, & Shuaib, A. Therapeutic strategies for the treatment of stroke. *Drug Discov. Today.* (2006).
- [82] Medhi, B, Aggarwal, R, & Chakrabarti, A. Neuroprotective effect of pioglitazone on acute phase changes induced by partial global cerebral ischemia in mice. *Indian J. Exp. Biol.* (2010). , 48(8), 793-9.
- [83] Pentón-rol, G, Marín-prida, J, Pardo-andreu, G, Martínez-sánchez, G, Acosta-medina, E. F, Valdivia-acosta, A, et al. C-Phycocyanin is neuroprotective against global cerebral ischemia/reperfusion injury in gerbils. *Brain Res. Bull.* (2011).
- [84] Ye, R, Kong, X, Yang, Q, Zhang, Y, Han, J, & Zhao, G. Ginsenoside Rd attenuates redox imbalance and improves stroke outcome after focal cerebral ischemia in aged mice. *Neuropharmacology.* (2011). , 61(4), 815-24.
- [85] Cheyuo, C, Jacob, A, Wu, R, Zhou, M, Qi, L, Dong, W, et al. Recombinant human MFG-E8 attenuates cerebral ischemic injury: its role in anti-inflammation and anti-apoptosis. *Neuropharmacology.* (2012). , 62(2), 890-900.
- [86] Yan, J, Zhou, B, Taheri, S, & Shi, H. Differential effects of HIF-1 inhibition by YC-1 on the overall outcome and blood-brain barrier damage in a rat model of ischemic stroke. *PLoS ONE.* (2011). e27798.
- [87] Le, H. T, Hirko, A. C, Thinschmidt, J. S, Grant, M, Li, Z, Peris, J, et al. The protective effects of plasma gelsolin on stroke outcome in rats. *Exp Transl Stroke Med.* (2011).
- [88] Broussalis, E, Trinka, E, Killer, M, Harrer, A, McCoy, M, & Kraus, J. Current therapies in ischemic stroke. Part B. Future candidates in stroke therapy and experimental studies. *Drug Discov. Today.* (2012).
- [89] Cook, D. J, Teves, L, & Tymianski, M. Treatment of stroke with a PSD-95 inhibitor in the gyrencephalic primate brain. *Nature.* (2012). , 483(7388), 213-7.
- [90] Arakawa, S, Nilupul, P, & Doonan, G. Neuroprotection in Stroke. *Advances in Clinical Neuroscience and Rehabilitation.* (2005). , 5(5), 10-11.
- [91] Fisher, M, Albers, G. W, Donnan, G. A, Furlan, A. J, Grotta, J. C, Kidwell, C. S, et al. Enhancing the development and approval of acute stroke therapies: Stroke Therapy Academic Industry roundtable. *Stroke.* (2005).
- [92] Collins, O, Macleod, V. E, Donnan, M. R, & Howells, G. A. DW. Evaluation of combination therapy in animal models of cerebral ischemia. *J Cerebr Blood F Met.* (2012). , 32(4), 585-97.

- [93] Sutherland, B. A, Sutherland, B. A, Minnerup, J, & Minnerup, J. MRCP JSB, Balami JS, et al. Neuroprotection for ischaemic stroke: Translation from the bench to the bedside. *Int J Stroke*. (2012). , 7(5), 407-18.
- [94] Antoniou, X, & Borsello, T. Cell Permeable Peptides: A Promising Tool to Deliver Neuroprotective Agents in the Brain. *Pharmaceuticals. Molecular Diversity Preservation International*. (2010). , 3(2), 379-92.