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Authors
Mizuma, Atsushi
Yenari, Midori A

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Clinical perspectives on ischemic stroke

Atsushi Mizuma, MD, PhD[1,2], **Midori A. Yenari, MD**[1]

[1]Department of Neurology, University of California, San Francisco and the San Francisco Veterans Affairs Medical Center, San Francisco, CA, USA

[2]Department of Neurology, Tokai University School of Medicine, Isehara, JAPAN

Abstract

Treatments for acute stroke have improved over the past years, but have largely been limited to revascularization strategies. The topic of neuroprotection, or strategies to limit brain tissue damage or even reverse it, has remained elusive. Thus, the clinical mainstays for stroke management have focused on prevention. The lack of clinical translation of neuroprotective therapies which have shown promise in the laboratory may, in part, be due to a historic inattention to comorbidities suffered by a majority of stroke patients. With the advent of more stroke models that include one or more relevant comorbidities, it may be possible to identify effective treatments that may translate into new treatments at the clinical level. In the meantime, we review comorbidities in stroke patients, modification of stroke risk factors and available acute stroke treatments in the clinic.

Keywords

clinical stroke; ischemic stroke; revascularization; tissue plasminogen activator; endovascular treatment; neuroprotection

1. Introduction

The past few years has shown remarkable advances in the treatment of acute ischemic stroke (IS), particularly as they relate to revascularization. Less progress has been made in the area of neuroprotection, and while there are many factors that may explain the gap between observations in preclinical studies and clinical trials, one important feature is that many laboratory models have not historically taken into account the many comorbidities suffered

*Correspondence to: Midori A. Yenari, MD, Professor, Department of Neurology, University of California, San Francisco and Veterans Affairs Medical Center, 4150 Clement Street, San Francisco, CA 94121, USA, TEL: (415)750-2011, FAX: (415)750-2273, Yenari@alum.mit.edu.

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by patients who are at risk for or suffer stroke. Laboratory studies often use healthy young male animals, but stroke typically occurs in patients of both genders with risk factors such as advanced age, atherosclerosis, diabetes, hypertension and other conditions. Thus, translating a potential therapy observed in the laboratory could be limited when moved to clinical trials. With this in mind, investigations have now turned to models of disease comorbidities in order to simulate a more realistic clinical scenario. By studying models of diabetes, hypertension and other conditions, it is now possible to extend testing of certain therapeutics that appear promising in healthy animals to those with relevant disease. Further, it is well known that gender can influence outcome from stroke as well as response to treatment, and these disparities should be studied and determined.

Here, we review the clinical perspective of stroke and how this might drive experimental studies. At the clinical level, the most effective treatment for stroke is prevention. Prevention is thought to entail reduction of stroke risk factors, but also prevention of future strokes once an initial stroke occurs (secondary prevention). After an acute stroke happens, there are few clinically proven acute treatments. Pharmacological thrombolysis and mechanical thrombectomy are currently the only widely accepted interventions. Pharmacological thrombolysis, typically with recombinant tissue plasminogen activator (t-PA) is restricted to patients that can be treated within 4.5 hours of symptom onset, while those who meet criteria for mechanical thrombectomy may receive this intervention out to 24 hours.[1)] Unfortunately, these treatments are still limited to a relatively short time frame with only about 6% of acute stroke patients eligible for intravenous t-PA and about 10% eligible for mechanical thrombectomy.[2, 3)]

Aside from offering acute intervention, it is important to identify the underlying cause of the stroke, also referred to as the stroke mechanism. Typical causes include atherosclerotic disease of the cerebral vessels or underlying cardiac disease which can predispose to thrombi that can then embolize. A third type of cerebrovascular disease involving the small end vessels of the brain is another common cause of ischemic stroke, and this is often observed in the setting of poorly controlled hypertension and diabetes. Finally, cryptogenic strokes, which include embolic strokes of undetermined sources (ESUS), constitute a substantial proportion of all strokes.[4)] Thus, an important part of the clinical management of stroke patients revolves around determining the cause of the stroke and modifying risk factors to prevent future occurrences.

2. Risk factors

Age is a major risk factor for IS. IS risk increases after the age of 45 years, and over 70% of strokes occur after the age of 65.[5)] Elderly patients also have several comorbid risk factors for IS which not only increase stroke risk, but also increases risk for bad outcome. Several comorbid medical conditions are known to increase risk for ischemic stroke. These include hypertension (HTN), diabetes mellitus (DM), dyslipidemia, tobacco use, and obesity/metabolic syndrome, and recreational drug use.[6–10)] There are also numerous genetic factors that increase stroke risk, some of which can be identified, and many others that are unknown. This review will focus on those which are generally thought to be modifiable either through lifestyle changes and/or pharmacological treatment.

Hypertension is a major modifiable risk factor for IS, and can lead to atherosclerosis and vascular damage, including disruption of the blood brain barrier (BBB), damage to the white matter, and post-stroke edema.[11, 12] Further, hypertension can be associated with lower treatment efficacy, especially in cases of larger infarcts. [12] Chronic hypertension leads to specific damage of the end vessels of the brain with disruption of the normal vascular architecture leading to a pathological condition known as lipohyalinosis. Lipohyalinosis is a hallmark of damage suffered by the small vessels of the brain by chronic hypertension and can predispose to occlusion and subsequent stroke. Thus, aggressive treatment of hypertension can prevent the development of lipohyalinosis and reduce stroke risk. One common strategy to reduce blood pressure and treat hypertension in stroke patients is through targeting angiotensin II. Angiotensin II raises blood pressure through vasoconstriction and can lead to the development of hypertension.[13] Hypertension can also lead to the overexpression of reactive oxygen species (ROS) in the arterial wall via type 1 angiotensin II receptors.[13] Suppression of the renin-angiotensin system using angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB) can contribute to the suppression of cerebral vascular remodeling, protection of the blood brain barrier (BBB), improvement of cerebral blood autoregulation, and suppression of oxidative stress.[14] In fact, treatment of hypertension has been shown to be a major factor in lowering stroke risk. Antihypertensive therapy using ACEI achieved a 28% relative risk reduction of recurrent IS as shown in the Perindopril Protection Against Recurrent Stroke Study (PROGRESS).[15] A meta-analysis similarly demonstrated the importance of treating hypertension and reducing recurrent IS.[16] ARBs have also been shown to reduce IS recurrence and improve outcome (ACCESS and MOSES studies).[17, 18]

While hypertension is a major stroke risk factor at the clinical level, few preclinical studies of acute neuroprotection studied potential stroke treatments in hypertensive animals. However, some animal studies have been carried out in hypertensive animals. In a meta-analysis by O'Collins et al [12], an exhaustive review of over 3,000 animal studies and over 500 potential treatments. Amongst the studies reviewed, the authors found that only 10% of studies addressed the role of hypertension. Overall, the authors failed to find any direct neuroprotective effect of the hypertensive agents studied, but also that hypertensive animals sometimes responded differently to potential therapies, compared to normotensive animals. While many of potential neuroprotective treatments were ineffective in hypertensive animals, the authors also found divergent responses, where certain potential therapies were only effective in normotensive models (thrombolysis, anesthetic agents), while other strategies were actually more effective in hypertensive models (therapeutic hypothermia). Thus, hypertension should be considered in preclinical translational studies of stroke.

A few clinical trials have also attempted to clarify a neuroprotective effect of anti-hypertensive agents in acute stroke, but have yet to demonstrate any efficacy. Early trials of calcium channel blockers such as nimodipine failed to show any neurological improvement, [19] although these studies were criticized for initiating treatment rather late (24–48 hours after stroke onset). More recently, the Superselective Administration of VErapamil During Recanalization in Acute Ischemic Stroke (SAVER-I) study assess the therapeutic potential of verapamil.[20] In this early phase I clinical trial, combined therapy of verapamil given immediately following thrombectomy in AIS patients was shown to be both safe and

feasible, without significantly increasing intracranial hemorrhage or other adverse events, but failed to show any benefit with respect to improved neurological outcome.

Hyperglycemia is well known to exacerbate stroke outcome,[21] and diabetes is a common comorbidity in stroke patients. Hyperglycemia has also been shown to increase inflammatory responses through the fueling of the NADPH oxidase (NOX) pro-inflammatory pathway and exacerbating oxidative stress through generation of superoxide.[22] It has also been shown to exacerbate inflammatory responses in experimental diabetic stroke models and in stroke amongst patients with diabetes. Compared to the normoglycemic state, the expression of several inflammatory molecules including proinflammatory cytokines, cell adhesion molecules (CAMs), chemokines, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), NOX, and NF-κB were increased by hyperglycemia.[23] This also led to increased leukocyte infiltration of the ischemic brain and activation of microglia. High serum levels of high mobility group box-1 (HMGB1) was also observed in diabetic rats and correlated to worse stroke outcomes.[24] In a clinical study, elevated serum HMGB1 levels has been associated with poor outcome in IS patients with DM.[25] Since glucose is required in order for NOX to generate superoxide, hyperglycemia itself can lead to increased oxidative stress.[26] Hyperglycemia is also known to increase brain hemorrhage in IS following treatment with tissue plasminogen activator (tPA).[27] However, at the clinical level, intensive glucose control in the acute setting has not been shown to improve outcome from IS.[28] Yet, treatments for long term glucose control in diabetes have shown some benefit in lowering stroke risk and improving stroke outcome. The UK Prospective Diabetes Study (UKPDS) showed that metformin reduced the incidence of large vessel events, compared to other treatments.[29] The PROactive study showed that pioglitazone, a peroxisome proliferator-activated receptor γ (PPARγ) agonist, reduced recurrent stroke risk significantly in patients with type 2 diabetes.[30] PPARγ agonists not only decrease serum glucose levels, but also inhibit inflammation. PPARγ activation in monocyte-derived macrophages is thought to influence macrophage polarization through an alternative or anti-inflammatory mechanism.[31] PPARγ agonists has also been shown to reduce ischemia-induced inflammation and hemorrhagic transformation (HTf) in experimental models.[32] Further, pioglitazone has also been shown to possess antioxidant and anti-apoptotic effects in experimental stroke models of diabetes.[33] Recent novel agents to treat diabetes such as dipeptidyl peptidase-4 (DPP-4) inhibitor, glucagon like peptide-1 (GLP-1) receptor agonist,[34] and sodium glucose cotransporter 2 (SGLT 2) inhibitor may also have a role in secondary prevention in large vessel diseases.[35] In particular, the DPP-4 inhibitor is thought to have pleiotropic effects against ischemic injury. In a recent study, sitagliptin administration has shown to suppress the pro-inflammatory NF-κB signaling pathway,[36] as well as anti-inflammatory, antioxidant, and anti-apoptotic effects in stroke models with diabetes.[36] To date, the neuroprotective effects of these diabetes treatments have not been assessed in clinical trials, although the GLP-1 receptor analogues are thought to reduce IS risk.[37] The SGLT2 and DPP-4 inhibitors have not been shown to be efficacious in recent clinical studies.[37, 38]

Hyperlipidemia is another major comorbidity contributing to stroke risk. Elevated serum lipid levels can lead to atherosclerosis and narrowing or occlusion of the cerebral arteries. Lipid-lowering-therapy (LLT) can significantly lower IS risk.[39] 3-Hydroxy-3-

methylglutaryl-coenzyme A (HMGA) reductase inhibitors (statins) are frequently used for treatment of dyslipidemia and are nearly routinely prescribed for secondary stroke prevention. The Stroke Prevention by Aggressive Reduction in Cholesterol Levels(SPARCL) study showed that atorvastatin treatment led to 16% relative risk reduction from IS.[40] Although the benefit of IS risk reduction with statins was not achieved with LDL-C levels < 120mg/dl in a previous study,[41] aggressive targeted LLT to reduce LDL-C levels to less than 70mg/dl led to 22% relative cardiovascular disease risk reduction.[42] Thus, statins have been the preferred lipid lowering agent for primary and secondary IS prevention.[43] Statins are also thought to have pleiotropic effects, in addition to their lipid lowering effects. Statins have been thought to have anti-thrombotic, anti-inflammatory, anti-oxidant, and neuroprotective effects.[44] Specifically, statins have been shown to up-regulate endothelial NOS (eNOS) through the inhibition of isoproterinoids.[45, 46] A variety of statins have been shown to reduce infarct volume and improve neurological deficit in experimental stroke. Rosuvastatin was also shown to reduce ischemic injury by inhibiting oxidative stress and inflammatory responses by reducing superoxide and NOX, inhibiting microglial activation, and downregulating inflammatory molecules (NF-κB, iNOS).[47] Recently, ezetimibe and a PCSK9 inhibitor have been also shown to have efficacy in combination with statins in cardiovascular disease;[48, 49] however, its benefit in IS prevention and neuroprotection is still unclear. In addition to LLT, ω3-polyunsaturated fatty acid (PUFA) supplementation as an addition to statin therapy may also prove beneficial in stroke prevention. In particular, eicosapentaenoic acid (EPA) has shown some benefit at the clinical level. The Japan EPA Lipid Intervention Study (JELIS) demonstrated the efficacy of EPA when added to statins pravastatin or simvastatin. In this study, investigators reported a 20% relative reduction in recurrent IS with EPA treatment among patients with a prior history of IS.[50] Its beneficial effect is thought to be through esolvin and protectin, both ω3-PUFA metabolites, which have anti-inflammatory and anti-oxidant properties.

Tobacco use is another significant risk factor which contributes to vascular disease, including stroke. A dose-response relationship between tobacco use in young IS patients has been described, although, interestingly, the relationship is less strong in older adults. Tobacco use leads to vascular endothelial damage and dysfunction through generation of ROS and activation of inflammation, both of which can increase atherosclerotic risk. Not only does tobacco use increase ROS generation, but it can also weaken antioxidant defense systems. Tobacco use promotes pro-inflammatory responses including leukocyte infiltration and activation of matrix metalloproteinases (MMPs) via cytokine signaling. Further, tobacco use can induce BBB dysfunction and worsen loss of cerebral blood flow during IS. In clinical studies, tobacco us was found to increase not only the incidence IS, but also HTf in the setting of anticoagulant use.[51, 52] Recently, current tobacco use has also been reported to increase the incidence of HTf in young patients (<65 years) with IS who have non-valular atrial fibrillation.[53]

Recreational drug use, while a significant public health problem for many reasons, is also associated with IS, particularly cocaine, methamphetamine, and cannabis.[54–56] The toxicity of these drugs can induce not only IS but also hemorrhagic stroke. Hemorrhagic stroke via cocaine and/or methamphetamine use may lead to hypertension, vascular fatigue as a consequence of hypertension and tachycardia, and necrotising angiitis.[55] In addition to

chronic vascular damage and acceleration of atherosclerosis, use of these substances can lead to IS from acute cerebral vasospasm and vasculitis.[54, 55, 57] Cannabis-associated stroke has been also reported, where most cases occurred after cannabis exposure or subsequent re-exposure.[57, 58] While half of these cases had concomitant risk factors such as tobacco and alcohol,[57] major causes of cannabis-related IS were thought to be due to acute cerebral angiopathy and vasospasm. [57, 58]

3. Stroke prevention: antiplatelet and anticoagulation, carotid revascularization

Stroke prevention can be broadly categorized into primary and secondary prevention. Primary prevention refers to a series of lifestyle modifications and treatments in patients who have stroke risk factors but have not suffered stroke. Secondary prevention refers to similar changes and treatments but applied to patients who have already suffered stroke or transient ischemic attack (TIA, or a temporary period of brain ischemia that does not lead to infarction). Many of these interventions overlap and will be discussed together.

Lifestyle changes that can reduce stroke risk include modifications in diet, exercise, tobacco and elicit drug use cessation.[59, 60] For primary stroke prevention, cessation of smoking, regular physical activity (≥30 min/day), a healthy diet, moderate alcohol consumption, and maintaining a body mass index $< 25 kg/m^2$ has been shown to reduce stroke risk by as much as 80% compared to no lifestyle modifications.[61] In contrast, the effectiveness of these modifications for secondary stroke prevention seems less robust.[62] While the consensus on a healthy diet has generally been interpreted to mean limiting intake of sodium, red meat and saturated fat intake, several other dietary factors have been reported to reduce stroke risk, such as diets rich in magnesium, flavonoids, lycopenes, fruits and vegetables, and chocolate.[60] Further, the Mediterranean diet was found to reduce cardiovascular risk in a randomized trial.[63] Intensive exercise has been also reported to have a benefit in secondary stroke prevention.[64] Thus, it is incumbent upon healthcare providers to emphasize lifestyle changes for stroke risk reduction.

Pharmacological approaches to reduce stroke risk often include agents which prevent or reduce thrombus formation. Anti-platelet agents are used for secondary prevention in most non-cardiogenic IS patients to prevent worsening of atherosclerotic disease. The main mechanism of IS reduction are through blockade of platelet aggregation and activation through the suppression of thromboxane A2 via cyclooxygenase-1 blockade and upregulation of cAMP via phosphodiesterase 3 or $P2Y_{12}$ receptor blockade. Aspirin, clopidgrel, dipyridamole, and cilostazol have all demonstrated efficacy for secondary prevention of IS through this anti-platelet effect. Prasugrel is newer antiplatelet agent, but has not been shown to be superior to conventional anti-platelet agents in recent study.[65] Ticagrelor, a recently approved anti-platelet inhibitor of the $P2Y_{12}$ ADP receptor in coronary disease, was shown to also have efficacy in primary and secondary IS prevention.[66–68] Dual antiplatelet treatment (DAPT) typically consists of aspirin plus clopidogrel has met with conflicting results. Early studies suggested that such combination therapy led to unacceptably high risks of major cerebral and gastrointestinal bleeding and a recent meta-

analysis indicated that such hemorrhage due to DAPT negated any antithrombotic benefit.[69, 70] It should be pointed out that these early studies used long term DAPT for months or even years. More recent studies of short term DAPT have now shown that this approach can further reduce stroke risk while not increasing the risk of significant hemorrhage (CHANCE, POINT).[71, 72] As such, it is now common practice to prescribe DAPT for periods of 21 or 90 days after non-cardiogenic IS.[73] It should also be noted that DAPT may increase HTf when used with t-PA in experimental stroke models,[74] In contrast, a recent study showed that combination therapy of aspirin or clopidogrel with cilostazol has been reported not to increase the incidence of HTf and to reduce relative risk of recurrent IS by 51% compared to single anti-platelet therapy.[75] Cilostazol is also thought to have pleiotropic effects such as an improvement of endothelial function by inhibition of smooth muscle cell proliferation and reduction of inflammation.[76, 77] In a recent experimental study, cilostazol was also shown to have a neuroprotective effect via reduction of inflammatory molecules, stabilization of the blood-brain barrier, and prevented apoptosis.[78]

Significant (≥70%) extracranial carotid artery stenosis is detected about 15% of IS patients. Revascularization, either via endarterectomy or endovascular approaches, of symptomatic stenotic carotids has been shown to significantly reduce stroke risk, especially if carried out within two weeks of the index transient ischemic attack (TIA) or stroke.[79, 80] While endarterectomy has long been the mainstay of revascularizing stenotic carotids, endovascular approaches include carotid artery angioplasty followed by stenting (CAS), which has the advantage of being less invasive and similarly efficacious.[81] Less clear is the role of carotid revascularization on asymptomatic carotids.[82]

In patients with a cardiogenic cause of stroke, anti-thrombotic therapy to prevent thrombus formation has been shown in several studies to substantially reduce recurrent stroke, particularly for atrial fibrillation.[83] The vitamin K antagonist warfarin has been the most widely studied and used, but recently, orally available direct thrombin and factor X inhibitors (also referred to as direct acting oral anticoagulants; DOAC) have been shown to be as effective and easier to manage than warfarin. Several clinical studies have shown that DOACs are noninferior to warfarin in the prevention of IS with a lower incidence of significant HTf. Further, the presence of cerebral microbleeds (CMBs), which may be an indication of underlying cerebral amyloid angiopathy, increases the risk for cerebral hemorrhage in association with anti-thrombotic therapy. The incidence of intracranial hemorrhage in the presence of CMBs during DOAC treatment has been reported to be less than anti-platelet and warfarin therapy. This is thought to be due to the ability of DOACs to avoid inhibiting factor VII, although hemorrhage risk is still higher compared to that amongst patients without CMBs. Thus, it is still not recommended to initiate DOAC or other anticoagulant treatment in this patient population.[84] ESUS are now increasingly thought to be due to occult atrial fibrillation (AF), in part, due to the availability of long term cardiac monitoring technology[85]; however, it is unclear whether these patients should be empirically anticoagulated.[86] DOACs were considered for secondary prevention of ESUS; however, a few studies (NAVIGATE-ESUS and RE-SPECT ESUS) have failed to show that this approach is effective.[87, 88] Other embolic sources such as aortic plaque, patent foramen ovale, and neoplasm have been identified as the etiology of ESUS where anticoagulation is not always the indicated treatment. Hence, documentation of occult AF will be important

prior to initiating anticoagulant therapy. Other beneficial effects of DOACs have been suggested in experimental studies. Dabigatran, a direct thrombin inhibitor, has been shown to inhibit microglial and astrocyte activation.[89] Edoxaban, a factor X inhibitor, has also been shown to have anti-inflammatory effects via suppression of PAI-1, MCP-1, and TNF-α.[90]

Some 'natural' approaches have been studied in stroke prevention as well, but have not been routinely implemented at the clinical level. Polyphenol supplementation has been proposed as a preventive agent for IS. In a previous study, polyphenol intake was thought to act as an antioxidant, leading to reduction in atherosclerosis.[91] It is also thought to have other beneficial properties such as regulating neurotrophin levels, especially nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Epigallocatechin gallate (EGCG), which is a polyphenol found in green tea, has gained interest for its putative antioxidant and neuroprotective effects via prevention of NF-κB activation, inhibition on PI3K/Akt signaling, and improvement of mitochondrial dysfunction.[92] EGCG also seems to the downregulate MMP-2 and MMP-9 and upregulate the endogenous t-PA inhibitor plasminogen activator inhibitor-1 (PAI-1). These latter observations suggest that EGCG may have a role as an adjunctive agent to t-PA by extending the therapeutic time-window for thrombolytic treatment while reducing other undesirable side effects of t-PA treatment such as severe HTf, brain edema and BBB disruption. Other polyphenols have demonstrated antioxidant effects, which have the potential to reduce stroke risk. Resveratol, a component of red wine, has been shown to inhibit phosphodiesterase and regulate cAMP, AMPK, and SIRT1 pathways during ischemic injury. Salvianolic acid has been shown to have neuroprotective effects dependent on mitochondrial connexin43 via PI3K/Akt pathway. Flavonol rich diets has been reported for a 14% relative risk reduction of IS. Flavonoids is main sources of apple polyphenol, which has been reported to reverse oxidative stress via P38 mitogen-activated protein kinase signaling pathway.[93, 94]

4. Gender and stroke

While gender itself is not necessarily a comorbidity for stroke, it does deserve mention as a risk factor. In general, it is becoming increasingly recognized that gender does influence the cause of a stroke occurs as well as recovery from it. While laboratory studies have shown that male animals tend to have larger infarcts and worse outcomes compared to female animals, at the clinical level, women have unique stroke risks such as contraceptive use, estrogen supplementation during menopause and pregnancy related stroke. Vascular reactivity related to sex hormones, especially estrogen, may explain these sex differences, and has been reported in several experimental studies.[95] Estrogen contributes to nitric oxide (NO) production via eNOS, suppression of ROS via improved mitochondrial function, and altering cyclooxygenase-dependent prostanoids such as vasodilating prostacyclin (PGI_2) and thromboxane activity (TxA_2). These differences all have the potential to affect stroke outcomes. Another reason that females may have relative neuroprotection from stroke may be due to reports of lower soluble epoxide hydrolase and higher epoxyeicosatrienoic acid levels in astrocyte and vascular endothelial cells after ischemia. Although estrogen has been shown neuroprotective effects in experimental female stroke models, female gender could also increase IS risk at the clinical level.[96] Coagulation factors such as factor Ⅶ and Ⅻ,

protein C and S, plasminogen activator inhibitor type 1, and anti-thrombin Ⅲ are affected by estrogen levels, which may increase as a result of pregnancy and oral estrogen intake. As such, higher estrogen levels may lead to a hypercoagulable state which can increase IS risk via pro-thrombotic mechanisms.[95] Further, several clinical studies have shown gender differences and IS subtype as well as responses to treatment and even stroke symptomatology. Women also suffer more cardioembolic stroke than men. While the incidence of AF is similar for both genders, women with non valvular AF (NVAF) have higher IS risk compared to men with the same.[97] Thus, it will be important to consider the effect of gender and response to future therapies for stroke.

5. Acute stroke treatment: revascularization and neuroprotection

Pharmacological recanalization with t-PA has been the mainstay for acute IS treatment for several decades. t-PA therapy has been shown to improve neurological outcome provided it is initiated within 4.5 h from symptom onset. In addition to rt-PA therapy, recent randomized controlled trials have demonstrated the efficacy of mechanical thrombectomy (MT) in large vessel stroke.[98] In several of these trials, pre-treatment with t-PA before MT intervention was superior to t-PA alone. A few studies also showed that MT could be extended to even 24h after stroke onset, provided imaging studies showed a large mismatch. The DAWN (6–24 h after stroke onset),[99] DEFUSE3 (6–16h after stroke onset),[100] and EXTEND (within 9h after stroke onset)[101] studies all evaluated endovascular methods of thrombectomy to acutely revascularize occluded large cerebral vessels that cause stroke. These studies have not only shown that acute revascularization can improve stroke outcome from longer time windows, but can also lengthen the therapeutic time windows for t-PA. These studies utilized imaging based criteria to identify appropriate candidates. In particular, those studies which showed longer time windows for thrombectomy used imaging to demonstrate a large, and thus salvageable ischemic penumbra in relation to the ischemic core (“mismatch”). Further, imaging criteria have allowed for the use of t-PA therapy in so-called “wake-up stroke”, where the time of stroke onset is unclear because the patient reports waking up with a neurological deficit after being neurologically intact at the time of sleep. Such cases may be pre-selected by mismatch from diffusion weighted (DWI) and FLAIR MRI.[1]

While the expansion of therapeutic time windows and improved outcomes have been shown in acute revascularization approaches, reperfusion injury (R/I) has the potential to worsen outcome, compared to no revascularization. While this phenomenon is well established in experimental stroke models, its existence in clinical stroke has been debated. Nevertheless, some have reported a hyperintense acute reperfusion marker on MRI which is thought to predict HTf and clinical worsening in some IS patients, and this has been suggested R/I in humans.[102] In experimental studies, R/I has been attributed to the introduction and generation of ROS when a previously occluded vessel is opened. This flood of ROS leads to inflammation. Inflammation then results in the generation of various damaging immune mediators, effector molecules and more ROS. ROS can also lead to apoptosis/necrosis via DNA/RNA damage, lipid peroxidation, and the reduction of ATP production. Further, t-PA treatment can promote extracellular matrix damage to lead to HTf. Hence, targeted R/I treatments in conjunction with t-PA and/or MT has the potential to further improve neurological outcomes.[103]

A few potential adjunctive agents have been explored at the clinical level. Edaravone is an antioxidant and ROS scavenger marketed as a neurovascular protective agent. At the clinical level, edaravone contributes to improving neurological function and reducing adverse events. In the PROTECT 4.5 trial, the efficacy and safety of combination therapy with edaravone and t-PA in stroke patients suggested that combination therapy might increase the numbers of patients with better outcomes, accelerated recanalization and reduced HTf. The YAMATO study showed that the timing of edaravone infusion did not affect the rate of early recanalization, symptomatic HTf, or favorable outcomes after t-PA therapy. However, early edaravone infusion in parallel with endovascular revascularization led to better functional outcomes at discharge, lower mortality, and lower incidence of HTf in a recent clinical trial. Edaravone has been already approved for the treatment of IS patients who present within 24 h of the onset of symptoms in Japan and other countries, but not the United States. Thus, the prospects of adding edaravone to t-PA and MT seem favorable.

Therapeutic hypothermia (HT) has already been shown to improve neurological outcomes in comatose survivors of cardiac arrest and neonatal hypoxic encephalopathy. While it has yet to be shown whether it has any role in patients with IS, major mechanisms for its neuroprotective effect seems to be related to its effects on multiple cell death pathways including inflammation, apoptosis, excitotoxicity and preservation of metabolic stores.[104] HT has also been shown to reduce BBB disruption and HTf in relation to t-PA use in experimental models.[105] Combination therapy with HT and t-PA also reduced HTf and endogeneous tPA expression, and has the potential to extend the time window for other acute therapies. Few clinical studies have been carried out in IS. The ReCCLAIM (Reperfusion and Cooling in Cerebral Acute Ischemia) and ICTuS studies assessed the combination therapy with rt-PA and HT in IS patients with large pretreatment infarcts, and both trials showed that this approach was safe and may even reduce reperfusion injury, as outcomes were improved compared to stroke patients who did not receive HT. The ICTuS2 study showed the safety and feasibility of both HT and HT with t-PA, although cooling increased the incidence of pneumonia. HT has been also combined with MT with selective brain cooling elicited by intra-arterial chilled saline infusion and was shown to be both safe and feasible.[106] The RECCLAIM-Ⅱ also examined MT with HT; however, this trial was stopped early for lack of funding.[107] A recent laboratory study also showed that the neuroprotective effect of HT differentially affects cells of neurovascular unit depending on the depth, duration and even timing of cooling.[108] Yet, clinical studies used a single target temperature with fixed duration (e.g. 24 hours). Thus, it may be important to design future clinical trials with adjusted temperature and cooling duration depending on targeted cell type(s) for neuroprotection versus vasculoprotection.

6. Conclusions

Disparities between experimental and clinical studies have been frequently related to several factors. The most common factors that often differ between IS patients and preclinical laboratory studies in animal models include age, gender, stroke severity, how well stroke models resemble clinical stroke, comorbidities and inherent species differences in the coagulation system. In many experimental studies of stroke models, young and healthy male animals have been used. On the other hand, most IS patients recruited to clinical trials are

elderly, have several vascular risk factors and are of both genders. Differences between human and rodent circadian rhythms are another recently identified factor that could affect the translation of preclinical stroke studies. In contrast to humans, rodents, the major species used in experimental stroke studies, are nocturnal, and daylight hours are thought to be rodents' "inactive phase" while their "active phase" occurs at night. Recent studies indicate that excitotoxicity and oxidative stress are increased during the "active phase", rather than the "inactive phase".[109] Yet, many experimental studies are carried out during the animals' "inactive phase", while clinical studies of acute stroke treatment have not routinely controlled for the human circadian phases, although it seems likely that most stroke patients enrolled in acute treatment trials may have been treated during the human "active phase" (day). Thus, it is also clear that the influence of circadian rhythm on neuroprotection should be considered.

Preclinical studies may not always study dose or dose-response in much detail, if at all. Even if the drug dose is appropriate for small animals such as mice and rats, it is sometimes inappropriate for large animals including humans. The toxicities may be also different depending on species, and studies of thrombolytics and anticoagulants often do not consider interspecies differences in the thrombotic and thrombolytic systems. Nevertheless, the recent development of stroke models in laboratory animals with typical vascular risk factors such as hypertension, diabetes, and dyslipidemia has shown that like human stroke patients, these animals respond less effectively or differently to potential interventions. With such models in hand, there may still be hope for the identification of viable neuroprotectants that may be translated to the clinical level.

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