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Review Article

A Review of Antithrombotic Agents for the Treatment of Ischemic Stroke

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Abstract

Ischemic stroke caused by a blockage in blood vessels that supplies blood to the brain, can lead to severe neurological disability or even death if not treated in a timely proper manner. Current treatment for ischemic stroke comprises Intravenous Thrombolytic Therapy (IVT) and Endovascular Therapy (EVT). However, the unmet medical needs lie in the high disability, narrow therapeutic window, low patient eligibility for operations, and recurrent ischemic stroke and bleeding complications. The development of antithrombotic agents is key to reducing complications of current treatment and secondary prevention of ischemic stroke. This review paper covers a wide range of most commonly used antithrombotic medications or advancements in antithrombotic agents, including warfarin, heparins (UFH and LMWH), direct oral anticoagulants (dabigatran, rivaroxaban, apixaban), aspirin, dual-antiplatelet therapies (clopidogrel/aspirin and dipyridamole/aspirin), and Glycoprotein (GP) inhibitors (tirofiban and anfibatide). This paper compares the efficacy and safety of antithrombotic agents and discusses the advancement and potential of GP inhibitors in treating ischemic stroke patients. Based on preclinical and clinical results, it was found that GP inhibitors have higher antithrombotic and neuroprotective efficacy and are relatively safe with a lower rate of bleeding complications among all agents. The novel Anfibatide, a snake venom-derived GPIbα inhibitor currently in the early clinical development stage, has shown superior efficacy and safety profile compared to other agents. It has great potential to fulfill the current unmet medical needs in ischemic stroke treatment. It is worth exploring the mechanism of GP inhibitors and their applications in ischemic stroke treatment.

Keywords: Antithrombotic agents; Antithrombosis; Antiplatelet; Anticoagulant; Ischemic stroke; Ischemia-reperfusion injury

Abbreviations: IVT: Intravenous Thrombolytic Therapy; rt-PA: Recombinant Tissue Plasminogen Activator; EVT: Endovascular Therapy; MT: Mechanical Thrombectomy; VKA: Vitamin K Antagonist; INR: International Normalized Ratio; PT: Prothrombin Time; AVS: Aortic Valve Stenosis; FFP: Fresh Frozen Plasma; rfVIIa: Recombinant Factor VIIa; UFH: Unfractionated Heparin; AT3: Antithrombin III; VTE: Venous Thromboembolism; HIT: Heparin-Induced Thrombocytopenia; LMWH: Low Molecular Weight Heparin; DVT: Deep Vein Thrombosis; RE-LY trial: Randomized Evaluation of Long-Term Anticoagulation Therapy trial; CYP enzymes: Cytochromes P450; AIS: Acute Ischemic Stroke; P-gp: P-glycoprotein; ICH: Intracranial Hemorrhage; DAPT: Dual Antiplatelet Therapy; CAD: coronary artery disease; ESPS 2 trial: European Stroke Prevent Study 2 trial; TIA: Transient Ischemic Attack; PRoFESS trial: Prevention Regimen for Effectively Avoiding Second Strokes trial; mRS: Modified Rankin Scale; MMSE: Mini-Mental State Examination; ESCAPIST trial: Efficacy and Safety of Tirofiban in Clinical Patients with Acute Ischemic Stroke trial; TTP: Thrombotic thrombocytopenic Purpura

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Background

Globally, stroke is the third-leading cause of death and disability combined and the second-leading cause of death. Ischemic stroke as the major stroke subtype accounts for almost 80% of total stroke cases [1]. It occurs when the blood supply to the brain is obstructed by vessel thrombosis, embolism, or stenosis [2]. Different vessel recanalization treatments are suggested to restore the blood flow of the brain occlusion region and minimize cerebral infarct volume. The current major treatments for ischemic stroke can be classified into two categories: Intravenous Thrombolytic Therapy (IVT) with Recombinant Tissue Plasminogen Activator (rt-PA) and Endovascular Therapy (EVT) i.e. angioplasty and Mechanical Thrombectomy (MT).

However, there is still an unmet medical need for ischemic stroke treatment. Both rt-PA and endovascular treatments exert high disability-adjusted life years. The mortality rate of patients after rt-PA treatment 3-6 months is nearly 20% and two-thirds of the patients resulted in various levels of disability. The DALY of post-MT ranges from 29% to 58% [3,4]. The therapeutic time window of rt-Pa treatment for acute ischemic stroke is only 4.5 h within stroke onset [5], with less than 3% of patients benefiting from the treatment. The advancement of MT extended the therapeutic window up to 24 hours for acute ischemic patients with the indication of large artery occlusion. But only a small percentage (estimated 10%) of patients are eligible for MT since not all patients have large artery occlusion and patients presenting onset later than 6 hours have a lower chance of qualifying for MT [6]. Reperfusion injuries and subsequent recurrence of vessel occlusion are also concerns over the current EVT approaches due to the lack of antithrombotic agents to suppress thrombus formation in injured vessels.

The high chance of disability, narrow therapeutic window, low patient eligibility for operations, and recurrence of ischemic stroke reflect the current treatment alone is not efficacious enough to treat ischemic stroke. The development of antithrombotic agents appears to be a key to reducing complications of the current treatment and secondary prevention of ischemic stroke. This paper summarizes and compares the marketed and novel antithrombotic agents to discuss the future development of antithrombotic therapy for ischemic stroke treatment.

Anticoagulants

Vitamin K Antagonist (VKA): Warfarin

Warfarin is a traditional orally administered anticoagulant. It is a vitamin K antagonist indicated to treat long-term anticoagulation following thrombotic events or prevention of thrombotic events, for example, venous thromboembolism, atrial fibrillation, mechanical and bioprosthetic heart valves, and post-myocardial infarction [7]. It is commonly prescribed for the prevention of cardioembolic stroke [8]. Warfarin inhibits the reduction of vitamin K-dependent coagulation proteins (including factors II, VII, IX, X, proteins C, S, and Z) by binding to the VKORC1 subunit of vitamin K epoxide reductase [8]. A decrease in active protein levels results in the prevention of embolism and thrombosis. Since warfarin targets vitamin K, the daily vitamin K intake of the patient has to be monitored and restricted.

Efficacy

The dosage of Warfarin ranges from 5 to 10 mg per day regarding the international normalized ratio (INR), which is a universal monitoring index based on Prothrombin Time (PT) [9]. The onset of action of Warfarin takes about 2-3 days and the anticoagulant effects are detectable between 2 and 5 days after medication cessation, thereby indicating a slow onset action and long duration for metabolization. In the BAATAF trial [10], 420 patients with atrial fibrillation and without mitral stenosis were treated with either warfarin (INR 1.5-2.7) or placebo. Ischemic stroke was set to be the primary endpoint. Results showed a 71% lower mean plasma level of prothrombin fragment (F_{1+2} level) in the warfarin-treated group than in the control group [10], indicating positive prevention of ischemic stroke with the warfarin treatment. However, studies also revealed that there are different levels of warfarin resistance among patients. Genetic polymorphism of VKORC1, which encodes for VKOR, and CYP2C9, which metabolize warfarin into inactive metabolites, causes pharmacokinetic and pharmacodynamic mechanisms of resistance respectively [11]. Polymorphism of VKORC1 accounts for a 30% of efficacy variation and that of CYP2C9 accounts for a 10% variation [8]. Due to the metabolic variation, the dose must be monitored and adjusted for a therapeutic range of 2-3 INR. Hence, the efficacy of warfarin in anticoagulation is selective among ischemic stroke patients.

Safety

The bleeding complication i.e. hemorrhage is often observed in warfarin-treated patients, including GI bleeding as a major Adverse Event (AE), and risk of Intracranial Hemorrhage (ICH) [8]. The risk of major bleeding in patients with acute ischemic cerebrovascular disease ranged from 2-13% during a follow-up of 60-30 months [12]. Warfarin-induced hemorrhage is found to be directly related to the INR level and the risk of hemorrhage increases when INR>5 [9]. Long-term use of warfarin also causes diarrhea, vomiting, and potential osteoporosis. bleeding, thrombocytopenia, recent GI bleeding, liver disease, etc., are contraindications for warfarin use [8]. In addition, warfarin prevents normal clotting. Long-term use of warfarin can impair the normal hemostasis of patients and increase the risk of hemorrhagic stroke [13]. It is also found that warfarin upregulates ERK1/2, leading to aortic valve stenosis (AVS; calcification of aortic valve) [14]. Due to the high risk of hemorrhagic and AVS complications in long-term and narrow therapeutic index (INR 2-3), patients undergoing warfarin treatment have to be closely monitored (for the prothrombotic time and INR) which affects their quality of life, and thus low patient adherence results.

Reversal of Anticoagulant Effects

To prevent an overdose of warfarin or the long-term hemorrhage risk, reversal agents are given to the patients, including administration of vitamin K, Fresh Frozen Plasma (FFP), Recombinant Factor VIIa (rfVIIa), and prothrombin complex concentrates [15,16]. However, with the continuous monitoring and adjustment of warfarin and reversal agents dosing, treating ischemic stroke becomes complicated and patients are less likely to follow the whole course of treatment.

Heparins: Unfractionated Heparin (UFH) and Low Molecular Weight Heparin (LMWH)

Unfractionated Heparin (UFH)

The basic mechanism of heparin is that heparin binds to antithrombin III (AT3; a peptide that inhibits several activated clotting factors) and augments the anticoagulant effect of AT3. UFH is one of the heparin types. The AT3/UFH complex induces conformational changes of clotting factors Xa and IIa (thrombin), leading to the inhibition of the two factors in a 1:1 ratio and

prevention of fibrin formation and thrombin-induced activation of platelets and factors V and VIII [9,17]. UFH is indicated for the treatment and prophylaxis of Venous Thromboembolism (VTE), thrombus prophylaxis in atrial fibrillation, and treatment of disseminated intravascular coagulation. Intravenous injection of UFH, either subcutaneous for prophylaxis use or continuous intravenous infusion, is indicated for treatment and prophylaxis of Venous Thromboembolism (VTE), thrombus prophylaxis in atrial fibrillation, and treatment of disseminated intravascular coagulation [9].

Effect

The International Stroke Trial, a multicenter, multinational, randomized open trial, examined the effectiveness of early subcutaneous UFH treatment in acute stroke patients. 90% of the patients were in the ischemic stroke category. 17% of the total patients had atrial fibrillation, while others did not. The finding showed that the risk of recurrent ischemic stroke within 14 days was low (<3.5%) and atrial fibrillation had no significant impact on this [18]. Compared to orally administered warfarin, UFH has a faster onset of action and has an immediate therapeutic efficacy when used intravenously; and has therapeutic efficacy reached within an hour when administered subcutaneously [9].

Safety

Hemorrhage is also a common side effect in UFH-treated patients. However, the risk of major bleeding in UFH is less than 3% in VTE treatment, much lower compared to that of warfarin treatment [12].

Heparin-Induced Thrombocytopenia (HIT), an extremely hypercoagulable state where heparins bind Platelet Factor 4 (PF4) forming the heparin-antibody immunocomplex, is another severe non-bleeding adverse event in UFH treatment [19]. HIT is defined as a platelet-count decline of more than 50% at 5 to 10 days after the start of heparin treatment and the HIT risk with UFH treatment is 2.6% [20]. Though the percentage seems low, HIT can lead to detrimental outcomes, including death, limb amputation/gangrene, thrombosis, and bleeding [21].

Reversal Agents

Protamine sulfate is the only approved reversal agent for UFH treatment. 1 mg IV of protamine reverses 100 units of UFH. Protamine sulfate has a fast onset and the neutralization of UFH only takes 5 mins. However, rapid administration may lead to severe hypotension and anaphylaxis [9,22].

Low Molecular Weight Heparin (LMWH): Dalteparin, Enoxaparin, Tinzaparin

LMWH is parenterally administered and is derived from UFH. LMWH has a smaller molecular size and a relatively different mode of action compared to UFH [23]. Dalteparin, enoxaparin, and tinzaparin are the major types of LMWH. LMWHs bind to AT3 and have a higher proportional impact on Xa versus IIa, in a 3:1 or 2:1 ratio. They are administered at a fixed dose according to the total body weight. Diet restriction or strict monitoring is not required in LMWH treatment [9]. In general, LMWH has advantages, such as high bioavailability, longer half-life, reduced heparin-antibody complex formation, and dose-independent clearance, over UFH [23].

Efficacy

In the Prevention of VTE after Acute Ischemic Stroke with LMWH (PREVAIL) study, the findings showed that enoxaparin performed slightly better than UFH in preventing venous thromboembolism in patients with ischemic stroke. The risk of VTE in enoxaparin-treated patients was reduced by 43% while that of the UFH-treated patients was only 18% (P<0.0001). This trial also showed that LMWHs or UFH did not adversely impact the patient functional or neurological outcomes, compared with placebo or aspirin [24]. LMWH reaches the peak level 2-4 hours after subcutaneous administration. LMWH presents a more predictable dose-response curve compared to that of UFH. However, LMWH with 3-4 hours of half-life is eliminated mainly via renal clearance, and thus patients with renal insufficiency require a dose reduction of LMWH [9].

Safety

Both Kase, C. S. et al. [24] and Moonis, M. et al. [25] studies pointed out that LMWH can reduce recurrent stroke in acute ischemic stroke patients but with an increase in the risk of intracranial and extracranial hemorrhage.

In addition, a meta-analysis study reviewing 19 randomized controlled trials revealed that the occurrence of Deep Vein Thrombosis (DVT), a common complication of ischemic stroke, in Acute Ischemic Stroke (AIS) patients within 14 days and 3 months were higher in the LMWH treatment group, with risk ratio favoring LMWH [26]. Hence, DVT is considered a risk of LMWH for AIS treatment.

Direct Oral Anticoagulants: Thrombin Inhibitor, Factor Xa Inhibitor

Thrombin Inhibitor (Dabigatran)

A thrombin inhibitor is a competitive antagonist of fibrin-bound or -unbound thrombin. It binds to the active site of thrombin and inhibits the conversion of inactive fibrinogen to active fibrin, which plays an important role in the blood coagulation pathway [8]. Dabigatran is one of the commonly used thrombin inhibitors. It is activated via esterase-catalyzed hydrolysis. Since it is also able to inhibit fibrin-bound thrombin, it is regarded as superior to UFH and LMWH. FDA approved the use of 150 mg of Dabigatran tablets and 75 mg tablets for severe renal impairment patients based on pharmacokinetic data [27]. No INR monitoring or diet modifications are required during dabigatran treatment and the half-life of it ranges from 14-17 hours [27].

Efficacy

Compared to warfarin, whose onset takes 4 days, the onset of dabigatran only takes 36-72 hours. In the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial, the efficacy of Dabigatran was compared with warfarin in treating patients with atrial fibrillation and increased risk for stroke. 18113 patients enrolled in the study and half of the patients had long-term use of vitamin K antagonists. Trial results showed the occurrence of stroke or systemic embolism and hemorrhagic stroke per year in 110 mg Dabigatran treatment was 1.53% and 0.12% (P<0.001); in 150 mg Dabigatran treatment was 1.11% and 0.26% (P<0.001); in warfarin treatment was 1.69% and 0.38%. 150 mg Dabigatran showed significantly greater efficacy in preventing the occurrence of stroke and embolism than warfarin [28].

Safety

Cytochromes P450 (CYP enzymes) are not involved in the

Table 1: Summary of effects/efficacy, safety, and restriction of the use of described antithrombotic agents.

Category	Drug	Effects/efficacy	Safety	Restriction of use
Anticoagulant (Heparin) (Direct oral anticoagulant)	Warfarin	1) Onset of action: takes 2-3 days; slow onset of action [10]. 2) Anticoagulant effects are detectable for 2-5 days after medication cessation; long duration of metabolization [10]. 3) Lower 71% of mean plasma level of prothrombin fragment; fair antithrombosis efficacy [10].	1) Common adverse event: GI bleeding and ICH [8]. 2) Long-term use causes diarrhea, vomiting, and potential osteoporosis, bleeding, thrombocytopenia, recent GI bleeding, and liver disease [8]. 3) Long-term use impairs normal hemostasis and increases the risk of hemorrhage stroke [13]. 4) Potentially cause aortic valve stenosis (AVS) [14].	1) Warfarin resistance [11] 2) Narrow therapeutic index (INR 2-3) [9]. 3) Continuous monitoring of diet and adjustment of reversal agent dosing according to INR [15,16].
	UFH	1) Risk of recurrent ischemic stroke with 14 days <3.5% [18]. 2) Immediate therapeutic efficacy; faster onset of action (within 1 hour) [9]. 3) Does not adversely impact patient function or neurological outcomes compared with aspirin [24].	1) Lower risk of major bleeding compared to warfarin [12]. 2) Occurrence of HIT – a detrimental SAE [19].	1) Cautious use of reversal agent (protamine); rapid administration leads to severe hypotension and anaphylaxis [22].
	LMWH	1) Risk of VTE was reduced by 43% vs 18% reduction by UFH [24]. 2) Does not adversely impact patient function or neurological outcomes compared with aspirin [24]. 3) Fast onset of action; plasma concentration peak at 2-4 hr after SC administration [9].	1) High incidence rate of DVT[26]	1) Patients with renal insufficiency require dose reduction.[9]
	Dabigatran	1) Greater efficacy in preventing stroke and embolism than warfarin [28].	1) Bleeding and esophagitis or esophageal injury are major side effects [26]. 2) Increase the risk of major bleeding in a dose-dependent manner [26].	
	Rivaroxaban	1) Slightly superior to warfarin in minimizing the occurrence of stroke or systemic embolism [31]. 2) Lower occurrence of myocardial infarction (0.9% per year) compared to warfarin (11.1% per year) [31].	1) GI hemorrhage and ICH as the most common SAEs but lower ICH rates than warfarin [31]	1) Patients with severe hepatic impairment cannot be prescribed [27]. 2) Dose adjustment is required for patients with renal insufficiency [27]. 3) Contraindicated for active pathological bleeding and severe hypersensitivity reactions [27]. 4) Drug interaction with P-gp, strong CYP3A4 inhibitors and inducers, and other anticoagulants during prophylaxis of DVT [27]. 5) Discontinuation of the drug increases the risk of recurrent thrombotic events.
	Apixaban	1) Slightly superior to warfarin in reducing stroke and systemic embolism occurrences [36]. 2) Able to reduce stroke/systemic embolism occurrences in elderly with renal deficiency [35]. 3) Superior to aspirin; occurrences of stroke (ischemic, hemorrhage, disabling or fatal stroke), myocardial infarction, and death were reduced by nearly or more than half compared to the aspirin group [36].	1) Reduced major bleeding occurrence by 20-50% in all age groups compared to the warfarin group [35,36]. 2) ICH rate of apixaban-treated patients was only one-third of the warfarin-treated patients [35,36].	1) Discontinuation of the drug increases the risk of recurrent thrombotic events [32].

Antiplatelet agents (DAPT) (GP inhibitor)	Aspirin	1) 42% overall stroke risk reduction in patients treated with aspirin in the SPAF-1 trial [39]. 2) Inferior to apixaban in terms of reducing the occurrence of stroke [36].	1) Increased major extracranial hemorrhage [42]. 2) Long-term use of aspirin significantly increases the rate of GI bleeding [8]. 3) Hemorrhage remains the common SAE and AE in aspirin therapy [8].	1) Aspirin resistance [40,8] 2) Numerous contraindications: patients with allergies to non-steroidal anti-inflammatory drugs, asthma, rhinitis, and nasal polyps due to bronchospasm, angioedema, or urticaria [8].
	Clopidogrel	1) Clopidogrel alone reduces stroke event rate per year to a larger extent than aspirin (CAPRIE trial: 7.15% in the clopidogrel group; 7.71% in the aspirin group [46]. 2) DAPT (Clopidogrel/Aspirin) is more effective than aspirin monotherapy in preventing recurrent ischemic/hemorrhage stroke in patients suffering from ischemic stroke/TIA [45].	1) Intracranial and extracranial bleeding events were more frequent in the DAPT groups than in the aspirin group [45] 2) CHRISMA, MATCH, and CHANCE: no significant added benefits from DAPT but an increased rate of AEs, SAEs, and death [8].	1) Clopidogrel resistance; 40% of clopidogrel users are resistant to clopidogrel; the frequency of clopidogrel-resistant patients with ischemic cerebrovascular disease is around 30% [48].
	Dipyridamole/aspirin	1) Aspirin alone reduced the risk of stroke or death by 13% (P = 0.016); dipyridamole alone reduced the risk by 15% (O = 0.015); and the combination reduced the risk by 24% (P < 0.001) [53]. 2) The dual therapy of dipyridamole/aspirin fails to demonstrate a neuroprotective function [52].	1) ESPS 2 trial: Compared to aspirin monotherapy, dual therapy (400mg dipyridamole + aspirin) increases the occurrence of headaches, bleeding, and gastrointestinal events [52]. 2) ESPRIT trial: 30% reduction in major bleeding complications (i.e. fatal and non-fatal hemorrhage in the extracranial and intracranial cavity) was observed in the dual therapy group (200mg dipyridamole) compared to the aspirin group [54].	1) Chronic use of aspirin leads to aspirin resistance and lower efficacy.
	Tirofiban	1) Association of mRS score improvements and tirofiban treatment was observed in multiple trials; reflecting a reduction in neurologic disability risk caused by AIS [57,58]. 2) Antiplatelet effect of tirofiban was found associated with a positive increase in the recanalization rate [57].	1) ESCAPIST trial: Bleeding events and sICH occurrence in tirofiban-treated patients were similar to that of aspirin-treated patients [57]. 2) ESCAPIST trial: occurrence of deaths within 90 days in the tirofiban group (0.6%) was much lower than in the aspirin group (3.9%) [58]. 3) Tirofiban is associated with drug-induced thrombocytopenia complication, a severe adverse event; thrombocytopenic purpura was observed in tirofiban-treated patients [60,61].	
	Anfibatide	(Based on preclinical study data) 1) Antiplatelet effect of Anfibatide was comparable to Ticlopidine and stronger than aspirin and dipyridamole [65]. 2) Anfibatide has superior antithrombosis efficacy than tirofiban [67]. 3) Anfibatide-treated MCAO rats had a larger extent of neurological improvements than tirofiban-treated MCAO rats [63]. 4) Anfibatide significantly reduces MDA and LDH expression levels in MCAO rat neuron cells and dose-dependently increases antioxidant activities [66].	1) No irritation or significant impact on blood pressure or pyrogenic changes [69]. 2) Low immunotoxicity risk [69]. 3) Lower ICH risk; hemorrhage volumes and bleeding complications were less severe in Anfibatide-treated subjects than those treated with tirofiban [63]. 4) Potential added benefits to TTP subjects; Anfibatide dramatically reduces thrombocytopenia rate [79].	

activation of Dabigatran, and thus reduce potential drug-drug interactions as many drugs utilize CYP enzymes. Bleeding and esophagitis or esophageal injury is side effects of dabigatran treatment. Dabigatran dose-dependently increases the risk of major bleeding. 110 mg of dabigatran results in a 2.71% per year prevalence in major bleeding; while 150 mg of dabigatran results in a 3.11% per year of prevalence. Life-threatening bleeding rates of 110 mg and 150 mg dabigatran twice a day are 1.22% and 1.45% [26].

Factor Xa Inhibitor: Rivaroxaban, Apixaban

Factor Xa is involved in both intrinsic and extrinsic activation pathways of blood coagulation. It catalyzes the conversion of prothrombin to active thrombin, which then up-regulates clotting, and platelet and endothelial activations. The ratio of catalytic conversion is around 1 Factor Xa to 1000 thrombin molecules [29]. Factor Xa inhibitors bind to S1 and S4 pockets of factor Xa and inhibit prothrombinase activity, reducing the formation of active thrombin. Rivaroxaban and apixaban are currently widely used DOACs.

Rivaroxaban

Rivaroxaban is indicated to reduce the risk of stroke and systemic embolism in patients with Non-Valvular atrial fibrillation

(NAF) and for the prophylaxis of Deep Vein Thrombosis (DVT). 10 or 20 mg oral dosage of rivaroxaban is prescribed to patients with different levels of NAF. 10 mg oral dose is prescribed for prophylaxis DVT. Patients with severe hepatic impairment cannot be prescribed [30]. Plasma rivaroxaban concentration peaks within 2-4 hours of oral ingestion and its half-life is within 7-11 hours. Constant monitory or dose adjustments are not required in rivaroxaban treatment to ensure the therapeutic INR levels [8]. However, dose adjustment is required when patients have a certain extent of renal impairment.

Efficacy

In the ROCKET-AF trial, the efficacy and safety of Rivaroxaban (20 mg once daily) and warfarin (INR 2.0-3.0) in treating patients with non-valvular AF and history or risk factors of stroke were compared. The findings revealed that the efficacy of rivaroxaban was not inferior to warfarin in minimizing the occurrence of stroke or systemic embolism (rivaroxaban: 1.7% occurrence per year; warfarin: 2.2% occurrence per year). In addition, myocardial infarction occurrence was lower in the rivaroxaban group (0.9% per year) compared to the warfarin group (1.1% per year). The efficacy of rivaroxaban in preventing ischemic stroke was slightly better than warfarin [31]. Rivaroxaban is an effective alternative to warfarin.

Safety

GI and intracranial hemorrhage are considered the most common SAE for factor Xa inhibitors. For rivaroxaban, the ROCKET-AF trial outcomes showed a similar risk of bleeding complications and rate of major bleeding in rivaroxaban (14.9% per year; 3.6% respectively) and warfarin (14.5% per year; 3.4% respectively) treatments. Major bleeding of GI occurred more in the rivaroxaban group. Regarding ICH rates, the rivaroxaban group was significantly lower than that of the warfarin group [31].

Rivaroxaban is contraindicated for active pathological bleeding and severe hypersensitivity reactions. It also displays drug interactions with combined P-glycoprotein (P-gp), strong CYP3A4 inhibitors and inducers, and other anticoagulants during prophylaxis of DVT [30]. It is also indicated that discontinuation of rivaroxaban increases the risk of recurrent thrombotic events and another anticoagulant has to be replaced with rivaroxaban to lower the risk of stroke.

Apixaban

Similar to rivaroxaban, apixaban is indicated to treat patients with nonvalvular atrial fibrillation to reduce the risk of stroke and systemic embolism. Oral administration of 5 mg twice daily is recommended for patients [32]. The maximum concentration of apixaban is reached within 3-4 hours after oral ingestion and it has a half-life of 12 hours. Unlike rivaroxaban, the dose adjustment of apixaban for renal-impaired patients is unknown but it is known that the renal elimination pathway of apixaban accounts for 25% of all pathways [33]. Constant monitoring and diet restriction are not required in apixaban therapy to ensure INR levels.

Efficacy

In the ARISTOTLE trial (n=18201), the efficacy and safety of apixaban were compared with warfarin for the reduction in stroke (ischemic or hemorrhagic) and other thromboembolic events in atrial fibrillation at different age groups [34]. The occurrences of stroke and systemic embolism (primary endpoint) were lower in the apixaban groups (1.25% in age 65-74; 1.56% in age ≥75) than that in the warfarin groups (1.73% in age 65-74; 2.19% in age ≥75). The study also showed favorable primary outcomes in elderly with a renal deficiency that the occurrences of stroke/systemic embolism at different Cockroft-Gault eGFR levels (>80, >50–80, >30–50, ≤30) were all lower in the apixaban group than the warfarin group [35].

In the AVERROES trial, patients with atrial fibrillation at increased risk of stroke and unsuitable for VKA therapy were randomly assigned to apixaban (5 mg twice daily) or aspirin (81-324 mg per day) to test for the efficacy and safety of apixaban in reducing stroke or systemic embolism and major bleeding. The efficacy results showed that the occurrences of stroke (ischemic, hemorrhage, disabling or fatal stroke), myocardial infarction, and death items in the apixaban group were reduced by nearly or more than half compared to the aspirin group with statistical significance [36].

These findings reveal that apixaban is efficacious in preventing and reducing the occurrence of stroke (ischemic and hemorrhagic) at a total of 10 mg dosage daily. It is a viable alternative to warfarin and aspirin therapies.

Safety

Same complications, indications, and contraindications as rivaroxaban. Major bleeding and Intracranial Hemorrhage (ICH) are the most common SAE in apixaban therapy. In the ARISTOTLE study, apixaban reduced major bleeding occurrence by 20-50% in all age groups compared to that of the warfarin group. The occurrence of ICH was much lower in 65 - <75 and ≥75 age groups treated with apixaban and the ICH rate in apixaban groups was only one-third of that in warfarin groups [35]. In the AVERROES trial, the occurrences of major bleeding, ICH, extracranial or unclassified bleeding, and GI bleeding were similar between the aspirin and apixaban groups [36]. Discontinuation of apixaban therapy without the substitution of another anticoagulant therapy also enhances the risk of ICH and stroke [32].

Antiplatelet Agents: Aspirin, Clopidogrel, Dipyridamole/Aspirin, Tirofiban, Anfibatide

Aspirin

Aspirin is known as a TXA_2 inhibitor, which is involved in platelet aggregation and vasoconstriction. It has been used to treat non-cardioembolic ischemic stroke prophylaxis for a long time. The mechanism of action of aspirin is that it acetylates COX1 enzyme active sites and prevents the conversion of arachidonic acid to prostaglandin endoperoxides, the transient intermediates of TXA_2. Declined TXA_2 level reduces platelet activation and thereby inhibits platelet aggregation and thrombus formation. Suppressed platelet aggregation and vasoconstriction improve the condition of ischemic stroke and reduce cerebral infarct size. The half-life of aspirin is within 2-3 hours, which is short, but the antiplatelet effect of aspirin is irreversible and lasts for up to 10 days depending on the platelet life [8]. Aspirin has antiplatelet effects and significantly reduces levels of inflammatory mediators and protects ischemic stroke patients from cerebral inflammation and neurological damage [37].

Efficacy

In the Antithrombotic Trialists' Collaboration (ATT) meta-analysis, a daily dose of 160-325mg and 75-150 mg aspirin sig-

nificantly reduced 26% and 32% of the occurrence of vascular events respectively, including myocardial infarction. stroke, or death [38]. The SPAF-1 trial also showed a 42% overall stroke risk reduction in patients treated with aspirin [39]. However, as mentioned previously, the AVERROES trial showed the inferiority of aspirin in reducing the occurrence of stroke as compared to apixaban. In addition, findings are showing an association between aspirin resistance and its chronic use and other factors like platelet sensitivity, genetic polymorphisms of COX1/2 enzymes, low bioavailability, etc. [40,8,41].

Safety

In Sandercock, P. A. et al. [42] meta-analysis study, three major trials (i.e. the Chinese Acute Stroke Trial, the International Stroke Trial, and the Multicentre Acute Stroke Trial-Italy) examining the antiplatelet effect in acute presumed ischemic stroke of aspirin and other antiplatelet drugs were compared. The findings revealed that the rate of symptomatic intracranial hemorrhage during the aspirin treatment period as compared to the control (without aspirin) was higher (OR=1.22; 95% CI, 1.00 to 1.50). Major extracranial hemorrhage during the aspirin treatment period was also higher than the control (OR=1.69; 95% CI, 1.35 to 2.11). Hemorrhage remains the common SAE and AE in aspirin therapy. Long-term use of aspirin has shown a significant rate of GI bleeding as a common complication. Aspirin also has numerous contraindications, including patients with allergies to non-steroidal anti-inflammatory drugs, asthma, rhinitis, and nasal polyps due to possible bronchospasm, angioedema, or urticaria [8]. Despite the short-term antiplatelet effectiveness, aspirin therapy is often being questioned about its safety and prescription limitations.

Clopidogrel

Clopidogrel generates active metabolite upon activation by the CYP3A enzyme and the active metabolite irreversibly binds to the platelet $P2Y_{12}$ (ADP) receptor, thereby inhibiting ADP-induced platelet aggregation [43]. The antiplatelet effect lasts around 7-10 days, depending on the affected platelet lifespan, due to the irreversible action of clopidogrel active metabolite. The recommended daily dose of clopidogrel is 75 mg once daily [44]. Clopidogrel can be used as mono- or Dual Antiplatelet Therapy (DAPT). More often, patients with acute ischemic stroke or transient ischemic attack are treated with clopidogrel and another antiplatelet drug, e.g. aspirin, for minimizing recurrent stroke.

Efficacy

In Yang, Y. et al. [45] study, 7 meta-analyses (including 133502 patients) on the efficacy of clopidogrel and/or aspirin in acute ischemic stroke patients were compared. The study showed that DAPT is more effective than aspirin monotherapy in preventing recurrent ischemic/hemorrhage stroke in patients suffering from ischemic stroke/TIA with Relative Risks (RR) equal to 0.75 or 0. 72 favoring DAPT treatment.

CAPRIE trial (n=19185), a randomized, blinded, international trial assessing the relative efficacy of clopidogrel 75 mg and aspirin 325 mg in risk reduction of a cluster of ischemic stroke, myocardial infarction, or vascular death. In terms of the first occurrence rate of the ischemic events, the average rate per year in the clopidogrel treatment group was 5.32% (939 events); while that in the aspirin group was 5.83% (1021 events). The relative risk reduction was 8.7% (95% CI 0.3-16.5), favoring clopidogrel treatment (p=0.043). In the subgroup of stroke alone, the stroke event rate per year in the clopidogrel group was 7.15%, lower than that in the aspirin group which was 7.71% [46].

Hence, clopidogrel is effective in preventing ischemic stroke and minimizing the risk of recurrent stroke as monotherapy, and even better as DAPT.

Safety

Berger, J. S. et al. [47] meta-analysis study included seven clinical trials of clopidogrel, including CREDO, CURE, CLARITY, COMMIT, and CHARISMA. Major bleeding events occurred less frequently in the clopidogrel group than in the placebo group, with nearly all ORs >1. However, studies on DAPT therapy showed the opposite result. In Yang, Y. et al. [45] meta-analysis, intracranial and extracranial bleeding events were more frequent in the DAPT groups than the aspirin group, with RR ≥1.40. CHRISMA, MATCH, and CHANCE all supported that there were no significant added benefits from DAPT but an increased rate of AEs, SAEs, and death [8].

In addition, clopidogrel resistance was observed in patients who had received clopidogrel for Coronary Artery Disease (CAD) or ischemic cerebrovascular disease. A study revealed that nearly 40% of clopidogrel users were resistant to clopidogrel and the frequency of clopidogrel-resistant patients with ischemic cerebrovascular diseases was 31.3% [48]. The mechanism of clopidogrel resistance is not fully understood but genetic polymorphism of CYP2C19 is found to be associated with the variability of patient responsiveness [49]. Increasing clopidogrel resistance results in reduced efficacy and thus increasing the risk of recurrent stroke events.

Dipyridamole/Aspirin

Another dual antiplatelet therapy with the combined use of dipyridamole and aspirin for ischemic stroke treatment. Dipyridamole is seldom used as monotherapy due to its unclear efficacy [50]. Adenosine transporter and phosphodiesterase-5 of platelets are inhibited by dipyridamole, leading to an increase in extracellular adenosine and intracellular cAMP and cGMP, thus reducing intracellular calcium levels and preventing platelet activation and aggregation [51]. It is also found that dipyridamole exerts anti-inflammatory characteristics and reduces cell death in the cerebral ischemia model [51]. Therefore, in combination with aspirin, the safety profile is greatly enhanced. 25 mg of aspirin plus 200 mg of dipyridamole twice a day is recommended for stroke patients [50]. The half-life of dipyridamole is about 13 hours; whereas the half-life of aspirin is 2-3 hours [52].

Efficacy

The European Stroke Prevent Study 2 (ESPS 2) trial included 6602 patients with prior stroke or Transient Ischemic Attack (TIA) who were randomly assigned to treatment with daily 50 mg aspirin alone, 400 mg modified-release dipyridamole alone, combined formulation of the two agents, or placebo. Aspirin alone reduced the risk of stroke or death by 13% (P=0.016); dipyridamole alone reduced the risk by 15% (O=0.015); and the combination reduced the risk by 24% (P<0.001) [53].

However, in terms of cognitive improvements, dual therapy of dipyridamole/aspirin fails to demonstrate a neuroprotective function despite the anti-inflammatory characteristics of dipyridamole. In the Prevention Regimen for Effectively Avoiding Second Strokes (PRoFESS) trial, 20332 patients who experienced ischemic strokes were recruited and randomly assigned to combined treatment of aspirin and extended-release dipyridamole,

clopidogrel, telmisartan, or placebo. The Modified Rankin scale (mRS) scores at 3 months after recurrent stroke, Barthel index score 3 months after the first recurrent stroke, distribution of patients with Mini-Mental State Examination (MMSE) score ≤24 points over time, and the number of patients with a decrease of 3 points or more in MMSE score from 1 month to penultimate visit were similar among the four groups. There was no evidence showing that dipyridamole/aspirin improved functional or cognitive functions in stroke patients [52].

Though the addition of dipyridamole has further reduced the risk of stroke compared to aspirin alone, dipyridamole does not possess a neuroprotective function in patients. As previously mentioned, chronic use of aspirin establishes resistance in patients and lowers the efficacy of the therapy. It is expected that a similar reduction in efficacy will appear in this dual therapy as well.

Safety

According to the ESPS 2 trial, headache, bleeding, and gastrointestinal events were the major adverse event in dual therapy. Compared to aspirin monotherapy, dual therapy increased the occurrence of headaches by 15%, bleeding by 6%, and gastrointestinal events by 7% [52].

Another trial ESPRIT, which was a randomized controlled trial comparing the efficacy and safety of aspirin versus dipyridamole/aspirin treatment after cerebral ischemia of arterial origin, showed around 30% reduction in major bleeding complications (including fatal and non-fatal hemorrhage in the extracranial and intracranial cavity) in the dual treatment group as compared to the aspirin group [54].

The difference in the bleeding occurrence of the two trials may due to the variation in dosage of dipyridamole since 400 mg of dipyridamole was used in ESPS 2 while only 200 mg of dipyridamole was used in the ESPRIT trial. Yet, there was a large proportion of patients (123 out of 1375 patients, nearly 10%) who discontinued treatment in ESPRIT due to the side effects of headaches. From the treatment mentioned before, dipyridamole/aspirin was the only one so far to indicate headaches as a major adverse event [54].

Tirofiban

Tirofiban and eptifibatide are currently the commonly used Glycoprotein (GP) IIb/IIIa inhibitors for Acute Ischemic Stroke (AIS). Tirofiban is a non-peptide platelet GP IIb/IIIa receptor antagonist which reversibly inhibits the final pathway of platelet aggregation due to its short half-life, about 2 hours [55]. Tirofiban is administered intravenously at a recommended initial rate of 0.4 μg/kg/min for 30 minutes and then continued at 0.1 μg/kg/min [55]. Over 90% of tirofiban is cleared via the renal pathway, and thus patients with renal impairment require dosage adjustments or may not be recommended for tirofiban treatment [56].

Efficacy

A meta-analysis summarized the efficacy and safety of Tirofiban as an acute ischemic stroke treatment study based on 14 relevant published papers. 11 out of the 14 studies showed a higher occurrence of 3-month modified Rankin Scale (mRS) 0-2 scores in the Tirofiban group versus placebo, with a total odds ratio 1.27 (95% CI 1.09 to 1.48). Five of the studies tested for the postoperative recanalization rate of tirofiban treatment versus placebo. All five studies showed an odds ratio favoring the tirofiban group and the total OR was 1.66 (95% CI 1.16 to 2.39). These findings reflected that tirofiban could reduce the risk of neurologic disability caused by acute ischemic stroke and the antiplatelet effect of tirofiban was associated with a positive increase in recanalization rate [57].

The Efficacy and Safety of Tirofiban in Clinical Patients with Acute Ischemic Stroke (ESCAPIST) trial showed consistent improvements in mRS score in tirofiban-treated patients compared to control (100 mg aspirin per day for 90 days). In addition, the trial showed a reduction in NIHSS score (at 24 h) from 6 in the control group to 3 in the tirofiban group, with a P value < 0.0001), indicating improvements in a neurological deficit [55].

Safety

Tang, L. et al. [57] meta-analysis study recorded fewer total occurrences of symptomatic ICH in the tirofiban group (860 events) compared to the placebo group (1590 events) (total OR=0.97; 95% CI 0.73-1.31). The total mortality rate at 3 months was in favor of the placebo (total OR=0.75; 95% CI 0.62 to 0.91). In the ESCAPIST trial, the bleeding events and sICH occurrence in tirofiban-treated patients were similar to that of aspirin-treated patients. However, the occurrence of deaths within 90 days in the tirofiban group (0.6%) was much lower than in the aspirin group (3.9%) [58]. In terms of bleeding and mortality rate, tirofiban was found relatively safer than the current anticoagulants.

However, it is also found that GP IIb/IIIa inhibitors are associated with drug-induced thrombocytopenia complication which occurs when the platelet count is low, and bleeding is not stoppable [59]. Exposure to tirofiban can immediately lead to sudden and severe thrombocytopenia. The exact mechanism of how the inhibitor causes thrombocytopenia is still unclear. It is also suggested that tirofiban, for example, alters GP receptors on platelets and creates a new antigen that is recognized and removed due to an immune reaction. Thrombotic Thrombocytopenic Purpura (TTP), a condition in that blood clots form in small blood vessels all over the body due to abnormal platelet, was also observed in tirofiban-treated patients [60,61]. The incidence rate of acute serious thrombocytopenia in patients treated with tirofiban in primary angioplasty is 0.2% - 0.5% [62]. Though the incidence rate is low, the occurrence can be a life-threatening condition.

Anfibatide

Anfibatide is a novel synthetic antiplatelet thrombolysin derived from snake venom and functions as a GPIb antagonist. It was found that Anfibatide significantly reduced GPIbα and vWF in the cerebral ischemic mouse model supporting that Anfibatide prevents binding between GPIb and vWF [63] and expression of P-selectin [64], thereby inhibiting platelet adhesion to the collagens exposed in the damaged subendothelial matrix and prevent recruitment of monocytes. Comprehensive preclinical studies are providing profound results on drug toxicology, safety, and its therapeutic effects in various animal models with AIS, ischemic-reperfusion injury, and prophylaxis use respectively. A phase I trial of Anfibatide in healthy human volunteers (NCT01588132) was also completed to test for the safety and tolerability of the drug.

Effects/efficacy

In the *in vitro* studies, the antiplatelet effect of Anfibatide was compared with that of ticlopidine, aspirin, and dipyridam-

ole in rat models, in which platelet aggregations were induced by ADP, collagen, blood coagulant, and arachidonic acid. The antiplatelet effect of Anfibatide was comparable to Ticlopidine and stronger than aspirin and dipyridamole [65]. In the *in vivo* studies, a histopathological assessment of MCAO mice v/w Anfibatide or tirofiban revealed the superior antithrombosis efficacy of Anfibatide than tirofiban, the number of microthrombus was reduced from over 15 to within the range of 5-10 in the Anfibatide group (P<0.01); while that in tirofiban group only showed slight reduction resulting in more than 10 microthrombi [66].

Anfibatide-treated subjects also showed neurological improvements and anti-inflammation. While tirofiban-treated MCAO rats only achieved Bederson neurological (4-point) score within 2-3 after treatment, the scores of Anfibatide-treated rats were ranging from 1 to 2 (P<0.01) [63]. Anfibatide-treated rats also had 4-fold intact cerebral cell number, ≥100 (P,0.01) compared to control MCAO rats; while tirofiban-treated rats had <100 intact cells [67]. Anfibatide-treated murine neurons have higher survivability, indicating the high neuroprotection ability of Anfibatide. Statistically significant reduction of inflammation markers specific to cerebral ischemia or I/R injury was observed that 0.02μmol/g Anfibatide was able to reduce the level of MDA and LDH expression in MCAO rat neuron cells and dose-dependently increase antioxidant activities, including the blood serum SOD and GSh-Px. The suppression of inflammatory mediators and neurological improvements of the animal model suggest that Anfibatide can minimize neurological damage caused by acute cerebral ischemia and ischemia-reperfusion injury [66].

Strong anti-platelet and inhibition on GPIbα-vWF binding are consistent between pre-clinical and the Phase I study. Immediate maximum inhibition was noted right after infusion of Anfibatide with human blood that plasma unbound Anfibatide could not be detected. For 6-8h cessation of the drug, the antiplatelet effect of Anfibatide cannot be detected reflecting the fast dissociation and reversible inhibitory mechanism of Anfibatide. The trial also supported that Anfibatide had no impact on fibrinolysis and bleeding time. The platelet count test at 6ug/mL Anfibatide was significantly lower than the control groups (P<0.01) [68].

Safety

Anfibatide does not provoke irritation in rabbit eyes or skin and has no significant impact on blood pressure and pyrogenic changes, indicating a low irritation risk and high biocompatibility of Anfibatide [69]. In terms of immunotoxicity, at 11.1U/kg Anfibatide dose which was far higher than the clinical dose for humans, no animal subjects, including guinea pigs, rats, and mice, were dead, and only mild to light allergic responses occurred [68]. The possible risks will be ICH and drug-induced TTP based on the recorded occurrence of these events in other antithrombotic drug treatments. However, hemorrhage volumes and bleeding complications were less severe in Anfibatide-treated subjects than those treated with tirofiban [63]. The estimated ICH risk of Anfibatide in humans is low or at least as safe as tirofiban. Regarding drug-induced TTP, Anfibatide was surprisingly found efficacious in mitigating spontaneous TTP in *Adamts13*$^{-/-}$ mice and treating Shigatoxin-induced TTP [70]. *Adamts13*$^{-/-}$ mice that received 60ng/g body weight of Anfibatide showed a dramatic reduction in thrombocytopenia rate (defined as a 30% decrease in platelet counts from baseline). Anfibatide dose-dependently improved thrombocytopenia-free survival and 60ng/g anfibatide was regarded as the optimal dose. Based on the pre-clinical studies, Anfibatide is safe and tolerable with relatively low ICH risk and potential benefits to thrombocytopenia/TTP subjects.

In the phase I trial, systemic adverse events, allergy, and antibody production are the measured safety parameters. No significant difference was found in the vital sign parameters, and no SAEs or allergic reactions were observed. Anti-anfibatide antibodies were not detected. Anfibatide has good biocompatibility in healthy volunteers. Anfibatide appears to be highly safe and tolerable in healthy humans [68].

Discussion

The effects/efficacy, safety, and restriction of the use of the mentioned antithrombotic agents are summarized in Table 1.

Warfarin and aspirin are the most traditional and widely accepted antithrombotic agents used. They are the basis of most antithrombosis research. Despite the fair efficacy in reducing the occurrence of stroke, limited neurological improvements, drug resistance, long-term complications, and risks, such as impairment of normal hemostasis and increasing risk of hemorrhage/bleeding, have been the major concerns over the usage of warfarin and aspirin in cerebral ischemia patients. These concerns are also the current unmet medical needs in cerebral ischemia treatment. Scientists have been trying to find a novel drug that can have higher efficacy in stroke reduction and neuroprotection; no drug resistance; and a higher safety profile. And thus, there are increasing numbers of antithrombotic agents on the market and under development including heparin, direct oral anticoagulants, DAPTs, and GP subunit inhibitors. Among these agents, GP inhibitors have become a research target and trend of the current ischemia drug development.

To explain the therapeutic significance and potential of GP inhibitors, further comparisons among the agents are necessary. From the Results, among the anticoagulants, apixaban seems to be the most superior agent as it shows better efficacy in stroke reduction than warfarin and aspirin regardless of age group. It also shows a reduction of major bleeding occurrence by at least 20%. While heparins may cause severe complications, such as HIT and DVT; dabigatran and rivaroxaban lead to significant bleeding side effects, including GI hemorrhage and ICH. There is also dose restriction of dabigatran and rivaroxaban among hepatic and renal insufficient patients. Therefore, regarding the lower risk of bleeding and fewer restriction of administration, apixaban is superior among the anticoagulants. However, as an anticoagulant, apixaban impacts the patient's normal hemostasis [71], and discontinuation of the drug without substitute anticoagulant treatment will increase incidences of thrombotic events. Thus, apixaban-treated patients tend to have prolonged long-term administration of apixaban and to be closely monitored with their hemostatic parameters, which lowers their quality of life. In addition, all the anticoagulants described fail to show significance in ischemic patient neurological improvements. Only UFH and LMWH are proven to have no adverse impact on the neurological outcomes of patients but still fail to show efficacy in protecting patients from neuron damage. To avoid interference with patient hemostasis and maximize neuroprotection, scientists have shifted the focus to antiplatelet agents.

For antiplatelet agents, DAPT and GP inhibitors are found to be superior to aspirin in terms of reduction in stroke risk. In several trials, clopidogrel and dipyridamole monotherapy treatments show greater reduction in stroke events than aspi-

rin treatment and increased reduction when they were used in combination with aspirin as DAPTs. However, the findings were controversial. In CHRISMA, MATCH, and CHANCE trials, clopidogrel/aspirin DAPT demonstrated no significant added benefits but an increased rate of AEs, SAEs, and deaths. While dipyridamole/aspirin DAPT also failed to demonstrate neuroprotective function despite the proposed anti-inflammatory effect of dipyridamole. Plus the drug resistance of clopidogrel and aspirin will largely reduce the anti-platelet efficacy of the DAPT treatments. In contrast, GP inhibitors are not associated with any drug resistance and have demonstrated significant neurological improvements in treated patients or animal models. Tirofiban-treated patients had significant mRS score improvement and an increase in recanalization rate. While *in vivo* rat study of Anfibatide demonstrated an anti-inflammatory effect and a positive association between Anfibatide treatment and neurological improvements. Both tirofiban and Anfibatide demonstrate high therapeutic efficacy in preventing ischemic stroke and neurological disability.

In terms of the safety of GP inhibitors, bleeding complications seem to be inevitable in all the antithrombotic agents but tirofiban and Anfibatide are relatively safe. The incidence rate of bleeding and hemorrhage events was found to be similar between tirofiban and aspirin-treated patients. While Anfibatide was found to have lower bleeding and ICH risk than tirofiban in the preclinical studies and its Phase I trial on healthy volunteers. However, thrombocytopenic purpura, observed in tirofiban-treated patients, is found to be a potentially severe adverse event of GP inhibitors. Though the incidence rate of TTP is very low the occurrence of TTP is life-threatening and may cause death. Interestingly, Anfibatide had shown potential benefits in treating TTP mice, and no TTP occurrence was found in the Phase I trial. But restricted to small sample sizes and limited clinical long-term safety results, it is still uncertain if Anfibatide does not cause TTP or can treat TTP in human patients.

In a nutshell, GP inhibitors can be a monotherapy for the recanalization of ischemic stroke patients or used as an adjuvant drug in combination with MT to minimize the chance of recurrent thrombosis and neurological disability due to reperfusion injuries. The current challenge is to minimize the bleeding side effects and establish a more profound long-term safety profile. Further investigations are necessary to evaluate the long-term safety and the actual efficacy in humans and determine the optimal dosing strategy for treating ischemic stroke patients. It is also worth looking into the different GP inhibition targets for more potential antithrombotic applications of them.

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