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Neutrophils in tPA-induced hemorrhagic transformations: Main culprit, accomplice or innocent bystander?

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ABSTRACT

The risk of intracerebral hemorrhage still greatly limits the use of tPA in stroke patients. Research is ongoing in order to identify the pathophysiological mechanisms at play, detect predictive biomarkers and discover new pharmacological targets to develop preventive or curative treatments. Going through experimental and clinical studies, this review focuses on the role of neutrophils as key predictive biomarkers for thrombolysis-induced hemorrhages and as pharmacological targets to limit their occurrence. To date, there are no established pharmacological modulators of neutrophils for ischemic stroke and its hemorrhagic complications. Several strategies are under evaluation, including lipid-lowering drugs, free radical scavengers, or minocycline, as well as non-pharmacological interventions such as physical exercise.

Contents

1. Introduction

Stroke is, after cardiovascular diseases and cancer, one of the major public health problems in industrialized countries. In 2013, about 6.4 million deaths were linked to stroke worldwide (Kim, Cahill, & Cheng, 2015). Stroke is the third cause of mortality, the second cause of dementia and the first cause of disability in adults. The main therapeutic strategy is the rapid restoration of blood flow in the ischemic area, which favors long-term recovery and helps to limit cognitive consequences (Powers et al., 2018). Pharmacologically, the principle of treatment is based on the acceleration and amplification of physiological fibrinolysis - i.e. the degradation of thrombus fibrin by plasmin - in order to limit the severity and duration of ischemia. The only authorized agent is recombinant tissue

Abbreviations: AT1R, Angiotensin-2 Type 1 Receptor; BBB, Blood Brain Barrier; ECM, Extra Cellular Matrix; GCSF, Granulocyte Colony Stimulating Factor; HDL, High Density Lipoprotein; HT, Hemorrhagic Transformation; ICAM-1, InterCellular Adhesion Molecule 1; MAC-1, Macrophage-1 antigen; MCAO, Middle Cerebral Artery Occlusion; MCP-1, Monocyte Chemoattractant Protein-1; MMP, Matrix MetalloProteinase; NET, Neutrophil Extracellular Trap; NIHSS, National Institutes of Health Stroke Score; NLR, Neutrophil – Lymphocyte Ratio; NURR1, Nuclear Receptor Related-1 protein; PARP, Poly-ADP Ribose Transferase; PDE3, Phospho-Di-Esterase 3; PMN, PolyMorphoNuclear cell; PPAR, Peroxisome Proliferator-Activated Receptor; ROS, Reactive Oxygen Species; TIMP, Tissue Inhibitor of MMP; TNFα, Tumor Necrosis Factor alpha; tPA, tissue Plasminogen Activator; VAP-1, Vascular Adhesion Protein-1; VCAM, Vascular Cell Adhesion Molecule.

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Plasminogen Activator (tPA, or alteplase), homologous to endogenous human tPA and sharing similar fibrinolytic properties.

Fibrinolysis (or thrombolysis) is beneficial if administered in the first few hours after the onset of cerebral ischemia under very specific conditions (Wardlaw, Murray, Berge, & Zoppo, 2014). Beyond the effects at the acute phase, thrombolysis also limits disability with decreased levels of dependency at 3 months. There are two major limitations restricting its use and efficacy. The first limitation stems from the risk of symptomatic cerebral hemorrhage. Hemorrhagic transformations (HT) are a well-known complication of stroke itself, but their frequency is increased when tPA is administered. Symptomatic cerebral hemorrhages were observed in the first 36 h after stroke in 6.4% of patients treated with tPA, but only in 0.6% of patients who received a placebo (NINDS rt-PA Stroke Study Group, 1995). The mortality rate in these patients is very high, with some studies reporting death rates up to 60% in patients who had developed intracranial hemorrhage (Gore et al., 1995). A meta-analysis of 27 clinical trials comparing cerebral fibrinolysis with different types of thrombolytic agents (10,187 patients) confirmed excess mortality at 3 months in patients who had received thrombolysis, due to a marked increase in the risk of symptomatic cerebral hemorrhage (Wardlaw et al., 2014). The risk for tPA-induced hemorrhages is time-dependent and accounts for its narrow therapeutic window. Treatment is deemed beneficial up to 4 h 30 min after symptom onset, the level of risk becoming excessive after 6 h. As a consequence, tPA is used to treat approximately only 10% of the eligible patients (Fugate & Rabinstein, 2014).

The second limitation is related to the resistance of the thrombus to tPA-induced lysis. The mechanisms of resistance to thrombolysis remain largely unknown. The analysis of thrombi gathered from stroke patients revealed that the characteristics of the thrombi played an important part in the response to tPA. The physical properties (shape, length, porosity) influence clot exposure to tPA and treatment efficacy (De Meyer et al., 2017). The composition of the clot, especially the red blood cell content, has also been linked to variable recanalization rates (Hashimoto et al., 2016). Finally, the overall effect of tPA could be altered if thrombus regeneration occurs through cellular inflammatory pathways, as the concept of thrombo-inflammation suggests (Tsivgoulis et al., 2018). This lack of recanalization drives the development of mechanical thrombolysis, i.e. thrombectomy. Several clinical trials recently demonstrated the efficacy of this therapeutic approach in combination with pharmacological thrombolysis, as confirmed by recent meta-analyses (Caranfa et al., 2018; Mistry et al., 2017; Tsivgoulis et al., 2018).

In this context of thrombolysis, thrombo-inflammation and HT, a role for polymorphonuclear cells (PMNs or neutrophils) was proposed (De Meyer et al., 2016; Ruhnau, Schulze, Dressel, & Vogelgesang, 2017). Neutrophils are recognized as key players in thrombo-inflammation at the acute phase of stroke (Price et al., 2004). They are one of the main sources of released molecules (pro-inflammatory cytokines, matrix metalloproteinases) that contribute to the breakdown of the neurovascular unit (Iadecola & Anrather, 2011; Jin, Yang, & Li, 2010; Moskowitz, Lo, & Iadecola, 2010). In addition, tPA is able to act directly on neutrophils to modify their level of activation and release activity (Carbone et al., 2015; Cuadrado et al., 2008).

The aim of this review is to assess and clarify the role of neutrophils in tPA-induced hemorrhages, and their potential as biomarkers and therapeutic targets. To do so, we conducted a comprehensive search on Medline, Pubmed and Embase for studies published until April 30th, 2018. The keywords used were stroke, cerebral ischemia, thrombolysis, tissue plasminogen activator, alteplase, hemorrhagic transformation, neutrophil, leukocyte, leukocyte-endothelium interactions, inflammation, and oxidative stress.

2. Neutrophils in the physiopathology of stroke

Discussing the role of neutrophils in thrombolysis requires beginning with a review of their role during stroke. In stroke, tissue damage and resulting neurological deficits originate from the implementation of various pathways, deleterious and protective. The vascular occlusion primes a complex sequence of events involving excitotoxicity, oxidative stress, inflammation (Iadecola & Anrather, 2011; Khoshnam, Winlow, Farzaneh, Farbood, & Moghaddam, 2017). Neutrophils appear as central players, as they directly and indirectly play a part and influence the course of these events, including the response to thrombolysis.

Neutrophils are the first circulating immune cells to be recruited after stroke onset. Within 15 min, neutrophils interact with activated endothelial cells. They slow down and roll at the surface of the endothelium through interactions of their surface integrin Macrophage-1 antigen (MAC-1) with endothelial P and *E*-selectins. This rolling phenomenon can be witnessed in pial vessels within 2 h. Neutrophils then attach to the endothelium through interactions with adhesion molecules, especially InterCellular Adhesion Molecule 1 (ICAM-1) and Vascular Cell Adhesion Molecule (VCAM). From here, they can extravasate, infiltrate the parenchyma and migrate towards the lesion following a gradient of chemokines and cytokines (Kim, Park, Chang, Kim, & Lee, 2016). In experimental stroke models, neutrophils can be detected in the microvascular compartment as early as 30 min after occlusion, reaching peak concentrations at 12 h (Garcia et al., 1994). As for infiltrated neutrophils, they can be detected in the ischemic parenchyma 6 h after stroke; their density is maximal 48 to 72 h after stroke (Kim et al., 2016; Zhang, Chopp, Chen, & Garcia, 1994). Post-mortem clinical data support these experimental data, evidencing ICAM-1 upregulation in blood vessels and neutrophil infiltration in the ischemic parenchyma (Lindsberg, Carpen, Paetau, Karjalainen-Lindsberg, & Kaste, 1996).

Given their phagocytic abilities, neutrophils are involved in the removal of cellular debris, mandatory for tissue regeneration. But they also play a direct part in tissue damage. Neutrophils have the capacity to synthesize and secrete pro-inflammatory mediators such as Tumor Necrosis Factor alpha (TNFα), thus promoting leukocyte infiltration and participating in the establishment of a sustained inflammatory reaction. They are also partially responsible for the oxidative phenomena majored by reperfusion as they are equipped with oxidative enzymes for phagocytosis. With this sudden influx of oxygen, the enzyme NADPH-oxidase produces superoxide in excess. Superoxide itself and Reactive Oxygen Species (ROS) derived from it act as intercellular messengers and prompt the expression of endothelial adhesion molecules. This overabundance of ROS also causes the formation of peroxynitrite by combination with nitric oxide, thus amplifying the oxidative stress and leading to enzyme dysfunction and cellular damage (Khoshnam et al., 2017).

Whether attached to the endothelium or infiltrated in the parenchyma, activated neutrophils degranulate in situ. The released enzymes, including elastase, proteases, collagenases, degrade the surrounding structures: in particular the extracellular matrix (ECM), tight and adherens junctions constituting the Blood-Brain Barrier (BBB). One family of enzymes is of particular interest. The Matrix Metallo-Proteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases able to degrade all components of the extravascular matrix (laminin, collagen and fibronectin). Two major isoforms are involved in stroke: MMP-2 and MMP-9. MMP-9 is secreted as an inactive pro-MMP-9 that is rendered active after cleavage by many enzymes (MMP-2, MMP-3, plasmin, urokinase-type plasminogen activator, tPA). Numerous studies have documented the role of MMP-9 in the breakdown of the BBB and in the formation of vasogenic edema, thus worsening stroke outcome (Rosell et al., 2006).

Besides contributing to inflammation, oxidative stress and structural degradation, neutrophils yet participate in another process: the no-reflow phenomenon, resulting from vascular dysfunction at different levels (Granger & Kvietys, 2017). The rolling, adhesion and infiltration of neutrophils at the surface of cerebral veins and venules has been linked to post-ischemic endothelial dysfunction (Palomares & Cipolla, 2011; Pétrault et al., 2005), as well as the disruption of the ECM and cellular junctions. But neutrophils are also involved in the formation of

thrombi occluding microvessels. They release tissue factors and proteases activating coagulation factors, thus leading to fibrin accumulation and clot formation (Ruhnau et al., 2017). Attached neutrophils also offer an interface for platelets to adhere. They interact, via their surface integrins, with activated endothelial cells, the exposed ECM and platelets (De Meyer et al., 2016). Recently, it was shown that their contribution to the constitution and stabilization of new thrombi relies on Neutrophil Extracellular Traps (NETs) (Michel & Ho-Tin-Noé, 2015). NETs constituted of extracellular strands of DNA associated with histones and proteins normally contained in granules such as myeloperoxidase, elastase, and MMPs. NETs are part of the defense mechanisms and released to entrap pathogens. Their role is complex (Bonaventura et al., 2018), but they have been shown to be major constituents of cerebral thrombi retrieved from stroke patients (Laridan et al., 2017).

3. Neutrophil involvement in tPA-induced hemorrhages

Thrombolysis exacerbates the risk of HT after stroke and symptomatic HT is thought to affect up to 7% of patients (Yaghi et al., 2017). Their definition relies on clinical and radiological criteria: symptomatic or asymptomatic, location and type of bleeding: petechial or parenchymal. Most events happen within 36 h (Yaghi et al., 2017). The pathophysiology of cerebral hemorrhage secondary to thrombolysis remains unclear and is still subject to discussion (Palomares & Cipolla, 2011; Yaghi et al., 2017). It appears to result from the combination of ischemia-reperfusion damages, in particular opening of the BBB and tPA pharmacological effects.

Experimental models have been developed in recent years to study the pathophysiological mechanisms of the hemorrhagic complications of thrombolysis with tPA. They are adapted from two well-established models of cerebral ischemia-reperfusion based on the temporary occlusion of the middle cerebral artery: Middle Cerebral Artery Occlusion (MCAO) models (Shearer, Douglas, Kirby, Tatlisumak, & Doyle, 2018). The occlusion can either be mechanical: introduction of an occluding filament, pulled out to allow reperfusion; or embolic: in situ injection of thrombin or of an autologous clot (Durukan & Tatlisumak, 2007). The principle is based on the administration, at different time points after the onset of cerebral ischemia, of tPA at various doses, bolus or continuous infusion. Rodent models are most frequently used and hemorrhagic complications, generally in the ischemic area, can be observed a few hours after thrombolysis. In terms of pathophysiology, the most accurate models are the thromboembolic ones (Aoki, Sumii, Mori, Wang, & Lo, 2002; Tejima, Katayama, Suzuki, Kano, & Lo, 2001). Post-reperfusion injections of tPA in mechanical MCAO models appeared not suitable to study hemorrhagic transformations, as the impact of clot thrombolysis is not being replicated (Aoki et al., 2002). Nevertheless, the injection of a solution resulting from the action of tPA on an autologous clot, replicating the phenomenon of thrombolysis ex vivo, got around this limitation and allowed to show that the clot itself and its breakdown by tPA play an essential role in the occurrence of post-thrombolysis hemorrhage (Gautier et al., 2003; Kahles et al., 2005).

The risk of thrombolysis hemorrhages increases with the duration of occlusion, reperfusion and vascular risk factors (Jickling et al., 2014). Reperfusion restores blood flow in injured vessels, extravasation ensuing because of the damaged BBB. As tPA accelerates reperfusion, it inevitably increases the risk of HT. Other tPA-related factors favoring HT are the coagulopathy linked to hypofibrinogenemia that can last up to 24 h after tPA treatment and direct blood vessel injury promoted by tPA (Kastrup et al., 2008; Yaghi et al., 2017). A dynamic MRI study showed that after ischemia, the vascular dysfunction depended on the timing of tPA administration. Hemorrhages were increasingly numerous as the administration of tPA was delayed, owing to a greater impact on BBB permeability (Zhang et al., 2014). A contributing role for neutrophils was proposed given their unique influence on the neurogliovascular unit during cerebral ischemia, and their ability to interact with tPA (del Zoppo, 2009; Roever & Levine, 2015) (Fig. 1).

Neutrophils directly contribute to the development of HT by promoting ischemic damages and partaking in the disruption of the BBB. Adherent neutrophils are early contributors to BBB disruption as they release MMPs among other products at the surface of blood vessels, and later on infiltrated neutrophils do the same in the parenchyma (Justicia et al., 2003). A few hours after experimental ischemic stroke, MMP-9 levels significantly increased; this phenomenon was aggravated and hastened in combination with thrombolysis with tPA (Lenglet et al., 2014). MMPs are thought to contribute to cerebral vascular impairment and tPA-related hemorrhages (Sumii & Lo, 2002; Tsuji et al., 2005; Turner & Sharp, 2016). The occurrence of post-thrombolysis hemorrhages was coincidental with an increase in cerebral vascular permeability and MMP-9 upregulation (Kahles et al., 2005). tPA increases in a dose-dependent manner the activities of plasma MMP-2 and MMP-9 (Golab, Kielbus, Bielewicz, & Kurzepa, 2015) and induced MMP expression by endothelial cells (MMP-3) and astrocytes (MMP-2) (Jickling et al., 2014). The detrimental role of MMP-9 during thrombolysis was recently corroborated as the plasma variations of MMP-9 were independently associated with death or symptomatic intracerebral hemorrhage (Inzitari et al., 2013). In addition, a clinical study on 327 stroke patients treated with tPA demonstrated that the MMP-9/Tissue Inhibitors of MetalloProteinases (TIMPs) ratios were predictors of intracerebral hemorrhages when elevated (Piccardi et al., 2015).

There are interactions taking place between neutrophils and tPA augmenting the risk of HT even more. tPA was responsible for an elevation in adherent and infiltrated neutrophils in an ischemia-reperfusion mouse model (Uhl et al., 2014). The pivotal interaction seems to be the triggering of neutrophil degranulation by tPA. This phenomenon, and the subsequent functional and structural degradation of the vascular compartment, was corroborated by several clinical studies. The analysis of brain and plasma samples confirmed that this phenomenon was accounting, at least partly, for the occurrence of post-thrombolysis hemorrhages (Carbone et al., 2015; Cuadrado et al., 2008; Rosell et al., 2008). A peak concentration of MMP-9 coming from neutrophil degranulation was detected in the first hours following the infusion of tPA in plasma collected from stroke patients who underwent thrombolysis (Carbone et al., 2015).

The role of neutrophils in thrombolysis hemorrhages was supported by experimental pharmacological modulation assays. Vinblastine-induced leuconeutropenia counteracted the tPA-induced massive infiltration of neutrophils – as well as the stroke-induced PMN infiltration - in the ischemic area. It also prevented tPA-induced hemorrhages and improved prognosis (Gautier, Ouk, Petrault, Caron, & Bordet, 2009;

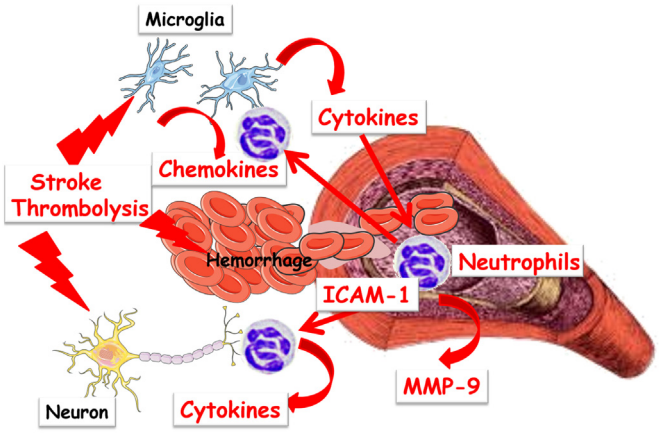

Fig. 1. Interplay between neutrophils, the neurogliovascular unit and the thrombolytic treatment at the acute phase of stroke: setting the stage for hemorrhagic transformation. ICAM-1, InterCellular Adhesion Molecule 1; MMP-9, Matrix MetalloProteinase 9.

Pétrault et al., 2005). Accordingly, neutropenia induced with an antibody specifically directed against neutrophils (mAbRP-3) just before stroke and thrombolysis prevented tPA-induced hemorrhages (Gautier et al., 2009). Inducing neutrophilia with Granulocyte Colony Stimulating Factor (GCSF) gave conflicting results. Whereas GCSF appeared clearly neuroprotective in experimental stroke models through vasculo- and angiogenesis mechanisms, the combination with tPA seems to exert contrasting effects depending on the experimental setting (Bråtane et al., 2009; Minnerup et al., 2008; Sevimli et al., 2009; Sobrino et al., 2010; Solaroglu, Digicaylioglu, Keles, & Zhang, 2015; Strecker et al., 2010). GCSF appeared to attenuate post-thrombolysis hemorrhages through vascular protection whereas neutrophilia elicited them through an increased release of MMP-9 from neutrophils (Dela Peña et al., 2015; Gautier et al., 2014). In a specific model of neonatal hypoxia ischemia, the beneficial neuroprotective effect of GCSF was observed only if neutrophilia was prevented (Doycheva et al., 2014).

Of course, HT do not result from the actions of neutrophils alone, and likely originate from the combined effects of several factors. Other early contributors are endothelial cells and pericytes, followed as the ischemic injury develops by neuron, astrocyte and microglia activation (Jickling et al., 2014). Oxidative stress plays a major part in HT, as does neuroinflammation (Wang, Li, Chen, & Wang, 2015). Another set of factors also plays a part in the occurrence and severity of HT: Vascular risk factors and clinical features at the acute phase of stroke, such as age, hypertension/blood pressure, diabetes/hyperglycemia, and the use of concomitant drugs are involved and their influence should not be underestimated (Lansberg et al., 2007; Larrue, von Kummer, Müller, & Bluhmki, 2001). Usually not or only partly replicated in preclinical studies, they are on the other hand easily assessable in the clinical setting (Menon et al., 2012).

4. The neutrophil as a biomarker of tPA-induced hemorrhages

The involvement of neutrophils in tPA-induced hemorrhages naturally led to consider them as potential biomarkers for HTs. Data already point towards a role for circulating neutrophils in stroke prognosis (Foerch, Montaner, Furie, Ning, & Lo, 2009). Pagram and collaborators found a strong correlation between circulating neutrophil counts and ischemic core volumes measured by MRI (Pagram, Bivard, Lincz, & Levi, 2016). The Caliber study, reviewing 7881 events, found an increased risk of stroke in patients with a high neutrophil count: HR = 1.36 [95% CI 1.17–1.57] (Shah, Denaxas, Nicholas, Hingorani, & Hemingway, 2017). Low neutrophil counts were associated with lower National Health Institutes Stroke Scores (NIHSS) and lesser risk for in-hospital mortality (Fang et al., 2017). Patients with a high neutrophil count after a minor or transient ischemic stroke were at higher risk of stroke recurrence in the 90 days following the first event (Zhu et al., 2018). In terms of hemorrhagic risk, white blood cell counts were significantly increased in the HT group after intra-arterial thrombolysis: OR = 1.097 [95% CI 1.012–1.190] (Xing et al., 2014). An increase in neutrophil counts after tPA administration and a high neutrophil count 24 h after stroke onset were predictive of an increased risk for death or major disability within 3 months (Shi et al., 2018).

Clinical studies also identified the Neutrophil – Lymphocyte Ratio (NLR) as a predictive marker of hemorrhagic transformations. A low NLR in the 24 h following stroke onset was associated with a better outcome at 3 months (Pagram et al., 2016; Qun et al., 2017;) and a lesser risk of short-term mortality (Shi et al., 2018; Tokgoz et al., 2013). Patients with NLR levels above 4.80 had a 3.71-fold increased risk for symptomatic intracerebral hemorrhages: adjusted OR = 3.71 [95% CI 1.97–6.98]. These parameters were also associated with a higher mortality and a poorer functional outcome 3 months after stroke (Maestrini et al., 2015). In another study, blood samples were collected over 48 h after tPA treatment in 189 Chinese stroke patients. A high NLR was associated with a high risk of hemorrhagic transformations: adjusted OR = 1.14 [95% CI 1.05–1.23], and patients with NLR levels above 10.59 had an 8-fold increased risk for symptomatic intracerebral hemorrhages: adjusted OR = 7.93 [95% CI 2.25–27.99] (Guo et al., 2016).

Neutrophils were identified as a main source of MMP-9 in areas of hemorrhage (Turner & Sharp, 2016). As MMP-9 is also expressed in neutrophils in peripheral blood (Tang et al., 2006), MMP-9 levels could be used as predictors of intracerebral hemorrhage in tPA-treated patients (Castellanos et al., 2007). In fact, high baseline plasma levels of MMP-9 were predictive of late hemorrhagic events (Montaner et al., 2003). In addition, NETs and their constituents appear as interesting biomarkers of the neutrophil activation and potentially useful in anticipating the response to tPA and stroke prognosis (Michel & Ho-Tin-Noé, 2015). NET constituents are easily measurable in plasma and tissue: citrullinated histone-3, nucleosomes, cell-free DNA, elastase and myeloperoxidase are among the most commonly used. These markers can be used to track neutrophil recruitment and activation in experimental ischemic stroke (Perez-De-Puig et al., 2015). In a cohort of stroke patients, plasma levels of NET markers were positively correlated with stroke severity and 1-year risk of death (Vallés et al., 2017). Furthermore, NETs seem to play an important part in the constitution of thrombi in ischemic stroke. Two studies have shown that NETs are an important part of their structure as constituents of the backbone on which cells and proteins can adhere. Their presence was associated with thrombolysis resistance, as tPA was less efficient on thrombi with a rich extracellular DNA and neutrophil content (Ducroux et al., 2018; Laridan et al., 2017). Further studies are required to assess the potential interactions between NETs and tPA, as data suggest that tPA could alter neutrophil anti-infectious properties and NET release in stroke patients (Vogelgesang et al., 2017).

Others markers could also be useful (Lu, He, Shen, & Cao, 2018). For example, tPA regulated the pro-inflammatory transcription factor Nuclear Receptor Related-1 protein (NURR1). This transcription factor, whose expression increases after thrombolysis, causes hemorrhagic injury in the rat, and patients with post-thrombolysis hemorrhages were found to have higher basal levels of this factor than those without hemorrhages (Merino-Zamorano et al., 2015). Likewise, the Vascular Adhesion Protein-1 (VAP-1) is involved in recruitment of neutrophils. Measuring its activity before tPA administration was demonstrated to be predictive of tPA-induced hemorrhages (Hernandez-Guillamon et al., 2010).

5. Reducing hemorrhagic transformations by neutrophil targeting

Reducing the risk of hemorrhages after thrombolysis could be a strategy to extend the otherwise limited therapeutic window of tPA, and may even pave the way for a broader use of tPA. Many pharmacological approaches have been tested with the aim of reducing the impact of stroke and hemorrhagic complications in general (Zhang, Zhang, & Chopp, 2012). As evident contributor to tPA hemorrhages, neutrophils could be valuable pharmacological targets. Strategies targeting neutrophils directly are limited, as discussed above, by difficulties to translate their use to the clinical setting (induced neutropenia, anti-neutrophil antibodies) or to demonstrate a clinical benefit (MMP-9 inhibitors for example). Conversely, indirect interventions targeting pathophysiological pathways involved in the recruitment and activation of neutrophils appear more valuable (Fig. 2).

5.1. Statins

The neurovasculoprotective potential of statins in ischemic stroke is well-established in experimental models as preventive or acute phase treatments (Ouk et al., 2014a; Potey et al., 2015). This benefit mainly relies on their anti-inflammatory effects, decreased neutrophil adhesion and infiltration, preservation of vascular properties (function and structure) and reduced microglial activity (Potey et al., 2015; Wang et al., 2006; Zhang et al., 2005). These effects rely at least partly on the

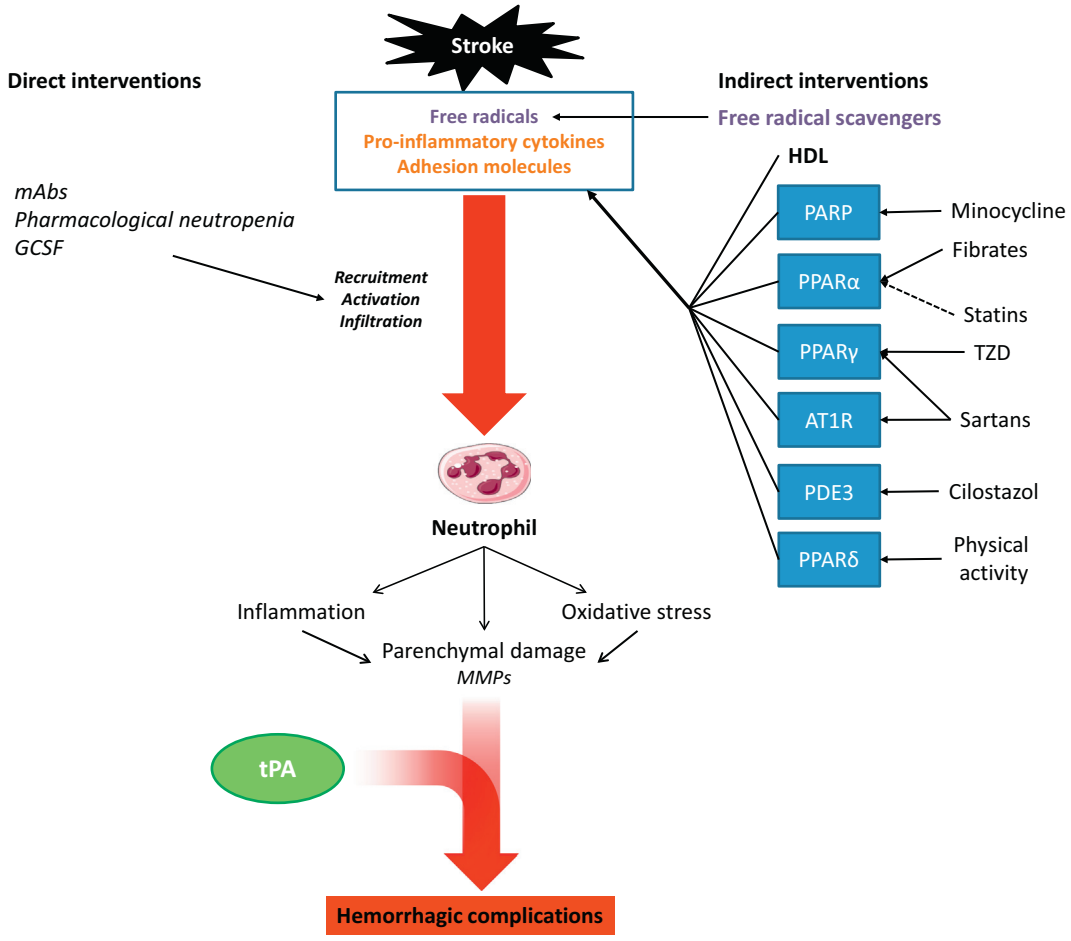

Fig. 2. Pharmacological interventions targeting the neutrophil as a key actor in the development of post-stroke tissue damage and hemorrhagic complications secondary to tPA treatment. AT1R, Angiotensin-2 Type 1 Receptor; mAbs, monoclonal antibodies, MMPs, Matrix Metalloproteinases, PARP, Poly-ADP Ribose Transferase; PDE3, Phospho-Di-Esterase 3; PPAR, Peroxisome Proliferator-Activated Receptor; TZD, Thiazolidinediones.

modulation of the nuclear receptor Peroxisome Proliferator-Activated Receptor (PPAR)-alpha and are independent of their lipid-lowering effects (Miedema, Uyttenboogaart, & Luijckx, 2012; Nardi et al., 2011, 2012; Rocco, Sykora, Ringleb, & Diedler, 2012).

In combination with tPA, data are controversial. In vitro experiments showed that lovastatin, even at high doses, did not have an impact on tPA thrombolytic activity (Kandadai, Meunier, Lindsell, Shaw, & Elkind, 2012). When hypertensive rats received simvastatin 15 min after the induction of ischemia and tPA 3 h later, no difference in terms of neuroprotection, MMP-2 and MMP-9 activities and rate and severity of hemorrhages compared to the placebo group treated with tPA only were found (Campos et al., 2013). In a thromboembolic model, atorvastatin administered before or concurrently to thrombolysis decreased neutrophil adhesion and infiltration, reduced the expression of MMP-9 and the risk of hemorrhage (Liu et al., 2006; Zhang et al., 2009). On the other hand, a preventive treatment with simvastatin increased significantly the incidence and volumes of tPA-induced hemorrhages in rabbits submitted to embolic cerebral ischemia (Lapchak & Han, 2009).

The potential benefits of statins in primary and secondary stroke prevention have been evaluated in several clinical studies (Amarenco et al., 2006; Phipps et al., 2013; Taylor, Ward, Moore, Burke, & Davey, 2013). Some studies have shown that the preventive use of a statin (before stroke) was associated with a better neurological and improved functional prognosis in thrombolyzed patients (Alvarez-Sabín et al., 2007; Engelter et al., 2011) whereas others failed to show any benefit or deleterious effect (Campos et al., 2013; Martinez-Ramirez et al., 2012; Meier et al., 2009; Miedema, Uyttenboogaart, Koopman, De Keyser, & Luijckx, 2010; Tsivgoulis et al., 2015). Among these studies, even if an association was found between previous statin use and hemorrhagic transformations, the 3-month prognosis was not altered (Martinez-Ramirez et al., 2012; Meier et al., 2009). Scheitz and collaborators (Scheitz et al., 2014; Scheitz, Nolte, & Endres, 2013) reported a long-term beneficial effect of statins yet found an increased and dose-dependent risk of thrombolytic hemorrhage associated with previous statin treatment. The underlying mechanisms remain largely unknown. Zhao and Zhang reported that a previous statin treatment had no impact on MMP-9 levels in thrombolyzed patients at 12, 24 and 72 h, with no change of the hemorrhagic risk or of the patients' course (Zhao & Zhang, 2014).

Data regarding the initiation of a statin treatment concomitantly to tPA at the acute phase of stroke is controversial. In some studies, there was a tendency towards improvement or a significant neurological and functional improvement (Cappellari et al., 2013; Geng et al., 2016; Tong, Hu, Zhang, Yan, & Lou, 2015), and a lower risk of mortality (Scheitz et al., 2016). The long-term benefit seemed to depend on the duration of the statin treatment, for at least three weeks after stroke (Tong et al., 2015). Others did not evidence a beneficial effect (Montaner et al., 2016), but overall, none raised safety concerns. The risk of hemorrhagic transformation was not increased in patients treated with the combination statin-tPA (Cappellari et al., 2013; Geng et al., 2016; Montaner et al., 2016; Scheitz et al., 2016). A relatively recent meta-analysis concluded that the use of statins at the acute phase was beneficial on the course of stroke, benefit that was lost if patients underwent thrombolysis (Ni Chroinin et al., 2013).

5.2. Fibrates

Fibrates are agonists of the nuclear receptor PPAR-alpha. The activation of PPAR-alpha at the acute phase of experimental ischemia, or a few days later as a secondary stroke prevention strategy, confirmed the value of fenofibrate, irrespectively of the treatment schedule, as a protector of the neurogliovascular unit as a whole (Deplanque et al., 2003; Ouk et al., 2009, 2014b). This protection was exerted through the modulation of the interactions between neutrophils, the vascular wall and microglia (decreased adhesion and rolling of neutrophils, decreased parenchymal and vascular inflammation, reduction in vascular dysfunction and infarct volume in particular). This modulation was associated with a significant reduction in the hemorrhagic risk following tPA administration. A concomitant reduction in the vascular adhesion and infiltration of neutrophils and in microglial activation was shown to be beneficial (Gautier et al., 2015). Nevertheless, there is no available clinical data on PPAR alpha modulation combined with tPA, and whether such an association could reduce the risk of hemorrhagic transformations has yet to be evaluated. Likewise, it is not known if PPAR alpha modulators alter could tPA thrombolytic properties.

5.3. Angiotensin II receptor type 1 blockers (sartans)

Angiotensin-2 Type 1 Receptor (AT1R) blockers, or sartans, exert beneficial effects in ischemic stroke via pharmacological mechanisms distinct from their on-label indication, hypertension. Sartans have been shown to modulate various pathophysiological pathways in experimental stroke, especially inflammation. Treatment with an AT1R blocker decreased the production of pro-inflammatory cytokines (TNFα, interleukin-1β), chemo-attracting factors such as Monocyte Chemoattractant Protein-1 (MCP-1), adhesion molecules (ICAM-1, P-selectin), and MMPs (Hosomi et al., 2005; Iwanami et al., 2010; Kasahara et al., 2010; Kozak et al., 2009).

The documentation of potential effects of sartans on neutrophils in stroke is scarce and a clinical effect has yet to be proved. Angiotensin-2 stimulates the expression of MMPs. It was shown that telmisartan, both an AT1R blocker and a PPAR gamma activator, just after ischemia was neuroprotective in a MCAO model, beyond its effect on blood pressure and through the reduction of MMP-9 and of the inflammatory response (Kono et al., 2015). Candesartan applied 3 h after embolic MCAO was neuroprotective and limited the incidence of secondary hemorrhages after delayed thrombolysis (tPA applied 6 h after stroke onset). MMP-9 activity was increased, and MMP-3 activity decreased (Ishrat, Pillai, Ergul, Hafez, & Fagan, 2013).

5.4. Thiazolidine-diones

Hyperglycemia is associated with reperfusion injury and promotes inflammation and oxidative stress. Acute hyperglycemia is associated with a poor prognosis in patients with ischemic stroke, and is a well-known risk factor of secondary hemorrhagic transformations (Ahmed et al., 2010; Poppe et al., 2009). The value of glycemia on admission is decisive and has a major influence on stroke outcome, tPA efficacy and risk of hemorrhagic transformations, whereas the history of glycemic control is of little value (Litke, Moulin, Cordonnier, Fontaine, & Leys, 2015).

Among the available anti-diabetic drugs, the thiazolidine-dione class is of particular interest in stroke. They are agonists of the nuclear receptor PPAR gamma. They modulate glycemia and multiple other pathways that grant them neuroprotective properties. Rosiglitazone limited the recruitment and extravasation of neutrophils to the ischemic area when administered preventively (Luo et al., 2006) or following experimental stroke (Tureyen et al., 2006). It was still protective and prevented neutrophilia 24 h after MCAO (Allahtavakoli, Moloudi, Arababadi, Shamsizadeh, & Javanmardi, 2009).

Rosiglitazone also proved to be beneficial when used as a combined treatment with tPA in a rat model of embolic stroke. It potentiated the protective effects of tPA, and increased the time window for safe tPA administration. In the ischemic area, MMP-9 enzymatic activity was reduced, and blood vessel structure preserved (Wang et al., 2009). It was also shown to reduce the occurrence of secondary hemorrhagic transformations induced by acute hyperglycemia in rats submitted to MCAO (Zhang et al., 2013). Clinical trials are needed to assess if these beneficial effects could be translated to humans. It would also be interesting to evaluate if PPAR gamma modulators modify the thrombolytic efficacy of tPA.

5.5. Free radical scavengers

Free radical scavengers were proposed as a joint therapy to tPA thrombolysis to counteract the rebound in oxidative stress that accompanies reperfusion. The main candidate NXY-059 was able to reduce the incidence of hemorrhagic transformations in studies based on a rabbit thromboembolic stroke model (Lapchak et al., 2002a; Lapchak, Araujo, Song, Wei, & Zivin, 2002b; Lapchak, Song, Wei, & Zivin, 2004). These results could not be replicated in patients. The SAINT-I and SAINT-II clinical trials combining tPA with NXY-059 failed to show beneficial effects, and the incidence of hemorrhagic transformations was not reduced either (Diener et al., 2008).

Edaravone by itself is a neuroprotectant authorized in Japan as an acute stroke treatment. When associated with tPA, edaravone amplified the thrombolytic efficacy of the treatment and provided more protection than tPA alone. The neuroprotective effects are various but notably involve a downregulation of MMPs, thus protecting the BBB (Kikuchi et al., 2017; Sun et al., 2014). Whether edaravone was administered during occlusion or concomitantly to tPA, the risk of hemorrhages was decreased, as were the expression and activity of MMPs (Yagi et al., 2009; Yamashita et al., 2009). It is not known if this effect on MMPs is related to an effect on neutrophils. Edaravone has been shown to diminish the production of ROS, especially of superoxide, in neutrophils harvested from patients at the acute phase of stroke (Aizawa et al., 2006). There is no available data on whether edaravone could be able to modulate neutrophil pro-inflammatory pathways. The synergistic effects of the edaravone – tPA combination were confirmed in vitro on plasma from stroke patients (Kikuchi et al., 2017). So far, clinical trials have failed to confirm beneficial effects either on the efficacy of thrombolysis or on the reduction of hemorrhagic complications (Aoki et al., 2017; Tsuruoka et al., 2014).

Another anti-oxidant, resveratrol, was also tested in combination with thrombolysis. The outcome of patients receiving the combined treatment was significantly better regardless of whether they benefited from early – less than 2 h after stroke onset – or delayed – 2 to 4 h – thrombolysis. In this group of patients, the plasma levels of MMP-2 and MMP-9 were significantly decreased compared to patients in the tPA/placebo group (Chen, Bai, Zhao, Sui, & Xie, 2016).

5.6. Minocycline

Minocycline, an antibiotic of the tetracycline family, has been shown to exert pleiotropic effects responsible for its neuroprotective properties in ischemic stroke, including marked anti-inflammatory effects. These effects are mediated, at least partly, by the inhibition of the enzyme Poly-ADP-Ribose Transferase (PARP). Several studies investigated the potential of a combined treatment associating minocycline and tPA. In vitro, there were no modifications of the fibrinolytic properties of tPA (Machado et al., 2009). Minocycline started before or simultaneously with tPA induced a reduction in the incidence and volume of hemorrhagic transformations in parallel with a global neuroprotective effect. The levels and enzymatic activities of MMP-2 and MMP-9, measured in plasma and brain samples, were decreased (Fan, Lo, & Wang, 2013; Machado et al., 2009; Murata et al., 2008). Fewer neutrophils were

found infiltrated in the peri-infarct areas 16 h after embolic MCAO and treatment with tPA and minocycline (Fan et al., 2013).

The clinical evaluation of minocycline in stroke is extensive, alone as an acute phase neuroprotectant or combined with tPA. The MINOS pilot study assessed the impact of minocycline started within 6 h of stroke onset in 60 patients treated for 3 days. Minocycline was well-tolerated and no severe hemorrhagic transformation was reported, even in the 44 patients treated with tPA (Fagan et al., 2010). In this subgroup of patients, the levels of plasma MMP-9 were significantly reduced compared to patients treated with tPA alone (Switzer et al., 2011). The ongoing Australian study WAIMATSS (Blacker et al., 2013) was designed to specifically evaluate if an acute treatment with intravenous minocycline (started within 6 h of symptoms onset, one dose every 12 h for 3 days) could reduce the incidence of tPA-induced hemorrhages. The preliminary results did not show any difference in the rates of hemorrhagic transformations (Blacker et al., 2015).

5.7. Cilostazol

In vitro, the Phospho-Di-Esterase 3 (PDE3) inhibitor cilostazol decreased the expression of endothelial adhesion molecules and subsequently neutrophil adhesion (Omi et al., 2004). Its neuroprotective effects are well established in ischemic stroke and involve a reduction of neutrophil infiltration in the ischemic area when administered at the acute phase of stroke (Lee et al., 2007; Park et al., 2007). Whether used as a preventive or as an acute phase treatment, cilostazol also exerts protective effects and protects against tPA-related hemorrhages. Under both treatment conditions MMP-9 activity was reduced (Hase et al., 2012; Ishiguro et al., 2010; Kasahara, Nakagomi, Matsuyama, Stern, & Taguchi, 2012).

As experimental data showed a neuroprotective effect in stroke, several clinical studies have been led evaluating this drug as an acute stroke treatment. Cilostazol tended to protect against complications and improve the functional prognosis at 3 months (Matsumoto, Shimodozono, Miyata, & Kawahira, 2011; Nakamura, Tsuruta, & Uchiyama, 2012; Shimizu, Tominaga, Ogawa, Kayama, Mizoi et al., 2013). To date, the potential benefits of associating cilostazol to tPA have not been assessed.

5.8. HDL

Besides the well-established correlation between high plasma levels of High Density Lipoproteins (HDL) and low cardiovascular risk, HDL exerts an anti-inflammatory effect in vivo and inhibits neutrophil adhesion and recruitment in a model of hemorrhagic shock (Cockerill et al., 2001). Similar effects were evidenced in rats subjected to embolic MCAO. HDL administered 3 or 5 h after occlusion helped to preserve the BBB against the ischemic injury, and inhibited neutrophil adhesion, recruitment and activation (Lapergue et al., 2010). When tPA was co-administered, the acute HDL treatment was still beneficial and modulated the same mechanisms. The rate of hemorrhagic transformations was significantly lower in animals treated with HDL combined with tPA (Lapergue et al., 2013). Interestingly, HDL do not alter the proteolytic activity of tPA and could be administered concurrently without modifying its thrombolytic efficacy (Lapergue et al., 2013). Clinical trials are needed to confirm the relevance of such a combination in ischemic stroke.

5.9. Non pharmacological interventions: physical activity

The beneficial influence of physical activity on outcome and recovery after ischemic stroke is linked to an upregulation of cerebral plasticity enhancing tissue repair and regeneration, and involves the activation of PPAR delta. Pre-stroke physical activity was linked to neuroprotection, via the promotion of anti-inflammatory pathways (Chrysohoou et al., 2005). It was shown to be responsible for a decrease in the vascular expression of ICAM-1, and in leukocyte infiltration in the ischemic parenchyma. A reduction in the expression of MMP-9 has also been reported. A similar effect on MMP-9 has been reported with post-stroke physical training (Pin-Barre & Laurin, 2015). Even if the benefits of physical activity in terms of recovery were not confirmed in a cohort of tPA-treated stroke patients (Decourcelle et al., 2015), there is no available clinical data on the impact on the safety profile of tPA. Whether the above-mentioned effects, evidenced experimentally, translate to a protective effect in the clinical setting towards tPA-induced hemorrhages remains unknown, and further studies are required.

6. Conclusion

Neutrophils are clearly accomplices in the occurrence of post-thrombolysis hemorrhages in stroke, primarily due to vascular alterations related to BBB disruption. Even if thrombectomy appears as a promising therapeutic option, tPA is still valuable despite the inherent risks. The next step is now to discover biomarkers and/or pharmacological agents that could be used to limit this risk in the clinical setting. Several reviews have addressed the matter of pharmacological modulation in this context (Jickling et al., 2015; Kanazawa, Takahashi, Nishizawa, & Shimohata, 2017), a recent one raising the question of the relevance of lipid-lowering drugs (statins and fibrates) as disease modifiers (medications that can have an overall influence on the course of the ischemic disorder and of thrombolysis) (Ouk et al., 2013). The latest recommendations rely on the reversion of the coagulopathy, the prevention of the extension of the hematoma by a close control of blood pressure and neurosurgical procedures (Yaghi et al., 2017). Pharmacological candidates targeting neutrophils require a thorough preclinical and clinical evaluation to confirm their relevance in the field of post-thrombolysis hemorrhages in ischemic stroke.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Ahmed, N., Dávalos, A., Eriksson, N., Ford, G., Glahn, J., Hennerici, M., ... SITS Investigators (2010). Association of admission blood glucose and outcome in patients treated with intravenous thrombolysis: Results from the Safe Implementation of Treatments in Stroke International Stroke Thrombolysis Register (SITS-ISTR). *Archives of Neurology 67*, 1123–1130.

Aizawa, H., Makita, Y., Sumitomo, K., Aburakawa, Y., Katayama, T., Nakatani-Enomoto, S., ... Kikuchi, K. (2006). Edaravone diminishes free radicals from circulating neutrophils in patients with ischemic brain attack. *Internal Medicine 45*, 1–4.

Allahtavakoli, M., Moloudi, R., Arababadi, M., Shamsizadeh, A., & Javanmardi, K. (2009). Delayed post ischemic treatment with Rosiglitazone attenuates infarct volume, neurological deficits and neutrophilia after embolic stroke in rat. *Brain Research 1271*, 121–127.

Alvarez-Sabín, J., Huertas, R., Quintana, M., Rubiera, M., Delgado, P., Ribó, M., ... Montaner, J. (2007). Prior statin use may be associated with improved stroke outcome after tissue plasminogen activator. *Stroke 38*, 1076–1078.

Amarenco, P., Bogousslavsky, J., Callahan, A., Goldstein, L.B., Hennerici, M., Rudolph, A.E., ... Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) Investigators (2006). High-dose atorvastatin after stroke or transient ischemic attack. *The New England Journal of Medicine 355*, 549–559.

Aoki, J., Kimura, K., Morita, N., Harada, M., Metoki, N., Tateishi, Y., ... YAMATO Study Investigators (2017). YAMATO study (tissue-type plasminogen activator and edaravone combination therapy). *Stroke 48*, 712–719.

Aoki, T., Sumii, T., Mori, T., Wang, X., & Lo, E. (2002). Blood-brain barrier disruption and matrix metalloproteinase-9 expression during reperfusion injury: Mechanical versus embolic focal ischemia in spontaneously hypertensive rats. *Stroke 33*, 2711–2717.

Blacker, D., Pollard, E., Bynevelt, M., Prentice, D., Kho, L., Ghia, D., ... Bates, T. (2015). Preliminary results of the intravenous minocycline combined with intravenous thrombolysis: A randomized pilot study of a strategy to reduce hemorrhagic transformation in acute ischemic stroke – The West Australian Intravenous Minocycline and TPA Stroke Study (WAIMATSS). *International Journal of Stroke 10*, 19.

Blacker, D., Prentice, D., Alvaro, A., Bates, T., Bynevelt, M., Kelly, A., ... Major, T. (2013). Reducing haemorrhagic transformation after thrombolysis for stroke: A strategy utilising minocycline. *Stroke 2013*, 1–7.

Bonaventura, A., Liberale, L., Carbone, F., Vecchié, A., Diaz-Cañestro, C., Camici, G., ... Dallegri, F. (2018). The pathophysiological role of neutrophil extracellular traps in inflammatory diseases. *Thrombosis and Haemostasis 118*, 6–27.

Bråtane, B., Bouley, J., Schneider, A., Bastan, B., Henninger, N., & Fisher, M. (2009). Granulocyte-colony stimulating factor delays PWI/DWI mismatch evolution and reduces final infarct volume in permanent-suture and embolic focal cerebral ischemia models in the rat. *Stroke 40*, 3102–3106.

Campos, M., García-Bonilla, L., Hernández-Guillamon, M., Barceló, V., Morancho, A., Quintana, M., ... Montaner, J. (2013). Combining statins with tissue plasminogen activator treatment after experimental and human stroke: A safety study on hemorrhagic transformation. *CNS Neuroscience & Therapeutics 19*(11), 863–870.

Cappellari, M., Bovi, P., Moretto, G., Zini, A., Nencini, P., Sessa, M., ... Paciaroni, M. (2013). The THRombolysis and STatins (THRaST) study. *Neurology 80*, 655–661.

Caranfa, J., Nguyen, E., Ali, R., Francis, I., Zichichi, A., Bosco, E., ... Kohn, C. (2018). Mechanical endovascular therapy for acute ischemic stroke: An indirect treatment comparison between Solitaire and Penumbra thrombectomy devices. *PLoS One 13*, e0191657.

Carbone, F., Vuilleumier, N., Bertolotto, M., Burger, F., Galan, K., Roversi, G., ... Montecucco, F. (2015). Treatment with recombinant tissue plasminogen activator (r-TPA) induces neutrophil degranulation in vitro via defined pathways. *Vascular Pharmacology 64*, 16–27.

Castellanos, M., Sobrino, T., Millán, M., García, M., Arenillas, J., Nombela, F., ... Dávalos, A. (2007). Serum cellular fibronectin and matrix metalloproteinase-9 as screening biomarkers for the prediction of parenchymal hematoma after thrombolytic therapy in acute ischemic stroke: A multicenter confirmatory study. *Stroke 38*, 1855–1859.

Chen, J., Bai, Q., Zhao, Z., Sui, H., & Xie, X. (2016). Resveratrol improves delayed r-tPA treatment outcome by reducing MMPs. *Acta Neurologica Scandinavica 134*, 54–60.

Chrysohoou, C., Pitsavos, C., Kokkinos, P., Panagiotakos, D.B., Singh, S.N., & Stefanadis, C. (2005). The role of physical activity in the prevention of stroke. *Central European Journal of Public Health 13*, 132–136.

Cockerill, G., McDonald, M., Mota-Filipe, H., Cuzzocrea, S., Miller, N., & Thiemermann, C. (2001). High density lipoproteins reduce organ injury and organ dysfunction in a rat model of hemorrhagic shock. *The FASEB Journal 15*, 1941–1952.

Cuadrado, E., Ortega, L., Hernández-Guillamon, M., Penalba, A., Fernández-Cadenas, I., Rosell, A., & Montaner (2008). Tissue plasminogen activator (t-PA) promotes neutrophil degranulation and MMP-9 release. *Journal of Leukocyte Biology 84*, 207–214.

De Meyer, S., Andersson, T., Baxter, B., Bendszus, M., Brouwer, P., Brinjikji, W., ... Liebeskind, D. (2017). Analyses of thrombi in acute ischemic stroke: A consensus statement on current knowledge and future directions. *International Journal of Stroke 12*, 606–614.

De Meyer, S., Denorme, F., Langhauser, F., Gueuss, E., Fluri, F., & Kleinschnitz, C. (2016). Thromboinflammation in stroke brain damage. *Stroke 47*, 1165–1172.

Decourcelle, A., Moulin, S., Sibon, I., Murao, K., Ronzière, T., Godefroy, O., ... Leys, D. (2015). Influence of previous physical activity on the outcome of patients treated by thrombolytic therapy for stroke. *Journal of Neurology 262*, 2513–2519.

Del Zoppo, G.J. (2009). Inflammation and the neurovascular unit in the setting of focal cerebral ischemia. *Neuroscience 158*, 972–982.

Dela Peña, I., Yoo, A., Tajiri, N., Acosta, S., Ji, X., Kaneko, Y., & Borlongan, C. (2015). Granulocyte colony-stimulating factor attenuates delayed tPA-induced hemorrhagic transformation in ischemic stroke rats by enhancing angiogenesis and vasculogenesis. *Journal of Cerebral Blood Flow and Metabolism 35*, 338–346.

Deplanque, D., Gelé, P., Pétrault, O., Six, I., Furman, C., Bouly, M., ... Bordet, R. (2003). Peroxisome proliferator-activated receptor-alpha activation as a mechanism of preventive neuroprotection induced by chronic fenofibrate treatment. *The Journal of Neuroscience 23*, 6264–6271.

Diener, H. -C., Lees, K.R., Lyden, P., Grotta, J., Davalos, A., Davis, S.M., ... for the SAINT I and II Investigators (2008). NXY-059 for the treatment of acute stroke: pooled analysis of the SAINT I and II trials. *Stroke 39*, 1751–1758.

Doycheva, D., Hadley, T., Li, L., Applegate, R., Zhang, J., & Tang, J. (2014). Anti-neutrophil antibody enhances the neuroprotective effects of G-CSF by decreasing number of neutrophils in hypoxic ischemic neonatal rat model. *Neurobiology of Disease 69*, 192–199.

Ducroux, C., Di Meglio, L., Loyau, S., Delbosc, S., Boisseau, W., Deschildre, C., ... Desilles, J. (2018). Thrombus neutrophil extracellular traps content impair tPA-induced thrombolysis in acute ischemic stroke. *Stroke 49*, 754–757.

Durukan, A., & Tatlisumak, T. (2007). Acute ischemic stroke: Overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. *Pharmacology, Biochemistry, and Behavior 87*, 179–197.

Engelter, S.T., Soinne, L., Ringleb, P., Sarikaya, H., Bordet, R., Berrouschot, J., ... Pezzini, A. (2011). IV thrombolysis and statins. *Neurology 77*, 888–895.

Fagan, S.C., Waller, J.L., Nichols, F.T., Edwards, D.J., Pettigrew, L.C., Clark, W.M., ... Hess, D.C. (2010). Minocycline to improve neurologic outcome in stroke (MINOS): A dose-finding study. *Stroke 41*, 2283–2287.

Fan, X., Lo, E.H., & Wang, X. (2013). Effects of minocycline plus tissue plasminogen activator combination therapy after focal embolic stroke in type 1 diabetic rats. *Stroke 44*, 745–752.

Fang, Y., Tong, M., Sung, P., Chen, Y., Chen, C., Tsai, N., ... Yip, H. (2017). Higher neutrophil counts and neutrophil-to-lymphocyte ratio predict prognostic outcomes in patients after non-atrial fibrillation-caused ischemic stroke. *Biomedical Journal 40*, 154–162.

Foerch, C., Montaner, J., Furie, K.L., Ning, M.M., & Lo, E.H. (2009). Invited article: Searching for oracles? Blood biomarkers in acute stroke. *Neurology 73*, 393–399.

Fugate, J., & Rabinstein, A. (2014). Update on intravenous recombinant tissue plasminogen activator for acute ischemic stroke. *Mayo Clinic Proceedings 89*, 960–972.

Garcia, J.H., Liu, K.F., Yoshida, Y., Lian, J., Chen, S., & Del Zoppo, G.J. (1994). Influx of leukocytes and platelets in an evolving brain infarct (Wistar rat). *The American Journal of Pathology 144*, 188–199.

Gautier, S., Ouk, T., Pétrault, M., Pétrault, O., Bérézowski, V., & Bordet, R. (2015). PPAR-alpha agonist used at the acute phase of experimental ischemic stroke reduces occurrence of thrombolysis-induced hemorrhage in rats. *PPAR Research 2015*, 246329.

Gautier, S., Ouk, T., Petrault, O., Caron, J., & Bordet, R. (2009). Neutrophils contribute to intracerebral haemorrhages after treatment with recombinant tissue plasminogen activator following cerebral ischaemia. *British Journal of Pharmacology 156*, 673–679.

Gautier, S., Ouk, T., Tagzirt, M., Lefebvre, C., Laprais, M., Pétrault, O., ... Bordet, R. (2014). Impact of the neutrophil response to granulocyte colony-stimulating factor on the risk of hemorrhage when used in combination with tissue plasminogen activator during the acute phase of experimental stroke. *Journal of Neuroinflammation 11*, 96.

Gautier, S., Petrault, O., Gele, P., Laprais, M., Bastide, M., Bauters, A., ... Bordet, R. (2003). Involvement of thrombolysis in recombinant tissue plasminogen activator-induced cerebral hemorrhages and effect on infarct volume and postischemic endothelial function. *Stroke 34*, 2975–2979.

Geng, J., Song, Y., Mu, Z., Xu, Q., Shi, G., Sun, Y., ... Li, Y. (2016). Early use of statin in patients treated with alteplase for acute ischemic stroke. *Acta Neurochirurgica. Supplement 121*, 269–275.

Golab, P., Kielbus, M., Bielewicz, J., & Kurzepa, J. (2015). The effect of recombinant tissue plasminogen activator on MMP-2 and MMP-9 activities in vitro. *Neurological Research 37*, 9–13.

Gore, J.M., Granger, C.B., Simoons, M.L., Sloan, M.A., Weaver, W.D., White, H.D., ... Topol, E.J. (1995). Stroke after thrombolysis. Mortality and functional outcomes in the GUSTO-I trial. Global use of strategies to open occluded coronary arteries. *Circulation 92*, 2811–2818.

Granger, D., & Kvietys, P. (2017). Reperfusion therapy—What's with the obstructed, leaky and broken capillaries? *Pathophysiology 24*, 213–228.

Guo, Z., Yu, S., Xiao, L., Chen, X., Ye, R., Zheng, P., ... Liu, X. (2016). Dynamic change of neutrophil to lymphocyte ratio and hemorrhagic transformation after thrombolysis in stroke. *Journal of Neuroinflammation 13*, 199.

Hase, Y., Okamoto, Y., Fujita, Y., Kitamura, A., Nakabayashi, H., Ito, H., ... Ihara, M. (2012). Cilostazol, a phosphodiesterase inhibitor, prevents no-reflow and hemorrhage in mice with focal cerebral ischemia. *Experimental Neurology 233*, 523–533.

Hashimoto, T., Hayakawa, M., Funatsu, N., Yamagami, H., Satow, T., Takahashi, J., ... Toyoda, K. (2016). Histopathologic analysis of retrieved thrombi associated with successful reperfusion after acute stroke thrombectomy. *Stroke 47*, 3035–3037.

Hernandez-Guillamon, M., Garcia-Bonilla, L., Solé, M., Sosti, V., Parés, M., Campos, M., ... Montaner, J. (2010). Plasma VAP-1/SSAO activity predicts intracranial hemorrhages and adverse neurological outcome after tissue plasminogen activator treatment in stroke. *Stroke 41*, 1528–1535.

Hosomi, N., Nishiyama, A., Ban, C.R., Naya, T., Takahashi, T., Kohno, M., & Koziol, J.A. (2005). Angiotensin type 1 receptor blockage improves ischemic injury following transient focal cerebral ischemia. *Neuroscience 134*, 225–231.

Iadecola, C., & Anrather, J. (2011). The immunology of stroke: From mechanisms to translation. *Nature Med 17*, 796–808.

Inzitari, D., Giusti, B., Nencini, P., Gori, A., Nesi, M., Palumbo, V., ... MAGIC Study Group (2013). MMP9 variation after thrombolysis is associated with hemorrhagic transformation of lesion and death. *Stroke 44*, 2901–2903.

Ishiguro, M., Mishiro, K., Fujiwara, Y., Chen, H., Izuta, H., Tsuruma, K., ... Hara, H. (2010). Phosphodiesterase-III inhibitor prevents hemorrhagic transformation induced by focal cerebral ischemia in mice treated with tPA. *PLoS One 5*, e15178.

Ishrat, T., Pillai, B., Ergul, A., Hafez, S., & Fagan, S. (2013). Candesartan reduces the hemorrhage associated with delayed tissue plasminogen activator treatment in rat embolic stroke. *Neurochemical Research 38*, 2668–2677.

Iwanami, J., Mogi, M., Tsukuda, K., Min, L.J., Sakata, A., Jing, F., ... Horiuchi, M. (2010). Low dose of telmisartan prevents ischemic brain damage with peroxisome proliferator-activated receptor-γ activation in diabetic mice. *Journal of Hypertension 28*, 1730–1737.

Jickling, G., Liu, D., Ander, B., Stamova, B., Zhan, X., & Sharp, F. (2015). Targeting neutrophils in ischemic stroke: Translational insights from experimental studies. *Journal of Cerebral Blood Flow and Metabolism 35*, 888–901.

Jickling, G., Liu, D., Stamova, B., Ander, B., Zhan, X., Lu, A., & Sharp, F. (2014). Hemorrhagic transformation after ischemic stroke in animals and humans. *Journal of Cerebral Blood Flow and Metabolism 34*, 185–199.

Jin, R., Yang, G., & Li, G. (2010). Molecular insights and therapeutic targets for blood–brain barrier disruption in ischemic stroke: Critical role of matrix metalloproteinases and tissue-type plasminogen activator. *Neurobiology of Disease 38*, 376–385.

Justicia, C., Panés, J., Solé, S., Cervera, A., Deulofeu, R., Chamorro, A., & Planas, A. (2003). Neutrophil infiltration increases matrix metalloproteinase-9 in the ischemic brain after occlusion/reperfusion of the middle cerebral artery in rats. *Journal of Cerebral Blood Flow and Metabolism 23*, 1430–1440.

Kahles, T., Foerch, C., Sitzer, M., Schroeter, M., Steinmetz, H., Rami, A., & Neumann-Haefelin, T. (2005). Tissue plasminogen activator mediated blood-brain barrier damage in transient focal cerebral ischemia in rats: Relevance of interactions between thrombotic material and thrombolytic agent. *Vascular Pharmacology 43*, 254–259.

Kanazawa, M., Takahashi, T., Nishizawa, M., & Shimohata, T. (2017). Therapeutic strategies to attenuate hemorrhagic transformation after tissue plasminogen activator treatment for acute ischemic stroke. *Journal of Atherosclerosis and Thrombosis 24*, 240–253.

Kandadai, M., Meunier, J., Lindsell, C., Shaw, G., & Elkind, M. (2012). Short-term high-dose effect of lovastatin on thrombolysis by rt-PA in a human whole-blood in vitro clot model. *Current Neurovascular Research 9*, 207–213.

Kasahara, Y., Nakagomi, T., Matsuyama, T., Stern, D., & Taguchi, A. (2012). Cilostazol reduces the risk of hemorrhagic infarction after administration of tissue-type plasminogen activator in a murine stroke model. *Stroke 43*, 499–506.

Kasahara, Y., Taguchi, A., Uno, H., Nakano, A., Nakagomi, T., Hirose, H., ... Matsuyama, T. (2010). Telmisartan suppresses cerebral injury in a murine model of transient focal ischemia. *Brain Research 1340*, 70–80.

Kastrup, A., Gröschel, K., Ringer, T., Redecker, C., Cordesmeyer, R., Witte, O., & Terborg, C. (2008). Early disruption of the blood-brain barrier after thrombolytic therapy predicts hemorrhage in patients with acute stroke. *Stroke 39*, 2385–2387.

Khoshnam, S., Winlow, W., Farzaneh, M., Farbood, Y., & Moghaddam, H. (2017). Pathogenic mechanisms following ischemic stroke. *Neurological Sciences 38*, 1167–1186.

Kikuchi, K., Setoyama, K., Kawahara, K., Nagasato, T., Terashi, T., Ueda, K., ... Maruyama, I. (2017). Edaravone, a synthetic free radical scavenger, enhances alteplase-mediated thrombolysis. *Oxidative Medicine and Cellular Longevity 2017*, 6873281.

Kim, A., Cahill, E., & Cheng, N. (2015). Global stroke belt: geographic variation in stroke burden worldwide. *Stroke 46*, 3564–3570.

Kim, J., Park, J., Chang, J., Kim, S., & Lee, J. (2016). Inflammation after ischemic stroke: The role of leukocytes and glial cells. *Experimental Neurobiology 25*, 241–251.

Kono, S., Kurata, T., Sato, K., Omote, Y., Hishikawa, N., Yamashita, T., ... Abe, K. (2015). Neurovascular protection by telmisartan via reducing neuroinflammation in stroke-resistant spontaneously hypertensive rat brain after ischemic stroke. *Journal of Stroke and Cerebrovascular Diseases 24*, 537–547.

Kozak, A., Ergul, A., El-Remessy, A.B., Johnson, M.H., Machado, L.S., Elewa, H.F., ... Fagan, S. C. (2009). Candesartan augments ischemia-induced proangiogenic state and results in sustained improvement after stroke. *Stroke 40*, 1870–1876.

Lansberg, M., Thijs, V., Bammer, R., Kemp, S., Wijman, C., Marks, M., ... DEFUSE Investigators (2007). Risk factors of symptomatic intracerebral hemorrhage after tPA therapy for acute stroke. *Stroke 38*, 2275–2278.

Lapchak, P., & Han, M. (2009). The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor simvastatin reduces thrombolytic-induced intracerebral hemorrhage in embolized rabbits. *Brain Research 1303*, 144–150.

Lapchak, P., Song, D., Wei, J., & Zivin, J. (2004). Coadministration of NXY-059 and tenecteplase six hours following embolic strokes in rabbits improves clinical rating scores. *Experimental Neurology 188*, 279–285.

Lapchak, P.A., Araujo, D.M., Song, D., Wei, J., Purdy, R., & Zivin, J.A. (2002a). Effects of the spin trap agent disodium- [tert-(butylimino)methyl]benzene-1,3-disulfonate N-oxide (generic NXY-059) on intracerebral hemorrhage in a rabbit large clot embolic stroke model: Combination studies with tissue plasminogen activator. *Stroke 33*, 1665–1670.

Lapchak, P.A., Araujo, D.M., Song, D., Wei, J., & Zivin, J.A. (2002b). Neuroprotective effects of the spin trap agent disodium-[(tert-butylimino)methyl]benzene-1,3-disulfonate N-oxide (generic NXY-059) in a rabbit small clot embolic stroke model: Combination studies with the thrombolytic tissue plasminogen activator. *Stroke 33*, 1411–1415.

Lapergue, B., Dang, B., Desilles, J., Ortiz-Munoz, G., Delbosc, S., Loyau, S., ... Michel, J. (2013). High-density lipoprotein–based therapy reduces the hemorrhagic complications associated with tissue plasminogen activator treatment in experimental stroke. *Stroke 44*, 699–707.

Lapergue, B., Moreno, J. -A., Dang, B.Q., Coutard, M., Delbosc, S., Raphaeli, G., ... Meilhac, O. (2010). Protective effect of high-density lipoprotein-based therapy in a model of embolic stroke. *Stroke 41*, 1536–1542.

Laridan, E., Denorme, F., Desender, L., François, O., Andersson, T., Deckmyn, H., ... De Meyer, S. (2017). Neutrophil extracellular traps in ischemic stroke thrombi. *Annals of Neurology 82*, 223–232.

Larrue, V., von Kummer, R.R., Müller, A., & Bluhmki, E. (2001). Risk factors for severe hemorrhagic transformation in ischemic stroke patients treated with recombinant tissue plasminogen activator: A secondary analysis of the European-Australasian Acute Stroke Study (ECASS II). *Stroke 32*, 438–441.

Lee, J., Park, S., Shin, H., Kim, C., Lee, W., & Hong, K. (2007). Poly(ADP-ribose) polymerase inhibition by cilostazol is implicated in the neuroprotective effect against focal cerebral ischemic infarct in rat. *Brain Research 1152*, 182–190.

Lenglet, S., Montecucco, F., Mach, F., Schaller, K., Gasche, Y., & Copin, J. -C. (2014). Analysis of the expression of nine secreted matrix metalloproteinases and their endogenous inhibitors in the brain of mice subjected to ischaemic stroke. *Thrombosis and Haemostasis 112*, 363–378.

Lindsberg, P.J., Carpen, O., Paetau, A., Karjalainen-Lindsberg, M. -L., & Kaste, M. (1996). Endothelial ICAM-1 expression associated with inflammatory cell response in human ischemic stroke. *Circulation 94*, 939–945.

Litke, R., Moulin, S., Cordonnier, C., Fontaine, P., & Leys, D. (2015). Influence of glycaemic control on the outcomes of patients treated by intravenous thrombolysis for cerebral ischaemia. *Journal of Neurology 262*, 2504–2512.

Liu, X., Zhang, Z., Zhang, L., Morris, D., Kapke, A., Lu, M., & Chopp, M. (2006). Atorvastatin downregulates tissue plasminogen activator-aggravated genes mediating coagulation and vascular permeability in single cerebral endothelial cells captured by laser microdissection. *Journal of Cerebral Blood Flow and Metabolism 26*, 787–796.

Lu, G., He, Q., Shen, Y., & Cao, F. (2018). Potential biomarkers for predicting hemorrhagic transformation of ischemic stroke. *The International Journal of Neuroscience 128*, 79–89.

Luo, Y., Yin, W., Signore, A., Zhang, F., Hong, Z., Wang, S., ... Chen, J. (2006). Neuroprotection against focal ischemic brain injury by the peroxisome proliferator-activated receptor-γ agonist rosiglitazone: PPAR-γ activation protects against cerebral ischemia. *Journal of Neurochemistry 97*, 435–448.

Machado, L.S., Sazonova, I.Y., Kozak, A., Wiley, D.C., El-Remessy, A.B., Ergul, A., ... Fagan, S.C. (2009). Minocycline and tissue-type plasminogen activator for stroke: Assessment of interaction potential. *Stroke 40*, 3028–3033.

Maestrini, I., Strbian, D., Gautier, S., Haapaniemi, E., Moulin, S., Sairanen, T., ... Bordet, R. (2015). Higher neutrophil counts before thrombolysis for cerebral ischemia predict worse outcomes. *Neurology 85*, 1408–1416.

Martinez-Ramirez, S., Delgado-Mederos, R., Marín, R., Suárez-Calvet, M., Sáinz, M.P., Alejaldre, A., ... Martí-Fàbregas, J. (2012). Statin pretreatment may increase the risk of symptomatic intracranial haemorrhage in thrombolysis for ischemic stroke: Results from a case–control study and a meta-analysis. *Journal of Neurology 259*, 111–118.

Matsumoto, S., Shimodozono, M., Miyata, R., & Kawahira, K. (2011). Effect of cilostazol administration on cerebral hemodynamics and rehabilitation outcomes in poststroke patients. *The International Journal of Neuroscience 121*, 271–278.

Meier, N., Nedeltchev, K., Brekenfeld, C., Galimanis, A., Fischer, U., Findling, O., ... Arnold, M. (2009). Prior statin use, intracranial hemorrhage, and outcome after intra-arterial thrombolysis for acute ischemic stroke. *Stroke 40*, 1729–1737.

Menon, B., Saver, J., Prabhakaran, S., Reeves, M., Liang, L., Olson, D., ... Smith, E. (2012). Risk score for intracranial hemorrhage in patients with acute ischemic stroke treated with intravenous tissue-type plasminogen activator. *Stroke 43*, 2293–2299.

Merino-Zamorano, C., Hernández-Guillamon, M., Jullienne, A., Le Béhot, A., Bardou, I., Parés, M., ... Montaner, J. (2015). NURR1 involvement in recombinant tissue-type plasminogen activator treatment complications after ischemic stroke. *Stroke 46*, 477–484.

Michel, J., & Ho-Tin-Noé, B. (2015). Thrombi and neutrophils. *Circulation Research 116*, 1107–1108.

Miedema, I., Uyttenboogaart, M., Koopman, K., De Keyser, J., & Luijckx, G.J. (2010). Statin use and functional outcome after tissue plasminogen activator treatment in acute ischaemic stroke. *Cerebrovascular Diseases 29*, 263–267.

Miedema, I., Uyttenboogaart, M., & Luijckx, G. (2012). Statin use and lipid profile in relation to safety and functional outcome after thrombolysis in ischemic stroke. *Expert Review of Neurotherapeutics 12*, 907–910.

Minnerup, J., Heidrich, J., Wellmann, J., Rogalewski, A., Schneider, A., & Schabitz, W. -R. (2008). Meta-analysis of the efficacy of granulocyte-colony stimulating factor in animal models of focal cerebral ischemia. *Stroke 39*, 1855–1861.

Mistry, E., Mistry, A., Nakawah, M., Chitale, R., James, R., Volpi, J., & Fusco, M. (2017). Mechanical thrombectomy outcomes with and without intravenous thrombolysis in stroke patients: A meta-analysis. *Stroke 48*, 2450–2456.

Montaner, J., Bustamante, A., García-Matas, S., Martínez-Zabaleta, M., Jiménez, C., de la Torre, J., ... STARS Investigators (2016). Combination of thrombolysis and statins in acute stroke is safe: Results of the STARS randomized trial (stroke treatment with acute reperfusion and simvastatin). *Stroke 47*, 2870–2873.

Montaner, J., Molina, C., Monasterio, J., Abilleira, S., Arenillas, J., Ribó, M., ... Alvarez-Sabín, J. (2003). Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. *Circulation 107*, 598–603.

Moskowitz, M.A., Lo, E.H., & Iadecola, C. (2010). The science of stroke: Mechanisms in search of treatments. *Neuron 67*, 181–198.

Murata, Y., Rosell, A., Scannevin, R.H., Rhodes, K.J., Wang, X., & Lo, E.H. (2008). Extension of the thrombolytic time window with minocycline in experimental stroke. *Stroke 39*, 3372–3377.

Nakamura, T., Tsuruta, S., & Uchiyama, S. (2012). Cilostazol combined with aspirin prevents early neurological deterioration in patients with acute ischemic stroke: A pilot study. *Journal of the Neurological Sciences 313*, 22–26.

Nardi, K., Engelter, S., Strbian, D., Sarikaya, H., Arnold, M., Casoni, F., ... Lipid Profile in Thrombolysis Study Group (2012). Lipid profiles and outcome in patients treated by intravenous thrombolysis for cerebral ischemia. *Neurology 79*, 1101–1108.

Nardi, K., Leys, D., Eusebi, P., Cordonnier, C., Gautier, S., Hénon, H., & Bordet, R. (2011). Influence of lipid profiles on the risk of hemorrhagic transformation after ischemic stroke: Systematic review. *Cerebrovascular Diseases Extra 1*, 130–141.

Ni Chroinin, D., Asplund, K., Asberg, S., Callaly, E., Cuadrado-Godia, E., Diez-Tejedor, E., ... Kelly, P.J. (2013). Statin therapy and outcome after ischemic stroke: systematic review and meta-analysis of observational studies and randomized trials. *Stroke 44*, 448–456.

NINDS (National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group) (1995). Tissue plasminogen activator for acute ischemic stroke. *The New England Journal of Medicine 333*, 1581–1587.

Omi, H., Okayama, N., Shimizu, M., Fukutomi, T., Nakamura, A., Imaeda, K., ... Itoh, M. (2004). Cilostazol inhibits high glucose-mediated endothelial-neutrophil adhesion by decreasing adhesion molecule expression via NO production. *Microvascular Research 68*, 119–125.

Ouk, T., Gautier, S., Pétrault, M., Montaigne, D., Maréchal, X., Masse, I., ... Nevière, R. (2014b). Effects of the PPAR-α agonist fenofibrate on acute and short-term consequences of brain ischemia. *Journal of Cerebral Blood Flow and Metabolism*(34), 542–551.

Ouk, T., Laprais, M., Bastide, M., Mostafa, K., Gautier, S., & Bordet, R. (2009). Withdrawal of fenofibrate treatment partially abrogates preventive neuroprotection in stroke via loss of vascular protection. *Vascular Pharmacology 51*, 323–330.

Ouk, T., Potey, C., Gautier, S., Bastide, M., Deplanque, D., Staels, B., ... Bordet, R. (2013). PPARs: A potential target for a disease-modifying strategy in stroke. *Current Drug Targets 14*, 752–767.

Ouk, T., Potey, C., Laprais, M., Gautier, S., Hanf, R., Darteil, R., ... Bordet, R. (2014a). PPARα is involved in the multitargeted effects of a pretreatment with atorvastatin in experimental stroke. *Fundamental & Clinical Pharmacology 28*, 294–302.

Pagram, H., Bivard, A., Lincz, L., & Levi, C. (2016). Peripheral immune cell counts and advanced imaging as biomarkers of stroke outcome. *Cerebrovascular Diseases Extra 6*, 120–128.

Palomares, S.M., & Cipolla, M.J. (2011). Vascular protection following cerebral ischemia and reperfusion. *Journal of Neurology and Neurophysiology*, S1.

Park, S., Lee, J., Kim, C., Rhim, B., Hong, K., & Lee, W. (2007). Beneficial synergistic effects of concurrent treatment with cilostazol and probucol against focal cerebral ischemic injury in rats. *Brain Research 1157*, 112–120.

Perez-De-Puig, I., Miró-Mur, F., Ferrer-Ferrer, M., Gelpi, E., Pedragosa, J., Justicia, C., ... Planas, A. (2015). Neutrophil recruitment to the brain in mouse and human ischemic stroke. *Acta Neuropathologica 129*, 239–257.

Pétrault, O., Ouk, T., Gautier, S., Laprais, M., Gelé, P., Bastide, M., & Bordet, R. (2005). Pharmacological neutropenia prevents endothelial dysfunction but not smooth muscle functions impairment induced by middle cerebral artery occlusion. *British Journal of Pharmacology 144*, 1051–1058.

Phipps, M., Zeevi, N., Staff, I., Fortunato, G., Kuchel, G., & McCullough, L. (2013). Stroke severity and outcomes for octogenarians receiving statins. *Archives of Gerontology and Geriatrics 57*, 377–382.

Piccardi, B., Palumbo, V., Nesi, M., Nencini, P., Gori, A., Giusti, B., ... Inzitari, D. (2015). Unbalanced metalloproteinase-9 and tissue inhibitors of metalloproteinases ratios predict hemorrhagic transformation of lesion in ischemic stroke patients treated with thrombolysis: Results from the MAGIC study. *Frontiers in Neurology 6*, 121.

Pin-Barre, C., & Laurin, J. (2015). Physical exercise as a diagnostic, rehabilitation, and preventive tool: influence on neuroplasticity and motor recovery after stroke. *Neural Plasticity 2015*, 608581.

Poppe, A., Majumdar, S., Jeerakathil, T., Ghali, W., Buchan, A., Hill, M., & Canadian Alteplase for Stroke Effectiveness Study Investigators (2009). Admission hyperglycemia predicts a worse outcome in stroke patients treated with intravenous thrombolysis. *Diabetes Care 32*, 617–622.

Potey, C., Ouk, T., Petrault, O., Petrault, M., Berezowski, V., Salleron, J., ... Gautier, S. (2015). Early treatment with atorvastatin exerts parenchymal and vascular protective effects in experimental cerebral ischaemia. *British Journal of Pharmacology 172*, 5188–5198.

Powers, W., Rabinstein, A., Ackerson, T., Adeoye, O., Bambakidis, N., Becker, K., ... American Heart Association Stroke Council (2018). 2018 Guidelines for the early management of patients with acute ischemic stroke: A guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke 49*, e46–e110.

Price, C.J.S., Menon, D.K., Peters, A.M., Ballinger, J.R., Barber, R.W., Balan, K.K., ... Warburton, E.A. (2004). Cerebral neutrophil recruitment, histology, and outcome in acute ischemic stroke: An imaging-based study. *Stroke 35*, 1659–1664.

Qun, S., Tang, Y., Sun, J., Liu, Z., Wu, J., Zhang, J., ... Ge, W. (2017). Neutrophil-to-lymphocyte ratio predicts 3-month outcome of acute ischemic stroke. *Neurotoxicity Research 31*, 444–452.

Rocco, A., Sykora, M., Ringleb, P., & Diedler, J. (2012). Impact of statin use and lipid profile on symptomatic intracerebral haemorrhage, outcome and mortality after intravenous thrombolysis in acute stroke. *Cerebrovascular Diseases 33*, 362–368.

Roever, L., & Levine, S. (2015). Cerebral hemorrhage following thrombolytic therapy for stroke: Are neutrophils really neutral? *Neurology 85*, 1360–1361.

Rosell, A., Cuadrado, E., Ortega-Aznar, A., Hernández-Guillamon, M., Lo, E., & Montaner, J. (2008). MMP-9-positive neutrophil infiltration is associated to blood-brain barrier breakdown and basal lamina type IV collagen degradation during hemorrhagic transformation after human ischemic stroke. *Stroke 39*, 1121–1126.

Rosell, A., Ortega-Aznar, A., Alvarez-Sabín, J., Fernández-Cadenas, I., Ribó, M., Molina, C., ... Montaner, J. (2006). Increased brain expression of matrix metalloproteinase-9 after ischemic and hemorrhagic human stroke. *Stroke 37*, 1399–1406.

Ruhnau, J., Schulze, J., Dressel, A., & Vogelgesang, A. (2017). Thrombosis, Neuroinflammation, and Poststroke Infection: The Multifaceted Role of Neutrophils in Stroke. *Journal of Immunology Research 2017*, 5140679.

Scheitz, J., MacIsaac, R., Abdul-Rahim, A., Siegerink, B., Bath, P., Endres, M., ... Bluhmki, E. (2016). Statins and risk of poststroke hemorrhagic complications. *Neurology 86*, 1590–1596.

Scheitz, J., Nolte, C., & Endres, M. (2013). Should statins be paused or discontinued after thrombolysis or acute intracerebral hemorrhage? No! *Stroke 44*, 1472–1476.

Scheitz, J.F., Seiffge, D.J., Tütüncü, S., Gensicke, H., Audebert, H.J., Bonati, L.H., ... Nolte, C.H. (2014). Dose-related effects of statins on symptomatic intracerebral hemorrhage and outcome after thrombolysis for ischemic. *Stroke 45*, 509–514.

Sevimli, S., Diederich, K., Strecker, J., Schilling, M., Klocke, R., Nikol, S., ... Schäbitz, W. (2009). Endogenous brain protection by granulocyte-colony stimulating factor after ischemic stroke. *Experimental Neurology 217*, 328–335.

Shah, A., Denaxas, S., Nicholas, O., Hingorani, A., & Hemingway, H. (2017). Neutrophil counts and initial presentation of 12 cardiovascular diseases. *JACC: Journal of the American College of Cardiology 69*, 1160–1169.

Shearer, J., Douglas, A., Kirby, B., Tatlisumak, T., & Doyle, K. (2018). Animal models of focal cerebral ischaemia and haemorrhagic transformation: Considerations in experimental stroke study design. *Current Vascular Pharmacology 16*(6), 534–546.

Shi, J., Peng, H., You, S., Liu, Y., Xu, J., Xu, Y., ... Liu, C. -F. (2018). Increase in neutrophils after recombinant tissue plasminogen activator thrombolysis predicts poor functional outcome of ischaemic stroke: A longitudinal study. *European Journal of Neurology 25* (687–e45).

Shimizu, H., Tominaga, T., Ogawa, A., Kayama, T., Mizoi, K., Saito, K., ... Mori, E. (2013). Cilostazol for the prevention of acute progressing stroke: A multicenter, randomized controlled trial. *Journal of Stroke and Cerebrovascular Diseases 22*, 449–456.

Sobrino, T., Millán, M., Castellanos, M., Blanco, M., Brea, D., Dorado, L., ... Castillo, J. (2010). Association of growth factors with arterial recanalization and clinical outcome in patients with ischemic stroke treated with tPA. *Journal of Thrombosis and Haemostasis 8*, 1567–1574.

Solaroglu, I., Digicaylioglu, M., Keles, G., & Zhang, J. (2015). New missions for an old agent: Granulocyte-colony stimulating factor in the treatment of stroke patients. *Current Medicinal Chemistry 22*, 1302–1309.

Strecker, J., Sevimli, S., Schilling, M., Klocke, R., Nikol, S., Schneider, A., & Schäbitz, W. (2010). Effects of G-CSF treatment on neutrophil mobilization and neurological outcome after transient focal ischemia. *Experimental Neurology 222*, 108–113.

Sumii, T., & Lo, E. (2002). Involvement of matrix metalloproteinase in thrombolysis-associated hemorrhagic transformation after embolic focal ischemia in rats. *Stroke 33*, 831–836.

Sun, Y., Morozov, Y., Yang, D., Li, Y., Dunn, R., Rakic, P., ... Kuan, C. (2014). Synergy of combined tPA-edaravone therapy in experimental thrombotic stroke. *PLoS One 9*, e98807.

Switzer, J.A., Hess, D.C., Ergul, A., Waller, J.L., Machado, L.S., Portik-Dobos, V., ... Fagan, S.C. (2011). Matrix metalloproteinase-9 in an exploratory trial of intravenous minocycline for acute ischemic stroke. *Stroke 42*, 2633–2635.

Tang, Y., Xu, H., Du, X., Lit, L., Walker, W., Lu, A., ... Sharp, F. (2006). Gene expression in blood changes rapidly in neutrophils and monocytes after ischemic stroke in humans: A microarray study. *Journal of Cerebral Blood Flow and Metabolism 26*, 1089–1102.

Taylor, F., Ward, K., Moore, T.H., Burke, M., & Davey, S.G. (2013). Statins for the primary prevention of cardiovascular disease. *Cochrane Database of Systematic Reviews 1*, CD004816.

Tejima, E., Katayama, Y., Suzuki, Y., Kano, T., & Lo, E.H. (2001). Hemorrhagic transformation after fibrinolysis with tissue plasminogen activator: Evaluation of role of hypertension with rat thromboembolic stroke model. *Stroke 32*, 1336–1340.

Tokgoz, S., Kayrak, M., Akpinar, Z., Seyithanoğlu, A., Güney, F., & Yürüten, B. (2013). Neutrophil Lymphocyte Ratio as a Predictor of Stroke. *Journal of Stroke and Cerebrovascular Diseases 22*, 1169–1174.

Tong, L., Hu, H., Zhang, S., Yan, S., & Lou, M. (2015). Statin withdrawal beyond acute phase affected outcome of thrombolytic stroke patients: An observational retrospective study. *Medicine 94*, e779.

Tsivgoulis, G., Kadlecová, P., Kobayashi, A., Czlonkowska, A., Brozman, M., Švigelj, V., ... Mikulik, R. (2015). Safety of statin pretreatment in intravenous thrombolysis for acute ischemic stroke. *Stroke 46*, 2681–2684.

Tsivgoulis, G., Katsanos, A., Schellinger, P., Köhrmann, M., Varelas, P., Magoufis, G., ... Alexandrov, A. (2018). Successful reperfusion with intravenous thrombolysis preceding mechanical thrombectomy in large-vessel occlusions. *Stroke 49*, 232–235.

Tsuji, K., Aoki, T., Tejima, E., Arai, K., Lee, S., Atochin, D., ... Lo, E. (2005). Tissue plasminogen activator promotes matrix metalloproteinase-9 upregulation after focal cerebral ischemia. *Stroke 36*, 1954–1959.

Tsuruoka, A., Atsumi, C., Mizukami, H., Imai, T., Hagiwara, Y., & Hasegawa, Y. (2014). Effects of edaravone, a free radical scavenger, on circulating levels of MMP-9 and hemorrhagic transformation in patients with intravenous thrombolysis using low-dose alteplase. *Journal of Stroke and Cerebrovascular Diseases 23*, 2894–2899.

Tureyen, K., Kapadia, R., Bowen, K., Satriotomo, I., Liang, J., Feinstein, D., & Vemuganti, R. (2006). Peroxisome proliferator-activated receptor-gamma agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents. *Journal of Neurochemistry 101*, 41–56.

Turner, R., & Sharp, F. (2016). Implications of MMP9 for blood brain barrier disruption and hemorrhagic transformation following ischemic stroke. *Frontiers in Cellular Neuroscience 10*, 56.

Uhl, B., Zuchtriegel, G., Puhr-Westerheide, D., Praetner, M., Rehberg, M., Fabritius, M., ... Fürst, R. (2014). Tissue-type plasminogen activator promotes postischemic neutrophil recruitment via its proteolytic and nonproteolytic properties. *Arteriosclerosis, Thrombosis, and Vascular Biology 34*, 1495–1504.

Vallés, J., Lago, A., Santos, M., Latorre, A., Tembl, J., Salom, J., ... Moscardó, A. (2017). Neutrophil extracellular traps are increased in patients with acute ischemic stroke: Prognostic significance. *Thrombosis and Haemostasis 117*(10), 1919–1929.

Vogelgesang, A., Lange, C., Blümke, L., Laage, G., Rümpel, S., Langner, S., ... Ruhnau, J. (2017). Ischaemic stroke and the recanalization drug tissue plasminogen activator interfere with antibacterial phagocyte function. *Journal of Neuroinflammation 14*, 140.

Wang, C., Ding, X., Noor, R., Pegg, C., He, C., & Shuaib, A. (2009). Rosiglitazone alone or in combination with tissue plasminogen activator improves ischemic brain injury in an embolic model in rats. *Journal of Cerebral Blood Flow and Metabolism 29*, 1683–1694.

Wang, S., Lee, S., Guo, S., Montaner, J., Wang, X., & Lo, E. (2006). Reduction of tissue plasminogen activator-induced matrix metalloproteinase-9 by simvastatin in astrocytes. *Stroke 37*, 1910–1912.

Wang, W., Li, M., Chen, Q., & Wang, J. (2015). Hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke: Mechanisms, models, and biomarkers. *Molecular Neurobiology 52*, 1572–1579.

Wardlaw, J., Murray, V., Berge, E., & del Zoppo, G.J. (2014). Thrombolysis for acute ischaemic stroke. *Cochrane Database of Systematic Reviews 7*, CD000213.

Xing, Y., Guo, Z., Yan, S., Jin, H., Wang, S., & Yang, Y. (2014). Increased globulin and its association with hemorrhagic transformation in patients receiving intra-arterial thrombolysis therapy. *Neuroscience Bulletin 30*, 469–476.

Yaghi, S., Willey, J., Cucchiara, B., Goldstein, J., Gonzales, N., Khatri, P., ... American Heart Association Stroke Council; Council on Cardiovascular and Stroke Nursing; Council on Clinical Cardiology; and Council on Quality of Care and Outcomes Research (2017). Treatment and outcome of hemorrhagic transformation after intravenous alteplase in acute ischemic stroke: A scientific statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke 48*, e343–e361.

Yagi, K., Kitazato, K.T., Uno, M., Tada, Y., Kinouchi, T., Shimada, K., & Nagahiro, S. (2009). Edaravone, a free radical scavenger, inhibits MMP-9-related brain hemorrhage in rats treated with tissue plasminogen activator. *Stroke 40*, 626–631.

Yamashita, T., Kamiya, T., Deguchi, K., Inaba, T., Zhang, H., Shang, J., ... Abe, K. (2009). Dissociation and protection of the neurovascular unit after thrombolysis and reperfusion in ischemic rat brain. *Journal of Cerebral Blood Flow and Metabolism 29*, 715–725.

Zhang, F.H., Lin, Y.H., Huang, H.G., Sun, J.Z., Wen, S.Q., & Lou, M. (2013). Rosiglitazone attenuates hyperglycemia-enhanced hemorrhagic transformation after transient focal ischemia in rats. *Neuroscience 250*, 651–657.

Zhang, L., Chopp, M., Jia, L., Cui, Y., Lu, M., & Zhang, Z.G. (2009). Atorvastatin extends the therapeutic window for tPA to 6 h after the onset of embolic stroke in rats. *Journal of Cerebral Blood Flow and Metabolism 29*, 1816–1824.

Zhang, L., Zhang, Z., Ding, G., Jiang, Q., Liu, X., Meng, H., ... Chopp, M. (2005). Multitargeted effects of statin-enhanced thrombolytic therapy for stroke with recombinant human tissue-type plasminogen activator in the rat. *Circulation 112*, 3486–3494.

Zhang, L., Zhang, Z.G., & Chopp, M. (2012). The neurovascular unit and combination treatment strategies for stroke. *Trends in Pharmacological Sciences 33*, 415–422.

Zhang, R., Chopp, M., Chen, H., & Garcia, J. (1994). Temporal profile of ischemic tissue damage, neutrophil response, and vascular plugging following permanent and transient (2H) middle cerebral artery occlusion in the rat. *Journal of the Neurological Sciences 125*, 3–10.

Zhang, Y., Wang, Y., Zuo, Z., Wang, Z., Roy, J., Hou, Q., ... Wintermark, M. (2014). Effects of tissue plasminogen activator timing on blood-brain barrier permeability and hemorrhagic transformation in rats with transient ischemic stroke. *Journal of the Neurological Sciences 347*, 148–154.

Zhao, H., & Zhang, Y. (2014). The effects of previous statin treatment on plasma matrix metalloproteinase-9 level in Chinese stroke patients undergoing thrombolysis. *Journal of Stroke and Cerebrovascular Diseases 23*, 2788–2793.

Zhu, B., Pan, Y., Jing, J., Meng, X., Zhao, X., Liu, L., ... CHANCE Investigators (2018). Neutrophil counts, neutrophil ratio, and new stroke in minor ischemic stroke or TIA. *Neurology 90*, e1870–e1878.