

Annexin A1: The dawn of ischemic stroke (Review)

CHEN TANG^{1*}, RUI LAN^{2,3*}, DONG-RUI MA⁴, MIN ZHAO^{2,3}, YONG ZHANG⁵, HONG-YU LI¹,
SHUANG LIU¹, BO-YANG LI¹, JIE-LI YANG¹, HUI-JIE YANG¹ and ZHEN-QIANG ZHANG⁴

¹Department of Encephalopathy, The First Affiliated Hospital of Henan University of Chinese Medicine, The First Clinical Medical College of The First Affiliated Hospital of Henan University of Chinese Medicine, Zhengzhou, Henan 450000, P.R. China;

²Department of Encephalopathy, The First Affiliated Hospital of Henan University of Chinese Medicine, Zhengzhou, Henan 450000, P.R. China; ³Collaborative Innovation Center of Prevention and Treatment of Major Diseases by Chinese and Western Medicine, Zhengzhou, Henan 450000, P.R. China; ⁴Academy of Chinese Medical Sciences, Henan University of Chinese Medicine, Zhengzhou, Henan 450000, P.R. China; ⁵Department of Traditional Chinese Medicine, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450000, P.R. China

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Abstract. Ischemic stroke is a prevalent clinical condition that poses a significant global challenge. Developing innovative strategies to address this issue is crucial. Annexin A1 (ANXA1), a key member of the annexin superfamily, performs various functions, such as inhibiting inflammatory factor release, promoting phagocytosis, and blocking leukocyte migration. Evidence indicates that ANXA1 plays a pivotal role in the pathogenesis of ischemic stroke. The present article reviews involvement of ANXA1 in anti-atherosclerosis, inflammatory processes, blood-brain barrier protection, platelet aggregation and anti-apoptotic mechanisms. The potential applications of ANXA1 in treating ischemic stroke are also explored.

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Correspondence to: Professor Zhen-Qiang Zhang, Academy of Chinese Medical Sciences, Henan University of Chinese Medicine, 156 Jinshui East Road, Jinshui, Zhengzhou, Henan 450000, P.R. China

E-mail: zhang_zhenqiang@126.com

Dr Rui Lan, Department of Encephalopathy, The First Affiliated Hospital of Henan University of Chinese Medicine, 19 Renmin Road, Jinshui, Zhengzhou, Henan 450000, P.R. China

E-mail: lanrui2121@163.com

*Contributed equally

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1. Introduction

Ischemic stroke is a pathological condition characterized by impaired blood supply to the brain, leading to local ischemia and hypoxia in the brain tissue, resulting in neurological deficits (1). Despite significant advancements in research, treatment of ischemic stroke remains a global challenge. Over 13 million individuals worldwide suffer from a new stroke and 5.5 million succumb to stroke each year. Stroke is the second leading cause of death and disability globally, with an associated economic burden exceeding \$891 billion and continuing to increase annually (2,3). Ischemic stroke represents the most prevalent form of stroke, accounting for 65.3% of new stroke cases globally; it is associated with a significant economic burden and social impact on a global scale (4). The primary treatment for ischemic stroke is intravenous or mechanical thrombolysis, which rapidly restores blood flow to the affected brain area and reduces disability. However, numerous patients miss the therapeutic window, and some who receive treatment still experience infarcts (5). Therefore, new therapeutic approaches are needed in the current situation. Pathophysiological changes following ischemic stroke involve inflammation, abnormal activation of immune cells, ionic imbalance and blood-brain barrier (BBB) dysfunction. Although extensive researches have been conducted on neurological damage after ischemic stroke, the exact mechanism remains unclear (6,7).

Atherosclerosis is a major etiological factor in the pathogenesis of ischemic stroke, and aortic atherosclerotic cerebral infarction is the most prevalent form of this condition. It is characterized by the formation of plaques within the arterial wall, which can lead to narrowing or complete obstruction of the blood vessels, precipitating an ischemic event (8). Following the onset of cerebral ischemia, the inflammatory response can

be initiated and amplified rapidly, resulting in a substantial elevation of inflammatory cytokines and exacerbation of brain tissue damage, which may lead to various complications. The BBB is instrumental in sustaining homeostasis of the central nervous system (CNS). The robust inflammatory response that follows a stroke impairs the integrity of the BBB, worsens the clinical course and negatively affects prognosis (9).

Annexin-A1 (ANXA1) is an important member of the annexin superfamily, consisting of 346 amino acids. It is widely distributed and expressed in eosinophils, neutrophils, monocytes, lymphocytes and endothelial cells as well as in the heart, brain, kidney, lung, vascular tissues and other cells (10,11). ANXA1 exerts a wide range of effects, including inhibition of cytokine release, blocking leukocyte recruitment, stimulation of phagocytosis, promotion of apoptosis and reduction of vascular permeability (12). ANXA1 has gained increasing attention in recent years owing to its significant role in various diseases, including ischemic stroke (Fig. 1). The present review aimed to summarize the roles of ANXA1 in various aspects of ischemic stroke, provide new insights into the mechanism of nerve injury following ischemic stroke, and explore the potential of ANXA1 as a novel therapeutic target for ischemic stroke.

The structure and biological function of Annexins. When members of the annexin family were first discovered in the late 1970s and the early 1980s, they were assigned a multitude of disparate nomenclature, each reflecting the individual biochemical properties of the respective proteins. However, as techniques for protein sequence analysis, cDNA sequencing, and gene cloning have advanced, researchers have recognized that these proteins share key biochemical properties, as well as gene structure and sequence features. To unify the terminology and resolve the confusion surrounding the naming of these proteins, the term ‘annexin’ was introduced (13). The term ‘annexin’ H was derived from the Greek word ‘annex’, meaning ‘union’ or ‘binding’, this nomenclature encapsulates the fundamental attribute of the Annexin family, namely their universal capacity to interact with biological membranes. Moreover, this designation reflects the collective objective of pioneering researchers who independently investigated these proteins, seeking a scaffolding protein capable of acting as a conduit between cellular structures.

Annexins are calcium-dependent phospholipid-binding proteins found in a wide variety of tissues and cells in eukaryotes. It is a large family comprising of 13 proteins with similar structural features (14,15). They are characterized by one or two homologous carboxyl-terminal ‘cores’, each consisting of four sequence repeats involved in membrane binding. Consequently, annexins are mainly differentiated by their relatively short non-homologous amino-terminal sequence (16). Members of the annexin superfamily are involved in a wide range of cellular activities, including cell division, apoptosis, vesicular transport, calcium signaling and growth regulation, contributing to overall cell functioning (13).

Annexins can be classified into five categories A-E, based on their molecular structure, evolutionary relationships and chromosomal localization. Annexin A is expressed in vertebrate cells, annexin B in invertebrate cells, annexin C in mononuclear eukaryotes and fungi, annexin D in plants, and

annexin E in prokaryotic cells (17). The annexin A subfamily comprises 12 members, specifically annexins A1-A11 and A13 (18). Notably, although typically numbered sequentially, annexin A12 is absent from the annexin A family, potentially due to gene loss or other evolutionary processes. Such discrepancies in gene nomenclature are not uncommon in biological classifications.

From a molecular perspective, members of the annexin A family consist of two main structural domains; a highly conserved C-terminal domain and a relatively variable N-terminal domain. The C-terminal domain, which serves as the central backbone of the proteins, contains four annexin repeat units (eight in ANXA6) that are tightly stacked through hydrophobic interactions, forming the characteristic ‘type 2’ calcium binding site that stabilize the overall protein structure (19). By contrast, the N-terminal domain, also known as the tail structural domain, exhibits significant variability within the annexin A family of proteins. This domain contains several post-translational modification sites unique to each family member, conferring a high degree of specificity in protein-protein interactions and binding to a wide range of ligands (20) (Fig. 2). As a result, the N-terminal domains of each member not only differ in spatial location but also exhibit specificity in biological function, enabling members of the annexin A family to play key roles in a wide variety of biological processes.

Evidence suggests that Annexins are strongly associated with various human diseases, including cardiovascular diseases, cerebrovascular diseases and cancer (21). ANXA1, a pivotal member of the annexin family of proteins, has been the focus of extensive researches and has been shown to exert regulatory effects on numerous biological processes, particularly those related to cerebral ischemia-reperfusion events (22). Formyl peptide receptors (FPRs), a family of G-protein coupled receptors including FPR1, FPR2, and FPR3, are the primary pathways through which ANXA1 exerts its biological effects (23). ANXA1 has been shown to exert reparative and regenerative effects on the nervous system via FPR2. Although the evidence is still limited, ANXA1 also displays a protective function in nerve conduction structures under both physiological and pathological conditions (24).

2. ANXA1 and ischemic stroke

ANXA1 exerts anti-arteriosclerosis effects. Atherosclerosis is a chronic inflammatory disease that occurs within blood vessel walls and progresses through various pathological stages. It is mainly characterized by the formation of fatty plaques on the walls of large and medium-sized arteries, particularly in regions with disturbed blood flow (25). At the site of the lesion, monocytes differentiate into macrophages when they phagocytose lipoproteins, transforming into foam cells and forming ‘fatty streaks’. In advanced atherosclerosis, defective clearance of apoptotic cells, excessive lipid loading, and accumulation of cholesterol crystals contribute to macrophage apoptosis and formation of a necrotic core. Infiltrating macrophages trigger an inflammatory response, leading to apoptosis of vascular smooth muscle cells, thinning of the fibrous cap, and degradation of the extracellular matrix. These processes

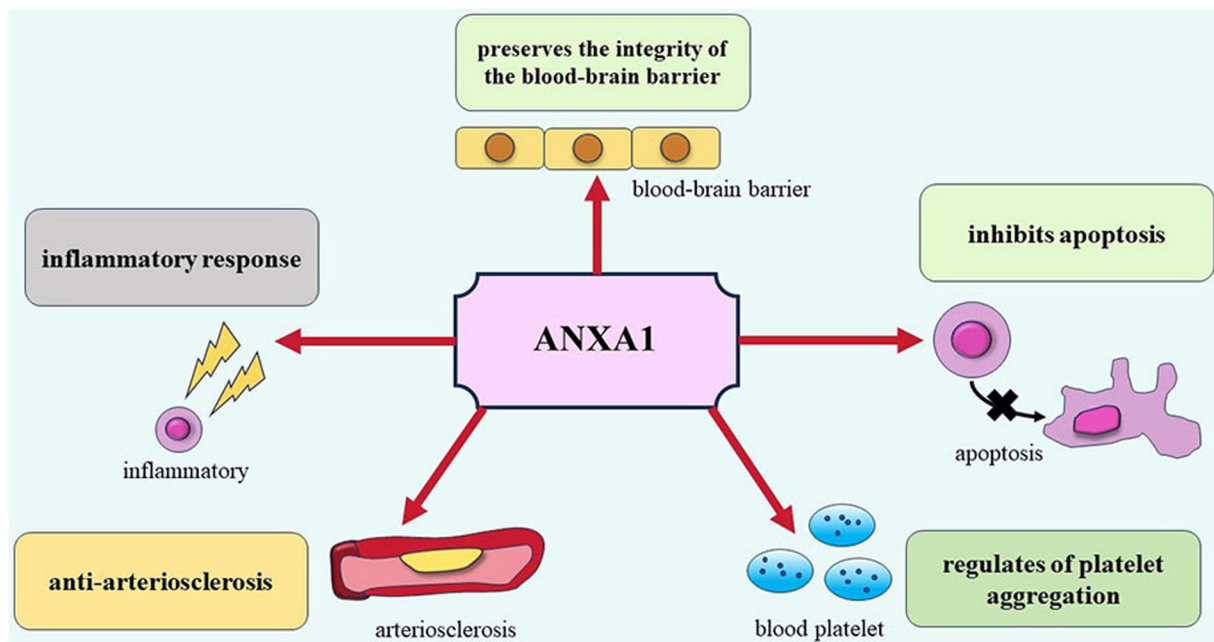


Figure 1. Mechanisms of ANXA1 in the treatment of ischemic stroke. ANXA1, Annexin A1.

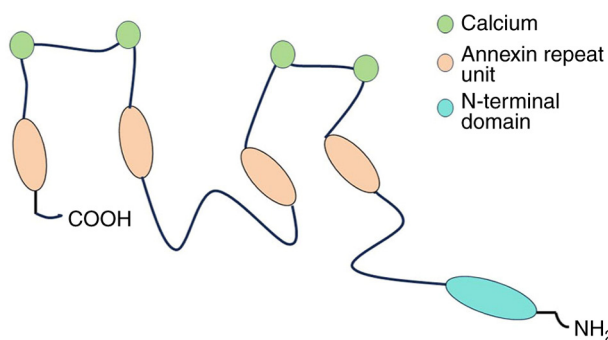


Figure 2. Structure of the Annexin A family.

ultimately destabilize the plaque and increase the risk of acute cerebrovascular events (26).

It has been revealed that ANXA1 expression is elevated in atherosclerotic plaques, which contain numerous apoptotic cells. Studies have shown that impaired clearance of apoptotic cells exacerbates the development of atherosclerosis, in which ANXA1 secreted by apoptotic cells binds to the FRP1 receptor on antigen-presenting cells (APCs) and helps APCs to invade dead cells, thereby promoting apoptotic cell clearance. ANXA1 may play a role in promoting apoptosis of inflammatory cells and influencing macrophage burial (27). Furthermore, a study analyzing 34 patients with carotid atherosclerosis found that ANXA1 is expressed in all plaques. Notably, ANXA1 expression in carotid plaques is significantly higher in asymptomatic patients than in those with neurological symptoms, suggesting that elevated ANXA1 expression levels may have a stabilizing effect on asymptomatic carotid plaques (28). In order to investigate the pharmacological effects of human recombinant ANXA1 (hrANXA1) on the formation and progression of atherosclerosis, Kusters *et al* (29) used a low-density lipoprotein gene-deficient (LDLR^{-/-}) mouse model and fed the mice a

Western Type Diet to induce atherosclerosis. The observation group was administered intraperitoneal injections of hrANXA1 protein at a dose of 1 mg/kg three times per week for six weeks. The control group was administered an equal volume of PBS. Subsequently, the total plaque area was quantified in the aortic arch and major arterial branches. The progression of arterial plaques was evaluated by immunohistochemistry, and the results revealed that in mice with pre-existing plaques, the observation group exhibited a reduction in plaque area of ~50% and a reduction in necrotic core volume of 76% in the observation group. The study demonstrated that hrANXA1 had a similar plaque-stabilizing effect, significantly reducing the progression of lesions to unstable plaques. Additionally, hrANXA1 can act as a bridging molecule between apoptotic cells and macrophages, contributing to increased phagocytosis by macrophages and a reduction in inflammatory factors (29). Macrophages play a multifaceted role in the inhibition of inflammation and clearance of cellular debris and apoptotic cells, and are instrumental in the onset, progression and regression of atherosclerosis (30). The aforementioned studies also indicated that the plaque-stabilizing effect of ANXA1 may be linked to macrophages.

Altered lipid metabolism is a key risk factor and characteristic manifestation of atherosclerosis. Several studies have indicated a correlation between ANXA1 and lipid metabolism (31-33). The aim of this study was to investigate the effects of ANXA1 deficiency on obesity and metabolism by establishing a diet-induced mouse obesity model by feeding a high-fat diet to ANXA1 knockout mice and wild-type mice (31). This was performed to investigate the effects of ANXA1 deficiency on obesity and metabolism. It was observed that the expression of key enzymes and proteins associated with adipose tissue lipolysis, including adipose triglyceride lipase, hormone-sensitive lipase and galectin-12, was significantly upregulated in wild-type mice. However, this was not observed in ANXA1-knockout mice, indicating

that ANXA1 deficiency may inhibit lipolytic processes and contribute to the development of obesity. Furthermore, plasma corticosterone levels were found to be significantly elevated in ANXA1 knockout mice, suggesting that ANXA1 deficiency may also influence the hypothalamic-pituitary-adrenal axis, thereby promoting the development of obesity (31). Another study also demonstrated an increase in ANXA1 expression in the subcutaneous fat of both young and elderly overweight patients (32). Similarly, a study conducted in Spain found higher levels of ANXA1 expression in obese children than in those of normal weight. Interestingly, despite increased expression, plasma levels of ANXA1 were negatively correlated with adipose markers and positively correlated with HDL cholesterol levels (33).

The relationship between diabetes and atherosclerosis is similarly robust, with multiple pathological pathways interlinking these two conditions. Evidence has demonstrated that patients with diabetes have a markedly elevated risk of atherosclerosis (34). ANXA1 is inextricably linked to blood glucose regulation. Additionally, Purvis *et al* (35) constructed an obese and insulin-resistant mouse model. They observed that ANXA1-knockout mice exhibited elevated blood glucose levels, impaired glucose tolerance and more pronounced insulin resistance. Following the administration of hrANXA1 via intraperitoneal injection for a period of six weeks, a notable reduction in both blood glucose levels and the rate of weight gain was observed. And the results showed the administration of hrANXA1 reduced blood glucose levels which may be related to the attenuation of the phosphorylation level at the Ser307 site on insulin receptor substrate-1. These findings indicate that ANXA1 has the potential to significantly reduce insulin resistance (35). Indeed, a considerable number of pharmaceutical agents employed in the treatment of diabetes are known to exert their effects by inhibiting of the Ras homolog gene family member A (GTPase RhoA). This includes metformin. Furthermore, evidence indicates that fasudil influences glucose metabolism by inhibiting the GTPase RhoA (36-38). It is of particular interest to note that RhoA activation is inhibited by ANXA1 (35), which provides further evidence of a close link between ANXA1 and glucose regulation.

Moreover, ANXA1 has significant effect on atherosclerosis. Activation of the lipoxin A4 receptor (ALX) serves as an endogenous anti-inflammatory effector, and FPR2 is a key signaling molecule that controls the cellular inflammatory response. Glucocorticoids have been shown to induce the release of ANXA1 in macrophages and neutrophils, activating ALX/FPR2 to exert anti-inflammatory effects (39). There is a robust correlation between the levels of cellular inflammatory factors and severity of atherosclerosis (40,41). This suggests that ANXA1 may positively affect atherosclerosis through its anti-inflammatory action. Additionally, Al-Kuraishy *et al* (42) discovered that ANXA1 inhibits integrin activation and myeloid cell accumulation in the arterial wall, reduces necrotic areas in the center of plaques, induces neutrophil apoptosis, reduces the risk of plaque exposure to deleterious neutrophilic intracellular contents, reduces the expression of endothelial cell adhesion molecules, and attenuates inflammatory responses, thereby reducing the incidence of atherosclerosis. The inflammatory response to arterial injury accelerates the growth of

neointima and can lead to restenosis of blood vessels. In the ANXA1-knockout mouse model, the accumulation of proliferating macrophages in the injured tissue exacerbates the growth of neointima, and then promotes the occurrence of vascular stenosis. de Jong *et al* (43) found that the levels of ANXA1 in plasma and at the lesion were negatively correlated with the size of neointima. Additionally, ANXA1 can inhibit the proliferation of macrophages by suppressing the production and release of macrophage colony-stimulating factor, thereby preventing the occurrence of arterial restenosis (43). AnxA1_{Ac2-26}, the mimetic peptide of ANXA1, significantly improves vascular remodeling parameters including reducing the thickness of the arterial intima and media, decreasing collagen deposition, and repairing elastic fiber breaks in ANXA1-deficient mouse models. ANXA1 notably slowed down the aging process of endothelial cells by inhibiting the expression of pro-senescence related molecules such as p53 and p21, and senescence-associated secretory phenotype factors tumour necrosis factor α (TNF- α) and interleukin-6 (IL-6). At the same time, it restores the proliferative and migratory abilities of endothelial cells by reducing DNA damage markers such as γ -H2AX, thereby promoting endothelial repair (44). The evidence indicates that ANXA1 may play a protective role in the repair of arterial injury and the prevention of restenosis.

ANXA1 plays a role in the inflammatory response. After the onset of ischemic stroke, the primary treatment goal is to restore blood flow to restore glucose and oxygen delivery to the ischemic brain tissue as quickly as possible. However, reperfusion exposes the infarct area to peripheral immune cells, which leads to immune activation and subsequent inflammatory damage. In addition, post-infarction, dead and dying cells stimulate the production of various inflammatory factors and chemokines, including CCR2, CCR5, CCR6, CXCL8 and CXCR2, further exacerbating the inflammatory response (45). Elevated levels of these inflammatory factors increase cerebral infarct areas, raise early mortality rates, and worsen patient prognosis (46).

Liu *et al* (47) investigated the effects of chloral hydrate on stroke in mice. They observed a significant increase in ANXA1 expression in chloral hydrate-treated mice during the acute phase of ischemic stroke, which was accompanied by a reduction in inflammatory factors such as TNF- α , IL-6 and interleukin-1 β (IL-1 β), effectively attenuating edema and damage to brain tissue following ischemia. These results were further verified using an ANXA1 inhibitor, confirming that ANXA1 plays a neuroprotective role by reducing the expression of inflammatory factors (47). The aforementioned finding is also consistent with another study suggesting that ANXA1 may play a pivotal role in the post-stroke inflammatory response (48).

Microglia, key regulators of the inflammatory response within the CNS, are activated by environmental stimuli following ischemic stroke. This activation leads to microglial polarization and shifts to different phenotypes, typically classified as pro-inflammatory or anti-inflammatory microglia (49,50). Proinflammatory microglia secrete a variety of inflammatory factors, including IL-1 β , IL-6, TNF- α and inducible nitric oxide synthase, which exacerbate the inflammatory response and accelerate the pathological progression

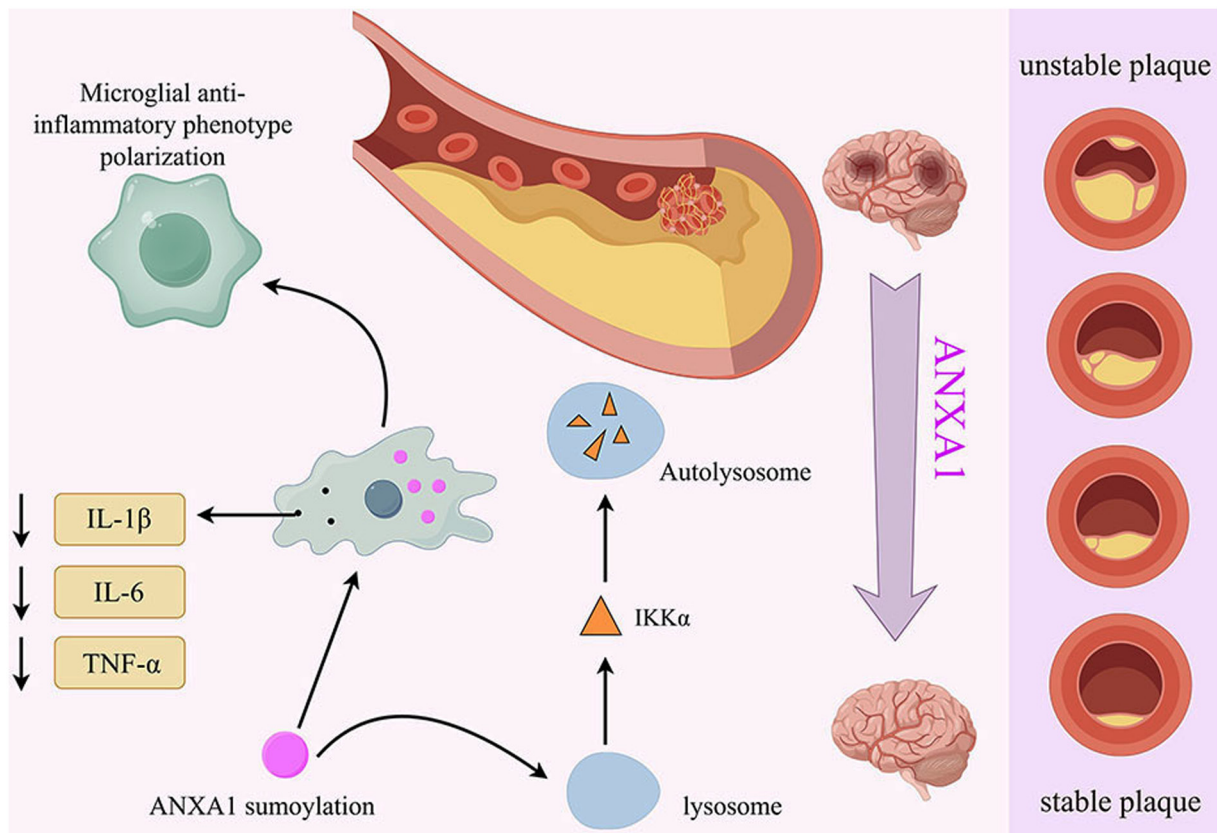


Figure 3. ANXA1 has been demonstrated to exert a stabilizing effect on arterial plaque. Furthermore, ANXA1 SUMOylation has been shown to promote the shift of microglia to an anti-inflammatory phenotype and to mediate $\text{IKK}\alpha$ degradation. ANXA1, Annexin A1; $\text{IKK}\alpha$, $\text{I}\kappa\text{B}$ kinase α ; IL, interleukin; TNF, tumor necrosis factor.

of cerebral ischemia. By contrast, anti-inflammatory microglia help to reverse the damage caused by cerebral ischemia by secreting relevant anti-inflammatory mediators (51).

ANXA1 is abundant in microglia and its biological function is tightly regulated by post-translational modifications (52), including SUMOylation a modification involving small ubiquitin-like modifier (SUMO) proteins. SUMOylated ANXA1 has been shown to effectively induce oxygen-glucose deprivation and reoxygenation-injured microglia to polarize towards an anti-inflammatory phenotype, thereby attenuating inflammatory stimuli and exerting neuroprotective effects (51,53). One research team discovered that Tat-Nuclear Translocation Signal (Tat-NTS), a peptide that increases ANXA1 SUMOylation, promotes microglia to adopt an anti-inflammatory phenotype. This process also selectively degrades $\text{I}\kappa\text{B}$ kinase α ($\text{IKK}\alpha$) through autophagy, blocking the activation of the NF- κB pathway triggered by cerebral ischemia-reperfusion injury. As a result, apoptosis of ischemic neurons was reduced, and neurological function was improved in experimental mice (54) (Fig. 3). Furthermore, another experimental study indicated that ANXA1 promotes the conversion of microglia to an anti-inflammatory phenotype and induces microglial migration to protect neurons from ischemic injury. This process is closely linked to the release of ATP and glutamate from injured neurons (55). Previous evidence also demonstrated a significant protective effect of ANXA1 against cellular edema and glutamate overload in the ischemic environment (56).

Leukocytosis is an important marker of the inflammatory response after stroke, in which neutrophils undergo conformational changes and migrate through the endothelium of the vessel wall. They are then attracted to ischemic tissue by chemokines, releasing pro-inflammatory factors, matrix metalloproteinases (MMPs), reactive oxygen species, and other signals that cause secondary damage, leading to altered BBB permeability and post-ischemic edema (57,58). ANXA1 can limit neutrophil recruitment and migration, inhibit the production of proinflammatory factors, and promote the clearance of apoptotic neutrophils, thereby reducing the inflammatory response (59). In an experiment, researchers observed cerebral microcirculation in mice with cerebral infarction and found that ANXA1-deficient mice had more leukocyte adherence in the small veins of the brain, larger infarcts, and worse neurological scores than the control group. The inflammatory indices significantly decreased after administration of an ANXA1 peptidomimetic, confirming that the anti-inflammatory circuits centered on ANXA1 have neuroprotective effects (60).

In addition to neutrophils, platelets also play a crucial role in the inflammatory response after stroke. Platelets are involved in several processes, including thrombosis and inflammation. The inflammatory response after ischemia-reperfusion leads to microvascular dysfunction and platelet adhesion to blood vessels, thereby increasing the risk of recurrent cerebrovascular events (61). Administration of ANXA1 via intravenous injection significantly increased blood flow in the cerebral arteries and veins, altered the thrombotic-inflammatory

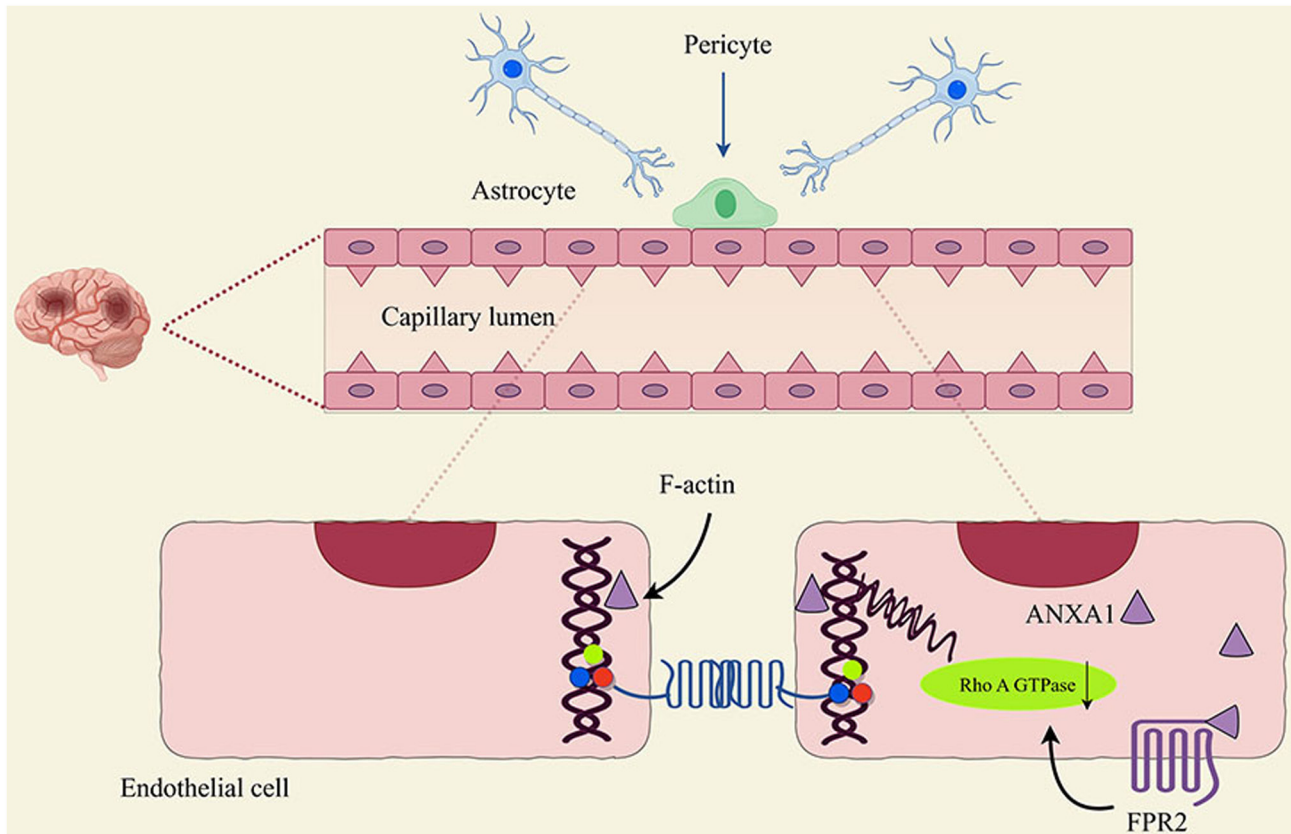


Figure 4. Two mechanisms through which ANXA1 regulates paracellular permeability of the blood-brain barrier have been identified. ANXA1, Annexin A1; FPR, formyl peptide receptor.

environment, and prevented thrombotic events after cerebral ischemia-reperfusion (62). Further findings revealed that ANXA1 inhibits thrombin-induced activation of signaling events and integrins, thereby reducing platelet aggregation and preventing thrombosis (62). It has been reported that ANXA1 is associated with cellular senescence and multiple inflammatory pathways, including the chemokine, NF- κ B and TNF signaling pathways. ANXA1 may prevent vascular aging by inhibiting inflammatory responses (44).

To date, substantial evidence has shown that ANXA1 can exert anti-inflammatory effects. However, it is noteworthy that ANXA1 has pro-inflammatory functions. The N-terminal structural domain of ANXA1 mediates anti-inflammatory effects, including the inhibition of leukocyte migration, whereas the core region of ANXA1 has been shown to promote endothelial cell aggregation and migration, resulting in pro-inflammatory effects. This suggests that ANXA1 contains two oppositely acting fragments (11). However, research on its pro-inflammatory effects in ischemic stroke is limited. Further studies are needed to explore its potential clinical applications, as the anti-inflammatory capacity and neuroprotective effects of ANXA1 could provide an effective therapeutic strategy for stroke.

ANXA1 preserves the integrity of BBB. The BBB is a dynamic component of cerebral blood vessels that plays a crucial role in regulating the permeation of solutes into the brain tissue and maintaining brain homeostasis (63). The cerebrovascular structure is unique in that there are very dense tight junctions

between adjacent endothelial cells, and the integrity of this structure is essential for maintaining the BBB function (64). In the pathological processes of numerous neurological diseases, destruction of the BBB leads to disease progression (65). After the onset of cerebral ischemia, BBB disruption occurs, leading to increased vascular permeability and leukocyte infiltration, resulting in cerebral edema (66). Simultaneously, BBB disruption promotes the occurrence of secondary brain injury and aggravates the incidence of post-stroke hemorrhagic transformation, which seriously affects the prognosis of patients (67).

ANXA1 functions as an anti-inflammatory messenger for glucocorticoids and is expressed in a wide range of cells within the brain, particularly in the endothelial cells of the cerebral microvascular system and at tight junctions in areas of intercellular contact (68). Glucocorticoids have been shown to upregulate ANXA1 expression and increase BBB tightness in the brain (69,70). Thus, ANXA1 may play a vital role in regulating BBB permeability. Cristante *et al* (71) found that ANXA1 co-regulates paracellular permeability of the BBB in two ways through interactions with the actin cytoskeleton and paracrine downregulation of RhoA GTPase activity by FPR2 (71) (Fig. 4), which is tightly correlated with maintaining the integrity and normal function of the BBB. Another study has found that ANXA1 maintains tight junctions between endothelial cells in BBB, prevents lipopolysaccharide (LPS) permeation through the BBB, and limits peripheral effects on the brain in pathological conditions (72). Further evidence has emerged to substantiate the protective effects of ANXA1

against ischemic stroke and other neurovascular diseases. While numerous studies have focused on the regulation of inflammation, there is evidence indicating its role in the cerebral vasculature (71). Other researchers administered hrANXA1 to mice with brain injuries via intravenous injection and evaluated the extent of brain edema, presence of neurological deficits and integrity of the BBB. The results demonstrated that in mice treated with hrANXA1, Evans blue staining extravasation and immunoglobulin G extravasation in the damaged cerebral hemispheres were markedly diminished, neutrophil infiltration and inflammatory factor levels were significantly attenuated, and cerebral edema and BBB disruption were significantly reduced (73). In animals lacking ANXA1 receptors, more severe BBB leakage has been observed after the onset of ischemia (60). One study found that hrANXA1 administration restored BBB function, which was associated with increased expression of tight junction proteins including occludin and claudin-5, decreased activity of MMPs, increased levels of MMP inhibitors and stabilization of F-actin (74). There is a clear sex difference in the incidence of neurovascular diseases including stroke, with women tending to have a lower incidence than men (75). Although numerous factors contribute to neuroprotection, estrogen plays a dominant role (76). Estrogen appears to play a role in protecting against oxidative stress. There is evidence that estrogen exerts specific protective effects on the BBB by regulating the expression of inter-endothelial tight and adhesion junction proteins. Furthermore, estrogen reduces the expression of adhesion molecules on the luminal surface of the endothelium and prevents leukocyte adhesion and migration during inflammatory response. Of particular interest is the fact that both the important effects of estrogen appear to be mediated by ANXA1 (77).

ANXA1 regulates of platelet aggregation. Platelets prevent bleeding after vascular injury and play a central role in maintaining hemostasis. However, abnormal platelet activation can lead to intravascular thrombosis, resulting in obstruction of blood flow and then triggering cardiovascular and cerebrovascular events in various pathological conditions, such as atherosclerotic plaque rupture (78). A previous study investigated the effects of AnxA1_{Ac2-26}, a mimetic peptide of ANXA1, on ischemia and reperfusion in mice. The results demonstrated that the adhesion of platelets to the endothelium increased in the ANXA1 knockout group at specific time points, namely 4 and 24 h after the onset of ischemia and reperfusion (79). In addition, the study also revealed that platelet aggregation was markedly attenuated in mice treated with ANXA1 via intravenous injection, accompanied by a significant increase in blood flow within the small cerebral arteries and veins. These findings indicate that AnxA1_{Ac2-26} reduces the bleeding time, regulates platelet aggregation, and mitigates the risk of thrombosis. Furthermore, the study found that the mechanism of thrombosis inhibition by AnxA1_{Ac2-26} was closely related to the regulation of the glycoprotein VI (GPVI) signaling pathway, reduction of α IIB β 3 activation and expression of P-selectin (79). Arachidonic acid (AA) and eicosanoids promote platelet aggregation and vasoconstriction. Macrophages deficient in ANXA1 have been shown to increase the production of AA and eicosanoids. Additionally,

a deficiency in ANXA1 results in a hypersensitive cellular response to LPS (endotoxin), which in turn promotes the activation of inflammatory vesicles, NLRP3, and increases the risk of thrombosis (80). These novel findings demonstrate that ANXA1 may improve cerebral ischemia-reperfusion injury by regulating platelet function suggesting that ANXA1 has great potential as a therapeutic agent for thrombophilia, opening a new avenue for the treatment of cerebral infarction.

ANXA1 inhibits apoptosis. Apoptosis is one of the major pathways that leads to cell death and plays a key role in ischemic brain injury. The high metabolic rate of neurons makes them vulnerable to injury. After the onset of cerebral ischemia-reperfusion, endogenous or exogenous apoptotic pathways are triggered, leading to neurons undergoing apoptosis in the ischemic penumbra or peri-infarct zone within hours or days (81). It can be reasonably deduced that targeted inhibition of pro-apoptotic factors would be an efficacious therapeutic strategy for ischemic stroke. Indeed, some researchers have already identified that acute ischemic stroke (AIS) promotes the translocation of ANXA1 from the neuronal cytoplasm to the nucleus, which in turn activates the neuronal apoptotic pathway and ultimately leads to cell death (82,83). This indicates that nuclear translocation of ANXA1 participate in neuronal apoptosis following AIS, which is of particular importance in the context of neurological injuries, and it has been shown that S100A11 can bind to ANXA1, inhibit nuclear translocation of ANXA1 and reduce apoptosis after ischemic stroke (84). Furthermore, evidence indicates that Sentrin/SUMO-specific protease 6 (SEN6) is tightened by the nuclear translocation of ANXA1 and the activation of p53-dependent apoptotic pathways, including increased BH3 interacting domain death agonist expression (Bid) and the activated caspase-3 pathway. Silencing SEN6 expression resulted in the inhibition of ANXA1 nuclear translocation, reduction in neuronal apoptosis, and improvement in neurological function following cerebral ischemia/reperfusion in mice (85). Another study established a cerebral ischemia-reperfusion injury induced by 60 min of middle cerebral artery occlusion in mice. The animals were then injected with a cell-penetrating peptide, fluorescein isothiocyanate (FITC)-Tat-NTS, labelled with FITC, into the unilateral lateral ventricle. The data showed that administration of Tat-NTS resulted in a significant reduction in ANXA1 levels within the nucleus accumbens, accompanied by inhibition of ANXA1 nuclear translocation (86). Further studies have demonstrated that the level of the pro-apoptotic protein Bid was significantly diminished in mice treated with the Tat-NTS peptide 24 h after reperfusion (86). Another study demonstrated that ANXA1 in mouse microglia was localized in the cytoplasm and exhibited a uniform distribution in the physiological state (54). Furthermore, ANXA1 expression was increased and shifted to the nucleus after MCAO. After Tat-NTS treatment, the nuclear translocation of ANXA1 was downregulated and promoted the conversion of microglia to an anti-inflammatory phenotype, thereby reducing the area of cerebral infarction areas in mice (54). These findings suggest that the Tat-NTS peptide may exert a profound neuroprotective effect by inhibiting ANXA1 nuclear translocation and reducing neuronal apoptosis.

3. Advances in the development of activators and molecular drugs

The development of ANXA1 activators and small-molecule drugs has made significant progress in recent years and has demonstrated considerable clinical potential, particularly in the fields of anti-inflammatory and neuroprotective therapies. Researchers have concentrated their efforts on the creation of peptide and non-peptide small-molecule drugs that imitate the biological activity of ANXA1, with the objective of treating cerebral ischemia, including Tat-NTS peptide, AnxA1_{Ac2-26} and hrANXA1, among others (73,79).

AnxA1_{Ac2-26}. AnxA1_{Ac2-26}, as an ANXA1 mimetic peptide, was mediated by the key resolution receptor, FPR 2/ALX. It has been demonstrated that AnxA1_{Ac2-26} administration could inhibit inflammation-induced microvascular thrombosis, and decrease platelet stimulation and aggregation by regulating α Ib β 3 and P-selectin in the cerebral microvasculature (79). Moreover, AnxA1_{Ac2-26} treatment reduced leukocyte adhesion and leukocyte-endothelial interactions in mice subjected to bilateral common carotid artery occlusion (87). Furthermore, a recent study has showed that AnxA1_{Ac2-26} administration downregulates cerebral thrombotic responses and mediates protein kinase B (Akt) and extracellular signal-regulated kinases (ERK1/2) to activate sickle cell disease neutrophils and enable resolution (88). At the start of reperfusion, Ac2-26 administration shifts microglia/macrophage polarization toward anti-inflammatory M2 phenotype, and improved cerebral ischemia-reperfusion injury in the *in vivo* and *in vitro* experiments through activating the AMPK-mTOR pathway by binding the FPR2/ALX (89).

Tat-NTS peptide. Tat-NTS peptide has previously been reported as a novel cell-penetrating peptide developed to prevent nuclear translocation of ANXA1. Tat-NTS peptide improves neuronal survival by reducing the transcriptional activity of p53 and activation of caspase-3 apoptosis pathway, downregulating expression of Bid following oxygen-glucose deprivation and reperfusion. In addition, Tat-NTS peptide administration significantly reduces infarct volume and improves neurological function after local brain ischemia (86). Interestingly, the latest study has demonstrated Tat-NTS regulates microglia ANXA1 function, and exerts neuroprotective effects following cerebral ischemia (54).

hrANXA1. Up to now, the studies on rANXA1 have mainly focused on traumatic brain injury and inflammatory brain diseases, but not been applied in cerebral ischemia related studies. The current studies have found that rANXA1 treatment notably protects neurons against brain damages induced by controlled cortical impact by alleviating BBB protection, reducing degradation of endothelial junction proteins and inhibiting inflammatory response through RhoA inhibition (73). Moreover, other evidence has supported that rANXA1 reduces neuro-inflammation by inhibiting the action of LPS-induced translocator protein-18KDa, which may be tightly related with the mechanisms of ANXA1 on inflammatory brain diseases (90). The aforementioned findings focus on clinical prospects, safety and efficacy of ANXA1, and provide

conceptual evidence that targeting ANXA1 may be a new therapeutic strategy for cerebral ischemia.

4. Conclusions and clinical prospects

Researches on Annexin are gradually increasing, and available evidence indicates that ANXA1 plays a multifaceted role in the development of ischemic stroke. This role encompasses anti-atherosclerotic effects, participation in inflammation, protection of the BBB, regulation of platelet aggregation and resistance to apoptosis.

So far, no relevant studies on ANXA1 in the treatment of cerebral ischemia have been found. However, ANXA1-related studies in stroke patients can be retrieved, which has great implications for clinical research and treatment. One study detects the protein and gene changes of the infarct core region of human brain and the corresponding contralateral brain region from the patients with ischemic stroke. The results indicated that ANXA1s, as one of the most influential molecules, are significantly dysregulated after stroke (91). Another clinical study revealed that significantly lower plasma levels of ANXA1 was detected in the patients with AIS during the onset stage compared with that in healthy controls (89). Decreased ANXA1 levels are recovered on 2-3 days post successful recanalization by endovascular thrombectomy. Furthermore, clinical evidence has demonstrated a negative correlation between ANXA1 levels and the Modified Rankin Scale score in patients three months postoperatively. These results showed that ANXA1 levels are positively correlated with clinical outcomes, indicating that plasma ANXA1 may be a promising biomarker to predict favorable prognosis of patients with AIS (89). Despite extensive studies on ANXA1, it is regrettable that its exploration has mainly focused on basic experiments. To date, there is still a lack of large-scale case-control trials for clinical application. In the field of ischemic stroke, studies related to ANXA1 have remained at the laboratory stage, and only a small number have been applied in clinical practice. Further studies are required to ascertain the safety of the clinical application of ANXA1 and its effect on patient prognosis. The present review summarizes the main mechanisms of ANXA1 in the treatment of ischemic stroke and highlights its potential as a novel therapeutic target and biomarker for stroke in the future. As research progresses, promising therapeutic strategies for ANXA1 may become available in the future.

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Authors' contributions

CT, RL and ZZ conceived the subject of the review, wrote and edited the original draft. DM reviewed and polished the manuscript. MZ reviewed the manuscript and made revisions regarding the intellectual content. YZ and HL wrote the original draft. SL and JY conducted a formal literature search and analyses. BL and HY completed the production of illustrations and made revisions to the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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