Brain, Behavior, & Immunity - Health 40 (2024) 100837

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

# Molecular mechanisms and therapeutic strategies for ferroptosis and cuproptosis in ischemic stroke

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ARTICLE INFO

*Keywords:*
Ferroptosis, Cuproptosis
Ischemic stroke
Mechanism

ABSTRACT

Ischemic stroke, as one of the most severe and prevalent neurological disorders, poses a significant threat to the health and quality of life of affected individuals. Stemming from the obstruction of blood flow, ischemic stroke, leads to cerebral tissue hypoxia and ischemia, instigating a cascade of pathophysiological changes that markedly exacerbate neuronal damage and may even culminate in cell death. In recent years, emerging research has increasingly focused on novel cell death mechanisms such as ferroptosis and cuproptosis. Mounting evidence underscores the independent roles of ferroptosis and cuproptosis in ischemic stroke. This review aims to elucidate potential cross-regulatory mechanisms between ferroptosis and cuproptosis, exploring their regulatory roles in ischemic stroke. The objective is to provide targeted therapeutic intervention strategies.

## 1. Introduction

Stroke is an acute cerebrovascular event caused by various factors that lead to the narrowing or blockage of cerebral blood vessels, or non-traumatic bleeding within the brain tissue, accompanied by clinical symptoms and signs (Murphy et al., 2020). Globally, stroke is the leading cause of adult disability and the second leading cause of death in middle-to high-income countries. Over the past decade, the incidence of ischemic and hemorrhagic strokes in these countries has increased to 85–94 cases per 100,000 individuals, with those aged 75 and above experiencing higher rates (1151–1216 cases per 100,000 individuals) (Murphy et al., 2020; Morotti et al., 2019). Additionally, 85% of stroke-related deaths and 87% of disability-adjusted life years lost due to stroke occur in low-income countries. In China, about 1.5 million new stroke cases are reported each year, with 70%–80% of survivors experiencing disabilities that hinder independent living (Lanas et al., 2021). Stroke can be categorized into ischemic and hemorrhagic types, with significant differences in their incidence rates. Ischemic strokes account for 62.4% of cases, while hemorrhagic strokes make up 27.9% (Montaño et al., 2021). Ischemic stroke, caused by blocked cerebral blood flow and resulting in hypoxic-ischemic damage to neural tissues, comprises about 71% of the global stroke burden (Qin et al., 2022). Hemorrhagic stroke involves bleeding within the brain tissue due to ruptured cerebral blood vessels.

Ischemic stroke, a critical and urgent medical condition, has received significant attention in the medical field (Patil et al., 2022). It results from insufficient blood supply to the brain, leading to neuronal hypoxia-ischemia, disrupted energy metabolism, and cell death. Research into neuronal cell death mechanisms has revealed that traditional modes of cell death, such as apoptosis, necrosis, pyroptosis, and apoptotic necrosis, do not fully explain the complex cellular outcomes in ischemic stroke (Zhang et al., 2022a; Khoshnam et al., 2017). Traditionally, cell death was categorized into apoptosis, pyroptosis, and necrosis. However, in ischemic stroke, cell death mechanisms are more complex and diverse (Krysko et al., 2008). Recently, ferroptosis and cuproptosis have emerged as new pathways of cell death, providing crucial insights into stroke pathology (Li et al., 2022a). This review examines ferroptosis and cuproptosis in ischemic stroke, focusing on metal ion regulation in neuronal cells and their potential therapeutic applications.

Under normal conditions, iron and copper are essential trace elements for cell survival. However, in ischemic stroke, their balance is significantly disrupted (Chen et al., 2020a). Ischemia reduces oxygen and nutrient supply to brain tissue, triggering various biological

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https://doi.org/10.1016/j.bbih.2024.100837
Received 15 January 2024; Received in revised form 10 July 2024; Accepted 1 August 2024
Available online 6 August 2024

reactions. These reactions include intracellular calcium overload, oxidative stress, and inflammation, which directly or indirectly affect metal ion homeostasis (Zhang et al., 2022b; Ghosh et al., 2019). Iron, an oxygen carrier and metabolic enzyme cofactor, is closely linked to ferroptosis, involving excessive accumulation of intracellular iron ions. It influences stroke pathophysiology by affecting iron homeostasis and lipid peroxidation (Gao et al., 2019). In stroke treatment, inhibiting ferroptosis can reduce brain damage. Compared to iron, copper's role in ischemic stroke is less studied. However, copper's role in cell survival and death regulation is significant (Jin et al., 2021). Cuproptosis involves abnormal copper ion regulation. Recently, Chen found that intracellular copper accumulation, essential for cell death, primarily functions in the tricarboxylic acid cycle of mitochondrial respiration (Chen et al., 2023a). They identified ferroxidase 1 as a key gene in copper-induced cell death.

To understand the roles of iron and copper in ischemic stroke, it is crucial to explore their dynamic distribution and regulatory mechanisms within cells (Chen et al., 2023a). Research shows a direct correlation between increased intracellular iron in ischemic stroke and mitochondrial dysfunction, oxidative stress, and related factors (Mu et al., 2021). Similarly, abnormal copper accumulation is closely linked to neuronal apoptosis and inflammatory response activation (Chen et al., 2022a). Investigating the distribution and interplay of iron and copper within cells is crucial for understanding metal ion regulation in ischemic stroke.

Studies suggest that modulating iron and copper homeostasis can effectively slow neuronal damage, showing potential therapeutic effects in experimental models. However, debates and unresolved questions remain regarding specific treatments targeting ferroptosis and cuproptosis, requiring further research for validation (Chang et al., 2021). Despite extensive literature on cell death mechanisms in ischemic stroke, detailed studies on the complex associations and mechanisms of ferroptosis and cuproptosis are still limited.

This research will explore the molecular mechanisms of iron and copper in ischemic stroke, their interactions with oxidative stress and inflammatory responses, and potential new therapeutic targets. Through comprehensive analysis, we aim to deepen the medical community's understanding of ischemic stroke pathogenesis and provide strong scientific support for future treatments.

## 2. Ferroptosis mechanisms and regulation

### 2.1. Iron accumulation in ischemic stroke

Ischemic stroke is a severe neurological disorder involving complex molecular processes (Qin et al., 2022). Among these, ferroptosis, a newly identified form of cell death, has gained significant attention in ischemic stroke research (Tang et al., 2020). Intracellular iron accumulation is a key feature of ferroptosis, crucial for understanding the pathology of ischemic stroke and identifying potential therapeutic targets (Chen et al., 2021a). This section examines iron accumulation in ischemic stroke, exploring its sources to provide an in-depth analysis of ferroptosis mechanisms and regulation.

In ischemic stroke, the blood supply to brain tissues is severely compromised, reducing oxygen and nutrient delivery and triggering a cascade of biological responses (Qin et al., 2022). Iron accumulation becomes particularly notable. Studies show a significant increase in iron content in the brain tissues of stroke patients, especially in the peri-infarct region. This is closely linked to factors like blood-brain barrier disruption, increased vascular permeability, and intracellular iron release (Zhang et al., 2020a; Urrutia et al., 2021). The breakdown of the blood-brain barrier is a pivotal factor in iron accumulation. Normally, the blood-brain barrier restricts iron ions from entering brain tissues. In ischemic stroke, a compromised blood-brain barrier allows free entry of iron, leading to excessive accumulation (Hussain et al., 2021; Wu et al., 2023). Increased vascular permeability also significantly contributes to iron accumulation. Iron accumulation patterns vary across brain regions and cell types, with microglia having one of the highest iron storage capacities, reflecting varying neuronal sensitivities to oxygen and nutrient changes.

Intracellular iron release significantly contributes to iron accumulation in ischemic stroke. Ischemia-induced cellular damage releases intracellular iron ions, accelerating iron accumulation (Zhang et al., 2022c). This is closely linked to mitochondrial dysfunction, as mitochondria are primary storage sites for intracellular iron. Impaired mitochondrial function releases iron, creating a cycle that further promotes excessive iron accumulation in ischemic stroke (Rolston et al., 2009; Chen et al., 2020b).

In ischemic stroke, intracellular iron sources are diverse. Besides exogenous hemoglobin entry, the brain contains substantial endogenous iron (Guo et al., 2023). During ischemic stroke, cell death and dissolution release endogenous iron stores into the environment, which neighboring cells absorb, contributing to iron accumulation (Tuo et al., 2022). Intracellular iron release is regulated by iron carriers, transport proteins, and other channels.

In summary, intracellular iron accumulation in ischemic stroke is a complex process involving multiple factors. Factors like blood-brain barrier disruption, increased vascular permeability, hemoglobin entry, and intracellular iron release interact dynamically. Understanding these mechanisms provides a deeper comprehension of ferroptosis in ischemic stroke, forming a theoretical basis for future therapies.

### 2.2. Mechanisms of intracellular iron regulation

In ischemic stroke, dysregulated intracellular iron homeostasis is crucial for cell survival and death. This section explores the mechanisms of intracellular iron balance, focusing on iron carriers and transport proteins, to reveal the regulation of iron-induced cell death in ischemic stroke (Guo et al., 2023; Jin et al., 2010). Iron is crucial for cellular metabolism, maintaining normal cell function, and responding to external stressors. Iron carriers are key regulators of intracellular iron balance (Gao et al., 2019). Internally, iron is regulated through pathways involving iron carriers and transport proteins. Within the cell, iron is primarily transported by transferrin. Transferrin transports iron in the bloodstream, forming complexes with free iron and entering cells through transport proteins on the membrane (Chung et al., 2003; Galy et al., 2023). Ferritin, responsible for iron storage, regulates intracellular iron by controlling ferritin synthesis and degradation (Galy et al., 2023; Ri et al., 2023). This mechanism is crucial for the cell's flexible iron utilization. Once iron enters the cell, it participates in various biological processes such as the respiratory chain of mitochondria and DNA synthesis (Gao et al., 2019). This requires precise regulation to maintain intracellular iron balance and normal function.

Ischemic stroke disrupts intracellular iron regulation, leading to excessive iron buildup (Guo et al., 2023). Inflammation and vascular damage from the stroke break down the blood-brain barrier, allowing free iron to enter brain tissue and further increase iron levels (Pei et al., 2015). Damaged and dying cells release iron, perpetuating this cycle. Mitochondrial dysfunction also exacerbates iron release, adding to intracellular accumulation. The brain's inherent iron, released by cell death, is absorbed by neighboring cells, worsening iron buildup (Fang et al., 2018). This review delves deeply into the mechanisms of iron regulation in ischemic stroke, offering a molecular-level understanding of iron-induced cell death. Disrupted iron regulation triggers cell death in ischemic stroke (Datta et al., 2020). Understanding these mechanisms enhances our grasp of stroke pathology and offers a basis for new therapeutic strategies. Exploring iron-induced cell death may reveal new treatment targets for ischemic stroke.

### 2.3. Cell signaling pathway for ferroptosis

**Fenton Reaction:** In ischemic stroke, disrupted oxygen supply leads to iron accumulation and activation of the Fenton reaction, producing

highly reactive oxygen species like hydroxyl radicals (•OH) (Yu et al., 2021). These radicals damage brain tissue by causing lipid peroxidation, protein oxidation, and nucleic acid fragmentation, leading to cell death.

**Oxidative Stress:** Oxidative stress is key in iron-induced cell death. It involves excess reactive oxygen species (ROS) from increased production and/or reduced degradation, crucial in ischemic stroke pathology (Chen et al., 2023b). Brain ischemia raises cellular calcium ions (Ca2+), extracellular glutamate, and arachidonic acid levels (Chen et al., 2023b). Increased ROS production and antioxidant depletion deactivate antioxidant systems (Snezhkina et al., 2019). Excess ROS cause cell dysfunction and death via lipid peroxidation and oxidative damage to proteins, DNA and RNA. Oxidative stress also activates transcription factor 4 (ATF4), whose overexpression induces cell death (Yu et al., 2021; Wang et al., 2023a). Elevated levels of ATF4 mRNA and translation post-oxidative stress lead to ATF4 overexpression, sufficient to induce cell death (Li et al., 2022b).

**Iron Regulatory Proteins:** Iron regulatory proteins, including transferrin, are vital in iron-induced cell death (Cardona et al., 2023). Ischemic stroke disrupts their regulation, causing iron accumulation and abnormal distribution, leading to cell death **[**Fig. 1**.]** .

Apoptotic Pathways: Iron-induced cell death also activates apoptotic pathways. Excess oxygen radicals and iron damage mitochondrial membranes, releasing apoptotic signals like cytochrome C, activating caspases, and causing cell apoptosis (Nakamura et al., 2019).

**Inflammatory Response:** Iron-induced cell death triggers inflammatory responses. Oxygen radicals and cell damage activate inflammatory pathways, releasing mediators that worsen cell damage in a vicious cycle (Chen et al., 2023c). Excessive release of inflammatory mediators further exacerbates cell damage, creating a vicious cycle.

The study identified MAP1LC3B, PTGS2, and TLR4 as potential diagnostic biomarkers of ischemic stroke through a random forest model and ROC analysis, underscoring the role of ferroptosis in stroke (Chen et al., 2021b).

Recent studies show iron oxide inhibitors can reduce ischemic infarct size, protect the blood-brain barrier (BBB), and restore neuronal function. Activation of PPAR and inhibition of AKT after stroke protect the BBB and promote myelin regeneration (Li et al., 2020; Du et al., 2022). Glutathione peroxidase-4 (GPX4), a key iron regulator, inhibits iron mutations, suggesting the PPARγ/AKT/GPX4 pathway as a target to inhibit iron oxidation (Wang et al., 2023b).

Traditional Chinese Medicine (TCM), especially An-Gong Niu-Huang Wan (AGNHW), has long been used to treat diseases, including cerebral infarction (Tsoi et al., 2019). Studies show AGNHW's effectiveness in treating cerebral infarction with phlegm-heat syndrome and its preventive potential (Wang et al., 2014). Clinical research indicates AGNHW reduces serum ferritin levels in stroke patients, suggesting it may inhibit ferroptosis. AGNHW reduces infarct size, protects the BBB, and lessens mitochondrial injury in stroke rats (Tsoi et al., 2019). It lowers ROS, lipid peroxidation (LPO), and Fe2+ levels, demonstrating its anti-ferroptosis effects (Su et al., 2019; Bai et al., 2023).

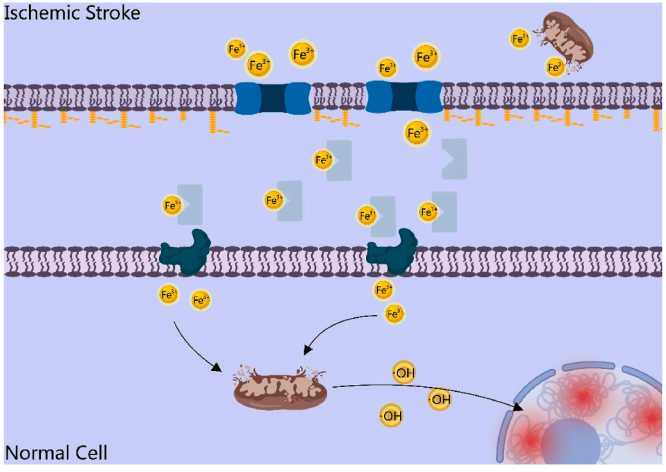


**Fig. 1.** Iron regulatory proteins like transferrin are critical in iron-induced cell death (Cardona et al., 2023). Ischemic stroke disrupts their regulation, causing iron accumulation and abnormal distribution, activating apoptotic pathways. Excess oxygen radicals and iron damage mitochondrial membranes, releasing apoptotic signals such as cytochrome C, activating caspases, and inducing cell apoptosis.

Liquid chromatography-mass spectrometry (LC-MS) identified key AGNHW components: bilirubin (cow bezoar), berberine (coptidis rhizoma), baicalin (scutellariae radix), and wogonoside (scutellariae radix) (Thomas et al., 2022). Network pharmacology was used to study AGNHW's anti-ferroptosis targets and mechanisms, identifying 393 candidate components for ischemic and hemorrhagic stroke (Zhao et al., 2023; Li et al., 2023a). GO and KEGG analysis suggested AGNHW treats cerebral infarction by regulating PI3K-AKT and PPAR pathways. Thirty-two targets related to ferroptosis in ischemic stroke were identified, with PPAR signaling and oxidative stress as potential regulatory pathways (Gu et al., 2021).

GO and KEGG analysis of AGNHW targets suggest it exerts anti-ferroptosis effects through PI3K-AKT and PPAR pathways (Chen et al., 2015). Protein-protein interaction (PPI) and clustering analysis identified key targets: PPARγ, AKT, and GPX4. Molecular docking and microscale thermophoresis (MST) experiments showed strong interactions between AGNHW components (bilirubin, berberine, baicalin, and wogonoside) and their targets (PPARγ, AKT, and GPX4) (Bai et al., 2023; Chen et al., 2015). This suggests a key role for AGNHW in regulating ferroptosis through the PPARγ/AKT/GPX4 pathway (Bai et al., 2023).

The PPARγ/AKT/GPX4 pathway inhibits neuronal ferroptosis, preserves the blood-brain barrier, and promotes myelin regeneration (Bai et al., 2023). AKT reduces post-ischemic neuronal damage. GPX4, a key regulator, suppresses ferroptosis, making the PPARγ/AKT/GPX4 pathway a target for inhibiting ferroptosis (Wang et al., 2014; Bai et al., 2023). PPARγ inhibits neuronal ferroptosis in models, protects the blood-brain barrier, and supports myelin regeneration, highlighting its therapeutic potential for CNS diseases.

AKT is known to reduce post-ischemic neuronal injury. The PI3K/AKT pathway, activated through phosphorylation, promotes the nuclear translocation and accumulation of Nrf2 (Bai et al., 2023). Activating Nrf2 helps prevent brain damage and increases the expression of xCT (SLC7A11) and glutathione, enhancing GPX4 activity (Saha et al., 2020). Given GPX4's role in inhibiting ferroptosis, the study used PC12 cells to investigate ferroptosis induction with erastin (Ma et al., 2022).

Results show erastin reduces cellular activity, while AGNHW serum enhances viability. LPO and Fe2+ levels are higher in the model group than the normal group (Sun et al., 2020; Yan et al., 2022). AGNHW serum increases PPARγ, ATK, and GPX4 mRNA expression compared to the model group. Protein expression of PPARγ, p-ATK/AKT, and GPX4 is lower in the model group but higher in the AGNHW group compared to the sham surgery group (Bai et al., 2023). These results suggest AGNHW's anti-ferroptotic effect in ischemic stroke by modulating the PPARγ/ATK/GPX4 pathway. Our findings align with previous literature (Wang et al., 2014; Bai et al., 2023).

We identified bilirubin, berberine, baicalin, and wogonoside in AGNHW serum as inhibitors of ferroptosis. Bilirubin may enhance pancreatic islet functionality, suggesting its potential in islet transplantation (Hansen et al., 1996). Berberine's increased glutathione peroxidase 1 expression may reduce neuronal ferroptosis in cerebral ischemia-reperfusion injury (Handy et al., 2022). Wogonoside may reduce liver fibrosis through SOCS1/P53/SLC7A11-mediated ferroptosis in hepatic stellate cells, offering a liver fibrosis treatment strategy (Handy et al., 2022). Baicalin prevents ferroptosis in ischemic stroke and primary cortical neurons (Liang et al., 2017). Thus, these compounds are potential ischemic stroke treatments.

AGNHW significantly improves neurological function, reduces infarct volume, and lessens brain tissue damage in ischemic stroke (IS) rats (Chen et al., 2022b). It increases cell survival and decreases LPO and Fe2+ in PC12 cells. In vivo and in vitro results show AGNHW combats ferroptosis via the PPARγ/ATK/GPX4 pathway (Bai et al., 2023; Ursini et al., 2020). In summary, AGNHW reduces cerebral infarction damage by activating the PPARγ/ATK/GPX4 pathway to inhibit ferroptosis (Bai et al., 2023). The signaling pathways of ferroptosis include Fenton reaction, oxidative stress, iron-regulatory proteins, apoptotic pathways, and inflammatory responses (Yu et al., 2021). Understanding this network aids in comprehending ferroptosis mechanisms in ischemic stroke. This study highlights AGNHW's role in inhibiting ferroptosis by activating the PPARγ/ATK/GPX4 pathway, reducing cerebral infarction damage (Bai et al., 2023). Research into ferroptosis mechanisms offers insights for developing therapeutic strategies to alleviate neural damage in ischemic stroke.

## 3. Cuproptosis: a double-edged sword for nerve cells

### 3.1. Physiological role of copper in the nervous system

Copper is essential in regulating enzymatic activity within the nervous system (Grubman et al., 2014). As a cofactor, it is involved in neurotransmitter synthesis, energy metabolism, and redox reactions (Tannous et al., 2021). This section reviews copper's physiological roles, especially its enzymatic regulation (Scheiber et al., 2014). laying the groundwork for understanding cuproptosis in ischemic stroke.

Copper is distributed throughout the brain and spinal cord, including neurons, glial cells, and blood vessels. Its balance is tightly regulated by transport and storage proteins (Scheiber et al., 2014). In blood, copper binds to transport proteins like ceruloplasmin, which regulate its ionic state (Chen et al., 2020a). Neurons use copper storage proteins, such as metallothioneins, to maintain homeostasis. Copper ions enter neurons via channels like CTR1 and are transported by ATP7A and ATP7B pumps (Mandal et al., 2020), ensuring proper copper levels in the nervous system.

Copper aids in synthesizing neurotransmitters by regulating enzymes like dopamine beta-hydroxylase (DBH) and tyrosine hydroxylase (TH), crucial for dopamine signaling (An et al., 2022). It is a part of redox enzymes like cytochrome C oxidase in the mitochondrial respiratory chain, vital for energy metabolism (Borodinsky et al., 2014). T As a cofactor for superoxide dismutase (SOD), copper helps eliminate superoxide anions, providing antioxidative defense, and maintaining normal nervous system function (Lennicke et al., 2021).

Copper is vital for neuronal development, synapse formation, and neuron migration by regulating growth factors and cell adhesion molecules (Chen et al., 2020a). Copper's physiological roles are multifaceted, involving neurotransmitter synthesis, energy metabolism, antioxidative defense, and synaptic transmission (Scheiber et al., 2014). Understanding these functions enhances our knowledge of copper's mechanisms in ischemic stroke (Borodinsky et al., 2014). Proper copper levels are essential, suggesting a systematic approach for therapeutic strategies targeting cuproptosis.

### 3.2. Cuproptosis in ischemic stroke

In ischemic stroke, the survival of neuronal cells is complex, with copper playing a crucial role (Tuo et al., 2022). Copper is vital for normal physiology, but during ischemic stroke, hypoxia and poor blood flow increase oxidative stress, leading to copper-mediated cell death (An et al., 2022). This section explores copper imbalance in ischemic stroke, its causes, and its effects on neurons.

Ischemic stroke triggers cellular changes, including copper ion release and accumulation. Damage to neurons can cause cell membranes to rupture, releasing copper and disrupting its balance (Tuo et al., 2022). Oxidative stress and damage affect copper-regulating proteins, such as metallothioneins, leading to improper copper levels (Rodrigo et al., 2013). Copper-regulating proteins, such as metallothioneins, typically play a crucial role in modulating the accumulation and distribution of copper. However, under conditions of ischemic injury, the expression of these proteins may be disrupted, leading to inappropriate copper accumulation (Gudekar et al., 2020). The inflammatory response and vascular damage triggered by ischemic stroke may result in the breakdown of the blood-brain barrier, facilitating the easier entry of copper from the blood into brain tissue. This disrupts the normal distribution of copper in the brain, exacerbating the state of copper imbalance.

Oxidative stress and inflammation from ischemic stroke contribute significantly to copper imbalance by activating intracellular copper regulation, causing improper release and accumulation (Chen et al., 2020a). This review examines the relationship between cuproptosis and oxidative stress, focusing on its triggers and regulatory mechanisms. Ischemic injury leads to cell membrane rupture, releasing copper and disrupting its interactions with cellular components. Ischemic stroke affects proteins that regulate copper, worsening the imbalance (Xue et al., 2023). This imbalance destabilizes redox reactions, increasing free radicals and oxidative damage, potentially leading to cell death.

Copper regulates enzymes involved in energy metabolism and antioxidation. Imbalance disrupts these pathways, affecting neuronal function (Chen et al., 2020a). It can also trigger inflammation, worsening neuronal damage and creating a vicious cycle of cell death (Chang et al., 2023). In ischemic stroke, copper imbalance is crucial to neuronal survival and death. Stress, cell membrane rupture, and impaired copper regulation profoundly affect neurons (Tuo et al., 2022). Cuproptosis, a form of cell death, is linked to oxidative stress mechanisms.

Mitochondria are essential for cellular energy metabolism and survival. Cuproptosis may impair mitochondrial function, disrupting the respiratory chain and ATP synthesis. Understanding copper imbalance mechanisms is vital to elucidating cuproptosis in ischemic stroke, providing a theoretical foundation for future therapies.

### 3.3. Effects and mechanisms of cuproptosis

Copper is crucial for normal nervous system function (Tapiero et al., 2003). In ischemic stroke, abnormal copper accumulation can cause neuronal cell death, making it essential to understand cuproptosis mechanisms (Tuo et al., 2022). Unlike other metals like iron, the mechanisms of copper-induced cell death are not well understood.

Todd R. Golub and Peter Tsvetkov have identified a novel copper-dependent cell death pathway, called copper-scaling disease. Copper interacts with fatty acylation components of the tricarboxylic acid cycle, causing excessive protein aggregation and loss of iron-sulfur cluster proteins, leading to protein toxicity and cell death (Tsvetkov et al., 2022). Elevated copper levels are seen in ischemic stroke patients, but more research is needed to determine if this induces neuronal cuprization and the role of Sirt1 in this process.

Evidence suggests copper ion carriers induce cell death by accumulating copper intracellularly, not by small molecules' direct impact. Golub and colleagues found that copper targets TCA cycle protein sulfur succinylation, with FDX1 as a key gene in this process. Copper also plays a role in atherosclerosis (Tsvetkov et al., 2022). Elevated serum copper is linked to higher atherosclerosis risk, but mechanisms are unclear. Copper deficiency may downregulate adhesion molecules (ICAM-1, VCAM-1) and raise cholesterol levels, increasing atherosclerosis risk (Tyrrell et al., 2021). This section examines cuproptosis mechanisms, focusing on oxidative stress, mitochondrial function, and other factors, to better understand cuproptosis in ischemic stroke.

Cuproptosis significantly increases oxidative stress. Copper, as a catalyst in redox reactions, can generate free radicals and damage cellular components when imbalanced, triggering apoptosis and necrosis (Chen et al., 2023d). It disrupts mitochondrial function, causing oxidative damage and impairing TCA cycle enzymes (Tsvetkov et al., 2022). Copper, a cofactor for mitochondrial enzymes like cytochrome *c*

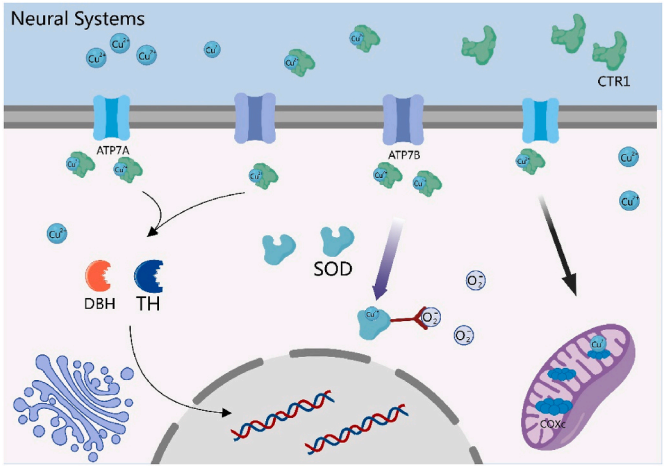


**Fig. 2.** Copper imbalance disrupts mitochondrial function, impairing TCA cycle enzymes and mitochondrial membranes, increasing oxidative damage and cell death. As a cofactor for cytochrome *c* oxidase and superoxide dismutase (SOD), copper imbalance reduces antioxidant defenses, exacerbating oxidative stress.

oxidase, participates in redox processes. Imbalance decreases mitochondrial function, increases electron leakage, ruptures membranes, and leads to cell death. As a cofactor for superoxide dismutase (SOD), copper imbalance depletes antioxidant defenses, making cells more vulnerable to oxidative stress (Itoh et al., 2009)[Fig. 2.].

Oxidative stress induced by ischemic stroke may lead to the release of copper ions from their normal binding forms. This process may increase intracellular oxygen free radical generation through pathways such as the Fenton reaction, triggering oxidative stress damage. The direct binding and regulation of copper with mitochondrial enzymes constitute a crucial mechanism in cuproptosis (Aruoma, 1998). Abnormal copper accumulation may alter the stability of the mitochondrial membrane, disrupt the redox balance within the mitochondria, and result in decreased intracellular energy production (Ruiz et al., 2021). Copper-regulating proteins, such as metallothioneins, play a key role in maintaining copper balance and preventing its toxic effects. Under conditions of cuproptosis, the function of these proteins may be impaired, leading to inappropriate copper accumulation (Okita et al., 2017). Inflammatory responses and vascular damage induced by ischemic stroke may result in the breakdown of the blood-brain barrier, facilitating the easier entry of copper from the blood into brain tissue. This process disrupts the normal distribution of copper in the brain, exacerbating the state of copper imbalance.

In comparison to existing literature, this paper delves more profoundly into the mechanisms of cuproptosis, with a particular emphasis on its relationships with oxidative stress and mitochondrial function. By meticulously analyzing the impacts of cuproptosis, a more comprehensive understanding is provided (Lombardo et al., 2023). Additionally, the dysregulation of copper-regulating protein functions and the effects of BBB disruption on cuproptosis are highlighted, offering novel avenues for future in-depth investigations (Deng et al., 2023). Cuproptosis, as a significant mechanism of neuronal cell death in ischemic stroke, involves disturbances in oxidative stress, mitochondrial function, and the depletion of antioxidant defense systems (Tuo et al., 2022). A thorough comprehension of the mechanisms of cuproptosis holds paramount significance in unveiling the pathophysiological processes of ischemic stroke, furnishing a more profound theoretical foundation for future therapeutic strategies.

## 4. Cross-cutting effects of ferroptosis and cuproptosis

Ischemic stroke represents a severe neurological disorder, with its pathophysiological mechanisms intricately entangled in complex cellular death processes (Qin et al., 2022). Throughout this progression, the interaction between two trace elements, iron and copper, has garnered considerable attention from researchers (Tatsumi et al., 2018). This paper delves into an in-depth exploration of the intersecting influences of iron-induced cell death and copper-induced cell death in ischemic stroke. The emphasis is particularly placed on their intracellular interactions, aiming to offer novel perspectives for furthering research in this field.

Firstly, it is essential to comprehend the fundamental roles of iron and copper in normal cellular functions. Iron is a crucial element necessary for maintaining normal cellular metabolism, participating in life activities such as oxygen transport, DNA synthesis, and cellular respiration (Mu et al., 2021). Simultaneously, copper, acting as a coenzyme and catalyst, engages in various redox reactions and enzyme activities, playing a vital role in maintaining intracellular homeostasis. In ischemic stroke, inadequate blood perfusion restricts cellular oxygen supply, disrupting the balance of intracellular redox reactions. Under such circumstances, the excessive accumulation of iron within cells leads to the generation of reactive oxygen species (ROS) through the Fenton reaction, resulting in lipid peroxidation and subsequent iron-induced cell death (Huang et al., 2023).

Research indicates a significant increase in iron deposition, lipid peroxidation, and neuronal death in the brains of adult rats in an ischemic stroke model. Unlike iron, copper exhibits a dual role in ischemic stroke. On the one hand, copper, by participating in the activity of antioxidant enzymes, inhibits the generation of free radicals, thereby mitigating oxidative stress-induced damage to cells. On the other hand, excessive copper may also contribute to the generation of oxygen free radicals, promoting cell death. The intricate interactions of iron and copper within cells may alter the distribution and concentration of metal elements, influencing cell fate, particularly in the context of ischemic stroke (Chen et al., 2020a; Huang et al., 2023).

In ischemic stroke, the cross-impact of iron and copper is particularly noteworthy. Excessive iron may inhibit the antioxidant function of copper, diminishing the dual role of copper and accelerating cell death (Guo et al., 2023; Zhang et al., 2020b). Simultaneously, iron and copper may form complex coordination structures within cells, inducing changes in the cellular microenvironment that affect cell metabolism and survival.

Ferroptosis and cuproptosis also exhibit some intersection in signaling pathways. Iron activates specific signaling pathways, such as the Fenton reaction and ferroptosis pathway, triggering cell death. Meanwhile, copper is involved in regulating cell apoptosis signaling pathways, such as the mitochondria-mediated pathway and death receptor signaling pathway. In ischemic stroke, the cross-interaction of iron and copper may impact the normal operation of these signaling pathways, leading to an irreversible increase in cell death. A profound understanding of the intersecting influences of ferroptosis and cuproptosis holds crucial clinical significance for the treatment and prevention of ischemic stroke. By fine-tuning the balance of iron and copper, there is a potential to design more precise intervention strategies, reducing the risk of cell death and enhancing both the survival rate and quality of life for patients.

Additionally, we explored the correlation between key genes and genes associated with iron deficiency and copper deficiency. This suggests that these key genes may be involved in the development of ischemic stroke (IS) by regulating mechanisms related to iron and copper deficiency. SRPK1 is a protein kinase involved in various signaling pathways and the regulation of gene expression. It plays a crucial role in neuronal growth and death by modulating the expression of apoptosis-related factors and cell cycle-related factors. BIRC2, an anti-apoptotic protein, functions in multiple biological processes such as cell apoptosis, immune response, and the cell cycle. Research indicates the significant role of BIRC2 in ischemia and hypoxia. Additionally, KLHL3 is a cell-skeletal-associated protein closely related to cell growth, differentiation, movement, and apoptosis. KLHL3 can inhibit the apoptotic

pathway post ischemic stroke, thereby reducing cell death. Through machine learning methods, three key genes (SRPK1, BIRC2, and KLHL3) were identified, and their significance was validated in other datasets and clinical patients (Huang et al., 2023).

Several studies have investigated the clinical significance of key genes in Acute Ischemic Stroke (AIS) by analyzing their correlation with NIHSS scores. The mRNA expression of SRPK1 and BIRC2 showed a positive correlation with NIHSS scores, whereas the mRNA expression of KLHL3 exhibited a negative correlation. Additionally, GPX4 serves as a significant marker for ferroptosis, while FDX1 is a crucial indicator for cuproptosis. Interestingly, the mRNA expression of SRPK1 and BIRC2 demonstrated a negative correlation with GPX4 mRNA expression, while the mRNA expression of KLHL3 showed a positive correlation. Furthermore, the mRNA expression of SRPK1 and BIRC2 positively correlated with FDX1, while KLHL3's mRNA expression exhibited a negative correlation (Huang et al., 2023). A recent study suggested that intracellular copper ions act as novel GPX4 inhibitors, and copper ion chelators can reverse erastin-induced GPX4 protein reduction (Chen et al., 2023a). Further research revealed that copper ions induce ferroptosis by binding to the cysteine residues C107 and C148 of GPX4, promoting the binding of GPX4 with the autophagy receptor Tax1 binding protein 1 (TAX1BP1), thereby increasing the ubiquitination of GPX4 (Huang et al., 2023).

This finding provides initial evidence for the relationship between cuproptosis and ferroptosis. The complexity of this iron-copper interaction remains incompletely understood in current research, necessitating deeper investigations to unveil the mutual influences between the two.

While there is extensive research in the literature on ferroptosis and cuproptosis, there remains a knowledge gap in unraveling the molecular mechanisms of their mutual influence. Future investigations should focus on a comprehensive understanding of the interaction between iron and copper in ischemic stroke, encompassing their reciprocal regulation within cells, temporal changes during pathological processes, and cross-impacts with other cellular signaling pathways (Li et al., 2020). Delving into these issues will enhance our comprehension of the interplay between iron and copper in ischemic stroke. Subsequent research should also delve into the specific mechanisms of iron and copper in ischemic stroke, providing more targeted points for novel therapeutic strategies (Tang et al., 2023).

This paper extensively explores the intracellular interactions of ferroptosis and cuproptosis in ischemic stroke, delving into multiple aspects such as the fundamental functions of iron and copper, metabolic abnormalities in ischemic stroke, cross-influences, and signaling pathways. This review not only deepens our understanding of the pathogenesis of ischemic stroke but also offers novel insights and directions for future research and treatment.

Additionally, research has revealed that during cerebral infarction, mitochondrial function is compromised, leading to an increased release of iron ions within the mitochondria. Iron ions, through the generation of oxidative stress, induction of cell apoptosis, and promotion of inflammatory responses, play a pivotal role in causing neuronal cell death, a critical factor in cerebral infarction (Khan et al., 2022). Furthermore, we have investigated the correlation between key genes and genes associated with ferroptosis and copper spore disease. For instance, the protein encoded by the HFE gene participates in regulating iron absorption and storage, and its mutations have been linked to hereditary hemochromatosis and neurological disorders (Barton et al., 2015). Studies suggest that HFE gene variations may increase iron accumulation in the brain, leading to iron-induced neuronal death. Transferrin receptor (TFRC) mediates cellular uptake of transferrin, thereby regulating intracellular iron levels (Kim et al., 2020). Aberrant expression of the TFRC gene has been confirmed to be associated with disrupted iron metabolism and cellular ferroptosis in some neurological disorders. Additionally, proteins encoded by the ATP7A and ATP7B genes are involved in copper transport and distribution, with ATP7A predominantly expressed in the small intestine and brain, and ATP7B in the liver (Linz et al., 2007). Mutations in these genes have been linked to the occurrence of copper spore disease, causing abnormal accumulation of copper ions in the body and impairing neurological function. This indicates that these key genes may be involved in the occurrence of cerebral infarction by regulating ferroptosis and melanin breakdown mechanisms.

## 5. Emerging treatment strategies and research prospects

### 5.1. Drug interventions for iron and copper

Ferroptosis and cuproptosis, as two crucial molecular mechanisms in ischemic stroke, have sparked substantial interest among researchers in terms of drug interventions (Li et al., 2023b). In recent years, drug interventions targeting iron and copper have gradually become a research focus [Table 1]. In the realm of ferroptosis, chelators like deferoxamine (DFO) have been studied, showing certain neuroprotective effects in animal experiments (Chen et al., 2023a; Li et al., 2023b). These drugs bind free iron ions, reducing iron accumulation and inhibiting ferroptosis. Leptin, a hormone derived from fat cells, inhibits glutamate release in the hippocampal CA3 region, attenuating ferroptosis induced by glutamate excitotoxicity. Leptin upregulates inflammatory factors, mediates GPX4 downregulation, accelerates iron overload, and ultimately leads to ferroptosis (Li et al., 2023c). Leptin has emerged as a potential target for treating ischemic stroke. Recent studies suggest that novel drugs such as Ferrostatin-1 and Liproxstatin-1, by modulating iron-dependent cell death pathways, exert protective effects against ischemic stroke. This research opens a new avenue, emphasizing the potential value of iron metabolism in stroke treatment. Resveratrol, playing a neuroprotective role in ischemic stroke (Zhang et al., 2019;

**Table 1**
Drug Interventions for Iron and Copper and their main mechaniams of actions.

| Drug name | Function |
|---|---|
| Ferrostatin-1 and Liproxstatin-1 | Modulating iron-dependent cell death pathways exerts protective effects against ischemic stroke |
| Resveratrol | It improves cognitive impairment by inhibiting hippocampal neuron ferroptosis through the activationof the Sirt1/Nrf2/GPx4 signaling pathway |
| Editing genes relatedto iron metabolism, such as Ferroportin and Transferrin Receptor | Modulates cellular iron intake and export, therebyinfluencing intracellular iron levels |
| Tetrathiomolybdate | By binding free copper and reducing its accumulation in the body, it is expected to exert similar protective effects in ischemic stroke |
| ATP7A and ATP7B | Regulates intracellular copper accumulation and distribution, affecting copper bioavailability |
| By designing small interfering RNA (siRNA) or microRNA (miRNA) | Selectively inhibits the expression of genes associated with iron and copper metabolism, thereby modulating intracellular levels of these metals |
| Antioxidants such as N-acetylcysteine and Melatonin | By reducing the generation of reactive oxygen species, it indirectly regulates intracellular iron and copper metabolism, alleviating cellular damage andenhancing cell survival |
| CRISPR-Cas9 | By designing specific RNA guide sequences, theCRISPR-Cas9 system can accurately cleave targetgenes within cells, thereby achieving gene deletion, repair, or replacement |
| Quercetin and Resveratrol | Enhances cellular antioxidant capacity by increasing the activity of intracellular antioxidant enzymes, thereby protecting thenervous system from ischemic stroke damage |

Park et al., 2019). Our previous research found that resveratrol pretreatment has similar effects to the ferroptosis inhibitor ferrostatin-1 in suppressing neuron-related Ferroptosis changes, such as iron overload, oxidative damage to the redox system, mitochondrial structural disruption, and upregulation of GPX4. Similarly, some researchs discovered that resveratrol improves cognitive impairment by inhibiting hippocampal neuron Ferroptosis through the activation of the Sirt1/Nrf2/GPx4 signaling pathway (Huang et al., 2023; Cao et al., 2018). Furthermore, recent evidence demonstrates that Sirt1 inhibits Ferroptosis through another crucial execution factor, SLC7A11, participating in neuroprotection against ischemic stroke both in vivo and in vitro (Su et al., 2021). Further research is needed to determine whether Sirt1 can directly inhibit Ferroptosis by deacetylating Ferroptosis-related molecules, thus exerting neuroprotective effects.

Like iron, copper also plays a dual role in ischemic stroke. Thus, regulating copper balance has become a crucial focus for treatment. Currently, some copper chelators such as Tetrathiomolybdate have been researched for treating copper metabolism-related diseases like Wilson's disease. These drugs, by binding free copper, reduce its accumulation in the body and are expected to exert similar protective effects in ischemic stroke. Additionally, natural products with antioxidant properties, such as Quercetin and Resveratrol, have attracted researchers' interest. These substances enhance cellular antioxidant capacity by increasing the activity of intracellular antioxidant enzymes, thereby protecting the nervous system from ischemic stroke damage. In the context of drug interventions for iron and copper, combination therapy might be a more effective strategy. Considering the intricate interactions of iron and copper within cells, simultaneous intervention for both trace elements holds the promise of comprehensively regulating the intracellular redox balance. Thus, conducting research on combination therapy for iron and copper drugs to find more ideal treatment approaches is a future development direction.

Future research should focus on elucidating the specific mechanisms of action of iron and copper in the nervous system, developing more effective drugs, and exploring the potential for combination therapies. This not only holds promise for providing new strategies in the treatment of ischemic stroke but also lays the groundwork for addressing other related neurological disorders. In-depth investigations into drug interventions for iron and copper are anticipated to become a highly promising therapeutic avenue in this field.

### 5.2. Gene editing and targeted therapy

On death and cuproptosis in ischemic stroke, as crucial pathological processes, have garnered widespread attention from researchers. When exploring emerging treatment strategies and research prospects, the application of gene editing and other targeted therapeutic methods to modulate the levels of intracellular iron and copper becomes an eagerly anticipated area. This paper delves into the principles, current applications, and future research prospects of these treatment strategies.

Gene editing technology, as a precise biological tool, has demonstrated immense potential in regulating intracellular levels of iron and copper. Among these technologies, CRISPR-Cas9, currently the most advanced and widely applied gene editing tool, finds extensive use in ischemic stroke research. By designing specific RNA guide sequences, the CRISPR-Cas9 system can accurately cleave target genes within cells, thereby achieving gene deletion, repair, or replacement. In the regulation of iron and copper levels, researchers attempt to edit genes associated with the metabolism of these trace elements to control intracellular iron and copper levels. For instance, editing genes related to iron metabolism, such as Ferroportin and Transferrin Receptor, can modulate cellular iron intake and export, thereby influencing intracellular iron levels. Similarly, editing genes related to copper metabolism, such as ATP7A and ATP7B, can regulate intracellular copper accumulation and distribution, affecting copper bioavailability. This provides researchers with a direct and effective means to precisely modulate intracellular iron and copper levels.

In addition to gene editing technology, various other targeted therapeutic approaches have found application in ischemic stroke research. Notably, RNA interference (RNAi) technology is a method that employs RNA fragments to selectively target specific genes, demonstrating potential therapeutic effects in regulating iron and copper metabolism. By designing small interfering RNA (siRNA) or microRNA (miRNA), researchers can selectively inhibit the expression of genes associated with iron and copper metabolism, thereby modulating intracellular levels of these metals. The advantages of this method lie in its relatively short treatment duration and lower therapeutic dosage, reducing unnecessary side effects. Furthermore, considering the oxidative stress characteristic of ischemic stroke, antioxidant therapy is also regarded as a crucial targeted strategy. Antioxidants such as N-acetylcysteine and Melatonin, by reducing the generation of reactive oxygen species, indirectly regulate intracellular iron and copper metabolism, alleviating cellular damage and enhancing cell survival.

## 6. Conclusions

In ischemic stroke, researchers have focused intensely on ferroptosis and cuproptosis as critical mechanisms of cell death. Investigating how these pathways operate in the nervous system during stroke provides valuable insights into the molecular basis of brain tissue damage. Understanding their roles could lead to new therapeutic strategies.

Ferroptosis, or iron-dependent cell death, is triggered by an excess of iron ions. During ischemic stroke, the accumulation of these ions in cells initiates this destructive pathway. Beyond directly harming nerve cells, ferroptosis exacerbates inflammation in later stroke stages. Research suggests it speeds up neuronal apoptosis by sparking oxidative stress, engaging mitochondrial and signaling pathways like NF-κB and p53. This process intensifies neural inflammation, reinforcing damage to brain tissue in a harmful cycle.

Conversely, cuproptosis has recently garnered attention for its role in ischemic stroke. Copper, essential in living organisms, is tightly regulated within the hypoxic-ischemic environment. Studies suggest its release correlates closely with nerve cell survival or demise during stroke. Cuproptosis hinges on copper ions' regulation of oxidative-reduction reactions and their impact on mitochondrial function, leading to nerve cell death. Moreover, it contributes to neuroinflammation, interacting with ferroptosis to create a complex cell death network post-stroke.

To deepen understanding of ferroptosis and cuproptosis in ischemic stroke, future research should focus on: Analyzing Cell Death Signaling Pathways: Clarifying molecular signaling in these pathways, including key factors and regulatory mechanisms.

Exploring New Therapeutic Strategies: Due to the crucial roles of ferroptosis and cuproptosis in strokes, it is crucial to develop innovative neuroprotective treatments. This involves exploring ways to prevent excessive iron and copper ion buildup, potentially vital for future therapies.

Studying Temporal and Spatial Dynamics of Cell Death: Given the fluctuating nature of stroke-related cell death, researchers can use advanced imaging and biosensors to monitor ferroptosis and cuproptosis processes in real time. Exploring the Link between Cell Death and Neural Repair: Investigating connections among ferroptosis, cuproptosis, and neural repair can unveil pathways that foster neural regeneration and recovery.

Treating ferroptosis and cuproptosis in ischemic stroke patients offers diverse possibilities. Future research may prioritize drug therapy, biotechnology, and interventions as potential focal points. For example, developing targeted iron chelators and copper regulators to modulate trace element balance could effectively mitigate cell death processes. Additionally, using gene editing techniques to enhance cellular repair mechanisms shows promise as a therapeutic approach.

Overall, delving deep into ferroptosis and cuproptosis mechanisms in

ischemic stroke, alongside exploring future research and treatment avenues, promises innovative insights and methodologies for stroke prevention and treatment. Progress in this area could lead to significant neurology and brain science advancements, ushering in more effective treatment approaches for ischemic stroke patients.

## Funding

This study was funded by Lu'an city science and technology plan project (2022lakj024) and Research project of teaching Hospital of Wannan Medical College (WK2023JXYY064).

## CRediT authorship contribution statement

**Jing Wang:** Writing – review & editing, Writing – original draft, Visualization. **Cunming Lv:** Writing – review & editing, Writing – original draft, Visualization. **Xinyu Wei:** Resources, Investigation. **Feng Li:** Writing – review & editing, Validation, Conceptualization.

## Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Data availability

No data was used for the research described in the article.

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