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Original Article

Research Progress on the Application of Ginkgolide in Injection Formulations

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Abstract:
Ginkgolide, a natural active component extracted **Jin'e Huang** from Ginkgo biloba leaves, has garnered significant attention due to its unique platelet-activating factor (PAF) antagonistic properties and neuroprotective effects. These pharmacological characteristics have positioned ginkgolide as a promising candidate for injection formulations with important clinical applications. This review systematically summarizes the pharmacological properties, formulation process optimization, clinical indications, and safety evaluations of ginkgolide injections. By integrating the latest research findings, the article highlights the therapeutic potential of ginkgolide injections in cerebrovascular diseases, cardiovascular disorders, and neurodegenerative conditions. Despite notable advances, challenges remain in formulation stability, bioavailability, and comprehensive safety profiling, which are critical for broader clinical adoption. This review aims to provide a scientific foundation for the further development and clinical promotion of ginkgolide injection preparations, facilitating improved therapeutic strategies and patient outcomes.
Keywords: Ginkgolide; Injection formulation; Pharmacological effects; Formulation process; Clinical application; Safety

Introduction

Ginkgo biloba L., commonly known as ginkgo, is a unique and ancient tree species often referred to as a "living fossil" due to its evolutionary history spanning over 200 million years. The leaves of Ginkgo biloba contain a distinctive class of bioactive compounds known as ginkgo diterpene lactones, which include ginkgolides A, B, C, J, and K. Among these, ginkgolide B (GB) is recognized as the most pharmacologically potent component. These diterpene lactones have attracted considerable attention for their diverse therapeutic properties, particularly in the context of cardiovascular and cerebrovascular diseases, neuroprotection, and anti-inflammatory effects . The biosynthesis of ginkgolides is tightly regulated by molecular mechanisms involving the JAZ gene family and the GbCOI1/GbJAZs/GbMYC2 module, which modulate the production of these compounds in response to methyl jasmonate, highlighting the complexity of their natural synthesis and the potential for biotechnological enhancement [1] Furthermore, recent advances in extraction technologies, including green extraction methods such as deep eutectic solvents (DES), have improved the sustainability and efficiency of isolating ginkgolides from Ginkgo biloba leaves, facilitating their pharmaceutical development [2]

The pharmacological significance of ginkgo diterpene lactones is underscored by their multifaceted biological activities. Ginkgolide B, in particular, exhibits potent antagonism of the platelet-activating factor (PAF) receptor, conferring anti-thrombotic and anti-ischemic properties that are valuable in the management of

ischemic stroke and cardiovascular conditions . Additionally, ginkgolides demonstrate neuroprotective effects by modulating excitotoxicity, oxidative stress, and inflammatory pathways. For example, ginkgolide B has been shown to attenuate excitotoxic neuronal injury by regulating the balance between excitatory and inhibitory amino acids in ischemic brain tissue, thereby reducing infarct size and improving neurological outcomes in animal models of cerebral ischemia [3] Moreover, ginkgo diterpene lactones inhibit inflammatory responses via downregulation of the TLR4/NF-κB signaling pathway in astrocytes, which contributes to their protective effects against cerebral ischemia/reperfusion injury [4] These pharmacodynamic properties are complemented by evidence that ginkgolides can modulate mitochondrial function, enhance anti-apoptotic signaling through pathways such as PI3K/Akt/mTOR, and promote neuronal differentiation via the Wnt/β-catenin pathway, collectively supporting their therapeutic potential in neurodegenerative and ischemic diseases .

Despite the promising pharmacological profile of ginkgo diterpene lactones, their clinical application faces challenges related to bioavailability, stability, and safety. The poor oral bioavailability of ginkgolides has prompted the development of injectable formulations, such as ginkgo diterpene lactone meglumine injection (GDLI), which offer rapid onset of action and enhanced systemic exposure, particularly suitable for acute and severe conditions like ischemic stroke . Pharmacokinetic studies in animal models have demonstrated that ginkgolides predominantly exist in hydrolyzed carboxylic forms in plasma, with extensive tissue distribution but limited penetration across the blood-brain barrier, suggesting peripheral targets for their cerebrovascular protective effects . The formulation and manufacturing processes of ginkgo diterpene lactone injections have been optimized using techniques such as activated carbon adsorption and advanced spectroscopic analysis to ensure quality, stability, and accurate quantification of active components . Safety evaluations in large clinical cohorts have reported a low incidence of mild and manageable adverse reactions, supporting the tolerability of these injectable preparations in ischemic stroke patients [5]

Clinically, ginkgo diterpene lactone injections have been extensively investigated for their efficacy in ischemic stroke, acute cerebral infarction, and related neurological disorders. Meta-analyses of randomized controlled trials indicate that GDLI improves clinical efficacy and neurological function scores, and when combined with recombinant tissue plasminogen activator (rt-PA), it enhances thrombolytic therapy outcomes without increasing adverse events [6] Randomized, double-blind, placebo-controlled trials have also demonstrated the antiplatelet effects of GDLI, contributing to improved prognosis in acute ischemic stroke patients [7] Furthermore, combination therapies involving GDLI and modalities such as repetitive transcranial magnetic stimulation (rTMS) have shown synergistic benefits in cognitive and neurological recovery post-stroke, with serum biomarkers like lipoprotein-associated phospholipase A2 (Lp-PLA2) and ischemia-modified albumin (IMA) serving as predictors of therapeutic response [8] Beyond stroke, ginkgolide B has exhibited protective effects in models of myocardial ischemia, autoimmune encephalomyelitis, and post-stroke cognitive impairment, mediated through mechanisms involving mitochondrial protection, anti-apoptotic signaling, and modulation of immune cell differentiation .

In summary, ginkgo diterpene lactones, particularly ginkgolide B, represent a class of bioactive compounds with significant therapeutic potential in acute and chronic cerebrovascular and

cardiovascular diseases. The injectable formulations of these compounds address pharmacokinetic limitations inherent to oral administration, enabling rapid and effective intervention in acute settings such as ischemic stroke. Ongoing research continues to elucidate their molecular mechanisms, optimize formulation technologies, and expand clinical indications, while safety monitoring remains integral to their clinical application. This review aims to comprehensively synthesize the pharmacological basis, formulation development, clinical efficacy, and safety considerations of ginkgo diterpene lactone injections, providing a foundation for future research and clinical practice.

1. Pharmacological Basis of Ginkgo Terpene Lactone Injection

1.1 Platelet-Activating Factor Antagonism

Ginkgo terpene lactones, particularly ginkgolides such as ginkgolide B, serve as potent antagonists of the platelet-activating factor (PAF) receptor, a critical mediator in platelet aggregation and thrombosis. These compounds competitively bind to PAF receptors on platelet membranes, effectively inhibiting PAF-induced platelet aggregation and thrombus formation. This antagonistic action is central to the therapeutic efficacy of ginkgo terpene lactone injections in ischemic conditions. Experimental evidence from animal models of cerebral ischemia-reperfusion injury demonstrates that administration of ginkgo terpene lactone injections significantly reduces cerebral infarct volume and diminishes microvascular thrombosis, indicating a protective effect against ischemic brain damage. For instance, in rat models subjected to middle cerebral artery occlusion (MCAO), treatment with ginkgo diterpene lactone meglumine injection (GDLMI) led to marked reductions in infarct size and neurological deficits, highlighting its efficacy in mitigating ischemic injury through PAF receptor blockade [9] Clinical meta-analyses further corroborate these findings, showing that ginkgo diterpene lactone meglumine injections improve neurological function scores and overall clinical efficacy in patients with acute ischemic stroke, with statistically significant improvements compared to control treatments [6,10] The combination of ginkgo terpene lactone injections with recombinant tissue plasminogen activator (rt-PA) thrombolytic therapy has been shown to enhance clinical outcomes, suggesting a synergistic effect in improving reperfusion and neurological recovery. Pharmacokinetic studies reveal that ginkgolides A, B, and C exhibit favorable plasma exposure and bioavailability profiles following intravenous administration, supporting their effective systemic action [11,12] Importantly, the platelet-activating factor antagonistic activity of ginkgolides is modulated by their chemical form; carboxylation in blood reduces their PAF antagonism, which should be considered in clinical applications [11] The safety profile of ginkgo terpene lactone injections is generally favorable, with no serious adverse drug reactions reported in clinical trials, underscoring their potential as adjunctive therapies in ischemic cerebrovascular diseases [6] Collectively, these data establish that the competitive inhibition of PAF receptors by ginkgo terpene lactones underlies their antithrombotic and neuroprotective effects, making them valuable agents in the management of ischemic stroke and related thrombotic disorders.

1.2 Neuroprotection and Anti-inflammatory Effects

Beyond their antiplatelet activity, ginkgo terpene lactones exhibit significant neuroprotective and anti-inflammatory properties that contribute to their therapeutic potential in cerebrovascular diseases. One key mechanism involves the inhibition of glutamate-mediated excitotoxicity, a major contributor to neuronal injury during ischemia. Ginkgo terpene lactones reduce excessive calcium influx into neurons by

modulating glutamate receptor activity, thereby attenuating calcium overload and subsequent oxidative stress-induced neuronal damage. Experimental studies in MCAO rat models have demonstrated that treatment with ginkgo diterpene lactone meglumine injection enhances antioxidant enzyme activities such as superoxide dismutase (SOD) and glutathione (GSH), while reducing malondialdehyde (MDA) levels, indicative of decreased lipid peroxidation and oxidative injury [9] At the molecular level, ginkgo terpene lactones downregulate the nuclear factor-kappa B (NF-κB) signaling pathway, leading to decreased expression of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). This anti-inflammatory effect mitigates the inflammatory cascade that exacerbates ischemic brain injury. In vitro studies further reveal that ginkgolide B activates the Akt/Nrf2 pathway, upregulating antioxidant proteins such as heme oxygenase-1 (HO-1) and quinone oxidoreductase 1 (Nqo1), which protect neurons from oxidative stress [13] The neuroprotective efficacy of ginkgolide B surpasses that of other ginkgolides and bilobalide, highlighting its therapeutic significance. Additionally, ginkgolide B has been shown to promote the proliferation of neural stem cells in vitro, suggesting a role in neural repair and regeneration post-injury. These multifaceted neuroprotective mechanisms, encompassing excitotoxicity inhibition, oxidative stress reduction, and inflammation suppression, position ginkgo terpene lactones as promising agents for ameliorating neuronal damage and enhancing recovery in ischemic stroke and other neurodegenerative conditions.

1.3 Cardiovascular and Cerebrovascular Protective Effects

Ginkgo terpene lactone injections also confer protective effects on the cardiovascular and cerebrovascular systems through multiple mechanisms. Vasodilation induced by ginkgo terpene lactones improves cerebral blood flow and tissue perfusion, which is critical in ischemic conditions. These compounds reduce blood viscosity and inhibit apoptosis of vascular endothelial cells, thereby preserving vascular integrity and function. In myocardial ischemia models, ginkgolide B administration decreases the release of myocardial enzymes such as creatine kinase and lactate dehydrogenase, markers of cardiac injury, and reduces infarct size through antioxidant and anti-apoptotic effects [12,14] Clinical evidence from multicenter studies indicates that ginkgo terpene lactone injections, when combined with conventional therapies, significantly lower the risk of recurrent cardiovascular and cerebrovascular events, underscoring their role in secondary prevention [10] Pharmacokinetic data support the sustained systemic presence of active ginkgolides following intravenous administration, facilitating their protective actions in target tissues [12] Furthermore, ginkgolide B's antagonism of PAF receptors contributes to the stabilization of myocardial and cerebral microcirculation, reducing ischemia-reperfusion injury and improving functional outcomes. The integration of these vascular protective, anti-thrombotic, and antioxidative mechanisms underlines the comprehensive cardiovascular and cerebrovascular benefits of ginkgo terpene lactone injections, making them valuable adjuncts in the management of ischemic heart and brain diseases.

2. Preparation Technology and Quality Research of Ginkgolide Injection

2.1 Extraction and Purification Process Optimization

The extraction and purification of ginkgolides, the active lactone components in Ginkgo biloba, are critical for producing high-quality injectable formulations with potent pharmacological effects. Traditional alcohol extraction methods combined with macroporous adsorption resin technology

have been demonstrated to efficiently enrich ginkgolides, achieving purities exceeding 90%. This approach leverages the selective adsorption properties of resins to concentrate ginkgolides from crude extracts, effectively removing many impurities and enhancing yield. However, the presence of allergenic compounds such as ginkgolic acids necessitates further refinement. The introduction of advanced techniques like supercritical fluid extraction (SFE) and membrane separation has significantly improved the recovery rates of lactone components while effectively eliminating sensitizing impurities. SFE, utilizing supercritical CO2, offers a green and efficient extraction method that preserves the bioactivity of ginkgolides and reduces solvent residues. Membrane separation further refines the extract by size exclusion and selective permeability, enhancing purity without harsh chemical treatments. More recently, high-speed counter-current chromatography (HSCCC) has been employed for the separation and purification of individual ginkgolide monomers. This technique is noted for its environmentally friendly profile, as it avoids solid supports and uses liquid-liquid partitioning, which enhances process controllability and scalability. HSCCC allows for the isolation of specific ginkgolide isomers with high purity and yield, facilitating the production of injectable formulations with consistent pharmacological profiles. Collectively, these optimized extraction and purification strategies not only improve the quality and safety of ginkgolide injections but also align with green chemistry principles, supporting sustainable pharmaceutical manufacturing. The integration of traditional and modern techniques ensures efficient enrichment, impurity removal, and monomer isolation, which are essential for meeting stringent quality standards and therapeutic efficacy requirements in clinical applications [15–17]

2.2 Formulation Stability and Safety Control

Ginkgolides, particularly ginkgolide B, exhibit chemical instability in aqueous solutions due to their lactone ring structures, which are prone to hydrolysis. This hydrolysis leads to ring opening, resulting in decreased biological activity and compromised therapeutic efficacy. Studies have shown that the hydrolysis rate is influenced by pH and temperature, with alkaline conditions accelerating degradation. To mitigate this, formulation strategies include the addition of stabilizing agents such as cyclodextrins, which can form inclusion complexes with ginkgolides, protecting the lactone ring from hydrolytic attack. Adjusting the pH of the injection solution to a mildly acidic range (approximately 4.5 to 5.5) further enhances stability by minimizing lactone ring opening. Ensuring the sterility and endotoxin-free status of ginkgolide injections is paramount, given their intravenous administration. Sterilization methods such as high-temperature autoclaving or aseptic filtration are employed to meet pharmacopeial standards. However, thermal sterilization must be carefully controlled to prevent degradation of sensitive ginkgolides. Hemolysis testing is a critical safety evaluation for injectable formulations; data indicate that ginkgolide concentrations below 0.5 mg/mL exhibit good hemocompatibility, whereas higher concentrations may induce red blood cell lysis, posing risks of hemolytic anemia or vascular complications. Therefore, concentration limits are established to balance therapeutic dosing with safety. Additionally, excipients such as solubilizers (e.g., Cremophor EL, PEG 400) and cryoprotectants (e.g., mannitol) are optimized to enhance solubility and stability without compromising safety. Freeze-drying (lyophilization) processes have been optimized to produce stable ginkgolide lyophilized powders with favorable reconstitution properties, ensuring long shelf life and ease of use. The freeze-drying cycle parameters, including shelf temperature and

chamber pressure, are meticulously controlled based on thermal analysis to prevent product collapse and maintain structural integrity. Overall, these formulation and process controls are essential to maintain the chemical stability, sterility, and safety of ginkgolide injections, thereby ensuring consistent clinical efficacy and patient safety [16,18,19]

2.3 Establishment of Quality Evaluation System

Robust quality evaluation systems are indispensable for ensuring the consistency, safety, and efficacy of ginkgolide injections. High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) has become the gold standard for quantitative analysis of ginkgolides in injection formulations. This technique offers high sensitivity, with detection limits reaching the nanogram level, enabling precise quantification of major active components such as ginkgolide A, B, and C. The method's specificity allows for the simultaneous detection of related impurities and degradation products, facilitating comprehensive quality control. Fingerprint chromatographic profiling is employed to characterize the overall chemical composition of the injection, including active ginkgolides and potential impurities like ginkgolic acids. This approach provides a holistic quality assessment, ensuring batch-to-batch consistency and detecting adulterations or deviations in raw materials. Moreover, biological activity assays, such as platelet-activating factor (PAF) antagonism tests, serve as complementary quality indicators. These bioassays reflect the pharmacodynamic consistency of the injection, correlating chemical composition with therapeutic potential. Incorporating bioactivity measurements into quality control frameworks enhances the predictive value of quality assessments for clinical efficacy. Recent advances also include the use of hyperspectral imaging combined with artificial intelligence algorithms to non-destructively monitor multiple quality attributes simultaneously, including chemical content, antioxidant activity, and anticoagulant properties. This innovative approach enables real-time, online quality monitoring during manufacturing, improving process control and product reliability. Collectively, integrating advanced chromatographic, spectrometric, and bioassay techniques establishes a comprehensive quality evaluation system that ensures the safety, potency, and consistency of ginkgolide injections, supporting their clinical application and regulatory compliance [15,17,20]

3. Clinical Applications and Safety Evaluation of Ginkgo Lactone Injection

3.1 Treatment of Cerebrovascular Diseases

Ginkgo lactone injection, predominantly containing active terpene lactones such as ginkgolides and bilobalide, has been extensively studied for its therapeutic effects in cerebrovascular diseases, particularly acute ischemic stroke and cerebral small vessel disease with cognitive impairment. Multiple randomized controlled trials have demonstrated that intravenous administration of ginkgo lactone injection at doses ranging from 10 to 20 mg per day significantly improves neurological deficit scores in patients with acute ischemic stroke. This neurofunctional improvement is likely attributable to the neuroprotective properties of ginkgolides, which modulate excitotoxicity by balancing excitatory and inhibitory amino acid neurotransmitters in the striatum, as evidenced by reductions in glutamate and aspartic acid levels and increases in GABA following treatment in animal models of middle cerebral artery occlusion [3] Moreover, ginkgo lactones exert antioxidative effects by attenuating lipid peroxidation and enhancing antioxidant enzyme activities, such as superoxide dismutase, thereby protecting erythrocytes and neuronal cells from ischemia-reperfusion injury [21] In patients with cerebral small vessel disease complicated by cognitive impairment, short-term use of ginkgo lactone

injection has been shown to enhance executive function and verbal memory, potentially through anti-inflammatory mechanisms that regulate hippocampal cytokines including IL-1β, IL-6, and TNF-α [22] Importantly, when combined with thrombolytic agents such as alteplase, ginkgo lactone injection does not increase the risk of hemorrhagic transformation, indicating a favorable safety profile in adjunctive therapy for ischemic stroke [23] Neuroimaging and biochemical studies further support the role of ginkgo lactones in promoting neuronal survival and functional recovery by activating signaling pathways such as Akt-CREB-BDNF, which are critical for neuroplasticity and repair after ischemic insult [23] Collectively, these findings underscore the clinical efficacy of ginkgo lactone injection in improving neurological outcomes in cerebrovascular diseases through multifaceted mechanisms including antioxidation, anti-excitotoxicity, anti-inflammation, and neurotrophic support.

3.2 Cardiovascular Diseases and Other Indications

Beyond cerebrovascular applications, ginkgo lactone injection has demonstrated beneficial effects in cardiovascular conditions such as coronary heart disease (CHD) with angina pectoris. Clinical studies reveal that intravenous administration of ginkgo lactones reduces the frequency of angina attacks and ameliorates electrocardiographic abnormalities, including ST-segment depression, likely through improved myocardial perfusion and anti-platelet aggregation effects . The platelet-activating factor (PAF) antagonism by ginkgolide B contributes to the stabilization of membrane phospholipids and reduction of ischemia-reperfusion myocardial injury, as shown in rat models where ginkgolide B decreased serum lactate dehydrogenase and preserved cardiac function [14] Preliminary investigations also suggest that ginkgo lactone injection may alleviate diabetic peripheral neuropathy by reducing pain and enhancing nerve conduction velocity, although further clinical trials are warranted to confirm these effects [24] Moreover, ongoing clinical research is exploring the potential of ginkgo lactones in preventing cerebral vasospasm following subarachnoid hemorrhage, leveraging their neuroprotective and vasodilatory properties [25] These diverse therapeutic indications highlight the broad pharmacological spectrum of ginkgo lactones, encompassing vascular protection, anti-inflammatory actions, and neurovascular modulation.

3.3 Adverse Reactions and Medication Precautions

Ginkgo lactone injection is generally well tolerated, with common adverse reactions including mild headache, nausea, skin rash, and injection site pain occurring in approximately 3% to 5% of patients; these events are typically transient and resolve without intervention [26] Serious adverse effects such as anaphylactic shock are rare but necessitate vigilance, especially in patients receiving high doses exceeding 20 mg/kg, which have been associated with thrombocytopenia [27] Contraindications include known hypersensitivity to ginkgo preparations, bleeding disorders, and pregnancy due to potential hemorrhagic risks. Given the influence of ginkgo lactones on platelet function and coagulation pathways, coagulation parameters should be monitored during therapy to mitigate bleeding complications [27] Additionally, ginkgo lactones may induce hepatic cytochrome P450 enzymes, particularly through bilobalide-mediated upregulation, which can attenuate the anticoagulant effects of warfarin, underscoring the importance of careful drug interaction assessment in patients on concurrent anticoagulant therapy [27] Overall, while ginkgo lactone injection exhibits a favorable safety profile, appropriate patient selection, dose regulation, and monitoring are essential to

optimize therapeutic outcomes and minimize risks.

Conclusion

Ginkgo biloba extract, particularly in the form of ginkgolide injection, has emerged as a multifaceted therapeutic agent with significant clinical implications in the management of acute and severe conditions such as ischemic stroke and coronary heart disease. From an expert perspective, its pharmacological profile—characterized by platelet-activating factor (PAF) antagonism, neuroprotection, and cardiovascular protection—positions it uniquely among current treatment modalities. This review underscores the importance of integrating diverse research findings to appreciate the compound's complex mechanisms and clinical potential fully.

The development of ginkgolide injection has been marked by continuous advancements in formulation technology and quality control measures. Innovations in purification techniques and stability optimization have enhanced the safety and efficacy of the product, while sophisticated quality evaluation systems, including HPLC-MS fingerprinting, have ensured batch-to-batch consistency and reliability. These technical improvements not only support regulatory compliance but also build clinician and patient confidence in the therapeutic use of ginkgolide injection.

Clinical evidence consistently demonstrates that ginkgolide injection is well tolerated at recommended dosages, with a favorable safety profile when used adjunctively with standard treatments. The synergistic effects observed in combination therapies highlight its potential to improve clinical outcomes in ischemic and cardiovascular pathologies. However, the risk of bleeding and hypersensitivity reactions, although relatively infrequent, necessitates vigilant monitoring and individualized risk assessment. Balancing these safety considerations with therapeutic benefits remains a critical aspect of clinical decision-making.

Looking forward, the trajectory of ginkgolide injection research should emphasize the development of long-acting formulations to enhance patient compliance and therapeutic consistency. Expanding its indications to encompass neurodegenerative disorders represents a promising frontier, given its neuroprotective properties. Moreover, the establishment of personalized medicine approaches—tailoring dosing regimens based on genetic, metabolic, and clinical parameters—could optimize efficacy while minimizing adverse effects. Such precision medicine strategies will require robust biomarker identification and validation through well-designed clinical trials.

In conclusion, ginkgolide injection exemplifies a successful translation of traditional pharmacological principles into modern clinical practice, supported by rigorous scientific validation and technological refinement. Its multifaceted actions and evolving clinical applications reflect a dynamic field where balancing efficacy, safety, and individualized care is paramount. Continued interdisciplinary research and innovation will be essential to fully harness its therapeutic potential and integrate it seamlessly into comprehensive treatment paradigms for cerebrovascular and cardiovascular diseases, as well as emerging indications in neurology.

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