Article

Hirudin: Pharmacology, pharmacokinetics, clinical applications, limitations, and future perspectives

WenJun Zhang
School of Life Sciences, Sun Yat-sen University, Guangzhou, China
E-mail: zhwj@mail.sysu.edu.cn, wjzhang@iaees.org

Received 30 March 2026; *Accepted 28 April 2026*; *Published online 5 May 2026*; *Published 1 March 2027*

Abstract
Hirudin is a 65-amino acid polypeptide originally isolated from the salivary glands of the medicinal leech *Hirudo medicinalis*, represents the most potent and specific natural inhibitor of thrombin discovered to date. Since its initial characterization in the 1950s, hirudin has evolved from a scarce natural product to a clinically available recombinant anticoagulant, with multiple derivatives approved for therapeutic use including lepirudin, desirudin, and bivalirudin. This review provides a comprehensive analysis of hirudin's discovery history, molecular structure, physicochemical properties, extraction and purification methodologies, pharmacokinetics, and extensive pharmacological activities. Beyond its established role as an anticoagulant, emerging evidence demonstrates that hirudin possesses diverse bioactivities including anti-inflammatory, antioxidant, neuroprotective, anti-fibrotic, wound-healing, anti-tumor, and anti-hyperuricemic effects. Clinical experience with hirudin and its derivatives spans acute coronary syndromes, heparin-induced thrombocytopenia, venous thromboembolism prophylaxis, and other thrombotic disorders. However, challenges including narrow therapeutic index, bleeding risk, renal dependence, and immunogenicity continue to limit broader application. This review synthesizes current knowledge while identifying critical gaps requiring further investigation, including optimization of dosing strategies, development of safer derivatives, expansion of non-anticoagulant therapeutic applications, and elucidation of molecular mechanisms underlying recently discovered bioactivities.

Keywords hirudin; thrombin inhibitor; anticoagulant; recombinant hirudin; lepirudin; desirudin; bivalirudin; pharmacology; clinical trials.

Network Biology
ISSN 2220-8879
URL: http://www.iaees.org/publications/journals/nb/online-version.asp
RSS: http://www.iaees.org/publications/journals/nb/rss.xml
E-mail: networkbiology@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Hirudin is an acidic polypeptide secreted by the salivary glands of *Hirudo medicinalis*, commonly known as the medicinal leech (Grzimek, 1974; Sawyer, 1986; Müller et al., 2016, 2017, 2020; Lukas et al., 2019; Chen et al., 2021; Wikipedia, 2026). As the strongest natural specific inhibitor of thrombin identified thus far,

hirudin has garnered sustained scientific and clinical interest since its discovery (Elliott and Tullett, 1984; Markwardt, 1994; Chen et al., 2021). The anticoagulant properties of leech extracts were first documented by Haycraft in 1884, with the active component subsequently designated "hirudin" by Jacoby in 1904 (Haycraft, 1883, 1884; Chen et al., 2021; Markwardt, 1991). However, the limited availability of natural hirudin constrained both research and therapeutic application for decades (Chen et al., 2021).

The advent of recombinant DNA technology in the 1980s revolutionized hirudin research and clinical development. In 1986, recombinant hirudin (r-hirudin) became available through genetic engineering, enabling large-scale production of biologically active hirudin with pharmacological properties closely resembling those of the natural compound (Chen et al., 2021; Rosenfeld et al., 1996). This technological breakthrough catalyzed extensive investigation into hirudin's therapeutic potential, culminating in the approval of several hirudin derivatives for clinical use (Chen et al., 2021; Markwardt, 2002; Nowak and Schrör, 2007).

Hirudin functions as a bivalent direct thrombin inhibitor, binding to both the catalytic active site and the anion-binding exosite I (fibrinogen recognition site) of thrombin (Greinacher & Warkentin, 2008; Rydel et al., 1990, 1991). This dual binding mechanism confers exceptional potency and specificity, distinguishing hirudin from indirect anticoagulants such as heparin that require antithrombin III as a cofactor (Markwardt, 1994; Johnson, 1994). Importantly, hirudin inhibits both free and clot-bound thrombin, a property that may confer clinical advantages in certain thrombotic conditions (Weitz et al., 1990; Greinacher & Warkentin, 2008).

Beyond its established anticoagulant and antithrombotic effects, accumulating evidence over the past two decades has revealed a broader pharmacological profile for hirudin and its derivatives (Sohn et al., 2001). These include wound repair promotion, anti-fibrotic activity, beneficial effects on diabetic complications, anti-tumor properties, anti-hyperuricemic effects, and neuroprotective actions in cerebral hemorrhage and ischemic stroke (Chen et al., 2021; Liu et al., 2020; Xia et al., 2023). The molecular mechanisms underlying these diverse bioactivities have been partially elucidated, revealing interactions with multiple signaling pathways including NF-κB, Nrf-2/HO-1, ERK1/2, and mTOR-regulated autophagy (Liu et al., 2020; Xia et al., 2023; Ma et al., 2023).

Despite these advances, hirudin's clinical application remains constrained by several limitations. Natural hirudin production is insufficient for large-scale therapeutic use, while recombinant hirudin exhibits a narrow therapeutic index with dose-dependent bleeding risk (Folkers et al., 1989; Chen et al., 2021; Direct Thrombin Inhibitor Trialists' Collaborative Group, 2002). Additionally, hirudin's predominantly renal elimination necessitates dose adjustment in patients with impaired kidney function (Fischer, 2002; Garcia et al., 2012). Immunogenicity, though weak, may lead to diminished or enhanced responsiveness upon repeated exposure (Greinacher & Warkentin, 2008).

This comprehensive review aims to synthesize the extensive body of literature on hirudin, spanning its discovery and structural characterization, extraction and production methodologies, pharmacokinetic properties, diverse pharmacological activities and underlying mechanisms, clinical applications and trial outcomes, safety considerations, and quality control standards. By systematically examining both established knowledge and emerging frontiers, this review seeks to provide a foundation for future research directions and therapeutic innovations involving hirudin and its derivatives.

2 Discovery and History

2.1 Early Observations and Initial Characterization

The medicinal use of leeches dates back to ancient civilizations, with records of leech therapy appearing in traditional medical texts across diverse cultures (Chen et al., 2021). In traditional Chinese medicine, leeches (Shuizhi; 水蛭) were documented in Shennong's Classic of Materia Medica as possessing the efficacy of

breaking blood stasis, expelling stagnation, and freeing the collateral vessels (Chinese Pharmacopoeia Committee, 2020; Chen et al., 2021; Fig. 1). However, the scientific investigation of leech-derived anticoagulant activity began in the late nineteenth century.

Fig. 1 *Hirudo medicinalis* (Dreamstime. 2026).

In 1884, Haycraft made the seminal observation that extracts from *Hirudo medicinalis* exhibited anticoagulant properties (Haycraft, 1884; Chen et al., 2021; Markwardt, 2002). This discovery marked the first scientific documentation of leech-derived anticoagulant activity. In 1904, Jacoby formally designated the active anticoagulant component as "hirudin" (Chen et al., 2021; Markwardt, 1991). Despite this early recognition, progress in isolating and characterizing hirudin remained limited for the subsequent half-century due to technical constraints.

2.2 Isolation and Structural Elucidation

A major milestone occurred in 1955 when Markwardt successfully obtained relatively pure hirudin from the salivary glands of *Hirudo medicinalis*, greatly facilitating subsequent research on thrombin inhibitors (Markwardt, 1955; Chen et al., 2021). This achievement enabled more detailed pharmacological characterization of hirudin's anticoagulant properties (Markwardt, 1970).

The complete amino acid sequence of hirudin was first described by Dodt and colleagues in 1984, representing a crucial advance in understanding the molecular basis of hirudin's biological activity (Dodt et al., 1984; Chen et al., 2021; Fig. 2). Hirudin was characterized as a single-chain polypeptide containing 65 amino acids with a molecular weight of approximately 7,000 daltons (Dodt et al., 1984; DrugFuture, 2024; Fig. 2). Several isoforms of hirudin were subsequently identified, including hirudin variant 1 (HV1, also designated hirudin-VV), hirudin variant 2 (HV2, or hirudin-IT), and hirudin variant 3 (HV3) (Wüstenhagen et al., 2020; Chen et al., 2021; Dodt et al., 1986).

The three-dimensional structure of hirudin and its interaction with thrombin were elucidated through X-ray crystallographic studies. The crystal structure of the hirudin-thrombin complex revealed that the amino-terminal domain of hirudin interacts with the active site of thrombin's catalytic triad residue serine-195, while the carboxy-terminal domain binds to the positively charged anion-binding exosite I (Rydel et al., 1990, 1991; Skrzypczak-Jankun et al., 1991; Fig. 2). Additional contact sites were identified that further enhance the binding affinity of hirudin to thrombin (Rydel et al., 1991). These structural insights provided the foundation for rational design of hirudin derivatives and synthetic thrombin inhibitors.

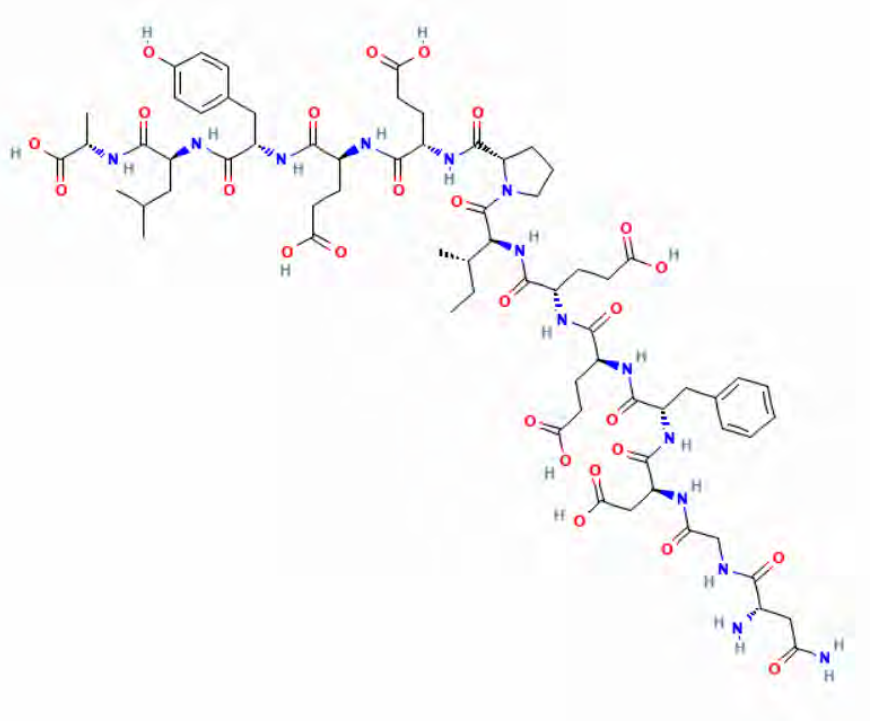

Fig. 2 Hirudin molecule (PubChem, 2004).

2.3 Recombinant Technology and Clinical Translation

The limited availability of natural hirudin from leech salivary glands constituted a major obstacle to both research and clinical development. The advent of recombinant DNA technology in the 1980s fundamentally transformed this landscape (Al-Badran and Al-Fadal, 2017). In 1986, recombinant hirudin (r-hirudin) was successfully produced through genetic engineering, with the cloned cDNA expressed in various host systems including Escherichia coli, Saccharomyces cerevisiae, and Pichia pastoris (Folkers et al., 1989; Haruyama and Wüthrich, 1989; Rydel et al., 1990, 1991; Rosenfeld et al., 1996; Johnson, 1994; Greinacher and Lubenow, 2001; Markwardt, 2002; Hsieh et al., 2014).

Recombinant hirudin differs from natural hirudin in one key structural aspect: natural hirudin contains a sulfated tyrosine residue at position 63, whereas recombinant hirudin lacks this post-translational modification (Folkers et al., 1989; Rydel et al., 1990, 1991; Marki et al., 1991). This desulfation results in an approximately two-fold reduction in potency without altering the specificity of thrombin inhibition.

The availability of recombinant hirudin enabled large-scale clinical investigation (Haruyama and Wüthrich, 1989; Folkers et al., 1989; Rydel et al., 1990, 1991). Early clinical studies demonstrated that hirudin provided more consistent anticoagulation compared with heparin, as gauged by activated partial thromboplastin time (aPTT) (Antman, 1994). Several hirudin derivatives subsequently received regulatory approval for clinical use, including lepirudin (Refludan), desirudin (Iprivask/Revasc), and the synthetic hirudin analog bivalirudin (Angiomax) (Chen et al., 2021; Greinacher & Warkentin, 2008).

The search for the development of hirudin from leech extract to genetically engineered products as an alternative anticoagulant has been extensively documented, with progress in molecular biology stimulating interest in the structure and function of hirudin (Markwardt, 2002). This historical trajectory illustrates the transformation of an ancient natural remedy into a modern biopharmaceutical agent.

3 Properties and Extraction

3.1 Sources of Hirudin

3.1.1 Natural Sources

Natural hirudin is secreted by the salivary glands of hematophagous leeches, primarily *Hirudo medicinalis* (Chen et al., 2021; Markwardt, 1994; Fig. 1). Other leech species have also been identified as sources of hirudin-like anticoagulant proteins, though *H. medicinalis* remains the most extensively studied source (Markwardt, 2002). The salivary glands of medicinal leeches produce hirudin as an evolutionary adaptation to prevent blood coagulation during feeding, enabling sustained blood ingestion.

Traditional Chinese medicine has utilized leeches (Shuizhi) for centuries, with documented applications in promoting blood circulation and removing blood stasis (Chen et al., 2021; Chinese Pharmacopoeia Committee, 2020). The recognition of leeches' therapeutic value in traditional medicine systems across multiple cultures provided early empirical evidence of hirudin's biological activity, preceding its scientific characterization by centuries (Chen et al., 2021).

3.1.2 Recombinant Production Systems

The limited yield of natural hirudin from leech salivary glands necessitated the development of recombinant production systems. Recombinant hirudin has been successfully expressed in multiple heterologous hosts, each offering distinct advantages and limitations (Rosenfeld et al., 1996; Markwardt, 2002).

Escherichia coli expression systems were among the earliest platforms developed for recombinant hirudin production. However, the absence of eukaryotic post-translational modification machinery and the tendency for inclusion body formation presented challenges for obtaining biologically active protein (Rosenfeld et al., 1996; Zhang et al., 2007).

Yeast expression systems have proven particularly successful for hirudin production. *Saccharomyces cerevisiae* has been employed for recombinant hirudin expression and secretion to the culture medium (Rosenfeld et al., 1996). The methylotrophic yeast *Pichia pastoris* has demonstrated remarkable efficiency, with one clone secreting recombinant hirudin at levels reaching 1.5 g/L (Rosenfeld et al., 1996). Using *P. pastoris* expression, recombinant hirudin was purified to greater than 97% purity with a recovery yield of 63%, and the purified product exhibited the predicted N-terminal amino acid sequence and thrombin inhibition kinetics comparable to hirudin isoforms produced in other heterologous systems (Rosenfeld et al., 1996).

Bacillus subtilis has also been explored as an expression host for recombinant hirudin (Rosenfeld et al., 1996). Optimization of fermentation conditions has been shown to dramatically increase recombinant hirudin expression levels across multiple expression systems (Rosenfeld et al., 1996; Zhang et al., 2007).

3.2 Chemical Structure

3.2.1 Primary Structure

Hirudin is a single-chain polypeptide composed of 64-66 amino acids, with a molecular weight of approximately 7,000 daltons (DrugFuture, 2024; Chen et al., 2021; Dodt et al., 1984). The primary structure of hirudin variant 1 (HV1) consists of 65 amino acid residues (Wüstenhagen et al., 2020; DrugFuture, 2024; Fig. 2). Several isoforms have been identified, designated as hirudin variant 1 (HV1, or hirudin-VV), hirudin variant 2 (HV2, or hirudin-IT), and hirudin variant 3 (HV3) (Chen et al., 2021; Dodt et al., 1986).

A key structural feature distinguishing natural hirudin from recombinant hirudin is the sulfation status of the tyrosine residue at position 63. Natural hirudin contains a sulfated tyrosine at this position, whereas recombinant hirudin produced in prokaryotic or yeast expression systems lacks this post-translational modification (Marki et al., 1991). Desulfation results in approximately two-fold reduction in antithrombin potency, without altering the specificity of thrombin inhibition.

3.2.2 Secondary and Tertiary Structure

The three-dimensional structure of hirudin is stabilized by three intramolecular disulfide bridges, which are essential for maintaining the proper folding and biological activity of the polypeptide (Dodt et al., 1986). The compact globular structure of the N-terminal domain contains the disulfide-bonded core, while the C-terminal domain adopts an extended conformation that facilitates interaction with thrombin's anion-binding exosite I (Rydel et al., 1990, 1991).

X-ray crystallographic analysis of the hirudin-thrombin complex has provided detailed insights into the molecular interactions underlying hirudin's potent inhibitory activity. The amino-terminal domain of hirudin interacts with the active site of thrombin's catalytic triad, specifically residue serine-195, while the carboxy-terminal domain binds to the positively charged anion-binding exosite I, also known as the fibrinogen recognition site (Rydel et al., 1990, 1991; Skrzypczak-Jankun et al., 1991). Additional contact sites have been identified that may further enhance the binding affinity of hirudin to thrombin (Rydel et al., 1991). These interactions result in an essentially irreversible complex formation.

3.2.3 Physicochemical Properties

Hirudin is characterized as a gray or white flaky substance or powder (DrugFuture, 2024). The isoelectric point (pI) of hirudin is approximately 3.9, reflecting its acidic nature due to the abundance of acidic amino acid residues (DrugFuture, 2024). Hirudin is soluble in water, physiological saline solution, and pyridine, but practically insoluble in alcohol, ether, acetone, and benzene (DrugFuture, 2024).

Stability studies indicate that hirudin deteriorates upon storage in sealed ampules, exposure to heat, and when maintained in solution with dilute acids (DrugFuture, 2024). These stability characteristics have implications for pharmaceutical formulation, storage conditions, and clinical handling of hirudin-based therapeutic products. Electricwala and colleagues (1990) conducted comprehensive physicochemical characterization of recombinant desulfatohirudin, providing essential data for pharmaceutical development.

3.3 Extraction and Purification

3.3.1 Natural Hirudin Isolation

The isolation of natural hirudin from leech salivary glands was first successfully achieved by Markwardt in 1955 (Markwardt, 1955; Chen et al., 2021). Subsequent improvements in isolation methodology were reported by Walsmann and Markwardt in 1985, enabling the preparation of hirudin with enhanced purity (Walsmann & Markwardt, 1985; DrugFuture, 2024). However, the inherent limitations of natural source extraction, including low yield and batch-to-batch variability, precluded large-scale therapeutic application (Markwardt, 2002; Chen et al., 2021).

3.3.2 Recombinant Hirudin Purification

The development of recombinant production systems necessitated robust purification methodologies to obtain hirudin of sufficient purity for research and clinical applications. Multiple chromatographic approaches have been developed and optimized for recombinant hirudin purification.

Hydrophobic chromatography and anion exchange chromatography have been successfully combined to achieve high-purity recombinant hirudin from fermentation broth (Zhu et al., 2021). This method effectively removes endotoxin and host cell proteins, yielding recombinant hirudin with high specific activity and purity suitable for pharmaceutical applications (Zhu et al., 2021).

Immobilized metal affinity chromatography (IMAC) has been identified as among the most efficient purification approaches with respect to purification fold and yield (Rosenfeld et al., 1996). Preparative high-performance liquid chromatography has also been employed for isolation of recombinant hirudin variant 2-Lys47 (rHV2-Lys47) produced by genetically engineered yeast strains (Rosenfeld et al., 1996).

A straightforward two-step chromatography procedure was developed for purifying recombinant hirudin expressed in P. pastoris, achieving greater than 97% purity with a recovery yield of 63% (Rosenfeld et al.,

1996). This process yielded multigram quantities of biologically active recombinant hirudin suitable for structure-function analyses and preclinical studies (Rosenfeld et al., 1996).

For recombinant hirudin produced in *S. cerevisiae*, purification schemes have been developed that include ammonium sulfate precipitation followed by chromatographic steps, providing evidence supporting the feasibility of scale production and clinical application of recombinant hirudin produced by genetic engineering methods.

3.3.3 Process Scale-Up Considerations

The translation of laboratory-scale purification protocols to industrial-scale production presents multiple challenges. Process development for the production of recombinant hirudin in S. cerevisiae has been systematically investigated, encompassing both upstream fermentation optimization and downstream purification (Rosenfeld et al., 1996). Factors influencing overall process yield include fermentation conditions, cell harvesting efficiency, and optimization of chromatographic parameters.

Decolorization steps may be required during purification of recombinant hirudin to meet pharmaceutical quality specifications (Lehman et al., 1993). The development of robust, scalable, and cost-effective purification processes remains essential for ensuring adequate supply of pharmaceutical-grade hirudin for clinical applications.

3.4 Pharmacokinetics

3.4.1 Absorption and Distribution

The pharmacokinetic properties of hirudin have been extensively characterized in healthy human subjects and various animal models. Following intravenous administration, hirudin exhibits first-order elimination kinetics after an initial distribution phase (Markwardt et al., 1984). The plasma concentration decline is most adequately described by a biexponential equation corresponding to a two-compartment model (Markwardt et al., 1984).

The volume of distribution of hirudin is relatively small, with a mean value of 12.9 liters calculated in healthy human subjects following intravenous administration (Markwardt et al., 1984). This limited distribution volume is consistent with the predominantly intravascular localization of hirudin and its primary action on circulating and clot-bound thrombin.

After subcutaneous injection, hirudin maintains low but sustained plasma levels (approximately 0.5 AT-U/mL) for a prolonged period (Markwardt et al., 1984). Maximum plasma concentrations following subcutaneous administration occur at approximately 128 ± 55 minutes in horses, with a terminal half-life of 561 ± 364 minutes (Feige et al., 2010). These pharmacokinetic characteristics support both intravenous and subcutaneous routes of administration, providing flexibility for different clinical scenarios.

3.4.2 Elimination and Half-Life

The plasma half-life of hirudin following intravenous administration ranges from 50 to 65 minutes in humans (Sciencedirect Neuroscience, 2024; Markwardt et al., 1984). The biologic half-life is approximately 2 hours (Sciencedirect Neuroscience, 2024). Studies in healthy human subjects have reported a mean elimination half-life of 0.84 hours (approximately 50 minutes) following intravenous administration of 1000 AT-U/kg (Markwardt et al., 1984).

Following subcutaneous administration, the half-life is prolonged, with reported values of 150-240 minutes in humans. In horses, elimination half-lives of 58 and 80 minutes were observed in two animals following intravenous injection, while subcutaneous administration resulted in a terminal half-life of 561 ± 364 minutes (Feige et al., 2010).

Hirudin is excreted predominantly by the kidneys, with up to 50% of the administered dose appearing in the urine in active form within 24 hours (Markwardt et al., 1984; Fischer, 2002). The renal clearance of hirudin

has important clinical implications, necessitating dose adjustment in patients with impaired kidney function to avoid accumulation and excessive anticoagulation (Fischer, 2002; Garcia et al., 2012).

Metabolic fate studies indicate that hirudin is catabolized predominantly in the kidney, whereas the thrombin-hirudin complex undergoes catabolism in both liver and kidney (Sciencedirect Neuroscience, 2024). This differential catabolism may influence the pharmacokinetic-pharmacodynamic relationship under various physiological and pathological conditions.

3.4.3 Pharmacokinetic-Pharmacodynamic Relationships

Hirudin prolongs coagulation parameters including thrombin time (TT), partial thromboplastin time (PTT), and prothrombin time (PT) in a dose-dependent manner that correlates with plasma hirudin concentration (Markwardt et al., 1984).

The relationship between hirudin plasma concentration and anticoagulant effect is relatively predictable, contributing to the more consistent anticoagulation observed with hirudin compared with heparin (Antman, 1994). However, inter-individual variability in pharmacokinetic parameters has been documented, supporting the need for monitoring in certain clinical settings.

Notably, hirudin does not prolong bleeding time when administered to normal volunteers at therapeutic doses, suggesting that the antithrombotic effect can be achieved without necessarily impairing primary hemostasis. This dissociation between anticoagulant effect and bleeding time prolongation may represent a favorable characteristic compared with other anticoagulants.

4 Pharmacological Activities and Mechanisms

Like other regulators for biological network (Huang and Zhang, 2012; Li and Zhang, 2013; Zhang, 2016a-b, 2017e, 2018, 2026, 2027a-d, 2028a-c), hirudin plays an important role in various aspects. Fig. 3 dipicts the pharmacological activities and mechanisms of hirudin.

4.1 Anticoagulant and Antithrombotic Effects

4.1.1 Mechanism of Thrombin Inhibition

Hirudin functions as the most potent and specific known inhibitor of thrombin, the serine protease that plays a central regulatory role in hemostasis and blood coagulation (Markwardt, 1994; Li et al., 1998). As a bivalent direct thrombin inhibitor, hirudin binds to both the catalytic active site and the fibrinogen-binding exosite I of thrombin, forming an essentially irreversible 1:1 stoichiometric complex (Greinacher & Warkentin, 2008; Rydel et al., 1990, 1991).

The amino-terminal domain of hirudin interacts with the active site pocket containing the catalytic triad of thrombin, while the carboxy-terminal domain binds to the positively charged anion-binding exosite I, which serves as the recognition site for fibrinogen and other thrombin substrates (Rydel et al., 1990, 1991; Skrzypczak-Jankun et al., 1991). This dual binding mechanism confers exceptional potency, with a dissociation constant in the picomolar range (Markwardt, 1994; Johnson, 1994).

Unlike heparin, which requires antithrombin III as a cofactor, hirudin inhibits thrombin directly and independently of other coagulation factors (Markwardt, 1994; DrugFuture, 2024). Furthermore, hirudin inhibits both free circulating thrombin and thrombin bound to fibrin clots, a property that may confer clinical advantages in preventing thrombus extension and re-thrombosis (Weitz et al., 1990; Breddin, 1994; Greinacher & Warkentin, 2008).

The formation of the hirudin-thrombin complex prevents fibrinogen cleavage to fibrin and inhibits all other thrombin-catalyzed reactions, including activation of clotting factors V, VIII, and XIII, as well as thrombin-induced platelet activation (Desirudin, 2024; Markwardt, 1994). This comprehensive inhibition of thrombin's multiple functions distinguishes hirudin from agents that selectively target only certain

thrombin-mediated processes (Binnie et al., 1990).

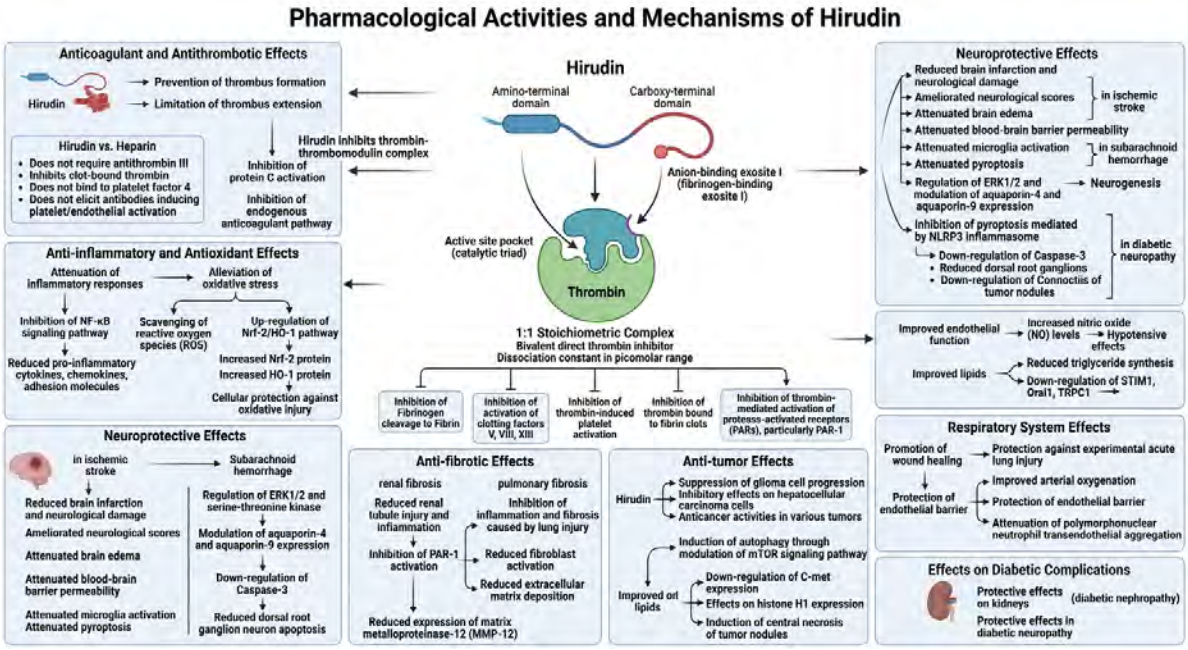

Fig. 3 Pharmacological activities and mechanisms of hirudin.

4.1.2 Antithrombotic Efficacy

Hirudin has been demonstrated to possess potent antithrombotic effects in numerous preclinical and clinical studies (Chen et al., 2021; Fenton et al., 1998; Li et al., 1998). The antithrombotic activity of hirudin derives from its ability to prevent thrombus formation and limit thrombus extension through inhibition of thrombin-mediated fibrin formation and platelet activation.

Experimental studies have shown that hirudin prevents thrombus formation after arterial injury, with two large clinical trials demonstrating marked reduction in acute clinical events (Clinical Trials, 2024). Hirudin is a specific thrombin inhibitor which acts independent of antithrombin III and is able to bind to fluid phase and clot bound thrombin, producing a relatively stable level of anticoagulation when compared with heparin (Recombinant Hirudins, 2012).

The antithrombotic efficacy of hirudin has been evaluated across multiple clinical indications, including acute coronary syndromes, venous thromboembolism, and prevention of thrombosis in patients undergoing surgical procedures (Greinacher & Warkentin, 2008; Direct Thrombin Inhibitor Trialists' Collaborative Group, 2002).

4.1.3 Comparison with Heparin

Several important distinctions exist between hirudin and heparin anticoagulants. Hirudin does not require antithrombin III for activity, inhibits clot-bound thrombin (which heparin cannot access), does not bind to platelet factor 4, and does not elicit antibodies that induce platelet and endothelial cell activation (Greinacher & Warkentin, 2008; Garcia et al., 2012). These properties make hirudin particularly suitable for patients with heparin-induced thrombocytopenia (HIT), a serious immune-mediated adverse reaction to heparin therapy.

However, hirudin also inhibits the ability of thrombin to activate fibrinolysis through complexing with thrombomodulin and thus activating protein C (Sciencedirect Neuroscience, 2024). This inhibition of the

endogenous anticoagulant pathway may partially offset the antithrombotic benefits of direct thrombin inhibition.

4.2 Anti-inflammatory and Antioxidant Effects

4.2.1 Anti-inflammatory Mechanisms

Beyond its anticoagulant properties, hirudin exhibits significant anti-inflammatory effects that have been documented in multiple experimental systems. Hirudin has been shown to possess anti-inflammatory properties in studies of cerebral ischemia and other pathological conditions (Xia et al., 2023).

In a rat model of cerebral ischemia, intracerebral hirudin injection reduced brain infarction and neurological damage, effects that were associated with attenuation of inflammatory responses (Xia et al., 2023). Hirudin treatment significantly ameliorated neurological scores and attenuated brain edema, blood-brain barrier permeability, inflammatory response, and microglia activation in subarachnoid hemorrhage rats (Hirudin Alleviates Early Brain Injury, 2025).

The anti-inflammatory effects of hirudin are mediated, at least in part, through inhibition of the nuclear factor-κB (NF-κB) signaling pathway. Studies in high glucose-treated rat dorsal root ganglion neurons demonstrated that hirudin inhibited activation of the NF-κB pathway (Liu et al., 2020). NF-κB is a central transcription factor regulating the expression of numerous pro-inflammatory cytokines, chemokines, and adhesion molecules (Huang and Zhang, 2012; Li and Zhang, 2013; Zhang, 2016a-b, 2017e, 2018, 2026, 2027a-d, 2028a-c).

In acute lung injury models, hirudin inhibited inflammation and fibrosis caused by lung injury and played a role in lung protection as an anti-inflammatory mediator (Effect of hirudin on acute lung injury, 2011). The protective effect of hirudin in acute lung injury may be partly related to protection of the endothelial barrier and attenuation of polymorphonuclear neutrophil transendothelial aggregation in lung (The protective effect of hirudin on acute lung injury, 2011).

4.2.2 Antioxidant Effects

Hirudin possesses antioxidant properties that contribute to its protective effects in various pathological conditions characterized by oxidative stress. In a rat model of cerebral ischemia, hirudin alleviated oxidative stress and enhanced neurogenesis in ischemic rats (Xia et al., 2023).

Studies in high glucose-induced oxidative stress models have elucidated the molecular mechanisms underlying hirudin's antioxidant effects. Hirudin treatment of dorsal root ganglion neurons exposed to high glucose resulted in scavenging of reactive oxygen species (ROS) and up-regulation of the nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf-2/HO-1) pathway (Liu et al., 2020). The Nrf-2/HO-1 pathway is a critical endogenous antioxidant defense mechanism that regulates the expression of multiple cytoprotective enzymes.

Quantitative analysis demonstrated that hirudin treatment reduced ROS levels in high glucose-exposed neurons (Liu et al., 2020). The expression of Nrf-2 protein in hirudin groups was significantly higher than in the high glucose group, while the expression of HO-1 protein in hirudin groups was also elevated compared with the high glucose group (Liu et al., 2020). These findings indicate that hirudin activates the Nrf-2/HO-1 antioxidant pathway, contributing to cellular protection against oxidative injury.

4.2.3 Combined Anti-inflammatory and Antioxidant Effects

The anti-inflammatory and antioxidant effects of hirudin often occur concurrently and synergistically in pathological conditions. In cerebral ischemia, hirudin alleviates cognitive deficits by attenuating oxidative stress and promoting hippocampal neurogenesis through the regulation of ERK1/2 and serine-threonine kinase in MCAO-subjected rats (Xia et al., 2023).

The ability of hirudin to simultaneously modulate inflammatory and oxidative stress pathways may confer

particular therapeutic advantages in conditions where both processes contribute to tissue injury, including ischemic stroke, diabetic complications, and acute lung injury.

4.3 Neuroprotective Effects

4.3.1 Protection in Ischemic Stroke

Hirudin has demonstrated beneficial effects in ischemic stroke through multiple mechanisms. Intracerebral hirudin injection reduced brain infarction and neurological damage in rats subjected to middle cerebral artery occlusion (MCAO) (Xia et al., 2023). Hirudin alleviated cognitive deficits by attenuating oxidative stress and promoting hippocampal neurogenesis through regulation of ERK1/2 and serine-threonine kinase signaling pathways (Xia et al., 2023).

The neuroprotective effects of hirudin in ischemic stroke involve both attenuation of oxidative stress and enhancement of neurogenesis in ischemic rats (Xia et al., 2023). These dual actions—protection of existing neurons and promotion of new neuron formation—suggest that hirudin may facilitate both acute neuroprotection and longer-term functional recovery following ischemic brain injury.

4.3.2 Effects in Cerebral Hemorrhage

The role of hirudin in cerebral hemorrhage represents a complex and clinically significant area of investigation. Research progress on the pharmacological effects of hirudin in preventing and treating cerebral hemorrhage has been summarized, with mechanisms including anti-thrombin activity, inhibition of apoptosis, reduction of oxidative stress response, decrease of inflammatory reaction, inhibition of aquaporin-4 expression, prevention of glial fibrillary acidic protein upregulation, inhibition of thrombospondin expression, and regulation of Na+, K+-ATPase activity (Research progress on hirudin in cerebral hemorrhage, 2024).

Edema formation has been linked to thrombin toxicity induced by blood clot at the acute stage of intracerebral hemorrhage (Recombinant hirudin treatment modulates aquaporin-4, 2024). Thrombin induces cell toxicity in neurons, microglia, and astrocytes. Recombinant hirudin treatment modulates aquaporin-4 and aquaporin-9 expression after intracerebral hemorrhage in vivo (Recombinant hirudin treatment modulates aquaporin-4, 2024).

In subarachnoid hemorrhage models, hirudin treatment significantly ameliorated neurological scores and attenuated brain edema, blood-brain barrier permeability, inflammatory response, microglia activation, and pyroptosis (Hirudin Alleviates Early Brain Injury, 2025). The neuroprotective effect of hirudin on early brain injury following subarachnoid hemorrhage is attributed to its ability to inhibit pyroptosis mediated by the NLRP3 inflammasome (Hirudin Alleviates Early Brain Injury, 2025).

4.3.3 Neuroprotection in Diabetic Neuropathy

Hirudin has demonstrated protective effects in models of diabetic neuropathy. The effects of hirudin on high glucose-induced oxidative stress and inflammatory pathways in rat dorsal root ganglion neurons have been investigated (Liu et al., 2020). The activity of dorsal root ganglion neurons can be promoted by hirudin under high glucose conditions (Liu et al., 2020).

The protective effects of hirudin on the inhibition of high glucose-induced dorsal root ganglion neuron damage mainly include scavenging reactive oxygen species, up-regulating the Nrf-2/HO-1 pathway, inhibiting activation of the NF-κB pathway, down-regulating the expression of Caspase-3, and reducing dorsal root ganglion neuron apoptosis (Liu et al., 2020). These findings suggest potential therapeutic applications for hirudin in diabetic peripheral neuropathy.

4.4 Anti-fibrotic Effects

4.4.1 Mechanisms of Anti-fibrotic Activity

Hirudin has been demonstrated to possess anti-fibrosis effects across multiple organ systems (Chen et al., 2021). The anti-fibrotic activity of hirudin is attributed to its ability to inhibit thrombin-mediated activation of

protease-activated receptors (PARs), particularly PAR-1, which play central roles in promoting fibroblast activation and extracellular matrix deposition.

In renal fibrosis models, hirudin has been shown to reduce renal tubule injury and inflammation in unilateral ureteral obstruction (UUO) mice (Recombinant Hirudin, 2024). The anti-fibrotic effects of hirudin in the kidney involve attenuation of inflammatory cell infiltration and reduction of pro-fibrotic cytokine expression.

4.4.2 Pulmonary Fibrosis

Hirudin inhibits inflammation and fibrosis caused by lung injury and plays a role in lung protection as an anti-inflammatory mediator (Effect of hirudin on acute lung injury, 2011). The ability of hirudin to attenuate both inflammatory and fibrotic responses in the lung suggests potential therapeutic applications in conditions such as idiopathic pulmonary fibrosis and acute respiratory distress syndrome.

In studies examining the effect of hirudin on acute lung injury, hirudin treatment reduced the expression of matrix metalloproteinase-12 (MMP-12), an enzyme implicated in extracellular matrix degradation and tissue remodeling (Effect of hirudin on acute lung injury, 2011). The correlation between inflammation factors and expression of protease-activated receptor-1 (PAR-1) was examined after hirudin pre-treatment (Effect of hirudin on acute lung injury, 2011).

4.5 Anti-tumor Effects

4.5.1 Glioma

Hirudin has demonstrated anticancer pharmacological effects through suppression of glioma cell progression (Ma et al., 2023). Hirudin inhibits glioma growth through mTOR-regulated autophagy (Ma et al., 2023). Glioma is the most common primary malignant brain tumor, and survival outcomes remain poor, highlighting the need for novel therapeutic approaches (Ma et al., 2023).

The molecular target and mechanism of hirudin's anti-glioma activity have been investigated, revealing that hirudin dose-dependently inhibits glioma cell proliferation and induces autophagy through modulation of the mTOR signaling pathway (Ma et al., 2023). These findings suggest potential applications of hirudin or hirudin-derived agents in neuro-oncology.

4.5.2 Hepatocellular Carcinoma

Hirudin has been shown to exhibit significant inhibitory effects on the proliferation of hepatocellular carcinoma cells. Studies using HepG2 and Huh-7 hepatocellular carcinoma cell lines demonstrated that hirudin suppressed cell proliferation in a concentration-dependent manner (Inhibitory Effect of Hirudin on Hepatocellular Carcinoma Cells, 2024).

In vivo studies using H22 hepatoma-bearing mice have further demonstrated the anti-tumor effects of hirudin (Anticancer effect of hirudin on H22 tumor cells, 2011). The mechanism may involve down-regulation of C-met expression in hepatoma cells (Anticancer effect of hirudin on H22 tumor cells, 2011). Additional studies have examined the effects of hirudin on histone H1 expression in mice bearing H22 hepatocarcinoma cell-derived tumors (Effects of Hirudin on Histone H1 Expressions, 2024).

4.5.3 Other Tumors

It has been reported that hirudin exhibits excellent anticancer activities in the treatment of various tumors, including human glioma, non-small cell lung cancer, and bladder cancer (Ma et al., 2023). The role of endogenous thrombin in tumor implantation, seeding, and spontaneous metastasis has been investigated, with hirudin inducing a considerable lag period in the appearance of tumor growth compared with phosphate-buffered saline treatment, though it had no effect on established tumor nodule growth in vivo or on tumor growth in vitro (Role of endogenous thrombin in tumor implantation, 2024). Hirudin treatment induced central necrosis of tumor nodules, and greater protection was noted with longer duration of treatment (Role of

endogenous thrombin in tumor implantation, 2024).

4.6 Wound Healing Effects

Hirudin has demonstrated potent wound repair effects in experimental studies (Chen et al., 2021; Recombinant Hirudin, 2024). Hirudin promotes wound healing in Sprague-Dawley rats after laser surgery at doses of 10 and 15 mg/kg (Recombinant Hirudin, 2024).

In vitro and in vivo studies have examined the inhibition of skin scar formation by hirudin (In vitro and In vivo Inhibition of Skin Scar by Hirudin, 2015). The results showed that hirudin can inhibit the proliferation of fibroblasts in human skin scars, and hirudin ointment demonstrated practical potential in treatment and prevention of animal skin scar hyperplasia (In vitro and In vivo Inhibition of Skin Scar by Hirudin, 2015).

The wound healing effects of hirudin are likely mediated through multiple mechanisms, including improved microcirculation through antithrombotic activity, modulation of inflammatory responses, and direct effects on fibroblast function and extracellular matrix remodeling.

4.7 Anti-hyperuricemic Effects

Hirudin has been reported to possess anti-hyperuricemia effects (Chen et al., 2021; Recombinant Hirudin, 2024). The anti-hyperuricemic activity of hirudin adds to its diverse pharmacological profile and suggests potential applications in the management of gout and other conditions associated with elevated uric acid levels. The mechanisms underlying this effect remain to be fully elucidated but may involve modulation of urate transporter expression or activity.

4.8 Effects on Blood Pressure and Lipid Metabolism

4.8.1 Blood Pressure Regulation

Hirudin has been shown to have effects on blood pressure regulation. In hypertensive patients, hirudin inhibited thrombin activity and protected vascular endothelium, effectively improving coagulation function and endothelial function (Effect of hirudin on coagulation and endothelial function in hypertensive patients, 2019). The improvement in endothelial function was associated with increased nitric oxide (NO) levels (Effect of hirudin on coagulation and endothelial function in hypertensive patients, 2019).

An antiprocoagulant complex isolated from lyophilized medicinal leeches exerted pronounced antithrombotic, thrombolytic, and hypotensive effects in experimental animals after intravenous injection (Biological activity and pharmacological properties, 1999). These findings suggest that hirudin may contribute to blood pressure reduction through improvement of vascular function and microcirculation.

4.8.2 Lipid Metabolism

Recombinant hirudin has been shown to significantly improve lipids and endothelial functions in apolipoprotein E knockout (ApoE-/-) mice, down-regulating expression levels of STIM1, Orai1, and TRPC1, and thus delaying the occurrence and development of atherosclerosis (Effect and mechanism of recombinant hirudin on atherosclerotic plaques, 2016).

Studies have indicated that hirudin can affect lipid metabolic pathways, increasing fatty acid oxidation and thereby enabling the liver to more fully utilize fat in energy metabolism, reducing triglyceride synthesis (Effect of hirudin on triglycerides, 2025). These lipid-modulating effects, combined with hirudin's anticoagulant and anti-inflammatory properties, suggest potential benefits in the prevention and treatment of atherosclerosis and related cardiovascular diseases.

4.9 Respiratory System Effects

Hirudin has demonstrated protective effects in acute lung injury models. Hirudin significantly limited lung ischemia-reperfusion injury-induced derangements in vascular permeability and intraalveolar inflammatory cell sequestration, resulting in improved arterial oxygenation after ischemia and 4 hours of reperfusion (Crosstalk Between Thrombosis and Inflammation, 2006). Thrombin promotes lung ischemia-reperfusion

injury, and hirudin protected against experimental acute lung injury (Crosstalk Between Thrombosis and Inflammation, 2006).

The protective effect of hirudin on acute lung injury with collaterals damaged by toxic stasis in mice has been examined, revealing that the pathophysiological mechanism of hirudin in the acute lung injury experimental model may be partly related to the protection of endothelial barrier and thus attenuating polymorphonuclear neutrophil transendothelial aggregation in lung (The protective effect of hirudin on acute lung injury, 2011).

4.10 Effects on Diabetic Complications

Hirudin has demonstrated beneficial effects on diabetic complications, including diabetic nephropathy and diabetic neuropathy (Chen et al., 2021; Research progress on diabetic nephropathy with leech, 2024). Hirudin demonstrates anti-coagulant, anti-fibrotic, anti-thrombotic, and anti-inflammatory properties, exhibiting significant protective effects on the kidneys (Research progress on diabetic nephropathy with leech, 2024).

The protective effects of hirudin in diabetic complications are likely mediated through multiple mechanisms, including attenuation of oxidative stress, inhibition of inflammatory pathways, reduction of fibrosis, and improvement of microcirculation. These multi-target actions may provide particular advantages in the complex pathophysiology of diabetic complications.

5 Clinical Trials and Outcomes

5.1 Clinical Applications and Formulations

5.1.1 Approved Hirudin Derivatives

Several hirudin derivatives have received regulatory approval for clinical use. Lepirudin (Refludan) is a recombinant hirudin consisting of 65 amino acids with a molecular weight of 6,979.5 kDa, which can directly inhibit the active site pocket and the fibrinogen binding site of free and clot-bound thrombin (Chen et al., 2021). Desirudin (Iprivask/Revasc) is identical in amino acid sequence to natural hirudin variant 1 except that it lacks a sulfate group on tyrosine at residue 63 (DrugFuture, 2024). Desirudin is approved for prevention of venous thromboembolism in patients undergoing elective hip replacement surgery (Desirudin, 2024).

Bivalirudin (Angiomax) is a synthetic 20-amino acid hirudin analog that retains the bivalent binding properties of hirudin but has a shorter duration of action and improved safety profile (Direct Antithrombins, 2024). Bivalirudin has the same binding sites to thrombin as hirudin but has a shorter pharmacological action and is safer for clinical use (Direct Antithrombins, 2024).

5.1.2 Novel Derivatives in Development

In recent years, a number of novel derivatives have been exploited because of the great demand for hirudin in physicochemical and clinical studies, including recombinant RGD-hirudin, boronophenylalanine-modified hirudin, neorudin, Annexin V-hirudin 3-ABD, and others (Chen et al., 2021). Advantages of these derivatives include reducing bleeding risks through targeting thrombus sites and increasing antithrombotic efficacy (Chen et al., 2021).

Recombinant neorudin is a developing anticoagulant drug for thrombotic diseases whose phase I clinical studies have been conducted, revealing that recombinant neorudin significantly increased Thrombin Time (TT) in both plasma surrounding the thrombus and peripheral blood, and reduced the wet weight of the thrombus (Recombinant neorudin, 2024).

5.2 Clinical Studies in Cardiovascular Disease

5.2.1 Acute Coronary Syndromes

Hirudin has been extensively studied in patients with acute coronary syndromes (ACS). Initial clinical trials showed promising results: hirudin, as compared with heparin, provided a more consistent level of

anticoagulation, as gauged by the activated partial thromboplastin time (About: Hirudin in acute myocardial infarction, 2024). Doses of intravenous hirudin and heparin were established, which should allow testing of the "thrombin hypothesis": that more potent inhibition of thrombin will translate into improved clinical outcome for patients with acute myocardial infarction (About: Hirudin in acute myocardial infarction, 2024).

The GUSTO IIb trial included patients with non-ST segment elevation ACS who received either unfractionated heparin (UFH) or hirudin (0.1 mg/kg bolus, 0.1 mg/kg/hr infusion). At 24 hours, the risk of death or nonfatal myocardial infarction was reduced in hirudin-treated patients compared with UFH-treated patients (1.3% versus 2.1%, respectively; P = 0.001) (Sciencedirect Neuroscience, 2024). The primary endpoint of death or nonfatal MI at 30 days was reached in 8.9% and 9.8% of patients, respectively (OR, 0.89; P = 0.006) (Sciencedirect Neuroscience, 2024). The risk of moderate bleeding was increased with hirudin treatment (8.8% versus 7.7%, respectively; P = 0.03) (Sciencedirect Neuroscience, 2024).

The OASIS-1 study included 909 patients with unstable angina or suspected MI without ST segment elevation who were randomly assigned to receive UFH, low-dose hirudin (0.2 mg/kg bolus, 0.1 mg/kg/hr infusion), or moderate-dose hirudin (0.4 mg/kg bolus, 0.15 mg/kg/hr infusion) (Sciencedirect Neuroscience, 2024). Hirudin reduced the incidence of the composite outcome of cardiovascular death, MI, or refractory angina at 7 days compared with UFH (OR, 0.57; 95% CI, 0.32 to 1.02) as well as the composite outcome of death, MI, or refractory/severe angina requiring revascularization at 7 days (OR, 0.49; 95% CI, 0.27 to 0.86) (Sciencedirect Neuroscience, 2024). Overall event rates were lowest in the moderate-dose hirudin group (Sciencedirect Neuroscience, 2024).

The favorable results in the OASIS-1 study prompted the large phase 3 OASIS-2 trial, which randomly assigned 10,141 patients with non-ST segment elevation ACS to receive a 72-hour infusion of either moderate-dose hirudin or UFH (Sciencedirect Neuroscience, 2024). The primary outcome (composite of death or MI at 7 and 35 days) was reported to occur in 3.6% of patients treated with hirudin and 4.2% of those treated with UFH (OR, 0.87; 95% CI, 0.75 to 1.01) (Sciencedirect Neuroscience, 2024).

However, despite these encouraging efficacy results, early randomized trials failed to demonstrate a clear net clinical benefit of hirudin compared with heparin because of a higher bleeding risk and only modest efficacy gains (John W. Eikelboom, 2024). Higher hirudin doses, when given with thrombolytic therapy, were associated with intracranial bleeding complications in the GUSTO IIA, TIMI 9A, and HIT III trials, necessitating premature halting of these studies (Gusto, 1994; Neuhaus et al., 1994; Antman, 1994).

5.2.2 Meta-analysis Findings

A comprehensive meta-analysis (Zhang, 2024) based on individual patients' data from randomized trials comparing direct thrombin inhibitors (hirudin, bivalirudin, argatroban, efegatran, or inogatran) with heparin included 35,970 patients from 11 randomized trials (Direct Thrombin Inhibitor Trialists' Collaborative Group, 2002; Shabareesh andKaur, 2016). Compared with heparin, direct thrombin inhibitors were associated with a lower risk of death or myocardial infarction at the end of treatment (4.3% vs 5.1%; odds ratio 0.85 [95% CI 0.77-0.94]; p=0.001) and at 30 days (7.4% vs 8.2%; 0.91 [0.84-0.99]; p=0.02) (Direct Thrombin Inhibitor Trialists' Collaborative Group, 2002).

This benefit was due primarily to a reduction in myocardial infarctions (2.8% vs 3.5%; 0.80 [0.71-0.90]; p<0.001) with no apparent effect on deaths (1.9% vs 2.0%; 0.97 [0.83-1.13]; p=0.69) (Direct Thrombin Inhibitor Trialists' Collaborative Group, 2002). A reduction in death or myocardial infarction was seen with hirudin and bivalirudin but not with univalent agents (Direct Thrombin Inhibitor Trialists' Collaborative Group, 2002). Compared with heparin, there was an increased risk of major bleeding with hirudin, but a reduction with bivalirudin (Direct Thrombin Inhibitor Trialists' Collaborative Group, 2002). There was no excess in intracranial hemorrhage with direct thrombin inhibitors (Direct Thrombin Inhibitor Trialists' Collaborative

Group, 2002).

Based on the results of this meta-analysis, hirudin appears to be more effective than unfractionated heparin in the treatment of patients with ACS, but it is associated with an increased rate of major bleeding (Direct thrombin inhibitors, 2024).

5.2.3 Coronary Angioplasty and Percutaneous Coronary Intervention

In randomized studies in patients with coronary artery disease, hirudin shows a slight decrease in angioplasty-associated acute ischemic complication without a higher risk of bleeding (Glusa, 1998). However, the beneficial effect on long-term outcome could not be demonstrated.

Two large clinical trials showed marked reduction in acute clinical events but no long-term benefits in reducing restenosis during angioplasty (Clinical Trials, 2024). These findings suggest that while hirudin provides acute antithrombotic protection during and immediately following percutaneous coronary intervention, it does not prevent the longer-term processes of neointimal hyperplasia and restenosis.

5.3 Heparin-Induced Thrombocytopenia

Hirudin has established a critical role in the management of heparin-induced thrombocytopenia (HIT), a serious immune-mediated adverse reaction to heparin therapy characterized by thrombocytopenia and paradoxical thrombosis. Hirudin does not bind to platelet factor 4, nor does it elicit antibodies that induce platelet and endothelial cell activation; thus it can be safely administered to patients with HIT (Sciencedirect Neuroscience, 2024; Greinacher & Warkentin, 2008).

The use of hirudin in the management of HIT is discussed extensively in the literature, with hirudin recognized as an important therapeutic option for patients who develop this complication (Sciencedirect Neuroscience, 2024; Greinacher & Warkentin, 2008). The most important adverse effects in this population are hemorrhages and the induction of anti-hirudin antibodies (Heparin-induced thrombocytopenia, 2024). Major hemorrhages were not significantly increased in patients with HIT compared with a historical control group, but prospective data comparing hirudin and heparinoids such as danaparoid are lacking (Heparin-induced thrombocytopenia, 2024).

A systematic review and meta-analysis examining the effectiveness and safety of non-heparin anticoagulants for the treatment of HIT included bivalirudin and other hirudins among the evaluated agents (Nilius et al., 2021). The pooled rates of platelet recovery ranged from 74% (bivalirudin) to 99% (fondaparinux), thromboembolism from 1% (fondaparinux) to 7% (danaparoid), major bleeding from 1% (DOAC) to 14% (bivalirudin), and death from 7% (fondaparinux) to 19% (bivalirudin) (Nilius et al., 2021). Confidence intervals were mostly overlapping, and results were not influenced by patient population, diagnostic test used, study design, or type of article (Nilius et al., 2021).

5.4 Venous Thromboembolism

Hirudin was shown to be slightly better than heparin in the prevention of venous thromboembolism in patients undergoing hip replacement (Agnelli & Sonaglia, 2000). In the treatment of established deep vein thrombosis, hirudin also demonstrated efficacy comparable or superior to heparin (Glusa, 1998).

Desirudin, a recombinant hirudin, is specifically approved for prevention of venous thromboembolism in patients undergoing elective hip replacement surgery (Desirudin, 2024). Bleeding complications reported with the use of desirudin in patients undergoing hip-replacement surgery are similar to those reported with heparin and enoxaparin (Desirudin, 2024).

5.5 Other Clinical Applications

5.5.1 Hemodialysis

Hirudin has been evaluated as an anticoagulant in experimental hemodialysis (Hirudin as anticoagulant in experimental hemodialysis, 2024). The use of hirudin in hemodialysis caused a long-lasting, dose-dependent

anticoagulant effect, characterized by the prevention of increasing pressure before the capillary dialyzer and reduced drop in fibrinogen and platelets during hemodialysis (Hirudin as anticoagulant in experimental hemodialysis, 2024).

The properties of hirudin that make it suitable for hemodialysis anticoagulation include its predictable dose-response relationship, lack of requirement for antithrombin III, and absence of cross-reactivity with heparin-induced antibodies.

5.5.2 Hirulog Clinical Evaluation

Hirulog (bivalirudin) has been extensively evaluated in clinical studies. In a randomized, placebo-controlled study involving 54 human volunteers, intravenous infusion of hirulog over 15 minutes showed a rapid, dose-dependent prolongation of activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) (Anticoagulant Activity of Hirulog, 2025). There was a corresponding dose-dependent increase in plasma hirulog levels (Anticoagulant Activity of Hirulog, 2025).

The peptide was rapidly cleared with a half-life of 36 minutes and a total body clearance rate of 0.43 L/kg/h (Anticoagulant Activity of Hirulog, 2025). Similar activity was observed following subcutaneous injection but with sustained pharmacodynamic and pharmacokinetic behavior (Anticoagulant Activity of Hirulog, 2025). There was a significant correlation between pharmacokinetic and pharmacodynamic variables for both intravenous ($r = 0.8$, $p < 0.001$) and subcutaneous administration ($r = 0.7$, $p = 0.002$) (Anticoagulant Activity of Hirulog, 2025).

Aspirin administration did not modify the peptide's activity, and hirulog infusion in subjects who had received aspirin was not associated with any significant changes in template bleeding time (Anticoagulant Activity of Hirulog, 2025). During prolonged intravenous infusions for up to 24 hours, hirulog exhibited sustained anticoagulant activity with no evidence for a cumulative effect (Anticoagulant Activity of Hirulog, 2025). In all phases of the study, hirulog administration was generally well-tolerated (Anticoagulant Activity of Hirulog, 2025).

5.6 Quality Control and Quality Standards

5.6.1. Analytical Methods

Quality control of hirudin and its derivatives relies on multiple analytical methodologies. Laboratory assays for the evaluation of recombinant hirudin have been developed, including a biochemically defined anti-IIa assay that may be useful in quality control since reagents are easily standardized (Laboratory Assays for the Evaluation of Recombinant Hirudin, 2024). However, the relevance of the results of the anti-IIa assay to clinical conditions remains to be determined (Laboratory Assays for the Evaluation of Recombinant Hirudin, 2024).

Ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) methods have been developed and validated for simultaneously determining novel recombinant hirudin derivatives (such as neorudin) and their active metabolites in human serum (Development, validation, and clinical pharmacokinetic application, 2024). The extraction recoveries and matrix effects at three quality control levels were satisfactory, and the stabilities during storage, preparation, and analysis were confirmed (Development, validation, and clinical pharmacokinetic application, 2024).

High-performance liquid chromatography (HPLC) has been employed for the determination of hirudin content, including measurement of hypoxanthine in Hirudo and determination of hirudin by thrombin-based assays (Quality comparison of Hirudo, 2008). These analytical methods are essential for ensuring batch-to-batch consistency and pharmaceutical quality of hirudin-based products.

5.6.2 Pharmacopoeial Standards

The Chinese Pharmacopoeia includes specifications for Hirudo (leech) and its processed products, including

methods for quality assessment of both water and alcohol extracts (Quality comparison of Hirudo, 2008). These standards provide a regulatory framework for ensuring the quality of leech-derived medicinal products in traditional medicine applications.

5.6.3 Purity and Characterization

Recombinant hirudin produced for pharmaceutical applications must meet stringent purity specifications. Typical purity requirements exceed 95% as determined by reverse-phase HPLC and SDS-PAGE analysis (Hirudin Pichia Pastoris, 2024). The removal of endotoxin and host cell proteins from recombinant hirudin fermentation broth is essential for producing material suitable for human administration (Zhu et al., 2021).

5.7 Safety Evaluation

5.7.1 Adverse Effects

The adverse effect profile of hirudin has been characterized in clinical studies and post-marketing surveillance. Reported adverse effects include fever, injection site bleeding, epistaxis, gastrointestinal bleeding, hematuria, abnormal liver function, heart failure, cough, and bronchospasm (Recombinant Hirudin Adverse Effects, 2024). These adverse effects primarily reflect the anticoagulant activity of hirudin and its impact on hemostatic function.

The most clinically significant adverse effect is bleeding, which is dose-dependent and represents the primary safety concern with hirudin therapy. In a rabbit ear bleeding model, hirudin produced more bleeding than heparin when the agents were used in doses that increased the APTT ratio to the same extent (Hirudin causes more bleeding than heparin, 2024). These studies highlight the pitfalls of extrapolating from experience with heparin when choosing tests to monitor new antithrombotics (Hirudin causes more bleeding than heparin, 2024).

Serious allergic reactions including anaphylaxis may occur with hirudins, with the highest risk on hirudin re-exposure or in patients with glomerular filtration rate less than 30 mL/min (Direct Thrombin Inhibitor, 2024). Bleeding complications and epidural hematoma are additional safety concerns, with high risk if lumbar puncture is performed while on direct thrombin inhibitors (Direct Thrombin Inhibitor, 2024).

5.7.2 Immunogenicity

Hirudin is a surprisingly weak immunogen, and its administration has exhibited no side effects, particularly on platelets (About: Hirudin, a new therapeutic tool?, 2024). However, hirudin does have weak immunogenicity, so that diminished (or, rarely, increased) responsiveness after repeated dosing is possible (Sciencedirect Neuroscience, 2024).

Anti-hirudin antibodies have been documented in some patients receiving recombinant hirudin therapy (Liebe et al., 2002). The formation of anti-hirudin antibodies may affect the pharmacokinetics and pharmacodynamics of hirudin, potentially leading to altered anticoagulant response.

5.7.3 Drug Interactions

Hirudin interacts with other medications that affect hemostasis. When combined with drugs that increase bleeding risk (such as oral anticoagulants), the anticoagulant effect is enhanced and the risk of bleeding increases (Recombinant Hirudin Adverse Effects, 2024). This necessitates careful monitoring and dose adjustment when hirudin is used in combination with other antithrombotic agents.

5.7.4 Special Populations

Recombinant hirudins are pregnancy category B drugs. Animal studies have not shown teratogenic effects, but hirudins can cross the placental barrier in rats; it is unknown whether hirudins cross the human placenta (Hirudin Derivative, 2024). The safety of hirudin in pregnancy has not been established through adequate and well-controlled studies.

Hirudin requires dose adjustment in patients with renal impairment due to its predominantly renal

elimination (Fischer, 2002; Garcia et al., 2012). Accumulation of hirudin in renal insufficiency increases the risk of bleeding complications, necessitating careful monitoring and dose reduction.

5.7.5 Reversal and Monitoring

Unlike heparin, which can be reversed with protamine sulfate, there is no specific antidote for hirudin. This lack of a reversal agent represents a significant limitation in clinical practice, particularly in settings of bleeding complications or emergency surgery. The effectiveness of hirudin anticoagulation may be assessed through measuring the PTT, although the relationship between PTT prolongation and clinical outcomes is not perfectly defined (Hirudin Derivative, 2024).

6 Limitations, Unmet Needs, and Future Research Directions

6.1 Identified Limitations and Challenges

6.1.1 Narrow Therapeutic Index and Bleeding Risk

The narrow therapeutic index of hirudin represents a fundamental limitation to its broader clinical application. The increased risk of major bleeding with hirudin compared with heparin, documented in multiple clinical trials and meta-analyses, underscores the challenge of achieving optimal antithrombotic efficacy while minimizing hemorrhagic complications (Direct Thrombin Inhibitor Trialists' Collaborative Group, 2002). The results from GUSTO IIA, TIMI 9A, and HIT III trials, which were prematurely halted due to intracranial bleeding complications when hirudin was combined with thrombolytic therapy, highlight this limitation.

6.1.2 Renal Dependence

The predominantly renal elimination of hirudin creates significant challenges for patients with impaired kidney function (Fischer, 2002; Garcia et al., 2012). Dose adjustment protocols have been developed, but the risk of accumulation and bleeding remains elevated in this population. The development of hirudin derivatives with alternative elimination pathways or reduced renal dependence would address an important unmet clinical need.

6.1.3 Lack of Specific Antidote

The absence of a specific reversal agent for hirudin distinguishes it unfavorably from heparin (which can be reversed with protamine) and direct oral anticoagulants for which specific reversal agents (such as idarucizumab for dabigatran and andexanet alfa for factor Xa inhibitors) have been developed. The development of an effective hirudin antidote would substantially enhance the safety profile and clinical utility of hirudin-based anticoagulants.

6.1.4 Immunogenicity

Although hirudin is generally a weak immunogen, the development of anti-hirudin antibodies can occur and may alter the pharmacokinetic and pharmacodynamic response to therapy (Sciencedirect Neuroscience, 2024; Liebe et al., 2002). Strategies to further reduce immunogenicity, including modifications to the hirudin molecule or novel formulation approaches, warrant investigation.

6.1.5 Short Half-Life

The relatively short half-life of hirudin (50-65 minutes following intravenous administration) necessitates continuous infusion for maintenance of therapeutic anticoagulation (Sciencedirect Neuroscience, 2024; Markwardt et al., 1984). Longer-acting hirudin derivatives or sustained-release formulations could improve convenience and potentially enhance therapeutic efficacy by providing more stable anticoagulation.

6.2 Areas Requiring Further Investigation

6.2.1 Mechanistic Studies of Non-anticoagulant Effects

While numerous bioactivities beyond anticoagulation have been documented for hirudin, including anti-inflammatory, antioxidant, anti-fibrotic, anti-tumor, and neuroprotective effects (Chen et al., 2021; Liu et al., 2020; Xia et al., 2023; Ma et al., 2023), the molecular mechanisms underlying many of these activities

remain incompletely understood. Systematic investigation of these mechanisms could reveal novel therapeutic targets and facilitate the rational design of hirudin derivatives optimized for specific indications.

6.2.2 Clinical Translation of Novel Indications

Many of the recently discovered pharmacological activities of hirudin have been demonstrated primarily in preclinical models. Translation of these findings to clinical applications requires rigorous evaluation through well-designed clinical trials. Priority areas for clinical investigation include neuroprotection in ischemic stroke and cerebral hemorrhage, anti-tumor therapy in selected malignancies, treatment of fibrotic diseases, and management of diabetic complications.

6.2.3 Optimization of Dosing Strategies

The optimal dosing of hirudin for various clinical indications remains incompletely defined. Further studies are needed to establish evidence-based dosing regimens that maximize therapeutic benefit while minimizing bleeding risk. Individualized dosing strategies based on patient characteristics (including renal function, age, body weight, and concomitant medications) may improve the risk-benefit profile.

6.2.4 Development of Novel Derivatives

The development of hirudin derivatives with improved pharmacological properties represents an active area of research. Advantages of newer derivatives include reducing bleeding risks through targeting thrombus sites and increasing antithrombotic efficacy (Chen et al., 2021). Continued exploration of structure-activity relationships may yield derivatives with enhanced selectivity, prolonged half-life, reduced immunogenicity, and alternative routes of administration.

6.2.5 Long-term Outcomes and Safety

Most clinical trials of hirudin have focused on relatively short-term outcomes. Longer-term safety and efficacy data are needed, particularly for chronic applications such as prevention of recurrent thrombosis or treatment of chronic diseases where hirudin's non-anticoagulant effects may be relevant.

6.2.6 Comparative Effectiveness Research

Direct comparisons between hirudin derivatives and newer anticoagulants, including direct oral anticoagulants, are limited. Comparative effectiveness research could help define the optimal positioning of hirudin-based therapies within the broader anticoagulant armamentarium.

6.3 Future Perspectives

6.3.1 Precision Medicine Approaches

The application of precision medicine principles to hirudin therapy could enhance both efficacy and safety. Pharmacogenomic factors influencing hirudin response, including polymorphisms in thrombin, coagulation factors, or drug metabolism pathways, warrant investigation. Biomarker-guided dosing strategies may enable more individualized and safer anticoagulation.

6.3.2 Novel Drug Delivery Systems

Innovative drug delivery systems could address several limitations of current hirudin formulations. Liposomal encapsulation, nanoparticle-based delivery, and sustained-release formulations may prolong half-life, improve bioavailability, enable oral administration, or achieve targeted delivery to specific tissues or pathological sites. Studies of hirudin stabilization and release from lipid-assemblies coated with hydrophobically modified dextran have demonstrated that coated liposomes stabilize hirudin and result in greater retention of its ability to inhibit thrombin's enzymatic activity (The stabilization and release of hirudin from liposomes, 2024).

6.3.3 Combination Therapies

The combination of hirudin with other therapeutic agents may produce synergistic benefits. Combinations with antiplatelet agents, thrombolytics, or agents targeting complementary pathways in inflammation, fibrosis, or tumor biology could expand therapeutic applications. However, careful evaluation of potential additive or

synergistic adverse effects, particularly bleeding risk, will be essential.

6.3.4 Biosimilar Development

As patents on original recombinant hirudin products expire, the development of biosimilar hirudin preparations may increase accessibility and reduce costs. Robust analytical and clinical comparability assessments will be necessary to ensure the safety and efficacy of biosimilar products.

6.3.5 Integration with Traditional Medicine

The long history of leech use in traditional medicine systems, particularly traditional Chinese medicine, provides a rich empirical foundation for investigating hirudin's therapeutic potential (Chen et al., 2021; Chinese Pharmacopoeia Committee, 2020). Systematic investigation of traditional applications using modern scientific methodologies may reveal novel therapeutic indications and mechanistic insights.

7 Conclusion

Hirudin, from its initial isolation from medicinal leech salivary glands to its current status as a clinically available recombinant anticoagulant, exemplifies the successful translation of a natural product into modern biopharmaceutical therapy. The unique mechanism of hirudin as a bivalent direct thrombin inhibitor confers potent and specific anticoagulant activity, with demonstrated clinical benefits in acute coronary syndromes, heparin-induced thrombocytopenia, and venous thromboembolism prophylaxis (Warkentin, 2004; Shabareesh and Kaur, 2016). The advent of recombinant DNA technology has enabled large-scale production of hirudin and facilitated the development of multiple derivatives with improved pharmacological properties.

Beyond its established role as an anticoagulant, emerging evidence reveals a remarkably diverse pharmacological profile for hirudin. Anti-inflammatory, antioxidant, neuroprotective, anti-fibrotic, wound-healing, anti-tumor, and anti-hyperuricemic effects have been documented, suggesting potential therapeutic applications extending far beyond thrombosis management. The molecular mechanisms underlying these activities are being progressively elucidated, revealing interactions with multiple signaling pathways and cellular processes.

However, significant challenges remain. The narrow therapeutic index of hirudin, its dose-dependent bleeding risk, predominant renal elimination, lack of a specific antidote, and weak immunogenicity continue to limit broader clinical application. Addressing these limitations through the development of novel derivatives, optimized dosing strategies, innovative drug delivery systems, and precision medicine approaches represents a critical research priority.

The rich history and ongoing scientific investigation of hirudin underscore the enduring value of natural products as sources of therapeutic innovation. As our understanding of hirudin's complex biology continues to expand, new opportunities for therapeutic application will undoubtedly emerge, potentially establishing hirudin and its derivatives as versatile agents for a spectrum of diseases extending well beyond the cardiovascular system.

References

Agnelli G, Sonaglia F. 2000. Prevention of venous thromboembolism. Thromb Research, 97(1): V49-62. https://pubmed.ncbi.nlm.nih.gov/10668808/

Al-Badran AI, Al-Fadal SA. 2017. Production and purification of constructed recombinant hirudin in BL21(DE3) strain. JBEI, 3: 62–70

Antman EM. 1994. Hirudin in acute myocardial infarction: safety report from the Thrombolysis and Thrombin Inhibition in Myocardial Infarction (TIMI) 9A trial. Circulation, 90: 1624-1630. doi:

10.1161/01.cir.90.4.1624

Binnie CG, Erickson BW, Hermans J. 1990. Inhibition of thrombin by synthetic hirudin peptides. FEBS Letters, 270(1-2): 85-89. https://www.sciencedirect.com/science/article/pii/001457939081240O

Breddin HK. 1994. Perspectives of the Clinical use of Hirudin. In: Anticoagulation (Doutremepuich C, eds). Springer, New York, NY, USA. https://doi.org/10.1007/978-1-4612-2668-0_8

Chen JR, Xie XF, Zhang HQ, et al. 2021. Pharmacological activities and mechanisms of hirudin and its derivatives - a review. Frontiers in Pharmacology, 12: 660757. https://doi.org/10.3389/fphar.2021.660757

Chinese Pharmacopoeia Committee. 2020. Pharmacopoeia of the People's Republic of China. China Medical Science Press, Beijing, China

Direct Thrombin Inhibitor Trialists' Collaborative Group. 2002. Direct thrombin inhibitors in acute coronary syndromes: principal results of a meta-analysis based on individual patients' data. The Lancet, 359(9303): 294-302. https://doi.org/10.1016/S0140-6736(02)07495-0

Dodt J, Müller HP, Seemüller U, Chang JY. 1984. The complete amino acid sequence of hirudin, a thrombin specific inhibitor: application of colour carboxymethylation. FEBS Letters, 165(2): 180-184. https://doi.org/10.1016/0014-5793(84)80165-9

Dodt J, Machleidt W, Seemüller U, Maschler R, Fritz H. 1986. Isolation and characterization of hirudin isoinhibitors and sequence analysis of hirudin PA. Biological Chemistry Hoppe-Seyler, 367(8): 803-811. doi: 10.1515/bchm3.1986.367.2.803

Dreamstime. 2026. Medicinal Leech-hirudo medicinalis during therapy sessions. https://www.dreamstime.com/medicinal-leech-hirudo-medicinalis-therapy-sessions-medicinal-leech-hirudo-medicinalis-image102712099. Accessed on April 18, 2026

DrugFuture. 2024. Hirudin. https://www.drugfuture.com/chemdata/hirudin.html

Electricwala A, Irons L, Wait R, et al. 1990. Physicochemical properties of recombinant desulfatohirudin. Thrombosis and Haemostasis, 63(3): 499-503. https://pubmed.ncbi.nlm.nih.gov/2402752/

Elliott JM, Tullett PA. 1984. The status of the medicinal leech Hirudo medicinalis in Europe and especially in the British isles. Biological Conservation, 29(1): 15-26. https://doi.org/10.1016/0006-3207(84)90011-9

Feige K, Dennler M, Kästner SBR, et al. 2010. Pharmacokinetics of recombinant hirudin in healthy horses. Equine Veterinary Journal, 36(2): 135-141. https://doi.org/10.2746/0425164044868666

Fenton JW, Ofosu FA, Brezniak DV, Hassouna HI. 1998. Thrombin and antithrombotics. Seminars in Thrombosis and Hemostasis, 24(2): 87–91. doi:10.1055/s-2007-995828

Fischer KG. 2002. Hirudin in renal insufficiency. Seminars in Thrombosis and Hemostasis, 28(5): 467-482. https://doi.org/10.1055/s-2002-35288

Folkers PJ, Clore GM, Driscoll PC, Dodt J, Köhler S, Gronenborn AM. 1989. Solution structure of recombinant hirudin and the Lys-47-Glu mutant: a nuclear magnetic resonance and hybrid distance geometry-dynamical simulated annealing study. Biochemistry, 28(6): 2601–2617. doi:10.1021/bi00432a038

Garcia DA, Baglin TP, Weitz JI, Samama MM. 2012. Parenteral anticoagulants: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest, 141(2 Suppl): e24S-e43S. https://doi.org/10.1378/chest.11-2291

Greinacher A, Lubenow N. 2001. Recombinant hirudin in clinical practice: Focus on lepirudin. Circulation, 103: 1479–1484. https://doi.org/10.1161/01.cir.103.10.1479

Greinacher A, Warkentin TE. 2008. The direct thrombin inhibitor hirudin. Thrombosis and Haemostasis, 99(5): 819-829. doi: 10.1160/TH07-11-0693https://doi.org/10.1160/TH07-11-0693

Grzimek B. 1974. Grzinek's Animal Life Encyclopedia (Vol. 1). Van Nostrand Reinhold Co, NY, USA

Haruyama H, Wüthrich K. 1989. Conformation of recombinant desulfatohirudin in aqueous solution determined by nuclear magnetic resonance. Biochemistry, 28(10): 4301–4312. doi:10.1021/bi00436a027

Haycraft JB. 1883. IV. On the action of a secretion obtained from the medicinal leech on the coagulation of the blood. Proceedings of the Royal Society of London, 36(228–231): 478–487. doi:10.1098/rspl.1883.0135

Haycraft JB. 1884. Über die Einwirkung eines Secretes des officinellen Blutegels auf die Gerinnbarkeit des Blutes. Archiv für Experimentelle Pathologie und Pharmakologie, 18: 209-217. doi: 10.1007/BF01833843

Hsieh YSY, Wijeyewickrema LC, Wilkinson BL, Pike RN, Payne RJ. 2014. Total synthesis of homogeneous variants of hirudin P6: A post-translationally modified anti-thrombotic leech-derived protein. Angew. Chem. Int. Ed. Engl., 53: 3947–3951. https://doi.org/10.1002/anie.201310777

Huang JQ, Zhang WJ. 2012. Analysis on degree distribution of tumor signaling networks. Network Biology, 2(3): 95-109. http://www.iaees.org/publications/journals/nb/articles/2012-2(3)/analysis-on-degree-distribution-of-tumor-signaling-networks.pdf

Johnson PH. 1994. Hirudin: clinical potential of a thrombin inhibitor. Annual Review of Medicine, 45: 165-177. https://doi.org/10.1146/annurev.med.45.1.165

Li JR, Zhang WJ. 2013. Identification of crucial metabolites/reactions in tumor signaling networks. Network Biology, 3(4): 121-132. http://www.iaees.org/publications/journals/nb/articles/2013-3(4)/crucial-metabolites-reactions-in-tumor-signaling.pdf

Li T, Wan C, Chen H. 1998. A novel high quality specific antithrombotic drug--hirudin. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi, 15(3): 306-310. https://pubmed.ncbi.nlm.nih.gov/12553260/

Liebe V, Brückmann M, Fischer KG, Haase KK, Borggrefe M, Huhle G. 2002. Biological relevance of anti-recombinant hirudin antibodies--results from in vitro and in vivo studies. Seminars in Thrombosis and Hemostasis, 28(5): 483-490. https://doi.org/10.1055/s-2002-35289

Liu W, Liang XC, Shi Y. 2020. Effects of hirudin on high glucose-induced oxidative stress and inflammatory pathway in rat dorsal root ganglion neurons. Chinese Journal of Integrative Medicine, 26(3): 197-204. https://doi.org/10.1007/s11655-019-2712-8

Lukas P, Wolf R, Rauch BH, Hildebrandt JP, Müller C. 2019. Hirudins of the Asian medicinal leech, Hirudinaria manillensis: Same same but different. Parasitol. Research, 18: 2223–2233. https://doi.org/10.1007/s00436-019-06365-z

Ma Y, Wu SB, Zhao FY, et al. 2023. Hirudin inhibits glioma growth through mTOR-regulated autophagy. Journal of Cellular and Molecular Medicine, 27(18): 2701–2713. https://pmc.ncbi.nlm.nih.gov/articles/PMC10494300/

Marki WE, Grossenbacher H, Grütter MG, Liersch MH, Meyhack B, Heim J. 1991. Recombinant hirudin: genetic engineering and structure analysis. Seminars in Thrombosis and Hemostasis, 17(2): 88-93. doi: 10.1055/s-2007-1002594

Markwardt F. 1955. Die Isolierung und chemische Charakterisierung des Hirudins. Hoppe-Seylers Z. Physiol. Chem, 308: 147–156.

Markwardt F. 1970. Hirudin as an inhibitor of thrombin. Methods in Enzymology, 19: 924-930. https://www.sciencedirect.com/science/chapter/bookseries/pii/0076687970190823?via%3Dihub

Markwardt F. 1991. Hirudin and derivatives as anticoagulant agents. Thrombosis and Haemostasis, 66(1): 141-152. https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0038-1646383

Markwardt F. 1994. The development of hirudin as an antithrombotic drug. Thrombosis Research, 74(1): 1-23. https://www.sciencedirect.com/science/article/pii/0049384894900329

Markwardt F. 2002. Hirudin as alternative anticoagulant-a historical review. Seminars in Thrombosis and Hemostasis, 28(5): 405-414. https://doi.org/10.1055/s-2002-35292

Markwardt F, Nowak G, Stürzebecher J, Griessbach U, Walsmann P, Vogel G. 1984. Pharmacokinetics and anticoagulant effect of hirudin in man. Thrombosis and Haemostasis, 52(2): 160-163. https://pubmed.ncbi.nlm.nih.gov/6523433/

Müller C, Haase M, Lemke S, Hildebrandt JP. 2017. Hirudins and hirudin-like factors in Hirudinidae: Implications for function and phylogenetic relationships. Parasitol Research, 116: 313–325. https://doi.org/10.1007/s00436-016-5294-9

Müller C, et al. 2016. More than just one: Multiplicity of hirudins and hirudin like factors in the medicinal leech, Hirudo medicinalis. Mol. Genet. Genomics, 291: 227–240. https://doi.org/10.1007/s00438-015-1100-0

Müller C, Lukas P, Böhmert M, Hildebrandt JP. 2020. Hirudin or hirudin-like factor—That is the question. FEBS Letters, 594: 841–850. https://doi.org/10.1002/1873-3468.13683.

Neuhaus KL, von Essen R, Tebbe U, Jessel A, Heinrichs H, Maurer W, Döring W, Harmjanz D, Kötter V, Kalhammer E, et al. 1994. Safety observations from the pilot phase of the randomized r-Hirudin for Improvement of Thrombolysis (HIT-III) study: a study of the Arbeitsgemeinschaft Leitender Kardiologischer Krankenhausärzte. Circulation, 90(4): 1638-1642. doi: 10.1161/01.cir.90.4.1638

Nilius H, Kaufmann J, Cuker A, Nagler M. 2021. Comparative effectiveness and safety of anticoagulants for the treatment of heparin-induced thrombocytopenia. American Journal of Hematology, 96(7): 805-815. doi: 10.1002/ajh.26194

Nowak G, Schrör K. 2007. Hirudin—The long and stony way from an anticoagulant peptide in the saliva of medicinal leech to a recombinant drug and beyond. Thromb. Haemost, 98: 116–119. https://doi.org/10.1160/TH07-05-0364

PubChem. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004. PubChem Compound Summary for CID 16132123, Hirudin (53-64); [cited 2026 Apr. 17]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Hirudin-_53-64

Rosenfeld SA, Nadeau D, Tirado J, Hollis GF, Knabb RM, Jia S. 1996. Production and purification of recombinant hirudin expressed in the methylotrophic yeast Pichia pastoris. Protein Expression and Purification, 8(4): 476-482. https://doi.org/10.1006/prep.1996.0127

Rydel TJ, Ravichandran KG, Tulinsky A, Bode W, et al. 1990. The structure of a complex of recombinant hirudin and human α-thrombin. Science, 249(4966): 277-280. https://doi.org/10.1126/science.2374926

Rydel TJ, Tulinsky A, Bode W, Huber R. 1991. Refined structure of the hirudin-thrombin complex. Journal of Molecular Biology, 221(2): 583-601. https://doi.org/10.1016/0022-2836(91)80074-5

Sawyer RT. 1986. Leech Biology and Behaviour (Vol 1-2). Clarendon Press, Oxford, UK

Shabareesh P, Kaur KJ. 2016. Structural and functional characterization of hirudin P6 derived novel bivalent thrombin inhibitors—Studying the effect of linker length and glycosylation on their function. Chem. Biol. Drug Des, 88: 129–141. https://doi.org/10.1111/cbdd.12742

Skrzypczak-Jankun E, Carperos VE, Ravichandran KG, Tulinsky A, Westbrook M, Maraganore JM. 1991. Structure of the hirugen and hirulog 1 complexes of α-thrombin. Journal of Molecular Biology, 221(4): 1379-1393. https://pubmed.ncbi.nlm.nih.gov/1942057/

Sohn JH, et al. 2001. Current status of the anticoagulant hirudin: Its biotechnological production and clinical practice. Applied Microbiology and Biotechnology, 57: 606–613. https://doi.org/10.1007/s00253-001-0856-9

Walsmann P, Markwardt F. 1985. On the isolation of the thrombin inhibitor hirudin. Thrombosis Research,

40(4): 563-569. https://www.sciencedirect.com/science/article/pii/0049384885902932

Warkentin TE. 2004. Bivalent direct thrombin inhibitors: Hirudin and bivalirudin. Best. Pract. Res. Clin. Haematology, 17: 105–125. https://doi.org/10.1016/j.beha.2004.02.002

Weitz JI, Hudoba M, Massel D, Maraganore J, Hirsh J. 1990. Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors. Journal of Clinical Investigation, 86: 385-391. https://doi.org/10.1172/JCI114723

Wikipedia. 2026. Hirudin. https://en.wikipedia.org/wiki/Hirudin. Accessed on April 17, 2026

Wüstenhagen DA, Lukas P, Müller C, et al. 2020. Cell-free synthesis of the hirudin variant 1 of the blood-sucking leech *Hirudo medicinalis*. Scientific Reports, 10: 19818. https://doi.org/10.1038/s41598-020-76715-w

Verstraete M, Nurmohamed M, Kienast J, Siebeck M, et al. 1993. Biologic effects of recombinant hirudin (CGP 39393) in human volunteers. Journal of the American College of Cardiology, 22(4): 1080-1088. https://doi.org/10.1016/0735-1097(93)90419-2

Xia XF, Li M, Wei RX, et al. 2023. Intracerebral hirudin injection alleviates cognitive impairment and oxidative stress and promotes hippocampal neurogenesis in rats subjected to cerebral ischemia. Neuropathology, 43(5): 362-372. https://doi.org/10.1111/neup.12897

Zhang CL, Yu AP, Jin JD, Wu CT. 2007. Research progress in hirudin fusion protein--review. Zhongguo Shi Yan Xue Ye Xue Za Zhi, 15(1): 215-218. https://pubmed.ncbi.nlm.nih.gov/17490558/

Zhang WJ. 2016a. Detecting connectedness of network: A Matlab program and application in tumor pathways and a phylogenic network. Selforganizology, 3(4): 117-120. http://www.iaees.org/publications/journals/selforganizology/articles/2016-3(4)/detecting-connectedness-of-network.pdf

Zhang WJ. 2016b. Selforganizology: The Science of Self-Organization. World Scientific, Singapore. https://doi.org/10.1142/9685

http://www.iaees.org/publications/journals/np/articles/2017-2(3)/classification-and-network-analysis-of-medicinal-functions.pdf

Zhang WJ. 2017e. Finding the shortest tree in the network: A Matlab program and application in tumor pathway. Network Pharmacology, 2(1): 13-16. http://www.iaees.org/publications/journals/np/articles/2017-2(1)/finding-the-shortest-tree-in-the-network.pdf

Zhang WJ. 2018. Fundamentals of Network Biology. World Scientific Europe, London, UK. https://doi.org/10.1142/q0149

Zhang WJ. 2024. MetaAnaly: The platform-independent computational tool for meta-analysis in the paradigm of new statistics. Network Biology, 14(2): 187-214. http://www.iaees.org/publications/journals/nb/articles/2024-14(2)/8-Zhang-Abstract.asp

Zhang WJ. 2026. Astaxanthin: Sources, biosynthesis, bioactivities, clinical applications, and future perspectives. Network Biology, 16(4): 515-524. http://www.iaees.org/publications/journals/nb/articles/2026-16(4)/3-Zhang-Abstract.asp

Zhang WJ. 2027a. Berberine in China: History, pharmacological research, clinical trials, industrialization application and market consumption. Network Pharmacology, 12(3-4): 9-14. http://www.iaees.org/publications/journals/np/articles/2027-12(3-4)/1-Zhang-Abstract.asp

Zhang WJ. 2027b. Curcumin: From molecules to markets. Ornamental and Medicinal Plants, 10(1-4): 1-5. http://www.iaees.org/publications/journals/omp/articles/2027-10(1-4)/1-Zhang-Abstract.asp

Zhang WJ. 2027c. Nicotinamide Adenine Dinucleotide (NAD+): Molecular mechanisms, clinical translation,

and industrial application. Selforganizology, 14(3-4): 25-33. http://www.iaees.org/publications/journals/selforganizology/articles/2027-14(3-4)/1-Zhang-Abstract.asp

Zhang WJ. 2027d. Baicalein: Molecular mechanisms, pharmacological properties, clinical applications, and challenges. Network Biology, 17(1): 1-19. http://www.iaees.org/publications/journals/nb/articles/2027-17(1)/1-Zhang-Abstract.asp

Zhang WJ. 2028a. Matrine: Molecular mechanisms, pharmacological properties, pharmacokinetics, clinical applications, and challenges. Selforganizology, 15(1-2): 1-20. http://www.iaees.org/publications/journals/selforganizology/articles/2028-15(1-2)/1-Zhang-Abstract.asp

Zhang WJ. 2028b. Nattokinase: Biological activities, pharmacological mechanisms, therapeutic applications, challenges and perspetives. Network Pharmacology, 13(1-2): 1-26. http://www.iaees.org/publications/journals/np/articles/2028-13(1-2)/1-Zhang-Abstract.asp

Zhang WJ. 2028c. Salvianolic acids: Molecular mechanisms, pharmacological properties, pharmacokinetics, clinical applications, and future perspectives. Ornamental and Medicinal Plants, 11(1-4): 1-19. http://www.iaees.org/publications/journals/omp/articles/2028-11(1-4)/1-Zhang-Abstract.asp

Zhu WJ, Li HJ, Chen P, Li HM. 2021. A kind of purification method of high-purity recombinant hirudin. Patent CN-113072639-A. https://pubchem.ncbi.nlm.nih.gov/patent/CN-113072639-A