Carrageenan-induced immunothrombotic models in rodents (Review)

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Abstract. Carrageenan (CGN)-induced immunothrombotic models are used in rodent thrombosis research. They enable both mechanistic dissection of inflammation-coagulation crosstalk and high-throughput screening of antithrombotic agents. The present narrative review summarizes current knowledge on modeling approaches, thrombotic mechanisms, quality determinants and translational relevance of the CGN model. CGN is a sulfated linear galactan that can be administered by intraperitoneal injection, tail vein injection or subcutaneous injection. Amongst these, intraperitoneal injection of κ-CGN (20-100 mg/kg in mice) is the most widely used due to its simplicity and superior reproducibility. Tail vein injection of CGN (1 mg/kg in rats) combined with ligation results in rapid model formation and is suitable for studying the intrinsic pathway of coagulation, whereas subcutaneous injection is mainly used for exploring mechanisms of local inflammatory thrombosis. By contrast, CGN combined with lipopolysaccharides can be used to establish a disseminated intravascular coagulation model, replicating consumptive coagulopathy and multi-organ microthrombosis. The thrombotic mechanisms of CGN involve Toll-like receptor 4/NF-κB pathway-mediated inflammatory cytokine release, upregulation of tissue factor and endothelial injury caused by the release of neutrophil extracellular traps following intraperitoneal CGN administration, direct activation of coagulation factor XII (which initiates the intrinsic coagulation pathway following tail vein administration) and direct activation of the platelet C-type lectin-like receptor 2 pathway. Model quality is influenced by multiple factors, including the subtype and molecular weight of CGN, animal strain (Kunming mice have the highest sensitivity), sex, age, administration parameters and observation time window (24-72 h). This model simulates the pathological process of sterile inflammation-driven thrombosis and shares similar mechanisms with clinical conditions, such as antiphospholipid syndrome and severe Coronavirus disease-19 coagulopathy. However, limitations exist in terms of anatomical site, hemodynamics, chronic etiology and species differences, which restrict its direct extrapolation to clinical settings. The adoption of a multi-model validation strategy and screening of target patient populations based on inflammatory markers are recommended to improve clinical translation.

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1. Introduction

A thrombus is a pathological solid mass formed by the abnormal aggregation of blood components within the cardiovascular system. This condition has become a major global public health threat (1). In total, ~16 million individuals worldwide succumb to thrombus-related diseases each year, with China accounting for 51% of these deaths (2).

According to Virchow's triad, thrombi can be classified into the following three categories according to their pathogenesis: Those associated with blood stasis; vascular endothelial injury; or a hypercoagulable state (3). In 2013, Engelmann and Massberg (4) first proposed the concept of immunothrombosis, which refers to inflammation- and immune-mediated thrombosis. This concept unifies stasis, endothelial injury and the hypercoagulable state at the level of immune and inflammatory molecular regulation, elucidating the cellular activation, molecular regulatory events and pathological outcomes involved in the development and progression of thrombosis.

Appropriate animal models are important tools for elucidating the mechanisms of thrombosis and evaluating the efficacy of antithrombotic drugs. Existing models include

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the renal vein ligation endothelial injury model (5), ferric chloride-induced model (6,7), mechanical vascular injury model (8), photochemically-induced model (9), local thrombin injection model (10) and the cerebral venous sinus thrombosis model (induced by semi-ligation combined with ferric chloride and thrombin) (3). These models correspond to individual components of Virchow's triad, closely mimic clinical triggers and provide relatively accurate thrombus localization. However, they do not fully represent the molecular mechanisms of inflammation-mediated thrombosis, nor do they deeply elucidate the molecular basis of the triad itself. In addition, the majority of the aforementioned require surgical procedures that are technically challenging and are not conducive to large-scale experimental implementation (3,5,8).

In studies of immunothrombosis models, lipopolysaccharide (LPS) and carrageenan (CGN) are two classic inflammatory inducers. LPS induces acute, systemic and lethal inflammation (11-13) and serves as the gold standard for sepsis models. By contrast, CGN induces chronic, localized and non-lethal inflammation, rendering it suitable for studies on the interaction between chronic inflammation and thrombosis (14-16). The differences between LPS and CGN in terms of Toll-like receptor 4 (TLR4) recognition patterns, downstream signaling networks and inflammatory regulatory mechanisms are gradually being elucidated.

The CGN-induced immunothrombosis model in rodents requires no complex surgery or specialized equipment (14). It has a well-defined mechanism, produces consistent results and is efficient for model generation. This model drives thrombus formation through an inflammatory response, thereby simulating the dual pathology of 'inflammation and thrombosis' (14-16). Since the timing and progression of thrombus formation can be directly observed on the surface of the tail, the model is widely used to investigate inflammation-thrombosis signaling pathways and molecular biomarkers (14-16), to study the pathology of disseminated intravascular coagulation (DIC) (17) and/or to screen potential targets for antithrombotic drugs.

At present, an increasing number of studies have investigated the mechanisms underlying CGN-induced thrombosis and CGN thrombosis models in rodents (14-16). However, to the best of our knowledge, no comprehensive review exists. Therefore, the present narrative review was conducted. The search covered major biomedical databases, including PubMed (https://pubmed.ncbi.nlm.nih.gov), Web of Science (https://www.webofscience.com) and China National Knowledge Infrastructure (CNKI; https://oversea.cnki.net), from their inception to April 2026. Original research articles and reviews focusing on CGN-induced thrombosis models in rodents were included, particularly those addressing modeling methods, mechanisms of thrombosis, model optimization and efficacy evaluation. Letters, case reports, conference abstracts, duplicated publications and literature unrelated to thrombosis research were excluded. Relevant literature was selected following review of the abstracts or full texts on a case-by-case basis.

The present review aimed to summarize the core molecular mechanisms of CGN-mediated immune-inflammatory thrombosis, strategies for optimizing dosing regimens in rodent CGN thrombosis models, standardized evaluation metrics and application scenarios. It also aimed to provide a standardized and reliable reference for the use of experimental tools to study the inflammation-tissue factor-extrinsic coagulation pathway, DIC pathology and the *in vivo* screening of anti-inflammatory and anticoagulant drugs. Current research gaps and limitations were also summarized, where the potential solutions were discussed to provide a theoretical basis and reference for future studies of immunothrombosis mechanisms and development of experimental models.

2. Animal model establishment and evaluation

CGN is a sulfated linear galactan that can be obtained from marine red algae of the genera *Eucheuma*, *Chondrus* and *Gigartina* through alkali extraction and purification (18,19), with a well-defined structure and wide applications. In 1962, Winter *et al* (20) first established the CGN-induced rat paw edema model. In 1973, Giroud and Timsit (21) first reported CGN-induced thrombosis (21). Since then, the model has been gradually refined and has become an important tool for screening antithrombotic drugs and studying inflammation-coagulation interactions (14-16).

CGN can induce tail vein thrombosis in rodents following intraperitoneal, subcutaneous or tail vein injection and can affect multiple organs, including the liver, lungs and kidneys (15). Additionally, CGN combined with LPS can be used to induce a DIC model (17). Common methods for establishing CGN-induced immunothrombotic models in rodents are listed in Table I.

Intraperitoneal injection of CGN to induce tail vein thrombosis. This is one of the most widely used models for antithrombotic drug screening (22-24). It is easy to perform, requires no anesthesia, allows direct quantification of ischemic necrotic areas in the tail, and has good reproducibility, making it suitable for medium-to-high throughput evaluation.

Modeling method. κ-carrageenan (κ-CGN) is commonly used, with a molecular weight of ~700 kDa (23). Commercial grade κ-CGN, such as Sigma-Aldrich C1013, has been reported, where a single intraperitoneal dose of 20-100 mg/kg is typically used (14,15,22-27). Experimental animals are mainly male institute of cancer research (ICR), C57BL/6, BALB/c, Kunming (KM) mice aged 6-8 weeks and weighing 18-30 g (13,14,23,26-30). However, male Wistar or Sprague-Dawley (SD) rats weighing 150-200 g can also be used (22,24). Observations are performed 24-72 h after model induction (22,24-30).

Control drugs. Commonly used positive controls are heparin (subcutaneous injection, 5,000 U/kg or oral administration, 20 mg/kg) (25,27,28) and aspirin (oral administration, 20-50 mg/kg) (14,15,24,30). Heparin exerts its anticoagulant effect by enhancing antithrombin III activity (25,27,28), whilst aspirin blocks platelet aggregation by inhibiting cyclooxygenase-1 (14,15,24,30). Both can reduce tail thrombus length in model animals.

Model quantification indicators and success criteria. The core indicator is the ratio of the tail thrombus (black necrotic area) length to the total tail length, with the relative thrombus length calculated as follows: Relative thrombus length=(thrombus length/total tail length) x100% (14,15,22-30).

Table I. Common methods used to establish carrageenan-induced thrombosis in rodents.

Approaches	Animal strain	Characteristics	(Refs.)
Intraperitoneal injection of CGN to induce tail vein thrombosis model: Animals were intraperitoneally injected with 20-100 mg/kg body weight CGN.	BALB/c mice, ICR mice, KM mice, SD rats, Wistar rats and C57BL/6 mice	The model is simple to establish, has good reproducibility and is widely used. It can induce systemic inflammatory responses. Disadvantages include potential local inflammation due to peritoneal irritation, which may cause non-specific inflammatory interference. In addition, thrombosis time is prolonged.	(14,15,22-30)
Tail vein injection of CGN to induce tail vein thrombosis model: CGN (1-3 mg/kg) was injected into the dorsal tail vein of rats, with the ligation at a site 12-13 cm from the tail tip for 10-20 min.	SD rats and Wistar rats	Thrombosis forms rapidly, making the model suitable for acute studies.	(31-35)
Subcutaneous injection of CGN to induce tail vein thrombosis model: CGN (0.4 mg; 10-40 μl) was injected into the subplantar region of the right hind paw of mice.	ICR mice and Swiss albino mice	A marked local inflammatory response is observed, allowing simultaneous observation of the association between inflammation and thrombosis. Thrombosis develops slowly with low mortality; poor reproducibility is a limitation.	(36-38)
Combined injection of CGN and LPS to induce DIC model: CGN and LPS were administered separately by intraperitoneal injections.	KM mice, ICR mice and BALB/c mice	Simulates sepsis-associated DIC with both systemic inflammatory response and coagulopathy.	(17,41)

CGN, carrageenan; ICR, Institute of Cancer Research; BALB/c, Bagg albino; C57BL/6, C57 black 6; KM, Kunming; SD, Sprague-Dawley; LPS, lipopolysaccharide; DIC, disseminated intravascular coagulation.

Normal mice have no blackening of the tail, whereas the relative thrombus length in the model group frequently reaches 60-85% (14,15,22-30). Histopathology (H&E staining) typically reveals mixed thrombi in the tail vein lumen, fibrin networks and vascular endothelial damage (23,25-27,29). Auxiliary indicators include four coagulation parameters [shortened activated partial thromboplastin time (APTT), prolonged prothrombin time (PT), shortened thrombin time (TT) and elevated fibrinogen (FIB)], decreased platelet count and elevated plasma TNF-α/IL-1β/IL-6/NF-κB (14,15,22-30). Success criteria include the following: i) visible black necrosis of the tail with a relative thrombus length ≥60%; ii) intravascular thrombus filling confirmed by H&E staining; and iii) coagulation abnormalities and elevated inflammatory factors (14,22-27).

Application scenarios. The model is widely used for the *in vivo* efficacy screening of antithrombotic drugs, including traditional Chinese medicine extracts, probiotics, microbial preparations, chemical compounds and natural polysaccharides (14,15,22,24-30), in addition to investigating thrombotic inflammatory mechanisms [NF-κB/NLR family pyrin domain containing 3 (NLRP3), high mobility group box 1 and C-type lectin-like receptor 2 (CLEC-2) pathways] (14,23,25-27,30) and the gut microbiota-thrombosis axis (25,27,28).

Recommended modeling protocol. Male ICR or BALB/c mice aged 6-8 weeks and weighing 20-25 g are recommended. κ-CGN should be prepared as a 1.0% solution in sterile saline and administered as a single intraperitoneal injection at 50 mg/kg (injection volume, 10 μl/g), with 48 h after model induction used as the endpoint. Positive controls can be heparin (oral administration, 20 mg/kg or subcutaneous injection, 5,000 U/kg) or aspirin (oral administration, 20 mg/kg) (15,23,24,29,30).

Tail vein injection of CGN to induce the tail vein thrombosis model. Tail vein injection of CGN activates the intrinsic coagulation cascade by activating coagulation factor XII (Hageman factor), whilst releasing inflammatory factors (31,32) that cause local vascular endothelial inflammation and damage, ultimately leading to thrombus formation (31,32).

Modeling method. κ-CGN is commonly used (31,33), with varying doses: Golakiya *et al* (31) used a single injection of 3 mg/kg; Nyansah *et al* (33) used 2 mg/kg; and multiple studies used 1 mg/kg (32,34,35). After injection, a nylon thread is used to ligate the tail 12-13 cm from the tip for 10-20 min to increase local blood stasis and the rate of thrombus formation (32,34). Experimental animals used are mostly male Wistar or SD rats weighing 225-300 g (32,33).

Control drugs. Heparin is the most widely used. Golaki *et al* (31) administered subcutaneous injections of 300 U/kg for 3 consecutive days, markedly prolonging APTT and restoring PT; Nyansah *et al* (33) observed that heparin reduced tail infarct length and microthrombus count. Other studies used aspirin (7.5-30 mg/kg) (34) or streptokinase (2,000 U/kg) (32) as positive controls.

Model quantification indicators and success criteria. Indicators include the following: i) Tail infarct length, measured at 2, 6, 24, 48 and 72 h post-injection as the length of the dark red infarcted area (34,35); ii) coagulation parameters, including PT, international normalized ratio, APTT, platelet count and fibrinogen (31); iii) fibrinolysis parameters, including fibrin degradation products (FDP) and D-dimer (31); iv) histopathology, including microthrombus count and inflammatory cell infiltration in the lungs, liver and mesentery (31,33); and v) an inflammation score, which is a semi-quantitative score (1-4) for tail redness, swelling, heat, and pain (33). Success criteria include: i) A visible dark red or reddish-brown infarct within 2-6 h post-injection, typically reaching 7-12 cm in length (32,33); ii) shortened PT and APTT, with elevated platelet count (31); and iii) histopathological evidence of microthrombi in microvessels and perivascular inflammatory cell infiltration (34).

Application scenarios. The model is widely used to evaluate the *in vivo* activity of anticoagulant and antithrombotic drugs, including sulfated polysaccharides (31), plant extracts (33), natural cysteine proteases such as ficin and papain (32,35) and water-soluble tomato concentrate (34). It is also used to assess anti-inflammatory effects (35) and effects on hemodynamics and microcirculation perfusion (32).

Recommended modeling protocol. Tail vein injection of κ-CGN (1 mg/kg) combined with tail ligation is recommended using male SD or Wistar rats (150-250 g). The tail should be ligated 12 cm from the tip for 10 min, where the animals should be maintained at 20-25˚C post-injection. Positive controls can be heparin (300 U/kg, subcutaneous injection) or aspirin (30 mg/kg, oral administration).

Subcutaneous injection of CGN to induce tail vein thrombosis model. The principle of this model is based on the local inflammatory response induced by the subcutaneous injection of CGN, which damages vascular endothelial cells and activates coagulation factor XII (Hageman factor) in the intrinsic coagulation pathway, leading to tail vein thrombosis (36,37).

Modeling method. Protocols reported in the literature vary. Zhang *et al* (38) used 4% ι-type CGN, with 10 μl injected subcutaneously into the right hind paw pad of male ICR mice (25-30 g). By contrast, Arslan *et al* (36) used 1% κ-type CGN, with 40 μl injected into the right hind paw pad of female Swiss albino mice (30-35 g). Kim *et al* (37) used 1% ι-type CGN, with 40 μl injected into the right hind paw of male ICR mice (25±2 g).

Control drugs. Zhang *et al* (38) used aspirin (50 mg/kg, oral administration) as a positive control. Arslan *et al* (36) used low-molecular-weight heparin (10 and 100 IU/kg, intraperitoneal injection), with the 100 IU/kg group showing significant antithrombotic effects. By contrast, Kim *et al* (37) used both aspirin (20 mg/kg) and heparin (100 IU/kg).

Model quantification indicators and success criteria. The main indicator is tail thrombus length, measured at 24, 48 and 72 h post-injection as the length of the black necrotic area (36-38). The thrombus appeared ~6 h post-injection as a wine-red lesion, with evident necrosis developing after 24 h (38). Zhang *et al* (38) reported a thrombus length of 53.5±6.9 mm at 48 h and 50.1±7.2 mm at 72 h, whereas Arslan *et al* (36) observed a thrombus length of ~6.74±0.75 cm at 72 h. Success criteria include persistent visible thrombotic necrosis in the tail, a formation rate of ~100% and stabilization of thrombus length after peaking at 48 h (36,37).

Application scenarios. The model is suitable for evaluating drug intervention effects on the intrinsic coagulation pathway, as well as for studying inflammation-related thrombus formation mechanisms (37).

Recommended modeling protocol. Male ICR mice (25-30 g) are recommended, with a single subcutaneous injection of 40 μl 1% ι-type CGN into the right hind paw pad. This protocol is currently less commonly used, as it induces prominent local inflammatory edema at the injection site. Anti-inflammatory effects of test compounds may indirectly reduce tail thrombus length, bringing potential false-positive outcomes for antithrombotic evaluation. Therefore, intraperitoneal and tail-vein injection protocols are more widely adopted for carrageenan-induced tail-thrombosis models.

Combined injection of CGN and LPS to induce DIC. In the sepsis DIC model, a single LPS injection has a high mortality rate (39), whilst cecal ligation and puncture is poorly reproducible (40).

Modeling method. Elsayed *et al* (41) first used λ-CGN as a priming agent in rats (10 mg/kg, intraperitoneal injection), followed 24 h later by intravenous injection LPS (500 μg/kg) as a provocative agent, inducing a DIC model characterized by multi-organ fibrin thrombi. Tang *et al* (17) systematically optimized the conditions, using κ-CGN instead of λ-type CGN, with intraperitoneal injection of κ-CGN (100 mg/kg) and LPS (50 μg/kg), thereby inducing stable DIC manifestations in KM mice. κ-CGN and LPS were dissolved separately in saline, with a 65˚C water bath used to aid the dissolution of κ-CGN, which were injected into the left and right sides of the abdomen, respectively (17). After model induction, the animals were housed at 16±1˚C to increase thrombotic tendency, following which the model stabilized after 12 h (17). Male and female KM mice showed similar responses (17). Amongst the strains assessed, KM mice exhibited the greatest prolongation of APTT and PT, with greater DIC susceptibility compared with ICR and BALB/c mice (17).

Control settings. Tang *et al* (17) established a κ-CGN-only group (without LPS), an LPS-only group (without κ-CGN) and a saline group. Within 24 h, only ~37.5% mice in the κ-CGN-only group showed tail thrombosis, whilst the LPS-only group did not exhibit tail thrombosis at all (17). However, neither alone was sufficient to reproduce DIC characteristics, where the combined injection produced a synergistic effect.

Model quantification indicators and success criteria. For coagulation indicators, APTT prolongation ≥2.3-fold and PT prolongation ($P<0.01$ compared with the normal group) are core parameters for determining a hypocoagulable state (17,41). In addition, a marked decrease in platelet count and fibrinogen

consumption provided direct evidence of DIC (17,41). Thrombus burden is quantitatively assessed by tail thrombus length (visual, expressed as a percentage of tail length) and fibrin thrombus count in organs, such as the liver, lungs and kidneys (phosphotungstic acid hematoxylin staining, per 0.25 mm^2 field) (17,41). Fibrinolysis inhibition is a key feature of sepsis DIC, manifested as elevated plasma plasminogen activator inhibitor-1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor levels (from 0.25 h post-injection), with relatively normal FDP/D-dimer levels (17). Success criteria include the following: i) Tail thrombus incidence ≥87.5% (17); ii) APTT prolongation ≥2.3-fold and PT prolongation ($P<0.01$) (17); iii) a marked, statistically significant decrease in platelet count ($P<0.01$ vs. normal control) (17); iv) fibrin thrombi in multiple organs, including the liver, lungs and kidneys, as assessed by hematoxylin-eosin (H&E) histopathological staining (17,41); and v) fibrinolysis inhibition, with statistically elevated PAI-1 ($P<0.05$ or $P<0.01$ vs. normal control) without marked changes in D-dimer (17).

Application scenarios. The model is primarily used to study the pathological mechanisms of sepsis-related DIC (42) and to evaluate the efficacy of anticoagulant and antithrombotic drugs. Recombinant human tissue factor pathway inhibitor has been shown to reduce organ thrombus count in this model (41).

Recommended modeling protocol. Male KM mice (26-32 g) are recommended. Intraperitoneal injections of κ-CGN (100 mg/kg) and LPS (50 μg/kg) should be administered separately (0.1 ml/10 g), followed by housing at 16±1˚C for 12 h after model injection.

Critical analysis of the model. The indicators and data in this section were derived from parameters reported in the original literature. In total, four types of CGN thrombosis modeling protocols will be summarized. A number of apparent discrepancies in the literature may result from the absence of a unified standardized operating procedure for this model, limiting comparability among experiments.

Heterogeneity of experimental parameters. Different studies have used multiple rodent strains, including ICR (37,38), BALB/c (14,17), C57B/6 (23), KM (17), SD (24,32) and Wistar (22,32). KM mice have greater sensitivity to inflammation and DIC stimulation compared with ICR mice (17). The dosage range of CGN is wide, with intraperitoneal injection of 20-100 mg/kg, tail vein injection of 1-3 mg/kg and subcutaneous administration using varying concentrations and injection volumes, without conversion to standardized doses. The majority of studies have used male animals to avoid sex hormone interference (14-16). However, female animals have also been used in subcutaneous and combined DIC models, where potent inflammatory stimulation may offset sex-related differences in thrombotic responses (17,36). Heparin and aspirin are commonly used positive controls, but there is no unified standard for their optimal administration dose and route. Heparin doses range from 300 to 5,000 U/kg (27,28,31,33), making it difficult to compare pharmacodynamic results across studies.

Analysis of the causes of apparent discrepancies. The variability in the success rate of κ-CGN thrombosis models arises from differences in strain, dose, ambient temperature and observation duration. The success rate of the conventional intraperitoneal injection of 50 mg/kg CGN in KM mice at room temperature for 24-72 h is ~100% (29). By contrast, the combined CGN and LPS DIC model used KM mice, 100 mg/kg CGN, a temperature of 16±1˚C and a 24-h observation period, which resulted in a success rate of only 37.5% (17). The apparent difference between these two sets of data is presumably due to the different animal strains, doses and rearing temperatures, in addition to the different observation periods. The early detection rate of tail cyanosis was low, where the proportion of thrombotic necrosis increased over time. Such parameter-dependent outcome variations have been previously reported in carrageenan-induced thrombosis rodent models, and the combined effects of these variables may ultimately result in differences in the success rates of CGN models rather than contradictory experimental conclusions. Coagulation indicators from tail vein CGN models suggest acute hypercoagulability (shortened PT and APTT), whilst CGN combined with LPS to induce DIC leads to consumptive hypocoagulability (prolonged PT and APTT) (17). These two phenomena are consistent with pathological stages, namely acute hypercoagulability in the CGN model and progression to consumptive hypocoagulability in the CGN + LPS DIC model (17). Most conventional carrageenan-only thrombosis studies preferentially employ male rodents, given known modulatory effects of sex-hormone milieu on inflammatory-thrombotic phenotypes. Direct head-to-head comparative data are still limited for pure CGN-challenge models. However, in the KM mouse combined DIC model, the potent prothrombotic effects of LPS and low temperature may mask the protective effect of sex hormones, resulting in no difference in response between sexes (17). Subcutaneous studies have used both κ and ι CGN and both male and female mice (36-38), Among these three publications, several only reported the injected solution volume without conversion to a weight-normalized standardized dose, which complicates cross-study result comparison (36-38).

Current shortcomings in the field. The aforementioned apparent discrepancies may be explained by differences in experimental variables and not contradictory experimental conclusions. However, they highlight a key limitation in the field, namely the absence of a unified operating standard. Varying modeling conditions have been applied based on their experimental objectives, resulting in important experimental details being incompletely reported in the literature, such that there remains to be no unified reference for positive controls. Data from different laboratories are difficult to compare, restricting experimental replication and meta-analysis of drug efficacy whilst hindering mechanistic and translational research.

3. Factors influencing the model

CGN subtypes and molecular weight. Based on differences in the position and number of sulfate groups on the sugar chain and molecular conformation, CGN can be divided into seven main subtypes, namely, κ, ι, λ, γ, ν, ξ and μ. Amongst these, the κ-, ι-and λ-carrageenans are the most commonly used in industrial production and research (18). The sulfate groups of CGN are key determinants of its physicochemical properties and biological activities. Previous studies using κ-, ι-, and λ-carrageenans purified by Sigma (such as C1013 and C1138)

demonstrated that their biological activities are positively associated with the degree of sulfation. Recognition and binding of sulfate groups by immune cells activate these cells to release inflammatory factors, thereby triggering inflammation and vascular injury (42).

Currently, κ-CGN is the main inducer used to establish CGN-induced immunothrombosis models. Multiple studies have used κ-CGN to establish tail thrombosis models in mice and rats, both of which have reported the successful induction of thrombosis (15,24,43-45). However, only two studies to date have used each of λ-CGN (46,47) or ι-CGN (37,38) for related experiments. There are marked differences in the number of sulfate groups and gel properties among the different subtypes of CGN (18). Biological-activity variations have been observed across CGN subtypes in *in vivo* thrombosis models (48,49). In 1981, Davidson *et al* (48) intraperitoneally administered 125 mg/kg λ-, κ-, and ι-CGN into SD rats and reported that all three subtypes could induce thrombocytopenia and coagulation disorders, with the effects ranked λ=κ>ι. In 1985, Bekemeier *et al* (49) further compared the thrombogenic abilities of the three subtypes and reported that κ-CGN exerted the greatest thrombotic activity (49).

In addition to subtype, molecular weight is another key factor influencing the biological effects of CGN. Based on differences in weight-average molecular weight (Mw), CGN can be further classified into three categories: Natural food-grade CGN (Mw, >100 kDa), moderately degraded CGN (Mw, 20-40 kDa) and the non-food-grade degradation product poligeenan (Mw, 20-30 kDa) (50,51). The proinflammatory activity and toxicity of CGN have been reported to increase as molecular weight decreases, with poligeenan exhibiting marked proinflammatory properties (52). Previous studies mostly used food-grade high-molecular-weight CGN (23) or commercially available κ-CGN without a specified molecular weight (14,15,27,29), where no studies using low-molecular-weight CGN for modeling were identified in the literature reviewed. High-molecular-weight CGN may therefore be suitable for stable model induction, whereas low-molecular-weight CGN may increase mortality due to its toxicity and may be unsuitable for drug efficacy screening. The specific effect of molecular weight on the quality of immunothrombosis models requires further systematic investigation.

Animal strains and sensitivity to each CGN subtype. Different strains of rodents exhibit varying sensitivity to CGN stimulation. A number of rodent strains have been successfully used to construct CGN-induced immunothrombosis models, primarily including SD rats (43), Wistar rats (45), BALB/c mice (53), ICR mice (54) and KM mice (38). Amongst these, KM mice are commonly used outbred laboratory animals in China and are characterized by high sensitivity to inflammatory and coagulation stimuli. By contrast, BALB/c inbred mice and ICR outbred mice are widely used albino laboratory mouse strains. These three mouse strains can be used for CGN-induced thrombosis modeling, but there are strain-specific physiological differences in thrombus formation and inflammatory responses (55-58).

In 2004, Posadas *et al* (55) found that the TLR4/NADPH oxidase 2 (NOX2)-mediated neutrophil inflammatory response in C57BL/6 mice was lower compared with that in CD1/ICR mice. A targeted comparative study in 2006 systematically compared model outcomes in two rat strains (SD rats and Wistar rats) and three mouse strains (KM mice, ICR mice and BALB/c mice) (56). The results showed that all five strains could successfully develop CGN-induced thrombosis, with clear thrombus observed in the tails of animals after model induction. Dynamic changes in hematological parameters were generally consistent, with marked decreases in peripheral blood leukocytes and platelets during the early stage of modelling. Both SD and Wistar rats showed rapid upregulation of serum inflammatory factors and IL-6 expression after model induction, with similar temporal secretion patterns of TNF-α and IL-6 (56). However, KM and BALB/c mice exhibited longer tail thrombus lengths and higher modeling rates compared with those in ICR mice (56). In 2026, Tang *et al* (17) reported findings consistent with previous studies, showing that in a sepsis-associated DIC model induced by combined injection of κ-CGN (100 mg/kg) and LPS (50 μg/kg) at 16±1˚C, KM and BALB/c mice had longer tail thrombus lengths compared with those in ICR mice, whereas KM mice had longer APTT and PT compared with those in ICR and BALB/c mice. This high susceptibility of KM mice is consistent with their reported greater inflammatory responses and higher basal oxidative stress levels compared with ICR mice (57). Additionally, KM mice reportedly exhibit greater lung injury severity during infection, highlighting their high sensitivity to systemic inflammatory challenges, whilst ICR mice show greater resistance (58). The exact mechanistic basis for this inter-strain difference was not fully elucidated in the original report, although genetic background, baseline oxidative stress and inflammatory responsiveness are presumed contributing factors.

Based on the available literature, commonly used rat and mouse strains can be used to establish CGN-induced immunothrombosis models. For practical reasons, rat models are typically preferred for studies involving tail vein injection.

Sex and age. The majority of published studies have used adult male animals for modeling, such as SD rats weighing 190-200 g (59) and ICR mice weighing 25-30 g (54). In particular, male animals are typically favored for experiments primarily to avoid potential effects of hormonal fluctuations during the estrous cycle on inflammation, coagulation responses and model reproducibility (38,43,60).

However, sex may influence model outcomes, since sex hormones, such as estrogen (61,62), have been shown to modulate vascular function and inflammatory responses (63). Clinical and experimental studies indicate a pronounced sex bias in the presentation of inflammatory and thrombotic diseases. Women tend to be more susceptible to chronic inflammatory autoimmune diseases (64), whereas acute inflammatory and thrombotic disorders (characterized by excessive and persistent innate immune activation) are associated with greater disease severity and higher mortality in men (65). In various conditions, such as ischemia-reperfusion-induced organ injury and acute respiratory distress syndrome, men tend to display higher incidence and severity compared with women (66-68). In addition to several factors in humans, such as lifestyle and environmental exposure, this sex difference is also widely observed in mammalian experimental models.

Experimental studies have indicated that following exposure to bacteria, inflammatory stimuli or ischemia-reperfusion intervention, male rodents sustain more pronounced tissue inflammatory injury, with markedly higher levels of neutrophil recruitment and tissue accumulation, compared with their female counterparts (69-71).

In 2015, following controlled studies on age-matched male and female Wistar rats and C57BL/6 mice, Madalli *et al* (72) reported that the intensity of acute inflammatory responses exhibited clear sexual dimorphism, with male animals showing stronger inflammatory responses compared with those in females. This effect was associated with the distinct sex-specific expression profiles of the chemokine C-X-C motif chemokine ligand (CXCL)5/CXCL6, which mediates sex differences in inflammatory infiltration, by promoting the increased release of bone marrow neutrophils and enhanced peripheral circulation migration in males.

Sex differences in the model are primarily mediated by sex hormone regulation, immune response typing and drug metabolism differences. The biological effects of estrogens and androgens are distinctly different. Estrogen tends to exert a protective effect on the cardiovascular system, by upregulating nitric oxide synthesis and inhibiting the renin-angiotensin system, thereby reducing the risk of vascular injury and thrombus formation (73,74). By contrast, androgens have a mainly pro-thrombotic effect, with testosterone enhancing platelet activity and exacerbating oxidative stress. This makes male mammals more prone to forming stable blood clots (74,75). However, there are also differences in immune response typing between male and female animals. Female rodents predominantly exhibit a T helper 2-type immune response (76,77), which can inhibit the progression of inflammatory vascular thrombosis. In comparison, male animals primarily show a T helper 1-type immune response (73,78), which tends to exacerbate microvascular occlusion and promote thrombus formation. Additionally, sex differences in drug metabolism can indirectly affect the model phenotype. Female animals have greater hepatic cytochrome P450 enzyme activity, leading to the increased clearance of CGN and a shorter half-life, effectively attenuating its pro-thrombotic effects (74).

Clinical settings associated with an increased incidence of venous thromboembolism in women, including pregnancy, oral contraceptive use and hormone therapy, are associated with changes in estrogen levels (79). Changes in estrogen levels during pregnancy and hormone therapy can alter the procoagulant state and increase thrombotic risk (80). However, the majority of studies on CGN-induced thrombosis models have only used male animals (32,34,35,37,38,53). Systematic assessment of the thrombotic effects of carrageenan under conditions of increased exposure, such as pregnancy simulation or exogenous estrogen administration, are lacking. This gap limits the application of the model to the study of thrombotic risk in females and the extrapolation of model findings to women with pulmonary embolism. Future studies should include animals at different stages of the estrous cycle, with exogenous estrogen intervention groups, to systematically evaluate the impact of sex hormone status on the thrombotic effects of CGN.

Age can affect the quality and reproducibility of the CGN-induced immunothrombosis model through effects on coagulation and inflammatory responses (55,74,81). There are notable differences in the coagulation-fibrinolysis system and inflammatory homeostasis among experimental animals of different ages (74,81). Young animals (<6 weeks old) have lower expression levels of coagulation factors II, VII, IX and anticoagulant protein C, with immature and unstable coagulation function, which can reduce model reproducibility (74). By contrast, adult animals (6-8 weeks old) have fully developed physiological systems, with the coagulation-fibrinolysis system in a stable dynamic equilibrium, making this a commonly used age range for thrombosis modelling. This is typically referred to as the 'gold standard' for model construction in multiple studies. Aged animals (>6 months old) exhibit aging characteristics, such as chronic low-grade inflammation and vascular sclerosis, which can amplify the thrombotic pathological phenotype, making them more suitable for mechanistic studies of age-related thrombotic diseases.

Age-related differences in inflammatory responses are important factors influencing model phenotypes. Following CGN administration, 3-5-week-old young mice have been reported to exhibit lower levels of neutrophil infiltration and myeloperoxidase activity, compared with those in 6-8-week-old adult mice, with CGN-induced inflammatory responses showing a clear age-dependent pattern (55). Bone marrow and alveolar macrophages in aged mice display increased inflammatory activation, with elevated NLRP3 inflammasome activation levels, which can exacerbate inflammatory responses by upregulating the caspase-1-dependent secretion of IL-1β and IL-18. This is closely associated with mitochondrial dysfunction and increased generation of reactive oxygen species (81). The sustained activation of the NLRP3 inflammasome pathway in aged mice, combined with exogenous polysaccharide stimulation from CGN and aging-related endogenous inflammation, may affect model specificity and bias experimental results. Future studies should systematically evaluate the effects of these variables, standardize animal sex or incorporate sex-stratified analyses and clearly define animal age ranges. Of note, terminologies such as '8-10 weeks of age' instead of 'adult' would improve model accuracy and reproducibility.

Route, dosage and vehicle system. Common routes of administration for this model include intraperitoneal injection, tail vein injection and subcutaneous injection, which differ in their applications, operational difficulty and model characteristics. Subplantar subcutaneous injection is primarily used to construct local inflammation-triggered thrombosis models (36,37,38). Published mouse protocols for this hind-paw subplantar CGN administration employ injection volumes ranging from 10 to 40 μl (36-38). Oversized injection volumes may exacerbate non-specific local tissue injury. Thrombus is generally confined to the tail vein, with mild systemic inflammation and low mortality. Tail vein injection is mostly used in rat modeling. Due to limited space for manipulation in the tail, experimental animals with a tail length >13 cm are preferred for modelling (33,34). To increase the thrombus formation rate, tail ligation can be performed either before (31,32,35) or immediately after (33) CGN

administration. Tail vein injection involves direct intravascular administration and rapidly induces thrombus formation, but it is difficult to operate, causes marked stress in animals and has high mortality. Intraperitoneal injection is currently the most commonly used route of administration (14,15,22-30), offering the advantages of simple operation and good reproducibility. Intraperitoneal administration induces systemic vasculitis, activates platelet aggregation and ultimately induces distal tail thrombus formation, whilst also inducing multi-organ microthrombus formation. This makes intraperitoneal administration suitable for studies on dynamic changes in the coagulation-fibrinolysis system.

Currently, the conventional administration vehicle for CGN modeling is normal saline, which is widely used (38,43). Consistent preparation of the vehicle is important for model reproducibility. Under appropriate vehicle conditions, modeling dosages vary among different routes of administration. Intraperitoneal injection is a common route for modeling, with multiple studies using 20-100 mg/kg κ-CGN administered intraperitoneally to induce tail thrombus formation (14,15,22-27). By contrast, tail vein injection is more difficult to operate but requires lower modeling dosages, with 1 mg/kg κ-CGN administered by tail vein injection sufficient to establish a rat thrombus model (32,34,35). The administration dosage is an important parameter determining model success and experimental result stability. An insufficient dose may fail to induce thrombus formation and inflammatory damage, whereas an excessive dose may cause severe systemic toxicity, increase animal mortality and interfere with experimental results. In studies of antithrombotic drug efficacy, 20 mg/kg κ-CGN administered intraperitoneally is typically used to construct a rat thrombus model (24), where a dose of 45 mg/kg is used to construct a mouse thrombus model (54).

Observation and sampling time window. The length of the black infarcted area at the tail tip is the main quantitative indicator for evaluating the degree of thrombosis in the CGN-induced rodent thrombosis model. Different observation and sampling time points correspond to distinct pathological features of inflammation, coagulation and thrombosis (15,24, 31,38,43,49,54,59).

Observation is commonly performed 24-72 h after model induction. The majority of studies have selected 24 and 48 h after model induction to measure tail thrombus length (24,38,43), though some studies have also quantified thrombosis at 72 h (38,82). Furthermore, to monitor the progression of thrombus formation, several studies have also used multiple observation time points of 2, 6, 24 and 48 h after model induction (54,59).

Sampling and outcome measures should be selected according to the specific time window and may include histopathological, coagulation and inflammatory assessments. Tail tissues can be assessed using H&E staining and Masson's trichrome staining for pathological examination (53), whereas blood and affected tissues, such as the liver and lungs, can be used for the assessment of coagulation parameters (PT, APTT, TT and FIB) and measurement of inflammatory cytokines (TNF-α, IL-1β and IL-6) (14,15,28,43). Overall, 24-72 h after model induction is the main time window for evaluating thrombotic injury and the efficacy of drug intervention. From 0 to 6 h after tail vein injection, animals may exhibit a number of acute inflammatory manifestations, such as local redness, swelling and increased skin temperature of the tail. Serum levels of inflammatory cytokines will typically increase between 6 and 24 h after model induction. Blood can be collected at multiple points (such as at 4, 8, 12 and 24 h) to monitor changes in coagulation function and hemorheological parameters. Visible thrombus formation in the tail typically occurs between 24 and 48 h after model induction, during which the length of the tail vein thrombus can be measured. In particular, 48 h after model induction is commonly used for pathological sampling of the tail, liver and lungs, allowing assessment of the thrombus structure and secondary organ damage. By contrast, at 72 h after model induction, the progression and repair of tail tissue necrosis can be observed (31,49).

Following intraperitoneal injection, tail thrombus can appear as early as 3 h after model induction. From 3 to 14 h, the thrombus appears dark red at the tail tip and gradually spreads toward the tail base. At 48-72 h, the lesion area changes from cyanosis to black (15). Histological examination in a study by Lee *et al* (15) indicated thrombus formation in the tail vein, pulmonary vein and hepatic veins (15). However, routine H&E staining alone cannot definitively determine whether a thrombus is located in an arterial or venous lumen.

The routinely recommended 48-h pathological sampling time point for CGN-induced rodent tail thrombosis model does not match the clinical time window for thrombolytic therapy in pulmonary embolism (thrombolysis indications are typically ≤14 days after symptom onset) (83). Conventional H&E staining also cannot effectively distinguish fresh thrombi (which are rich in platelets and fibrin) from organized thrombi, which are rich in collagen and smooth muscle cells. This limitation restricts the application of this model to studies of the pathogenic mechanism of chronic thromboembolic pulmonary hypertension (CTEPH). A key pathological feature of CTEPH is thrombus organization and pulmonary vascular remodeling. If the research objective involves CTEPH or treatment responses according to thrombus age, then it is recommended to add long-term sampling time points at 7, 14, and 28 days after model induction, combined with Masson's trichrome staining or immunohistochemistry, such as α-smooth muscle actin and CD31, to identify the degree of thrombus organization.

Miscellaneous factors. Environmental factors, such as temperature, humidity and light, may also affect model outcomes. During model establishment, laboratory temperature is typically maintained at 20-25˚C, since lower temperatures may exacerbate tail ischemia. Humidity is controlled at 40-70% and the light-dark cycle is set to 12 h, with free access to food and water. A previous study reported that environmental static magnetic fields reduced the length of tail blackening in rat thrombosis models, decreased *in vitro* thrombus mass and reduced wet and dry thrombus weights, suggesting a potential antithrombotic effect (43). These findings suggest that specific environmental factors may interfere with model outcomes and therefore require strict control during experiments.

Model selection and standardization recommendations. Based on the aforementioned analysis of administration routes, animal strains, sex, age and dosage aforementioned,

the following standardized recommendations are proposed for different experimental objectives.

For routine antithrombotic drug screening. Intraperitoneal injection of κ-CGN (50 mg/kg) in 6-8-week-old male ICR or BALB/c mice is recommended, with 48 h after model induction used as the observation endpoint and relative tail thrombus length as the primary evaluation index (14,15,22-27). This protocol is simple to perform and has good reproducibility, making it suitable for medium-to-high throughput drug screening.

For intrinsic coagulation pathway studies. Tail vein injection of κ-CGN (1 mg/kg) combined with tail base ligation for 10-20 min in male SD or Wistar rats (150-250 g) is recommended (32,34,35). This protocol can rapidly activate the coagulation factor XII (FXII) contact activation pathway, making it suitable for studying the intrinsic coagulation pathway and evaluating anti-FXII targeted drugs.

For sepsis-associated DIC modelling. Intraperitoneal injection of κ-CGN (100 mg/kg) combined with LPS (50 μg/kg) in male KM mice (26-32 g), followed by housing at 16±1˚C for 12 h after model induction, is recommended (17). This protocol can reproduce consumptive coagulopathy and multi-organ microthrombi, making it suitable for studying the pathological mechanisms of DIC and evaluating anticoagulant drugs.

Critical parameters requiring strict control. The CGN subtype should be clearly specified (κ-type is preferred, Sigma C1013 or equivalent), where the molecular weight and batch information should be recorded. The animals should ideally be 6-8 weeks old, with sex clearly specified and considered in the analysis. Environmental temperature should be controlled at 22-25˚C except for the DIC model, with the humidity set at 40-70% with a 12 h light-dark cycle. The observation time window should be set according to the research objective, with acute thrombus evaluation primarily at 24-48 h and chronic or organization-related studies extended to 7-28 days.

4. Comparative medicine and translation of the model

An ideal animal model of thrombosis should reproduce relevant aspects of the pathophysiological process of human thrombus formation and help predict clinical efficacy. The rodent CGN immunothrombosis model is an inflammation-thrombosis model used in antithrombotic drug research due to its unique triggering mechanism and pathological features. However, there are differences between rodent thrombus models and human thrombotic diseases in terms of triggers and pathological mechanisms, such that the model cannot reproduce all types of human thrombotic diseases. Therefore, its research value and limitations should be considered from comparative medicine and translational medicine perspectives.

Comparative medical analysis with human thrombotic diseases in terms of triggers and mechanisms. In rodent thrombosis models, intraperitoneal injection of CGN activates TLR4 receptors on the surface of peritoneal macrophages through the specific recognition of the sulfate groups and α-1,3-galactoside epitopes of CGN (84,85), upregulating proinflammatory factors, including TNF-α and IL-1β and tissue factor (TF), through the TLR4/NF-κB pathway (84,86). Activated macrophages release inflammatory mediators (87) that recruit and activate neutrophils (88). Under severe inflammatory stress, TF expression in neutrophils is upregulated (89), where upon activation, neutrophils undergo a respiratory burst and release ROS, proteases and neutrophil extracellular traps (NETs). CGN has been demonstrated to induce NET release by viable neutrophils in a mouse model of acute peritonitis, with neutrophil elastase and myeloperoxidase expressed on DNA fibers (88). These processes can directly damage vascular endothelial cells and basement membrane (88,90). After endothelial injury, vascular collagen is exposed, providing binding sites for coagulation factors, where endothelial cells can also upregulate TF expression under inflammatory stimulation (15). In addition, monocytes are considered a major cellular source of TF in circulating blood (91). Macrophages, monocytes, neutrophils and endothelial cells therefore provide multiple cellular sources of TF where, together with inflammatory cytokine release, endothelial injury and NET release, can combine to initiate the extrinsic coagulation pathway, promoting thrombus formation. Vascular endothelial injury is a key initiating feature of the model. The model may reproduce aspects of inflammation-driven endothelial dysfunction observed in conditions, such as coronavirus-19 disease (COVID-19), sepsis, autoimmune diseases and post-traumatic inflammation. TLR4 is highly conserved in recognizing LPS (92), but response to LPS-like substances differs between humans and rodents. Nauseef (93) previously reported that human and mouse neutrophils differ in terms of oxidative burst intensity, granule components, receptors and NET generation capacity, resulting in species differences in the initiation threshold of NETosis, NET structure and *in vivo* clearance pathways. These differences consequently may affect thrombus formation kinetics.

After the intravenous injection of CGN into the tail vein, its highly sulfated polysaccharide structure carries a strong negative charge that can specifically bind to basic amino acid residues on the surface of circulating FXII molecules, through electrostatic interactions (94,95). This binding induces conformational rearrangement of FXII, driving its spontaneous activation into activated factor XII (FXIIa), a non-enzymatic physical activation process that does not depend on calcium ions or physiological cofactors (94,96). As the initiating factor of the intrinsic coagulation pathway, FXIIa sequentially activates downstream coagulation factors, such as factor XI, initiating and amplifying the intrinsic coagulation cascade, ultimately inducing thrombus formation (97,98). Previous studies have demonstrated that rodents and humans exhibit differences in complement-system architecture and the abundance of certain circulating coagulation factors. Notably, basal hemostatic phenotypes are conserved: Human congenital factor XII deficiency results in prolonged activated partial thromboplastin-time without clinical bleeding (99), and FXII-deficient rodents also show normal baseline hemostasis. Nevertheless, under pathological inflammatory-thrombotic challenge such as contact-activation stimuli, rat experiments indicate that FXII participates prominently in multiple inflammatory and coagulation cascades (31,32), whereas the *in vivo* contribution of FXII to pathological thrombus formation in human patients remains less well defined (100). These interspecies differences in immune cell composition and function

are important considerations in model translation. Therefore, if the candidate drug targets NETosis or FXII, the predictive application results of the model cannot be directly extrapolated to human clinical efficacy.

In addition to the aforementioned intrinsic and extrinsic coagulation pathways, the model also includes a direct platelet activation pathway. κ-CGN can also directly bind to the CLEC-2 receptor on the platelet membrane, inducing platelet aggregation and granule release without relying on collagen, further accelerating thrombus formation (23). At the molecular level, human recombinant CLEC-2 protein can also directly bind to κ-CGN (23). Notably, this recombinant-protein binding does not guarantee equivalent cellular functional responses; robust functional evidence for κ-CGN-driven activation of intact human platelets remains lacking (23).

The CGN thrombus model manifests as shortened APTT, elevated FIB, and progressively increasing D-dimer levels. Following combined administration of CGN and LPS, it progresses to consumptive coagulopathy, with prolonged PT/APTT and progressively decreasing platelet counts (17,41), reproducing features of the coagulation abnormalities observed in clinical DIC.

Slow blood flow in the distal tail vein may contribute to the preferential development of thrombosis in the tail. Due to the slower blood flow velocity at the distal end of the rodent (101,102), CGN injected into the tail or inflammatory mediators in the circulation can accumulate locally, thereby inducing platelet aggregation and fibrin deposition and ultimately forming a thrombus (33). By contrast, areas with faster blood flow in the systemic circulation may be less prone to similar focal thrombus formation (33). Therefore, distal tail blood stasis may contribute to the preferential location of thrombosis in this model. Clinical conditions associated with venous stasis, such as prolonged bed rest, postoperative immobilization and venous compression by tumor, share this hemodynamic feature with the model.

Correspondence between model thrombus classification and clinical thrombus. Tail vein thrombi induced by CGN alone are red thrombi primarily composed of red blood cells and fibrin (15,27), similar to the composition of clinical venous thrombi. A 2025 clinical MRI study confirmed that deep vein thrombosis thrombi contain abundant red blood cells and fibrin (103).

Following high-dose CGN administration, microvascular thrombi in various organs may be platelet-rich white microthrombi or mixed inflammatory microthrombi (15), whereas multi-organ microcirculatory thrombosis associated with clinical sepsis and severe infection can also involve platelet-rich white microthrombi or mixed inflammatory microthrombi. Autopsies of patients with meningococcal sepsis revealed widespread pulmonary microvascular thrombi consisting of platelets and white blood cells (104). Purpura fulminans associated with *Escherichia coli* sepsis also manifested as platelet-rich white thrombi in skin microvessels, accompanied by protein C deficiency and DIC (105). In septic shock, endothelial dysfunction and coagulation-fibrinolysis imbalance further promote the formation of mixed inflammatory microthrombi (106). Histologically, septic microvascular thrombi frequently contain platelets, white blood cells and fibrin, which are consistent with mixed inflammatory microthrombi (107,108). The consumptive coagulopathy and multi-organ microthrombi induced by the combined CGN and LPS (17,41) share features with sepsis-associated DIC and COVID-19-related coagulation disorders.

Model evaluation from the perspective of translational medicine

Simulation validity for human diseases. The model most closely represents sterile inflammation-driven thrombosis, a pathological mechanism relevant to several diseases. Clinically relevant examples include immune-mediated thrombosis, such as antiphospholipid syndrome (109), inflammatory bowel disease-associated thrombosis (110) and severe COVID-19 coagulopathy (111). In these diseases, abnormal activation of the innate immune system contributes to thrombosis. The model may also represent certain stages of acute infection-induced DIC, particularly the phase dominated by inflammation with widespread microvascular thrombosis.

However, the model has several limitations in terms of its representation of human disease.

Anatomical location. Human thrombotic disease commonly involves deeper and larger vessels, which differ mechanically and anatomically from the tail vein-based thrombi in the model. Thrombus formation can also be observed in lung tissue in the model (15). In the literature, pathological examinations frequently describe 'pulmonary vein thrombosis' or 'hepatic vein thrombosis', which are considered *in situ* thrombi based on pathogenesis. However, routine H&E staining alone cannot definitively distinguish whether a pulmonary thrombus represents an embolus resulting from detachment of a tail vein thrombus or an *in situ* pulmonary thrombosis (112). Routine histopathological examination can reveal thrombus morphology and location but cannot trace its origin. Furthermore, no relevant studies employing thrombus-tracking techniques, such as fluorescent labeling of tail vein thrombi or serial tissue sectioning to trace thrombus migration, were retrieved in the systematic literature search. Since most clinical pulmonary emboli originate from deep venous thrombi that detach and migrate to the pulmonary arteries (113), the inability to establish whether pulmonary thrombi in this model are embolic limits its translational application to pulmonary embolism research. Future studies may consider using fluorescent or radioactive labeling of tail vein thrombi combined with *in vivo* imaging techniques to clarify the source of pulmonary thrombi.

Hemodynamics. The blood flow in the tail veins is slow, with a component of venous stasis, which differs from the thrombus formation mechanism under high shear stress in the clinical arterial system (114). Clinical pulmonary embolism may involve acute right ventricular dysfunction, manifested as an increased right ventricular/left ventricular end-diastolic diameter ratio (RV/LV ratio), decreased tricuspid annular plane systolic excursion (TAPSE) and reduced right ventricular fractional area change (115). Elevated serum B-type natriuretic peptide (BNP) or N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels can also reflect increased right ventricular wall tension (116). However, these hemodynamic and biomarker measures were not retrieved as routine

outcome measures for κ-CGN-induced thrombosis models in the present focused literature search. However, these hemodynamic and biomarker measures were not identified as routine outcome measures in the CGN-induced thrombosis models in the focused literature search (14-16). Their absence limits the assessment of hemodynamically relevant pulmonary embolism and pulmonary embolism-associated right ventricular dysfunction using this model. Future studies could include echocardiography to assess RV/LV ratio, TAPSE and measurement of serum BNP/NT-proBNP levels after tail vein injection of carrageenan to evaluate pulmonary embolism-related changes in right ventricular function.

Chronic etiology. Human thrombosis is often based on chronic processes, such as atherosclerosis (117), whereas this model is induced acutely and lacks underlying chronic vascular pathology (118).

Key barriers to clinical extrapolation of the model

Non-physiological triggers. Food-grade CGN is poorly absorbed from the gastrointestinal tract into the bloodstream (50). The model uses exogenous CGN administration to induce thrombosis, which does not reproduce the spontaneous primary trigger of human thrombosis. In particular, it does not reproduce atherosclerotic plaque rupture and is therefore unsuitable as a sole model for confirmatory efficacy evaluation of drugs targeting cardiogenic or atherosclerotic thrombosis.

Lack of systematic validation with standard clinical anticoagulants for pulmonary embolism. In previous studies, positive control drugs are mostly heparin (subcutaneous injection, 300-5,000 U/kg) or aspirin (oral gavage, 20-50 mg/kg) (24,25,27,28,31,33), representing the classic standards in anticoagulant and antiplatelet therapy, respectively (119,120). Low-molecular-weight heparin has also been used as a positive control (121). Nevertheless, systematic parallel comparison of this model with direct oral anticoagulants (DOACs), such as rivaroxaban, apixaban and dabigatran, remains limited. DOACs are used in the clinical prevention and treatment of pulmonary embolism (122). The lack of pharmacodynamic bridging data with DOACs limits direct comparison of model efficacy outcomes with clinical treatment strategies. Future studies could include DOACs as positive controls to enhance the clinical translational relevance of the model.

Acute thrombus course. The conventional observation window of the model is mostly 1-3 days, whilst clinical deep venous thrombosis involves a prolonged course of organization, recanalization and post-thrombotic syndrome (123). Short-term models do not permit evaluation of long-term organ damage and sequelae.

Lack of arterial thrombus phenotype. This model mostly induces venous and microvascular thrombosis and does not reproduce arterial plaque rupture-associated thrombosis. It is therefore unsuitable as a sole model for confirmatory efficacy evaluation of antithrombotic drugs for stroke or myocardial infarction.

Species differences. Differences in neutrophil function and coagulation factors between humans and rodents may affect the translation of efficacy findings from rodent models to humans (93,99).

Model optimization and precision translation strategies

Model combination strategy based on comparative pathology. A single model should not be used in isolation. Findings from the CGN immunothrombosis model (inflammation-coagulation model) can be evaluated alongside findings from the ferric chloride thrombosis model (oxidative damage-arterial thrombosis), the inferior vena cava ligation stasis model (venous thrombosis) and the laser injury thrombosis model (precise microvascular thrombosis) for validation (124). If a candidate drug shows consistent efficacy across these mechanistically distinct models, this may increase confidence in its translation potential.

Selection of species and strain for stratified validation. During initial screening and mechanism exploration, rodent models can be used to investigate the role of a target within the relevant pathway. In addition, when resources permit, candidate compounds validated in rodents may undergo further evaluation using similar inflammation-thrombosis models, such as endotoxin-induced DIC models, in minipigs or non-human primates, which may more closely represent aspects of human cardiovascular anatomy, coagulation and immune function (125).

Precision translation according to human disease type. The application of this model should be focused on thrombosis driven primarily by innate immune activation. In clinical trial design, patient stratification based on inflammatory markers, such as elevated C-reactive protein, D-dimer and neutrophil levels, may be considered (126). Therefore, data obtained from animal models sharing a relevant mechanism, such as TLR4/inflammatory pathway activation, may be applied to appropriately selected patient populations to improve precision in clinical translation.

5. Conclusion

Since Giroud and Timsit (21) first reported in 1973 that carrageenan could induce thrombosis, the CGN-induced rodent immunothrombosis model has undergone >50 years of development and optimization and is now used to assess systemic inflammation, endothelial injury, coagulation cascade activation and multi-organ microthrombi. The critical analysis and discussion of translational limitations in the present review indicate that the model has both specific applications and important limitations that should be considered according to the research objective.

From the perspective of mechanistic investigation and screening applications, this model has several practical advantages. Tail thrombosis is visually quantifiable and multiple measures of coagulation, inflammation and histopathology can be assessed, making it suitable for the preliminary screening of anti-inflammatory and anticoagulant candidate drugs, such as natural products, small molecules and probiotics. The model also provides an *in vivo* platform for initial target validation that can be used for mechanistic studies of sterile immunothrombosis and sepsis-related DIC. Intraperitoneal injection of κ-CGN (50 mg/kg in mice) is a widely used and data-rich protocol, with its operational simplicity and reproducibility supporting medium-to-high-throughput screening of antithrombotic drugs.

However, the limitations and applicability of this model should be clearly defined, such that experimental conclusions should not be extrapolated to all human thrombotic diseases. The model relies on exogenous seaweed polysaccharide stimulation and lacks primary pathological conditions, such as human atherosclerosis and chronic vascular degenerative damage. In addition, thrombosis is mainly confined to superficial tail veins and organ microvessels, making it difficult to reproduce high-shear arterial thrombosis (127). There are species differences between humans and rodents in neutrophil function and coagulation factors (93,99). Furthermore, the model primarily represents acute thrombotic lesions over a period of several days and does not reproduce long-term pathological processes, such as thrombus organization and post-thrombotic syndrome (118,128). Therefore, it is not suitable as a sole model for confirmatory efficacy studies of conditions, such as atherosclerotic myocardial infarction and cerebral infarction or as the sole basis for clinical translation.

Based on findings summarized in the present review, this model is most optimally suited to preliminary screening and mechanistic exploration, particularly for studies of inflammation-mediated venous thrombosis, severe infection-related coagulopathy and pathological mechanisms relevant to antiphospholipid syndrome. If the research objective involves arterial thrombosis, long-term thrombotic lesions or confirmation of candidate drug efficacy, the model should be cross-validated with other systems, such as the ferric chloride vascular injury model, inferior vena cava ligation stasis model and large animal DIC models, to address the translational limitations of a single model.

At present, numerous areas in this field require further investigation. The dose-response relationship of different CGN subtypes and molecular weights has not been systematically compared, studies including both sexes are limited, unified standardized operating guidelines have not been established and systematic validation data for positive control drugs (such as DOACs) are lacking. Future controlled side-by-side male-female comparative experiments are warranted to clarify sex-dependent thrombotic outcomes in pure carrageenan-only thrombosis models. Future research could focus on three areas, namely raw material quality control, standardized modeling procedures and multi-model validation. The scope of model application should be clearly defined, distinguishing between basic mechanism screening and confirmatory clinical translation, whilst taking advantage of its throughput and ease of use and accounting for its species, pathological and anatomical limitations. The model may thereby remain useful for basic immunothrombosis research and early drug screening.

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XM and SL conceived and designed the study. XM and DW performed data collection and conducted the literature search. XM wrote the manuscript. All authors assisted with manuscript revisions. SL approved the manuscript. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript

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