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REVIEW

Animal models of hematogenous *Staphylococcus aureus* osteomyelitis in long bones: a review

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Louise Kruse Johansen
Henrik Elvang Jensen

Department of Veterinary Disease Biology, Faculty of Health and Medical Science, University of Copenhagen, Frederiksberg, Denmark

Abstract: Hematogenous osteomyelitis (HO), especially due to *Staphylococcus aureus*, is primarily reported in children and occurs when blood-borne bacteria settle in the metaphysis of a long bone and mediate an inflammatory response. The literature contains several reports on animal models aiming to simulate pediatric HO, in order to investigate the pathogenesis and for therapeutic use. In these models, osteomyelitis lesions develop subsequently to bacteremia, which can be induced by either intravenous or intra-arterial inoculation of bacteria. Intravenous inoculation is not optimal because of the ethical aspects of the extensive systemic reaction and the unpredictable identity of bones being infected. Also, intravenous inoculation often has to be combined with the induction of artificial bone necrosis in order to have macroscopic lesions. In contrast, models based on intra-arterial inoculation and subsequent development of local osteomyelitis, are the most accurate and predictable way to extrapolate to pediatric cases of HO. The most commonly used animal species for modeling of HO are rabbits, chickens, and mice, whereas, less frequently, dogs, rats, and pigs have been applied. The use of intra-arterial inoculation, without simultaneous artificial bone necrosis for the development of HO lesions has only been used in porcine models. Because of the similarity of human and porcine physiology, metabolic rate, and size, porcine models of HO are advantageous. Therefore, porcine models based on the intra-arterial induction of osteomyelitis are the most refined HO models.

Keywords: hematogenous osteomyelitis, animal models, *Staphylococcus aureus*

Introduction

Hematogenous osteomyelitis (HO) is an infection of bone and bone marrow caused by blood-borne microorganisms, usually *Staphylococcus aureus*, and is most commonly present in children.[1] Although HO is optimally cured with antibiotics and surgery, the potential complications of HO in childhood are severe and sometimes lifelong.[1] In the western world, many aspects, including the incidence, diagnosis, and treatment of pediatric HO, have changed owing to vaccination programs, high standard imaging techniques, and a general good health care system. Therefore, chronic HO is now rarely seen in this part of the world, whereas many children from developing countries still suffer both physically and socially from chronic HO (Figure 1).[2,3] Various animal models of osteomyelitis have been developed by the use of the direct injection of bacteria into the medullary cavity often in combination with a sclerosing agent.[4] However, the disease produced by this method is not similar to the development of osteomyelitis seen in children and infants, in which the pathogenesis particularly is hematogen.[1] This paper is a literature review of (1) HO in children focusing on the mechanisms behind blood-borne seeding of

Correspondence: Louise Kruse Johansen
Department of Veterinary Disease Biology, Faculty of Health and Medical Science, University of Copenhagen, Ridebanevej 3, 1870 Frederiksberg C, Denmark
Tel +45 2216 5332
Email louise-k@sund.ku.dk

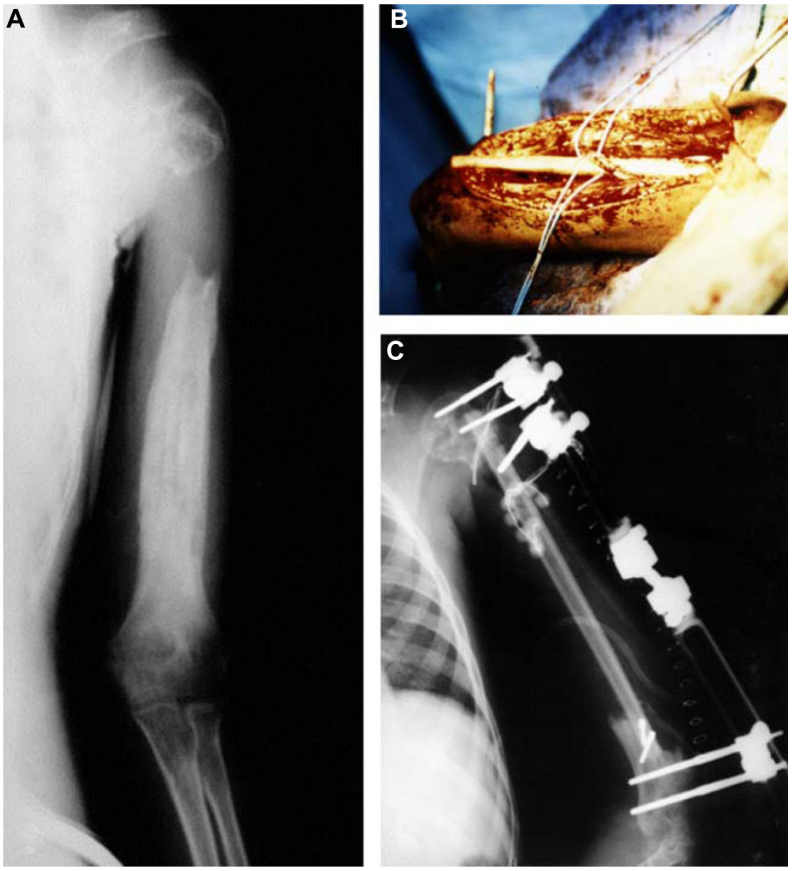

Figure 1 Chronic HO in humerus of an 11-year-old Angolan girl.
Notes: (**A**) Chronic HO of humerus, the proximal part of the bone is completely destroyed. (**B**) The right fibula is dissected free. (**C**) The fibula is inserted into the remaining part of the debrided humerus to build a foundation for new bone.
Abbreviation: HO, hematogenous osteomyelitis.

bacteria to long bones, and (2) a report of animal models based on bacterial inoculation into the blood stream in order to mimic HO.

Inclusion and exclusion criteria

The keywords "h(a)ematogenous osteomyelitis," "osteomyelitis," "p(a)ediatric," "bone infection," and "animal models" were searched individually and combined in the following databases: PubMed, Web of Science and Google Scholar. The articles were selected in two groups concerning general aspects of osteomyelitis and animal models, respectively. The titles and abstracts identified by the above-mentioned searches were downloaded and evaluated by using the inclusion/exclusion criteria listed in Table 1. Thereby, only papers regarding HO in long bones and animal models of osteomyelitis inoculated into the blood stream were enclosed. This resulted in consensus between the first section about HO in children and the animal models described in the last section of the present review.

Epidemiology of hematogenous osteomyelitis in children

In some studies, a decline in the incidence of HO among children has been reported,[5,6] whereas others report either no change or an increase.[7–9] HO is estimated to account for

Table 1 Inclusion and exclusion criteria

Main areas	Number of articles included	Inclusion	Exclusion
Hematogenous osteomyelitis	50	Long bone Pediatrics Acute bone infection Chronic bone infection Biofilm formation	Implant-associated osteomyelitis Traumatic osteomyelitis Vertebral osteomyelitis
Animal models of osteomyelitis	28	*Staphylococcus aureus*	Intramedullary inoculation

up to 1% of all pediatric hospitalizations in industrialized nations (Table 2).[10] Development of a chronic disease stage of HO with severe morbidity and disability occurs especially for children living in developing countries (Table 2).[3] *S. aureus* is the predominant cause of pediatric HO[9,11] and is implicated in 50%–90% of cases.[9] A broader spectrum of causative organisms is found particularly in infants and children less than 4 years of age, where streptococci and Gram-negative bacteria such as *Haemophilus influenzae* and *Escherichia coli* are responsible for up to 60% of cases, with the remainder being caused by *S. aureus*.[9,12] HO is often caused by methicillin-susceptible *S. aureus* bacteria, although the incidence of community-acquired methicillin-resistant *S. aureus* bacteria (CA-MRSA) has increased in many countries.[1,13,14] The actual rate of CA-MRSA in pediatric HO has not been calculated, but CA-MRSA is increasingly isolated from cases of HO.[14] Some paper reports that CA-MRSA-caused HO is associated with a more aggressive course of disease with severe clinical symptoms.[15] Multiresistant Gram-negative bacteria are also becoming an emergent problem; however, they have literally only been described in cases of adult vertebral HO following urinary tract infections or intravenous drug use.[16] Furthermore, multiresistant *Acinetobacter baumannii* have increasingly been isolated from US soldiers and local citizens from Afghanistan and Iraq following an infection of severe war-related bone trauma.[17] However, it is tempting to speculate that these organisms also are involved in cases of HO in children living in this part of the world (Middle East and Western Asia). *H. influenzae* type B and *Mycobacterium tuberculosis* remain major causes of HO in many less developed countries[1,9,18,19] and fungi, eg, *Candida* spp. and *Aspergillus* spp., are also rare but important causes of HO in immunocompromised patients and neonates.[1,20]

Table 2 Epidemiology, risk factors, and pattern of bone distribution of pediatric HO

Epidemiology		
Incidence of acute and subacute HO		
Developed countries	1.94–13/100,000[9]	
Developing countries	43/100,000 (Polynesia[11]) 200/100,000 (Aborigines[6])	
Incidence of chronic HO		
Developing countries	Estimated 12 million[3]	
Mean age	6.6[9]	
Male: female	1.8:1[9]	
Risk factors (%) for HO in children	Unknown 47.0[9]	
	Blunt trauma 29.4[9]	
	Recent systemic infection 37.4[9]	
Pattern of bone distribution (%) of HO in children		
Femur	26.9%	HO involves most often only one bone[9]
Tibia	26.6%	
Pelvis	9.2%	
Humerus	8.1%	
Foot	7.7%	
Forearm	4.8%	
Calcaneus	4.6%	
Vertebra	3.8%	
Fibula	3.7%	
Other	3.6%	

Abbreviation: HO, hematogenous osteomyelitis.

Long bones as an infectious focus

There are four categories of bones: long bones, short bones, flat bones, and irregular bones. Characteristically, pediatric HO involves long bones of the extremities (Table 2).[1,9] Most often only one bone is involved; however, in neonates, 50% of cases involve multiple bones.[9] The diaphysis of long bones is composed primarily of dense cortical bone, whereas the metaphysis and epiphysis are composed of a trabecular bone meshwork surrounded by a relatively thin shell of dense cortical bone. The difference between cortical and trabecular bone tissue anatomy is important for the typical development of HO and the definition of chronicity. Because cortical lesions develop more slowly and can be difficult for the organism to repair, HO is clinically defined as chronic when the disease has occurred for several months or dead cortical bone tissue can be identified.[3] Because of the fast growth of the long bones in children, the associated vascular systems are also developing fast.[21,22] This leads to discontinuous and weak vessels and can explain why HO in childhood occurs in long bones, compared with the situation in adults, where the vertebra is most often involved.[23] The fact that long bones of the extremities are extra exposed to small blunt trauma due to childhood activities, creating a locus of minor necrosis, has also been speculated as a reason (Table 2).[9,24]

Pathogenesis and disease development

The metaphysis of long bones is most frequently involved in pediatric HO, and the anatomy of the metaphyseal region seems to be a major cause for this localization.[1] However, primary epiphyseal osteomyelitis does also occur.[25,26] The nutrient artery ends in the metaphysis as narrow capillaries that make sharp loops near the growth plate and enter a system of large venous sinusoids (Figure 2A and 3).[1,21] On the top of the loops, blind-ended vessels named capillary sprout tips are present (Figure 3). These vessels are lined with an attenuated endothelium with no underlying basement

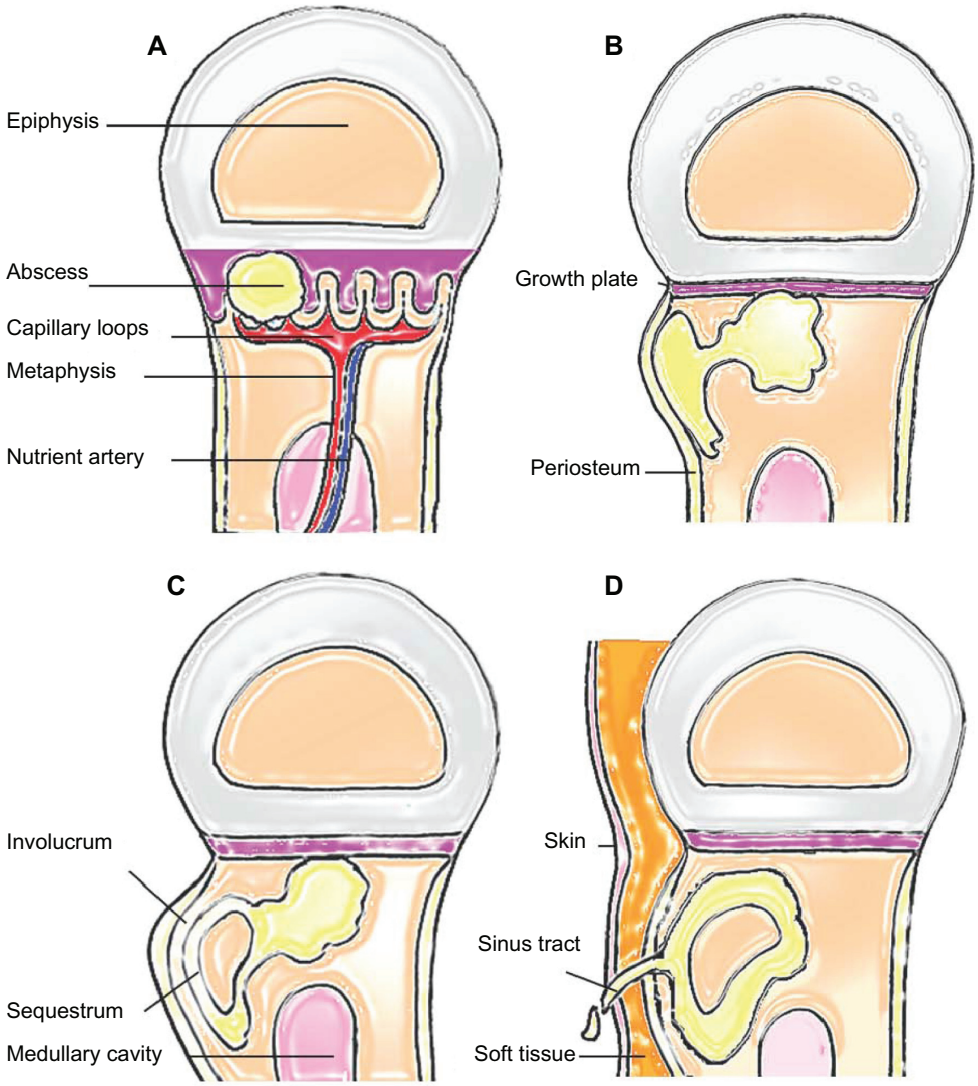

Figure 2 The pathogenesis of hematogenous osteomyelitis.
Notes: (**A**) Terminal branches of the metaphyseal artery form loops at the growth plate and are the area of osteomyelitis initiation. (**B**) The bone lesion spreads transversely to the cortex and elevates the periosteum. (**C**) A segment of devitalized cortical bone (sequestrum) develops and the elevated periosteum produces new bone to form the involucrum. (**D**) The infection erodes the periosteum and soft tissue and a draining sinus is present.

membrane and invade up the hypertrophic chondrocytes of the growth plate.[27] The metaphyseal capillaries share the same ultrastructure features as the sprout tips; however, the endothelial cell lining is more continuous and the basement membrane more developed toward the metaphyseal area of bone deposition (Figure 3).[27] It is generally accepted that this loop structure leads to a slowing and stasis of blood flow in the sinusoidal veins, which appear to be the first region where blood-borne bacteria localize.[28,29] This localization of bacteria causes thrombosis in the sinusoids and the arterial side of the metaphyseal loops is secondarily (retrograde) thrombosed.[28,29] However, the initiation of infection may also result from specific binding of *S. aureus* to the cartilage exposed at the sprout tips (Figure 3).[22,30,31] *S. aureus* displays receptors for components of the bone matrix and cartilage, including fibronectin,[32] collagen,[33] and bone sialoglycoprotein.[34] Electron micrographic studies have demonstrated that injected carbon particles and erythrocytes can escape through the endothelial gaps, and, therefore, it is possible that bacteria also pass during an episode of bacteremia.[35] When the sinusoids or the end-capillary tips are obstructed, necrosis will occur. Recently, in a porcine model of HO, *S. aureus* bacteria were detected both at the top of capillary loops and deeper in the metaphysis 12 hours after intravenous inoculation.[30] This indicates that both situations (sprout tips and sinusoids) of initial localization of bacteria are feasible.

The cytology of the metaphysis may also favor bacterial colonization. The vessels of the metaphysis have a poorly

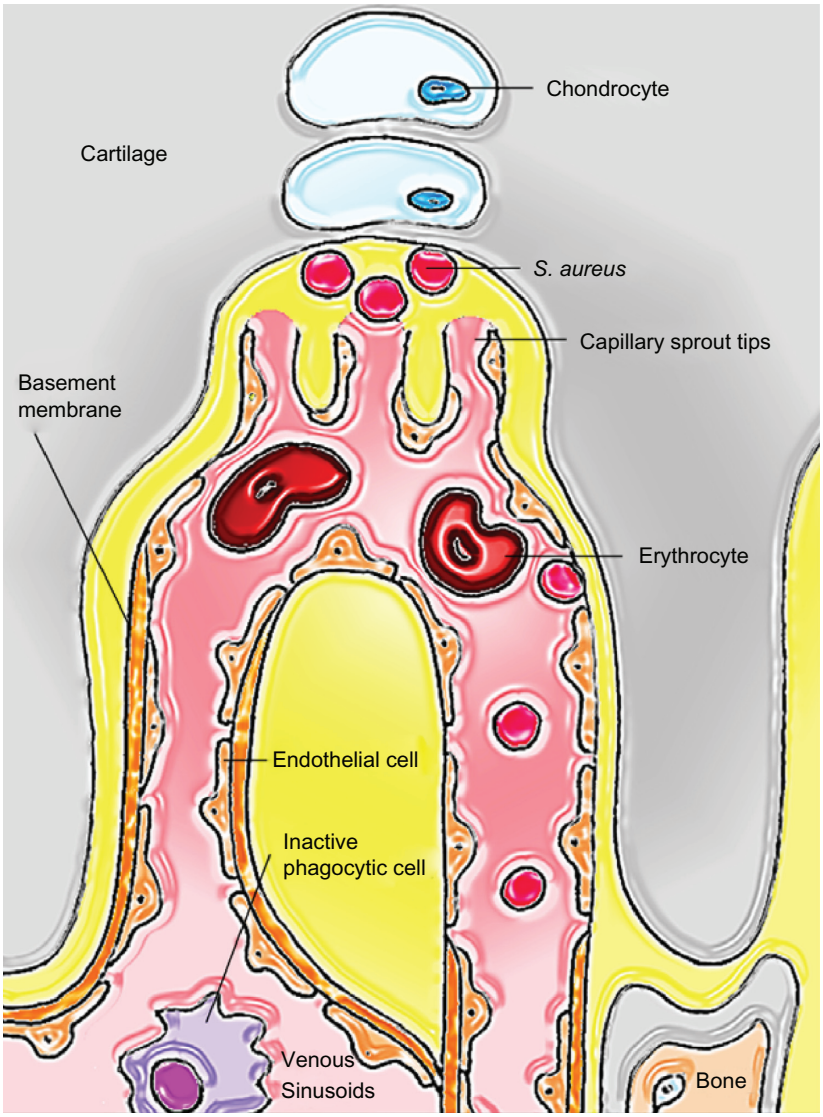

Figure 3 Metaphyseal microvasculature favoring settling of blood-borne *Staphylococcus aureus.*
Notes: Blood-borne *Staphylococcus aureus* bacteria are in favor of adherence to cartilage of the growth plate and initiate osteomyelitis owing to the interrupted lining by endothelial cells and the absence of a basement membrane present at the capillary sprout tips.
Abbreviation: *S. aureus, Staphylococcus aureus*.

developed reticuloendothelial system, because the metaphyseal capillaries lack phagocytic lining cells, and the sinusoidal veins contain functionally inactive phagocytic cells.[29] Accompanying the initial thrombosis, an acute inflammatory response mediated by a number of local and systemic cytokines and mediators of inflammation, eg, interleukin-1, interleukin-6, cyclooxygenase-2, and tumor necrosis factor alpha, are present.[36,37] These mediators influence the homeostatic balance of bone turnover, increasing osteoclast differentiation and bone resorption, and thereby driving bone destruction.[37]

In addition to the inadequate blood supply, the inflammatory response further compromises the medullary circulation, and areas of dead trabecular bone develop.[38,39] From the metaphysis, the infection may proceed toward the cortex through Haversian and Volkmann canal systems (Figure 2B).[1] A subperiostal accumulation of pus will lift the periosteum from the surface of the bone and thereby compromise the periosteal and endosteal circulations, and large areas of dead cortical bone may be formed (Figure 2C).[40] The dead cortical bone is gradually detached from the living bone to form a large sequester.[23,39,40] The complete separation may take from 2 weeks to 6 months.[23] In infants, the infection may spread to the epiphysis and joint through capillaries that cross the growth plate.[21,28] In children of more than 1 year of age, the growth plate is avascular and the infection is almost confined to the metaphysis.[21,28] However, the physis might be damaged by direct invasion and chondrolysis, and if the growth plate is intra-articular, as proximally in the femur, joint involvement can occur without damage of

the epiphysis.[26] Dead bone of both trabecular and cortical origin will be absorbed.[23,39] Trabecular bone is absorbed rapidly during 2–3 weeks leaving a cavity behind,[39] whereas necrotic cortical bone is removed more slowly (more than 6 months).[23] However, cortical sequesters may be too voluminous to be absorbed, which necessitates surgical involvement.[3] An involucrum, which is defined as a surrounding envelope of new bone, will develop around the cortical sequester, [23] and the function of this structure is to encapsulate the infection (Figure 2C). The new bone is produced from the surviving fragments of periosteum and endosteum in the region of the infection.[23] An involucrum is irregular and often perforated by openings through which pus may track into the surrounding soft tissues or drain to the skin surfaces (Figure 2D).[23]

Persistence of infection

Bacteria display two life forms during growth and proliferation.[41] In one form, the bacteria appear as single, independent cells (planktonic); and, in the other form, bacteria are organized in sessile aggregates.[41] The latter form is commonly referred to as the biofilm mode of growth. These two life forms have serious implications for bacterial infections in humans including HO.[42] The most common etiology of osteomyelitis is *S. aureus,*[9,11] which is a well-known potent biofilm-forming bacterium,[43] leading to the perception that biofilm formation is associated with osteomyelitis.[42] Acute HO is assumed to involve planktonic bacteria (Figure 4) and is generally treatable with antibiotics, although successful treatment depends on accurate and

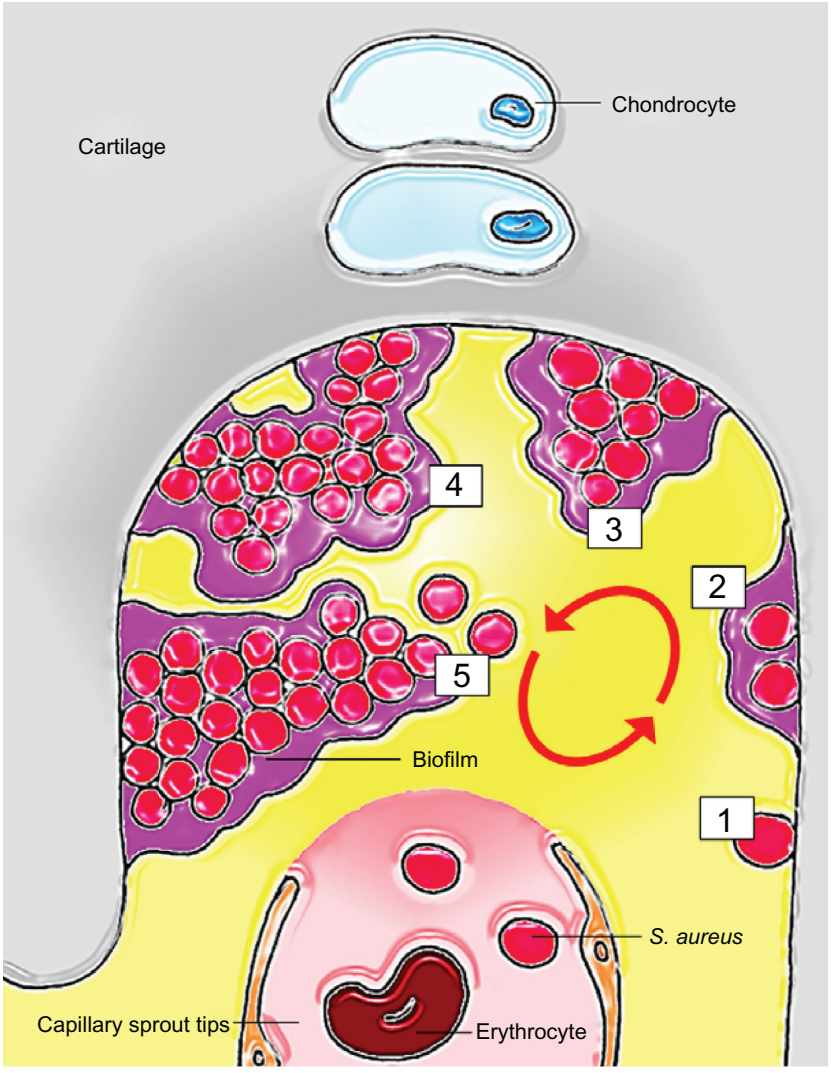

Figure 4 Biofilm formation in hematogenous osteomyelitis.
Notes: When *Staphylococcus aureus* bacteria escape from the blood stream through the capillary sprout tips of the metaphysis, they can grow as a biofilm on cartilage and bone tissue. Five stages of biofilm development exist:[47,48] (**1**) initial attachment, (**2**) irreversible attachment, (**3**) maturation I, (**4**) maturation II, and (**5**) dispersion.
Abbreviation: *S. aureus, Staphylococcus aureus*.

fast diagnosis.[44,45] However, in the cases where the bacteria succeed in forming a biofilm within the human host, the infection often turns out to be untreatable and will develop into a chronic state.[3,46] HO is induced when the infecting bacteria anchor to the cartilage of the primary spongiosa (Figure 4). After that, the bacteria start the production and excretion of extracellular matrix substances, which is the first step in biofilm formation.[47] The definition of a biofilm is as follows: a microbially derived sessile community, typified by cells that are attached to a substratum, interface, or each other, are embedded in a matrix of extracellular polymeric substance, and exhibit an altered phenotype with regard to growth, gene expression, and protein production.[48] Growing as a biofilm benefits facilitation of resistance, such as elimination by antimicrobial drugs and host phagocytic clearance. [41] The resistance to antibiotics is mediated through very low metabolic levels and radically downregulated rates of bacterial division of the entrenched microorganisms.[49] Furthermore biofilms act as a diffusion barrier to slow down the infiltration of some antimicrobial agents.[42,50] Finally, bacteria growing as a biofilm have the potential for dispersion via detachment (Figure 4).[47] Microcolonies may detach and spread to other regions of the host to attach and promote more biofilm formation.[47] Sequesters of chronic HO are an optimal nonliving surface for the attachment of *S. aureus* and, thereby, for the formation of biofilms.[42] This, coupled with the host's inability to resorb the dead bone, results in a very complicated infection to treat. Therefore, biofilm formation is assumed to be the cause of failed antibiotic treatment of HO and development of a chronic disease stage where surgical intervention or revision is necessary. Numerous in vitro studies have characterized the nature of biofilm formation. However, if biofilms are commonly accepted to be the primary reason for unsuccessful treatment of osteomyelitis, a greater understanding of how to prevent and diagnose biofilms in vivo is necessary. To obtain this understanding, it is important to rely on appropriate animal models. Animal models of osteomyelitis are an important tool for studying the initiation and development of biofilms within HO. On the other hand, if a biofilm infection has already been established, an animal model can be used to investigate how to diagnose the infecting bacteria and subsequently how to treat these infections. This has been recently illustrated; biofilm antigens present during an osteomyelitis infection were identified in a rabbit osteomyelitis model.[51–53] The upregulation of these biofilm antigens was confirmed by microarray analyses[51] and may have great potential as targets for novel diagnostic modalities and vaccines. Additionally, biofilm formation on bone trabeculae has been demonstrated shortly after injection of *S. aureus* in a porcine HO model,[39] which may explain why antibiotic therapy sometimes fails in patients diagnosed at an early disease stage.

Animal models of hematogenous osteomyelitis

Animal models of HO are essential not only for exploring the pathogenesis, but also for developing preventive and effective therapeutic strategies. Furthermore, the use of animal models of HO is important, because the clinical presentation has a marked variability that hampers controlled clinical studies. The multiple variables, eg, patient age, route of infection, anatomical location, and disease state, however, can be managed and controlled in animal models. The history of HO models, ie, models inoculated by injection of bacteria into the blood stream, is reviewed, and the basic considerations of model design are addressed. The overall pros and cons of the different models are summarized in Table 3, and details about inoculation doses, bacterial strains, and animal species are summarized in Table 4.

Rabbit models

The first models of HO were developed in the late 19th century[54,55] and were based on the intravenous inoculation of rabbits with attenuated *S. aureus* bacteria[55] or bacteria described as micrococcus.[54] In general, the rabbits did not survive for more than a few days after inoculation, and postmortem examination demonstrated abscesses in the liver, kidneys, or spleen along with occasional bone lesions. Two more reports based on the intravenous injection of rabbits followed in the first half of the 20th century.[56,57] However, in these models, only a few osteomyelitis lesions were found that did not mimic the human conditions.[56,57] In 1941, Scheman et al[58] reported on a new osteomyelitis model in rabbits based on the tibial intramedullary injection of sodium morrhuate as a sclerosing agent, in combination with bacteria, or followed by later intravenous bacterial inoculation. Rabbits that did not die of sepsis developed progressive chronic osteomyelitis lesions within the tibia and survived for several weeks.[58] This study established the basis for the creation of contained osteomyelitis models and led to the development of a number of models based on intramedullary injection of sclerosing agents.[4] Sclerosing agents are used to facilitate the formation of bone infections, because the agents result in sclerosis of the vessels in the

Table 3 Pros and cons of existing animal models of HO

Existing animal models	Pros	Cons
IA inoculated large animals (pigs and dogs)[39,61,62,75–77]	• Predictable development of bone lesion • Many physiological similarities to humans • Can be used for both antibiotic and surgical treatment experiments	• Time-consuming inoculation procedure • Expensive to purchase and house
IV inoculated pigs[30]	• Fast inoculation procedure • Many physiological similarities to humans	• Non predictable osteomyelitis • Systemic side effects • Expensive to purchase and house • Short experimental time frame
IV inoculated rodents[66,67–74]	• Economical • Fast inoculation procedure • Tolerate broad-spectrum antibiotic therapy	• Non predictable osteomyelitis • Systemic side effects • A high number of bacteria are needed to induce osteomyelitis • Difficult to allow surgical manipulation • IV administration of antibiotics is difficult
IV inoculated rabbits[24,54,55,57–60,66]	• Economical • Fast inoculation procedure • Large enough to allow some surgical manipulation	• Non predictable osteomyelitis • Systemic side effects • A high number of bacteria are needed to induce osteomyelitis • Do not uniformly tolerate broad-spectrum antibiotic therapy • The size precludes evaluation of some large surgical procedures
IV inoculated chickens[31,63–65,84,85]	• Economical • Fast inoculation procedure • Large enough to allow some surgical manipulation	• Non predictable osteomyelitis • Systemic side effects • Immune system different from human

Abbreviations: HO, hematogenous osteomyelitis; IA, intra-arterial; IV, intravenous.

medullary cavity and subsequent tissue necrosis.[58] In 1943, Weaver and Tayler[59] succeeded in developing macroscopic osteomyelitis lesions after intravenous inoculation of rabbits, although the infection rate was only 38% and nonosseous lesions appeared. Inspired by the results obtained by Scheman et al,[58] and in order to confirm the concepts of a locus minoris resistentiae, rabbit models based on a 3-point press or trauma of the proximal part of the tibia followed by intravenous inoculation were developed in the late 1980s.[24,60] In these studies, rabbits that only received intravenous bacteria had occasional small foci of histologically confirmed osteomyelitis in several bones, but animals with fracture and subsequent bacteremia developed contained, macroscopic osteomyelitis lesions of the traumatized tibia in nearly all cases.[24,60]

Dog models

Currently, only one dog model by Deysine et al for HO has been described in 1976[61] and 1983,[62] who used adult mongrel dogs for development of experimental osteomyelitis. The dogs were anesthetized, and their tibial nutrient arteries were isolated with the use of a dissecting microscope. This was followed by injection of 20% barium sulfate (acting as a sclerosing agent) in combination with the bacterial inoculum. The infection was progressive and led to the death of many of the animals within 48 hours after inoculation, and 30% had complications of the wound created for isolation of the artery.[61] Osteomyelitis lesions consisting of medullary destruction, pathological fractures and periosteal reaction developed in all the surviving animals. However, the progressiveness resulted in the death of the dogs between 4 and 16 weeks after inoculation.[61] A longer period of survival was archived in the study from 1983 in which it was planned that 10 dogs would survive for 2 years following inoculation. This was achieved with half of the animals, who exhibited pathological fractures, skin fistulae, sequestration, and active bone remodeling resembling chronic HO in humans.[62]

Chicken models

Since 1983, Emslie, Nade, and Speers have reported on several experiments in which 29-day-old chickens have been used for the development of osteomyelitis based on the inoculation of bacteria into a wing vein.[31,38,63–65] The animals were euthanized at different time points up to 8 days after inoculation. The success rate was high, with nearly all chickens developing microscopic and macroscopic osteomyelitis following 12 and 24 hours of

Table 4 Animal models of hematogenous *Staphylococcus aureus* osteomyelitis

Year	Author	Modification prior to infection	Animal species	Strain of *S. aureus*	Origin of *S. aureus* strain	CFU injected*
Route of inoculation						
Intravenous inoculation						
1884	Rodet[54]	–	Rabbit	Micrococcus	?	?
1894	Lexer[55]	–	Rabbit	Attenuated *S. aureus*	Human osteomyelitis	?
1938	Thompson[57]	–	Rabbit	OH 172	Human osteomyelitis	?
1941	Scheman[58]	+	Rabbit	? (not reported)	Human osteomyelitis	?
1943	Weaver[59]	–	Rabbit	*S. aureus*	Human osteomyelitis	?
1966	Kadyrov[4,66,**]	–	Rabbit, rat	*S. aureus*	–	–
1971	Koiunderliev[4,**]	–	Rabbit	*S. aureus*	–	–
1972	Holland[4,**]	–	Rabbit	*S. aureus*	–	–
1983	Emslie[63–65]	–	Chicken	6/42E/53/77/83A/84[a]	Avian infection	$10^{7,b}$
1985	Speers[31]	–	Chicken	6/42E/53/77/83A/84[a]	Avian infection	10^{8}–10^{9}
1986	Alderson[84]	–	Chicken	6/42E/53/77/83A/84[a]	Avian infection	10^{8}
1988	Whalen[60]	+	Rabbit	ATCC-25932	Laboratory strain	$10^{7,b}$
1989	Morrissy[24]	+	Rabbit	ATCC-25932	Laboratory strain	$10^{6,c}$
1990	Daum[85]	–	Chicken	Type 8 capsular isolate	Human arthritis	10^{7}
1995	Heinz[67]	+	Rat	*S. aureus* Phillips	Human osteomyelitis	10^{8}
1997	Matsushita[73]	–	Mouse	M138	Human infection	10^{6}
1999	Chadha[68]	+	Mouse	LS-1	Mouse pathogen	10^{7}
1999	Yoon[69]	+	Mouse	LS-1	Mouse pathogen	10^{7}
2002	Elasri[72]	–	Mouse	UAMS-1	Human osteomyelitis	10^{7}
2002	Elasri[72]	–	Mouse	UAMS-237	Mutation of UAMS-1	10^{7}
2003	Blevins[70,71]	–	Mouse	RN6390	Human infection	10^{8}
2003	Blevins[70,71]	–	Mouse	UAMS-957	RN6390	10^{8}
2003	Blevins[70,71]	–	Mouse	UAMS-1	Human osteomyelitis	10^{8}
2003	Blevins[70,71]	–	Mouse	UAMS-155	Mutation of UAMS-1	10^{8}
2003	Blevins[70,71]	–	Mouse	UAMS-929	Mutation of UAMS-1	10^{8}
2003	Blevins[70,71]	–	Mouse	UAMS-930	Mutation of UAMS-1	10^{8}
2003	Blevins[70,71]	–	Mouse	UAMS-969	Mutation of UAMS-1	10^{8}
2010	Jensen[30]	–	Pig	S54F9	Porcine lung abscess	10^{8b}
2012	Horst[74]	–	Mouse	5860	Human osteomyelitis	10^{6}
Intra-arterial inoculation						
1976	Deysine[61,62]	+	Dog	52,80,81[a]	Human infection	10^{5}
2011	Johansen[75]	–	Pig	S54F9	Porcine lung abscess	$10^{4,b}$
2012	Johansen[39]	–	Pig	S54F9	Porcine lung abscess	$10^{4,b}$
2012	Johansen[39]	–	Pig	NCTC 8325-4	Human infection	$10^{4,b}$
2012	Johansen[39]	–	Pig	UAMS-1	Human osteomyelitis	$10^{4,b}$

Notes: *The reported number of CFUs injected is only an approximate number. In cases of dose–response studies, the reported dose is the one associated with the highest frequency of osteomyelitis; **the information is obtained from a review:[4] [a]phage type; [b]CFU/kg body weight; [c]CFU/100 g body weight.
Abbreviations: CFU, colony-forming unit; *S. aureus, Staphylococcus aureus*.

inoculation, respectively.[63] The lesions were comparable to HO of hematogenous origin, with bacterial localization in metaphyses of long bones. Moreover, the histology of the lesions supported the presence of endothelial gaps in the tips of the growing metaphyseal vessels.[31] Another finding in the chicken model was the presence of vascular tunnels passing through the growth plate, allowing bacterial access to the epiphysis, as seen in infants.[63] In their series of experiments they investigated the pathogenesis of the disease,[63] the surrounding blood supply of a HO lesion,[38] the ultrastructural adherence of *S. aureus* to bone,[31] the effects of drilling and cutting on HO lesions,[65] and the therapeutic effect of specific antibiotics.[64]

Rat models

In 1966, Kadyrov et al[66] induced HO in rats by intravenous bacterial injection. Later on, in 1995, a new model was developed by inoculation of a sclerosing agent locally into the mandible and tibia of rats followed by inoculation of varying doses of bacteria into the tail vein.[67] The rats were euthanized

after 2 weeks and examined. Animals that received both a sclerosing agent and bacteria developed osteomyelitis, which was characterized by gross pathology, histopathologically and radiographically. However, animals undergoing surgery without admission of a sclerosing agent, but receiving only a bacterial inoculum, could not be consistently infected. None of the animals died during the experiment, but bacterial seeding to the spleen and liver occurred.[67]

Mouse models

Two murine copies of the rabbit model created by Morris[24] and Whalen[60], based on upper tibial epiphyseal injuries and subsequent intravenous bacterial injection were reported in 1999.[68,69] Abscess formation was seen after 10 days in the proximal tibia, and, histologically, local inflammation was evident at the fracture site. Control mice developing only bacteremia subsequent to tail vein inoculation did not develop osteomyelitis.[68,69] These studies aimed to explore the influence of bacterial bone infections on the immune system, especially on T-cell immunity. Other publications using mice exposed to only intravenous injection of bacteria for development of HO without artificial trauma have aimed at testing the virulence of different *S. aureus* strains.[70–73] In these studies, only histopathological bone lesions have been reported with no information regarding examination for, eg, systemic side effects or the presence of nonosseous lesions.[70–72] However, in a recently published paper, mice exposed to tail vein inoculation survived for 56 days and developed chronic HO, reproducing most features of the human disease.[74]

Pig models

The first porcine model of HO was based on inoculation of bacteria into the ear vein with euthanization of the animals after 6, 12, 24, and 48 hours.[25] Microscopic osteomyelitis was seen in all animals after 12 hours, and the study provided reliable information about the initiation of the disease and the associated bone pathomorphology.[25] However, pneumonia occurred as a systemic side effect. In 2010, a porcine intra-arterial inoculated model was developed.[75] This study explored the optimal dose of bacteria for development of contained osteomyelitis, which was found between 500 and 50,000 colony-forming units (CFU)/kg body weight.[75] The pigs were inoculated in the right brachial artery; however, this sometimes resulted in cellulitis, and only microscopic osteomyelitis lesions were seen in the bones supplied by the artery.[75] The former model was refined in 2011 by a shift toward inoculation into the femoral artery.[39,76] In this model, no side effects were seen, and the pigs developed macroscopic osteomyelitis lesions in the femur and tibia ipsilateral to the site of inoculation.[39,76] The femoral intra-arterial porcine model was described in a series of four publications reporting the optimal technique for femoral intra-arterial inoculation,[76] heterogeneity between different strains of *S. aureus* and visualization of biofilms within HO lesions,[39] the expression of cyclooxygenase 2 in HO lesions,[36] and the porcine models' potential for refinement of surgical procedures.[77]

Points of evaluation when designing animal models of HO

Animal species

The ideal animal species for modeling of HO should have molecular, cellular, structural, and mechanical features akin to human bone, a temperament allowing easy housing and handling, a low cost, toleration of antibiotic treatment, and a sufficient size to endure medical and surgical interventions that reflect clinical practice. Despite the development of several HO models, no such animal model exists, and the different models imply compromise and prioritization. Concerning bone anatomy, femoral cortical and lumbar trabecular bone tissues from dogs, pigs, chickens, and rats have been compared with those from human cadavers. Canine and porcine femoral bone has similar mineral proportions as in humans, with rat bone being the most different.[78] The anatomy of the avian and mammalian growth plate has also been proven different.[79] Furthermore, the size of long bones in pigs and dogs is advantageous because they will allow refinement of surgical procedures, eg, excision of abscesses and application of prostheses/medical devices.[80] Porcine and canine bony geometry can also accommodate human orthopedic equipment.[77,81] The small size of the long bones of both rabbits and chickens precludes the evaluation of many surgical procedures,[65] whereas evaluation of surgical procedures in rats and mice is extremely difficult to perform and impossible to accomplish with human orthopedic equipment. However, their small size allows for easy pulverization of the bones for quantitative microbiology.[82]

If the animal model is used for testing of antibiotic treatment, the gastrointestinal physiology of the different species will affect the results. A major drawback of using rabbits is their pseudoruminant gastrointestinal system, which preludes testing of many antibiotics.[30] In contrast, rats and mice tolerate broad-spectrum antibiotics with a minimum of side effects.[82] Because a pig is an omnivorous animal, it provides an adaptable model to evaluate the efficacy of oral and systemic antibiotic treatment.[83]

Chickens, rabbits, rats, and mice may be housed and fed more easily than dogs and pigs, thereby allowing larger numbers and more statistically powerful conclusions. Finally, the housing of dogs and pigs is expensive in comparison with the housing of small laboratory animals and chickens.

Route of inoculation

Experimental induction of HO can be established following intravenous or intra-arterial inoculation of bacteria. The reliability of the models relies on the ability to deliver the pathogen predictably and hence create local infection without creating sepsis or metastatic infection. This is difficult to accomplish in the intravenous inoculated HO models. Because HO most often occurs in a single bone, HO established by inoculation into the arterial supply to selected bones should be preferred. Apart from the porcine studies on intra-arterial inoculation,[39,75–77] this route has only been used for the development of HO in a canine model.[61,62] In the last reported porcine intra-arterial inoculated model, no complications were reported due to the inoculation technique per se.[39,77] In that model, the inoculation technique was based on bacterial injection into the femoral artery using a retrograde modified Seldinger technique (nonpercutaneous).[76] From a surgical point of view, intra-arterial inoculation in the femoral artery is manageable because of its large diameter and uncomplicated anatomical access.[76]

S. aureus strain and number of bacteria used for induction of HO

Some animal models of HO are based on bacterial strains isolated from the same animal species, whereas, in others, human strains sometimes isolated from patients with osteomyelitis are used (Table 4). The origin of the strain is likely to influence the outcome of the lesions induced, because of potential host specificity and the different expression of virulence factors.[39] It has been suggested that the inoculation dose of the human strains needs to be increased in order to obtain comparable lesions, as with an animal strain from the same species.[39] In all previous studies of HO, the intravenously inoculated models received bacterial doses above 10^6 CFU (Table 4) to obtain high osteomyelitis frequencies when inoculated with human strains (Table 4). Despite these high intravenous inoculation doses, trauma or sclerotic agents have often been necessary in order to induce macroscopic osteomyelitis in rabbits, rats, and mice (Table 4). A small inoculum is needed for development of HO in dogs and pigs; however, this has only been demonstrated within dogs in combination with artificial bone necrosis.[61,62]

Age of the experimental animal

The age of the animals used for modeling of HO in long bones is essential, because the disease commonly occurs in children. However, the age of the used animals was not discussed in the majority of the present papers listed in Table 4. In the dog model,[61,62] it is only reported that adult animals were used and, in the rat model, the age has not been reported at all.[67] The only information about the difference in development of HO between young and adult experimental animals was reported by Weaver and Tayler in 1943.[59] They found that rabbit best suited for development of HO was between 6- and 8-weeks-old and that attempts in adult animals were met with complete failure.[59] Rabbits of 8 weeks were also used in the models developed by Morrisy and Haynes[24] and Whalen et al.[60] The murine models have been based on female mice that were 8- to 10-weeks-old.[66,68–74] Experimental mice are weaned and sexually mature at this age, which might influence the inability to establish large macroscopic lesions in most of these models.[66,68–73] The porcine[30,75] and avian[63–65] HO models were developed in growing animals and demonstrated the presence of transphyseal vascular tunnels, which are comparably found in infants.

Conclusion

The development of HO in children is complex, and the initial situation going on in the metaphysis of long bones (Figure 3) cannot be replicated or compared to direct intramedullary inoculation of bacteria. Therefore, animal models of HO should be based on infection due to bacteremia. However, looking at the history of animal models of osteomyelitis based on bacterial injection into the blood stream, these models are few and often unsatisfactory regarding present experimental animal welfare. Many questions of HO in children still need to be answered, regarding biofilm understanding and optimal strategies for both antibiotic and surgical therapy. In this perspective, replacement of the small rodent and rabbit models with large animal models that closely mimic the human situation of HO, and allow the evaluation of both antibiotic and surgical intervention, should be preferred, ie, the canine and porcine models. Goats and sheep would also be suitable animals for testing of human orthopedic devices because of the large intramedullary canal. Literally, goats and sheep have been used for modeling of osteomyelitis, although they were inoculated traumatically into the tibial medulla.[86–88] The success of these models indicates that goats and sheep could be used for modeling of HO, although the ruminant gastrointestinal system might be a drawback in pharmaceutical studies. Pigs and dogs are expensive laboratory animals; however,

a reliable method of delivering the desired pathogen to the area of interest, as with the refined femoral intra-arterial inoculation technique,[76] enables a reduction in animal numbers. Therefore, it can be concluded that the femoral intra-arterial inoculated porcine model should be favored as the most optimal animal model of HO according to the 3 Rs (replacement, refinement, and reduction) pronounced by Russel and Burch in 1959.[89] However, because pharmaceutical products necessitate preclinical testing in more than one animal or species, further development of reliable small animal model, like the recent developed murine model,[74] is also essential.

Disclosure

The authors report no conflicts of interest in this work.

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