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Chronic Staphylococcal Osteomyelitis: An Experimental Model[1]

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INTRODUCTION

Chronic osteomyelitis continues to be a serious clinical problem. For example, despite antibiotic treatment, chronic osteomyelitis has been observed to develop in 15 to 29 percent of patients with acute hematogenous osteomyelitis (1, 2). Chronic osteomyelitis has been noted to develop with greater frequency in patients with osteomyelitis secondary to postoperative infection, i.e., particularly in patients undergoing open reduction including internal fixation of fractures (1, 3–6). Little is known about the pathogenesis of chronic osteomyelitis, particularly those predisposing factors which might be more likely to lead to the development of a chronic rather than an acute lesion. The lack of a good experimental model for human osteomyelitis is most certainly responsible, at least in part, for the slow progress of investigative efforts into this serious disease. It is well known that chronic osteomyelitis of a long bone is a difficult lesion to produce in laboratory animals (7). Most animals will die within a few weeks after inoculation of bacteria into bone or else they will rapidly clear the bone marrow of infection. A number of investigators (3, 7, 8, 9) were able to produce acute osteomyelitis in experimental animals but, other than Mitra (9), were either unable to keep their animals alive, or did now show that the acute process became chronic. Recently Norden (10), employing a model initially described by Scheman and colleagues (7), reported observations on infected rabbit tibias over 180 days. To our knowledge, no one has maintained an active infection for longer periods.

The present report describes our observations on a model of chronic staphylococcal osteomyelitis maintained in the rabbit tibia for periods up to one and one-half years. The pathologic and radiologic appearance of the present model is similar

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to that observed in human disease, particularly the chronic osteomyelitis which complicates open reduction and internal fixation of fractures of long bones.

MATERIALS AND METHODS

New Zealand white adult female rabbits weighing three to six kilograms were used and injected with one of two different strains of *Staphylococcus aureus.* (1) *Staphylococcus aureus,* Giorgio strain (11) is hemolytic, coagulase-positive, penicillin resistant, and is Phage type (6, 7, 47, 53, 54, 75, 77, 81, 83, 84).

(2) *Staphylococcus aureus,* Phage type 80-81 is also hemolytic, coagulase-positive and penicillin resistant. Overnight (18 hours) cultures were prepared by inoculating a single colony from a blood agar plate into ten milliliters of beef heart infusion broth. Cultures were centrifuged for one hour at 3000 r.p.m. and then decanted. The bacterial sediment was resuspended in a volume of sterile normal saline equal to the discarded supernatant. Serial tenfold dilutions in normal saline were made from each inoculum, incubated in agar pour plates for 24 hours, and the number of colonies were recorded as previously described (12). A measured volume of the resuspended cells, which regularly contained 2×10^8 bacteria per milliliter, was used as the inoculum for most experiments. Occasionally, inocula containing 2×10^9 cells per milliliter were prepared by resuspending the bacterial sediment in one milliliter of sterile normal saline. In some experiments, inocula containing 2×10^4, 2×10^5, 2×10^6, and 2×10^7 cells per milliliter were prepared from tenfold serial dilutions of the overnight culture resuspended in normal saline.

The animals were lightly anesthesized. Under aseptic conditions, the skin was incised directly down to bone exposing about two-thirds of the anteromedial shaft of the tibia.

Group I. In the first group of 30 rabbits (inoculation only), the periosteum was elevated at the proximal end of the tibia and through a $\frac{3}{32}$ inch drill hole the inoculum was injected in a volume of 1.0 milliliter.

Group II. In a second group of 22 rabbits (fracture, inoculation and rodding), the tibia was fractured by a three-pronged clamp. The clamp consistently produced a simple fracture in the middle one-third of the shaft. There was a slight variation in the shape of the fractures but the injury was uniform. The leg was then prepared aseptically and as in Group I, the inoculum was injected through the drill hole into the medullary cavity in a volume of 1.0 milliliters. A stainless steel pin was then inserted into the medullary cavity. The pin consisted of a slightly curved $\frac{3}{32}$ inch stainless steel rod of 8–18 S.M.O. stainless steel (supplied by the Zimmer Company, Warsaw, Indiana) cut to a length that extended to within a few millimeters of the ankle joint.

Group III. A third group of 13 rabbits (inoculation and rodding) was treated identically except their tibias were not fractured prior to inoculation. The stainless steel pin was inserted into the medullary cavity as in Group II.

Group IV. Four control animals were also studied. Three were given a midshaft fracture and an intramedullary rod and no inoculation, and one received rodding only with no fracture and no inoculation.

Cultures of biopsy specimens of the medullary cavity of the tibia were obtained aseptically at specific intervals from six weeks up to 18 months after staphylococcal inoculation. Under aseptic technique a drill hole was made into the medullary cavity; the fragment of soft tissue and bone were cultured on blood agar plates and

TABLE 1

THE EFFECT OF INOCULUM SIZE ON THE NORMAL RABBIT TIBIA FOLLOWING DIRECT INOCULATION OF *Staphylococcus aureus*

Inoculum no. organisms	Number of rabbits	Tibial cultures (months after inoculation)						No. rabbits with osteomyelitis
		2	3	4	6	9	12	
Giorgio								
2×10^5	2	0/2[a]	0/2	0/2				0
2×10^6	2	0/2	0/2	0/2				0
2×10^7	3	0/3	0/3	0/3				0
2×10^8	6	0/6	0/6	0/6				0
2×10^9	6	5/6—died within 24 hours						0
		1/6—died on 30th day. Staphylococci (Giorgio) recovered from tibia[b]						0
	19							
80–81								
2×10^6	4		0/4		0/3	0/2	1/2	1
2×10^8	7	5/7—died within 4 days						0
		1/7—sacrificed on 18th day. Staphylococci (80–81) recovered from tibia[b]						0
	11							
		1/7—sterile tibial cultures from serial biopsies over 6 months						0
Group I total—30								

[a] Number of rabbits with *Staphylococcus aureus* recovered from tibia per total number cultured.
[b] No x-ray or histological evidence of osteomyelitis.

thioglycollate broth. Bacterial growth was subcultured and identified as the original inoculum only when it was hemolytic, coagulase-positive, penicillin-resistant and conformed to the same phage pattern as the original inoculum. Roentgenograms of the tibias were obtained at the same time of biopsy and culture in many animals. At the conclusion of the study the rabbits were killed by an intravenous administration of an overdose of Nembutal, cultures taken as before, and the inoculated tibias removed, fixed and decalcified with Bouin's solution, imbedded in paraffin, and stained with hematoxylin and eosin for histological study.

RESULTS

In order to determine the susceptibility of the normal rabbit tibia to staphylococcal infection, the right tibia of 30 normal rabbits was directly injected with either the Giorgio or phage type 80–81 strains of *Staphylococcus aureus* (Table 1). The tibias of those animals (Group I) receiving 2×10^5, 2×10^6, 2×10^7, or 2×10^8 Giorgio staphylococci became sterile, and no sustained infections could be detected by tissue examination, culture or radiographically in any of the tibiae studied. When 2×10^9 Giorgio staphylococci were inoculated, the animals did not survive, most of them dying within 24 hours. Most of those rabbits inoculated with 2×10^8 staphylococci Phage type 80–81 died, (Table 1). This is in contrast to the experiments with 2×10^8 Giorgio staphylococci (see above) which did not affect the normal rabbit. Therefore, four additional rabbits received the lower inoculum of 2×10^6 organisms directly into the right tibia. Biopsy cultures were sterile in these animals at three, six, and nine months after inoculation. Phage type 80–81 staphylococci were recovered in only one of 11 tibial biopsy cultures, and then at 12 months

TABLE 2

THE EFFECT OF FRACTURE AND RODDING ON THE DEVELOPMENT OF CHRONIC OSTEOMYELITIS IN THE RABBIT TIBIA INOCULATED WITH *Staphylococcus aureus* (Giorgio)

Inoculum (no. organisms)	Number of rabbits	Tibial cultures (months after inoculation)								No. rabbits with osteomyelitis[b]
		1	2	3	4	6	9	12	18	
2×10^4	4		0/4[a]	0/4	0/4					0
2×10^6	2		0/2	0/2	0/2					0
2×10^8	16	8/8	2/3	7/7	7/7	2/2	6/10	4/9	8/11	14
Group II total	22									

[a] Number of rabbits with *Staphylococcus aureus* recovered from tibia per total number cultured.
[b] Osteomyelitis-histology, x-ray, and culture evidence.

after challenge in an animal that had a sterile tibial biopsy culture three months after challenge (Table 1).

In order to determine the effect of fracture and rodding on the susceptibility of the rabbit tibiae to staphylococcal infection, the right tibia of 22 rabbits was inoculated with the Giorgio strain of *Staphylococcus aureus* after fracture and rodding (Table 2). Initially, four rabbits received 2×10^4 and two rabbits received 2×10^6 staphylococci. All biopsy cultures in these six animals were sterile.

Therefore, 16 rabbits received 2×10^8 Giorgio staphylococci into the right tibia after fracture and rodding (Group II) and had serial tibial biopsies, obtained over 18 months. Chronic staphylococcal osteomyelitis developed in 88 percent of these animals. The staphylococcus was recovered from 26 of 27 biopsies obtained during the first six months after inoculation. Subsequently, staphylococci were cultured from 60 percent of the tibial biopsies at nine months, 45 percent at one year, and 72 percent at eighteen months after inoculation. A total of 57 biopsies were performed in the group of animals, and 44 or 78 percent were positive for Giorgio staphylococci on culture. *Staphylococcus aureus* was recovered from the medullary cavities of 88 percent of these animals at some time during the study. Clinically, the rabbits developed fever from the third to the eighth day after inoculation and shortly thereafter signs of inflammation appeared in the injected leg. This inflammation progressed to abscess formation with spontaneous drainage of grossly purulent exudate which eventually healed spontaneously in all animals, but which persisted in some for as long as 90 days.

X-rays at about four to six weeks showed a radiolucent line or gap between the metal rod and the tibial cortex. As the lesion progressed, the medullary cavity increased in size at the location of the infection. Occasionally the entire cortex on one side would disappear and form a hole in the cortex. After three to six months, a collar of new bone formed around the rod. Adjacent to the bony collar an irregularly dense laceworks of intramedullary bone was seen, and occasionally an area of periosteal new bone was formed. If a large cloaca developed in the tibial cortex, less periosteal new bone formation was the rule. The same sequence of changes occurred in the group with and without fractures. In the fracture group there was more abundant callus formation and all of the fractures healed. Usually a circumferential network of bone formed around an area of thick fibrous scar tissue. This thick layer of fibrous tissue surrounded the metal rod and a cavity filled with masses of polymorphonuclear leucocytes. Openings in this enclosure formed sinus tracts through the cortex to the subcutaneous tissue and usually drained through the skin.

TABLE 3

THE EFFECT OF RODDING WITHOUT FRACTURE ON THE DEVELOPMENT OF CHRONIC OSTEOMYELITIS IN THE RABBIT TIBIA INOCULATED WITH *Staphylococcus aureus* (80–81)

Inoculum (no. organisms)	Number of rabbits	Tibial cultures (months after inoculation)							No. rabbits with osteomyelitis
		2	3	4	6	9	12	18	
2×10^8	5	2/2[a] (3/5 died within 24 hours)		1/1			2/2		2
2×10^6	8	2/2	2/2	4/4	3/3	2/2	5/5		8
Group III total	13								10
0	4[b]	0/4	0/1		0/4	0/2	0/4	0/4	0
Group IV total	4								

[a] Number of rabbits with Staphylococcus aureus recovered from tibia per total number cultured.
[b] Three rabbits had tibial fracture and rod, one had rod only. No bacterial inoculation.

In order to determine the effect of rodding without fracture on the susceptibility of the rabbit tibia to staphylococcal infection, the right tibia of 13 rabbits was inoculated with the 80–81 strain of *Staphylococcus aureus* after rodding (Table 3). In Group III where the tibia was not fractured but where a rod was inserted, three of the five animals which received an inoculum of 2×10^8 staphylococci died within 24 hours and 80–81 staphylococci were recovered from marrow cavity cultures. The remaining two animals, however, developed chronic staphylococcal osteomyelitis. Since 2×10^8 staphylococci resulted in a high mortality rate, eight additional rabbits were given an inoculum of 2×10^6 staphylococci of the 80–81 strain into the rodded tibia which consistently cause chronic staphylococcal osteomyelitis. Cultures from tibial biopsies were positive for 80–81 staphylococci in every instance throughout the study period of one year. The clinical picture and x-ray findings in all ten animals with chronic osteomyelitis was the same as that observed in the rabbits studied in Table 2. None of the control animals developed osteomyelitis (Table 3).

COMMENTS

The results of this study indicate that chronic staphylococcal osteomyelitis can be produced in the rabbit tibia in the presence of a metallic implant. This model closely approximates the condition of chronic osteomyelitis in patients treated with intramedullary nails (1). As in human disease, this model of osteomyelitis in the rabbit results in fever, local signs of early inflammation, abscess formation, spontaneous drainage and healing, but with slowly progressive destruction of the tibial shaft manifested by periosteal reaction, cavity formation at the metal-bone interphase, and frequent sequestra and new bone formation. Although the draining sinuses heal, the tibia continues to show evidence of severe chronic osteomyelitis and staphylococci can easily be cultured from the tibial marrow cavity for as long as 18 months after inoculation. In the present study, both strains of staphylococci tested were capable of producing chronic osteomyelitis but at different doses of inoculum.

The present observations, as well as those of others (7), also indicate that the normal rabbit tibia is extremely resistant to staphylococcal infection and rapidly recovers from a direct inoculation of as many as 10^8 organisms of the Giorgio strain. However, a tenfold increase in the inoculum (10^9) proved to be rapidly

lethal for all animals studied. In contrast, 10^8 Giorgio staphylococci (one-tenth of the lethal dose), which were unable to infect the normal tibia, were capable of producing chronic osteomyelitis in the rabbit tibia in the presence of fracture and an intramedullary nail. In the present study, it was difficult to definitively separate the role of fracture and intramedullary rods in the pathogenesis of chronic staphylococcal osteomyelitis, since it is not possible to study fractured tibias without pinning. Nevertheless, the present observations that an inoculum of 10^6 staphylococci of the 80–81 strain (one-one hundredth of the lethal dose), which did not infect the normal rabbit tibia, did result in chronic osteomyelitis in the rodded tibia which was not fractured, strongly suggests that fracture is not the major factor. Serial tibial x-rays indicated that the fractures appeared to heal quickly by periosteal new bone formation, while the infection maintained itself adjacent to the rod and would drain into the surrounding soft tissues. This observation correlates with our clinical experience that a long bone fracture in a patient with an infected intramedullary rod may heal despite the presence of continued infection, as long as wound drainage can be maintained.

Previous models of experimental osteomyelitis initially described by Scheman *et al.* (7) and then adopted by others (3, 10), have been based on the induction of vascular thrombosis with the sclerosing agent, sodium morrhuate. The part played by vascular obstruction alone is probably very important but remains difficult to assess (1). It is clear that intramedullary injections of sodium morrhuate produce a certain amount of aseptic necrosis of bone which is apparently necessary or at least responsible in part for the inception and propagation of infection in this tissue (7). We specifically avoided the use of a sclerosing agent and elected to use an intramedullary pin in order to minimize and possibly eliminate the role of aseptic necrosis. No roentgenographic changes of aseptic necrosis were observed in rabbits rodded but not inoculated with staphylococci although serial histologic sections showed new bone formation. Although we were unable to maintain an intact metal to bone surface for microscopic study as it was necessary to remove the rod in order to section the bone, those areas that were studied showed only polymorphonuclear leucocytes present near normal bone substance. These observations suggest that the stainless steel rod provides a surface or location where the organism may become inaccessible to the phagocytic activity of adjacent cells and possibly other host defenses. The role of foreign bodies in enhancing the susceptibility of surrounding tissue to infection by producing a slight delay in host defenses is well known (6, 6a). This may be a significant factor in the successful production of chronic staphylococcal osteomyelitis in the present model.

Postoperative infections constitute serious complications of internal fixation devices and present difficult problems in the management of these patients. In certain circumstances the internal device has been removed, while in others it has been left in place (1). Both of these approaches have been combined with local drainage, sequestrectomy, high dosage parenteral antibiotics, closed irrigation with antibiotic detergent mixtures, or regional perfusion with antibiotics in various combinations (1). Nevertheless, the relative importance of each of these therapeutic modalities in humans remains to be determined. Since the present experimental model closely simulates the same disease in humans, including the isolation of the organism from bone for as long as 18 months after onset of infection, it can be used to evaluate each of these therapeutic procedures in a controlled system designed to determine their specific value in the treatment of chronic staphylococcal osteomyelitis.

SUMMARY

The inoculation of *Staphylococcus aureus* into the tibial marrow cavity of rabbits following tibial fracture and rodding, as well as into the rodded tibia without fractures, results in chronic staphylococcal osteomyelitis in a high percentage of these animals. Staphylococci were easily recovered from the tibial marrow cavity for as long as 18 months after onset of the infection. This model closely resembles the human disease in which chronic osteomyelitis develops as a complication of internal fixation devices, and provides a reliable method for evaluating various specific approaches to the treatment of this difficult problem in management.

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