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Preliminary Clinical Assessment of a Gentamicin-Loaded Monoolein Gel Intended to Treat Chronic Osteomyelitis

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Abstract: The aim of this study was to perform a preliminary clinical assessment of efficacy and safety of a biodegradable gentamicin-loaded monoolein gel. An open single dose, one treatment study including 19 patients with chronic osteomyelitis caused by a microorganism sensitive to gentamicin was conducted. After surgical curettage of the infected bone, the dead space was filled in with the gentamicin-loaded monoolein gel. To prevent post-operative septicaemia, a systemic antibiotherapy was prescribed for 3 days following the operation. Clinical, biological and radiological follow-up was performed to assess the efficacy and the safety of the treatment. After a follow-up period ranging from 2 to 12 months, all 19 patients included in the study felt well. Eighteen patients recovered from chronic osteomyelitis without adverse events. The wound of one patient whose bone was exposed did not scar over after 10 months. However, it was no longer infected. In conclusion, gentamicin-loaded monoolein gel was efficacious in treating chronic osteomyelitis without side-effects.

Key words: Clinical assessment, gentamicin, monoolein, implant, chronic osteomyelitis

INTRODUCTION

Osteomyelitis is a severe infection of bone and its marrow that can arise from a variety of mechanisms. Injury (often an open fracture) and postoperative infection are part of the etiology in the adult patient. Rarely, adult patients develop osteomyelitis after hematogenous seeding of long bones during an episode of bacteremia (Parsons and Strauss, 2004). Osteomyelitis often results in the formation of sequestra or new apposition of bone (Lew and Waldvogel, 1997). Although empirically understood, there is no generally accepted definition of chronic osteomyelitis. Numerous functional definitions have included a variety of criteria: clinical or radiographic evidence of infection over 6 weeks, relapse or persistence of infection after appropriate antibiotic therapy and infections associated with foreign bodies or vascular abnormalities (Parsons and Strauss, 2004). The chronicity of osteomyelitis is multifactorial. The relative avascular and ischemic nature of the infected region and sequestrum produce an area of lowered oxygen tension as well as an area that antibiotics can not penetrate (Wirganowicz, 1999). Chronic osteomyelitis is a frequent event, especially in

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immunocompromised, diabetic, or sickle-cells disease patients (Springer *et al*., 2007). It is frequent in Africa and some developing countries. For example, the frequency of chronic osteomyelitis is about 5% of the diseases in the orthopaedic and bone surgery Service of Ouagadougou Academic Hospital/Burkina Faso (Nacoulma *et al*., 2007).

Gentamicin-impregnated polymethylmethacrylate (PMMA) beads have been used commonly for many years for the treatment of chronic osteomyelitis. Local antibiotic concentrations with these beads are clearly higher than those achieved by intravenous application and they additionally avoid toxicity resulting from high plasma levels (Sivakuma and Rao, 2002; Mendel *et al*., 2005). However, treatment with PMMA beads has some disadvantages. They are not biodegradable and so a second operation is required to remove them. To avoid cost, pain and other risks associated with the necessary second surgical operation for extracting non-biodegradable delivery systems, biodegradable implants using polymers such as poly (D,L-lactide) or poly (D,L-lactide-co-glycolide) carriers were investigated (Zhang *et al*., 1994). Although these polymeric delivery systems allow prolongation of the drug release, they are solid, non bioadhesive and often show marked burst effects due to a high proportion of non-encapsulated drug (Mauduit *et al*., 1993; Schmidt *et al*., 1995).

These limitations of polymeric implants in osteomyelitis management have led us to develop a gentamicin-loaded monoolein gel. Previous studies demonstrated that the gel is biodegradable, has suitable physico-chemical and drug sustained-release properties for use as an implant for the treatment of chronic osteomyelitis. Biocompatibility and toxicity tests performed *in vitro* and *in vivo* revealed that the gel could be safe for clinical trial.

The aim of the present study was to evaluate the clinical efficacy and safety of the gentamicin-loaded monoolein gel we developed.

PROTOCOL

The clinical trial of the gel was conducted at the orthopaedic and bone surgery Service of Ouagadougou Academic Hospital (Burkina Faso). It was carried out over 12 months (from July 2006 to July 2007).

The Assessed Product

The assessed product was a gentamicin-loaded monoolein gel containing gentamicin sulfate (5%), monoolein (80%) and water (15%). It was a liquid crystalline gel and became very viscous in contact with aqueous body fluids at 37°C. The gel was a sustained-release implant. It was sterile and apyrogenic.

Patient Recruitment

Patients admitted to the trial had chronic osteomyelitis. The diagnosis of osteomyelitis was made on the basis of clinical, biological and radiographic evidence of infection. A total of 19 patients (16 men and 3 women), aged from 5 to 50 years old, were recruited. Exclusion criteria included pregnancy or breast-feeding, severe disease requiring concomitant antimicrobial therapy, hypersensitivity to aminosides and resistance of the isolated pathogen to gentamicin. Patients with impaired renal function (serum creatinine >120 μmol L^{-1}) or with diabetes were also excluded. Patients received no antibiotic therapy for 7 days before the beginning of the treatment.

Treatment

All patients who were included in the trial received the same treatment sequence. After a surgical incision, the affected soft and hard tissues were widely excised. The involucrum was then fenestrated and all the necrotic bone and sequestra were excised to leave healthy bleeding bone, often in the shape

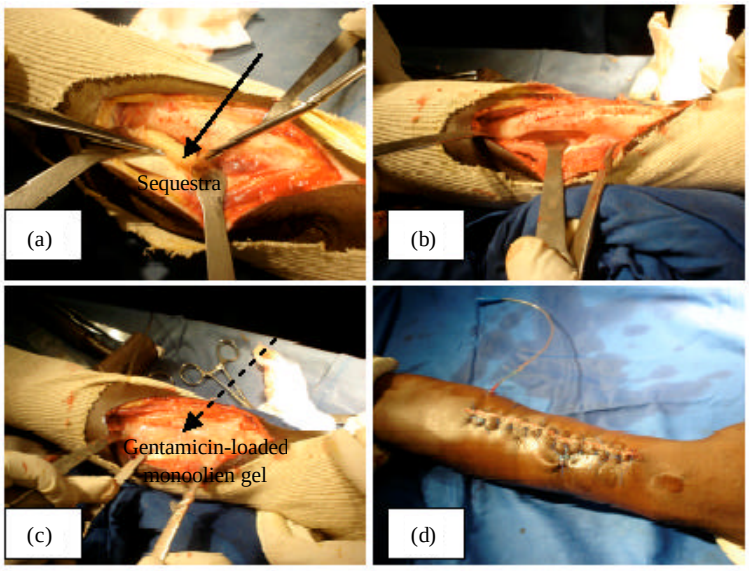

Fig. 1: Sequestra removing (a), curettage (b), dead space filling by the gentamicin-loaded monoolein gel (c) and surgical wound sutured surgical wound (d)

of a gutter. The dead space was cleaned by sterile normal saline and filled with the gentamicin-loaded monoolein gel. A suction drain was placed at the bottom of the gutter. The wound was stitched and packed using iodine polyvidone-soaked gauzes (Fig. 1). A short course of antibiotics was prescribed postoperatively. Each patient received intravenous (IV) gentamicin (at a unique administration of 80 and 160 mg, respectively for children and adults) and lincomycin (300 and 600 mg every 12 h, respectively for children and adults) for 3 days after the surgical operation. As an analgesic, intravenous paracetamol was administered for the 3 first days at 15 mg kg^{-1} every 8 h. On the third day after surgical operation, the suction drain was removed. The surgical wound was cleaned every 3 days with iodine-soaked gauze until healing. Surgical stitches were removed 21 days after the surgical operation.

Follow-Up and Outcome

Isolation and susceptibility to gentamicin of (a) causative pathogen(s) were obtained before the initiation of therapy. Bacterial culture was performed if pus occurred. A complete blood count, erythrocyte sedimentation rate, serum glucose, creatinine, aspartate amino-transferase, alanine amino-transferase, triglycerides and bilirubins were performed before the initiation of therapy, on days 5 and 45, then at 3, 6 and 12 months after the beginning of therapy. Radiographic images of the infected bone were also obtained at these periods.

Patients were examined clinically for purulent drainage, irritation at the site of implantation, erythema, local heat, body temperature, diuresis, auditive acuteness, dizziness and for any adverse event. The clinical examinations were made twice a week during the first month, then on day 45 and 3, 6 and 12 months after surgical operation. Any serious adverse event related to the study medication resulted in the patient's immediate withdrawal from the study.

Outcomes during the post-treatment follow-up period were classified by two independent physicians as follows: recovery, defined as the absence of clinical, biological or radiological evidence

of infection throughout the post-treatment follow-up; improvement, if signs of the infection were markedly reduced at the end but the wound did not scar over; failures, defined by the presence of pain, swelling, erythema or purulent wound; relapse, when a medical event with isolation of the same pyogenic microorganism from a clinically significant site occurred after healing of the wound.

Ethics

The study was conducted in accordance with the principles stated in the Declaration of Helsinki and approval was obtained from the Ethics committee for Human health Research of Burkina Faso (No. 2006/016/CERS/BF). Before starting the study, the patients were informed about the aim and design of the clinical trial and written consent was obtained.

RESULTS

A total of 19 patients with chronic osteomyelitis were treated. All patients had infected necrotic bone within a compromised soft tissue envelope. There were 16 male and 3 female with an age range of 6-50 years (median 24 years). The duration of their disease ranged from 2 to 11 years (median 5 years). Before being included in this study, all patients affirmed that they had previous treatment based on antibiotics by local and/or systemic route, but without any success. Five pathogen germs were isolated from the patients with a predominance of *Staphylococcus aureus* (Table 1). All pathogens were susceptible to gentamicin. Comorbid disease (sickle-cell disease with haemoglobin SC) was present in 2 patients. The White Blood Cell (WBC) levels were normal in all patients except one who had 12000 WBC mm^{-3}. The hemoglobin concentration in blood ranged from 9.5 to 13.5 g dL^{-1} (median, 12.1 g dL^{-1}). The erythrocyte sedimentation rates at the first and second hour were respectively over 7 mm (from 15 to 52 mm, median 22 mm) and 13 mm (from 20 to 90 mm, median 43 mm). Radiographic images showed sequestra in all patients.

The doses of the gentamicin-loaded monoolein gel used to fill dead spaces ranged from 30 to 110 g (median 55 g) in adults (over 14 years old). With the two children included in the study, 30 and 70 g of the gel was needed to fill dead spaces.

Table 1: Results of treatment with gentamicin-loaded monoolein gel in 19 patients with chronic osteomyelitis

Patient age (year)	Infection site(s)	Organism isolated	Duration of the disease (years)	Duration of the follow-up (months)	Results (recovery, improvement, failure, relapse)
22	Ulna	*Escherichia coli*	3	12	Recovery
31	Ulna	*Escherichia coli*	11	12	"
24	Tibia	*Salmonella* sp.	5	11	"
18	Ulna	*Proteus mirabilis*	4	11	"
6	Fibula	*Staphylococcus aureus*	3	11	"
23	Tibia	*Proteus mirabilis*	3	10	"
5	Radius	*Staphylococcus aureus*	2	10	"
	Tibia	*Staphylococcus aureus*	2	10	
27	Humerus	*Staphylococcus aureus*	2	10	"
50	Tibia	*Staphylococcus aureus*	7	10	Improvement
23	Tibia	*Escherichia coli*	5	9	Recovery
26	Femur	*Staphylococcus aureus*	3	9	"
31	Femur	*Staphylococcus aureus*	6	9	"
17	Femur	*Staphylococcus aureus*	3	8	"
26	Tibia	*Salmonella* sp.	5	4	"
18	Humerus	*Proteus mirabilis*	4	4	"
22	Femur	*Pseudomonas aeruginosa*	6	3	"
30	Tibia	*Proteus mirabilis*	8	3	"
20	Tibia	*Escherichia coli*	7	2	"
46	Crane	*Staphylococcus aureus*	6	2	"
Median 24			5	10	

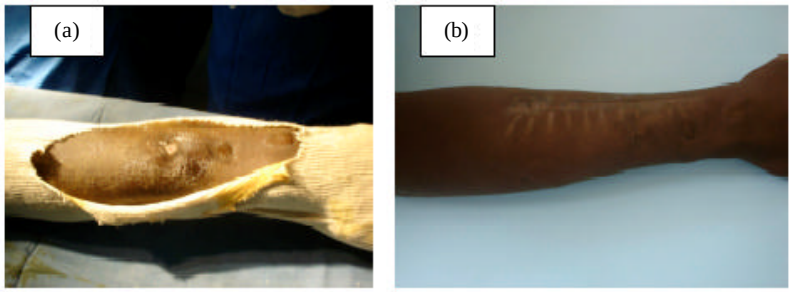

Fig. 2: Osteomyelitis of a forearm before treatment (a) and 35 days after treatment (b) with gentamicin-loaded monoolein gel

All patients were alive and felt well. The quantity of blood in the suction drain was about 10 mL. Between 6 and 12 days after the surgical operation, a non-purulent liquid flew along wounds. A bacteriological culture of this liquid proved that it was sterile. Except for one patient, wounds of all patients scarred over after between 30 and 70 days (median 42 days) (Fig. 2, Table 1). The treated bone of the non-recovered patient was exposed; however, the surgical wound and bone were neither infected nor purulent. During the biological monitoring on the 45th day post-operation, erythrocyte sedimentation rates became normal (below 7 and 13 mm, respectively at the 1st and 2nd hour). Patients remained apyretic except one who became feverish on day 6 post-operative. Further investigation demonstrated that this fever was caused by malaria contracted during the hospitalisation. Administration of quinine enabled his body temperature to be normalized.

No sign of adverse events was observed. The hemoglobin concentration had slightly decreased on day 5 post-operative. Serum glucose, creatinine, aspartate amino-transferase, alanine amino-transferase, triglycerides and bilirubins values were normal during the follow-up investigations. Radiographic images showed no sign of bone lesion.

DISCUSSION

To the best of our knowledge, this is the first published report of a clinical trial with a drug-loaded monoolein gel as an implant.

The short course of antibiotics administered intravenously after the operation aimed to prevent septicaemia from the septic site. The gentamicin-loaded monoolein gel could take over from these by releasing gentamicin at an efficacious concentration at the septic site. This therapeutic protocol was also used by other researchers (Méani and Romano, 1994) to treat chronic osteomyelitis locally with an antibiotic-impregnated implant, where the systemic administration of antibiotic lasted 5 days.

The filling of the dead space by the gel prevented haemorrhage at the surgical operation site; so avoiding post-operative blood transfusion and constitution of haematoma in the bone. This is one of the advantages of the assessed gel. Indeed, haematoma does not facilitate sterilisation of an infectious site (Hall *et al*., 1983, Fitzgerald *et al*., 1985). The mechanism of the haemostatic effect of the gel could be a mechanic compression of vessels that were damaged during the surgical operation.

The non-purulent and aseptic liquid flowing from the surgical wound (between days 6 and 12) may have derived from the biodegradation of the gel. Besides, our previous study of the gel in animal revealed that it was degradable *in vivo*.

The clinical, biological and radiological signs in all patients suggested that the gentamicin-monoolein gel was efficacious in treating chronic osteomyelitis. The single case of non-recovery (but

improvement) could have been due to the exposure of the treated bone, where there was no soft tissue to accelerate the skinning. As the bone was not infected after treatment, skin grafting could be envisaged in this case.

There was no need for long-term systemic antibiotherapy or a second operation to remove the implanted gel. Therefore, the treatment of chronic osteomyelitis with the gel was comfortable for the patients. It was also cheap.

Other results from chronic osteomyelitis treatment were obtained with other therapeutic protocols. The classical treatment of chronic osteomyelitis, based on both long-term systemic antibiotherapy and surgical debridement, had failure rates of approximately 25-30% (Blaha *et al*., 1993; Nelson *et al*., 1993). A study (carried out in the orthopaedic and bone surgery Service where we did our clinical trial) reported that only 33/65 (i.e., 50.8%) patients recovered from chronic osteomyelitis using long-term (more than 3 months) systemic antibiotherapy (gentamicin and lincomycin) and surgical debridement without implants. Adverse events (such as nausea, dizziness, auditive acuteness decrease) to this long-term systemic administration of antibiotics (which was thus costly and uncomfortable) were reported. The failure rate of gentamicin-impregnated polymethylmetacrylate (PMMA) in the local treatment of chronic osteomyelitis was approximately 10-15% (Klemm, 1993; Garvin *et al*., 1994). It also has the disadvantage that it is non-degradable and a secondary surgery is required to remove it after scarring over has occurred (Bucholz *et al*., 1981; Langlais *et al*., 1988; Klemm, 1993; Miclau *et al*., 1993; Kanellakopoulou and Giamarellos-Bourboulis, 2000).

The complete blood count, blood chemistry profile and clinical follow-up confirmed the non-toxicity of the gel at therapeutic doses.

Gentamicin would not be released at a toxic level in the blood, but at an efficacious dose at the infectious site. Indeed, gentamicin-related adverse events such as kidney damage, dizziness, auditive acuteness decrease and minor allergic reactions (Elisenber *et al*., 1987; Medicines Complete Browser, 2004) were not observed in any of the patients.

Lack of irritation and bone necrosis at the implantation suggested that the gel was biocompatible. The lack of adverse events attributable to the gel was not totally unexpected. All agents (gentamicin, monoolein) used to make the gel are known to be well tolerated (Ganem-Quintanar *et al*., 2000; Rowe *et al*., 2003).

The safety margin of the assessed gel was large. Doses of gel ranging from 30 to 110 g were efficacious without provoking damage in adult patients. It was also efficacious and safe in children at doses ranging from 30 to 70 g.

The follow-up period of maximum 12 months may seem small. However, Waldvogel *et al*. (1970) concluded that 95% of recurrences of chronic osteomyelitis occur within the first 12 months after surgery.

In conclusion, gentamicin-loaded monoolein gel appears to be an effective, safe and well-tolerated product for the local treatment of patients with chronic osteomyelitis. The treatment of chronic osteomyelitis with the gel is expected to be cheap and comfortable for the patients. As the gel has been shown to be efficacious and safe, in the future multi-centre randomized controlled trial study including more patients would be conducted and the period of follow-up extended.

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