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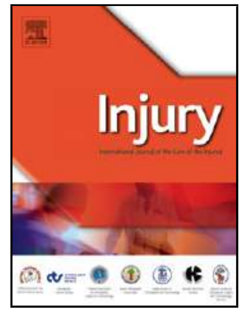

Title: Comparison of the Use of Antibiotic-loaded Calcium Sulphate and Wound irrigation-suction in the Treatment of Lower Limb Chronic Osteomyelitis

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Comparison of the Use of Antibiotic-loaded Calcium Sulphate and Wound irrigation-suction in the Treatment of Lower Limb Chronic Osteomyelitis

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Research highlights

1. The first study to compare antibiotic-loaded calcium sulphate and irrigation-suction in treating chronic osteomyelitis.

2. Present high infection recurrence and docking obstruction rate of irrigation-suction.

3. Present potential inductivity of calcium sulphate.

ABSTRACT

Aims: We sought to compare the efficacy of antibiotic-loaded calcium sulphate with wound irrigation-suction in patients with lower limb chronic osteomyelitis. **Patients and Methods:** Adult patients with lower limb chronic osteomyelitis treated at our hospital by means of segmental bone resection, antibiotic-loaded calcium sulphate implantation or wound irrigation-suction, followed by bone transport with external fixator from January 2011 to July 2015 were retrospectively evaluated. The clinical presentation, laboratory results, complications, docking obstruction, infection recurrence were compared. **Results:** There were totally 74 patients met the inclusion criteria. Docking obstruction rate and infection recurrence were higher in the irrigation group with significant difference. The success rate of the first operation was 90.74% in the calcium sulphate group compared with 45% in the irrigation group. Postoperaton leakage of the incision happened more in the calcium sulphate group, but it wasn't a risk factor for docking obstruction and infection recurrence. Patients in the calcium sulphate group had shorter hospital stay and systemic antibiotic treatment, also with less external fixator index. **Conclusions:** The findings of our study suggest that antibiotic-loaded calcium sulphate implantation for lower chronic limb osteomyelitis was a more successful method than wound irrigation-suction, it greatly decreased infection recurrence and docking obstrucion. Postoperative leakage after implantation didn't worsen patient's outcome.

Keywords: chronic osteomyelitis, calcium sulphate, irrigation, infection, docking obstrcution

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INTRODUCTION

Chronic osteomyelitis of the lower limb remains a challenge for both orthopaedic surgeons and patients. Microorganisms especially Staphylococcus aureusis cause inflammation to the cortical, trabecular bone, bone marrow or periosteum[13, 36]. However, despite thorough and aggressive bone excision, re-infection may still occur from dormant organisms which form biofilms to resist antibiotics and the immune system[24]. So the questions have arisen on how to fully eradicate microorganism. Continuous irrigation-suction and pulsating irrigation have been used by many surgeons to compliment the debridement of infected tissues, it mainly function by mechanical cleansing to remove foreign bodies, sequestra and other necrotic tissue, thus decrease bacterial colonization of the surgical site[2]. Besides, antibiotic can be added into the irrigation solution to increase local concentration. Its initial purpose is the same with local antibiotic carrier at present --- to reduce the rate of infection recurrence. Several studies have reported success in using the methods of wound irrigation and mechanical wound cleansing techniques to reduce local bacterial burden[1, 23, 26].

The antibiotic doses needed to treat biofilm infections are usually 100 to 1000 times higher than those required for treating planktonic bacterial infections[6, 18, 22]. So, to eradicate the infection radically, besides surgery as the cornerstone, maintenance of local high enough concentration of antibiotic is also vital. To achieve it, local antibiotic carrier was proved to be the most effective. Among the varieties of local carriers, calcium sulphate is now the most widely used practice. Calcium sulphate is biocompatible, with characteristics of suitable biodegradability, low immunogenicity, good tolerance, and controlled drug release[4, 21]. These characteristics are also the advantages of calcium sulphate over other carriers. Many research had compared calcium sulphate and other local carriers, but only a handful of research had compared it with wound irrigation-suction in the treatment of lower limb osteomyelitis.

In our comparative-cohort study, we aim to find out whether antibiotic-loaded calcium sulphate is advantageous over wound irrigation-suction in the treatment of lower limb osteomyelitis.

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PATIENTS AND METHODS

Inclusion/Exclusion Criteria

This comparative-cohort study focused on patients with lower limb chronic osteomyelitis that were treated in our hospital. Chronic osteomyelitis is defined as the presence of radiographic osteolytic changes in the long bones of patients with a background history of local onset of infection[13]. Data were collected retrospectively between January 2011 and June 2015. Inclusion criteria were adult patients (age≥18 years), chronic osteomyelitis of femur or tibia treated with segmental bone resection followed by bone transport with circular or unilateral external fixator without acute bone shortening during surgery. Exclusion criteria were cases accompanied with deformity correction, associated severe dysfunction of liver or kidney, diabetes with uncontrolled blood glucose.

Surgical Management

In the calcium sulphate group, antibiotic-loaded calcium sulphate was used to fill in the defect, including part of upper and lower medullary cavity. In the irrigation group, one irrigation tube was placed in the upper medullary cavity, and one to two suction tubes were placed in the lower medullary cavity. Whether an additional procedure of osteotomy for bone transport performed either as a one-stage or two-stage surgery was up to the infection level and the surgeon's decision.

Postoperative Treatment

The patients of both group were given intravenous antibiotics and regular wound care. Bone transport to cover the bony defect were started one week after the osteotomy with a speed of 1mm/day for the calcium sulphate group. Patients in the irrigation group were given continuous irrigation and suction of the infected region and medullary cavity with normal saline, which contain antibiotics for 1-2 weeks, and bone transport performed with the same protocol as the calcium sulphate group. The external fixation index (time spent in an external fixator) is calculated for both groups.

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Statistical Analysis

Data were collated using Microsoft Excel (Redmond,Washington) and analysed using SPSS v20 (SSPS Inc., Chicago, Illinois). Comparisons of continuous variables were tested by independent-samples T-test or Wilcoxon rank sum test. The differences between the rates of infection were tested by Chi-square test or Fisher exact test (2-tailed). P value of < 0.05 was considered statistically significant.

RESULTS

Between January 2011 to July 2015, 153 patients with lower limb chronic osteomyelitis were treated with either antibiotic-loaded calcium sulphate or wound irrigation-suction, of which 71 patients who did not undergo bone transport and 8 children were excluded. Therefore, 74 patients were chosen in accordance with the inclusion criteria. 72.97% of them were managed by antibiotic-loaded calcium sulphate and 27.03% were managed by wound irrigation-suction.

Patients' Profile, Etiology and Risk Factors Comparison

Comparison of the patients' profile, etiology and risk factors are demonstrated and summarized in Table 1-3. The results showed no significant difference between the two groups, indicating comparability of them. Most of the patients were middle-aged ones, and male patients with tibia involved were common. More than 70% of them suffered from sinus, while less than 5% of them were febrile before surgery. The major initial cause of osteomyelitis was trauma. For the anamnesis, most of them were healthy.

Preoperative and postoperative laboratory results

Laboratory results of preoperative and postoperative laboratory are summarized in Table 4. Data of preoperative WBC, ESR and CRP were collected from the first test as an in-patient before giving antibiotic. Data of postoperative WBC, ESR and CRP were collected from the final test within two weeks following surgery. Only the CRP in the

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calcium sulphate group decreased after surgery while other indicators increased, though it was not significant after statistical validation.

Pre- and Postoperative Data comparison

The comparison data did not differ significantly between the two groups (Table 4). Most patients were afebrile after surgery. Wound leakage at the incision site after surgery occurred more commonly in the calcium sulphate group as expected. 3 out of these 19 cases with leakage had positive culture resultsof staphylococcus aureus infection, and only in 1 of this cases recurrence of infection developed. Our results showed that the infection recurrence rate is lower in the calcium suphate group as compared to the irrigation group (6 cases). External fixator index comparison showed that patients in the calcium sulphate group spent less time with the external fixator. (Table 5)

Overview of all patients' outcome

The whole treatment process and patients details of those who needed additional treatment are shown in Fig.1. In the calcium sulphate group, 49 patients (90.74%) achieved complete healing after the index (osteotomy) operation; 1 patient (1.85%) had reinfection two months after operation, and was conservatively treated with wound care and intravenous antibiotics. 4 patients (7.41%) needed an additional docking site consolidation procedure. In the irrigation group, 9 patients (45%) achieved completehealing after the index operation. 6 patients (30%) developed re-infection between 1 to 9 months with 4 patients having to undergo antibiotic-loaded calcium sulphate implantation to solve the problem. Docking site obstruction was just much higher in the irrigation group (25%) when compared to the calcium sulphate group (1.8%).

DISCUSSION

At present, many methods are available to manage lower limb chronic osteomyelitis however it continues to be challenging and often the recurrence rates remain high.

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Infection eradication and bone defect reconstruction remain to be the main challenge in treating chronic osteomyelitis[17]. The Masquelet technique is recognized as two-stage surgery to reduce the initial hospital stay and allows patient for an easier rehabilitation period. The role of irrigation in the treatment of chronic osteomyelitis is well-known and proved to be an effective method. Interestingly, our study showed that antibiotic-loaded calcium sulphate implantation was a more effective method for treating lower limb osteomyelitis. It decrease infection recurrence due to a high local concentration of antibiotics above the minimum inhibitory concentration, which suggestively able to penetrate biofilm formation[7, 21, 34].

Surgery remains the cornerstone of the management of osteomyelitis[29]. Besides surgical treatment, antibiotic therapy is indispensible[17]. When local carrier of antibiotic is used, the outcomes are better than systemic therapy or surgical treatment alone. Especially local high concentration of antibiotic is crucial to destroy the residual organism in biofilm thus help to reduce infection recurrence as is proven in our study[20]. Wound irrigation and suction provides a low concentration of antibiotic from the irrigated saline solution therefore may lead to poorer outcome[9, 12].

Docking site is where the transported segment meets the target segment[14], it is a frequent source of problems, especially docking obstruction. Docking obstruction is a condition where the transported bone is unable to perch nicely. This complication commonly presents in bone transport procedures therefore result in additional surgery and delay of the healing time by several months. In our study, we found docking obstruction occurred more in the irrigation group, thus further debridement of intervening tissues and freshening of the osteotomized bone surfaces are required[15, 28]. When nothing is filled into the gap between the resected segments, the medullary cavity might be sealed by fibrocartilageinous tissue, and the gap would be occupied by granulation tissue or scar[14, 27], thus block the docked ends to meet and union. Moreover, under the circumstance of continuous irrigation, tissues would be swelling, leading to reduced activity of the osteocyte; with the mechanical cleansing of the flowing solution, the local bone marrow mesenchymal stem cells and local bone growth factors would be washed away. The negative factors together mentioned

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above result in docking obstruction. Further more researches are needed to perform to verify the influence of irrigation-suction on docking site.

Options to prevent docking obstruction are available. Hatzokos I et al shared their experience in using autologous bone marrow combined with demineralized bone matrix to improve docking site consolidation. However, autologous bone graft contributes to donor site morbidity[10]. Other ways like refreshing to docked ends, acute shortening, application of pulsed electromagnetic fields[3], low intensity ultrasound[5] are useful to avoid obstruction. Nikolaos Giotakiswhile et al considered that spontaneous union on docking site is infrequent[8], and Tetsworth K et al even considered docking site modification as a standard transport treatment protocol for bone transport[31], while our study found a high success rate of one-staged union with the use of calcium sulphate.

We reviewed all the patients' imaging materials and found that when the cavities were filled with calcium sulphate, there would be membranes forming around the fillers, and the osteotomized bone surfaces appear to be roughened, indicating that there may be continuous osteogenesis stimulated by calcium sulphate (Fig.2a-2c). In the irrigation group, no membrane-like structure were formed around the docking site with the osteotomized bone surfaces appeared smooth. We hypothesized that it is caused by lack of osteogenesis (Fig.3a-3c). We concluded several possible explanations for the higher docking obstruction rate in the irrigation group as follows: a lack of osteogenesis stimuli at the docking contact site; a void left in the cavity post irrigation resulting in overgrowth of intervening granulation tissues which can interfere with the bony contact; and negative influence on callus formation from continuous irrigation[33].

Figure 4a-4b indicated the pathological examination of proliferous fibrous tissue and new vessels in the induced membrane surrounding calcium sulphate. This findings supported by previous studies which reported an induced membrane reaction of calcium sulphate in the treatment of calcaneal osteomyelitis[11] and description of properties of the induced membrane performed on rats[16]. At present, scholars have different opinions on the osteoinductivity of calcium sulfate, more and more evidence indicates the potential ability of it. As an inorganic compound, calcium sulfate itself doesn't contain any induced substance, but it may react with the surrounding tissue to

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generate or activate the process of osteogenesis. Calcium sulphate can reduce local PH value, leading to demineralization of the surrounding host bone, thus the level of bone growth factors such as β-TGF、PDGF、BMP is getting higher than normal[32], and these factors significantly stimulate bonemesenchymal stem cells (BMSCs) growth. Local high level of calcium also devotes to osteogenesis by activating calcium-sensing receptor (CASR) of osteoblast[30, 35], which can enhance activity of osteoblast when activated.

Postoperative leakage is common when calcium-based bone substitute are implanted[25], however it did not increase the recurrence of infection[19]. Our study shows a high occurrence up to 35.2% in the calcium sulphate group which similar to other studies[20]. We concluded several explanation for leakage as follows: there were not enough soft tissue to cover and contain the calcium sulphate; the lesion was too superficial, especially for tibia; and calcium sulphate was squeezed out of the cavity during bone transport. Though outcome were not affected, it increase patients' distress during the treatment process as they do mimic the presence of recurrence of infection with the substance leaking out of their wounds. Wound irrigation-suction has similar complication, usually caused by blockage of the suction pipe.

There are several limitations of this study which are small samples size, non-randomized trial, and absence of the general photograph of the membrane around calcium sulphate. A larger sample size and a well conducted randomized controlled clinical trial will greatly assist in the verification of such a study. Basic science study is encouraged to discover type of membrane and its properties induced by calcium suphate.

Conclusion

Our study demonstrated better outcomes of antibiotic-loaded calcium sulphate in the treatment of lower limb chronic osteomyelitis than wound irrigation-suction. This technique can improve prognosis of such condition and offer a patient-friendly treatment.

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Declearation

I confirm that the authors have no competing interests.

Statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Fig.1. The whole treatment process of all the patients. Number of patients and surgical procedure type at the first procedure(dark blue) and those who required a second procedure(orange).

Fig.2a-2c. Patient's X-ray films from calcium sulphate group.The red solid arrows show the membrane induced by calcium sulphate.The yellow solid arrows show the rough docking site with a lot of callus.

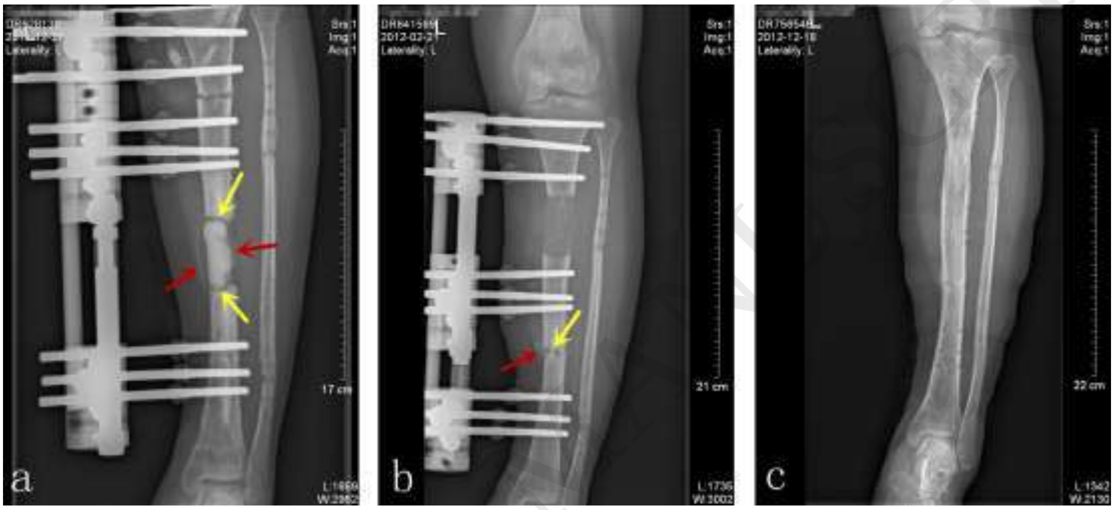

Fig.3a-3c. Patient's X-ray films from irrigation group.The blue solid arrow shows the irrigation and suction pipes.The green solid arrow shows the smooth docking site with little callus.

Fig.4a-4b. Patient's pathological examination of the membrane induced by calcium sulphate from calcium sulphate group(Samples was obtained from this patient's additional debridement procedure).The red solid arrows show new vessels in the membrane,and the blue solid arrows show the multiple proliferous fibroblasts.

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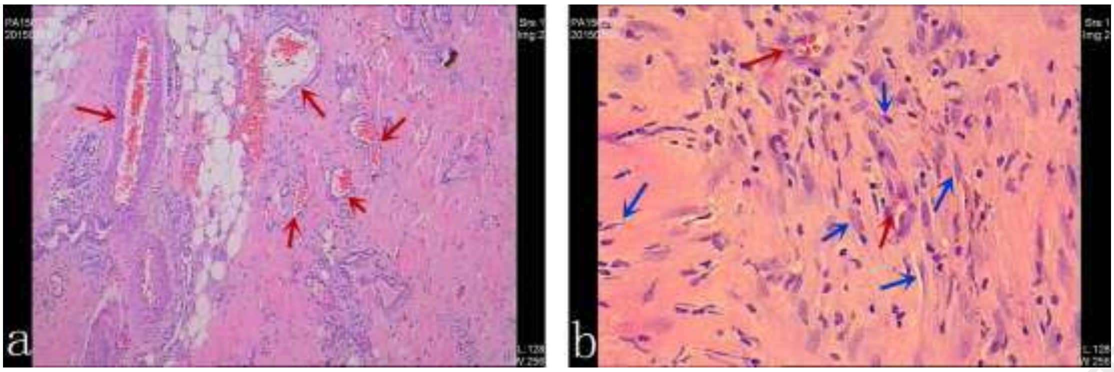

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TABLE 1.Patient Demographic Characteristics

	Calcium sulphate Group	Irrigation Group	P-Value
No. of patients	54	20	
Mean age(yrs)*	39.35 (35.40 to 43.30)	39.60 (33.11 to 46.09)	0.947
Sex†			0.379
Male	50	17	
Female	4	3	
Site(Femur/Tibia) †			1.000
Femur	16	6	
Tibia	38	14	
Side†			1.000
Right	29	11	
Left	25	9	
Sinus‡	39 (72.22%)	18 (90.00%)	0.131
Fever‡	3 (5.56%)	1 (5.00%)	1.000
Mean Symptom duration(months)*	11.23 (8.75 to 13.71)	9.54 (5.13 to 13.95)	0.483
Cierny-Mader classification†			0.231
Stage 1-medullary osteomyelitis	3	0	
Stage 2-superficial osteomyelitis	0	1	
Stage 3-localized osteomyelitis	15	4	
Stage 4-diffuse osteomyelitis	36	15	
Number of previous surgeries§	2 (1)	2 (1)	0.357

Remarks:*The values were given as the mean,with the 95% CI inparentheses.†The values were given as the number of patients. ‡The values were given as the number of patients, with the percentage in parentheses.§The values were given as the median, with the interquartile range in parentheses.

TABLE 2. Etiology of Osteomyelitis

	Calcium sulphate Group (N=54)	Irrigation Group(N=20)	P Value
Hematogenous spread†	1 (1.85%)	2 (10%)	0.176
Superficial wound†	3 (5.56%)	1 (5%)	1.000
Trauma†	50 (92.59%)	17 (85%)	0.379
Falling height	9	4	
Traffic accident	36	11	
Gunshot wound	1	1	
Heavy pound injury	4	1	

Remarks: †The values were given as the number of patients, with the percentage in parentheses.

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TABLE 3. Systemic Risk Factors

	Calcium sulphate Group (N=54)	Irrigation Group (N=20)	P Value
Without systemic risk factors†	45 (83.33%)	19 (95%)	0.270
With systemic risk factors†	9 (16.67%)	1 (5%)	0.270
Smoking	5	0	
Drinking	3	0	
Hypertension	3	1	
Chronic hepatitis	3	0	
Diabetes	1	0	
Tuberculosis	2	0	

Remarks: †The values were given as the number of patients, with the percentage in parentheses.

TABLE 4. Laboratory Results

	Calcium sulphate Group (N=54)	Irrigation Group (N=20)	P Value
Pre-operative†			
White blood-cell count(x10^9/L)	7.71 (7.09 to 8.32)	6.77 (6.02 to 7.52)	0.093
Erythrocyte Sedimentation Rate(mm/h)	27.04 (20.91 to 33.18)	27.35 (14.17 to 40.53)	0.961
C-reactive protein(mg/L)	14.22 (10.20 to 18.24)	9.20 (4.06 to 14.35)	0.171
Post-operative†			
White blood-cell count(x10^9/L)	7.86 (7.12 to 8.59)	8.57 (7.54 to 9.60)	0.291
Erythrocyte Sedimentation Rate(mm/h)	30.37 (23.54 to 37.19)	37.66 (18.84 to 56.49)	0.981
C-reactive protein(mg/L)	10.63 (7.88 to 13.39)	15.02 (5.97 to 24.07)	0.208

Remarks: †The values were given as the mean with the 95% CI in parentheses.

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TABLE 5. Operation and Post-operative Data Comparison

	Calcium sulphate Group (N=54)	Irrigation Group (N=20)	P Value
Surgery time(mins)†	140.65 (127.66 to 153.63)	144.35 (119.88 to 168.82)	0.773
Bleeding volume(ml) †	167.22 (143.69 to 190.75)	176.00 (132.27 to 219.73)	0.581
Length of bone defect(mm) †	86.11 (76.72 to 95.51)	82.25 (66.17 to 98.33)	0.669
Febrile‡	5 (9.26%)	1 (5%)	1.000
Leakage‡	19 (35.19%)	2 (10%)	0.042
Hospital stay(days) †	21.61 (19.01 to 24.21)	30.65 (23.05 to 38.25)	0.004
Systemic antibiotic treatment duration(days) †	15.81 (13.84 to 17.79)	25.10 (18.03 to 32.17)	0.002
Follow-up(months) †	28.50 (7.25)	30.50 (14.75)	0.460
External Fixator Index(days/cm) †	41.85 (36.57 to 47.13)	53.62 (44.32 to 62.92)	0.024
Docking obstruction‡	4 (7.41%)	6 (30%)	0.020
Autogenous iliac bone graft	1	5	
Refreshing	3	1	
Infection Recurrence‡	1 (1.85%)	6 (30%)	0.001
Within 12 months	1	6	
Within 24 months	1	6	

Remarks: † The values were given as the mean, with the 95% CI inparentheses. ‡ The values were given as the number of patients with the percentage in parentheses.

ACCEPTED MANUSCRIPT