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ORIGINAL STUDY

Elution of ciprofloxacin from acrylic bone cement and fibrin clot : An *in vitro* study

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The purpose of this study was to investigate the release of ciprofloxacin from acrylic bone cement and fibrin clot.

Under sterile conditions, bone cement and fibrin clot were individually mixed with ciprofloxacin. Ten specimens of each complex were placed in 1 ml of nutrient broth and incubated at 37°C. The nutrient broth was changed daily, and the removed samples were stored at -70°C until the antibiotic concentration in each sample was determined by a microbiological method. The maximum level in bone cement specimens was obtained at the second day (80.80 µg/ml) and its diffusion was rapid at first, decreasing gradually over a period of 365 days. Fibrin clot biodegradable specimens released high concentrations of ciprofloxacin (1.52-49.91 µg/ml) *in vitro* for the period of time needed to treat bone infections (i.e. 65 days).

We conclude that the high release of ciprofloxacin *in vitro* from acrylic bone cement and fibrin clot is very promising since the obtained levels are much higher than the required minimal inhibitory concentration (MIC) against the implicated pathogens in soft tissue and bone infections. The *in vivo* relevance of the obtained results requires carefully performed studies in animal models.

Keywords : bone cement ; fibrin clot ; ciprofloxacin ; chronic osteomyelitis ; drug delivery system.

INTRODUCTION

One of the most serious problems in orthopaedic surgery is chronic osteomyelitis. Chronic osteomyelitis is a difficult infection to treat and eradicate. Long-term parenteral antibiotics with multiple surgical debridements are often required for effective treatment *(5,24)*. Therefore, it is understandable that continuous efforts are being made to improve one or other element in the treatment of chronic osteomyelitis.

The use of local antibiotic delivery systems has become an accepted method that continues to evolve for a variety of reasons. There has been an explosion of new technologies designed to facilitate the delivery of antibiotics in new and creative ways. The primary reason for using these local antibiotic delivery vehicles is the ability to achieve very high local concentrations of antibiotics without associated systemic toxicity. In the typical infected wound

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environment, which frequently has zones of avascularity, the ability to achieve high levels of antibiotics in these otherwise inaccessible areas is highly desirable (*4*). Additional reasons for use of these delivery vehicles include the desire to treat remaining planktonic organisms and sessile organisms in biofilms more effectively with high concentrations of antibiotics (*11*).

An ideal local antibiotic delivery system would provide a more efficient delivery of higher levels of antibiotics to the site of infection and yet minimise the risks of systemic toxicity associated with traditional methods of intravenous antibiotics (*10*). Antibiotic loaded bone cement represents the current gold standard for local antibiotic delivery in orthopaedic surgery (*19*). Additional methods have included adding antibiotics to bone graft (*16*) or other naturally occurring polymers whereby the antibiotic is absorbed to the surface of these materials and is then released into the wound environment. This category includes antibiotic-loaded collagen sponge (*1*), fibrin clot (*13,17*), and other commercially available systems that use clotted blood products.

One of the primary advantages of a biodegradable system is the avoidance of secondary surgical procedures to remove foreign material, such as bone cement, once antibiotic elution has ceased. Although there are investigators actively involved in the use of these materials, their use as local antibiotic delivery vehicles is not as common as the use of antibiotic-loaded bone cement.

The purpose of this study was to investigate the pharmacokinetics of ciprofloxacin elution from fibrin clot and bone cement in an *in vitro* model. Ciprofloxacin (a 4-fluoroquinolone) was chosen for study because it has high intrinsic activity against most pathogens in bone and soft tissue infections, including nosocomial, multiresistant isolates.

MATERIALS AND METHODS

Production of bone cement specimens

We prepared ten compounds of the bone cement ciprofloxacin complex as follows. Under sterile conditions 150 mg of pure substance of ciprofloxacin (Bayer, Leverkusen, Germany) was added to 6 g (ratio 1:40) to the polymer powder of bone cement (Simplex-Howmedica, Rutherford, New Jersey) and mixed for 1 minute. Subsequently, 3 ml of liquid monomer was added. After thorough mixing and kneading, the bone cement specimens were placed in standard diameter sterile glass tubes, and left to cool and solidify at 37°C.

Production of fibrin clot specimens

We prepared ten compounds of the fibrin clot ciprofloxacin complex as follows. A Tissucol kit (Immuno AG, Vienna, Austria) was used in the various steps of the preparation of the fibrin clot. Preliminary tests first determined the highest possible concentration per unit volume and the conditions under which the precipitation of fibrinogen take place. This could be achieved only when 15 mg of pure substance of ciprofloxacin were dissolved in 1ml of sterile water for injection ; this was then mixed with 2 ml of fibrinogen and finally with 2 ml of clotting solution (500 I.U./ml thrombin and 3000 KIE aprotinin). Each specimen (with a total volume of 5 ml for each clot) was placed in a standard diameter sterile glass tube. Despite the high concentration of thrombin, polymerisation occurred after only approximately 2 minutes.

Each test tube had a free surface of 200 mm^2. Tubes were sterilised by UV light, and they were closed firmly to prevent any contamination.

One milliliter of sterile Mueller-Hinton broth (Oxoid Ltd, London, UK) was added to the free surface of each mixture, which was then left to incubate at 37°C. At 24 hour intervals the entire amount of broth was removed in a sterile manner and kept refrigerated at -70°C until determination of the ciprofloxacin levels. It was then replaced after thorough washing of the interior of the test tube by sterile pyrogen-free water. This process was continued until decomposition of the fibrin clot mixture was apparent (about 65 days) and for one year (365 days) for the bone cement mixture. Ciprofloxacin levels (expressed in micrograms per milliliter) were determined by a microbiological agar well diffusion assay on Mueller-Hinton agar (Oxoid Ltd) with the application of *Escherichia coli* 14 (ICB 40-04) as an indicator strain. Drug levels were estimated by a standard curve created with known ciprofloxacin concentrations and plotted on semi-logarithmic paper (*3*).

RESULTS

On each day of sampling the mean or the median values for the ten test tubes of bone cement ant for

Table I. — *In vitro* release of ciprofloxacin after incorporation in acrylic bone cement (PMMA)

Day of Assay	Mean (± SD) ciprofloxacin concentration (µg/ml)
1	33.00 ± 6.76
2	80.80 ± 9.12
3	56.04 ± 6.33
4	48.02 ± 4.72
5	39.34 ± 5.43
10	23.88 ± 6.33
20	12.80 ± 1.48
30	9.17 ± 2.04
45	6.01 ± 0.47
60	4.53 ± 0.61
100	2.11 ± 0.17
200	1.05 ± 0.36
300	0.88 ± 0.30
365	0.57 ± 0.17

Table II. — *In vitro* levels of ciprofloxacin after incorporation in fibrin clot

Day of Assay	Mean (± SD) ciprofloxacin concentration (µg/ml)
1	49.91 ± 4.87
2	33.37 ± 3.95
3	32.27 ± 4.01
4	26.98 ± 3.12
5	24.40 ± 3.05
10	19.68 ± 2.80
20	16.08 ± 2.73
30	15.40 ± 2.60
45	12.06 ± 1.95
60	2.11 ± 0.78
65	1.52 ± 0.80

the ten test tubes of fibrin clot complex was determined (*18*). The mean values (± standard deviation) of ciprofloxacin release from bone cement and fibrin clot were calculated (tables I and II).

The results in table I indicate that ciprofloxacin levels released *in vitro* from bone cement samples ranged from 10- to 620-fold of its minimum inhibitory concentrations (MICs) for the common causative pathogens of chronic osteomyelitis (*6,8*). The maximum level of ciprofloxacin in bone cement samples was obtained on the second day (80.80 ± 9.12 µg/ml). The diffusion of ciprofloxacin from the bone cement-antibiotic complex progressed rapidly after the second day, decreasing gradually over a period of 365 days. The levels of ciprofloxacin obtained on the last day of the experiment are about 10 times higher than the MICs values for pathogens implicated in bone and soft tissues infections.

The results in table II indicate that the maximum levels of ciprofloxacin after the incorporation of antibiotic into fibrin clot was obtained on the first day (49.91 ± 4.87 µg/ml). Thereafter, a step-wise decrease over time was observed. The fibrin clot-ciprofloxacin complexes usually disintegrated after 65 days. The ciprofloxacin levels released *in vitro* from fibrin clot samples ranged 30- to 380-fold of its MICs for the common causative pathogens of chronic osteomyelitis (*6,8*).

The elution of ciprofloxacin from bone cement and fibrin clot samples is shown in fig 1. A large proportion of ciprofloxacin was released in nutrient broth during the first 5 days from bone cement samples and during the first 15 days from fibrin clot samples. Nevertheless antibiotic continued to leak from bone cement for one year and from fibrin clot for two months.

DISCUSSION

Musculoskeletal sepsis is a chronic debilitating disease. In particular, osteomyelitis is more difficult to treat than other infectious disorders because systemically administered antibiotics fail to reach the site of infection owing to relative avascularity. Infection in the bone can persist despite thorough debridement, sequestrectomy, saucerisation and long-term systemic antibiotic therapy. With the increasing popularity of internal fixation, the problem of infected osteosynthesis is increasing.

The principle of depot administration of antibiotic is based on a well-accepted fact that the drug leaches out locally over a period of time. The resulting bactericidal tissue concentration at the site of infection is therefore higher than the concentration achievable by systemic antibiotic administration (*21,23*).

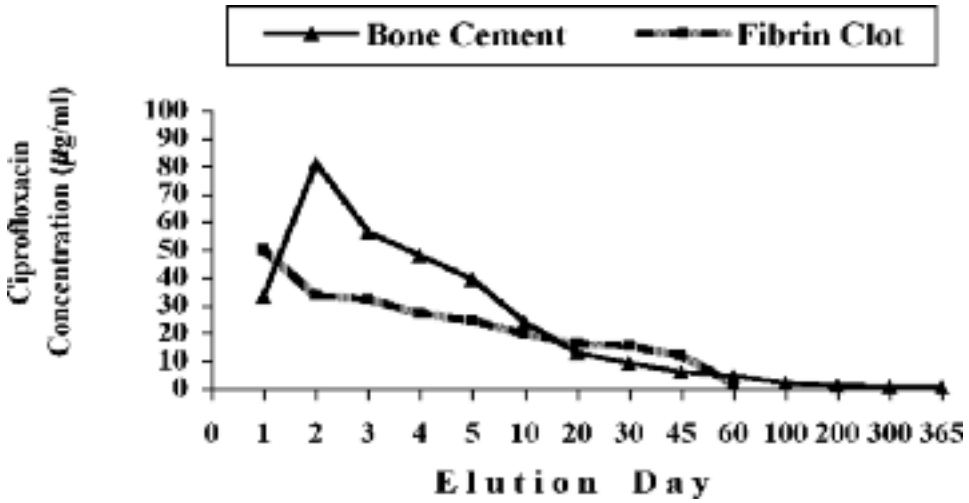

Fig. 1. — *In vitro* elution of ciprofloxacin from acrylic bone cement (PMMA) and fibrin clot

Local antibiotic delivery systems have improved the management of complex wounds in musculoskeletal surgery. When a thorough debridement is augmented with high sustained local antibiotic concentration, the extension of bone and soft tissue contamination to a regional infection may be prevented (*2*). Antibiotic bone cement beads are often used to sterilise and temporarily fill dead space following debridement surgery (*2,5*). The beads are surgically implanted in the debrided bone and are covered with soft tissues. The bone cements beads are left in place for 3 to 4 weeks and are then surgically removed (*2,5,7*).

The antibiotic used in the beads should provide serum concentrations above the breakpoint sensitivities for 3 to 4 weeks, have adequate granulation tissue and bone concentrations in a clinical setting, and not produce high serum drug concentrations. Currently, antibiotics used for bead delivery must be in powdered form and are selected to correspond to the sensitivities of the wound pathogens. It has been shown that a ratio at least 3.6 g of antibiotic per 40 g of acrylic bone cement is desirable for effective elution kinetics and sustained therapeutic levels of antibiotic (*20*). Many investigators use as much as 6 to 8 g of antibiotic per 40 g of bone cement when preparing antibiotic-loaded beads or spacers used in the presence of active infection (*12*). The disadvantages associated with using bone cement beads include a necessary secondary surgery to remove the beads (*2*) and a less than optimal antibiotic elution profile (i.e. only 50% of the antibiotic is eluted from the beads by 4 weeks).

In this study, ciprofloxacin powder was added to the polymer powder in a ratio of 1 g of antibiotic per 40 g of acrylic bone cement. The bone cement ciprofloxacin complex maintained adequate *in vitro* antibiotic concentrations for 365 days.

There are many biodegradable materials that have been evaluated as local antibiotic delivery systems, and some have been evaluated clinically. The materials being evaluated include bone graft and bone graft substitutes, protein based compounds, and synthetic polymers (*13,14,17,19*). A wide variety of antibiotics have been studied in combination with fibrin clot such as cefotaxim, cefazolin, teicoplanin and gentamicin (*9,15,22*). Most studies have evaluated the effect of adding antibiotic solutions to the strength of the resultant fibrin clot and the release characteristics of the antibiotic from the fibrin clot. In previous *in vitro* studies, the fibrin clot antibiotic compounds released adequate antibiotic concentration for a maximum period of 4 to 6 days (*9,15,22*). In the present study, a fibrin

clot ciprofloxacin complex released adequate ciprofloxacin concentrations for a period of at least 60 days (60 to 65 days).

The results of comparing the elution of bone cement and fibrin clot specimens are shown in fig 1. These results indicate that the elution characteristics of ciprofloxacin from fibrin clot compounds resemble those from bone cement samples. However, the ciprofloxacin bone cement complexes were kept for much longer (365 days) than the ciprofloxacin fibrin clot complexes, which usually disintegrated after 60 days.

Fibrin clot ciprofloxacin compounds have the advantage of averting the need for surgical removal, and avoiding a second surgery decreases anaesthesia risk and nosocomial infection rates associated with surgery.

The results of this study suggest that the release of ciprofloxacin from fibrin clots and from acrylic bone cement is very promising, since the levels obtained in the immediate environment are much higher than the MIC values of pathogens implicated in bone and soft tissue infections. The main limitation of this study is that it was conducted in a closed *in vitro* system, which would not disclose any negative effects of the compounds that might occur *in vivo*. Further *in vivo* research will help determine more predictable antibiotic release from bone cement and fibrin clot, degradation rates for fibrin clot antibiotic compounds and determine if the compounds produce toxic serum drug concentrations.

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