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In vitro elution of moxifloxacin and fusidic acid by a synthetic crystallic semihydrate form of calcium sulphate (Stimulan™)

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ABSTRACT

Stimulan™ was evaluated in vitro as a biodegradable carrier for local delivery of moxifloxacin and fusidic acid. Moxifloxacin or fusidic acid was mixed with calcium sulphate at a ratio of 95:5 to prepare five replicas per antibiotic. In vitro elution was estimated daily using a high-performance liquid chromatography (HPLC) system. Elution of moxifloxacin lasted for 31 days. Eluted concentrations reached their peak on Day 13 (mean level 745 μg/mL); the lowest eluted concentration was detected on Day 30 (mean level 367 μg/mL). Elution of fusidic acid lasted for 14 days. Eluted concentrations reached their peak on Day 6 (mean value 249.5 μg/mL); the lowest eluted concentration was detected on Day 13 (mean value 10.9 μg/mL). The presented results revealed that Stimulan™ may allow adequate in vitro elution of moxifloxacin and fusidic acid. The latter results support the application of this system in experimental models of osteomyelitis.

1. Introduction

Chronic osteomyelitis is a common, difficult-to-treat infection despite advances in operation techniques and more than 50 years of experience with antibiotic therapy [1]. The goal of therapy is to eradicate the infection and to restore function. Most cases of osteomyelitis in adults require a combination of medical and surgical therapy for successful eradication of the offending pathogens. Systemic treatment does not often lead to high local tissue concentrations of antibiotic and therefore local deposition of antimicrobial agents has become increasingly popular. Several biodegradable and non-biodegradable substances have been employed as the vehicle for delivery [2]. Polymethylmethacrylate (PMMA) beads are the major representative of non-biodegradable carrier systems; however, they require surgical removal upon completion of drug release. Biodegradable carriers do not necessitate surgical removal. The semihydrate form of calcium sulphate ($CaSO_4$), commonly known as plaster of Paris, has been used for decades to fill defects in bone. It has also lately been proposed as a delivery system for the administration of antibiotics in musculoskeletal infection [3].

Stimulan™ is a synthetic biocompatible bone graft material from calcium sulphate. It is completely reabsorbed and replaced by new bone. It is produced using a synthetic process resulting in 100% purity with no traces of potentially toxic impurities, which have been associated with naturally occurring mineral sources of calcium sulphate. The purpose of the present study was to develop an in vitro system of elution of moxifloxacin and fusidic acid using Stimulan™ as a delivery system. These antimicrobials were selected due to their considerable in vitro activity against staphylococci, which are common pathogens of chronic osteomyelitis.

2. Materials and methods

Stimulan™ (Biocomposites, Keele, UK) was mixed with moxifloxacin (Bayer, Berlin, Germany) or fusidic acid (Boehringer Ingelheim, Ingelheim, Germany) at a ratio of 95:5 up to a total weight of 3 g for each antibiotic. One millilitre of sterile normal 0.9% NaCl was added and distributed into sterile vials (160 mm × 100 mm) to prepare five vials per applied antibiotic. Vials were left at room temperature for 15–30 min for solidification. One millilitre of Mueller–Hinton broth (Trek Diagnostic Systems, East Grinstead, UK) was added over the free surface of each mixture and replaced every 24 h. The vials were incubated at 37 °C. On each consecutive day, the eluent was removed, transferred to a sterile plastic tube and replaced with 1 mL of broth. This procedure was repeated until optical degradation of the prepared mixture. Eluents were stored at −70 °C until analysis.

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Concentrations of moxifloxacin were estimated by modification of a previously described method [4,5] using ciprofloxacin as an internal standard. Briefly, 250 μL of sample were diluted with 250 μL of displacing reagent and centrifuged for 5 min at 2700 × *g*. The displacing reagent consisted of 0.5% sodium dodecyl sulphate (SDS) (Merck, Darmstadt, Germany) and 20% acetonitrile (Merck) in 75 mM sodium phosphate buffer (pH 7.5). Ten microlitres of the supernatant were injected onto an Agilent 1100 series high-performance liquid chromatography (HPLC) system (Agilent Technologies, Waldbronn, Germany) with the following elution characteristics: Nucleosil® 100-5 C18 (4.6 mm × 250 mm, 5 μm) column under 37 °C; mobile phase consisting of a buffer (pH 3.0) composed of 25 mM Na_3PO_4 and 10 mM SDS and acetonitrile 99% at a 35/65 ratio with a flow rate of 1 mL/min; ultraviolet (UV) detection at 293 nm. Ciprofloxacin (Bayer) was added to all samples at a concentration of 2 μg/mL. The retention time of moxifloxacin was 2.8 min and its concentration was estimated by a standard curve created with known concentrations of moxifloxacin. The lower detection limit was 0.03 μg/mL and the interday coefficient of variation of the assay was 0.1%. All determinations were performed in duplicate.

Concentrations of fusidic acid were determined in duplicate by a modification of a previously described assay [6]. A volume of 200 μL of the eluent was mixed with 200 μL of acetonitrile 99% and centrifuged for 20 min at 13 000 × *g* at 4 °C.

One hundred microlitres of the supernatant were injected onto an Agilent 1100 series HPLC system with the following characteristics of elution: ZORBAX Eclipse XDB-C18 (4.6 mm × 150 mm, 5 μm) column under 37 °C; mobile phase consisting of 10 mM K_3PO_4 buffer (pH 3.0), acetonitrile 99% and methanol 70% at a 30/50/20 ratio with a flow rate of 1 mL/min; UV detection at 235 nm. The retention time of fusidic acid was 11.5 min and its concentration was estimated by a standard curve created with known concentrations of fusidic acid. The lower detection limit was 0.15 μg/mL.

Results were expressed as mean ± standard deviation (S.D.). The area under the curve (AUC) for each vial was determined by the linear trapezoidal rule.

3. Results

Elution of moxifloxacin was studied for a total of 31 days. After that time interval the prepared mixture was fully destroyed. Eluted concentrations reached their peak on Day 13, equal to a mean level of 745 μg/mL. The lowest eluted concentration was detected on Day 30, equal to a mean level of 367 μg/mL (Fig. 1). Mean ± S.D. AUC of the elution of moxifloxacin over this 31-day period was 14 484.2 ± 4102.8 μg day/mL.

Elution of fusidic acid was studied for a total of 14 days. After that time interval the prepared mixture was fully destroyed. Eluted concentrations reached their peak on Day 6, equal to a mean value of 249.5 μg/mL. The lowest eluted concentration was detected on Day 13, equal to a mean value of 10.9 μg/mL (Fig. 2). Mean ± S.D. AUC of the elution of fusidic acid over this 14-day period was 1290.4 ± 532.4 μg day/mL.

4. Discussion

Chronic osteomyelitis presents significant therapeutic difficulties. Long-term antimicrobial treatment is hampered by tissue penetration issues as well as the development of adverse events. Surgical intervention is usually necessary. Thorough research has focused on the development of antibiotic-loaded implants, incorporating antibiotics for potential use in the treatment of bone infections [2]. Staphylococci are the main pathogens implicated as

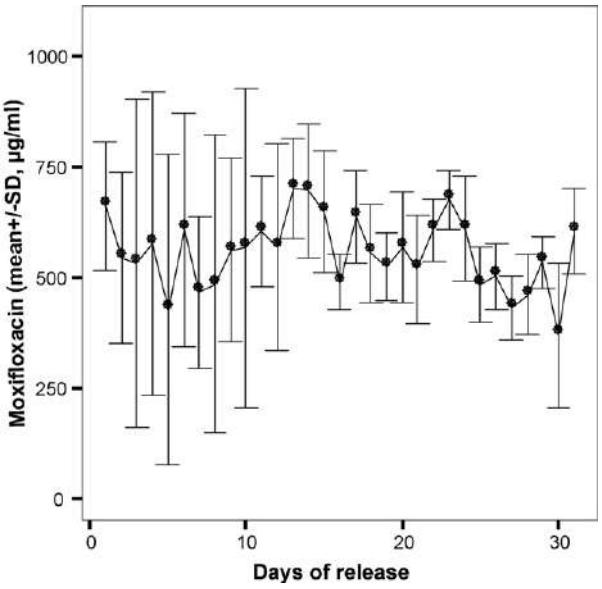

Fig. 1. Elution of moxifloxacin by Stimulan™. S.D., standard deviation.

the causative agent in bone and joint infections [7]; the emergence of methicillin resistance has created a need for new antibiotics for treating osteomyelitis. Former studies by our group have shown that local implantation of a biodegradable system of poly D-,L-dilactide releasing pefloxacin was very potent for the eradication of experimental osteomyelitis caused by meticillin-resistant *Staphylococcus aureus* (MRSA) [8]. Based on the aforementioned beneficial effect of quinolones, a novel biodegradable carrier was developed in vitro eluting either moxifloxacin or fusidic acid. The carrier was calcium sulphate (Stimulan™) commonly used as a bone graft to fill bone cavities resulting from disease, trauma or surgery. Its main characteristics are 100% purity and its ability for biodegradation. Moxifloxacin is a fourth-generation quinolone with similar activity to that of ciprofloxacin and pefloxacin against Gram-negative bacteria but with enhanced activity against MRSA [9]. A recent

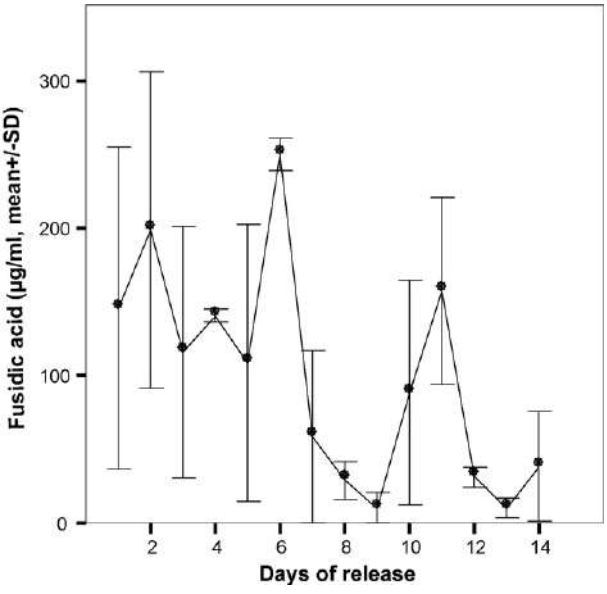

Fig. 2. Elution of fusidic acid by Stimulan™. S.D., standard deviation.

experimental study in rats showed superior activity of systemically administered moxifloxacin compared with vancomycin for the eradication of femoral osteomyelitis caused by MRSA [10]. Fusidic acid has also been proposed as a promising agent for the management of bone infections owing to its considerable antistaphylococcal activity [11].

Results revealed that the prepared system achieved elution of moxifloxacin at concentrations well above 400 μg/mL for a period of 29 days (Fig. 1). The estimated AUC of moxifloxacin for the latter time of elution might be considered as evidence of the total amount of drug released, as proposed elsewhere [12]. Moreover, the AUC/minimum inhibitory concentration (MIC) ratio has been considered as the most critical pharmacodynamic parameter for the activity of a fluoroquinolone against a bacterial pathogen [13]. Values >125 of the ratio should be achievable to eradicate a microorganism. Taking into account the AUC of moxifloxacin eluted from Stimulan™ and the MIC_{90} values (MIC for 90% of organisms) for MRSA [14], it is evident that the developed system may be effective for the eradication of chronic osteomyelitis caused by MRSA.

Few attempts are reported in the literature of the development of systems for local delivery of moxifloxacin. Collagen shields have been applied for its intraocular administration in humans, delivering concentrations well below those described here for Stimulan™ [14,15]. Norian skeletal repair system was also evaluated as an in vitro carrier for moxifloxacin by our group [16]. Release lasted for almost 400 days but the eluted concentrations were lower than those achieved with Stimulan™ as a carrier.

The applied carrier in the present study allowed for continuous release of fusidic acid lasting for 14 days (Fig. 2). Eluted concentrations over the first 6 days were >100 μg/mL and remained above 50 μg/mL over most of the period of release. The MIC_{90} for MRSA is reported to be 0.5 μg/mL [17], which makes the developed system of release promising for the eradication of MRSA osteomyelitis. However, fusidic acid is not a popular candidate for local release systems. Only one study has recently been published where it was incorporated in microspheres of poly(lactide-co-glycolide), where it proved effective for the management of experimental osteomyelitis caused by MRSA in rats [18].

Calcium sulphate has been applied as a carrier in various studies for the in vitro elution of a variety of antimicrobials, including vancomycin, teicoplanin, clindamycin, gentamicin and daptomycin [3,19]. Peak elution of all the tested agents was found within the first 2 days. Elution of vancomycin, teicoplanin, clindamycin and gentamicin lasted only for 10 days, whereas that of daptomycin lasted for 28 days. However, released concentrations of daptomycin ranged between 5 μg/mL and 7 μg/mL after Day 4 [19], which are far lower than those achieved in the present study for both moxifloxacin and fusidic acid.

The present results revealed that Stimulan™ may allow adequate in vitro elution of moxifloxacin and fusidic acid. The latter results support the application of this system in experimental models of osteomyelitis.

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