JCEI /
Journal of Clinical and Experimental Investigations
2014; 5 (2): 194-199
doi: 10.5799/ahinjs.01.2014.02.0388

ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Fibrin sealant as a carrier for sustained delivery of antibiotics

Antibiyotiklerin uzun süreli ilaç salınımı için bir taşıyıcı olarak fibrin yapıştırıcı

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ABSTRACT

Objective: To evaluate the activity and sustained release of antibiotics from fibrin sealant against common strains of ocular bacteria.

Methods: Vancomycin, ceftazidime, moxifloxacin and lomefloxacin were incorporated into fibrin sealant in the shape of discs. Each antibiotic disc and control fibrin disc without drug was tested in vitro against standard bacterial strains of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae* and *Pseudomonas aeroginosa*. After 24 hours of incubation at 37 °C, the discs were transferred to new plates of bacteria and triplicated for each antibiotic.

Results: All antibiotic discs demonstrated detectable activity after 24 hours. Vancomycin had the longest duration of activity (4 days) on the *S. pneumonia* grown plate. The moxifloxacin discs showed a prolonged inhibition of *S. aureus* and S. pneumonia for 3 days and inhibited the other strains for 2 days.

Conclusion: Fibrin sealants provided prolonged drug delivery, which indicates that antibiotic-loaded fibrin clots could be useful for early ocular postoperative care and treatment. *J Clin Exp Invest 2014; 5 (2): 194-199*

Key words: fibrin sealant, sustained release, antibiotic, drug delivery, bacteria

ÖZET

Amaç: Sık görülen oküler bakteri suşlarında antibiyotiklerin fibrin yapıştırıcıda etkinlik ve sürekli salınımlarının değerlendirilmesi.

Yöntemler: Vankomisin, seftazidim, moksilofloksasin ve lomeflokasasin disk şeklinde fibrin yapıştırıcılara katıldı. Her bir antibiyotik diski ve ilaçsız kontrol fibrin diski in-vitro olarak *Stafilokok aureus, Stafilokok epidermidis, Streptokok Pnömonia ve Psödomonas aeruginosa* standart bakteri suşlarında test edildiler. 37°C'de 24 saatlik inkübasyon sonrasında diskler yeni bakteri pleytlerine transfer edildiler ve bu işlem her antibiyotik için üç kez tekrarlandı.

Bulgular: 24 saat sonra tüm antibiyotik diskleri saptanabilir etkinlik gösterdiler. *Streptokok Pnömonia*'nın ürediği pleytte vankomisin en uzun sureli (4 gün) etkinliğe sahipti. Moksilofloksasin diskleri *S. aureus* ve *S. pnömonia* için 3 gün ve diğer suşlara 2 gün uzamış inhibisyon etkisi göstermiştir.

Sonuç: Fibrin yapıştırıcılar uzun süreli ilaç dağılımı sağlamaktadırlar. Bu özellik antibiyotik yüklü fibrin pıhtıların erken postoperatif koruma ve tedavi için kullanışlı olabileceğini göstermektedir.

Anahtar kelimeler: Fibrin yapıştırıcı, uzun sureli salınım, antibiyotik, ilaç dağılımı, bakteri

INTRODUCTION

Topical administration of ocular antibiotics is the current common route of ocular therapy. Despite of its easy administration, there are problems with the rapid drainage and penetration of the drugs [1]. Although, there are developments in relation to this drug route, such as functionalized polymers, peptides and nano-particles, it requires repetitive administration or higher drug doses to achieve sufficient concentration at the posterior segment [2-4]. Topical administration may lead to insufficient antimicrobial effects, fluctuations in ocular drug concentrations and poor compliance because of frequent instillation [2,5]. Also, fortified antibiotics for severe ocular infections include higher concentration of the drugs. In contrast, the subconjunctival, posterior sub-tenon, retrobulbar, intracameral and intravitreal injection of the drugs enables practitioners to administer less of the antibiotic. These drug delivery methods provide drug diffusion through sclera or vitreous for a prolonged time [6]. In addition recently numerous ophthalmic vehicles for anterior segment were developed and there is also growing interest in drug delivery systems to posterior segment

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Received: 12.08.2013, Accepted: 20.03.2014

which obtain long-term sustained release of drugs [5,7]. Nowadays, biodegradable antibiotic loaded nanoparticles and polymeric drug delivery devices are reported to be used as an alternative delivery method of ocular antibiotics [8,9]. However, these methods are not broadened. Other classic antibiotic delivery methods have some limitations like risk of endophthalmitis, retinal detachment, vitreous hemorrhage and cataract in intravitreal injection, low drug bioavailability and corneal toxicity in frequent topically administrations [10,11].

Fibrin sealants have hemostatic and adhesive properties, and have been used increasingly in general surgical procedures [12]. For the last 20 years also ophthalmic surgical procedures have been employed the use of fibrin sealants for sutureless technique of pterygium and drug delivery [13-15]. Two liquid components of the fibrin sealant are mixed to form a fibrin clot that is biocompatible, biodegradable and suitable for mixing with a drug [15,16]. Several drugs, such as chemotherapeutics, antimicrobials and growth factors, were reported to be encapsulated with fibrin sealant [17-19]. Although fibrin sealants have the potential to provide a slow release system for antibiotic chemotherapy, the features of the chemotherapy drugs might change the biochemical interactions of the drug-sealant mixture, resulting in variations in the drugs' release times [20]. The sealant mixtures might also be affected by the products of pathogen bacteria, such as bacterial toxins and enzymes, which can degrade the fibrin matrix faster and release the drug rapidly. The recent studies related to orthopedics and general surgery reported on the efficacy of a single antibiotic that was impregnated in a fibrin sealant against commonly seen pathogens [13,21]. Marone et al. have been demonstrated antibacterial activity of the release of the four ocular antibiotics from fibrin sealant [22]. However, *Staphylococcus epidermidis was the only bacterial strain analyzed in their study. There is need to be examined the potential for* the delivery of antibiotics by fibrin sealant on more bacterial strains.

The aim of this study is to investigate the antibacterial effect of common and new ocular applicable antibiotics impregnated into fibrin clots against four common ocular bacterial pathogens and to monitor their sufficient antibacterial effect time.

METHODS

In this study lyophilized human fibrin (Tisseel Lyo ®, Baxter, Vienna, Austria), standard bacterial strains of *Staphylococcus aureus* (ATCC 65389), *Staphylococcus epidermidis* (ATCC 12218), *Streptococcus pneumoniae* (ATCC 49619) and *Pseudomonas aeroginosa* (ATCC 27853) and the antibiotics vancomycin (Vancomycin ®, Abbott France S.A., Rungis Cedex, France), ceftazidime (Fortum ® , GlaxoSmithKline, Izmit, Turkey), moxifloxacin (Vigamox, Alcon, TX, USA) and lomefloxacin (Okacin, Novartis, Istanbul, Turkey) were used. To prepare the fibrin glue with antibiotics, a thrombin solution and antibiotic solution were mixed and this mixture was transferred to a flat-bottomed 96 well plate. Later, it was mixed with fibrin component which contains fibrinogen, factor XIII and aprotinin was prepared according to the manufacturer's instructions. The final antibiotic concentrations were determined based on the commonly used concentrations in the antibiotic discs. The final concentrations of the antibiotic fibrin clot discs were 30 µg for vancomycin and ceftazidime and 5 µg for moxifloxacin and lomefloxacin. The dimensions of discs were 6 mm of diameter and 4 mm of height. The antimicrobial effects of the vancomycin, ceftazidime, moxifloxacin and lomefloxacin including fibrin glue discs were tested by using the Kirby-Bauer disc diffusion method against *S. aureus*, *S. epidermidis*, *S. pneumoniae* and *P. aeroginosa* standard bacterial strains. Freshly grown bacterial colonies were prepared as suspensions equal to 0.5 McFarland turbidity and spread on Mueller-Hinton agar media, or 5% sheep blood agar medium for *S. pneumoniae*, with a sterile swab. Vancomycin, ceftazidime, moxifloxacin and lomefloxacin loaded fibrin discs and control fibrin discs without drug were placed on the plates. The plates were incubated for 24 hours at 37 °C and any antibiotic fibrin discs that were not completely dissolved were transferred to new medium plates. This process was repeated after 24, 48, 72 and 96 hours. The diameter of the inhibition zones around the discs were measured daily and the average for the three discs was recorded. These experiments were performed triplicate and the mean values of inhibition zones were analyzed.

RESULTS

The antibacterial effects of all four antibiotics were detectable, and the drugs achieved similar inhibition zones during the triplicate experiments for each antibiotic-sealant disc after 24 hours (Table 1-4). No zone of inhibition was observed around the control fibrin discs without drug. The zone of inhibition in the *S. epidermidis* plate was measurable with moxi-

floxacin on the second day, but the others showed activity only for the first day. In the *S. aureus* plate, the moxifloxacin-loaded discs had detectable zones after 3 days. Prolonged antimicrobial activity for S. pneumonia was observed with vancomycin (4 days) and moxifloxacin (3 days) discs. On the *P. aeroginosa* grown plate, all of the antibiotics except vancomycin (which is not selective) were released from the sealant, inhibiting bacterial growth for a period of 2 days. The inhibition zone diameters of all antibiotic-loaded fibrin sealant discs that were detectable on the second day decreased approximately 50% or more after 24 hours. All fibrin clots dissolved after three days on the *P. aeroginosa* plate; the vancomycin-loaded sealants also dissolved on all plates after four days.

Table 1. Antibacterial activity of antibiotic loaded fibrin sealant discs against *Staphylococcus aureus* (average of triplicate experiments)

	Diameter of inhibition zone (mm)			
Antibiotic	**24 h**	**48 h**	**72 h**	**4 days**
Moxifloxacin (5 µg)	23	10.66 ± 0.77	3.66 ± 0.47	0
Lomefloxacin (5 µg)	16.66 ± 0.47	0	0	0
Vancomycin (30 µg)	13.66 ± 0.47	0	0	0
Ceftazidime (30 µg)	22.33 ± 0.57	0	0	0

Table 2. Antibacterial activity of antibiotic loaded fibrin sealant discs against *Staphylococcus epidermidis* (average of triplicate experiments)

	Diameter of inhibition zone (mm)			
Antibiotic	**24 h**	**48 h**	**72 h**	**4 days**
Moxifloxacin (5 µg)	21.66 ± 0.57	11.33 ± 1.52	0	0
Lomefloxacin (5 µg)	18 ± 1	0	0	0
Vancomycin (30 µg)	13.33 ± 0.57	0	0	0
Ceftazidime (30 µg)	24.33 ± 1.15	0	0	0

Table 3. Antibacterial activity of antibiotic loaded fibrin sealant discs against *Streptococcus pneumoniae* (average of triplicate experiments)

	Diameter of inhibition zone (mm)			
Antibiotic	**24 h**	**48 h**	**72 h**	**4 days**
Moxifloxacin (5 µg)	22.66 ± 0.57	7.66 ± 0.57	2 ± 1.73	0
Lomefloxacin (5 µg)	5.66 ± 0.57	0	0	0
Vancomycin (30 µg)	16.66 ± 0.57	14.66 ± 0.57	5.33 ± 4.61	2 ± 1.73
Ceftazidime (30 µg)	26	20.33 ± 0.57	0	0

Table 4. Antibacterial activity of antibiotic loaded fibrin sealant discs against *Pseudomonas aeroginosa* (average of triplicate experiments)

	Diameter of inhibition zone (mm)			
Antibiotic	**24 h**	**48 h**	**72 h**	**4 days**
Moxifloxacin (5 µg)	20.33 ± 0.57	3.33 ± 0.57	0	0
Lomefloxacin (5 µg)	16.66 ± 0.57	0.63 ± 0.05	0	0
Vancomycin (30 µg)	0	0	0	0
Ceftazidime (30 µg)	24.66 ± 0.57	0.6 ± 0.1	0	0

DISCUSSION

This study shows that fibrin sealant has the potential to be used as a sustained release vehicle for ocular drug delivery. Prolonged antibacterial activity was observed for all antibiotic-impregnated fibrin sealants, but the elution of all agents at a therapeutic concentration decreased by 50% or more after 24 hours. The bolus from the antibiotic-impregnated fibrin sealant was possibly released from the peripheral layers of the sealant on the first day, so the bolus might have been in a gel or liquid phase. Although the proposed procedure must be optimized to sustain the release of antibiotics and to prevent the initial rapid release, this study demonstrates that antibiotic-impregnated fibrin sealants, particularly vancomycin and moxifloxacin-impregnated sealants, are useful for ocular postoperative care and the treatment of bacterial diseases via local administration.

Tissue adhesives have been successfully used for about 30 years for their hemostatic and adhesive properties [12,23,24]. After the FDA approved fibrin tissue adhesives in 1998, their spectrum of usage broadened. Recently, fibrin sealant was used in the ocular surgery of pterygium, strabismus, bleb revision, amnion membrane transplantation and keratoplasty [25-28]. Fibrin sealants include 2 liquid components of fibrinogen and thrombin that must be mixed to form a fibrin clot at the site of the application [16]. The fibrin clot provides adhesion between the corresponding tissues and has the potential to depot a drug that can be incorporated in liquid components just before the clot formation [15]. Therefore, the accumulation of a drug in a fibrin clot makes it possible to use the clot as a drug delivery vehicle.

The most common pathogenic bacteria in ocular infections are *S. aureus*, S. pneumonia, *S. epidermidis* and *P. aeroginosa* [29]. Higher doses of effective antibiotics are useful for prophylaxis or for the treatment of ocular infections that they can be applied only locally with the minimum side effects and less frequency of administration. The delivery and activity of the four commonly used antibacterials released from fibrin sealant were tested for ocular infections.

Although fibrin sealants have previously been used to deliver chemotherapeutics to tumor sites, the number of reports about the encapsulation and slow release of antibiotics from sealants has recently increased [21]. Simpson at al. showed that a carboplatin-impregnated fibrin sealant maintained a two-week drug release, whereas Tredwell at al. reported a mere 2-day release of cefazolin [13,19]. Therefore, a drug's ability to depot in a fibrin sealant matrix and its elution from the sealant is possibly related to the drug's interaction with the fibrin material. Some studies recently demonstrated that hydrophobic antibiotics release from the sealant over the course of a week, whereas hydrophilic ones release in a shorter time [13,20,21]. Antibiotics used in this study also were hydrophilic drugs that may accelerate the elution of drugs. On the other hand, the elution time of antibiotic-loaded fibrin sealants may change as the pathogen bacteria change the environmental conditions. Bacterial toxins, bacterial enzymes and the antimicrobial responses of organisms can affect the release time of the antibiotics from the sealant. In Marone at al.'s study, the antibacterial activity of vancomycin-impregnated fibrin sealant persisted for 2 days on the *S. aureus* grown plate [22]. The present study likewise recorded a short activity time for *S. aureus* (one day) but In contrast, the antibacterial activity time extended to 4 days on the S. pneumonia grown plate. Thus, the difference in this release time possibly comes from the fibrinolytic effects of *S. aureus* toxins. In the current study, all of the antibiotic-fibrin sealant mixtures were completely dissolved on the *P. aeroginosa* grown plate at the end of the fourth day. Therefore, it can be suggested that the release time of the antibiotic from fibrin sealant may be associated with the environment of the local treatment site. Quicker fibrinolysis of antibiotic loaded-fibrin sealant hinders the sealant's ability to retain the drug, thus eradicating the slow release. However, at least 2 days of persistent antibacterial activity from a drug-loaded fibrin sealant might be enough to inhibit sensitive pathogenic bacteria for early postoperative care. This prolonged, local treatment of antibiotics can also decrease the frequency of topical application so that the local dose is stable and so that practitioners do not need to touch the eye lids often. After the local treatment period, topical therapy at a lower frequency should be started for maintenance.

There are some limitations to this study; the elution of the antibiotic-impregnated sealants in liquid was not tested, so the authors do not know the real quantity of the released antibiotic from sealants, even in the cases where the antibiotic is still in the core of the sealant on the plate. In vivo, releasing times may increase because of the resultant drug. Moreover, the present study did not incorporate different concentrations of the antibiotics in the fibrin sealant, which might change the releasing times,

so further studies should be designed to discover which drug concentrations do not inhibit clot formation.

The present data indicate that the moxifloxacin release over minimal inhibitory concentrations (MIC) from fibrin sealant is sufficient for three days against *S. aureus* and S. pneumonia grown plates. The inhibition zones of moxifloxacin were measured after at least two days for all bacterial isolates tested. Moxifloxacin might be preferable for antibiotic-impregnated fibrin sealants for ocular empiric treatment or for postoperative care. The other antibiotics tested in this study may be used after the pathogen has been determined. A sensitive, controlled-release antibiotic, such as vancomycin, seems to be effective against S. pneumonia.

The results of this study demonstrate that antibiotic delivery through a sustained release system of fibrin sealant may be effective against common ocular infections in vitro. Especially, moxifloxacin impregnated fibrin sealant in the environments of four common ocular pathogens suggests that it possibly has potential to be effective for one or two days longer than the other antibiotics studied, which might be useful for early ocular postoperative care and treatment.

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