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DIFFUSION OF ANTIBIOTICS IN INTERVERTEBRAL DISC

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Abstract

Delivering charged antibiotics to the intervertebral disc is challenging because of the avascular, negatively charged extracellular matrix (ECM) of the tissue. The purpose of this study was to measure the apparent diffusion coefficient of two clinically relevant, charged antibiotics, vancomycin (positively charged) and oxacillin (negatively charged) in IVD. A one-dimensional steady state diffusion experiment was employed to measure the apparent diffusion coefficient of the two antibiotics in bovine coccygeal annulus fibrosus (AF) tissue. The averaged apparent diffusion coefficient for vancomycin under 20% compressive strain was $7.94 \pm 2.00 \times 10^{-12} \text{ m}^2/\text{s}$ ($n=10$), while that of oxacillin was $2.26 \pm 0.68 \times 10^{-10} \text{ m}^2/\text{s}$ ($n=10$). A student's t-test showed that the diffusivity of vancomycin was significantly lower than that of oxacillin. This finding may be attributed to two factors: solute size and possible binding effects. Vancomycin is approximately 3 times larger in molecular weight than oxacillin, meaning that steric hindrance likely plays a role in the slower transport. Reversible binding between positive vancomycin and the negative ECM could also slow down the rate of diffusion. Therefore, more investigation is necessary to determine the specific relationship between net charge on antibiotic and diffusion coefficients in IVD. This study provides essential quantitative information regarding the transport rates of antibiotics in the IVD, which is critical in using computational modeling to design effective strategies to treat disc infection.

Keywords

apparent diffusion coefficient; transport; antibiotics; annulus fibrosus; intervertebral disc

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest regarding the authorship and/or publication of this research article.

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INTRODUCTION

Intervertebral disc (IVD) infection is a dangerous, devastating, and debilitating condition with growing incidence related to the aging population, an increase in spinal surgeries, hematogenous spread, particularly in immunosuppressed patients (e.g., HIV, cancer, rheumatic disease patients), and the rise of intravenous (IV) drug abuse. Currently, disc infections are difficult and costly to treat, often requiring weeks of inpatient care and IV administered drugs. Successful treatment requires antibiotic levels in the IVD above the minimally inhibitory concentrations (MIC). However, the avascular nature of the IVD, coupled with the charged nature of the disc extracellular matrix (ECM), make delivery of antibiotic drugs, which are often charged molecules, to the tissue problematic in practice.

A number of experimental studies have indicated that electrical charge plays a significant role in the penetration of antibiotics into the IVD (Conaughty et al., 2006; Gibson et al., 1987; Riley et al., 1994; Scuderi et al., 1993; Tai et al., 2002; Thomas et al., 1995). Overall, these studies either directly suggested or indicated that positively charged antibiotics have easier access to or higher uptake in the discs than negatively charged ones. We have developed a computational model of the human IVD able to predict the kinetics of antibiotic penetration into the IVD (Zhu et al., 2016). Our numerical prediction was in agreement with these empirical findings, with positively charged drugs having higher concentrations and uptakes than their negatively charged counterparts. However, this computational analysis relied on estimates for antibiotic diffusion coefficients, since quantitative values are not available in the literature. To more accurately predict drug penetration and concentration profiles in the disc, more information is needed on the value of the diffusion coefficient and net charge of relevant drugs in IVD tissues.

To our knowledge, no previous study has quantified the diffusivity of antibiotics in the IVD. Such information is necessary in order to employ computational modeling to design effective strategies to treat disc infection while maintaining drug levels above MIC. To this end, we will measure the apparent diffusion coefficient of antibiotics in IVD tissue. The diffusion coefficient of two clinically relevant antibiotics, vancomycin (positively charged) and oxacillin (negatively charged), in bovine coccygeal annulus fibrosus (AF) tissues were investigated in this study.

MATERIALS AND METHODS

Specimen Preparation:

AF specimens were prepared from the bovine coccygeal IVD. Using a corneal trephine, 6 mm cylindrical punches were cored in the axial direction from the AF section of the IVD. Using a sledge microtome with freezing stage, the cylindrical specimens were cut to a final height of 1 mm, as measured using a custom, current-sensing micrometer. A total of 20 specimens were prepared, for a sample size of $n=10$ for each molecule investigated. All samples were prepared from the middle AF region of the IVD. Several specimens were prepared from each disc and then divided between the two antibiotics groups.

Diffusion Measurement:

The apparent diffusion coefficients of vancomycin and oxacillin were measured using a one-dimensional quasi-steady state experiment, similar to our earlier studies (Jackson et al., 2012; Jackson et al., 2008; Kleinhans et al., 2015; Yuan et al., 2009). A custom diffusion chamber was used, consisting of two acrylic solution chamber halves, separated by a specimen holder in the middle. The specimen was held in place with two rigid porous plates (hydrophilic polyethylene, 50–90 µm pore size, Small Parts, Inc., Miami Lakes, FL) to inhibit swelling, and sealed with an o-ring. The compressive strain was controlled by changing of a spacer placed between the two chamber halves. For all experiments, the compressive strain was controlled at 20%.

At the start of the experiment, an initial one hour incubation period was used to facilitate equilibration of the antibiotic in the tissue specimen. During this time, phosphate buffered saline (PBS) solution was filled in the downstream chamber, while a PBS solution containing antibiotic was filled in the upstream chamber. For oxacillin, a concentration of 100 mM oxacillin in PBS was used upstream, while, for vancomycin, a concentration of 10 mM vancomycin in PBS was used. These concentrations were based on preliminary studies for protocol optimization. A magnetic stir rod was placed in each chamber and the diffusion apparatus was placed on a magnetic stir plate at room temperature (~23°C).

Following the one hour incubation, the downstream chamber was emptied and rinsed with fresh PBS solution. At the start of the experiment, 500µL of fresh PBS solution as filled in the downstream chamber; the upstream chamber still contained a solution containing antibiotics as described above. The upstream and downstream chambers were both continuously stirred with a magnetic stir bar and stir plate. At 30 minute intervals, the contents of the downstream chamber were collected for concentration measurements. The downstream chamber was once again rinsed with PBS, and the experiment was repeated for 10 consecutive intervals of 30 minutes each (for a total of 300 minutes). Preliminary studies showed that this was adequate time to reach steady state in the tissue. Representative experimental curves for both antibiotic molecules are shown in Figure 3.

Following sample collection, the concentration of antibiotic in the samples from the downstream chamber, as well as the final concentration of antibiotic in the upstream chamber, was determined using the Folin-Ciocalteu reagent colorimetric assay with calibration curve. The absorbance was measured at 750 nm using a BioTek plate reader and Gen5 software.

The apparent diffusion coefficient, D_{app} , of antibiotic in AF tissue was determined based on the average antibiotic concentration at steady state (i.e., average concentration of last three measurements) using the following relationship:

$$D_{app} = \frac{V_{down} C_{down} h}{(C_{up} - C_{down}) At}$$

where V_{down} is the volume of the downstream chamber, C_{up} and C_{down} are the upstream and downstream antibiotic concentrations, respectively, h is the thickness of the tissue specimen, A is the diffusion area, taken as 50% of the cross sectional area of the tissue, due to the 50% open area of the porous plates, and t is the time interval.

Statistical Analysis:

A student's t-test was used to determine if the diffusion coefficients of the two antibiotics in AF tissues were significantly different using Excel software (Microsoft, Inc., Seattle, WA). The significance level was set at $p < 0.05$. Results are expressed in mean $\pm$ standard deviation.

RESULTS

The molecular weights, net charges, and averaged diffusion coefficient for vancomycin and oxacillin in bovine coccygeal AF under 20% compressive strain are shown in Table 1. For each molecule, a total of 10 samples ($n=10$) were measured. A student's t-test showed that the apparent diffusivity of vancomycin was significantly lower than that of oxacillin.

DISCUSSION

In this study, we investigated the apparent diffusivity of two clinically relevant antibiotics in IVD tissues. While numerous studies have done on the penetration of various antibiotics in the disc, to our knowledge, this is the first study to quantify the diffusion coefficients of such molecules in disc tissues. Positively charged vancomycin had a significantly lower apparent diffusion coefficient than negatively charged oxacillin in bovine coccygeal AF tissues. Because the ECM of the IVD is negatively charged, positively charged antibiotics are more capable of penetrating the disc, as shown in our recent computational study confirming this phenomenon (Zhu et al., 2016). The smaller diffusion coefficient for vancomycin found in this study may be attributed to two factors: (1) solute size effects; and (2) possible binding effects.

There is a substantial size difference when comparing the vancomycin molecule to the oxacillin molecule, with oxacillin being approximately 1/3 the molecular weight of vancomycin, see Table 1. By comparison, previous studies have shown that the diffusivity of fluorescein (MW=332 Da) in bovine coccygeal AF is on the same order of magnitude ($\sim 1 \times 10^{-10} \text{ m}^2/\text{s}$) as similarly sized oxacillin (Travascio and Gu, 2007), while larger dextran molecules (3K+ Da) have much smaller diffusion coefficients ($\sim 10^{-12} - 10^{-11} \text{ m}^2/\text{s}$) in articular cartilage (Jackson and Gu, 2009). Ignoring electrostatic interactions, solute size is a major factor affecting the diffusion coefficient in hydrogels and disc tissues (Gu et al., 2004), given that it is directly related to steric hindrance (Maroudas, 1970, 1976). Previous studies have shown that solute diffusion coefficients decrease with increasing solute size in cartilaginous tissues (Jackson and Gu, 2009). Here, we found that the larger vancomycin molecule had a slower diffusion coefficient than oxacillin; however, additional factors may also contribute to this finding.

The smaller value of the diffusion coefficient for positively charged vancomycin could also be attributed to possible binding interactions with the tissue. That is, weak, reversible

binding between the drug molecule and the charged ECM could slow the diffusion process (Garcia et al., 2003). This could be a contributing factor to the lower diffusivity seen for vancomycin as compared to oxacillin, which would not be expected to bind to the negatively charged ECM. Although binding might slow the initial diffusive process into the tissue, such interactions could increase the equilibrium uptake into the tissue, and allow for a more sustained local delivery of the antibiotic in the tissue (Bajpayee et al., 2014). This may also explain the higher uptake of positively charged antibiotics into the disc as found by numerous investigators in the literature (Conaughty et al., 2006; Gibson et al., 1987; Riley et al., 1994; Scuderi et al., 1993; Tai et al., 2002; Thomas et al., 1995).

The limitations of this study are as follows: Bovine coccygeal discs were used to carry out these experiments; in future studies, we will investigate the diffusion of charged solutes in human lumbar IVD, which is more physiologically relevant. Furthermore, investigating the effects of loss of proteoglycan content and concomitant decreased fixed charge density, as occurs in disc degeneration (Lyons et al., 1981), on diffusion coefficient of charged molecules in the tissue is essential for designing effective treatment strategies. Future studies will investigate the effects of other contributing factors on transport properties; in particular, we will quantify the binding effects on the diffusion of antibiotics in IVD. In addition, we will also investigate the effect of direction on the antibiotic diffusion in the IVD (e.g., axial vs. radial), given that previous studies have shown that solute diffusivity in AF is anisotropic (Hsu and Setton, 1999; Jackson et al., 2006; Jackson et al., 2012; Jackson et al., 2008; Travascio and Gu, 2007; Travascio et al., 2009). Nevertheless, given that these are the first studies to report quantitative data on antibiotic apparent diffusion coefficients in the IVD, we believe the results are valuable and represent a launching point for more comprehensive characterization of antibiotic transport in the disc.

In summary, this study represents, to our knowledge, the first quantitative characterization of antibiotic diffusion in IVD tissues. Our results show that negatively charged oxacillin has a higher diffusion coefficient than positively charged vancomycin in bovine coccygeal AF. In order to more fully understand the transport kinetics of charged antibiotics in IVD tissues, we will examine the effects of electrostatic interactions in comparably sized molecules (i.e., to eliminate size effects) as well as the effects of binding on transport rates in future studies. We will also quantify the net charge of additional relevant antibiotics, which is currently lacking in the literature. In total, this research provides valuable new quantitative information on antibiotic transport in IVD, which is necessary for the design of effective strategies to treat the dangerous and costly condition of disc infection via computational modeling.

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Figure 1:
Schematic showing location, orientation, and size of specimens collected from the AF region of bovine coccygeal IVDs.

Figure 2:

Schematic of custom diffusion chamber used in 1-D steady state diffusion experiment to calculate the apparent diffusivities of antibiotics in bovine AF. The specimen is sealed between two porous plates and on the radial edge by an o-ring. The level of compression for the specimen is controlled by the size of the metal spacer between the two chamber halves; all specimens were measured at 20% compressive strain. Solute moves from the upstream chamber, through the tissue, to the downstream chamber during the experiment.

Figure 3:
Representative experimental curves for oxacillin and vancomycin samples. The experiment was carried out for 300 minutes, with concentration measurements taken every 30 minutes. The concentration at equilibrium was taken from the averaged concentrations in the final 120 minutes of the experiment (see box outline).

Table 1:

Molecular Weight, Net Charge, and Measured Apparent Diffusivities of Vancomycin and Oxacillin in Bovine Annulus Fibrosus

	Molecular Weight	Net charge	Apparent diffusivity in AF at 20% compressive strain
Vancomycin	1449 g/mol	+1	$7.94 \pm 2.00 \times 10^{-12} \text{ m}^2/\text{s}$
Oxacillin	401 g/mol	-1	$2.26 \pm 0.68 \times 10^{-10} \text{ m}^2/\text{s}$