The In Vitro Elution Characteristics of Vancomycin from Tendons

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Abstract

Background Infection after ACL reconstruction is uncommon but catastrophic. Prophylactic graft saturation in vancomycin reportedly reduces infection rates.

Questions/purposes We characterized vancomycin elution from soaked tendons. Specifically, the effect of rinsing was studied. We also determined how vancomycin concentration in the soak solution and tendon dimension influenced this elution rate, and examined whether the vancomycin amount released was lower than osteoblast and chondroblast toxic concentrations.

Methods Bovine tendons were wrapped in sterile gauze swabs presoaked with 5-, 2.5-, or 1.25-mg/mL vancomycin solutions. After 10 minutes, rinsed and unrinsed tendons were placed in 100 mL agitated 37°C phosphate-buffered saline (PBS). One-milliliter samples taken at 10 minutes and 1, 6, 12, 24, and 72 hours were analyzed by high-performance liquid chromatography.

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Results The maximum elution rate occurred between 10 minutes and 1 hour, with no lag between experiment initiation and drug appearance in the solution. Rinsing affected the initial amount in solution but had little influence on drug release after 10 minutes. Vancomycin diffusion rates were dependent on soak solution concentration at all sampling intervals. The vancomycin amount released or eluted did not increase after the 1-hour interval. At 24 hours, concentrations were 45 ± 12 , 16 ± 1 , and 9 ± 3 µg/mL for the 5-, 2.5-, and 1.25-mg/mL solutions, respectively. Higher elution rates were observed in larger-volume tendons.

Conclusions Soaked tendon grafts can act as reservoirs for vancomycin, with the amount released and elution profile dependent on rinsing, tendon volume, and soak solution concentration. Vancomycin elution was lower than previously reported osteoblast and chondroblast toxicity concentrations and above the minimum inhibitory concentration for *Staphylococcus*.

Clinical Relevance Presoaking ACL reconstruction autografts with vancomycin may reduce the risk of ACL reconstruction infection without the risk of local or general toxicity.

Introduction

Rupture of the ACL is a common sporting injury, usually occurring as a result of a noncontact pivot event. Surgical reconstruction of the ACL typically is required to stabilize the joint and decrease the risk of meniscal tearing. ACL reconstruction techniques continue to evolve, with improving outcomes with time. Graft choices vary among institutions but include autografts, such as hamstring tendons or the middle third of the patella tendon, and allografts.

Septic arthrosis is a rare but potentially devastating complication after ACL reconstruction, with rates reported between 0.14% and 1.7% [5, 9, 13, 15–17]. The choice of graft selected for ACL reconstruction may increase the risk of infection, with several studies reporting a higher incidence of infection with hamstring tendon autografts compared with bone-patella-tendon-bone autografts and allografts [4, 10, 11].

Infection typically results from coagulase-negative *Staphylococcus* that contaminates the harvested avascular graft, despite administration of prophylactic intravenous antibiotics. The patient often presents late with moderate pain and swelling, an increasing C-reactive protein, and elevated erythrocyte sedimentation rate. Poor outcomes such as reduced function compared with that of peers, arthrofibrosis, pain, early osteoarthritis, and graft failure requiring revision often result.

Vancomycin is a glycopeptide antibiotic with a high volume of distribution and is water soluble. It has a low allergy rate, is heat stable, and has low resistance rates among gram-positive bacteria. It is less toxic to local tissues than tobramycin, cefazolin, and gentamicin. The above attributes make it ideal for use as a locally delivered, intraarticular antibiotic prophylaxis agent. It principally is used for methicillin-resistant *Staphylococcus aureus* (minimum inhibitory concentration [MIC] = 0.5 mg/L); however, it imparts time-dependent bactericidal activity against numerous gram-positive bacteria, including *Streptococcus* (MIC = 0.25 mg/L) and *Enterococcus* spp (MIC = 2.0 mg/L) [1].

Vancomycin has been used routinely for local antibiotic infusion into joints, and in vitro studies have provided some evidence of its low osteoclast and chondrocyte toxicity [2, 6]. Wrapping hamstring tendon autografts in gauze presoaked in 5 mg/mL vancomycin hydrochloride solution before insertion is currently undergoing clinical trials in ACL reconstruction procedures. Preliminary research indicates this use of vancomycin reduces infection rates (confirmed by negative culture) postoperatively (relative risk, 3.134; 95% confidence interval [CI], 2.847–3.453) (Unpublished data. CJ Vertullo; 2009).

We therefore characterized the elution of vancomycin from tendons treated using the same above-mentioned protocol currently undergoing trials during surgery and determined what parameters influenced the rate and extent of observed drug elution. The primary aim was to evaluate the effect of rinsing on the rate and extent of vancomycin elution from tendon tissue. The secondary aims were to determine whether the rate and extent of drug elution were dependent on the concentration of the vancomycin solution and whether tendon dimension influenced the rate and extent of vancomycin release. In addition, we examined whether the amount of

vancomycin released was less than the concentration shown to be toxic to osteoblasts.

Materials and Methods

This was an in vitro study investigating the amount of drug release and elution profiles of vancomycin from freshly excised calf tendons obtained from a local abattoir. Tendons were stored on ice during transport and used on the same day of excision. Tendons were stripped and dissected by scalpel into 1- and 5-cm-long sections each with a diameter of 0.8 cm. Tendon volumes were determined by liquid displacement. Soak solutions were prepared by dissolving and diluting the contents of 500 mg DBL[®] vancomycin hydrochloride vials (Hospira, Sydney, Australia) with an appropriate volume of sterile normal saline to produce final concentrations of 5, 2.5, and 1.25 mg/mL. Sterile gauze swabs (10 cm × 10 cm × eight ply) (Smith and Nephew, Mt Waverley, Australia) were immersed in soak solutions for 1 minute. Tendon grafts were wrapped tightly in impregnated gauze swabs and left to stand for 10 minutes. At the end of this period, tendons either were flushed with 5 mL normal saline before elution studies or left unrinsed.

The protocols used for this elution study were adapted from previously described methods [7, 8, 14]. Tendons subsequently were placed in covered chambers containing either 50 or 100 mL PBS (pH 7.4). Chambers were agitated gently and kept at 37°C. One-milliliter samples were taken at 10 minutes and then at 1, 6, 12, 24, and 72 hours. An equivalent volume of PBS was returned to each chamber to maintain volumes. Samples were stored immediately at –20°C and analyzed by high-performance liquid chromatography (HPLC) within 7 days of collection.

The validated analytic HPLC method used in this study was adapted from the published methods of Backes et al. [3]. The system consisted of a Varian Prostar 240 Solvent Delivery Module, Varian Prostar 430 Autosampler, and Varian Prostar 335 Photodiode Array Detector (Agilent Technologies, Santa Clara, CA, USA). The stationary phase consisted of a 250- × 4.6-mm, 5-μm particle size Gemini[®] II C18 column (Phenomenex Inc, Torrance, CA, USA) with temperature set at 30° ± 1°C. An injection volume of 20 μL was used for each analysis and vancomycin was detected at dual wavelengths of 210 and 280 nm. Samples were vortex-mixed briefly, centrifuged (10,000 g, 15 minutes), and filtered through a 0.45-μm polytetrafluoroethylene syringe filter (Phenomenex) before injection. Buffer consisted of 0.05 mol/L phosphate buffer (pH 6). Mobile phase components A and B consisted of buffer-acetonitrile-methanol (91:5:4) and buffer acetonitrile-methanol (84:8:8), respectively. Chromatography-grade

acetonitrile and methanol were obtained from Chromasolv (Sydney, Australia). Elution of vancomycin was achieved using the following gradient conditions at a flow rate of 1.2 mL/minute: 100% A for 2 minutes, to 100% B in 12 minutes, held for 16 minutes, and then returned to 100% A for 5 minutes. The total run time for each sample was 35 minutes. Linear calibration curves, from 2.5 to 100 µg/mL, had linearity coefficients of 0.99 or more. The lower detection limit of this HPLC assay was 0.1 µg/mL.

The amount of antibiotic released from each tendon per sampling interval was calculated as follows: the measured antibiotic concentration of each sample at each interval was multiplied by the constant volume of the chamber (ie, 100 mL), yielding the total antibiotic released into the chamber during that interval. The cumulative amount of vancomycin released by each tendon was calculated using the equation:

$$Q = \left\{ C_n V_c + \sum_{i=1}^{n-1} C_i S \right\}$$

where Q = the cumulative amount of vancomycin released; C_n = the concentration of vancomycin determined at the n^{th} sampling interval; V_c = the volume of the chamber; S = the volume of the sampling aliquot; and $\sum_{i=1}^{n-1} C_i$ = the sum of vancomycin concentrations determined at sampling intervals 1 to $n - 1$.

The elution rate for each tendon was calculated at each sampling interval by dividing the amount of antibiotic released by each tendon at each interval by the length of the interval. Results are represented as the means $\pm$ SDs of replicate experiments. GraphPad InStat Version 3.10 (GraphPad Software Inc, San Diego, CA, USA) was used for all statistical calculations. P values less than 0.05 were considered significant. Statistical comparisons were made by one-way ANOVA and two-tailed t test where relevant.

Results

No lag time was observed between the initiation of the experiment and the appearance of vancomycin in solution for any of the conditions tested. The maximum rates of elution were observed between the 10-minute and 1-hour sampling intervals for all conditions investigated (Fig. 1). The amount of vancomycin released from unrinsed tendons was uniformly higher than that recorded in tendons that had been rinsed before elution experiments (Table 1). However, with the exception of the 10-minute interval ($p < 0.001$), the differences in calculated elution profiles for rinsed and unrinsed tendons decreased in value and significance throughout the experiment (Fig. 1A). Unrinsed

Fig. 1A–C The graphs show the elution rate of vancomycin from tendons comparing (A) rinsed and unrinsed tendons; (B) tendons wrapped in gauze presoaked in 5-, 2.5-, and 1.25-mg/mL vancomycin solution; and (C) 2.5- x 0.8-cm and 1.0- x 0.8-cm tendons wrapped in gauze presoaked in 5-mg/mL vancomycin solution. All values were obtained from triplicate experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and ### $p < 0.05$ for comparison between 2.5- and 1.25-mg/mL vancomycin solutions.

tendons resulted in 4 ± 0.6 mg vancomycin being detected at the 10-minute sampling interval. In comparison, rinsed tendons eluted only 0.3 ± 0.2 mg vancomycin at the same time ($p < 0.05$, two-tailed t test).

Table 1. Cumulative amount of vancomycin released from tendons at each sampling interval

Tendon dimensions (length x diameter) (cm)	Rinsed/unrinsed	Vancomycin concentration (mg/mL)	Cumulative amount of vancomycin released (mg)*				
			10 minutes	1 hour	6 hours	12 hours	24 hours
2.5 × 0.8	Rinsed	1.25	0.3 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.9 ± 0.3
2.5 × 0.8	Rinsed	2.5	0.9 ± 0.3	1.1 ± 0.1	1.2 ± 0.2	1.4 ± 0.1	1.6 ± 0.1
2.5 × 0.8	Rinsed	5	1.2 ± 0.4	3.4 ± 1.5	4.1 ± 1.2	4.3 ± 1.1	4.5 ± 1.2
1 × 0.8	Rinsed	5	0.8 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.7 ± 0.1	1.7 ± 0.2
2.5 × 0.8	Unrinsed	5	4.0 ± 0.6	5.2 ± 0.6	5.3 ± 0.3	5.8 ± 0.2	6.2 ± 0.1

* Values are expressed as mean ± SD.

The cumulative amount of antibiotic released was shown to increase at all sampling intervals with increasing concentrations of vancomycin soak solution used (Table 1). The amount of vancomycin released at the 24-hour interval was 4.5 ± 1.2 , 1.6 ± 0.1 , and 0.9 ± 0.3 mg when 5-, 2.5-, and 1.25-mg/mL vancomycin soak solutions were used in the protocol, respectively. Calculated elution profiles increased with the increasing concentrations of vancomycin soak solution used (Fig. 1B). With the exception of the 10-minute interval for the 5- and 2.5-mg/mL vancomycin soak solutions ($p > 0.05$), differences in elution profiles were observed throughout the experiment ($p < 0.001$).

The dimensions of the tendon were shown to influence the cumulative amount of vancomycin released (Table 1) and the calculated elution rate (Fig. 1C). The amount of vancomycin measured at the 10-minute interval was more than 1.5-fold higher for the 2.5- × 0.8-cm tendon than for the 1- × 0.8-cm tendon. Amounts were more than 2.5-fold higher at all other sampling intervals tested. Different elution profiles were observed for the two tendon dimensions tested, with higher ($p < 0.01$) elution rates measured for tendons with larger volumes.

The cumulative vancomycin concentrations of 45 ± 12 , 16 ± 1 , and 9 ± 3 µg/mL were measured during the 24-hour period for the 5-, 2.5-, and 1.25-mg/mL vancomycin soak solutions, respectively. These maximum concentrations were less than the accepted toxic concentration of 1000 µg/mL [6].

Discussion

Infection after ACL reconstruction is catastrophic; however, prophylactic graft saturation in vancomycin has been reported to reduce infection rates (Unpublished data. CJ Vertullo; 2009). We investigated the elution of vancomycin from tendons that had been wrapped in gauze presoaked in different concentrations of vancomycin solution and evaluated influencing parameters.

This study has two major limitations. First, animal tendon tissue was used to investigate the various factors influencing

the rate and extent of vancomycin elution. This model may not truly represent the interaction between human collagen tissue and vancomycin. Second, this study is an in vitro model of the in vivo release of antibiotic from a tendon graft into a chamber. It does not characterize the behavior of vancomycin once released into the joint synovial fluid.

Rinsing tendons was shown to reduce the amount of vancomycin detected in the chamber, particularly at the 10-minute interval. This reduction could be attributed to the removal of vancomycin soak solution coating the external surface of the tendon. After rinsing, elution of vancomycin from tendons was still observed. This, together with the detection of increasing amounts of vancomycin in solution throughout the experiment, provides some evidence of the potential distribution of vancomycin into the tendon. Further evidence is provided by the nonlinear elution rates observed for the different concentration soak solutions tested. Increasing the concentration of the soak solution increased the quantity of vancomycin detected in the chamber and calculated elution rates. This effect is evident throughout the experiment and is not confined to the initial 10-minute interval. This would suggest tendons, prepared using the described protocol, are in fact reservoirs for vancomycin release.

The elution rates, however, do decline rapidly after the 1-hour interval, suggesting vancomycin is unlikely to accumulate over longer periods of time as a result of continued elution, even in a static environment. The reduction in elution profile is likely to be attributable to the decreased availability of vancomycin in the tendon to maintain the concentration gradient to favor diffusion into the chamber rather than solute saturation of the chamber solution. The measured concentrations of vancomycin, for all of the conditions tested, were well below the reported solubility of this extremely water-soluble antibiotic [12]. In addition, experiments conducted in chambers with reduced volume (50 mL) showed no differences in vancomycin elution profiles when compared with 100-mL chambers (results not shown).

The dimensions of the tendon were shown to influence the amount of vancomycin released and calculated elution

rates. The amount released and elution rates increased with larger tendons. This suggests tendons of larger dimension provide for a greater reservoir of vancomycin for drug elution. Larger tendons also eluted drug for longer periods of time; however, the differences in amounts of vancomycin released after 1 hour were small.

Despite this difference, vancomycin release during the first 10 minutes and cumulatively during the 24-hour period was lower than the concentration ($> 1000 \mu\text{g/mL}$) shown to be toxic to osteoblasts [6]. Therefore, in addition to decreasing infection rates, vancomycin released from tendons should not affect healing time. However, this needs to be confirmed in vivo.

Using the described protocol, tendons elute vancomycin into the environment in which they are placed. The amount of drug released and elution profiles for vancomycin from tendons are dependent on rinsing, size of tendon, and concentration of vancomycin soak solution used in the protocol. The elution of vancomycin is not sustained for long periods of time and therefore should not pose a risk to osteoclasts and chondrocytes owing to accumulation of the drug locally. Caution should be exercised regarding rinsing the tendon. Vancomycin concentrations were shown to be higher immediately when tendons were not rinsed. Further in vitro and in vivo studies are required to determine local vancomycin concentrations after insertion of tendons prepared using this protocol and to evaluate the clinical benefits and risks of using vancomycin in this procedure.

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