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Biomechanical and microbiological effects of local vancomycin in anterior cruciate ligament (ACL) reconstruction: a porcine tendon model

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Abstract

Introduction Although there is increasing evidence for the successful use of local vancomycin applied by soaked compresses during ACL reconstruction, there are still little data on its microbiological and biomechanical effects. Furthermore, exact dosage of vancomycin with respect to tendon stability and microbiological effectivity is still unknown.

Materials and methods 63 porcine flexor digitorum profundus tendons were harvested under sterile conditions from fresh cadaver legs. After contamination with *Staphylococcus epidermidis* (*S. epidermidis*), tendons were wrapped into sterile compresses moistened with different concentrations of vancomycin for 10 or 20 min. Sterile sodium chloride was used for control. After treatment, tendons were rolled onto blood-agar plates to test for residual bacterial contamination and tested for maximum load and stiffness using a uniaxial testing device with cryo-clamps for tendon fixation. Agar plates were checked after 1 week of culture at 36 °C for signs of bacterial growth.

Results When applying vancomycin for only 10 min, bacterial contamination was found in all dosage groups ranging from 28.6% contamination ($n=2$ of 7 tendons) when using 10 mg/ml up to 85.7% ($n=6$ of 7 tendons) when using 1 mg/ml. Applying vancomycin-soaked compresses for 20 min, bacterial contamination was still found in the groups using 1 mg/ml and 2.5 mg/ml (contamination rate 85.7 and 42.9% respectively). When using 5 mg/ml and 10 mg/ml, no bacterial contamination could be perceived after 7 days of culture. With regard to biomechanical properties, no differences were found regarding maximum load or Young's modulus between groups.

Conclusions This study showed no signs of biomechanical impairment of porcine flexor tendons after the use of vancomycin wraps with concentration ranging from 1 to 10 mg/ml for 10 or 20 min at a time zero testing. Contamination with *S. epidermidis* was cleansed in 100% of tendons when using at least 5 mg/ml of vancomycin for 20 min.

Keywords ACL · Anterior cruciate ligament · Vancomycin · Infection

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Introduction

Surgical site infection (SSI) is a feared but at the same time possibly avoidable complication in all surgical procedures. In anterior cruciate ligament (ACL) reconstruction, the overall rate of deep infection is reported between 0.14 and 1.8% in different studies with coagulase-negative Staphylococcus (*Staphylococcus epidermidis*) being the most common pathogen [1–4]. Although the infection rates are relatively low, the fact that ACL reconstruction is a common procedure with 46 per 100,000 persons/year in Germany results in a relevant amount of SSIs [5, 6]. In most cases of SSI, the graft can be maintained, but the occurrence of SSI after ACL reconstruction includes the

risk of flexion contraction, higher grades of knee joint laxity and osteoarthritic changes in the long term [2, 7, 8]. Additionally, SSI results in revision surgery, prolonged use of antibiotics and prolonged hospitalization for the patient [2, 7, 9].

Avoidance strategies for SSI include several pre- and intraoperative measures such as preoperative antibacterial showers, not shaving patients' legs but clipping the hairs to avoid micro-lesions of the skin, the preoperative administration of a single dose of antibiotics and intraoperative measures such as not touching the skin with the graft or a meticulous surgical technique and short operation times [3, 10]. Other attempts to reduce the rate of SSI during ACL reconstruction included the addition of antibiotics, e.g., gentamicin, to the irrigation fluid [11].

Recently, the use of a vancomycin wrap to cover the graft has been reported to significantly reduce the rate of SSI in ACL reconstruction in retrospective studies [3, 10]. This seems of special interest when using autologous hamstring tendon grafts for the ACL reconstruction as the use of hamstring tendons seems to be associated with an approximately three–eight times higher infection rates when compared to the use of patellar tendon grafts (BTB) [12, 13].

Although clinical evidence suggests using vancomycin wraps in ACL reconstruction, no data on biomechanical effects of the local application of vancomycin as well as data on dosage and time of administration are currently available.

Therefore, the aim of the present study was to evaluate the biomechanical effects of local vancomycin on tendons as well as establish dosage recommendation in a porcine tendon model using *S. epidermidis* for bacterial contamination.

Methods

Tendon preparation

Fresh porcine flexor digitorum profundus tendons were harvested from porcine feet under sterile conditions and used in the following model as they compare to human semitendinosus tendons regarding their biomechanical properties [14]. Porcine feet were obtained from a local butcher as secondary products directly after the animals were slaughtered. Tendons were freed from any residual tissue and cut to a length of 6 cm. Tendons were then weighed to precisely account for even small changes in diameter between tendons and thus allow exact comparison of the biomechanical results. Tendons were then put into a sterile microbiological nutrition solution for 20 min. This solution was cultured for 1 week at 36 °C to check for any residual contamination after the preparation process which might bias the results.

Method of contamination

Tendons were taken out of the nutrition solution and put into a watery solution containing a defined concentration of *S. epidermidis* of 1×10^4 colony forming units (CFU) for 30 min. This concentration was used as it was the lowest concentration which resulted in a reproducible infection. The non-biofilm-forming, vancomycin-sensitive, genome-sequenced strain ATCC® 12228™ was used in all experiments.

Local vancomycin application

After infection, tendons were divided into nine different groups. The treatment groups used different concentrations of vancomycin for either 10 or 20 min starting with 1 mg/ml and increasing the dosage over 2.5, 5 mg/ml up to 10 mg/ml. Sterile physiological sodium chloride solution (NaCl) was used as a control group. In all cases, 10 ml of antibiotic solution or sodium chloride solution was used to soak sterile compresses which were wrapped around the tendons.

After tendons were treated, they were rolled onto blood-agar Petri discs for microbiological cultivation and directly afterwards subjected to biomechanical testing. The blood-agar discs were cultivated at 36 °C for 1 week and then checked for bacterial growth. All experiments were done under laminar air flow using a standard laboratory bench and sterile OR instruments.

Biomechanical testing

Biomechanical testing was performed using the Instron® 5566 machine (Instron, Germany). To ensure exact measurement and to prevent tendon slippage, tendons were fixed to the machine using a cryo-clamp device system (Fig. 1) as described by Domnick et al. [14] (Fig. 1).

Tendons were tested for maximum load (uniaxial, 20 mm/min) with an accuracy of ±0.4% regarding load with the machine being calibrated for loads up to 10 kN and a distance resolution of 0.057 µm according to the manufactures manual. 1.5 cm of tendon was fixed to the cryo-clamp with 3 cm of free tendon remaining in between. Elongation and load were continuously recorded during testing. Data recording and Young's modulus calculation were performed using the Blue Hill® material testing software (Version 2.17, Instron, Germany).

Statistical analysis

Statistical analysis was performed using a logistic regression model for the evaluation of correlation between duration

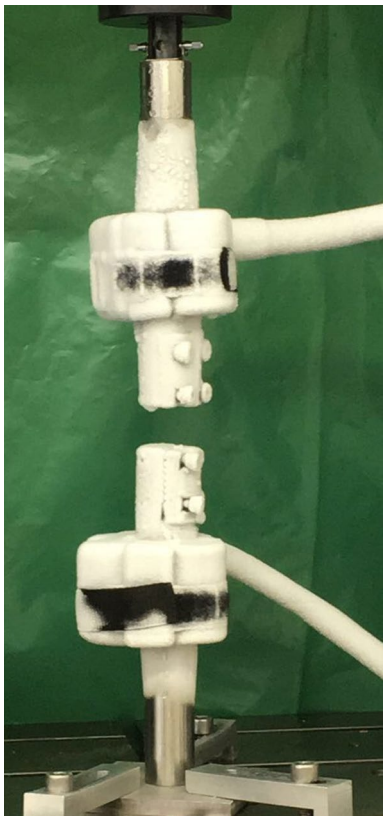

Fig. 1 Experimental setup for the cryo-clamp device to prevent tendon slippage from the clamps

and dosage of vancomycin and residual contamination. For comparison of biomechanical properties one-way ANOVA was used. Significance level was set at $p < 0.05$.

Results

Biomechanical results

Failure mode in all cases was rupture of the tendon. No slippage out of the cryo-clamp system was observed. Mean maximum load adjusted for tendon weight per length (in g/cm) for the control group was 2650 ± 437, 2562 ± 194 N for group 1 (1 mg/ml vancomycin for 10 min) and 2736 ± 409 N for group 8 (10 mg/ml vancomycin for 20 min). Comparisons of maximum load and e-modulus are given in Figs. 2 and 3, respectively.

Statistical analysis of biomechanical properties showed no significant differences regarding maximum load ($F(8,54) = 1.7450$, $p = 0.11$) and Young's modulus ($F(8,54) = 0.6860$, $p = 0.70$). Complete data of each group are given in Table 1.

Microbiological results

With regard to the microbiological effects, positive cultures for *S. epidermidis* were found in all groups except for 5 mg/ml vancomycin applied for 20 min and 10 mg/ml vancomycin applied for 20 min. Complete microbiological data of each group are given in Table 2.

The logistic regression analysis showed statistical significance within the full model ($\chi^2 = 19.086$, $p < 0.001$ with *df* 2). The overall prediction success was 76.8% (84.4% for negative microbiological culture and 66.7% for positive microbiological culture). When regarding the individual variables "duration of application" and "dosage of vancomycin", only the effect of "dosage" was statistically significant ($p = 0.001$) with "duration of application" closely failing statistical significance ($p = 0.068$). The respective odds ratios were 0.660 (95% KI 0.512–0.849) for "dosage" and 0.886 (95% KI 0.778–1.009) for "duration of application".

Discussion

The most important finding of the present study was that local vancomycin applied to porcine flexor tendons via soaked compresses cleansed contamination of *S. epidermidis* in 100% of tendons when concentrations of 5 mg/ml or more were used for 20 min without adverse effects on biomechanical properties of the tendons.

The present study showed a significant effect of dosage on the microbiological results, which in itself is a logical result. But the parameter "duration of application" failed statistical significance. This might be an effect of too small a sample size as represented by the confidence intervals.

Still, the duration of application remains worthy of discussion. The reduced contamination after prolonged application of vancomycin might be biased by the method of contamination used in this model as tendons were soaked in a bacterial solution. In a clinical setting, the method of contamination is more likely a superficial contamination while the graft gets into contact with the patient's skin or is prepared on a back table. Therefore, transference of these results into the clinical setting might seem questionable. But as the tendons were rolled onto agar plates to check for contamination, mainly bacteria remaining on the tendon surface would get detected. The duration of application should also be considered from an additional point of view. After application of local vancomycin, tendons act as a reservoir for the antibiotic and the drug is washed out of the tendons over time [15]. In summary, we recommend to apply the vancomycin as early and for as long as possible but without delaying surgery despite the missing statistical significance within this regression analysis.

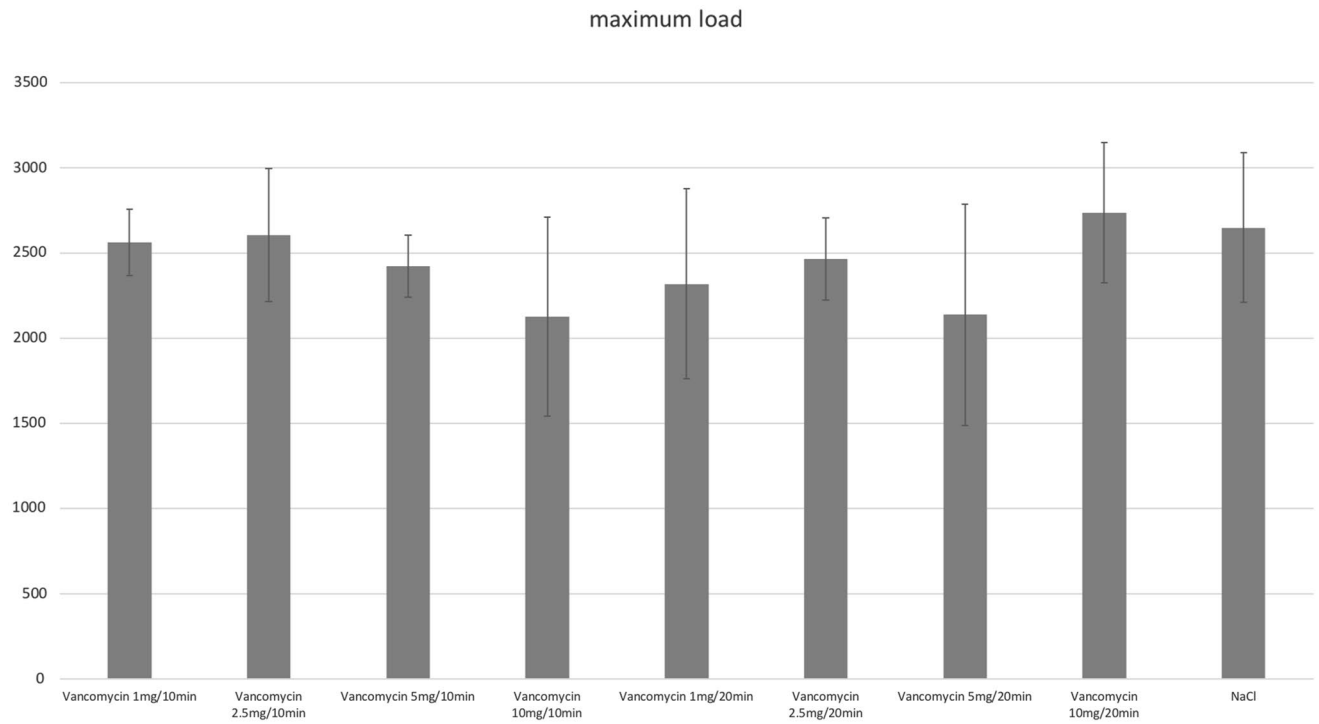

Fig. 2 Comparison of maximum load (N) adjusted for tendon weight to length ratio (g/cm). Data given as mean ± standard deviation. No significant differences were found between groups

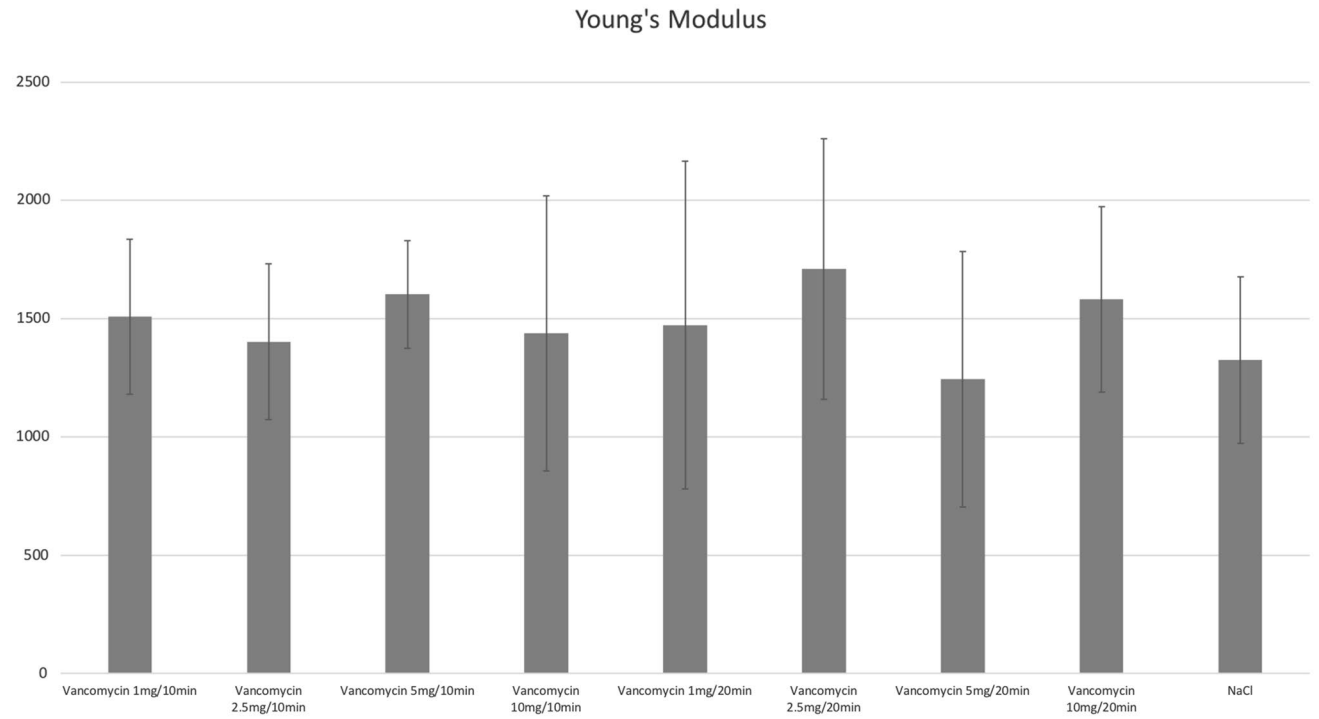

Fig. 3 Comparison of Young's modulus (MPa) adjusted for tendon weight to length ratio (g/cm). Data given as mean ± standard deviation. No significant differences were found between groups

Table 1 Biomechanical data

Group	*n*	Max. load (N)	Young's modulus (MPa)
(1) 1 mg/ml for 10 min	7	2562 ± 195	1508 ± 328
(2) 2.5 mg/ml for 10 min	7	2606 ± 391	1402 ± 328
(3) 5 mg/ml for 10 min	7	2423 ± 182	1601 ± 227
(4) 10 mg/ml for 10 min	7	2126 ± 584	1438 ± 581
(5) 1 mg/ml for 20 min	7	2319 ± 556	1472 ± 692
(6) 2.5 mg/ml for 20 min	7	2467 ± 240	1710 ± 549
(7) 5 mg/ml for 20 min	7	2139 ± 649	1244 ± 539
(8) 10 mg/ml for 20 min	7	2737 ± 409	1581 ± 391
(9) NaCl—control	7	2650 ± 437	1324 ± 351

Data given as mean ± standard deviation. Data were adjusted for tendon length to weight ratio (g/cm)

Table 2 Microbiological data

Group	*n*	*n* pos. culture	Percentage
(1) 1 mg/ml for 10 min	7	6	85.7
(2) 2.5 mg/ml for 10 min	7	4	57.1
(3) 5 mg/ml for 10 min	7	3	42.9
(4) 10 mg/ml for 10 min	7	2	28.6
(5) 1 mg/ml for 20 min	7	6	85.7
(6) 2.5 mg/ml for 20 min	7	3	42.9
(7) 5 mg/ml for 20 min	7	0	0
(8) 10 mg/ml for 20 min	7	0	0
(9) NaCl—control	7	7	100

Although the biomechanical testing revealed no significant differences with respect to maximum load or Young's modulus in this model, other possible harms of the local use of vancomycin need to be discussed. Eriksson and Karlsson [16] have recently published an article comprising the possible harms and benefits of local vancomycin in ACL surgery. Two of the main concerns were possible toxicity of vancomycin and potential development of vancomycin-resistant bacteria. With respect to local toxicity, several in vitro studies using different cell lines showed a high margin of safety for vancomycin. In an in vitro model using chondrocyte and evaluating different antibiotics, no toxic effects on chondrocytes by vancomycin could be perceived when using vancomycin in a relatively low dosage of 16 µg/ml [17]. In a similar in vitro study using an osteosarcoma cell line, doses of 1000 µg/ml had little to no effect on cell viability, while doses of 10,000 µg/ml caused toxic reactions [18]. But as osteosarcoma cells are possibly more resistant than "normal" cells, these high margins are questionable. Another study evaluating similar dosages but using chondrocytes and osteoblasts showed a toxic threshold for vancomycin of 2000 µg/ml [19].

To estimate the local vancomycin concentrations in vivo, Grayson et al. [15] created an elution model using hamstring tendons soaked with vancomycin. These experiments showed that a standard hamstring graft of 2.5 cm intraarticular length and 0.8 cm diameter soaked with 5 mg/ml vancomycin would result in 45 µg/ml to 62 µg/ml estimating a joint volume of 100 ml [15]. These studies suggest a high margin of safety when using vancomycin as a local antibiotic drug.

The present study can give no insight into the potential risk of resistance development against vancomycin after local application. But the use of local vancomycin is not a completely new idea, as it has been in use in other disciplines such as cardiovascular/thoracic or spine surgery for some time. Currently, no data on resistance development in these disciplines are available, but resistance development as a general future risk must be taken into consideration [16]. But, on the other hand, resistance development is also a possible risk in the setting of SSI and the need of prolonged use of antibiotics to cure the infection.

The present study has some limitations. As stated above, the method of contamination in the presented model is most likely different from the way contamination of tendons occurs in the OR, which might bias our results. But prospective in vivo studies evaluating vancomycin dosage or duration of application are not feasible due to the overall low infection rates in ACL surgery. Therefore, using an in vitro model despite such limitations seems appropriate. Additionally, the sample size used is probably too small to provide recommendations on the duration of vancomycin application. Another limitation is the fact, that this model only accounts for an acute mode of tendon failure, as this model cannot give insight into possible adverse effects regarding tendon remodeling, vascularization or tendon–bone integration in vivo.

Conclusion

This study showed no signs of biomechanical impairment of porcine flexor tendons after the use of vancomycin wraps with concentration ranging from 1 to 10 mg/ml for 10 or 20 min at a time zero testing. As expected, lower infection rates were seen in groups using higher concentrations of vancomycin and with prolonged application. Although the parameter "duration of application" failed statistical significance, we still recommend early application of vancomycin for as long as possible but without delaying the surgical procedure, as contamination with *S. epidermidis* was cleansed in 100% of tendons when using at least 5 mg/ml of vancomycin for 20 min.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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