

In-vitro controlled mechanical testing of tendons from living donors

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Original article

Ropivacaine alters the mechanical properties of hamstring tendons: In vitro controlled mechanical testing of tendons from living donors



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ABSTRACT

Objective: Intraarticular or periarticular injection of ropivacaine (RI) is an element of current knee surgery practices. The goal of this study was to determine the effects of RI on the mechanical properties of hamstring tendons. We hypothesized that RI would have a detrimental effect on the mechanical properties of periarticular soft tissues

Methods: A tensile test to failure was performed on 120 hamstring tendon segments harvested during ACL reconstruction surgery in 120 patients. Two sets of tensile tests were done. The first evaluated the effect of RI itself on the mechanical properties of tendons: 30 samples were soaked for 1 hour in a 2% RI solution and compared to 30 samples soaked in a saline solution (control group). The second evaluated the effect of RI concentration on the mechanical properties of hamstring tendons: 30 samples were soaked for 1 hour in a 2% RI solution and 30 samples were soaked in a 7.5% RI solution.

Results: In the first test, 29 samples from each group were analyzed as two samples (one in each group) failed at the grip interface. The specimens exposed to 2% RI had lower ultimate tensile strength ($\Delta = 4.4$ MPa, $P = 0.001$), strain energy ($\Delta = 13$ MPa, $P = 0.001$) and Young's modulus ($\Delta = 1.6$ MPa, $P = 0.02$) than the specimens in the control group. There was no significant difference in the strain at failure between groups ($\Delta = 5\%$, $P = 0.3$). In the second test, one specimen from the 7.5% RI group failed during the preloading and was excluded. There was no significant difference in terms of the load at failure and ultimate tensile stress ($\Delta = 0.45$ MPa, $P = 0.6$) and strain energy ($\Delta = 0.49$ MPa, $P = 0.49$) between the two groups. There were significant differences in terms of elongation at failure ($\Delta = 28\%$, $P = 0.0003$) and Young's modulus ($\Delta = 2.6$ MPa, $P = 0.005$), with the specimens exposed to 7.5% RI undergoing greater deformation and having a lower Young's modulus.

Discussion: While local RI injections are widely performed in clinical practice, the results of this in vitro study point to short-term alterations of the mechanical properties of hamstring tendons. If these results hold in vivo, this could lead to weakness of the soft tissues exposed to this product, particularly the tendons and ligaments around the injection area.

Level of evidence: Experimental study. Level 1

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1. Introduction

Postoperative pain after joint surgery, whether performed by arthroscopy or as an open procedure, is a major concern for patients and surgeons [1–3]. While there are no studies specifically recommending intraarticular injection of local anesthetics, ropivacaine (RI) is often injected inside or around the joint, or administered by the perineural route to manage pain [3–5]. RI is an amide local

anesthetic (LA). Recent publications have described negative effects on chondrocytes following in vitro joint injection [6–9]. Studies have also shown toxic effects on the mesenchymal and fibroblastic tissues involved in healing and postoperative recovery [10–13]. Haasters et al. [11] showed that RI has a harmful effect on tendon cells, with an elevated apoptosis rate after 6 hours of incubation [11,12].

We hypothesized that RI would have a detrimental effect on the mechanical properties of periarticular soft tissues. We had a dual primary objective. First, to assess the toxicity of RI by comparing the mechanical properties of fresh human hamstring tendons soaked for 1 hour in a 2% RI solution to tendons soaked in a saline solution. Second, to assess the RI concentration effect by comparing

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Table 1
Demographics of the patients in the various groups.

Demographic data	Control group (n = 30)	Ropivacaine 2% A (n = 30)	P	Ropivacaine 2% B (n = 30)	Ropivacaine 7.5% (n = 30)	P
Age (years)	30 ± 8 (18–44)	29 ± 7 (18–42)	0.7	31 ± 8 (18–43)	29 ± 7 (18–42)	0.7
Gender (F/M)	43/17	43/17	1	41/19	41/19	1
Sports level (UCLA)	9 ± 1 (8–10)	9 ± 1 (8–10)	1	9 ± 1 (8–10)	9 ± 1 (8–10)	1
Professional athletes	2	2	NA	2	2	NA
Specimens (mm)	8 ± 5 (6–12)	7 ± 4 (5–10)	0.5	8 ± 4 (5–11)	8 ± 5 (5–12)	0.6

Data are expressed as mean ± standard deviation (minimum–maximum); P was significant when <0.005.

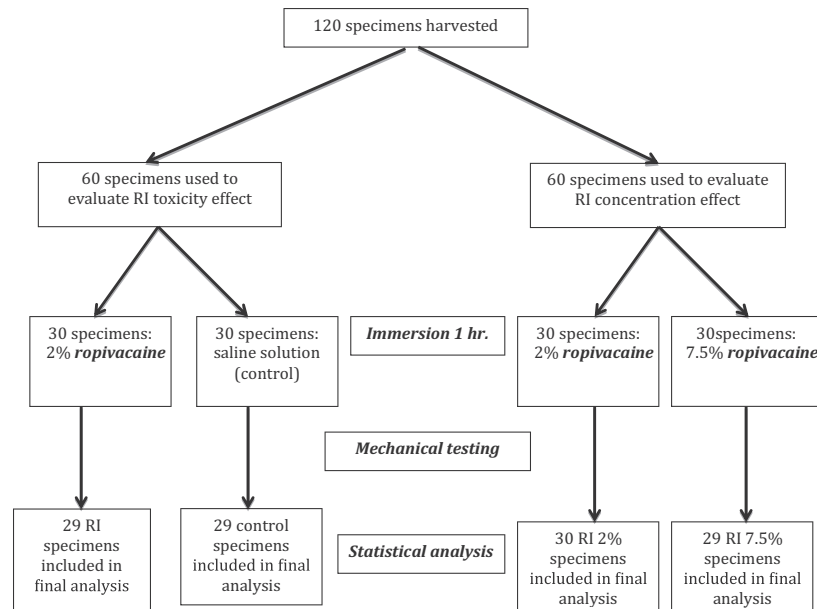


Fig. 1. Flow chart for the study procedures.

the mechanical properties of fresh human tendons soaked in a 2% versus a 7.5% RI solution.

2. Methods

Biomechanical testing was carried out with 120 hamstring tendon segments harvested during ACL reconstruction surgery in 120 patients. The mean patient age was 29.7 ± 8.8 years; 71% were men and 29% were women (Table 1).

Digital calipers (Absolute Digimatic[®], MitutoyoTM, Kanagawa, Japan) with an accuracy of $U \pm 0.03$ ($k = 2$ mm) were used to create identical, 3 cm long samples; the diameter was based on the width of the proximal gracilis tendon. Two sets of tensile tests were done (Fig. 1). The first evaluated the effect of RI on the mechanical properties of tendons: 30 samples were soaked for 1 hour in a 2% RI solution and compared to 30 samples soaked in a saline solution (the RI diluent). The 1 hour soaking period corresponded to the “toxic period” of specimens after ACL surgery, during which more than 50% of the product is active [14]. The half-life of intraarticular RI has not been reported, however the intravenous half-life is 1 to 2 hours. The second set of tests evaluated the effect of RI concentration on the mechanical properties of hamstring tendons: 30 samples were soaked for 1 hour in a 2% RI solution and 30 samples were soaked in a 7.5% RI solution.

All specimens were carefully dried after the soaking step and frozen at -20°C until the mechanical testing, according to a previously validated protocol [15]. To minimize bias, the specimens were assigned to each group after being stratified on patient age (± 3 years), gender and sports level (UCLA score). All patients were high-level athletes (UCLA score > 8); the eight specimens from professional athletes were separated into two per group. All patients

provided written consent, and the study was approved by our hospital’s ethics committee (No. 2012-015724-11; 26 January 2013).

2.1. Mechanical testing

Mechanical testing was performed on a universal testing machine (Instron 5566-A, Instron[®], Norwood MA, USA) (Fig. 2). After the specimens were thawed for 1 hour, the ends of each tendon were placed in two grips (1 cm from each end). The mechanical properties were determined on the tissue between the two grips (1 cm) using a previously validated method [16]. Tendons were preconditioned with 10 cycles of 1 mm extension. The interface of each tendon/grip was marked with China ink to monitor potential tendon sliding. All tendons were subjected to a tensile test to failure [17]. The instance and method of failure were recorded for each specimen. The maximum load and stiffness were determined from the load–elongation curves using Bluehill 3 acquisition software (Instron[®], Norwood, MA, USA). The ultimate tensile strength (UTS) was calculated by assuming the specimens had a cylindrical shape [18]. The following parameters were calculated: UTS, elongation at failure, Young’s modulus and strain energy.

A specimen was excluded from the final analysis if it failed at the grip interface or inside the grip, slipped during the test, or failed during preconditioning or before 150 N of tensile load had been applied.

2.2. Statistical analysis

The Kolmogoroff-Smirnoff test was used to determine the normality of the data distribution. Parametric tests were used to compare normally distributed variables (demographic data and

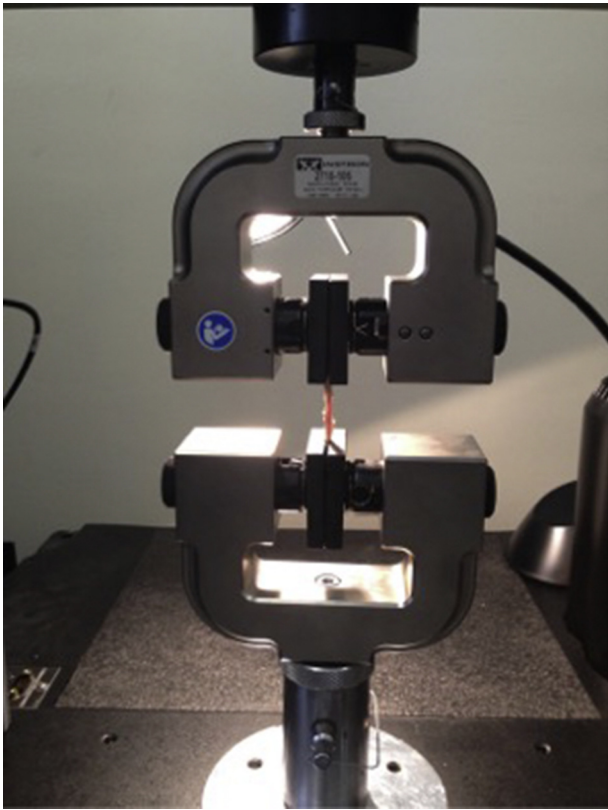


Fig. 2. Materials testing system used for the tensile load to failure tests.

mechanical properties) between the groups. Non-parametric tests were used to compare the elongation at failure, since this data was not normally distributed. Two-tailed tests were carried out using PASW Statistics version 20 (SPSS, IBM Inc., Chicago, Illinois). The significance threshold was set at $P < 0.05$. Since we could not find any published data on the mechanical properties of “fresh” human hamstring tendons, sample size calculations were based on the test results of the first 20 specimens. These calculations showed that at least 28 tendons were needed in each group to show a clinically significant 70 N difference in the failure load between the groups ($\alpha = 0.05$; $\beta = 0.8$ SD for failure load of 80 N). Consequently, we included 30 patients per group, which allowed 2 specimens to be excluded if necessary.

3. Results

3.1. RI versus control

In the first series, 29 samples from each group were analyzed as two samples (one in each group) failed at the grip interface. The specimens exposed to 2% RI had lower ultimate tensile strength ($\Delta = 4.4$ MPa, $P = 0.001$), strain energy ($\Delta = 13$ MPa, $P = 0.001$) and Young's modulus ($\Delta = 1.6$ MPa, $P = 0.02$) than the specimens in the control group (Table 2). There was no significant difference in the elongation at failure between groups ($\Delta = 5\%$, $P = 0.3$).

3.2. RI concentration

In the second series, one specimen from the 7.5% RI group failed during the preloading and was excluded. There was no significant difference in terms of ultimate tensile stress ($\Delta = 0.45$ MPa, $P = 0.6$) and strain energy ($\Delta = 0.49$ MPa, $P = 0.49$) between the two groups. There were significant differences in terms of elongation at failure ($\Delta = 28\%$, $P = 0.0003$) and Young's modulus ($\Delta = 2.6$ MPa, $P = 0.005$),

with the specimens exposed to 7.5% RI undergoing greater deformation and having a lower Young's modulus.

4. Discussion

The main finding of this study is that ropivacaine alters the mechanical properties of human hamstring tendons in vitro. Intra- or periarticular injection of a local anesthetic is regularly performed to improve postoperative analgesia after knee surgery. Its therapeutic benefits for controlling pain have been clearly demonstrated [2,3]. However, several warnings have been issued related to the cytotoxic effects of local anesthetics on chondrocytes, mesenchymal cells and fibroblasts [6–13].

Our objective was to determine whether RI, which is known to be cytotoxic, affects the macroscopic properties of soft tissues. Our hypothesis was confirmed—RI affects the mechanical properties of human hamstring tendons. Although RI did not affect tendon elongation relative to a saline solution. In comparison to published results describing the load at failure of tendons harvested from cadavers [5,19], our values appear low; however, our results correspond to the mechanical properties of isolated tendons tested in a similar configuration (3 cm length) without reinforcement (i.e., suture). In addition, we used fresh tendons from living donors, while most tests were done with animal or cadaver specimens [17–20]. Mechanical properties of ligaments and tendons are related to various morphological parameters [19]. Potential confounding external factors in our study were controlled by pairing our patients based on activity level (UCLA score, professional athletes), sex and age (± 3 years) before the groups were created.

Our results on the effects of RI on the mechanical properties of tendon tissue tend to support in vitro findings of cell toxicity [5,7,8,11,13]. Piper and Kim [8] were the first to describe the toxic effects of local anesthetics (bupivacaine) on human chondrocytes in vitro; however, RI did not increase cell apoptosis in comparison to the control saline solution. Grishko et al. [7] published the opposite results – they showed that lidocaine, bupivacaine, and ropivacaine led to mitochondrial dysfunction and apoptosis of human chondrocytes in vitro. Fedder et al. [12] explored the effect of local anesthetics on human fibroblasts; they concluded that continuous intraarticular injection of lidocaine, bupivacaine and ropivacaine leads to severe cell damage. An in vitro study on the effect of local anesthetics on stem cells from hamstring tendons showed that bupivacaine (0.5%) and ropivacaine (0.75%) have considerable cytotoxic effects on human tendon progenitor/cell stems [11]. The authors concluded the viability of progenitor cells harvested from in vitro ligament reconstruction grafts was compromised when using bupivacaine and ropivacaine. Recently, Lehner et al. [20] evaluated the effect of bupivacaine on mechanical properties of rat Achilles tendon. They found that bupivacaine had a dose-dependent and time-dependent negative effect on the viability of tendon cells in vitro. A single in vivo peritendinous injection of bupivacaine can trigger apoptosis of endotenon cells, increase metalloprotease activity and alter the quality of tendon collagen after only 6 hours [20]. These results can help to explain our study's findings. The collagen and extracellular matrix that gives structure to soft tissues [21] immediately loses 17% of the normal tendon architecture when bupivacaine is administered [20].

Our second objective was to evaluate the potential effect of various ropivacaine concentrations on the mechanical properties of hamstring grafts. There was no difference between 7.5% and 2% RI on the load at failure and UTS. However, the strain energy and elongation at failure were greater in the specimens exposed to 7.5% ropivacaine. This concentration effect was known on a cellular level, as Fedder et al. [12] found no difference in fibroblast viability when exposed to three different ropivacaine concentrations

Table 2
Mechanical properties of specimens in the various groups.

Mechanical properties (at failure)	Control group (n=30)	Ropivacaine 2% A (n=30)	P	Ropivacaine 2% B (n=30)	Ropivacaine 7.5% (n=30)	P
Ultimate tensile strength (MPa)	13 ± 7.1	8.2 ± 3.8	0.001	8.3 ± 4	8.8 ± 3.7	0.6
Strain (%)	77 ± 31	82 ± 29	0.367	69 ± 17	97 ± 32	0.0003
Energy (MPa)	30 ± 24	16.5 ± 9.5	0.001	17.4 ± 9.8	15.5 ± 9.6	0.49
Young's modulus (MPa)	5.9 ± 3.7	4.3 ± 2.9	0.019	4.2 ± 2	3.24 ± 3.3	0.04

Data are expressed as mean ± standard deviation; P was significant when <0.005.

(0.31, 0.62 and 1.25 mg/mL). This effect is also present in hamstring tendon cells, since Haaster et al. [11] concluded that 0.5% RI led to moderate loss of viable cells, while 0.75% RI triggered significant apoptosis and reduction in metabolism of the remaining cells. Lehner and al. [20] also described a concentration-dependent effect and time-dependent effect of bupivacaine, another amide local anesthetic.

Our study has several limitations. First, an in vitro preparation does not reproduce intraarticular or periarticular conditions. The tendons were submerged directly in the RI solution or the control saline solution at concentrations equal to standard intraarticular or periarticular administration. Since the half-life of RI topical injection is estimated at 1 to 2 hours [14–22], we limited the tendon immersion time to 1 hour. Another limitation is that the impact of blood or synovial fluid was not considered; this may have caused us to overestimate the cytotoxic effect. Ropivacaine was used in this study because this amide local anesthetic is often used in current surgical practices by anesthesiologists and surgeons. Also, RI is said to be the least toxic of all the amide-type local anesthetics [8,11,13]. Lastly, the mechanical test used in this study has never been described for human tendons, but was validated on colon specimens by Massalou et al. [16]. To prevent bias during the analysis, all tests were done by a trained engineer following a specified protocol. The testing on more than 100 fresh human tendon samples harvested using strict inclusion/exclusion criteria was blinded and controlled.

This study demonstrates only the immediate effect of RI on hamstring tendons. Given that we did an in vitro analysis on non-vascularized specimens, we could not analyze short-term remodeling in the tissue and potential adaptation of the collagen and extracellular matrix to RI exposure. This information is needed to understand the relationship between cell toxicity and the mechanical effects of RI. We can only presume that RI deeply modifies the micro-architecture of tendon tissue, thereby altering its mechanical properties. It is essential to determine how these substances penetrate and alter the graft tissue and change its configuration [20].

It is also important to note that our specimens (ex vivo proximal gracilis tendon) only provide us with an approximation of the general effect of ropivacaine on other tendons. We are currently doing a histological and mechanical study of other tissues.

Despite these limitations, our study shows the potential harmful effects of RI administration on the mechanical properties of tendons. Our findings must be confirmed by histological and clinical studies to understand the cause of the harmful mechanical effects of RI on periarticular tissues, but also to analyze the in vivo consequences of these tissue modifications.

Disclosure of interest

Jean-Noël Argenson is an educational consultant for Zimmer/Biomet.

Sébastien Parratte is an educational consultant for Newclip, Zimmer/Biomet and Arthrex.

Matthieu Ollivier, Jaafar Sbihi, Abderrahmane Sbihi and Martine Pithieux declare that they have no competing interest.

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