Original Article

Dexamethasone Does not Compensate for Local Anesthetic Cytotoxic Effects on Tenocytes: Morphine or Morphine Plus Dexamethasone May Be a Safe Alternative

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Purpose: The purposes of this in vitro study were to investigate whether the addition of dexamethasone can compensate for any cytotoxic effects of the amide-type local anesthetics (LA) bupivacaine and ropivacaine and whether morphine and morphine-6-glucuronide (M6G) may be a safe alternative for peritendinous application. **Methods:** Biopsies of human biceps tendons ($n = 6$) were dissected and cultivated. Cells were characterized by the expression for tenocyte markers, collagen I, biglycan, tenascin C, scleraxis, and RUNX via reverse transcriptase-polymerase chain reaction and immunohistochemistry. Tenocytes were incubated with bupivacaine, ropivacaine, morphine, M6G, or a saline control with and without addition of dexamethasone for 15, 60, or 240 min. Cell viability was determined by quantifying the presence of adenosine-triphosphate. **Results:** Significant time-dependent cytotoxic effects were observed for LA after all exposure times. After 15, 60, and 240 minutes, cell viability decreased to 81.1%, 49.4% and 0% ($P < .001$) for bupivacaine and to 81.4%, 69.6%, and 9.3% ($P < .001$) for ropivacaine compared to saline control. Dexamethasone did not compensate for these cytotoxic effects. Cell viability was not affected after 15, 60-min exposures to morphine and M6G but decreased significantly ($P < .001$) after 240 minutes compared to saline control. However, in combination with dexamethasone, tenocyte viability was significantly increased at all times for morphine ($P < .01$) and at 15 and 60 minutes for M6G ($P < .01$). **Conclusions:** The results showed that amide-type LA have a time-dependent cytotoxic effect on human tenocytes in vitro, which could not be compensated for by dexamethasone, whereas morphine and M6G had no cytotoxic effects on tenocytes after 15 and 60 minutes. The addition of dexamethasone to morphine and M6G had a positive effect on viability, which increased significantly compared to the opioids. **Clinical Relevance:** It is known that amide-type local anesthetics used for local joint analgesia have chondrotoxic side-effects. The combined application of morphine and dexamethasone may be a safe alternative.

Introduction

Intraarticular, periarticular, and peritendinous injections with amide-type local anesthetics (LA) and/or corticosteroids are performed for analgesia and to inhibit inflammation in patients with substantial joint pain (postoperative, degenerative, and inflammatory diseases). Although analgesia is required for early rehabilitation and prevention of joint stiffness, studies have proven that amide-type local anesthetics have chondrotoxic side effects, whose intensity depends on the actual active substance, time of exposure, and concentration.[1-9] Currently, intraarticular injection is only performed under strict indication and is becoming less common, whereas postoperative intra-articular infusion of a local anesthetic via a pain pump has been abandoned because of devastating cases of

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glenohumeral chondrolysis.[10-17] Recent research has confirmed a similar cytotoxic effect of LA on tenocytes in vitro.[6,18-21,22] In vivo studies evaluating the cytotoxic profile of LA in tendons are limited and present partially conflicting results after periarticularly injected LA. Whereas Friel et al. found no effects on rotator cuff tendons in rabbits after continuous subacromial bupivacaine infusion (.25 % with epinephrine for 48 h) after 2 weeks , Lehner et al. demonstrated that a single injection of .5% bupivacaine caused short-term changes in rat Achilles tendons.[23,24] Similarly, Nuelle et al. noted significant tenotoxicity of the supraspinatus tendon after a single injection of a combination of bupivacaine in low concentration (.06%) and corticosteroids into the subacromial space in adult dogs at day 7.[25]

Corticosteroids still play a major role in the management of all kinds of inflammatory disease, especially in the musculoskeletal system.[26-29] In multiple clinical trials, the addition of corticosteroids extended the duration of the analgesic effect of LA in regional blocks (brachial plexus).[30-34] Corticosteroid adjuvants in periarticular analgesic injections are of interest; in fact, several clinical studies have reported a slight reduction in postoperative pain or prolongation of analgesic effect with the addition of corticosteroids to periarticular and intra-articular LA.[35-42]

Alternatives to LA with fewer side-effects are urgently needed. The mode of action of opioid drugs differs from that of amide-type local anesthetics and may be a safe and potent alternative for particular application. Morphine has been proven to provide sufficient analgesic effect in periarticular application [43,44] and intraarticular application.[45-50] Experimental trials have shown that morphine is significantly less chondrotoxic[1,51,52] and tendotoxic[20] compared to LA.

Morphine-6-glucuronide, an active metabolite of morphine, is an effective analgesic with a slower onset but and a longer analgesic effect/duration of action compared to morphine when administered intravenously or subcutaneously. Side effects, most importantly postoperative nausea and vomiting, occur less frequent after M6G treatment.[53] Studies suggest that the metabolite M6G instead of morphine itself is the major contributor of analgesic effect via μ-opioid receptors after administration of morphine to patients, irrespective of the route of administration.[53,54]

In the present study, we investigated whether the addition of dexamethasone can compensate for any cytotoxic effects of the amide-type LA (bupivacaine and ropivacaine) and whether opioids (morphine and morphine-6-glucuronide) may be a safe alternative for peritendinous application.[1]

Our hypothesis is, that morphine and M6G do not reduce the viability of the primary human tenocytes, in contrast to the LA bupivacaine and ropivacaine and that the addition dexamethasone can compensate for the cytotoxicity caused by the LA.

Materials and Methods

Study Population

The study was approved by the local Ethics Committee of the Heinrich-Heine-University Düsseldorf (#3506). Informed consent was gathered prior to the study initiation from the patient, caregiver, or legal representative. The tendon samples were obtained from patients without preexisting illnesses (i.e., metabolic syndrome, diabetes, coronary heart diseases, acute or chronic infections, and cancer), who were scheduled to undergo arthroscopic surgery for rotator cuff repair or open shoulder surgery, such as hemiarthroplasty following humeral head fractures. Exclusion criteria were the presence of substantial degenerative tendon changes, infections, or previous surgery at the site of biopsy. Proximal explants of human long biceps tendons were obtained from 6 patients in total (2 male and 4 female), with an average age of 48.5 ± 18.9 years (range: 24-75 years). A case number estimation for unrelated samples and continuous targets was performed using the program "jumbo" from the University of Münster, Münster, Germany.[55] For the power analysis, we used our previous study as a guide, in which the effect of LA and morphine on the viability of chondrocytes was tested.[1] The case number estimation resulted in a maximal case number of 5; we decided to take $n = 6$.

Tenocyte Harvesting and Culture

Visually intact proximal explants of the long biceps tendons were washed 3 times in sterile PBS (Gibco, Deisenhofen, Germany). Tenocytes were harvested by cell migration, as previously described.[56,57] The tendons were dissected into 30-60 mm^3 fragments and cultivated in culture flasks using Dulbecco's Modified Eagle Medium (DMEM; Gibco) with 4.5 g/L glucose, 20% fetal bovine serum (Gibco), 100 units/mL penicillin, 100 μg/mL streptomycin and 2 mM glutamine (Gibco) culture conditions analogous to chondrocytes, as previously described.[1] The culture conditions were 8.5% CO_2 at 37°C. No further growth or differentiation factors were added. The medium was changed twice per week. Tenocytes continuously migrated from the tendon fragments and adhered to the culture flask. Immediately before the cells displayed a confluent monolayer (defined as passage zero, or P0), they were trypsinized (.05% trypsin/.02% ethylenediaminetetraacetic acid (EDTA) (Gibco) counted and either cryopreserved or subcultured directly into passage one (P1). Cryopreservation of cells (P0) was performed in 90% FCS + 10% DMSO (Gibco) at −80°C in 5 of 6 patients. For the experiments, the cells were thawed

and subcultured as P1. The cells of all 6 patients were subsequently subcultured into passage two (P2), which was used for the experiments.

Cell Characterization

The tenocytes of P2 of all 6 patients were characterized by their expression via RT-PCR and protein profiles via immunocytochemisty for five typical tenocyte markers, collagen I, biglycan, tenascin C, scleraxis, and RUNX. GAPDH was used as a housekeeping gene.

Reverse Transcriptase Polymerase Chain Reaction

In order to control the tenocyte genotype, RNA was extracted from 6 wells ($\sim 7.4 \times 10^4$ tenocytes in total) using the RNeasy mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. RNA concentration was measured by a photometer (NanoDrop, Peqlab, Erlangen, Germany). For PCR, the One-Step RT-PCR Kit (Qiagen) was used in a thermocycler (Allignet Technologies, Ratingen, Germany), according to the manufacturer's instructions. The PCR program consisted of the reverse transcription at 50°C for 30 minutes, the initial PCR activation at 95°C for 15 minutes, 35 times (35×) the following three-step-cycle: 1) denaturation at 94°C for 30 seconds, 2) annealing at 55°C for 30 seconds, and 3) extension at 72°C for 60 seconds with a final extension at 72°C for 10 minutes. PCR products were separated via agarose gel electrophoresis. The primers for Aggrecan, Biglycan, Decorin, Collagen I and Tenascin C were used as described before.[58] GAPDH (5′-ctc aag atc agc aat gcc, 3′-gat ggt aca tga caa ggt gc) was used as housekeeping gene.

Immunocytochemical Staining

In order to control the tenocyte phenotype, the cells were immunocytochemically stained for tenocyte markers. The cells of the second passage were seeded onto 24-well plates in a cell density of 3×10^3 cells/cm^2 and cultured for 4 days. After fixation with 4% buffered paraformaldehyde (Rotifix, Carl Roth, Germany), endogenous peroxidase was blocked with .3% hydrogen peroxide for 30 minutes. After washing, the fixed cells were incubated with the primary monoclonal or polyclonal antibodies at 5°C. The monoclonal antibodies were collagen I, (1:100 dilution, AbDSerotec, Puchheim, Germany), biglycan (1:200 dilution, Abcam, Cambridge United Kingdom), and tenascin C (1:100 dilution, Acris Antibodies, Hiddenhausen, Germany). The polyclonal antibodies were Runx 2 (1:500 dilution, rat-anti-human; R&D Systems, Minneapolis, MN) and scleraxis (1:100 dilution, rabbit-anti-human; Acris Antibodies). The respective negative controls were incubated in comparable concentrations of either mouse IgG1, IgG2a (Vector Laboratories, Burlington, CA), polyclonal rat or rabbit serum in antibody diluent (Dako, Agilent, Santa Clara, CA) at 5°C. After washing, a secondary anti-mouse or anti-rat biotin-labeled antibody (Vector Laboratories) was added for 60 minutes at RT. The antibody-antigen complex was visualized using streptavidin peroxidase (Vector Laboratories) and diaminobenzidine (Sigma-Aldrich, Steinheim, Germany) as chromogen.

Experimental Setup

Tenocytes of $n =$ six donors (P2) were seeded onto 24-well plates (Nunc, Darmstadt, Germany) in a concentration of 3×10^3 cells/cm^2 using the DMEM, as described before. At day 4, the cells were incubated with the substances, and finally, they were fixed for immunocytochemistry or conserved for PCR. The cell supernatant was removed for incubation, and the cells were exposed to the following substances for increasing incubation times (15, 60, and 240 minutes) (Table 1). The cells were incubated with the anesthetics bupivacaine (5 mg/mL; Bucain-Actavis, Actavis, Langenfeld, Germany), ropivacaine (7.5 mg/mL; Naropin, AstraZeneca, Wedel, Germany), morphine (10 mg/mL, diluted with saline; Merck Serono Darmstadt, Germany), morphine-6-glucuronide (M6G; 5 mg/mL; diluted with saline; Sigma-Aldrich, Deisenhofen, Germany) or saline as a control. Furthermore, dexamethasone (2.5 mg/mL; Lipotalon, Merckle Recordati, Ulm, Germany) was also added to the cells. Combinations of dexamethasone and local anesthetic were diluted 1:10 (dexamethasone: local anesthetic) to mimic clinical practice.[6] Dexamethasone alone was diluted with saline. These concentrations of the LA have been used in previous and comparable in vitro studies with chondrocytes[1,7,52,59] and tenocytes[18-20,60], as well as intra-articular and periarticular[38,45,61,62] in clinical studies. Furthermore, these are concentrations that can be used for anesthesiologic field blocks with LA in surgery.[63,64]

Table 1. Incubation Scheme and the Concentrations Used

	Without dexamethasone			with dexamethasone 0.23 mg/ml = 0.023 %		
Incubation time final concentration	15 min	60 min	240 min	15 min	60 min	240 min
Bupivacaine 5 mg/ml = 0.5 %	X	X	X	X	X	X
Ropivacaine 7.5 mg/ml = 0.75 %	X	X	X	X	X	X
Morphine 0.5 mg/ml = 0.05 %	X	X	X	X	X	X
Morphine-6 glucuronide 0.5 mg/ml = 0.05 %	X	X	X	X	X	X
control saline NaCl 9 mg/ml = 0.09 %	X	X	X	X	X	X

For each concentration, time point, and patient, $n = 6$ wells were used. After incubation with the anesthetics, the cells were washed with PBS and cultured further in a fresh medium without additives. Three days after incubation, cell viability was measured in $n = 6$ wells per substance and time point for each of $n = 6$ donors (= 36 wells in total per condition) (Table 1).

To exclude any side effects, the final anesthetic and dexamethasone solutions in culture medium were controlled for osmolarity (Osmometer, Knauer, Oberursel, Germany) and pH value. Cell culture medium is designed to have osmolarity in the range of 260 and 320 milliosmoles (mOsm), basically to mimic the osmolarity of serum at 290 mOsm/kg.[65] For ropivacaine and bupivacaine, the values were 301.7 and 290.0 mosmol/L. After the addition of dexamethasone, the values were 303.7 and 288.7 mosmol/L. Morphine and M6G yielded values of 313.7 and 292.7 mosmol/L. The addition of dexamethasone did not change osmolarity, and the pH values were in the range of 6.0−6.5.

Cell Viability Measurement

Cell viability was assessed using the CellTiter-Glo luminescent cell viability assay (Promega, Mannheim, Germany), as described before.[1,66] This assay quantifies the presence of adenosine triphosphate (ATP), which identifies the metabolically active cells. Luminescence produced by the luciferase-catalyzed reaction of luciferin and ATP was measured using a multilabel plate reader (VICTOR3; PerkinElmer LAS, Rodgau-Jügesheim, Germany).

One major advantage of this method is that it is fast, the background interference (autofluorescence from compounds, media, and cells) is low, and it generally provides a much broader dynamic range and higher sensitivity. In brief, the medium was removed, 50 µL PBS and 150 µL CellTiter-Glo reagent was added into each well. After an incubation period of 20 minutes at room temperature, the luminescent signal was recorded in counts per second. ATP standard curves were plotted with defined ATP concentrations (25-4000 nM) for each measurement, and the number of cells was calculated with standard measurements performed for each patient with defined numbers of tenocytes from the monolayer culture (standard curves with 7.8×10^2, 1.5×10^3, 3.12×10^3, 6.2×10^3 1.25×10^4, 2.5×10^4, 5×10^4, and 1.0×10^5 cells). The mean intra-assay coefficient of variability of the CellTiter-Glo luminescent cell viability assay was 1.28 % in the preliminary experiments ($n = 3$; .88-1.62 %). The mean inter-assay coefficient of variability of the CellTiter-Glo luminescent cell viability assay of the main experiments was 2.49 % ($n = 5$, .93-3.35%).

Statistics

The statistical analyses were performed using SPSS software (SPSS 27.0, Chicago, IL; Microsoft Excel, Redmond, WA). Data were expressed as means ± SD for cell viability. For the statistical comparisons between the different independent groups, the nonparametric Mann−Whitney *U*-test was used to compare cell viability after incubation with the different substances. To determine the influence of dexamethasone on the incubation with LA (bupivacaine/ropivacaine) or morphine/M6G, the *t*-test for equality of means for independent samples was used. The level of significance was set at $P < .05$.

Results

Cell Proliferation and Characterization

The average generation time of the first two passages of cells was 3.46 ± 1.00 days; the range was 2.84-5.45 days for $n = 6$ donors. The cells were characterized as tenocytes by the expression of the tenocyte markers biglycan, runx, scleraxis, collagen I, and tenascin C on the protein level, as shown via immunohistochemical staining of the typical tenocyte marker (Fig 1). Also, on the mRNA level, the expression of the tenocyte markers could be detected in 5 of the 6 patients. For one patient, the amount of mRNA was not sufficient for all PCRs (Fig 2).

Cell Viability After Exposure

There was a significant time-dependent decrease in the tenocytes' cell viability after exposure to amide-type local anesthetics (bupivacaine and ropivacaine) (Fig 3). After 15, 60, and 240 minutes of incubation with bupivacaine, cell viability decreased to 81.1 ± 18.9 % ($P < .001$), 49.4 ± .22 % ($P < .001$) and .0 % in relation to the saline control (=100 %). Ropivacaine caused a similar decrease in tenocyte viability after short-term incubation but did not lead to complete cell death after long-term incubation. After incubation for 15, 60, and 240 minutes, cell viability decreased to 81.4 ± 18.2 % ($P < .001$), 69.6 ± 14.0 % ($P < .001$) and 9.2 ±3.1 % ($P < .001$) in relation to the saline control.

There was no statistical difference in cell viability after incubation with morphine and M6G for 15 and 60 minutes compared to the saline control (morphine: 96.1 ± 16.1% and 100.5 ± 15.3%, M6G: 97.0 ± 19.0% and 99.2 ± 18.4%). After 240 minutes, the viability decreased to 78.6 ± 6.0% (morphine, $P < .001$) and to 86.1 ± 8.5 % (M6G, $P < .01$) compared to saline control. The further time-dependent decrease at 240 minutes, compared to viability after 15 and 60 minutes, was only significant for morphine (Fig 3).

Exposure to dexamethasone alone (without other additives) had no significant effect on viability after any exposure time ($t = 15$: 101.6 ± 20.5 %; $t = 60$: 104.2 ±17.3%; $t = 240$: 99.3 ± 19.7 % compared with saline as control.

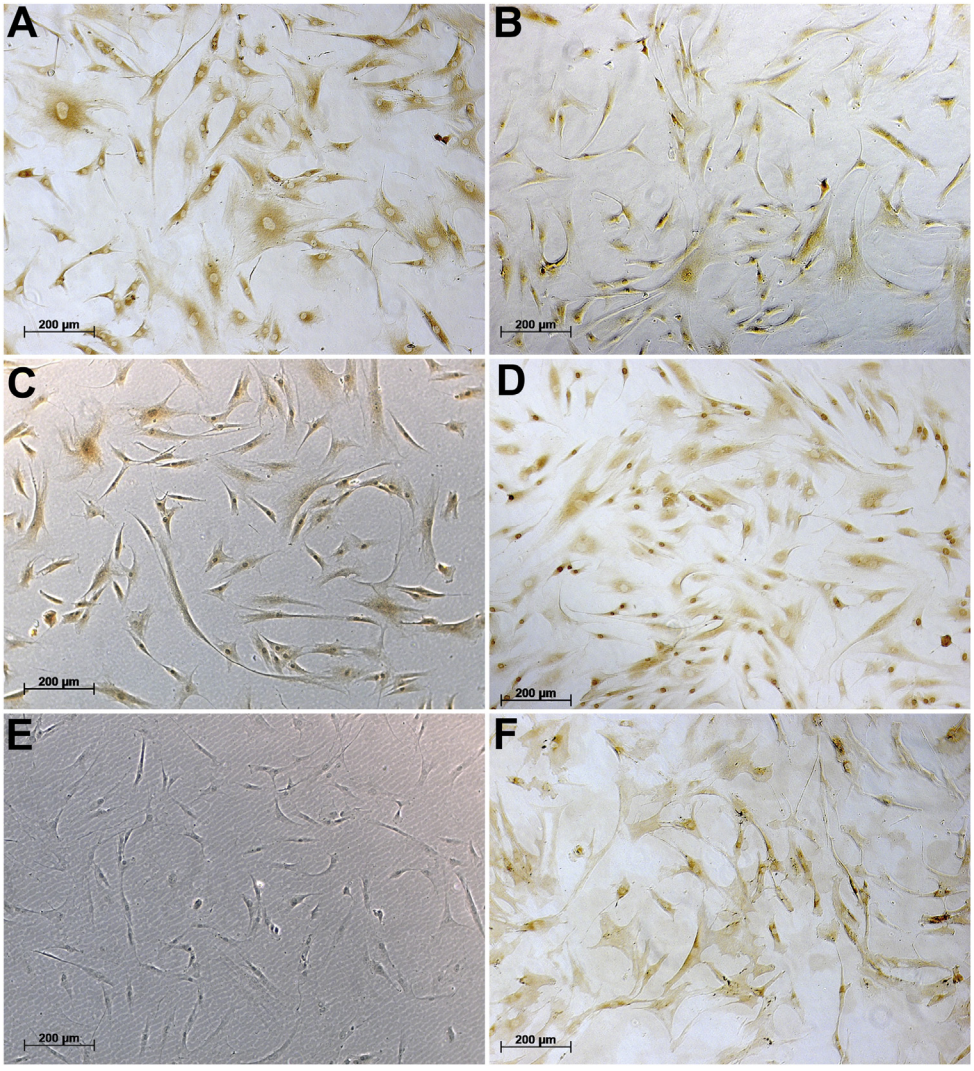

Fig. 1. Immunohistochemical staining of tenocyte markers: biglycan (A), Runx (B), scleraxis (C), collagen I (D), unstained control (E), tenascin C (F).

The cytotoxic effects of bupivacaine and ropivacaine were not compensated for by the addition of dexamethasone. In contrast, after incubation for 15 minutes, the viability of the tenocytes decreased significantly, with the addition of dexamethasone compared to when they were incubated with the anesthetics alone (bupivacaine: 81.1 ± 19.0 % vs 50.1 ± 22.5 % [$P < .001$], ropivacaine 81.4 ± 18.2 % vs 69.5 ± 19.2 % [$P < .05$]) (Fig. 4A). After a more prolonged incubation (60 and 240 minutes), there was no significant difference in the viability with or without the addition of dexamethasone. (Fig 4, B and C).

The addition of dexamethasone to morphine and M6G had a positive effect on the viability of tenocytes, which increased significantly for all incubation times compared to the opioids alone. It was even higher than the saline control (=100%) for most time points (Fig 4, A–C). The values for morphine and dexamethasone were 110.7 ± 18.5% ($P < .01$), 116.5 ± 23.2% ($P < .001$), and 112.4 ± 19.2% ($P < .001$) after 15, 60, and 240 minutes and with >100% higher than the saline control. Also combined incubation of M6G and dexamethasone displayed increased cell viability with 111.1 ± 21.3% ($P < .01$), 110.6 ± 16.4 % ($P < .001$), and 100.0 ± 20.1% after 15, 60, and 240 minutes compared to the saline control (=100%).

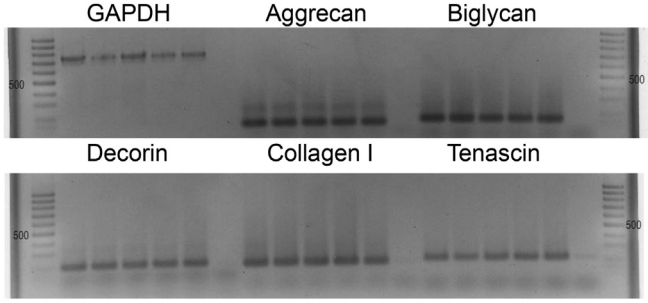

Fig 2. Expression profile of typical tenocyte markers. In the cells of all five patients tested, the tenocyte markers were expressed in comparable intensities.

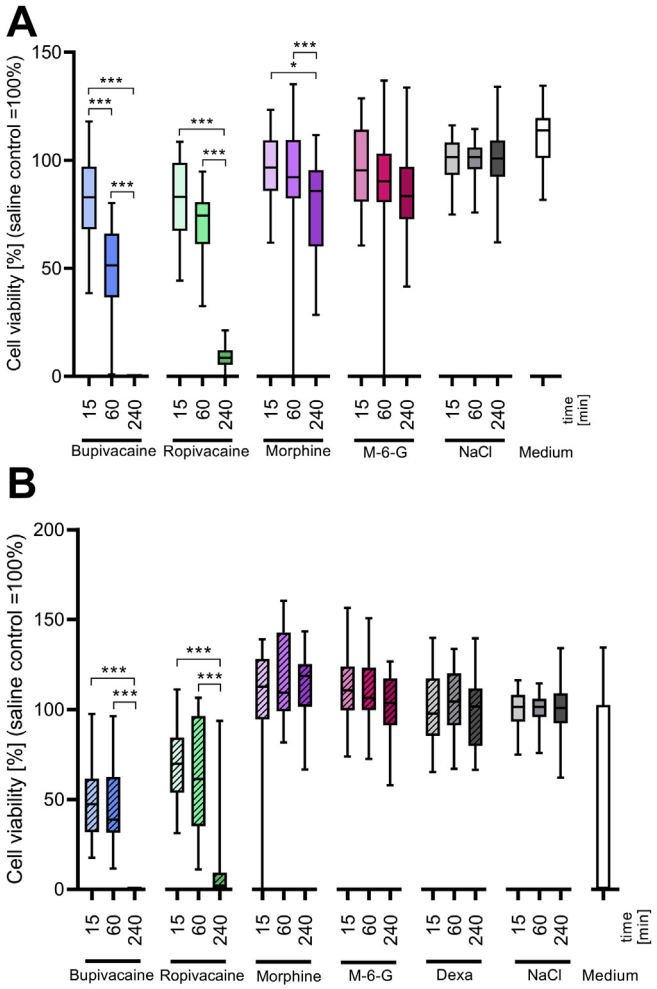

Fig 3. Cell viability after exposure to all substances. (A) Influence of different incubation times (15, 60, and 240 minutes) with anesthetics on the viability of tenocytes in relation to the saline control (=100 %). (B) Influence of different incubation times (15, 60, and 240 minutes) with anesthetics combined with dexamethasone on the viability of tenocytes in relation to the saline control. The box plots span the interquartile range. The vertical line inside the box represents the mean. The whiskers extend to the highest and lowest observations. Experiments reveal the means ± SD of $n = 6$ wells per patient ($n = 6$ patients) for each time point and substance, amounting to a total of 36 wells per time point and substance, except for morphine and M6G with $n = 5$ patients at 60 minutes with a total of 30 wells. Statistical significances are expressed as $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

Discussion

The tested opioid drugs, morphine and M6G, showed no cytotoxic effects on human tenocytes after short-time exposure of up to 60 minutes, as cell viability was as high as in the saline control. After long-time exposure to opioids for 240 minutes, the slightly decreased cell viability was counterbalanced by the addition of dexamethasone. We observed severe cytotoxic effects of LA after incubation for 240 min, resulting in complete cell death in bupivacaine, and nearly complete cell death in ropivacaine, which could not be compensated for by the addition of dexamethasone. Dexamethasone alone had no significant impact on the viability of the tenocytes compared to saline control in our experiments.

In our study, we observed the absence of relevant cytotoxic effects of opioids after short-term exposure. These findings are in line with the results of the study by Haasters et al., who did not find any adverse effect for morphine (.25 mg/mL, 120 minutes) on the viability of human tendon stem/progenitor cells from hamstring tendons.[20] However, in contrast to Haasters, we observed a low, but significant, decrease in viability after longer exposure (240 minutes) to both opioids. This might be due to the different time points for viability measurement (Haasters after 0-6 hours, our study after 72 hours), delayed cytotoxic effects, and the double concentration of morphine (.5 mg/mL) used in our study. So far, there have been no studies showing the effect of M6G on tenocytes. In previous experiments on human chondrocytes, morphine, as well as M6G (both .5 mg/mL) did not affect viability after 240 minutes of incubation.[1] Other in vitro studies confirmed this neutral effect of morphine on chondrocyte viability in cocultures of canine cartilage and synovial tissue explants[52] and on human chondrocytes.[67]

We detected a time-dependent cytotoxicity of LA on tenocyte viability in vitro. Our results confirm previous findings, which demonstrated that 6 hours of exposure to bupivacaine (5.0 mg/mL) and ropivacaine (7.5 mg/mL) resulted in total cell death of human hamstring tenocytes. The cytotoxic effects were concentration- and time-dependent.[20] Other studies have also reported cytotoxic effects of bupivacaine (5.0 mg/mL) on human rotator cuff tenofibroblasts after 24 hours of exposure, as well as of ropivacaine (7.5 mg/mL).[18,21] Lower concentrations of bupivacaine up to .05 mg/mL and 24 hours incubation time had no toxic effect, whereas concentrations of more than 2.5 mg/mL were cytotoxic.[6,21] It can be concluded that the cytotoxic effect of LA on tenocytes depends on the substance (bupivacaine > ropivacaine), concentration and time of exposure. The same has been demonstrated for chondrocytes in various studies.[1,3,6,7]

In our in vitro study, the time-dependent negative effect on cell viability of both bupivacaine and ropivacaine was particularly distinct after the long incubation time of 240 minutes. For bupivacaine, this reduction of tendon cell viability could not observed in vivo, as shown in the study of Lehner et al., who compared cell viability after bupivacaine treatment in vitro and in vivo. In vitro, the rat tendon-derived cells were treated with bupivacaine (.5% for 10 minutes), while in vivo, the rats received a single peritendinous injections into the Achilles tendon.[24]

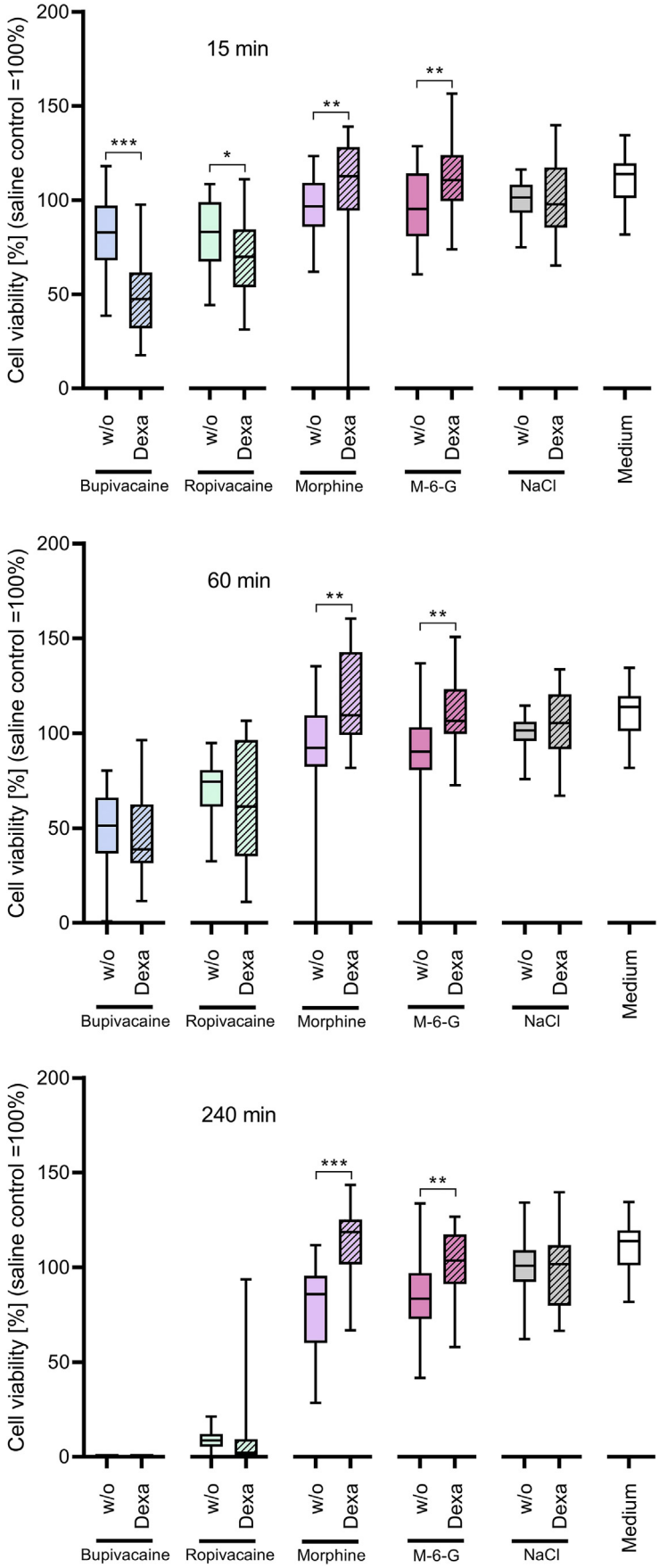

Fig 4. Influence of the addition of dexamethasone. Addition of dexamethasone to tenocytes after incubation with anesthetics for 15 minutes (A), 60 minutes (B), and 240 minutes (C). The box plots span the interquartile range. The vertical line inside the box represents the mean. The whiskers extend to the highest and lowest observations. Experiments reveal the means ± SD of $n = 6$ wells per patient ($n = 6$ patients) for each time point and substance amounting to a total of 36 wells per time point and substance, except for M6G and M with only $n = 5$ patients after 60 minutes of exposure. Statistical significances are expressed as $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

Treatment of rat tendon-derived cells had detrimental effects on cell viability, which could be reduced by N-acetyl-L-cysteine or reduction of extracellular calcium. In vivo, single peritendinous injections had impairing effects on cells within areas of loose connective tissue and elicited considerable, although only temporary, functional damage. It could be shown that bupivacaine induces mitochondrial dysfunction, as well as overproduction of reactive oxygen species (ROS), which cause necrosis or apoptosis.[3,24] Other studies also showed that the cytotoxic ROS-mediated effect is potentiated by a higher level of extracellular calcium.[68-70] The reason for the different observations in vivo and in vitro is most likely the missing extracellular matrix, which may provide protection for tenocytes, thus mitigating the damaging effects observed using in vitro monolayer cell culture models, as postulated by Sherb et al.[22]

In the present study, dexamethasone alone in a concentration of .23 mg/mL did not significantly affect the viability of tenocytes regardless of the duration of the exposure time. This observation is in line with previous studies with a similar experimental setup, which reported results for .25 mg/mL dexamethasone and 24 hours of incubation[6] and for .8 mg/mL and 30 minutes of incubation.[66]

Our results demonstrated that the tendotoxic effect of bupivacaine and ropivacaine could not be compensated for by the addition of dexamethasone. On the contrary, after incubation for 15 minutes, the tenocytes' viability decreased significantly with the addition of dexamethasone compared to incubation with the LA alone. However, after incubation for 60 minutes with LA and dexamethasone, no differences between the combination and the LA alone were detected. This corresponds to previous findings, in which the combined incubation with dexamethasone and ropivacaine (.8 mg/mL and 5.0 mg/mL) for 30 minutes also reduced the viability of bovine tenocytes significantly compared to ropivacaine alone.[66]

In all probability, it is the different modes of action by which LA and opioids lead to analgesia that are responsible for the distinct difference in the cytotoxic effects on tenocytes. The opioids morphine and M6G act directly via the μ-opioid receptor as signaling agonists.[71,72] Morphine has several different roles in cell protection and the modulation of cell death. In a review by Tegeder et al.,[73] several studies reported on the protective and proliferating effect of morphine on different cell types (i.e., immune cells, neurons and glia, endothelial cells and fibroblasts, tumor cells) at low concentrations, while relatively high concentrations in vitro, as well as chronic clinical opioid treatment can lead to inhibition of cell growth. So far, no studies have been published on whether these findings also apply to human tenocytes.

Regarding the mode of action, amide-type local anesthetics influence sodium channels in the cell membrane, leading to an induction of mitochondrial dysfunction, as well as overproduction of ROS, DNA damage, and apoptosis.[3,68,69]

Regarding the clinical application of morphine, morphine was only added to a multimodal drug injection and admitted periarticularly. In the most recent study, a mixture of steroids, local anesthetics, NSAIDs, and epinephrine with or without morphine (.1 mg/kg) was injected periarticularly into randomly assigned patients ($n = 100$) after total hip arthroplasty. The results suggested that the addition of morphine to the multimodal cocktail injection after total hip arthroplasty was not effective for relieving postoperative pain, alleviating swelling, or improving range of motion.[74]

Also, after total knee arthroplasty, the effect of morphine added to periarticular multimodal drug injection (PMDI) or spinal anesthesia on pain management and functional recovery was investigated in $n = 100$ patients in total. The data revealed that the efficacy of morphine added to periarticular multimodal drug injection was limited and that of morphine added to spinal anesthesia disappeared within 20 h postoperatively. Adding morphine to PMDI or spinal anesthesia did not improve functional recovery and caused some adverse effects.[75]

Limitations

There are some limitations to this study. The tenocytes derived from a limited number of healthy individuals. Therefore, interindividual variation in tendon quality and susceptibility to cytotoxic agents cannot be excluded. The age range of the patients and was high (24-75 years). In clinical practice, mean patient age could be higher, resulting in even greater local anesthetic cytotoxicity.

The exposure time and the number of applications in a clinical setting can vary substantially between different individuals, physicians, and locations, due to many heterogeneous factors. We cannot exclude regeneration of the cells after a single exposure, nor can we exclude long-term detrimental effects of a single application to the cells due to the experimental setup.

Another limitation in this study is that only one concentration per local anesthetic was used in the experiments, although lower concentrations have also been used in clinical practice and studies that have lower cytotoxicity profiles (i.e., .25% bupivacaine and .2% ropivacaine).[19,21,66] We focused on the concentrations that have been used in both clinical practice[6,45,61,62] and previous studies[18-20,60] and that are used for anesthesiological field blocks with LA. We used these known cytotoxic concentrations to be able to investigate possible compensatory effects of the addition of corticosteroids.

Conclusions

The results showed that amide-type local anesthetics have a time-dependent cytotoxic effect on human tenocytes in vitro, which could not be compensated for by dexamethasone. Morphine and M6G, on the other hand, were found to have no cytotoxic effects on tenocytes after 15 and 60 minutes of exposure. The addition of dexamethasone to morphine and M6G had a positive effect on the viability of tenocytes, which increased significantly compared to the opioids alone.

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