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INFECTION

Influence of ceftriaxone on human bone cell viability and in vitro mineralization potential is concentration- and time-dependent

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Aims

In orthopaedic and trauma surgery, implant-associated infections are increasingly treated with local application of antibiotics, which allows a high local drug concentration to be reached without eliciting systematic adverse effects. While ceftriaxone is a widely used antibiotic agent that has been shown to be effective against musculoskeletal infections, high local concentrations may harm the surrounding tissue. This study investigates the acute and subacute cytotoxicity of increasing ceftriaxone concentrations as well as their influence on the osteogenic differentiation of human bone progenitor cells.

Methods

Human preosteoblasts were cultured in presence of different concentrations of ceftriaxone for up to 28 days and potential cytotoxic effects, cell death, metabolic activity, cell proliferation, and osteogenic differentiation were studied.

Results

Ceftriaxone showed a cytotoxic effect on human bone progenitor cells at 24 h and 48 h at concentrations above 15,000 mg/l. With a longer incubation time of ten days, subtoxic effects could be observed at concentrations above 500 mg/l. Gene and protein expression of collagen, as well as mineralization levels of human bone progenitor cells, showed a continuous decrease with increasing ceftriaxone concentrations by days 14 and 28, respectively. Notably, mineralization was negatively affected already at concentrations above 250 mg/l.

Conclusion

This study demonstrates a concentration-dependent influence of ceftriaxone on the viability and mineralization potential of primary human bone progenitor cells. While local application of ceftriaxone is highly established in orthopaedic and trauma surgery, a therapeutic threshold of 250 mg/l or lower should diminish the risk of reduced osseointegration of prosthetic implants.

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Keywords: Ceftriaxone, Cytotoxicity, Human bone progenitor cells, Osteogenic differentiation

Article focus

- Investigation of acute and subacute cytotoxic effects of ceftriaxone in vitro.
- Study the influence of different concentrations of ceftriaxone on the mineralization potential of bone progenitor cells in vitro.

Key messages

- Ceftriaxone showed a cytotoxic effect on human bone progenitor cells at 24 h and 48 h above 15,000 mg/l, but subtoxic effects already above 500 mg/l upon exposure for ten days.
- In vitro mineralization after 28 days of culture was negatively affected at ceftriaxone concentrations above 250 mg/l.

Strengths and limitations

- This study investigated acute and subacute effects of ceftriaxone on bone progenitor cells at different timepoints up

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to 28 days of in vitro culture, and reports that ceftriaxone demonstrates a negative effect on cell viability and on mineralization at elevated concentrations.

- Chosen endpoint measurements report cell states at one specific point of time, thus possibly missing out on potential effects of ceftriaxone on dynamic processes such as gene expression.

Introduction

Local application of antibiotics is commonly used in orthopaedic and trauma surgery, as it allows for high drug concentrations in compartments poorly accessible to systemic administration.[1-4] Furthermore, it offers the unique possibility to reach certain therapeutic thresholds required for antibiotic-mediated biofilm eradication.[5-7] Biofilm formation is one of the most common causes of treatment failure in implant-associated infections.[6–10] High concentrations of antibiotics may however become toxic for local cells, as demonstrated in vitro for many antibiotics.[11–19] These studies focused on short-term cytotoxicity, with up to 96 hours of exposure, but cytotoxicity thresholds associated with prolonged drug exposure are likely lower. Studies with prolonged exposure, however, are rare.[20] Holtom et al[13] assessed the influence of three different fluoroquinolones on proliferation and extracellular matrix mineralization of osteoblast-like cells, showing a concentration-dependent reduction in calcium deposition after 14 days. Notably, Rathbone et al[20] studied the effect of 21 different antibiotics on viability and osteogenic differentiation of human osteoblast cells after 14 days of exposure and showed that cell number and alkaline phosphatase activity (ALP) are influenced by the type of antibiotic as well as the concentration applied. However, both studies presented only limited investigations on the expression of osteogenic markers and proteins known to be required for bone turnover.[21] Besides those two studies, the influence of cefuroxime on extracellular matrix ossification was evaluated after 56 days of exposure, but only qualitatively.[19] The influence of antibiotics is not only concentration-dependent, but is also affected by the origin of cells used. Primary cells are clinically more relevant, but also more heterogeneous; a sophisticated isolation procedure is needed and they are often not widely available.[22,23] Being more readily available and showing a higher reproducibility, cell lines have been frequently employed in previous studies.[8,11–14,16,17] However, they are known to show different characteristics and responses to stimuli compared to primary cells,[24] thus limiting the clinical relevance of most studies currently available.

Ceftriaxone is a third-generation cephalosporin antibiotic agent. Despite being a beta-lactam antibiotic, inhibiting cell wall synthesis,[25] it appears to have higher in vitro activity against biofilm from staphylococci than vancomycin, daptomycin, fosfomycin, as well as tigecycline.[26] Staphylococci cause the large majority of orthopaedic device-associated infections.[27-29] Recently published data indicate a particular and promising release profile of ceftriaxone from calcium sulphate,[30] a resorbable antibiotic carrier commonly used in the treatment of bone and joint infections.[31-34] With the extended antibacterial spectrum of ceftriaxone, this would open new treatment possibilities. However, to the best of our knowledge, there are only limited data available regarding cellular toxicity and potential influence on osteogenesis of this drug.[11,19,20]

In this study, acute cytotoxicity and subtoxic effects of ceftriaxone on primary human bone progenitor cells as well as its subsequent potential influence on mineralization was investigated in vitro. At timepoints ranging from 24 hours to 28 days, the influence of ceftriaxone on cell viability and proliferation rate, as well as osteogenic differentiation at the gene and protein level, was investigated, demonstrating concentration- and time-dependent effects. This study thus provides important insights regarding the influence of ceftriaxone on osteoblasts and their osteogenic activity, which is relevant for the management of bone and joint infections.

Methods

Human bone progenitor cell culture. Human bone progenitor cells (HBCs) were isolated from pieces of trabecular bone prepared from human bone marrow samples of patients undergoing orthopaedic surgery as described previously.[35] Informed consent was obtained in accordance with the local ethics committee (EKSG 08/14). The cells were culture expanded in proliferation medium (α-MEM, 22561021; Thermo Fisher Life Technologies, Switzerland) supplemented with 10% fetal calf serum (FCS) (F-9665; Sigma-Aldrich, Switzerland), 1% penicillin/streptomycin/neomycin solution (P-4083, Sigma-Aldrich) and 20 µL fibroblast growth factor 2 (FGF-2) (F-0291; Sigma-Aldrich) at 37°C in a humidified 5% CO_2 atmosphere. The medium was exchanged every three to four days until cells reached approximately 90% confluence. Cells were harvested, resuspended in osteogenic differentiation medium (α-MEM, 10% FCS, 1% PSN, 44 µM L-ascorbic acid 2-phosphate (A-8960, Sigma-Aldrich), 2 mM β-glycerophosphate (G-9422, Sigma-Aldrich), 10 nM 1,25 dihydroxyvitamine D3 (D-1530, Sigma-Aldrich), and 10 nM dexamethasone (D-4902, Sigma-Aldrich)), containing different concentrations of ceftriaxone (Rocephin, Roche, Switzerland) and seeded in multiwell plates as well as 35 mm glass bottom dishes (P35G-0.170-14-C, MatTek Cooperation, USA). Cells seeded in osteogenic medium and proliferation medium without ceftriaxone supplementation served as control.

Influence of ceftriaxone on cell viability, metabolic activity, and proliferation. Lactate dehydrogenase (LDH) and 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethonyphenol)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assays were used to determine cytotoxicity and metabolic activity after one and two days, whereas metabolic activity for subtoxic effects was assessed by a PrestoBlue assay (A13262, Thermo Fisher) after

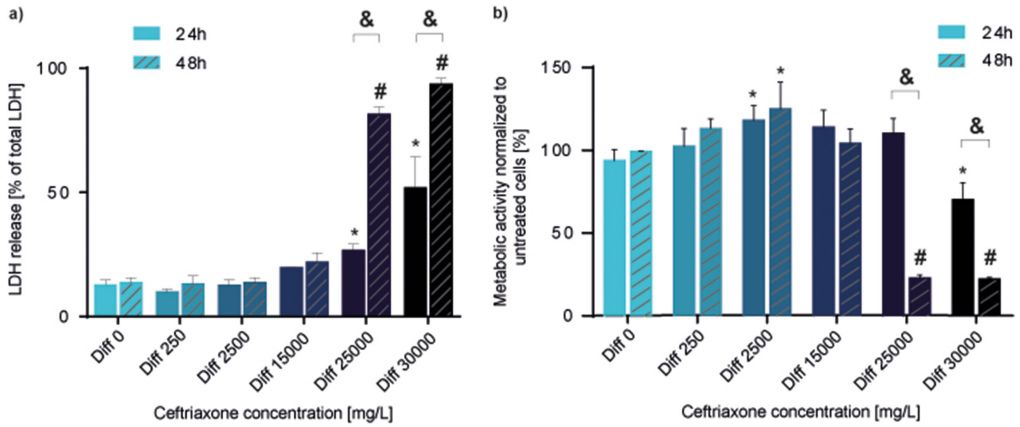

Fig. 1

a) Acute cytotoxicity and b) metabolic activity of ceftriaxone on human bone progenitor cells (HBCs) after 24 hours and 48 hours exposure at various concentrations (mean and standard deviation, n = 3). Statistically significant differences compared to Diff 0 are indicated with "*" at 24 hours, "#" at 48 hours, and "&" between 24 hours and 48 hours (* at 25,000 mg/l a)), p = 0.0104, * at 30,000 mg/l a) p < 0.0001, * at 30,000 mg/l b) p = 0.017, &p < 0.0001, #p < 0.0001, two-way analysis of variance). LDH, lactate dehydrogenase.

ten days. The 5-ethynyl-2'-deoxyuridine (Edu) assay (Click-iT Edu Alexa Fluor 488 Imaging Kit, C10337; Invitrogen, Thermo Fisher) was implemented for measuring proliferation of HBCs after ten days. In brief, HBCs were cultured for one and two days in osteogenic differentiation medium containing indicated ceftriaxone concentrations in multiwell plates (3.5×10^3 cells/mL). Cadmium sulfate ($CdSO_4$) was used at five different concentrations as positive control (data not shown). Measurements were taken using a Mithras Plate reader as detailed in the Supplementary Material.[2] When culturing HBCs for ten days, subtoxic effects and metabolic activity were determined using LDH and PrestoBlue assay, respectively. The proliferation assay was performed at day 10 using EdU according to the manufacturer's instruction. Details are provided in the Supplementary Material.

Influence of ceftriaxone on osteogenic differentiation. The effect of different ceftriaxone concentrations on osteogenic markers (alkaline phosphatase, collagen I, and osteocalcin) was investigated using reverse transcription-polymerase chain reaction (RT-PCR). The method was applied, as previously described,[36] with minor modifications as detailed in the Supplementary Material.

To assess the expression of osteogenic markers on the protein level, the samples were stained against alkaline phosphatase (ALP; B4-78, Developmental Studies Hybridoma Bank, USA), collagen type I (Col-I; C2456, Sigma-Aldrich), actin cytoskeleton (Alexa Fluor 546 Phalloidin, A22283, Thermo Fisher), and nuclei (DAPI; 4',6-diamidino-2-phenylindole, D9542, Sigma-Aldrich, Buchs, Switzerland) after fixation at day 10.

Collagen was quantified at day 14 by Soluble Collagen Assay Sircol Kit (S1000, Biocolor Life Science Assays, UK) following the manufacturer's instruction. DNA quantification with Hoechst 33258 (94403, Sigma-Aldrich) was performed at the same time according to the manufacturer's protocol. Detailed descriptions of all assays and primer sequences are provided in the Supplementary Material and Supplementary Table i, respectively.

After 28 days, mineralization was assessed using the Calcium Assay Kit (KA4081, Abnova, UK) according to the manufacturer's instructions. Metabolic activity measurement was performed at the same timepoint using a PrestoBlue assay. Details are provided in the Supplementary Material.

Statistical analysis. Analysis of acute cytotoxicity and metabolic activity at days 1 and 2 were performed using a two-way analysis of variance (ANOVA) followed by Sidak's post-hoc test for multiple comparison. Subtoxic effects, cell proliferation, and gene expression at day 10 as well as Col-I protein and DNA concentration at day 14 were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Analysis of mineralization and metabolic activity at day 28 were performed using one-way ANOVA followed by Dunnett's post-hoc test for multiple comparison. The bar graphs in the figures show means and standard deviations (SDs). A p-value < 0.05 was considered as statistically significant.

Results

Influence of ceftriaxone on cell viability, metabolic activity, and proliferation. Analyzing acute cytotoxicity of different ceftriaxone concentrations on HBC after 24 hours and 48 hours did not show a negative effect up to a concentration of 15,000 mg/l (Figure 1a). However, a significantly increased LDH release could be observed at both timepoints at 25,000 mg/l and 30,000 mg/l. The metabolic activity of HBCs showed a slight increase with ceftriaxone concentrations from 0 mg/l ranging to 2,500 mg/l at 24 hours and 48 hours, followed by a remarkable decrease at concentrations of 30,000 mg/l after 24 hours of incubation and at 25,000 mg/l after 48 hours of culture (Figure 1b).

Assessing potential subtoxic effects of ceftriaxone at longer incubation times, LDH release, metabolic activity, and cell proliferation were measured. A continuously

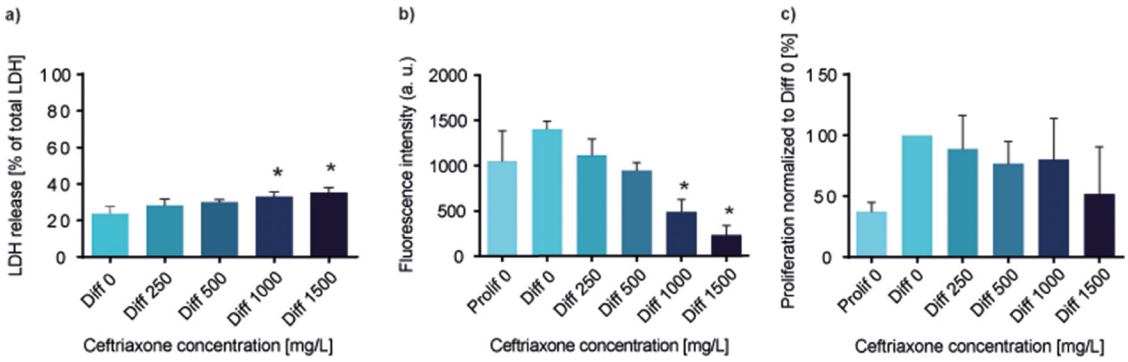

Fig. 2

a) Subtoxic effects and influence of different concentrations of ceftriaxone on b) metabolic activity and c) proliferation of human bone progenitor cells (HBCs) cultured in proliferation medium (Prolif) or osteogenic differentiation medium (Diff) for ten days (mean and standard deviation, n = 3). Statistically significant differences compared to Diff 0 are indicated with * (lactate dehydrogenase (LDH) 1,000 mg/l, p = 0.024, LDH 1,500 mg/l p = 0.006, fluorescence intensity (FI) 1,000 mg/l, p = 0.00041, FI 1,500 mg/l, p < 0.0001,0, one-way analysis of variance). a.u., arbitrary units.

rising LDH release could be observed with increasing concentrations of ceftriaxone with osteoblasts cultured for ten days, showing statistically significant differences at 1,000 mg/l (p = 0.024, two-way ANOVA) and 1,500 mg/l (p = 0.006, two-way ANOVA) (Figure 2a). Measured metabolic activity also demonstrated a concentration-dependent effect on cell viability after ten days (Figure 2b). Values were highest for the osteogenic control without ceftriaxone supplementation, and lowest at a ceftriaxone concentration of 1,500 mg/l. Assessing the cell proliferation rate in osteogenic medium supplemented with ceftriaxone, a reduction to a mean of 86.9% (SD 21.60) and 80.4% (SD 27.45) could be observed for concentrations between 250 mg/l and 1,000 mg/l, which dropped to 51.6% (SD 32.03) at 1,500 mg/l, when compared to control cell cultures without ceftriaxone (Figure 2c). Notably, cells cultured in proliferation medium showed significantly lower values than cells cultured in osteogenic differentiation medium, whereas no significant differences could be observed for the proliferation rate.

Influence of ceftriaxone on osteogenic differentiation. At the gene expression level, *osteocalcin* and *ALP* showed no clear trend after ten days for ceftriaxone concentrations up to 1,000 mg/l. A notable decrease could only be observed at 1,500 mg/l of ceftriaxone, but this was not found to be statistically significant (Figure 3a). On the other hand, *Col-I* expression revealed a concentration-dependent decrease with increasing ceftriaxone concentrations (Figure 3a). The messenger RNA (mRNA) levels of *Col-I* at 0 mg/l ceftriaxone were 2.6-fold higher than at concentrations of 1,000 mg/l (p = 0.028, one-way ANOVA), and 16.8-fold higher than at 1,500 mg/l (p = 0.002, one-way ANOVA). Statistically significant differences could also be observed between 250 mg/l and 1,500 mg/l (p = 0.005, one-way ANOVA) as well as between 500 mg/l and 1,500 mg/l (p = 0.030, one-way ANOVA).

Immunohistochemical staining against ALP showed high expression levels in HBCs cultured for ten days in osteogenic medium supplemented with ceftriaxone concentrations of up to 1,000 mg/l, compared to proliferation control (Figure 3b). At 1,500 mg/l, almost no ALP staining could be observed, but cell numbers were also greatly reduced. When staining for the actin cytoskeleton, cells were well spread out, showing prominent actin fibres at ceftriaxone concentrations up to 1,000 mg/l, whereas HBCs cultured at 1,500 mg/l ceftriaxone displayed reduced spreading (Figure 3b). Staining against extracellular Col-I of HBCs cultured for 14 days in osteogenic differentiation medium supplemented with increasing ceftriaxone concentrations showed, compared to proliferation control, a higher protein expression at concentrations up to 500 mg/l. At 1,000 mg/l, the Col-I signal was noticeably decreased, whereas at 1,500 mg/l, almost no extracellular Col-I as well as greatly diminished numbers of cells were observed (Figure 4a). When quantifying Col-I levels, a concentration-dependent decrease could be seen with increasing ceftriaxone concentration, going from 49.25 µg/ml (SD 15.17) at 0 mg/l to 14.80 µg/ml (SD 3.24) at 1,500 mg/l (Figure 4b). A similar trend could be observed for total DNA concentration. However, statistically significant differences in both Col-I levels and DNA concentration were only observed at 1,500 mg/l, when compared to cells cultured in osteogenic medium without ceftriaxone supplementation (0 mg/l).

To assess the in vitro mineralization potential of HBCs in presence of increasing concentrations of ceftriaxone, the total amount of Ca^{2+} was quantified after 28 days of culture (Figure 4c). A distinct concentration-dependent decrease in mineralization could be seen at mean concentrations of 500 mg/l (1,183.7 ng Ca^{2+} per well, SD 806.8) and higher, being statistically significant at 1,000 mg/l (91.6 ng Ca^{2+} per well, SD 64.7; p = 0.013, one-way ANOVA) and 1,500 mg/l (43.0 ng Ca^{2+} per well, SD 54.4; p = 0.012, one-way ANOVA). Metabolic activity, as determined by the PrestoBlue assay, showed no clear trend between 0 mg/l and 500 mg/l, however with a notable, significant decrease at ceftriaxone concentrations at 1,000 mg/l (p= 0.008, one-way ANOVA) and higher.

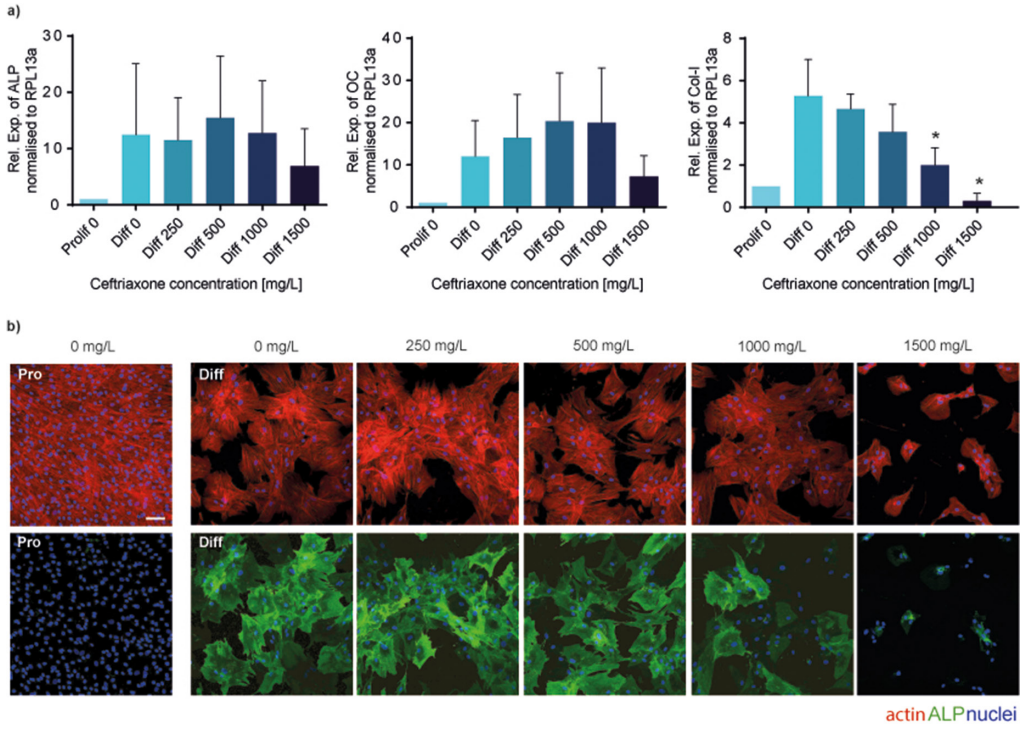

Fig. 3

a) Gene expression analysis of human bone progenitor cells (HBCs) cultivated in proliferation medium (Prolif) or osteogenic differentiation medium (Diff) for ten days, both supplemented with increasing concentrations of ceftriaxone (mean and standard deviation (SD), n = 3). Messenger RNA (mRNA) expression levels of alkaline phosphatase (*ALP*), osteocalcin *(OC)*, and collagen type I (*Col-I*) were normalized to Prolif with *RPL13a* as housekeeping gene. Statistically significant differences compared to Diff 0 are indicated with * (p = 0.028 at 1,000 mg/l and p = 0.002 at 1,500 mg/l, one-way analysis of variance). b) Immunohistochemical staining demonstrating the influence of ceftriaxone supplementation on ALP expression. ALP is illustrated in green, actin cytoskeleton in red, and nuclei in blue. HBCs were cultured in osteogenic differentiation (Diff) and proliferation medium (Pro) for ten days (scale bar 100 µm). Rel. Exp., relative expression.

Discussion

Local application of antibiotics is an attractive option to enhance the treatment efficacy in bone and joint infections, particularly orthopaedic device-associated infections,[2,3,6,37,38] but increased concentrations should not impair osteoblast viability and their ability to induce bone formation. Ceftriaxone has a promising spectrum of activity and release profile from calcium sulphate, and was investigated here for its effect on bone cell viability and in vitro mineralization potential.

During our assessment of cytotoxicity and metabolic activity of HBCs subjected to increasing concentrations of ceftriaxone, increasing cell death and declining metabolic activity could be observed at concentrations of 25,000 mg/l and above after 24 hours and 48 hours (Figure 1). This concentration dependence is in agreement with previous reports showing an acute toxic effect on osteoblasts for various antibiotic families including aminoglycosides,[8,12,14,16,20] cephalosporins,[19,20] glycopeptides,[8,11,20] lincosamides,[18] rifamycins,[15] tetracyclines,[39] and quinolones.[8,13,17,20] However, such high concentrations of ceftriaxone may not be obtained realistically when applied locally with calcium sulphate as carrier material.[30]

However, as the healing of an implant-associated infection or of a fracture-related infection is a lengthy process, potential subtoxic effects on HBCs may impair osseointegration or fracture healing in the long run. For example, Rathbone et al[20] assessed viability after a culture time of ten and 14 days and reported decreased osteoblast cell numbers at a concentration above 10 mg/l for cefotaxime and cefazolin, and above 100 mg/l for cefepime, whereas higher antibiotic concentrations of 1,000 mg/l elicited an acute cytotoxic effect after 72 hours.[19] These results illustrate that different drugs can have distinct toxicity thresholds, even when they belong to the same antibiotic family. Ceftriaxone is also a cephalosporin, and when culturing HBCs with increasing ceftriaxone concentrations for ten days, we observed concentration-dependent negative effects on cell viability and metabolic activity. While the observed effects showed a gradual response, statistically significant differences could only be observed at concentrations above 500 mg/l (Figure 2a). Similarly, cell proliferation was also slightly reduced with increasing concentrations of ceftriaxone. The most marked decrease could be seen at 1,500 mg/l, however without reaching statistical significance. Notably, this concentration is much higher than the approximately 100 mg/l obtained from the continuous release from calcium sulphate beads containing 8% weight per weight of different antibiotic powders in vitro.[30] It is also higher than previous

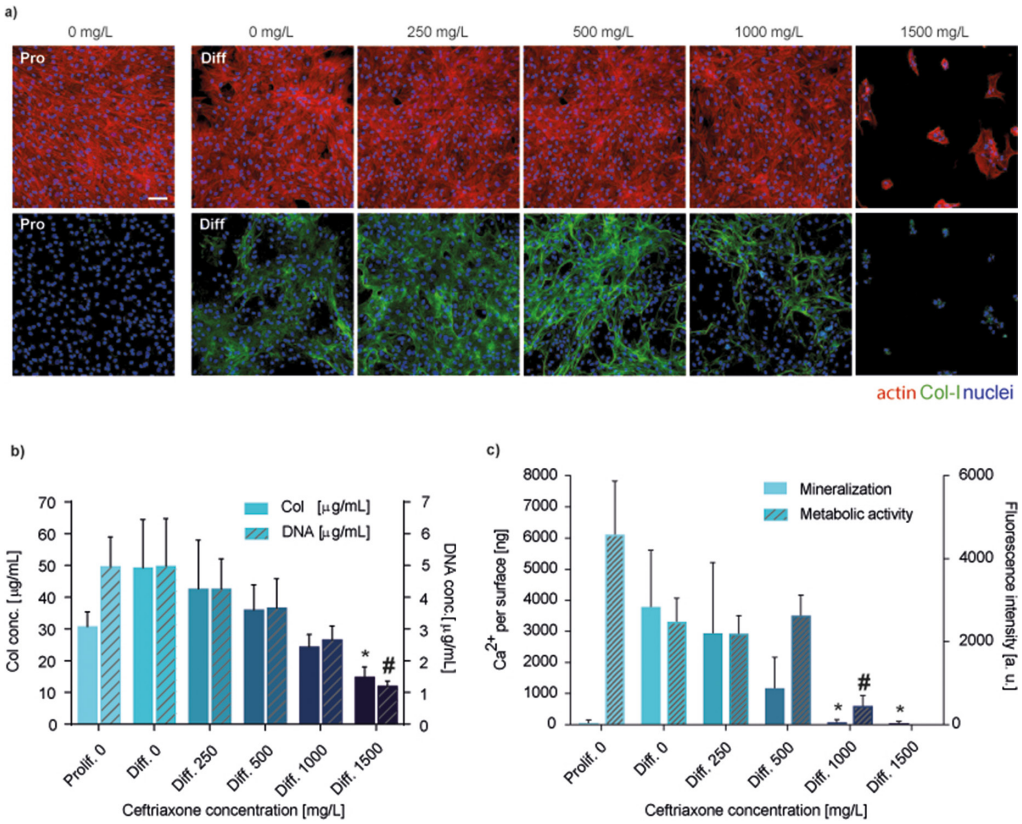

Fig. 4

a) Immunohistochemical staining demonstrating osteogenic differentiation of human bone progenitor cells (HBCs) cultivated in proliferation medium (Pro) or osteogenic differentiation medium (Diff) supplemented with increasing concentrations of ceftriaxone. Staining for extracellular collagen I (green) and nuclei (blue) after 14 days of exposure. The scale bar indicates 100 µm. b) Osteogenic differentiation of HBCs cultured in proliferation medium (Prolif) or Diff supplemented with increasing concentrations of ceftriaxone. Collagen (Col) and DNA concentrations are indicated dependent on ceftriaxone concentration. Cells cultured in Prolif served as control (mean and standard deviation (SD), n = 3). Statistically significant differences compared to Diff 0 are indicated with * ($p = 0.016$, one-way analysis of variance (ANOVA)) at Col-I and # for DNA content ($p = 0.006$, one-way ANOVA). c) Osteogenic differentiation of HBCs cultured in Prolif or Diff supplemented with increasing concentrations of ceftriaxone. Quantified calcium concentrations dependent on ceftriaxone concentrations after a culture period of 28 days. Cells cultured in Prolif served as control (mean and SD, n = 3). Statistically significant differences compared to Diff 0 are indicated with * (1,000 mg/l, $p = 0.013$, 1,500 mg/l, $p = 0.012$, one-way ANOVA) for mineralization and # for metabolic activity (1,000 mg/l, # 0.008, one-way ANOVA). a.u., arbitrary units.

reports with other cephalosporins.[11,40] Edin et al[11] showed that a concentration of 200 mg/l cefazolin significantly decreased osteoblast proliferation, whereas Duewelhenke et al[40] observed the same effect only at a concentration of 400 mg/l. Both concentrations are well below the observed threshold of 1,500 mg/l. This might be due to differences in the antibiotic agent as well as in the osteoblastic cells and culture conditions used. HBCs cultured in proliferation medium also showed a lower proliferation rate than HBCs cultured in osteogenic differentiation medium without ceftriaxone supplementation, which is likely due to cells reaching confluency well before the analysis timepoint.

Osseointegration of prosthetic implants is essential to obtain stable and functional arthroplasties. Differentiated osteoblasts have to produce non-mineralized matrix at the interface that subsequently mineralizes over time to constitute a bone structure.[41] The same process is required for successful fracture healing. Investigating the influence of ceftriaxone on the expression of osteogenic markers after ten days showed no clear tendency for *osteocalcin* and *ALP* up to a ceftriaxone concentration of 1,000 mg/l, but a marked decrease was observed at 1,500 mg/l (Figure 3a). A similar trend could be seen on the protein level, where ALP expression was reduced at 1,000 mg/l and almost completely absent at 1,500 mg/l of ceftriaxone (Figure 3b). As was reported for the effects of antibiotics on cell proliferation,[13] the expression of osteogenic markers also appears to be dependent on the type of drug. Additionally, the chosen timepoints can lead to differences in the reported results.[42] To this end, Naal et al[18] showed that clindamycin resulted in increased ALP activity at a low concentration of 10 µg/ml at 24 hours and 48 hours, whereas at the highest concentration of 500 mg/l, ALP activity was significantly decreased at each timepoint up to 72 hours. Rathbone et al[20] also showed a clear negative effect of cefazolin on ALP activity of human osteoblasts at 100 mg/l after ten and 14 days in culture.

Bone has a complex structure of organic and inorganic components, where Col-I is abundant in the

extracellular matrix of bone with a proportion of 17 to 20 wt%.[43] When determining the influence of ceftriaxone on Col-I expression after ten and 14 days of culture, *Col-I* gene and protein expression showed a concentration-dependent decrease, being most prominent at 1,000 mg/l and above. Notably, Burkhardt et al[44] showed a concentration-dependent decrease of collagen synthesis in their in vivo study, which is in agreement with our in vitro findings.

Studying the influence of different concentrations of ceftriaxone on in vitro mineralization, we could observe a concentration-dependent decrease at concentrations higher than 250 mg/l, with the lowest Ca^{2+} values being measured at 1,000 mg/l and 1,500 mg/l. Interestingly, metabolic activity was not affected by ceftriaxone supplementation at concentrations up to 500 mg/l, as evaluated by the PrestoBlue assay, whereas matrix mineralization was already markedly reduced at this concentration. Our reports are in good agreement with a previous study by Salzmann et al,[19] in which extracellular matrix calcification of cefuroxime-treated human osteoblasts was decreased after 56 days at 250 mg/l and completely inhibited at 1,000 mg/l. Interestingly, clindamycin treatment was also reported to result in decreased extracellular matrix calcification at however a much lower concentration of 50 mg/l after 56 days,[18] further highlighting the differences in the response depending on the type of antibiotic.

In summary, local application of antibiotics is commonly used in orthopaedic and trauma surgery, as it enhances the possibility of reaching high local concentrations in otherwise hard-to-reach compartments, while simultaneously reducing systematic side effects. Subtoxic effects of ceftriaxone could be shown here only at concentrations above 500 mg/l. Slightly lower cytotoxicity was observed for ceftriaxone as reported for other cephalosporins. Our results thus suggest that ceftriaxone has no cytotoxicity, no negative influence on osteoblast proliferation, metabolic activity, or osteogenic gene expression at concentrations up to 100 mg/l, a level that was shown to be reached by a continuous release from calcium sulphate. Furthermore, extracellular matrix calcification and collagen synthesis are not impaired by this concentration of ceftriaxone, collectively indicating that its local application represents a safe treatment for bone and joint infections without inhibiting osteogenesis. In vitro models should incorporate prolonged culture times in order to observe sub-lethal toxicity and inhibitory effects on bone matrix formation, as orthopaedic device-associated and fracture-related infections require prolonged antibiotic treatment, as well as bone formation for fracture healing or proper integration of joint arthroplasty components. While local application of ceftriaxone is well established in orthopaedic and trauma surgery, the risk of reduced osseointegration of prosthetic implants can be diminished by adhering to a therapeutic threshold of 250 mg/l or lower.

Supplementary material

Additional details on the methods used, a list of the primers used for reverse-transcription polymerase chain reaction, and brightfield microscopy images of human bone progenitor cells cultured in different concentrations of ceftriaxone at day 10.

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