International Orthopaedics (SICOT) (2011) 35:1719–1723
DOI 10.1007/s00264-010-1178-2

ORIGINAL PAPER

Toxicity of antiseptics against chondrocytes: What is best for the cartilage in septic joint surgery?

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Received: 14 November 2010 / Revised: 20 November 2010 / Accepted: 20 November 2010 / Published online: 16 January 2011

Abstract In septic joint surgery, the most frequently used antiseptics are polyhexanide, hydrogen peroxide and taurolidine. The aim of this study was to examine the effects of these antiseptics on viability of human chondrocytes. Our hypothesis was that antiseptics and supplemental irrigation with sodium chloride lavage are less toxic on human chondrocytes than treatment with antiseptics only. Primary human chondrocytes were isolated and cultured from six donated human knee joints. Polyhexanide, hydrogen peroxide or taurolidine were added to the cultures. Toxicity analysis was performed by visualisation of cell structure using light microscopy and LDH activity. The determination of vital cells and total cell numbers of chondrocytes treated with antiseptics partly followed by irrigation with sodium chloride solution was performed by using Casy Cell-Counter. Light microscopic data revealed a defect in cell structure after addition of antiseptics. We showed a significant increase of LDH enzyme activity after the treatment with polyhexanide or taurolidine. After treatment with antiseptics followed by sodium chloride solution a significant increase of vital and total cell numbers resulted in comparison with the chondrocytes that were only treated with antiseptics. The data show that treatment with polyhexanid, hydrogen peroxide or taurolidine induces cell death of human chondroctes in vitro. The application of sodium chloride solution after the treatment with polyhexanide and hydrogen peroxide possibly has a protective effect on chondrocyte viability.

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Introduction

Although the application of local antiseptics is a common treatment of acute joint infections, the outcome is still an unresolved problem [1]. The most frequently used antiseptics in local joint and wound infections are polyhexanide, hydrogen peroxide and taurolidine. The most frequent bacteria responsible for wound and joint infection are staphylococci, streptococci, and gram negative bacteria [2–5]. Various studies have shown that shoulder and knee joint infections often result from infiltration with local anaesthetics, glucocorticoids or hyaluronic acid contrary to hip joint infection that is rarely infiltrated for diagnostic or therapeutic reasons [2, 6, 7]. Whilst there is consensus on a staged operative treatment such as repeated aspirations or lavage, the intra-articular application of antiseptic substances remains controversial [8–10]. On the one hand, the treatment of local wound with antiseptics induces tissue toxicity, on the other hand it is evident that mechanical elimination of bacteria through joint and tissue lavage and surgical debridement can be supported by antiseptic substances. This results from the fact that in contrast to antibiotic substances they do not differentiate between eukaryontic and procariontic cells, so that tissue toxicity of antiseptic substances should be determined for every

tissue coming into contact. There is an ongoing conflict between elimination of bacteria and toxicity to chondrocytes [11, 12]. Literature reviews show insufficient data about the effects of antiseptics on human cartilage. In this study we investigate the toxic cell damage of human chondrocytes after treatment with polyhexanide, hydrogen peroxide or taurolidine. We postulated that antiseptics and supplemental irrigation with sodium chloride lavage would be less toxic on human chondrocytes than treatment with antiseptics only.

Materials and methods

Tissue culture plasticware were obtained from TPP (Trasadingen, Switzerland). Culture medium, phosphate buffer saline (PBS), trypsin and foetal calf serum (FCS) were purchased from Biochrom (Berlin, Germany). All other reagents were obtained from Sigma-Aldrich (Deisenhofen, Germany).

Chondrocyte isolation and culture

Chondrocyte isolation was performed as described before [13]. Cartilage was obtained from human donors with knee osteoarthritis not presenting any evidence of sepsis. Experimental protocols were approved by the local ethics committee. Cartilage was minced and digested in medium containing 1 mg/ml pronase (Sigma-Aldrich, Deisenhofen, Germany) for 30 minutes at 37°C. Next, digestion medium was discarded and the tissue was digested with medium containing 1 mg/ml clostridial collagenase (Sigma-Aldrich, Deisenhofen, Germany) at 37°C over night. Digested solution was then filtered (70 μm Nylon, BD Falcon, Bedford, Germany) and centrifuged at 1200 rpm for eight minutes. The supernatant was discarded and the cell pellet was washed three times with phosphate buffer saline (PBS). Then chondrocytes were suspended in DMEM Hams-F12 with 10% FBS, 1% penicillin/streptomycine and cultured at 37°C, 95% air and 5% CO^2 and experiments were performed immediately.

Chondrocytes treatment and detection of cell structure

Human chondrocytes, cultured and grown on 24-well plates at a density of sub-confluence, were added to 100 μl undiluted solution of concentrated 0.04% polyhexanide (Charité, Berlin, Germany), 3% hydrogen peroxide (Charité, Berlin, Germany) and 0.5% taurolidine (Boehringer Ingelheim, Ingelheim, Germany) for 30 minutes. PBS treated chondrocytes were used as control. Immediately after incubation time, the results were interpreted using light microscopy analysis (Axiovert 40C Light Microscope, lens 10 x 0.25, ocular 10×18 Zeiss, Göttingen, Germany). The view fields were then digitised by a digital camera (Canon EOS 500D, 15.1 Megapixels).

Activity of lactate dehydrogenase

LDH activity is a marker of advanced cell death. Release of LDH exposure indicates the loss of membrane plasma integrity as a possible marker of increased cell necrosis at this stage. Chondrocyte monolayers were challenged with polyhexanide, hydrogen peroxide or taurolidine. PBS was used as negative control and 2% Triton X 100 was used as positive control. Chondrocytes were treated for ten, 20 and 30 minutes. LDH activity in the supernatant was determined by the colorimetric measurement of the reduction of sodium pyruvate in the presence of NADH and expressed as the percentage of total enzyme activity liberated from chondrocytes in the presence of the antiseptics.

Determination of total cell numbers and vital cells

Isolated human chondrocytes were counted using Casy Cell-Counter and grown on 24-well plates at a density of 2×104 cells per well. After one day, chondrocytes were treated with 100 μl of 100% polyhexanide, hydrogen peroxide or taurolidine, PBS (negative control). The treatment lasted 30 minutes. Then, one half of the treated cells were additionally washed twice with 100 μl of 0.9 % physiological sodium chloride. Subsequently, all cells were suspended in growth medium for 48 hours. After removal of growth medium all cells were detached with 100 μl trypsin. The detection of living and total cell numbers was determined by the Casy Cell-Counter and Analyser System (Schärfe-System, Reutlingen, Germany).

Statistical analysis

A nonparametric Wilcoxon matched-pairs test was used as indicated in the legends. A p value of <0.05 was considered to be significant, a p value of <0.001 was considered to be highly significant.

Results

Human chondrocytes that were treated with polyhexanide, hydrogen peroxide or taurolidine showed increased number of cells with defects in the cell structure after an incubation time of 30 minutes. There were no defective chondrocytes when cultured without antiseptics (Fig. 1a). Chondrocytes incubated with hydrogen peroxide were globular, shrunken and showed loss of cell contacts (Fig. 1b). In contrast,

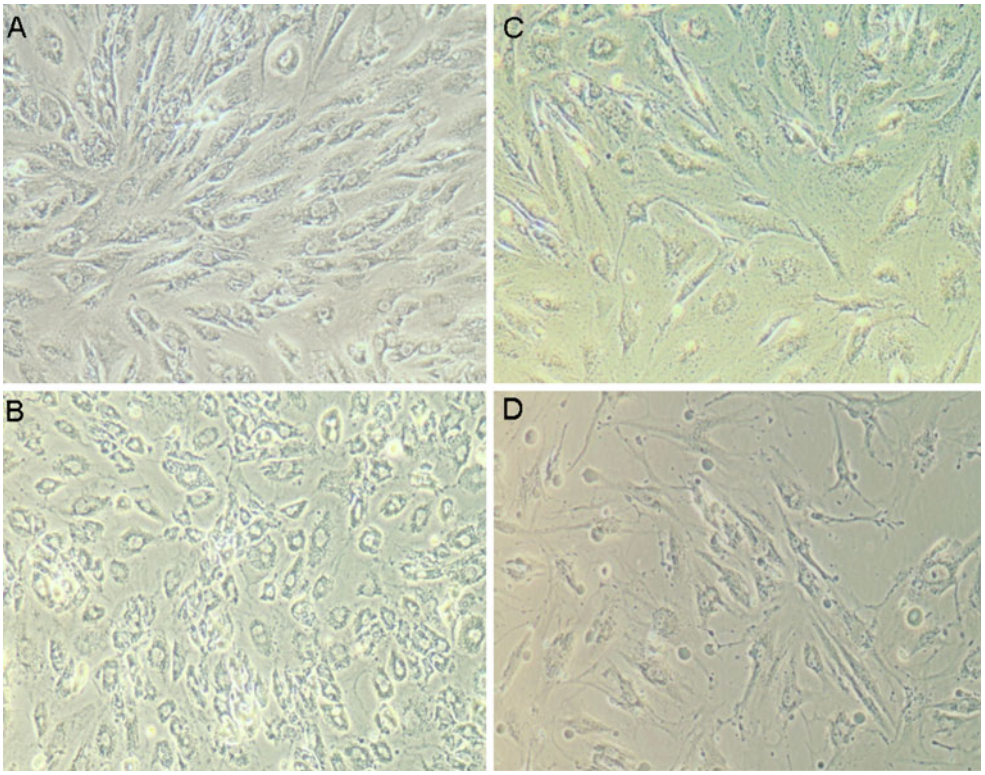

Fig. 1 Chondrocyte damage after exposure to antiseptics. Chondrocytes were treated for 30 minutes with polyhexanide, hydrogen peroxide or taurolidine and were analysed by light microscopy. **a** Untreated control chondrocytes. **b** Chondrocytes treated with hydrogen peroxide. **c** Chondrocytes incubated with polyhexanide. **d** Chondrocytes incubated with taurolidine. One representative is shown from at least three independently performed experiments

chondrocytes treated with polyhexanide or taurolidine were swollen and showed a defective cell structure (Fig. 1c, d).

LDH activity was analysed in the supernatant of each cell culture. Compared to controls (chondrocytes treated with PBS), we noted significantly increased LDH activity after as little as ten minutes and after 20 and 30 minutes of treatment with polyhexanide or taurolidine, indicating early cell necrosis, $p<0.001$, respectively (Fig. 2). Triton X100 was used as a known mediator of cell necrosis and induced, as expected, a high LDH activity. LDH activity was not detected at each time point after the treatment with hydrogen peroxide. The determination of vital cells and total cell numbers of antiseptic treated chondrocytes showed a significant decrease of vital cells (control 10.43 per 100 $\mu l \times 10^3$, $p<0.05$) (Fig. 3), and total cell numbers (control 14.17 per 100 $\mu l \times 10^3$, $p<0.05$) (Fig. 4). The examination of antiseptic treated chondrocytes with or without subsequent irrigation with sodium chloride showed a significant increase of living cells when the treatment with polyhexanide or hydrogen peroxide was followed by irrigation with sodium chloride, with $p<0.05$ (Fig. 3). There were no effects of irrigation with physiological sodium chloride after the treatment with taurolidine on number of live cells (Fig. 3). In contrast, the total cell numbers of chondrocytes showed significant increase when antiseptic treatment was followed by irrigation with sodium chloride in all cases, with $p<0.05$, respectively (Fig. 4).

Discussion

Currently, the therapy of wound and joint infections remains an unsolved problem. In contrast to treatment using antibiotics which provide a therapeutic window based on different mechanisms of action, antiseptics act through their more or less undifferentiated cell toxicity. Despite their importance in eradication of bacteria, the damage of antiseptic application to cartilage tissue is still insufficiently investigated. In our experiment a temporary irrigation of cartilage tissue in septic joint lavage or surgery was simulated by using antiseptics. After antibiotics, the additional local treatment with antiseptics plays an important role in eliminating bacteria to eradicate an infection. We investigated three of the most frequently used antiseptics in septic surgery. Our hypothesis was that antiseptics and supplemental irrigation with sodium chloride lavage are less toxic on human chondrocytes than treatment with antiseptics alone.

After incubation with polyhexanide or taurolidine, the qualitative microscopical analysis showed a considerable number of swollen chondrocytes with a defective cell structure. This may indicate cell necrosis as the end-stage of toxic cell damage [14]. In contrast hydrogen peroxide incubated chondrocytes were shrunken, globular, and showed losses of cell contact probably resulting from apoptotic cell death [14, 15]. Next, we could show a significant increase of LDH activity after as little as ten minutes incubation with

polyhexanide and taurolidine. There was absolutely no LDH activity induction after incubation with hydrogen peroxide at any time point investigated. Increased LDH activity after the treatment of human chondrocytes with polyhexanide or taurolidine indicates a loss of membrane plasma integrity as possible marker of cell necrosis [15, 16]. The absence of LDH activity after treatment with hydrogen peroxide could possibly support the hypothesis that hydrogen peroxide induced less cell necrosis and has less toxic potential than polyhexanide or taurolidine. This is supported by a study of Lo and Kim that demonstrated apoptosis of chondrocytes after incubation of different concentrations of hydrogen peroxide. The data showed apoptotic cells after six hours incubation time at a concentration of 4 mM hydrogen peroxide [17].

In our study, the investigation of total cell numbers and cell viability showed that human chondrocytes were negatively affected after the treatment with all three antiseptics in comparison to the control. Similar results were shown by Ince et al. In this study, human osteoblasts and endothelial cells were incubated with different concentrations of polyhexanide for six hours. As low a concentration of 0.0006 percent of polyhexanide demonstrated a significant decrease of total cell number and viability [18]. By comparison, antiseptic treated chondrocytes with and without the use of sodium chloride proved that cell viability of polyhexanide or hydrogen peroxide treated chondocytes followed by irrigation with sodium chloride was significantly higher than cell viability of polyhexanide or hydroxin peroxide treated chondrocytes without subsequent irrigation. There were no significant differences between single taurolidine treated chondrocytes and taurolidine treated chondrocytes with subsequent irrigation. The total cell numbers of chondrocytes treated with all three antiseptics showed significant increase when irrigated with sodium chloride.

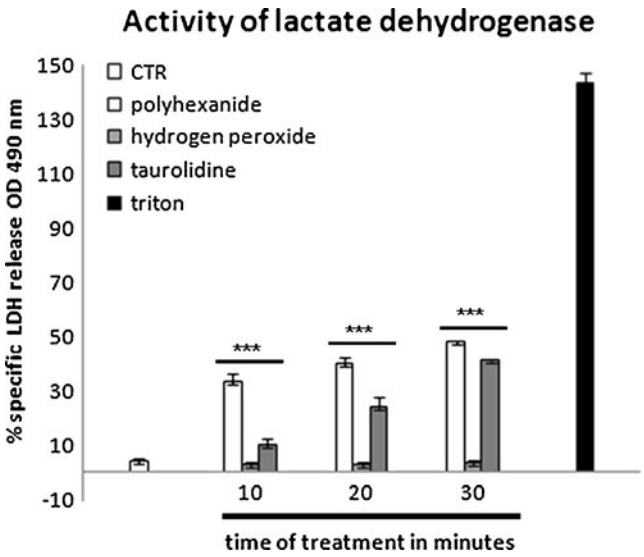

Fig. 2 LDH activity following treatment with antiseptics. Chondrocytes were incubated with polyhexanide, hydrogen peroxide or taurolidine. After the indicated time points supernatants were analysed for LDH activity by ELISA, n=6. Values given as mean ± SEM. Nonparametric Wilcoxon matched-pairs test, p as compared to CTR, ***p<0.001. The results are averaged for six independently performed experiments

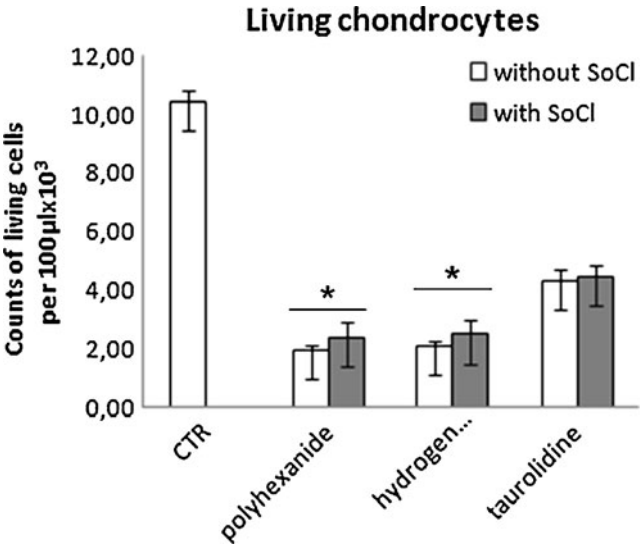

Fig. 3 Chondrocyte viability after treatment with polyexanide, hydrogen peroxide or taurolidine. Antiseptic incubated chondrocytes were compared to antiseptic incubated chondrocytes with subsequent irrigation with sodium chloride, n=6. Values given as mean ± SEM. A nonparametric Wilcoxon matched-pairs test, p as compared to CTR, *p <0.05

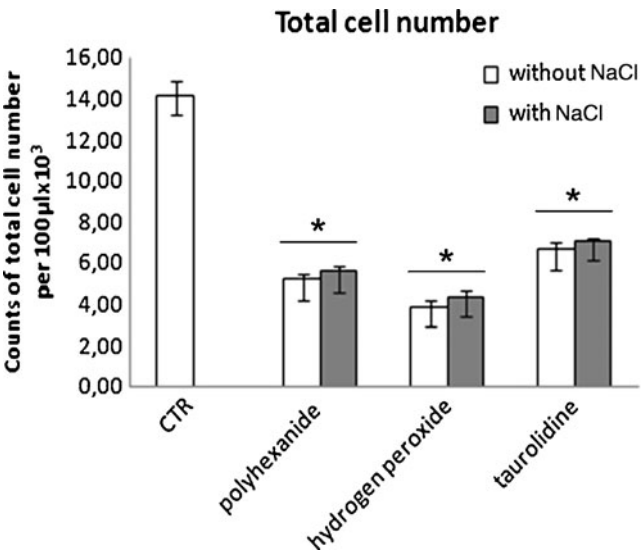

Fig. 4 Total cell numbers of chondrocytes after treatment with polyexanide, hydrogen peroxide or taurolidine. Antiseptic incubated chondroctes were compared to antiseptic incubated chondrocytes with subsequent irrigation with sodium chloride, n=6. Values given as mean ± SEM. A nonparametric Wilcoxon matched-pairs test, p as compared to CTR, *p<0.05

In in vitro studies with human cells, uncontrollable influences may play an important role such as deviation temperature, barometric pressure or contamination. Therefore, our cell model may have limitations. The results of our study do not represent the in vivo situation, as in vitro results show higher cell toxicity than in vivo examinations based on the direct chondrocyte incubation. Antiseptic solutions in vitro do not have to pass different barriers such as chondrocytes matrix.

In summary, polyhexanide, hydrogen peroxide or taurolidine induce cell death of human chondrocytes. Additionally, we could show that the subsequent application of sodium chloride has a positive effect on cell viability after treatment with polyhexanide and hydrogen peroxide, but not with the use of taurolidine.

Disclosure statement The authors have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the results and views presented in this article.

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