Preferred Use of Polyhexanide in Orthopedic Surgery

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abstract

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In orthopedic and trauma surgery, the most frequently used antiseptic is polyhexanide. Its favored application is based on prepossessing tissue compatibility in contrast to various antiseptics and a high antimicrobiological effect. Recent studies showed toxic effects of this antiseptic on human chondrocytes. The aim of this study was to further analyze the toxic and apoptotic effects of polyhexanide on primary human chondrocytes. The hypothesis of this study was that polyhexanide induces apoptosis on human chondrocytes.

Primary human chondrocytes were isolated and cultured from human donors with osteoarthritis of the knee who underwent total arthroplasty and had no indication of infection. Polyhexanide at a standard concentration of 0.04% was added to the monolayer cultures. Early and late apoptotic cells were analyzed by flow cytometric detection of annexin V, active caspases, and 7AAD, and by fluorescence microscopy using annexin V and propidium iodide staining. Flow cytometric analysis demonstrated an increase of annexin V and active caspases expression of human chondrocytes after incubation with polyhexanide. Fluorescence microscopy demonstrated a high number of annexin V positive and propidium iodide negative early apoptotic cells. The data show that polyhexanide promotes apoptosis on primary human chondrocytes in vitro, which may indicate the use of polyhexanide in septic joint surgery.

Figure: To detect apoptosis by fluorescence microscopy, chondrocytes were treated with 0.04% polyhexanide (A) or phosphate buffered saline (B) (control) for 30 minutes and stained with annexin V and propidium iodide. Apoptotic chondrocytes are shown as annexin V positive (green fluorescence) and propidium iodide negative or positive (red fluorescence).
One of the most common complications in orthopedic surgery is joint infection. Acute inflammation of a knee, hip, or shoulder is an orthopedic case of emergency and requires immediate operative and nonoperative therapy. It is evident that mechanical elimination of bacteria through lavage and surgical debridement can be supported by antiseptic substances. However, the problem is that antiseptic joint lavage induces considerable tissue toxicity. In contrast, antiseptic substances. However, the problem is that antiseptic joint lavage induces considerable tissue toxicity.1-3 In contrast to antibiotic substances, which eliminate just prokaryotic cells, antiseptics damage all cells (eu-karyotic and prokaryotic cells) that they come in contact with. Nevertheless, the local use of antiseptic is a standard procedure in septic joint surgery. One of the most commonly used antiseptics is polyhexanide. Although literature reviews have yielded controversial data about the effects of polyhexanide on human chondrocytes, its preferred use in septic joint surgery seems undisputed.4,5

Recent studies have shown the toxic effect of polyhexanide on human chondrocytes or chorion cells.4-6 In most cases, polyhexanide was the antiseptic with high prepossessing tissue compatibility and a high antimicrobial effect in contrast to various antiseptics.5,6

The mechanisms of how antiseptics induce cell death remain unclear. The examination of the kind of cell death, especially the differentiation between cell necrosis and apoptosis, could play an important role for tissue compatibility and cell regeneration. Cell necrosis is an unorganized and nonphysiologically induced destruction of tissue, with damage of the surrounding tissue. In a progressive stage, it leads to a systemic inflammatory reaction.7-9

In contrast, apoptosis is a physiological and systematic process of cell death. Characteristics of cell apoptosis are cell shrinking, absence of inflammatory reaction, activation of caspases, and DNA fragmentation.7-9 In addition, apoptosis or programmed cell death is a fundamental biological process in the maintenance of cellular homeostasis and plays an important role in physiological cell removal or inflammatory joint diseases.10 Upregulated chondrocyte apoptosis after exposure to antiseptics may play a role in the initiation and progression of joint diseases and may influence cartilage repair mechanisms.

A review of the literature revealed insufficient data about the effects of antiseptics on human cartilage and how they induce cell death. Therefore, the aim of this study was to analyze the apoptotic effects of polyhexanide on human chondrocytes.

**MATERIALS AND METHODS**

Tissue culture plasticware was obtained from TPP (Trasadingen, Switzerland). Culture medium, phosphate buffered saline, trypsin, and fetal bovine serum were obtained from Biochrom (Berlin, Germany). All other reagents were obtained from Sigma-Aldrich (Deisenhofen, Germany).

**Chondrocyte Isolation and Culture**

Chondrocyte isolation was performed from 3 human donors with knee osteoarthritis who underwent knee arthroplasty and had no indication of infection. Experimental protocols were approved by the local ethics committee.

Cartilage was minced and digested in medium containing 1 mg/mL pronase (Sigma-Aldrich) 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) at 37°C overnight.

Digested solution was filtered (70 μm Nylon; BD Falcon, Bedford, Germany) and centrifuged at 1200 rpm for 8 minutes. The supernatant was discarded, and the cell pellet was washed 3 times with phosphate buffered saline. Chondrocytes then were suspended in Dulbecco’s Modification of Eagle’s Medium/Hams-F12 with 10% fetal bovine serum and 1% penicillin/streptomycin and cultured at 37°C, 95% air and 5% carbon dioxide. Experiments were performed immediately.

**Chondrocyte Treatment**

Human chondrocytes were cultured on 24-well plates at a density of subconfluence. Undiluted polyhexanide (100 μL) at a standard concentration of 0.04% (Charité, Berlin, Germany) was added to the monolayer cultures for 15 and 30 minutes each. Phosphate buffered saline-treated chondrocytes were used as a control.

**Detection of Apoptosis by Fluorescence Microscopy**

Chondrocytes, cultured on chamber slides with a density of 2×10^4 cells/cm², were treated with 0.04% polyhexanide for 30 minutes each. Phosphate buffered saline-treated chondrocytes were used as a negative control. After washing, cells were stained with annexin V (20 μL/mL) and propidium iodide (20 μL/mL) for 15 minutes. Documentation was performed immediately by fluorescence microscopy (microscope CKX 41; Olympus, Tokyo, Japan).

**Apoptosis Analyzed by Flow Cytometry**

Chondrocytes, cultured up to 80% subconfluence on 6-well plates, were incubated with 0.04% polyhexanide for 15 and 30 minutes. Analysis of early- and late-phase apoptosis was performed by flow cytometric analysis of annexin V, active caspases, and 7AAD (CaspACE FITC-VAD-FMK; Promega, Madison, Wisconsin; Annexin-V Apoptosis Detection Kit; BD Biosciences, Franklin Lakes, New Jersey) according to the manufacturer’s protocol. Cells were stained with 10 μM of FITC-VAD-FMK for 25 minutes at 37°C, washed, and re-suspended in binding buffer. Annexin V and 7AAD were added (1:20), and cells were gently mixed and incubated for 15 minutes at room temperature in the dark. Binding buffer was added, and flow cytometric analysis was performed immediately. Cytometric analysis was evaluated using LSR II (BD...
Early apoptotic cells were defined as annexin V positive and 7AAD negative, or active caspases positive and 7AAD negative. Late apoptotic cells were defined as annexin V positive and 7AAD positive, or active caspases positive and 7AAD positive. All apoptotic cells (early and late apoptotic cells) were defined as all annexin V positive or all active caspases positive (including the 7AAD positive late apoptotic cells), respectively.

Statistical Analysis
Statistical analysis was performed using analysis of variance with Dunnett’s multiple comparison post hoc test. A P value <.05 was considered significant.

RESULTS
Analysis of different stages of apoptosis was evaluated by annexin V and propidium iodide staining examined by fluorescence microscopy of human chondrocytes treated with 0.04% polyhexanide. Evaluation showed an increased number of annexin V positive and propidium iodide negative (apoptotic) cells after an exposure time of 30 minutes (Figure 1A). Chondrocytes treated with phosphate buffered saline (negative control) showed no relevant numbers of apoptotic or necrotic cells (Figure 1B).

Annexin V and 7AAD detection examined by cytometric analysis showed an increased number of early apoptotic cells (annexin V positive and 7AAD negative) after 15 and 30 minutes of treatment with polyhexanide compared to phosphate buffered saline-treated control cells (P<.05) (Figure 2). A statistically significant increased frequency of early apoptotic chondrocytes was confirmed at the single cell level. Frequencies of late apoptotic cells (annexin V positive and 7AAD positive) were not increased (Figure 2). In addition, the expression of active caspases was analyzed on the single cell level by flow cytometry (Figure 3). An increase of early apoptotic chondrocytes (active caspases positive chondrocytes not stainable with 7AAD) was demonstrated when

Figure 1: To detect apoptosis by fluorescence microscopy, chondrocytes were treated with 0.04% polyhexanide (A) or phosphate buffered saline (B) (control) for 30 minutes and stained with annexin V and propidium iodide. Apoptotic chondrocytes are shown as annexin V positive (green fluorescence) and propidium iodide negative or positive (red fluorescence).

Figure 2: Early and late phase of apoptosis demonstrated by annexin V and 7AAD staining. Chondrocytes were treated with 0.04% polyhexanide for 15 (A) and 30 minutes (B) and analyzed by flow cytometry. Early apoptotic chondrocytes are shown as annexin V positive and 7AAD negative (% of chondrocytes). Late apoptotic chondrocytes are shown as annexin V positive and 7AAD positive (% of chondrocytes). All apoptotic chondrocytes are shown as all annexin V positive cells including 7AAD positive (% of chondrocytes). n = 3; mean ± standard error of mean; 1-way analysis of variance with Dunnett’s multiple comparison post hoc test. P as compared to control, *P<.05. Abbreviations: CTR, control; Ann V, annexin V.
treated with polyhexanide for 15 and 30 minutes compared to the control (phosphate buffered saline-treated chondrocytes). Late apoptotic cells (active caspases positive and 7AAD positive) also were not significantly increased compared with the control.

**DISCUSSION**

The most optimal therapy of joint infections remains an unsolved problem. Next to antibiotic treatment, the mechanical elimination of bacteria through joint and tissue lavage and surgical debridement can be supported by antiseptic substances. In our experiments, temporary irrigation of cartilage tissue in septic joint lavage or surgery was simulated by using polyhexanide, one of the most frequently used antiseptics. We examined and analyzed the apoptotic effects of polyhexanide on human chondrocytes after exposure times of 15 and 30 minutes.

The present study focused on antiseptic-induced cell apoptosis, which may play a key role in the initiation and progression of postinfectious arthritis of the joint, even after complete eradication of the infection. Various studies have indicated that a disbalance in chondrocyte homeostasis, induced by antiseptics or antibiotics, results in a degradation of cartilage tissue.

Our hypothesis was that polyhexanide induces apoptosis on human chondrocytes. Our study clearly demonstrated the induction of apoptotic events after treatment with polyhexanide by flow cytometric analysis of annexin V, active caspases, and 7AAD as well as by analysis of annexin V and propidium iodide staining in fluorescence microscopy. We found significantly increased levels of early apoptotic human chondrocytes when treated with polyhexanide.

In comparison to nonphysiologically induced cell destruction by cell necrosis, apoptosis is the physiological process of cell death. One difference between apoptosis and necrosis is the absence of inflammation with damaging surrounding tissue. In addition, apoptosis is a fundamental biological process in the maintenance of cellular homeostasis and plays an important role in physiological cell removal.

Therefore, although cell necrosis could lead to the damage of more deeply located chondrocytes via the accompanying inflammation, an advantage of apoptotic cell death could be the protection against cell damage of the surrounding deeper tissue of human cartilage in the joints. Thus, although both necrosis and apoptosis result in the death of chondrocytes, we hypothesize that the apoptotic cell death of chondrocytes treated with polyhexanide could be an advantage for the long-term outcome in terms of osteoarthritis development after septic arthritis.

Recent studies showed toxic effects of antiseptics in human chondrocytes. In previous studies, we compared the antiseptics polyhexanide and hydrogen peroxide in terms of cell toxicity. The studies showed that both antiseptics induce a significant decrease of vital cell numbers and inhibit proteoglycan synthesis of human chondrocytes, which possibly result in chondrocyte loss and cartilage degradation after an exposure to antiseptics.

Schaumberger et al and Kalteis et al demonstrated that polyhexanide is an antiseptic with good tissue compatibility and a high antimicrobiological efficacy.
after exposure to human chondrocytes and chorion cells compared with various others commonly used antiseptics. All of these studies did not distinguish between apoptosis and necrosis, although the differences are fundamental and may result in different long-term outcome for the cartilage and thereby the joint.

The toxicity of antiseptics against human tissue is still an unsolved problem. For that reason, treatment with an antiseptic inducing mainly apoptosis and not necrosis could play an important role in the prevention of negative long-term effects to the joint.

The results of our study do not represent an in vivo situation, as in vitro results show higher cell toxicity than in vivo examinations based on direct chondrocyte incubation. Antiseptic solutions in vitro do not have to pass through different barriers such as the synovial membrane and chondrocyte matrix, or lose antiseptic effects after contact with blood. Therefore, our in vitro cell model may have limitations. Nevertheless, we consider this tool to be suitable to investigate general effects.

Polyhexanide is an antiseptic with excellent tissue compatibility and sufficient antimicrobiological potential. This study showed that polyhexanide induces apoptosis on human chondrocytes and thus may influence cartilage repair mechanisms. This study showed polyhexanide to be an antiseptic that induces apoptosis in chondrocytes but exhibits low toxicity via necrosis. Thus, polyhexanide may be one of the most eligible antiseptics for the treatment of septic joints. Nonetheless, after such treatment, the joint should be thoroughly irrigated with sodium chloride or a comparable solution to remove any remaining antiseptic from the cartilage surface.

REFERENCES