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Doi: 10.7126/cumudj.438506 *RESEARCH ARTICLES*

EVALUATION OF CYTOXICITY OF QMIX, ETHYLENE DIAMINTETRAACETIC ACID AND CHLORHEXIDINE ON HUMAN OSTEOBLAST CELL LINE

Human Osteoblast Hücre Hattı Üzerinde Qmix, Etilen Diamintetraasetik Asit ve Klorhheksidin'in Sitotoksisitesinin Değerlendirilmesi

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ABSTRACT

Objectives: In this study, the time-dependent toxic effects of QMix ™, ethylene ediaminetetraacetic acid and chlorhexidine irrigation solutions on human osteoblast cells were as evaluated.

Methods and Materials: Human osteoblast cells were grown as monolayer cultures at 37ºC in an atmosphere of 5% CO_2 in air and 100% relative humidity. Cells were exposed to ethylene diaminetetraacetic acid (EDTA), chlorhexidine (CHX) and QMix™ for 4 hours and 24 hours. Cell viability was assessed by a 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide kit (XTT) assay. The differences in the mean viability of human osteoblast cells were evaluated statistically.

Results: There was a statistically significant difference between the mean percentage of viable cells in the test solutions and control group, both after 4 hour ($p<0.001$) and 24 hour exposure ($p=0.004$). The mean percentage of viable cells decreased statistically significantly with the increase in the time of exposure in the EDTA, CHX and QMix™ groups ($p<0.05$). After 4 hours' exposure, the EDTA and QMix showed statistically a less toxic effect than did CHX ($p<0.05$). There was no statistically significant difference between the toxicity of the irrigation solutions after 24 hours' exposure ($p>0.05$).

Conclusion: All irrigation solutions tested showed various toxic effects on the human osteoblast cell line. The increase in exposure time also increased the toxicity of irrigation solutions on the human osteoblast cell line.

Keywords: Chlorhexidine; Ethylene Diaminetetraacetic acids; Toxicity; QMix™.

ÖZ

Amaç: Bu çalışmada, QMix ™, etilen diamintetraasetik asit ve klorheksidin irrigasyon solüsyonlarının human osteoblast hücreleri hattı üzerindeki zamana bağlı toksik etkisi değerlendirdi.

Materyal ve metod: Human osteoblast hücreleri, %5 CO_2 ve %100 bağıl nem içeren bir ortamda 37ºC'de tek tabakalı olacak şekilde kültüre edildi. Hücreler 4 saat ve 24 saat boyunca etilen diamintetraasetik asit (EDTA), klorheksidin (CHX) ve QMix ™ 'e maruz bırakıldı. Hücre canlılığı 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide kiti (XTT) ile değerlendirildi. Human osteoblast hücrelerinin ortalama yaşayabilirliğindeki farklılıklar istatistiksel olarak değerlendirildi.

Bulgular: Deney solüsyonlarındaki ve kontrol grubundaki canlı hücrelerin ortalama yüzdesi arasında hem 4 saatlik ($p<0,001$) hem de 24 saatlik ($p=0,004$) uygulamanın sonrasında istatistiksel olarak anlamlı fark görüldü. Canlı hücrelerin ortalama yüzdesi, EDTA, CHX ve QMix™ grubunda uygulama süresinin artması ile istatistiksel olarak anlamlı derecede azaldı ($p<0,05$). 4 saat süresince uygulamanın ardından CHX'e göre EDTA ve QMix istatistiksel olarak anlamı derecede daha az toksik etki gösterdi ($p<0,05$). 24 saat süresince uygulamanın ardından ise irrigasyon solüsyonlarının toksisitesi arasında istatistiksel olarak anlamlı fark bulunmadı ($p>0,05$).

Sonuç: Bu çalışmada kullanılan irrigasyon solüsyonları human osteoblast hücre hattı üzerinde çeşitli derecede toksik etki gösterdi. Uygulama süresinin artması kullanılan irrigasyon solüsyonlarının human osteoblast hücre hattı üzerindeki toksisitesini de arttırdı.

Anahtar Kelimeler: Klorheksidin, Etilen diamintetraasetik asit, Toksisite, QMix ™.

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INTRODUCTION

The inability to completely clean root canals by mechanical shaping has increased the interest in irrigation solutions.[1] There are many irrigation solutions with different contents in endodontics, but none of these solutions can provide all the properties expected from an ideal irrigation solution. For this reason, efforts to determine the ideal solution are continuing.[2-4]

EDTA is a commonly-used chelating agent in endodontic treatment. It is used to remove the smear layer by acting on the inorganic components of dentin.[5] However, EDTA is used in combination with NaOCl in endodontic treatment because of a lack of sufficient antibacterial activity against endodontic bacteria when used alone.[6, 7]

Another irrigation solution commonly used in endodontic treatment is chlorhexidine (CHX). Being the most effective member of the bisguanide group, CHX is a cationic detergent with a broad antimicrobial spectrum.[8] The substantivity which allows the antimicrobial effect of CHX to persist even after application, gives it a unique feature advantage.[9] In addition, this feature prevents the formation of resistant microorganisms, and provides a great advantage in endodontic treatment.[10, 11]

QMix™ is a new solution containing EDTA, CHX, and a detergent. This newly-developed solution has the ability to remove the smear layer through the use of EDTA, contains the antimicrobial and substantivity properties of CHX, and has alow surface tension due to its detergent content. Thus, all the positive properties of the included solutions are collected in QMix™.[12-14]

One of the most studied topics in dentistry is to find the most appropriate materials that can be used in treatment, and to use them in the most appropriate way. In this sense, biocompatibility is accepted as one of the basic requirements when it comes to the use of any dental restorative material in clinical practice.[15, 16]

The lack of biocompatibility with regard to the materials used, leads to the possibility that degeneration may occur in terms of structure, proliferation, adhesion and enzyme systems, and therefore in all vital functions of the cells in the related tissue.[17] Furthermore, the biological properties of the materials used have a significant effect on the success of endodontic treatment.[18] For this reason, we aimed to investigate the biocompatibility of the EDTA, CHX and QMix™ used in root canal irrigation *in vitro*. The null hypothesis of the study tested was that there is no difference among the tested irrigation solutions in terms of toxicity.

MATERIALS AND METHODS

Cell cultures

Human osteoblast (hFOB 1.19; American Type Culture Collection, Manassas, VA; #ATCC CRL-11372) cell lines were obtained from commercial sources for these studies. Cells were cultured in Dulbecco's Modified Eagles Medium (DMEM; Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO, USA), penicillin (100 U/ml; Sigma, St. Louis, MO, USA), and streptomycin (100 g/ml; Sigma, St. Louis, MO, USA) at 37 °C in a humidified atmosphere of 5% CO2 in air. The culture medium was changed every 3 to 4 days.

96-well plastic tissue culture plates (Linbro, Flow Laboratories Inc, McLean, VA) were filled with 200 µl of medium containing 2x104 hGFs in each well. The plates were then incubated at 37 °C in a humidified atmosphere of 5% CO2 and 95% air for 24 hours to permit attachment of the cells to the plates (Figure 1). After 24 hours, the medium was removed, and the hGFs were rinsed three times with 200 µl phosphate buffered saline (PBS). All manipulations of the specimens were performed under a laminar flow hood

(NUAIRE, Plymouth, MN) to avoid contamination from outside organisms.

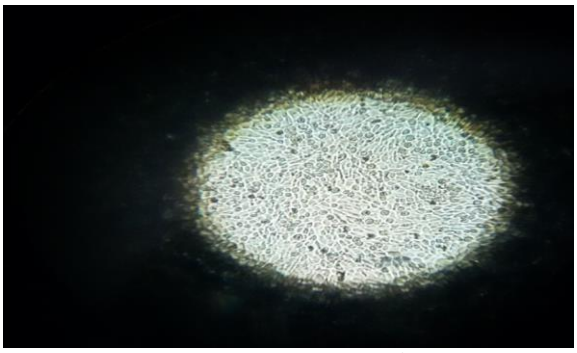

Figure 1: Inverted microscope images of cultured human osteoblast cells before processing.

The study groups were identified as follows:

Group1: Control (Fetal bovine serum)

Group 2: 2% CHX (Drogsan, Ankara, Turkey)

Group 3: 17% EDTA (Imident Med, Konya, Turkey)

Group 4: QMix™ (DENTSPLY Tulsa Dental Specialties, Tulsa, OK, USA)

Cytotoxicity assay

2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazoliumhydroxide kit (XTT) (AppliChem, Darmstadt, Germany) was used for cytotoxicity testing. An XTT solution was prepared by mixing the XTT agent (Labeling reagent)/activation agent (electron coupling reagent) at a 50/1 ratio. The intensity of the orange resulting from formazan is proportional to the number of live cells (Figure 2). The cell viability was determined by an assessment of the intensity of the orange color observed at the end of the incubation period, which was done using a micro plate reader (Multiskan™ FC MicroplatePhotometer, Ther moScientific, USA) in the reference range of 490 nm. The incidence of live cells was calculated using the following formula: Cell viability (%) = (sample/negative control)* 100 (OD 490 nm).

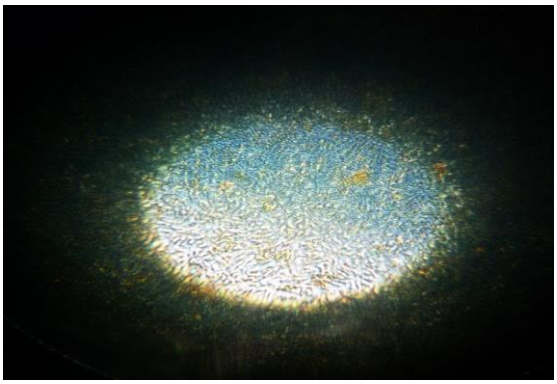

Figure 2: Inverted microscope image of the formazan crystals formed after XTT assay applied after application of test solutions on human iosteoblast cells.

At the end of the first 4 hours, the XTT solution was added to the plate to measure the 4-hour effect, and the viability of the cells in each test group was analyzed through the use of an ELISA reader (Multiskan ™ FC Microplate Photometer, Thermo Scientific, USA).

The same procedure was also applied to measure the 24-hour effect at the end of the first 24 hours, and the viability of the cells was analyzed. Thus, XTT outputs showing cell viability obtained by living and dead cells at 4 and 24 hours were obtained. For each irrigation solution used and for the control group, 5 specimens were prepared.

Statistical analysis

The data were analyzed using SPSS 13.0 (SPSS Inc, Chicago, IL) statistical software. The results of the XTT assays was calculated as percentages relative to the control (100% = no toxicity). The results were submitted to the Kolmogorov-Smirnov's test to evaluate the normal distribution. It was found that the data did not show a normal distribution. Therefore, the cytotoxicity data were analyzed using Kruskal-Wallis and Mann-Whitney's tests. The level of significance was set at 0.05.

RESULTS

The toxic effect of the test solutions on the human osteoblast cell line based on exposure time is shown in Table 1.

Table 1: The viability of human osteoblast cells after exposure to EDTA, CHX and QMix™ solutions for 4 hours and 24 hours.

	Control	EDTA	CHX	QMix™
	Mean ±*SD*	**Mean ±*SD***	**Mean ±*SD***	**Mean ±*SD***
4 hours	99.4 ± 0.8^{Aa}	73 ± 8.6^{Ab}	53.6 ± 7.3^{Ac}	57.2 ± 6.5^{Abc}
24 hours	98.6 ± 2.1^{Aa}	38.6 ± 5.7^{Bb}	35 ± 4.8^{Bb}	29 ± 6.5^{Bb}

Results are expressed as the percentage of cell viability relative to control group. Data are shown as a mean ± standard deviation. Different superscript uppercase letters in the same column indicate a statistically significant difference (p<.05). Different superscript lowercase letters in the same row indicate a statistically significant difference (p<.05).

As a result of the study, when the exposure time was 4 hours, there was a significant difference in the toxicity of the solutions (p=0.001). When the exposure time was 4 hours, CHX was more toxic than EDTA (p=0.008). However, there was no significant difference between EDTA and QMix™ (p=0.16) and CHX and QMix™ (p=0.421). When the exposure time was 24 hours, all solutions were more toxic than the control group (p=0.004). However, there was no statistically significant difference in the toxicity between EDTA and CHX (p=0.31), between EDTA and QMix™ (p=0.56), and between CHX and QMix™ (p = 0.151).

DISCUSSION

Not only the antibacterial and smear removal properties of the irrigation solutions but also the biological effect of the surrounding tissues have an important role in the success of endodontic treatment.[19] In this study, the time-dependent cytotoxicity of three different endodontic irrigation solutions (EDTA, CHX, and QMix™) was evaluated. As a result of the study, there was a statistically significant difference among the test solutions in terms of toxicity. For this reason, the null hypothesis of this study was not accepted.

No matter how much prevention is attempted during endodontic treatment, there is a risk that the solutions used may extrude from apical to periapical tissues. As a result, if the solutions are not biocompatible, they can cause damage to the surrounding tissue or delay healing in the event of an existing pathology.[20]

Osteoblast cells are important in the regeneration of bone tissue, so damage to osteoblast cells may delay the healing of periapical pathology.[21, 22] For this reason, a human osteoblast cell line is preferred in this study.

In vitro cell culture studies assess systemic, local, and other reactions that can be caused by dental materials in animal and human experimental models, thus providing information on the biocompatibility of materials. *In vitro* cell, culture cytotoxicity assays are commonly used in biocompatibility studies because they are reliable, reproducible, controllable, simple and provide short-term results.[19, 23] Various methods have been used for the evaluation of cytotoxicity, including the evaluation of flow cytometry, MTT or XTT, WST-1, WST-8 assay and lactate dehydrogenase (LDH) activity.[24] In this study, the XTT test method which is reliable and easy to use in the evaluation of cytotoxicity, was used. This test method has been used in many studies evaluating *in vitro* toxicity.[25]

In this study, except for the control group, increased exposure time with regard to all test solutions increased toxicity. Vouzara *et al*.[26] reported that an increase in the exposure time of EDTA and CHX on human lung fibroblasts cell line (6, 24 hours) increased toxicity. Li *et al*.[27] reported that CHX toxicity on the murine macrophage cell line increased with the increase in exposure time (1, 2, 4 hours). Similarly, Giannelli *et al*.[28] reported that an increase in exposure time (1, 5, 15 minutes) increased the toxicity of CHX on osteoblastic, endothelial and fibroblastic cell lines. AlKahtani *et al*.[29] reported that the toxicity of QMix™ on the human bone marrow mesenchymal stem cell line increased with increased exposure time. The result of these studies is compatible with those of our study.

Similar to the results of our study, Mollashahi *et al*.[30] have shown that the toxicity of EDTA and QMix™ solutions on human

apical papilla cell lines increased with increased exposure time, whereas in the case of the CHX group, toxicity did not increase with an increase in exposure time. This is in contrast to our study findings. This can be explained by differences in the cell line, concentration of the solution, evaluation assay, and exposure time in the two studies.

As a result of our study, when the exposure time was 4 hours, CHX and QMix™ showed higher toxicity on the osteoblast cell linethan did EDTA, and in the control group. When the exposure time was 24 hours, the toxicity of the test solutions was not statistically significant. Prado *et al*.[31] and Vouzara *et al*.[26] reported that CHX was more toxic than EDTA on the human lung cell line when the exposure time was 4 hours and on the Balb/c3T3 cell line when exposure times were 6 and 24 hours, respectively. Trevino *et al*.[32] reported that CHX is more toxic than EDTA on Human Stem Cells of the Apical Papilla in organotype root canal models. In contrast to the results of our study, Mollashahi *et al*.[30] found that EDTA and QMix™ were more toxic than CHX, and that EDTA was more toxic than QMix™ on the stem cell of human apical papilla cell line. This may be due to method differences involving different exposure times and different cell lines. Although the intrinsic mechanism leading to the high toxicity of CHX is not completely known, this may be related to the inhibition of DNA and protein synthesis, mitochondrial activity, and cell proliferation.[33] The low toxicity of EDTA can be explained by the release of dentin-derived growth factors, which areimportant for the survival, proliferation, and differentiation of cells, as noted in various studies.[32]

CONCLUSION

All the irrigation solutions used in this study showed toxic effects when compared to the control group. Increasing the contact time of the solutions used in the study with the osteoblast cells leads to increased toxicity. When exposure time was 4 hours, CHX was found to be more toxic than the other solutions. But further *in vivo* and *in vitro* investigations are needed to obtain more information about the biocompatibility of these solutions.

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None declared.

Conflict of Interest

None declared.

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