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Research / Araştırma Makalesi

Cytotoxic Effect of Dentin Desensitizers on Bovine Pulp Derived Cell Viability

Dentin Hassasiyet Gidericilerin Sığır Pulpasından Türetilen Hücre Canlılığı Üzerindeki Sitotoksik Etkisi

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ÖZ

Aim: Present study evaluated the cytotoxicity of dentin desentisizing agents on bovine pulp-derived cells (bDPCs).

Materials and Methods: Transfected bDPCs cells were exposed to original, ½ and ¼ dilutions of Shield Force Plus, Gluma and Teethmate Desensitizer for 24 h. Culture medium was used as a control group. The bDPCs viability was tested by MTT after 24 hours of exposure. Data were analyzed using the one-way analysis of variance (ANOVA) and the Tukey HSD tests.

Results: Neither of the Shield Force Plus and Teethmate Desensitizers change the survival rate of bovine pulp-derived cells when compared to the control ($p>0.05$). Gluma Desensitizer have cytotoxic effects on bovine pulp-derived cells at all dilutions ($p<0.05$).

Conclusion: Shield Force Plus and Teethmate Desensitizer were more biocompatible than Gluma on bDPCs.

Keywords: desensitizers, cytotoxicity, MTT

ABSTRACT

Amaç: Mevcut çalışmada sığır pulpasından elde edilmiş hücreler üzerinde dentin hassasiyet giderici ajanların sitotoksisitesi değerlendirdi.

Gereç ve Yöntemler: Sitotoksisite testi için, SV40 (Simian virüsü 40) büyük T antijeni ile transfekte edilmiş sığır pulpasından elde edilmiş hücreler Shield Force Plus, Gluma ve Teethmate Desensitizer'in farklı dilüsyonlarına (orjinal, % ½ ve % ½) 24 saat boyunca maruz bırakıldı. Kontrol grubu olarak tam hücre kültür ortamı kullanıldı. Sığır pulpasından elde edilmiş hücrelerin canlılığı MTT testi ile belirlendi. Veriler tek yönlü varyans analizi (ANOVA) ve Tukey HSD testleri kullanılarak analiz edildi.

Bulgular: Shield Force Plus ve Teethmate Desensitizer grupları, kontrol ile karşılaştırıldığında sığır pulpasından elde edilmiş hücreler sağkalımını değiştirmedi ($p>0.05$). Gluma, tüm konsantrasyonlarda sığır pulpasından elde edilmiş hücreler üzerinde sitotoksik etki gösterdi ($p<0.05$).

Sonuçlar: Shield Force Plus ve Teethmate Desensitizer, sığır pulpasından elde edilmiş hücreler üzerine Gluma'dan daha biyouyumludur.

Anahtar Kelimeler: hassasiyet gidericiler, sitotoksisite, MTT

Introduction

Dentin hypersensitivity (DH) is a sort of pain that is in response to thermal, tactile, chemical or osmotic stimuli without any other tooth pathology. The pain of DH classically has features as fast onset, short, and sharp. Theory about DH reveals that it is based on the stimulus of wet tubules of dentin and as a result of this to the activation of nociceptor at pulp/dentin border area. Some A-ð fibers and intradental myelinated A-ß fibers are thought to response to the stimuli which is displacing the fluid in dentin tubules and cause to the characteristic short, sharp pain of DH. Human studies demonstrated that low dentin resistance and high dentinal fluid conductivity with open dentin tubules are important feature of DH. For DH, the initiation of lesion can be stimulated with such forces leading slowly loss of intact tooth hard tissues. Despite the main factor is erosion, co-effect with abrasion is presumably the most seen event. These factors cause to wear of dentin and opening tubule. The difference in composition and flow of the saliva, can further progress the DH by influencing the accumulation rate of natural minerals on the surfaces containing open dentinal tubules. Furthermore, the location level of the gingival margin, toothbrushing with extreme hand forces and drinking high acidic beverages would make individual prone to DH.[1]

To get long term effective treatment or intercept further or new improvement of DH, it is quite a lot necessary to clear predisposing factors. The control of foods and beverages are included to this. Tooth wear generally caused by bruxism, and it is suggested that the use of an occlusal guard may be appropriate. Treatment of gingivitis and periodontitis can be predisposing factors for DH owing to the secondary dentinal exposure that may result. This should be predicted during treatment and for successful management of DH before, after and during to the treatment of gingival diseases, appropriate measures should be taken. Most frequently used treatment for DH is application of desensitizing agents, and it is conservative too. Especially when cervical hard tissue loss and cervical exposure is limited or unnoticeable. Agents used to treat DH aims to suppress nerve impulses by mechanical and chemical blockage of dentin tubules or by stopping nociceptive transduction occurring within the nerve terminal complex. According to application methods desensitizing therapy may separate to categories as at home or in the office. For professional usage, desensitizing products can be found as varnishes, gels, glass ionomers, dentin adhesives and resin sealants alongside with low level laser techniques. Generally, all treatment options should begin with choices that are non-invasive, safe and inexpensive to implement.[2]

Agents that are used to treat DH are materials which are directly in touch with dentin. Thus, toxic substances can leach to pulp within tubules of dentin. Repetitive and longtime usage of desensitizing agents is suggested to get therapeutic effects generally. For this reason, these products should have a clinically acceptable biocompatibility. Dentin desensitizing agents should be tested in terms of cytotoxicity before launching. Some of these products contain strong cytotoxic chemical components like fluoride, glutaraldehyde and HEMA (Hydroxyethyl methacrylate). Glutaraldehyde demonstrates its cytotoxic effects in wide concentration spectrum area. HEMA can obstruct the reproduction of dental pulp cells. Nonetheless, a part of dental materials that contain those ingredients with hazardous effects may have acceptable effects on in vivo tooth pulp.[3]

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With this study evaluation the in vitro cytotoxicity of different types of dentin desensitizing agents on bovine pulp-derived cells (bDPCs) is aimed. Our hypothesis on this study is that: there is no difference in cytotoxicity between tested dentin desensitizing agents.

Materials and Methods

Desensitizing agents were prepared with sterile tubes in biosafety cabinet for the cell culture tests. In each group, 10 µL desentisizing agent was dropped in 15 mL sterile tube and shaken gently. Then the Shield Force Plus was light cured from the base of tube with 2mm distance with light curing unit (BlueLEX GT-1200, Monitex) for 10 s. Culture medium (10 mL) was added per tube and incubated for 24 h in 5% CO_2 at 37°C. The material containing culture medium were sterile filtered. Besides the original concentration, 50% and 25% diluted dilutions were prepared with the culture medium.

Transfected bDPCs were routinely cultivated as described previously.[4] The bDPCs were added at a density of 5000 cells/well to cell culture plates and held on incubator for 24 h (37°C). 200 µL of material containing culture medium was added to the experimental groups, the same amount of culture medium was added to the experimental group, then incubated again for 24 h. bDPCs viability was assessed by methyltetrazolium test (MTT). Cell culture wells were bathed with phosphate buffered saline. Then, a 200-µL freshly prepared MTT solution (0.5 mg/mL in culture medium) was added to each well and plates held on incubator for 2 h at 37°C. The cells were then bathed with phosphate buffered saline. Blue formazan precipitate was extracted from mitochondria using 200 µL dimethyl sulphoxide in a shaker at room temperature for 30 min. With spectrophotometer absorption at 540 nm was mesured Optical readings from control group cultures accepted as 100% and the experimental group culture viability calculated accordingly. Total 12 wells used for each group (n = 12). Data were analyzed using SPSS (IBM) software with the one-way analysis of variance (ANOVA) and the Tukey HSD tests.

Results

Neither of the Shield Force Plus and Teethmate Desensitizer groups significantly reduced bDPCs survival when compared to the control group ($p>0.05$). But, Gluma showed cytotoxic effects on bDPCs at all dilutions when compared to the control and other experimental groups ($p<0.05$).

Table. Composition and manufacturers of tested desensitizing agents.

Materials and Manufacturers	Ingredients
Tokuyama Shield Force Plus Tokuyama Dental Corp.	Phosphoric acid monomer, Bis-GMA, 3G (TEGDMA), HEMA, water, alcohol, camphorquinone
Gluma Desensitizer Heraeus Kulzer	Glutaraldehyde, hydroxyethyl methacrylate, and purified water
Teethmate Desensitizer Kuraray Noritake Dental Inc.	Powder: Tetracalsium phosphate, Dicalcium phosphate anhydrous Liquid: Water, Preservative

The cell viability distribution of the other groups is shown in the graph by accepting the cell viability of the control group as 100% (Graph). According to ISO standards, viability below 70% is considered cytotoxic. All concentrations of Gluma are below 70%. Only the undiluted concentration of Shield Force Plus decreased the cell viability below 70%. Cell viability of all dilutions of the Teethmate desensitizer group is above 70%.

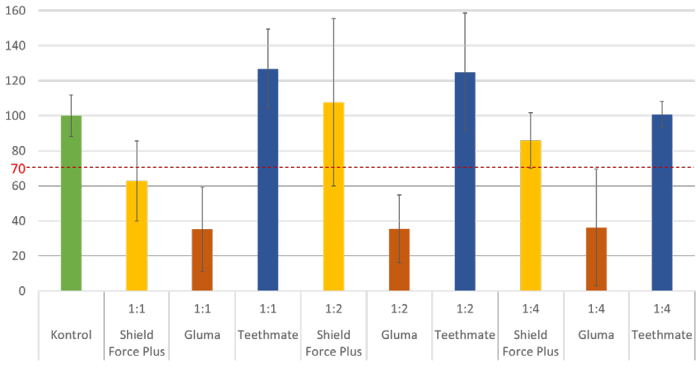

Figure. Cell viability after exposure to 100%, 50% and 25% dilutions of tested desensitizers.

Discussion

According to the findings obtained from our study, undiluted extracts of desensitizer agents except from tetracalsium phosphate (TCP), Dicalcium phosphate anhydrous containing desensitizing agent (Teethmate Desensitizer) showed cytotoxic effects on bovine dental-pulp derived cells. In this case, our null-hypothesis, "there is no difference in cytotoxicity between dentin desensitizing agents" was rejected.

Gluma Desensitizer contains glutaraldehyde (5%) and HEMA (hydroxyethyl methacrylate, 35%). Glutaraldehyde is used as disinfectant and sterilizing agent against bacteria and viruses. Glutaraldehyde causes a decrease in dentin permeability by causing coagulation of plasma proteins in the tubule fluid.[5] HEMA, a low molecular weight hydrophilic monomer, easily penetrates the dentin tissue, affecting odontoblast viability and physiological activity.[6] However, it has been reported that the cytotoxicity of HEMA is dependent on time and concentration.[7] In our study, the severe cytotoxic potential seen in whole concentrations (even 25%) of Gluma may be due its content of glutaraldehyde and HEMA. Similar to our study, Eyüboğlu et al.they showed that Gluma, Smart Protect and Systempsitizer containing glutaraldehyde have toxic effects for cells.[8]

Some of the desensitizing agents contains resin monomers have similar content to dentin adhesive agents. Resins can effectively seal dentinal tubules by forming a hybrid layer and may provide a more durable and long-lasting dentin desensitization effect.[9] These agents, after curing with light, penetrate into the tubule, make resin extensions and provide closure in the tubules,[10] so they can be considered successful in the treatment of DH. However, negative effects of bonding agents on fibroblast cells were found in in vitro studies.[11] Ratanasathien et al.[12] reported

that the cytotoxicity of monomers was ranked as Bis-GMA>UDMA>TEGDMA (3G)>HEMA. Of the desensitizers we tested in the study, Shield Force Plus contains Bis-GMA, TEGDMA (3G) and HEMA, and only its undiluted extract showed cytotoxic effects. This relatively moderate toxicity may be due to monomers that did not participate in the polymerization reaction.

Compounds such as $CaCO_3$, $Ca(OH)_2$, CaF_2, CaC_2O_4 or some calcium phosphates have been used to relieve sensitization by tubule blockage with calcium crystals. Teethmate Desensitizer contains TCP and anhydrous dicalcium phosphate to occlude clinically exposed dentinal tubules. It can transform into biological apatite within hours after the local application of Teethmate Desensitizer on dentin.[13,14] In our study, the Teethmate Desensitizer group did not affect cell viability. The reason why the Teethmate Desensitizer group is not cytotoxic may be that its content is derived from hydroxyapatite, which is the basic original material of dental hard tissues.

Conclusion

The ingredients of dentin desensitizers may affect their biocompatibility. Glutaraldehyde and resin contents increase the cytotoxicity of the desensitizing agents.

Değerlendirme / Peer-Review

İki Dış Hakem / Çift Taraflı Körleme

Etik Beyan / Ethical statement

Bu çalışma herhangi bir kongre veya sempozyumda sunulmamıştır.

Çalışma herhangi bir tez çalışması değildir.

Bu çalışmanın hazırlanma sürecinde bilimsel ve etik ilkelere uyulduğu ve yararlanılan tüm çalışmaların kaynakçada belirtildiği beyan olunur.

This study has not been presented in any congress or symposium.

The study is not any thesis work

It is declared that during the preparation process of this study, scientific and ethical principles were followed and all the studies benefited are stated in the bibliography.

Benzerlik Taraması / Similarity scan

Yapıldı – ithenticate

Etik Bildirim / Ethical statement

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Yazar Katkıları / Author Contributions

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Veri Toplanması | Data Acquisition: MÜ %20 HEÜ %30 ENB %50
Veri Analizi | Data Analysis: MÜ %40 HEÜ %40 ENB %20
Makalenin Yazımı | Writing up: MÜ %20 HEÜ %20 TK %60
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