The rabbit gingival tissue response to retraction liquids and tetrahydrozoline

Odgovor gingivalnog tkiva kunića na retrakciona sredstva i tetrahidrozolin

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Original Article

Abstract

Background/Aim. Retraction agents for temporary vertical and lateral suppression of gingival tissue as well as bleeding control and fluid flow in the gingival sulcus are expected to have maximal efficiency without irreversible damage of local tissue and adverse systemic effects. The research started from the assumption that tetrahydrozoline is a biologically more acceptable means of gingival retraction than commercially available preparations. The aim of the study was to comparatively analyse the inflammatory effects of different retraction materials and tetrahydrozoline. Methods. The effect of retraction liquid on the basis of aluminum chloride and epinephrine and tetrahydrozoline hydrochloride on gingival tissue of rabbits was investigated. The application time in the rabbit’s gingival sulcus was 7 minutes. Tissue biopsy was performed after an hour, a day, and 7 and 30 days. Tissue preparations were analyzed under a microscope. Results. The obtained results indicate a reversible damage of gingival tissues as a result of local application of aluminum chloride- and epinephrine-based retraction agents. Their use led to acute inflammatory response after an observation period of 1 and 7 days. After 30 days reparation of damaged tissue was observed. The use of tetrahydrozoline resulted in a visibly weaker inflammatory response. Conclusion. Retraction liquids insertion led to an acute inflammatory response of gingival tissue which in time assumed a chronic character. The inflammatory response to the administered tetrahydrozoline was significantly lower with complete reparation of gingival tissue. Taking this fact into account it is recommended as a potential retraction agent.

Key words: gingivitis; tissues; inflammation; animals, laboratory.

Introduction

Regular impression taking is a prerequisite for construction of high-quality fixed prosthetic appliance, thus allowing maximum accuracy possible at the contact site of biological tissue and restoration margin and ensuring integrity of periodontal structures. If a preparation margin is set at the level of or below the gingival margin, it is necessary to make it accessible to impression material by reversible temporary shift in apical direction.

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One of the most commonly used clinical methods for gingival retraction is a mechanical-chemical method which involves the use of special cotton cords of different thickness, impregnated with a solution (retraction agent) \(^1\). The role of retraction agent implies temporary vertical and lateral suppression of gingival tissue as well as bleeding control and fluid flow in gingival sulcus \(^2,3\). For this purpose, vasoconstrictors (epinephrine) and astringents (aluminum chloride, aluminum sulfate, zinc chloride) are currently used. The applied retraction agent is expected to have maximal efficiency without irreversible damage of local tissue and adverse systemic effects \(^4\).

On the other hand, some literature findings suggest that these gingival retraction agents may cause systemic reactions and local damage to periodontal tissues \(^5-7\). Systemic effect is related to epinephrine, especially if it is applied to damaged marginal gingiva and greater number of teeth, because it is contraindicated in patients with cardiovascular diseases, hyperthyroidism and diabetes \(^8,9\). Since astringents act by precipitation of proteins and show very low cellular permeability they cause no systemic effects. Astringents of moderate concentrations cause irritation of surrounding tissue, and those of high concentrations cause caustic effect, which is especially important if one takes into account the fact that there is inadequate dose control \(^4\).

Sympathomimetic vasoconstrictors also show retraction activity and are commercially available as nasal and olfactory decongestives \(^7\). Thus, these preparations having tetrahydrozoline as active component are also advantageous in dental prosthetics. In this study tetrahydrozoline was assumed to represent biologically more acceptable retraction agent when compared to commercially available products.

The aim of the study was to compare the effects of different commercially available retraction agents and tetrahydrozoline-based preparation on gingival tissue of rabbits.

**Methods**

The study included 3 commercially available retraction agents and tetrahydrozoline-based agent (Table 1).

Experimental studies were carried out in accordance with the Helsinki Declaration (Approval of the Ethics Committee of the Faculty of Medicine in Niš, No. 01-2066-2).

The experiment involved 32 experimental male rabbits, 8 weeks of age and 1.8–2.2 kg of weight. The animals were divided into 4 experimental groups, 8 rabbits for each tested material. Each animal was administered 10 mg/kg intramuscular anesthesia Zoletil\(^\text{™}\) (Virbac).

<table>
<thead>
<tr>
<th>Agent</th>
<th>Chemical content</th>
<th>Manufacturer</th>
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<tbody>
<tr>
<td>Retrargin(^8)</td>
<td>25% aluminium chloride hexahydrate, pH = 0.8</td>
<td>Galenika, Serbia</td>
</tr>
<tr>
<td>Gingiva Liquid(^9)</td>
<td>10% aluminium chloride hexahydrate, pH = 1.8</td>
<td>Roeko, Italy</td>
</tr>
<tr>
<td>Surgident(^\text{™}) retraction solution</td>
<td>8% epinephrine -HCl, pH =2.5</td>
<td>Sigma Dental Systems</td>
</tr>
<tr>
<td>Visine(^\text{™}) Original</td>
<td>0.05% tetrahydrozoline hydrochloride, pH = 5.6</td>
<td>Emasdi GmblH, Germany</td>
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</tbody>
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<thead>
<tr>
<th>Manufacturer</th>
<th>Tetrahydrozoline-based preparation on gingival tissue of rabbits.</th>
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**Results**

The tissue samples of clinically healthy gingiva of the negative controls that underwent no surgical procedures showed normal histological image of the gingiva (Figure 1a). On histological preparations of the control gingiva of a false treatment type, 1 h and a day after the treatment mild inflammatory reaction was visible (Figure 1b). After 7 and 30
days from the false treatment examined gingivae were not histologically different from the negative controls.

The gingival samples treated with different retraction agents showed different degrees of inflammatory reaction. One hour after removal of a retraction cord, gingival tissue showed slight inflammatory changes compared to the controls. All the tissue samples had foci of inflammatory infiltrate with a reduced amount of collagen fibers (Figure 2).

A day after removal of a retraction cord, inflammatory infiltration was more prominent in the tissue samples treated with retraction agents in relation to those treated with tetrahydrozoline (Visine®) (Figure 3).

After a 7 day observation period, the tissue samples treated with retraction agents showed signs of extensive acute inflammation. More intense degradation of collagen fibers was observed after application of epinephrine-based drugs (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Treatment</th>
<th>Observation period</th>
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<tbody>
<tr>
<td>1st</td>
<td>NC (u)</td>
<td>TA (u)</td>
</tr>
<tr>
<td>2nd</td>
<td>NC (u)</td>
<td>TA (l)</td>
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<tr>
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<td>NC (u)</td>
<td>TA (u)</td>
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<td>6th</td>
<td>NC (u)</td>
<td>TA (l)</td>
</tr>
<tr>
<td>7th</td>
<td>NC (u)</td>
<td>TA (u)</td>
</tr>
<tr>
<td>8th</td>
<td>NC (u)</td>
<td>TA (l)</td>
</tr>
</tbody>
</table>

TA - tested retraction agent; NC - negative control (intact control); FT – false treatment.

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Fig. 1 – a) The tissue samples of the intact controls (the negative controls) had normal amount of collagen fibres; b) Negative control preparations after one day biopsy showed mild focal inflammatory infiltrates that were later replaced with healthy connective tissue (HE, x200).

Fig. 2 – Histopathological findings after a 1-hour biopsy.

a) Retrargin® (25% aluminium chloride hexohydrate); b) Surgident® (8% epinephrine-HCl); c) Visine® (0.05% tetrahydrozoline hydrochloride). All tissue samples presented foci of inflammatory infiltrate with reduced amounts of collagen fibres (HE x100).
agent. Tetrahydrozoline-based preparation showed the least inflammatory effect in this case, where tissue fibrosis was observed on a histopathological preparation (Figure 4).

With increasing duration of observation period, there occurred tissue fibrosis, and inflammation became chronic. The newly formed fibrous tissue was the sign of defect reparation in the treated tissue. Complete reparation occurred only in case of tetrahydrozoline application (Figure 5).

Figure 6 shows different amounts of collagen in the tissue structure observed under the polarization microscope after a 7-day observation period. A small amount of collagen observed after application of retraction agents was the sign of more intense acute inflammatory response.

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Discussion

In order to make an adequate impression of the marginal line area located in or below the gingival level it is necessary to dilate and dry the gingival sulcus. The consequent ischaemia is reversible and is accompanied by reactive hyperpermia limited to a 17-minute period upon cord removal. Prolonged ischaemia might lead to tissue damage and necrosis. Changes that occur after retraction procedure usually last from 1 to 2 weeks. Still, infection or serious tissue damage may develop within this period. Changes occur at the junction line of gingiva and connective tissue and may result in periodontitis, apical migration of epithelial junction and alteration of cement surface. After a period of tissue reparation clinically acceptable apical migration of marginal gingiva must not exceed 0.1 mm.

Jokstad has shown that the retraction effect of epinephrine- and aluminum-salt-based astringents is almost equal. On the other hand, there is a wide range of adverse systemic reactions to absorption of epinephrine, which significantly reduces its indication area. Therapeutic effect of epinephrine is vasoconstriction of blood vessels, leading to increased blood pressure and heart rate. The risk increases if epinephrine in retraction agent is combined with local anaesthetic, endogenous secretion in a stressful situation, or at greater damage of gingival tissue during tooth preparation. In this study, retraction agents were administered to healthy gingival sulci, without previous tooth preparation, so as not to damage the tissue during preparation and thus jeopardize the objectivity of the results. Retraction was the result of local absorption of a retraction agent and the degree of resorption depended on the degree of tissue damage as well.

Previous studies have shown that retraction agents damage epithelium, sulcus epithelium as well as connective tissue in vitro and in vivo conditions. Changes in the periodontal tissue may be the result of mechanical damage of epithelium during application of retraction cord, but are more often related to the effect of the applied retraction agent. From the clinical point of view, the use of retraction cord without retraction agent indicates lower therapeutic effect.

The obtained results show that careful application of retraction cord cause no inflammatory changes in gingival tissue. The study results indicate a reversible damage to gingival tissue as a result of local application of aluminum-chloride- and epinephrine-based retraction agents. There were no significant changes in tissue structure 1 h after retraction agents removal. However, their use led to acute inflammatory response after an observation period of one and seven days. After thirty days reparation of damaged tissue was observed. These results are consistent with the findings of and .

Tetrahydrozoline belongs to the group of sympathomimetic vasoconstrictors or α-adrenergic agonists and is commercially available as nasal and olfactory decongestants. Systemic reactions to the use of these products are very rare, given that the maximum recommended doses are significantly higher than those required for effective gingival retraction. Studies by Bowles et al. showed a satisfactory clinical effect of tetrahydrozoline, strong local vasoconstrictive effect and absence of systemic reactions. Clinical study conducted by Tardy et al. demonstrated greater retraction efficiency of tetrahydrozoline in relation to epinephrine without adverse effects.

An in vitro study by Kopač et al. found significantly lower damage of cell cultures treated by tetrahydrozoline compared to aluminum chloride. Retraction agents represent acidic solutions with pH values from 0.8 to 3, the parameter which is considered to be major cause of periodontal tissue damage. Conversely, pH value of tetrahydrozoline is 5.6, so it is considered biologically acceptable from that point of view. An in vitro study of Nowakowska et al. showed high cell viability values of human gingival fibroblasts after treatment with tetrahydrozoline-HCl based gels. On the other hand, the authors demonstrated cytotoxic activity of astringent retraction agents.

Inflammatory changes occurred as the result of application of tetrahydrozoline were of significantly lower intensity compared to the retraction agents based on aluminum chloride and epinephrine, and resulted in a complete tissue reparation after a 1-month observation period. Tetrahydrozoline proved to be biologically acceptable in relation to the investigated retraction agents. As biocompatibility is considered to be an essential feature of dental materials, clinical use of tetrahydrozoline is recommended.

Conclusion

All the examined retraction agents led to an acute inflammatory infiltration of gingival tissue in rabbits, which eventually became chronic. The inflammatory response to the administered tetrahydrozoline was significantly lower with complete reparation of tissue. Taking this fact into account it is recommended as a potential retraction agent.

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REFERENCES


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