Effects of Sildenafil on Dental Pulp: Immunohistochemical and Ultrastructural Evaluation

Efectos de Sildenafil en la Pulpa Dental: Evaluación Inmunohistoquímica y Ultraestructural

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SUMMARY: Sildenafil is a strong peripheral vasodilator and is used to treat cardiovascular and neurosurgery. The purpose of this study was to investigate the immunohistochemical and ultrastructural effects of sildenafil on dental pulp of rats. The study was performed with adult female Wistar-Albino rats. Control group (n= 7) were fed on standard laboratory diet until surgery. The study group (n= 7) were administered sildenafil orally with orogastric tube 10 mg·kg⁻¹ once a day for 30 days. Each rat was anesthetized and incisor teeth were removed. This study examined the immunohistochemical and ultrastructural effects of sildenafil on the dental pulp in rats. The relaxation from the vessel, endothelial cell hyperplasia, moderate degeneration of collagen fibers were observed to cause degenerative changes in odontoblast with sildenafil. In the pulp tissue long-term use sildenafil is thought to cause degeneration and new vessel formation.

KEY WORDS: Sildenafil; Dental pulp; Morphological changes; Immunohistochemical study; Ultrastructural study.

INTRODUCTION

Sildenafil is known as a selective and potent inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase-5 (PDE-5). PDE-5 catalyzes the hydrolysis of cGMP. Inhibition of PDE-5 causes increased concentration of cGMP and cyclic adenosine monophosphate (cAMP) (Bella et al., 2007). PDE5 inhibitors are the treatment of erectile dysfunction [sildenafil (Viagra), vardenafil (Levitra), and tadalafil (Cialis), udenafil (Zydena)] and idiopathic pulmonary hypertension [sildenafil (Revatio)], although several other potentials have also been identified, such as memory improvement, anticancer therapy and treatment of heart diseases (Glossmann et al., 1999). The effect is seen as leading to vasodilation by increasing cyclic Guanosine Monophosphate (cGMP) by means of Nitric Oxide (NO) (Langtry & Markham, 1999; Robson et al., 2004; Salcido, 2008; Sarifakioglu et al., 2004). The effect on bone healing is an interesting current topic and there are studies showing benefits in the treatment of fractures (Akgül & Alemdaroglu, 2008).

The regulation of angiogenesis and collateral vascular formation is a complex process that involves stimulators, inhibitors and modulators. Most angiogenic factors bind to specific receptors on the endothelial cells and induce basement membrane breakdown, endothelial cell migration and proliferation. In angiogenesis, several cytokines play important roles, but the vascular endothelial growth factor (VEGF) is considered to be vital (Arras et al., 1998). VEGF binds to receptors on the endothelial cells, which results in their growth, proliferation, and migration (Ferrara, 1999).

The aim of this study was to investigate the effects of sildenafil pulp tissue with immunohistochemical and ultrastructural methods.

MATERIAL AND METHOD

In literature, the reported dose of sildenafil to be administered varies from 3–20 mg·kg⁻¹ per day in animal models. A dose of 10 mg·kg⁻¹ sildenafil achieves possible systemic protective effects in rats. Higher doses (over 20 mg·kg⁻¹) may have greater vasodilatory effects but they may
also have side effects such as hypotension, diminished tissue perfusion and excessive anti-inflammatory response whereas doses <10 mg·kg⁻¹ are less or not effective at all (Irkorucu et al., 2008, 2009). For this reason, we chose to administer sildenafil as a single daily dose of 10 mg·kg⁻¹. Rat incisor teeth were placed in 10 % formaldehyde solution for 2 weeks then fixed in Bouin’s solution for 2 days. The fixed bone samples were decalciﬁed in a 10 % acetic acid, 0.85 % NaCl and 10 % formalin solution. Paraffin blocks were then prepared in a standard manner. The four sections were taken 6–7 mm in thickness at 20 mm intervals from the paraffin blocks. These sections were stained with haematoxylin eosine and evaluated separately.

**Immunohistochemical Analysis**

**VEGF Immunohistochemistry Stain.** Antigen retrieval process was performed twice in citrate buffer solution (pH 6.0); the first for 7 min, and later for 5 min, boiled in microwave oven at 700 W. They were allowed to cool to room temperature for 30 min and washed twice in distilled water for 5 min. Endogenous peroxidase activity was blocked in 0.1 % hydrogen peroxide for 20 min. Ultra V block (Cat. No: 85-9043, Invitrogen, Carlsbad, CA, USA) was applied for 10 min prior to the application of primary antibodies (vWF antibody, rabbit-anti-vWF, 1/800, ab6994, Abcam) overnight. Secondary antibody (Cat. No: 85-9043, Invitrogen, Carlsbad, CA, USA) was applied for 20 min. Slides were then exposed to streptavidin-peroxidase for 20 min. As a chromogen, diaminobenzidine (DAB Invitrogen, Carlsbad, CA, USA) was applied for 20 min. No inflammatory cells except a few macrophage were observed in the pulp tissue. Many fibroblasts and undifferentiated mesenchymal cells were identiﬁed (Fig. 1e). Control slides were prepared as mentioned above but with omitting the primary antibodies. After counterstaining with hematoxylin and washing in tap water for 8 min and in distilled water for 10 min, the slides were mounted with Entellan.

**Ultrastructural examination.** In control pulp, subodontoblastic layer of the capillary was found to be located close to the odontoblast. Collagen fiber bundles could be seen at the periphery of some ﬁbroblasts of the control group. Many mitochondria and secretory vesicles showed in odontoblasts. No inﬂammatory cells except a few macrophage were observed in the pulp tissue. Many ﬁbroblasts showed weak expression. However, VEGF showed positive reaction for degenerative odontoblast cells in the pulp dentin border (Fig. 1d).

**Immunohistochemical examination.** Superﬁcial zone of dental pulp: ﬁbroblasts and endothelial cells were positive (Fig. 1c). In the experimental group, the thin vascular wall endothelial cells and connective tissue ﬁbroblast cells showed weak expression. Expression of VEGF in odontoblasts was observed. Depending on the dilatation of blood vessels, thinning and separation in the basal membrane, also showed an increase in inflammatory cell inﬁltration (Fig. 1b).

**DISCUSSION**

Sildenafil has signiﬁcant effects on endothelial cell function and cellular apoptosis. Sildenafil has been shown to prolong erection in aged rats via AKT-dependent eNOS phosphorylation. Phosphorylation of eNOS is coincident with enzyme activation and increased eNO release (Zhang et al., 2003). In rabbit models of cardiac ischaemia-reperfusion, sildenafil has been shown to reduce the area of ischaemia (Ockaili et al., 2002). In the developing tooth, VEGF and VEGFR-2 are expressed in odontoblasts and the inner enamel epithelium and may regulate odontoblast development and the differentiation of inner enamel epithelium to ameloblasts (Aida et al., 2005; Miwa et al., 2008). VEGF expression in pulp ﬁbroblasts and odontoblasts of human teeth is higher in immature than mature permanent teeth, suggesting a role of VEGF in tooth maturation (Wang et al., 2007). Zhang et al. used sildenafil treatment (2 mg/kg · p.o.) in a rat model of brain ischemia and found out that sildenafil promoted angiogenesis via VEGF. In our study,
Fig. 1. a) Normal appearance of dental pulp (H-E staining, Bar 100 µm). b) Sildenafil group; Degeneration of the odontoblast cells (yellow arrow), dilation of blood vessels and congestion (star), thinning and separation in the basal membrane (arrow) (H-E staining, Bar 100 µm). c) VEGF expression in the control group by immunohistochemistry. VEGF expression of fibroblasts (yellow arrow) and endothelial cells (red arrow) in dental pulp (VEGF immun-staining, Bar 100 µm). d) Sildenafil group; Weak VEGF expression in fibroblast and endothelial cells (arrow); VEGF positive expression of degenerative odontoblast cells in the pulp dentin border (yellow arrow) (VEGF immun-staining, Bar 100 µm). e) In ultrastructural section of control group; Many mitochondria and secretory vesicles in odontoblasts (yellow), regular capillary vessels of subodontoblastic layer (red arrow) (Uranyl acetate and lead citrate staining, Bar 2 µm). f) Dilatation and congestion in the capillary, basal membrane thinning (red arrow), endothelial cell hyperplasia (yellow arrow), mild degeneration of collagen fibers of the connective tissue (star), hyperplasia and vacuolar changes in odontoblast cells (light arrow) (Uranyl acetate and lead citrate staining, Bar 0.2 µm).
the pulp tissue of rat treated sildenafil, increase blood vessel dilation also result in a reduction in VEGF expression has triggered the formation of new blood vessels.

For Yaman et al. (2011), sildenafil without causing any changes to the application of alveolar bone and gingiva pulp underline that caused the increase vascularity. Also gingiva flap surgery, gingiva injury, dental pulp, or is stated to be subject to the terms of sildenafil may be useful effects such as dental trauma. In this study, the thin vascular wall endothelial cells and connective tissue fibroblast cells showed weak expression. However, VEGF showed positive reaction for degenerative odontoblast cells in the pulp dentin border. Due to vascular dilatation, degenerative effect on endothelial cell hyperplasia and odontoblasts, also reduction in VEGF protein induced the formation of new vessels. Ultrastructural examination of the pulp tissue of sildenafil group; Dilatation and congestion in the capillary, basal membrane thinning, endothelial cell hyperplasia, mild degeneration of collagen fibers of the connective tissue was observed. Also hyperplasia and vacuolar changes were observed in odontoblast cells.

As a result, sildenafil, relaxation from the vessel, endothelial cell hyperplasia and moderate degeneration of collagen fibers were observed to cause degenerative changes in odontoblast. In the pulp tissue long-term use sildenafil is thought to be caused degeneration and new vessel formation.


RESUMEN: El sildenafil es un vasodilatador periférico importante y se utiliza para tratar enfermedades cardiovascular y en neurocirugía. El propósito de este estudio fue investigar los efectos inmunohistoquímicos y ultraestructurales del sildenafil sobre la pulpa dental de ratas. El estudio se realizó con ratas Wistar albina, hembras adultas. El grupo de control (no: 7) fue alimentado con una dieta estándar de laboratorio hasta que se realizó la cirugía. El grupo de estudio (no: 7) fue tratado con sildenafil por vía oral y sonda orogástrica 10 mg·kg⁻¹ una vez al día durante 30 días. Cada rata fue anestesiada y se extrajeron los dientes incisivos. Se examinaron los efectos inmunohistoquímicos y ultraestructurales del sildenafil sobre la pulpa dentaria. Con la administración de sildenafil se observó la relacionamiento de los vasos, la hiperplasia de las células endoteliales y una degeneración moderada de fibras colágenas causando cambios degenerativos en los odontoblastos. En el tejido pulpar, el uso de sildenafil a largo plazo puede causar la degeneración y neoformación de vasos.

PALABRAS CLAVE: Sildenafil; Pulpa dental; Cambios morfológicos; Estudio inmunohistoquímico; Estudio ultraestructural.

REFERENCES


