Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

EFFICACY OF COMBINATION OF BIODENTINE AND SIMVASTATIN AS A PULP CAPPING MATERIALS IN VITAL PULPOTOMY IN DOG'S MOLARS: AN EXPERIMENTAL STUDY

NOHA ELSAYED FATHI ABDOU

Department of Pediatric dentistry, Preventive Dentistry and Dental Public Health, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt. Email: noha_elsayed1@dent.suez.edu.eg
Department of Pediatric dentistry, Preventive Dentistry and Dental Public Health, Faculty of Dentistry, Aswan University, Aswan, Egypt.

ENAS M HEGAZY

Department of Oral Biology, Faculty of Dentistry, Suez Canal University,
Email: enas_soliman@dent.suez.edu.eg, ORCID: 0000-0001-6131-3535

MOHAMED A.M. HASHEM

Anesthesiology and radiology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. Email: dr.mohamed_hashem@vet.suez.edu.eg,

MOHAMED S. FARAG

Department of Pediatric dentistry, Preventive Dentistry and Dental Public Health, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt. Email: Msherifsfarag@gmail.com

SHAIMAA MOHAMED MAHFOUZ OMER

Department of Pediatric dentistry, Preventive Dentistry and Dental Public Health, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt. Email: Shaimaa_Mahfouz@dent.suez.edu.eg,
ORCID: 0000-0002-8479-6485.

Abstract

Purpose: This study examined the histological effectiveness of using Biodentine and Simvastatin as pulpotomy agents in dog molars. **Methods:** A total of 64 teeth were used out of 4 healthy mongrel dogs. The 16 canine molars were separated into four equal groups in each dog, group A; without any treatment, group B; with Biodentine, group C; treated with Simvastatin and group D; treated with a combination of Biodentine with Simvastatin. At each predetermined interval, the dogs were sacrificed (4 and 9 weeks). The samples were then prepared for histological evaluation. **Result:** The data revealed that after 4 and 9 weeks, there was no or mild inflammatory reaction and complete dentine bridge formation for the Biodentine treated group, while Simvastatin treated group showed severe inflammatory reaction necrosis and absent bridge with a thin layer of hard tissue extending. The combination of the Biodentine and Simvastatin treated group showed mild to moderate inflammatory reaction with complete dentin bridge. **Conclusion:** The histological analysis from the current study suggested that using Simvastatin alone as a pulpotomy agent failed to preserve the pulp vitality and in the formation of continued dentin bridge sealing the exposure site of the pulp of dog's teeth while adding biodentin to Simvastatin as a combination can represent a good pulpotomy agent based on the relative good histological results.

Keywords: Biodentine, Dog's molars, Histological analysis, Pulpotomy, Simvastatin.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

INTRODUCTION

Pulpotomy is one of the most widely used clinical techniques for treating carious exposed pulps in primary teeth that are symptom-free. The rationale is based on the radicular pulp tissue's capacity to recover after the surgical amputation of the damaged or infected coronal pulp [1].

The rationale behind the regeneration approach is the preservation of healthy pulp tissue while producing reparative dentine, which is accomplished using a variety of biomaterials and medications, including calcium hydroxide (CH), mineral trioxide (MTA), aggregate, and calcium-enriched mixture (CEM) cement [2]. Other materials, including BioAggregate, Endosequence Root Repair Material (ERRM), Biodentine, and Theracal, have also been studied for pulp capping [3,4].

Biodentine is a unique tricalcium silicate-based (Ca_3SiO_5) inorganic cement with dentine-like mechanical properties; when used as pulp capping material, it is thought to have a positive impact [5,6]. When in contact with pulp, biodentine can encourage mineralization and produce reactive dentine and a dense dentine bridge. Biodentine promotes cell growth and the expression of osteopontin and dentine sialoprotein in pulp tissue[7]**.**

Simvastatin is a medication that inhibits 3-hydroxy-3-methylglutaryl coenzyme, a reductase, and lowers cholesterol. Its widespread, long-term use has demonstrated that it is a cheap medicine with a high level of safety. It is reported to have pleiotropic effects, including the promotion of angiogenesis and bone formation and anti-inflammatory qualities [8]. It also controls neuronal cell survival and boosts neurogenesis [9].

Dental pulp stem cells (DPSC) treated with Simvastatin at a concentration of 1 mol/L improved their expression of angiogenesis growth factors and odontoblastic differentiation markers, their ALP activity, and the production of mineralized nodules [10]. Additionally, a recent animal study discovered that treatment with Simvastatin at 1 mol/L improved DPSC-induced pulp regeneration in pulpotomised teeth [11]. Furthermore, DPSC-induced pulp regeneration in teeth with pulpotomies improved in an animal investigation when Simvastatin at 1 mol/L was used as a therapy [11]. These data point to Simvastatin's potential as a pulp capping substance.

The study's null hypothesis was that there is no discernible difference between Biodentine and Simvastatin used alone or in combination as a pulpotomy agent in canine molars. Therefore, this research assessed the effectiveness of using Biodentine and Simvastatin as pulpotomy agents in doges' molars.

MATERIALS AND METHODS

Study design:

The Ethical Committee approved this study of the Faculty of Dentistry, Suez Canal University (No.2020-293).

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

Sample size determination:

Four pathogen-free male mongrel dogs, 1-year-old, bodyweight 10-12 kg with a total number of 64 maxillary and mandibular posterior teeth in all dogs were selected. With a 0.55 value of effect size using an alpha (α) level of 0.05 and Beta (β) level of 0.05, i.e., power= 95%, the estimated minimum sample size (n) was a total of 64 samples,16 samples for each group.

Experimental Procedures:

1-Pre-operative care and housing:

Considering the veterinary measures, animals were housed in the faculty of Veterinary animal house, Suez Canal Univ., under hospital conditions. The dogs were housed in separate kennels that were sprayed with Neocidol (1/ 1000) (Neocidol, Zgro Pharmaceutical, Australia) to guard against external and internal parasites; subcutaneous injection of Ivermectin (Paramectin Super, Sweda Pharmaceutical, Egypt) at a dose rate of 0.1mg/kg was administrated once for each dog. Animals were fed a regular diet and received normal water under 12 hour's light/12-hour dark cycle. Temperature and humidity were managed to provide an environment that maintained the dogs in their thermo-neutral comfort zone. For dogs housed indoors, a temperature range of 18 to 29 °C was supported.

2-Anesthesia:

Each dog was premeditated with a mixture of Atropine Sulphate (El Nile Pharmaceutical, Egypt) (0.05 mg\kg body weights) and Diazepam (Chimidarou Tehran, Iran) (1.0 mg\kg body weight) intravenously to reduce salivation and resist any sudden arrhythmia. Injection intravenously of Xylazine (Xyla Ject, Adwia Pharmaceutical, Egypt) 1.0 mg/kg body weight in combination with Ketamine (Ketamor, Amoun Pharmaceutical, Egypt) at a dose rate of 10 mg/kg body weight [12].

3-Pulpotomy procedures:

- The operative area was cleaned with 3 % tincture iodine, and teeth were cleaned and polished with pumice paste to eliminate plaque and calculus. A dry field was established with the placement of gauze and cotton roll in the mucobuccal fold and rubber dam if possible, and to control saliva, the high-speed evacuation was used.

- Pulpotomies were performed in the selected teeth using a high-speed carbide bur (size 010) locked to a high-speed hand piece (40,000rpm) connected to the high-speed motor with air and water coolant to avoid heat generation; using an excavator with a sharp, sterile spoon; the coronal pulp was severed.

Normal saline was used to irrigate the pulp chamber (El Fath Pharmaceutical, Cairo, Egypt), and hemostasis was performed by using a sterile, damp cotton pellet for 2 to 3 minutes, then dressing the wound with material appropriate for the type of group: Group

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

A: included 4 (lower left posterior teeth) without any treatment as a negative control group. Group B: included 4 (lower right posterior teeth) treated with Biodentine (Septodont, Saint-Maur-Des-Fosses Cedex France). According to the manufacturer's instructions, Biodentine is available as a premeasured capsule; a capsule was placed in an amalgamator for 30 seconds. The amalgam carrier was used to introduce the mixture into the pulp chamber, uniformly placed over the floor of the pulp chamber, and compacted with a condenser. Group C: included 4 (upper left posterior teeth) that were treated with Simvastatin (Al Debeiky Pharmaceutical, Cairo, Egypt). Simvastatin powder 1.5 mg was mixed with distilled water to get a homogenous paste. The past was then delivered into the pulp chamber using a plastic instrument and compacted with a condenser. Group D: included 4 (upper right posterior teeth) that were treated with a combination of Biodentine and Simvastatin. 1:1 mixture of Biodentine and Simvastatin paste was mixed using one measurable instrument. The mixture was delivered into a pulp chamber using a plastic instrument and compacted with a condenser. Finally, an intermediate glass ionomer restorative material was used to fill all cavities (Riva self-cure, SDI, Australia).

Post-operative care:

Each animal was immediately returned to its kennel and fed on a regular soft diet, and analgesics were given to minimize the pain and discomfort after the procedures. Antibiotics were administered to prevent further infection. By the end of each experimental period, the animal was euthanatized intravenously using an overdose (0.5 gm) of 10% solution of thiopental sodium (Thiopenal, Epico Pharmaceutical, Egypt). After scarifying, both jaws were removed surgically from each dog to evaluate the pulp response for capping materials histologically.

Histological preparation:

After scarifying, block sections of both jaws were removed from each dog, and the blocks were sectioned into smaller segments containing one tooth. Immediately after, roots were sectioned approximately 5mm from the apex with a high-speed handpiece using a fine diamond bur and copious water cooling. This facilitated rapid and immediate penetration of the fixation solution (10% neutral buffered formalin) for ten days. The specimens were then further prepared for routine histological analysis and demineralized in (EDTA) for 5 months.

The histological analysis was done using a light microscope with a digital camera. All images were captured by the digital camera and transferred to the computer system by Leica software (Leica queen), and data were analyzed by ImageJ software (fuji 64).

The histopathological changes in pulp tissue were interpreted according to the criteria that Faraco and Holland established [13] (Table 1).

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

Table 1: Criteria of histopathological changes of pulp tissue and formed dentinal bridge.

Score	Pulp inflammation	The thickness of dentinal bridge	Continuity of the dentinal bridge	Morphology of dentinal bridge
1	Absent or very few inflammatory cells	>0.25 mm	Complete dentin bridge formation	Dentin or dentin associated with irregular hard tissue
2	Mild, defined as an average of <10 inflammatory cells	0.1–0.25 mm	Partial/incomplete dentin bridge formation extending to more than one-half of the exposure site	Only irregular hard tissue deposition.
3	Moderate, defined as an average of 10–25 inflammatory cells	<0.1 mm	Initial dentin bridge formation extending to not more than one-half of the exposure site	Only a thin layer of hard tissue deposition
4	Severe, defined as an average >25 inflammatory cells	Partial or absent bridge.	No dentin bridge formation.	No hard tissue deposition

Statistical Analysis:

Data were collected, calculated, tabulated, and statistically analyzed using the following statistical tests. One-way ANOVAs were used to compare groups. Bonferroni as a post hoc test was performed for pair-wise comparison.

Results

When comparing between three treated groups, there was a statistically significant difference (p<0.001) in the intensity and extensity of pulp inflammation at both evaluation periods (4 and 9 weeks). According to our findings, the Biodentine treated group only had inflammatory cells near the Dentin Bridge or area of pulp exposure. There was no or a minor inflammatory reaction overall. In contrast, Simvastatin treated group showed a severe inflammatory reaction, and all the pulp was infiltrated or necrotic. The combination of the Biodentine and Simvastatin treated group showed mild to moderate inflammatory reaction, and inflammatory cells might be next to the Dentin Bridge or area of pulp exposure or observed in part of the pulp Figure (1) Table (2).

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

Table 2: pulp inflammation.

	Score	Biodentine		Simvastatin		Combination		X^2	P value
		No.	%	No.	%	No.	%		
4 weeks	Score 1	5	62.5	0	0	0	0	27.74	<0.001** 0.0003**
	Score 2	3	37.5	0	0	3	37.5		
	Score 3	0	0	3	37.5	5	62.5		
	Score 4	0	0	5	62.5	0	0		
9 weeks	Score 1	7	87.5	0	0	0	0	38.85	<0.001** 0.0001**
	Score 2	1	12.5	0	0	6	75		
	Score 3	0	0	1	12.5	2	25		
	Score 4	0	0	7	87.5	0	0		
X^2		26.0		26.0		21.0			
P value		0.0005037**		0.0005037**		0.00377**			
The test used chi-square at P<0.05 ****;** means a significant difference									

In comparing those three treated groups, there was a statistically significant difference (p<0.001) in thickness, continuity, and morphology of the formed dentinal bridge at both evaluation periods (4 and 9 weeks). Most of the samples in the biodentine-treated group displayed complete dentine bridge formation with a thickness greater than 0.25 mm. In contrast, in the Simvastatin group, most samples showed partial or absent bridges with a thin layer of hard tissue extending. In combination with Biodentine and Simvastatin treated group, most samples showed partial/incomplete dentin or dentin associated with irregular hard tissue formation extending to more than one-half of the exposure site Figure (2) Table (3,4,5).

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

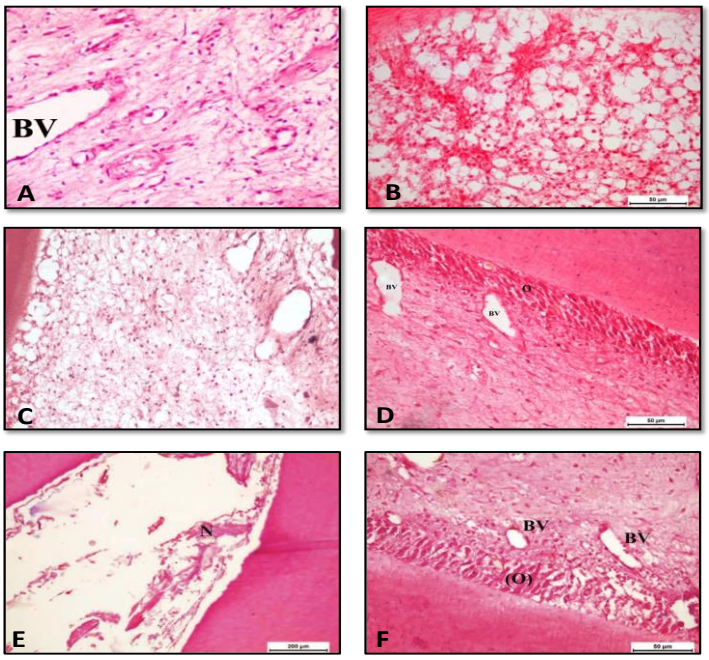

Fig. 1: Photomicrograph showing pulp tissue reaction among different treated groups; (A, B &C) at first observation period (four weeks): A) pulp tissue of the teeth treated with Biodentine showed few inflammatory cells in addition to dilatation of blood vessel (BV), B) simvastatin treated group showed severe inflammation accompanied with destruction of the odontoblastic layer, C) combination of Biodentine and Simvastatin treated group showing moderate inflammatory cell reaction. (D, E &F) at the second observation period (nine weeks): D) Biodentin treated group, the pulp tissues and odontoblastic layer (O) appeared almost normal. However, some blood vessels (BV) dilatation was noted, E) simvastatin treated group showed destruction of the odontoblastic layer in addition to signs of complete necrosis (N), F) combination of Biodentine and Simvastatin treated group showing mild inflammation, odontoblastic layer (O) appeared with some vacuolization.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

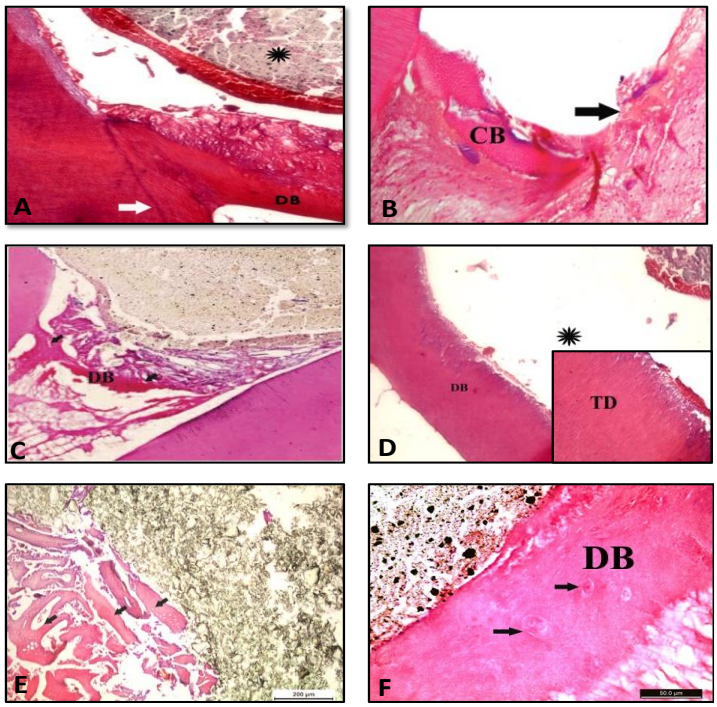

Fig. 2: Photomicrograph dentine bridge formation (A, B &C); at four weeks of Biodentine treatment, showing the formation of a thin dentin bridge (DB) sealing the exposure site continued with the lateral wall of the pulp. The black star represents the remaining restorative material, (B) Simvastatin treated group, showing the calcified bridge (CB) formation limited to the exposure site's margin. In contrast, the rest of the exposure site was covered by fibrous tissue (arrow), C) a combination of biodentin and Simvastatin, showing a thin dentin bridge (DB) with cellular inclusion (arrows), sealed the exposure site. (D, E &F); at nine weeks; (D) Biodentine treated group at nine weeks, showing the formation of thick dentin bridge (DB) sealed at the exposure site (*), (E) simvastatin treated group showing an incomplete bridge formed of scattered calcific tissue (arrows) denoting failure of intact calcified bridge formation, F) h combination of Biodentine and Simvastatin showing the formation of thick dentin bridge (DB) with cellular inclusion (arrows) sealed at the exposure site.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

Table 3: Thickness of dentinal bridge

		Biodentine		Simvastatin		Combination		X^2	P value
		No.	%	No.	%	No.	%		
4 weeks	Score 1	4	50	0	0	1	12.5	20.95	0.002** <0.001**
	Score 2	3	37.5	0	0	5	62.5		
	Score 3	1	12.5	3	37.5	2	25		
	Score 4	0	0	5	62.5	0	0		
9 weeks	Score 1	6	75	0	0	2	25	26.42	0.0002** <0.001**
	Score 2	2	25	0	0	5	62.5		
	Score 3	0	0	2	25	1	12.5		
	Score 4	0	0	6	75	0	0		
X^2		17.0		21.0		14.0			
p-value		0.0174*		0.00377**		0.152			
Test used: chi-square at P<0.05 **; means a significant difference									

Table 4: Continuity of the dentinal bridge

		Biodentine		Simvastatin		Combination		X^2	P value
		No.	%	No.	%	No.	%		
4 weeks	Score 1	5	62.5	0	0	0	0	27.00	<0.001** 0.0001**
	Score 2	3	37.5	0	0	6	75		
	Score 3	0	0	6	75	2	25		
	Score 4	0	0	2	25	0	0		
9 weeks	Score 1	6	75	0	0	0	0	31.018	<0.001** 0.00008**
	Score 2	2	25	1	12.5	7	87.5		
	Score 3	0	0	5	62.5	1	12.5		
	Score 4	0	0	3	37.5	0	0		
X^2		21.0		18.3		29.0			
P value		0.00377**		0.01071**		0.0001447**			
Test used: Chi square at P<0.05 **; means significant difference									

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

Table 5: Morphology of dentinal bridge

		Biodentine		Simvastatin		Combination			
		No.	**%**	**No.**	**%**	**No.**	**%**		
4 weeks	**Score 1**	6	75	0	0	3	37.5	16.42	0.012**
	Score 2	2	25	1	12.5	3	37.5		
	Score 3	0	0	5	62.5	2	25		
	Score 4	0	0	2	25	0	0		
9 weeks	**Score 1**	7	87.5	0	0	3	37.5	13.78	0.032**
	Score 2	1	12.5	3	37.5	4	50		
	Score 3	0	0	4	50	1	12.5		
	Score 4	0	0	1	12.5	0	0		
X^2		29.0		12.0		8.00			
***p*-value**		0.0001447**		0.32		0.65			
Test used: Chi square at *p*<0.05 **; means significant difference									

Discussion

Because the mechanism of dentin synthesis and induction in humans and dogs is the same, dogs were used for this study [14]. The pulp size also offers an appropriate sample for histopathological analysis. In addition, dogs have a good number of teeth, enabling the comparison of multiple materials or techniques on the same dog[15]. The selected dogs' ages ranged from 12 to 18 months to ensure that all teeth in their mouth were permanent. The experiment was carried out on premolars and the molars as they are relatively larger, so a large number of histological specimens could be obtained from them**.**

Our results revealed an absence or mild inflammatory reaction at both evaluation periods (4 and 9 weeks) for Biodentine treated group. This may be attributed to the anti-inflammatory repairing capacity, high biocompatibility, and bioactivity of Biodentine. This current result was compatible with Nowicka et al. [16], who concluded that no inflammation process in human dental pulp capped with Biodentine and Mineral trioxide aggregate, and agreed with De Rossi et al. [17], who concluded that no pulpal or periapical inflammatory response in dog's molars treated with Mineral trioxide aggregate and Biodentine. Moreover, this agrees with Khatab and Deraz [18], who concluded that Biodentine treated teeth showed normal soft tissue organization of pulp tissue with mild inflammation after three months.

This result was contrary to Hoseinifar et al. [19] cited that after histological evaluation of the human pulp response to direct pulp capping, the Biodentine group displayed significantly higher inflammation when compared to the MTA, CEM cement, or control groups. In addition, De Rossi et al. [17] recorded that high Biodentine alkalinity may cause neighboring cells, tissue proteins, and a few microorganisms present in exposed areas to denaturation, resulting in chronic and mild inflammation. As the material is set, changes

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

in pH and cell injuries subside with an improvement of inflammatory intensity and extensity.

In the present study, Simvastatin treated group showed severe chronic and acute inflammatory reactions, and all the pulp was infiltrated with inflammatory cells or necrotic; the considerable increase in the percentage of apoptotic cells brought on by the cytotoxic effects of statins may be the cause of this [20,21].

Our results agreed with the observations of Aminabadi et al. [22] who found that in all inves tigated Simvastatin groups compared to the calcium hydroxide group, there was more pulpal inflammation and necrosis, also Jia et al. [11] concluded that Simvastatin suppressed dental pulp stem cells (DPSC) growth slightly. However, Xu et al. [23] demonstrated that Simvastatin had no effect on DPSC proliferation at lower concentrations and that at higher concentrations, it caused cell death. Other cell types, such as osteoblastic cells, vascular smooth muscle cells, and neuronal cells, also displayed comparable behaviors. On the other hand, our finding was contrary to several studies [10,24–26] that showed the beneficial action of Simvastatin on DPSC differentiation and its potent anti-inflammatory action that can enhance pulpal regeneration.

The complex of both Biodentine and Simvastatin showed mild to severe chronic and acute inflammation. Inflammatory cells may have been present near the dentin bridge, in the area where the pulp was exposed, or in some of the pulp (in one-third or more of the pulp); these may be due to the presence of Biodentine that had high biocompatibility and bioactivity properties which might suppress the cytotoxic effect of Simvastatin. On the other hand, Varalakshmi et al. [27] concluded that combining Smv+MTA showed lower cell growth, decreased Alkaline Phosphatase activity, and decreased levels of mineralization markers than combining Smv+αTricalcium phosphate cement and suggested that statin can be delivered locally using a-TCP as a pulp capping material to hasten the production of reparative dentin.

This study's findings demonstrated the development of a dent in bridge at the point where the pulp and pulp capping material meet; this development may be interpreted as a positive response to stimulation and an indication of healing, as agreed by Hamdy et al., 2018 [28], who suggested that healing process in vital pulp treatment requires the foundation of hard tissue bridge which is crucial for the protection of the pulp from other stimuli, prevention of secondary pulp infection, and avoiding obliteration of the pulp. Moreover, the crucial pulp therapy's main success determinant is compact hard tissue development without bacterial invasion. Several studies 12/23/2022 2:43:00 PM recorded that the existence of a dentinal bridge does not guarantee the pulp's health nor shield it from microbial threats. But it might also indicate healing or a response to annoyance.

Our histological findings demonstrated that all treatment groups had significantly different dentin thickness, continuity, and morphology at four and nine weeks. In the Biodentine treated group, most of the samples had complete dentine bridge creation with thickness

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

>0.25mm along with the commencement of tubular dentin and odontoblast cell production, consistent with previous studies[7,29–31].

Our histological analysis showed that samples treated with Simvastatin had a thin layer of hard tissue covering no more than half of the exposure site, which may be related to the cytotoxic impact of statins that kill dental pulp stem cells (DPSCs), odontoblasts, or cells that resemble odontoblasts, preventing exposure site from repairing as recorded by Okamoto et al. [32] and concluded that after eight weeks, hard tissue production at all doses of Simvastatin (0.1, 1, and 10 mol/L) was minimal and that DPSCs pretreatment with Simvastatin at a dosage of 1 mol/L produced a disproportionately higher amount of mineralized tissue than other preparations. Levels of Simvastatin. This was similar to Aminabadi et al. [22], who concluded that statin treatment results in a lower rate of hard tissue formation than the widely used calcium hydroxide. This result was contrary to Min et al. [33], who suggested that Simvastatin effectively promoted reparative dentinogenesis and regenerated damaged dental pulp tissues. Moreover, Jia et al. [11] concluded that Simvastatin promotes DPSC-induced pulp and dentin regeneration following pulpotomy and stimulates DPSCs mineralization both in vivo and in vitro.

The majority of samples in the group that received both Biodentine and Simvastatin treatment exhibited partial or incomplete dentin or dentin associated with irregular hard tissue formation, extending to more than half of the exposure site but not completely closing the exposure site with a thickness of 0.1-0.25mm. In our opinion, it might be due to the presence of Biodentine, which has high biocompatibility and bioactivity properties and might suppress the cytotoxic effect of Simvastatin that may induce DPSCs that regenerate dentin.

CONCLUSION

-Biodentine showed a very high histological success rate.

-Simvastatin alone as a pulpotomy agent failed to preserve the pulp vitality and form the continued dentin bridge.

-Combination of Biodentine and Simvastatin showed a good histological result.

References

1. Fuks AB, Eidelman E. Pulp therapy in the primary dentition. Curr Opin Dent. 2013;1:556–63.
2. Parisay I, Ghoddusi J, Forghani M. A Review on Vital Pulp Therapy in Primary Teeth. Iran Endod J. 2015;10:6–15.
3. Chang S-W, Lee S-Y, Kum K-Y, Kim E-C. Effects of ProRoot MTA, Bioaggregate, and Micromega MTA on odontoblastic differentiation in human dental pulp cells. J Endod. 2014;40:113–8.
4. Shayegan A, Jurysta C, Atash R, Petein M, Abbeele AV. Biodentine used as a pulp-capping agent in primary pig teeth. Pediatr Dent. 2012;34:e202-208.
5. Jalan AL, Warhadpande MM, Dakshindas DM. A comparison of human dental pulp response to calcium hydroxide and Biodentine as direct pulp-capping agents. J Conserv Dent. 2017;20:129–33.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

6. Jeanneau C, Laurent P, Rombouts C, Giraud T, About I. Light-cured Tricalcium Silicate Toxicity to the Dental Pulp. J Endod. 2017;43:2074–80.
7. Tran XV, Gorin C, Willig C, Baroukh B, Pellat B, Decup F, et al. Effect of a calcium-silicate-based restorative cement on pulp repair. J Dent Res. 2012;91:1166–71.
8. Liu C, Wu Z, Sun H-C. The effect of simvastatin on mRNA expression of transforming growth factor-beta1, bone morphogenetic protein-2 and vascular endothelial growth factor in tooth extraction socket. Int J Oral Sci. 2009;1:90–8.
9. Miron VE, Rajasekharan S, Jarjour AA, Zamvil SS, Kennedy TE, Antel JP. Simvastatin regulates oligodendroglial process dynamics and survival. Glia. 2007;55:130–43.
10. Karanxha L, Park S-J, Son W-J, Nör JE, Min K-S. Combined Effects of Simvastatin and Enamel Matrix Derivative on Odontoblastic Differentiation of Human Dental Pulp Cells. Journal of Endodontics. 2013;39:76–82.
11. Jia W, Zhao Y, Yang J, Wang W, Wang X, Ling L, et al. Simvastatin Promotes Dental Pulp Stem Cell-induced Coronal Pulp Regeneration in Pulpotomized Teeth. J Endod. 2016;42:1049–54.
12. Inc VT. Anesthesia and Analgesia for Veterinary Technicians 4th edition | 9780323055048, 978-0-323-05504-8 [Internet]. VitalSource. 2007 [cited 2022 Sep 25]. Available from: https://www.vitalsource.com/products/anesthesia-and-analgesia-for-veterinary-john-thomas-philip-lerche-v9780323055048
13. Faraco IM, Holland R. Response of the pulp of dogs to capping with mineral trioxide aggregate or a calcium hydroxide cement. Dent Traumatol. 2001;17:163–6.
14. Yamamura T. Differentiation of pulpal cells and inductive influences of various matrices with reference to pulpal wound healing. J Dent Res. 1985;64 Spec No:530–40.
15. El Ashry SH, Abu-Seida AM, Bayoumi AA, Hashem AA. Regenerative potential of immature permanent non-vital teeth following different dentin surface treatments. Exp Toxicol Pathol. 2016;68:181–90.
16. Nowicka A, Lipski M, Parafiniuk M, Sporniak-Tutak K, Lichota D, Kosierkiewicz A, et al. Response of human dental pulp capped with biodentine and mineral trioxide aggregate. J Endod. 2013;39:743–7.
17. De Rossi A, Silva LAB, Gatón-Hernández P, Sousa-Neto MD, Nelson-Filho P, Silva RAB, et al. Comparison of pulpal responses to pulpotomy and pulp capping with biodentine and mineral trioxide aggregate in dogs. J Endod. 2014;40:1362–9.
18. Khatab A, Deraz EM. Clinical, Radiographical and Histopathological Evaluation of Biodentine Versus Formocresol In Primary Teeth Pulpotomy. Egyptian Dental Journal. The Egyptian Dental Association (EDA); 2019;65:3199–212.
19. Hoseinifar R, Eskandarizadeh A, Parirokh M, Torabi M, Safarian F, Rahmanian E. Histological Evaluation of Human Pulp Response to Direct Pulp Capping with MTA, CEM Cement, and Biodentine. J Dent (Shiraz). 2020;21:177–83.
20. Campos-Lara M, Mendoza-Espinoza JA. Cytotoxic evaluation of fluvastatin and rosuvastatin and effect of fluvastatin in the hela cell cycle. African Journal of Pharmacy and Pharmacology [Internet]. 2011 [cited 2022 Sep 29];5. Available from: https://www.readcube.com/articles/10.5897%2Fajmr10.342
21. Glynn SA, O'Sullivan D, Eustace AJ, Clynes M, O'Donovan N. The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, simvastatin, lovastatin and mevastatin inhibit proliferation and invasion of melanoma cells. BMC Cancer. 2008;8:9.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 65 Issue 12 | 2022
DOI 10.17605/OSF.IO/DGEQ8

22. Asl Aminabadi N, Maljaei E, Erfanparast L, Ala Aghbali A, Hamishehkar H, Najafpour E. Simvastatin versus Calcium Hydroxide Direct Pulp Capping of Human Primary Molars: A Randomized Clinical Trial. J Dent Res Dent Clin Dent Prospects. 2013;7:8–14.
23. Xu L, Wang C, Shen WW, Qi R. [Effects of simvastatin nano-liposomes on osteogenic differentiation of bone marrow stromal cells]. Beijing Da Xue Xue Bao Yi Xue Ban. 2014;46:883–8.
24. Dianat O, Mashhadiabbas F, Ahangari Z, Saedi S, Motamedian SR. Histologic comparison of direct pulp capping of rat molars with MTA and different concentrations of simvastatin gel. J Oral Sci. 2018;60:57–63.
25. Jung JY, Woo SM, Kim WJ, Lee BN, Nör JE, Min KS, et al. Simvastatin inhibits the expression of inflammatory cytokines and cell adhesion molecules induced by LPS in human dental pulp cells. Int Endod J. 2017;50:377–86.
26. Lee S-Y, Min K-S, Choi G-W, Park J-H, Park S-H, Lee S-I, et al. Effects of simvastain and enamel matrix derivative on Portland cement with bismuth oxide-induced growth and odontoblastic differentiation in human dental pulp cells. J Endod. 2012;38:405–10.
27. Varalakshmi PR, Kavitha M, Govindan R, Narasimhan S. Effect of statins with α-tricalcium phosphate on proliferation, differentiation, and mineralization of human dental pulp cells. J Endod. 2013;39:806–12.
28. Hamdy M, Fayyad DM, Eldaharawy MHA, Hegazy E. Physical properties of different Pulp Capping Materials and Histological Analysis of their effect on Dogs'Dental Pulp Tissue Healing. Egyptian Dental Journal. The Egyptian Dental Association (EDA); 2018;64:2657–67.
29. Laurent P, Camps J, About I. Biodentine(TM) induces TGF-β1 release from human pulp cells and early dental pulp mineralization. Int Endod J. 2012;45:439–48.
30. Nowicka A, Wilk G, Lipski M, Kołecki J, Buczkowska-Radlińska J. Tomographic Evaluation of Reparative Dentin Formation after Direct Pulp Capping with Ca(OH)2, MTA, Biodentine, and Dentin Bonding System in Human Teeth. J Endod. 2015;41:1234–40.
31. Zanini M, Sautier JM, Berdal A, Simon S. Biodentine induces immortalized murine pulp cell differentiation into odontoblast-like cells and stimulates biomineralization. J Endod. 2012;38:1220–6.
32. Okamoto Y, Sonoyama W, Ono M, Akiyama K, Fujisawa T, Oshima M, et al. Simvastatin induces the odontogenic differentiation of human dental pulp stem cells in vitro and in vivo. J Endod. 2009;35:367–72.
33. Min K-S, Lee Y-M, Hong S-O, Kim E-C. Simvastatin promotes odontoblastic differentiation and expression of angiogenic factors via heme oxygenase-1 in primary cultured human dental pulp cells. J Endod. 2010;36:447–52.