



In vivo Histological Evaluation of Effect of Direct Pulp Capping with BMP7 with and without Laser Therapy

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Abstract

Background: Direct pulp capping is defined as the capping of exposed vital pulp with a bioactive material to maintain dental pulp viability and facilitate the formation of reparative dentin. Application of laser irradiation in vital pulp therapy has been proposed as another alternative to pharmacotherapeutic techniques. Bone morphogenetic proteins have been proposed as potential capping agents in direct pulp-capping.

Aim: Histological evaluation of effect of bone morphogenic protein (BMP-7) on pulp regeneration with and without laser therapy.

Materials and methods: forty rabbit incisors were divided into control groups (10 teeth) were pulp capped by mineral trioxide aggregate (MTA), while experimental groups (30 teeth):1- laser therapy was applied 2-bone morphogenic protein (BMP applied, 3-Laser irradiation and BMP were used. The teeth then extracted and prepared for the histological sections after 1 and 4 week healing periods. The histological sections then studied by measuring the predentin thickness, inflammatory cell response score, and odontoblasts score of the pulp tissue.

Results: Effect of applied agents showed variable response of pulp tissue on histological examination. The predentin thickness recorded highest values in 1week duration, high significant differences was recorded between (1 and 4 weeks). There was no significant difference between the BMP group and BMP /Laser groups.

Conclusion: As illustrated by the obtained results laser therapy did not add prominent effect on efficacy of BMP in term of acceleration of healing process of pulp tissue which was responsive to BMP.

Key words: Pulp regeneration, Laser therapy, Bone morphogenic protein.

INTRODUCTION

The dental pulp is a unique, specialized loose connective tissue that contains mainly interstitial fibroblasts in cell-rich zone in the center of the pulp and odontoblasts that align dentin surface in the periphery (the odontoblast layer). Stem/progenitor cells reside among interstitial fibroblasts and perhaps adjacent to blood vessels. The primary goal of regenerative endodontics is to restore the vitality and functions of the dentin-pulp complex, as opposed to filing of the root canal with bioinert materials^[1]. Direct pulp capping is the most indicated in pulps exposed by mechanical agents or traumas. It involves the application of a biomaterial directly over the exposed pulp, in order to stimulate the differentiation of new odontoblasts from stem cells of the pulp. This, in turn, enables a restorative reaction through the formation of tertiary reparative dentin, maintaining pulp vitality and its normal functions^[2, 3]. The biomaterials used in vital pulp therapy should have the property of stimulating the formation of reparative dentin, maintaining pulp vitality, and providing effective bactericidal and/or bacteriostatic action and pulp sealing^[4, 5].

The strategies of regeneration depend on the state of the dental pulp: the stage of inflammation and volume of damaged and infected tissues. For less important lesions, it is possible to perform an indirect or direct capping on the pulp with the biomaterials^[6].

Direct pulp capping procedure is a therapeutic application of a drug on exposed tooth pulp in order to ensure the closure of the pulp chamber and to allow the healing process to take place^[7].

For more significant lesions, drilling to access the endodontic space is needed to apply the biomaterials in the coronal part or deeper in the root pulp. When the tooth is immature, the induction of a calcified barrier becomes

required to close the incompletely formed injured root (apexification).

The odontoblasts are able to respond to injury (e.g., caries, cavity preparation) and up-regulate its secretory activity leading to deposition of reactionary dentin. The nature of the signaling process from this stimulus may be rather variable and has been hypothesized to result from the release of growth factors and other bio-active molecules from the dentin matrix during injury^[8]. The important feature of this response is that there is no cell renewal and the odontoblasts have to survive the injury. This is in contrast to reparative dentinogenesis, where the intensity of the injury is of a magnitude that results in odontoblast death and cell renewal by a new generation of odontoblast-like cells^[8, 9]. The dental pulp contains progenitor/stem cells, which can proliferate and differentiate into dentin-forming odontoblasts. Dentin formation following pulp capping is known to involve differentiation of odontoblast-like cells that form reparative dentin and biosynthetic activity by surrounding primary odontoblasts. A variety of bioactive molecules may participate in the signaling of reactionary dentinogenesis, although relatively few have been characterized. Members of the TGF- β family, including TGF- β 1, TGF- β 3, and BMP-7, are capable of up-regulation of secretion^[10, 11].

Various laser systems have been increasingly and successfully applied in direct pulp capping. Lasers offer excellent characteristics in terms of hemostasis and decontamination for field preparation during direct pulp capping treatment. Clinicians should consider the characteristics of each wavelength, the emission mode, irradiation exposure time, power, type of laser tip, and the distance between the laser tip and the surface being irradiated^[12].

Bone Morphogenic Protein (BMP)

Growth factors can regulate a variety of cellular processes; they are intercellular signaling molecules promoting cell migration, proliferation, differentiation and maturation depending on their type ^[13]. Bone morphogenic proteins (BMPs) comprise a subgroup of the TGF β superfamily and are involved in many biological activities including cell proliferation, differentiation, and apoptosis ^[14]. BMP2, BMP4, BMP7 and BMP11 are of clinical significance due to their role in inducing mineralization ^[15, 16, 17].

MATERIALS AND METHODS

Materials: BMP7 (Human recombinant BMP7 ab50100, USA), Ketamine HCL (Germany), MTA (Poland).

Study Design

Total samples of 40 rabbit teeth were selected for the induction of pulp exposure and removal of the coronal pulp tissue then the experimental procedure proceeded after appropriate irrigation with sterile saline and hemostasis of the pulp tissue achieved using sterile cotton pellets and saline solution ^[5]. The collected teeth were divided into groups according to the applied agent for the healing periods (1 and 4 weeks) as follow:

1. Control group (C): (10 teeth) where only blood clot filled the pulp cavity and sealed coronally by (MTA) material.
2. Laser group (L): (10 teeth) pulp exposed to laser (808 nm, 10 W, continuous wave for 2 s per 1 mm) then sealed by MTA.
3. Bone morphogenic protein group (B): (10 teeth), where pulp regeneration procedure carried out by addition of BMP-7 (100 ng/ml), and (MTA) sealing the coronal access.
4. Bone morphogenic protein /laser group (B/L): (10 teeth), where pulp regeneration procedure carried out by diode laser exposure to the remained pulp tissue (808 nm, 10 W, continuous wave for 2 s per 1 mm), addition of BMP-7 (100 ng/ml), and (MTA) sealing the coronal access.

The samples were collected and prepared for routine histological examination.

Pulp capping procedure:

The surgical procedure was performed under a well sterilized condition and gentle operative technique. Every animal was weighted to calculate the dose of general anesthesia which was given to it. The micro engine with its hand piece and surgical bur (2mm) were prepared for operation. The general anesthesia was induced by Intra muscular injection of xylazine 2% (0.4 mg/kg B.W.), plus ketamine HCL 50mg (40 mg/kg- B.W.). Teeth cleaned with alcohol and covered with a piece of cotton damped in 75% alcohol and hydrogen peroxide for 10 minutes. Pulp chamber was opened under aseptic conditions with #4 round bur, by intermittent drilling under irrigation with sterile normal saline. Saline solution and sterile cotton pellets were used to control hemorrhage ^[18]. A guide hole was made until pulp was reached, then after pulp exposure

removal of coronal part was done using a barbed broach for a distance of 3mm depth. Irrigation of normal saline was then performed until hemostasis achieved, then the cavity dried gently by cotton. Procedure proceeded as follows:

1. By using laser handpiece, tip No. (E4), bioactivation wavelength of (940nm) was applied to the remaining pulp tissue for 2 seconds - For groups L, and B/L.
2. By using the micropipette, a 100um drop of BMP solution was applied to the cavity - For groups B, and B/L.
3. After mixing of the powder and liquid of MTA material, it is applied by using ash instrument to seal the cavity - For all groups.

Histomorphometric analysis

a. Predentin thickness analysis.

The stained sections were viewed under Trinocular research microscope (Olympus BX51). The distance between the odontoblastic cell layers of the pulp to the border line of the dentin was considered for the measurement of the predentin thickness. Images of the predentin width were captured using a 3 chip CCD camera (Proview, Media Cybernetics) with $\times 40$ apochromatic objective. All captured images were stored in a hard disk, and measurements (in microns) were carried out on these images using the tools of the Image measurement – ImageJ software ^[19].

b. Inflammatory cell response.

Dental pulp was examined for the inflammatory cell response at power $\times 10$ of magnification according to the following:

- Grade 1. Absent or very few inflammatory cells.
- Grade 2. Mild or average number less than 10 inflammatory cells.
- Grade 3 Severe inflammatory lesion appearing as an abscess or dense infiltrate involving one-third or more of the coronal pulp.
- Grade 4. Completely necrotic pulp ^[20].

c. Scores odontoblastic cell layer.

The dental pulp of the sample was examined at power $\times 40$ of magnification according to the following scores:

- 1 Palisade pattern of cells.
- 2 Presence of odontoblast cells and odontoblast-like cells.
- 3 Presence of odontoblast-like cells only.
- 4 Absent ^[21].

Statistical Analysis

Data were analyzed using SPSS (statistical package of social science) software version 24. The following statistical data analysis approaches were used in order to analyze and assess the results of this study:

1. Graphical presentation by using Line charts.
2. Inferential data analysis to accept or reject the statistical hypotheses, which included the following:

- Analysis of Variance (ANOVA) for equality of means of several independent Groups with least significant difference (LSD and Games-Howell) Methods.
- General Linear model (ANOVA) for two or three factors.

RESULTS

Histological findings

One week duration

Microphotograph view of control group after one week of pulp capping with MTA material shows inflammatory infiltration, alongside the odontogenic layer, odontoblast like cells, fibroblasts, (figure 1).View of laser group shows odontoblasts which appear disoriented (figure 2). After one week of BMP application the histological examination shows extensive inflammatory reaction invading pulp tissue, zone of predentin deposited by odontoblasts like cells differentiated from pulp stem cells (figures 3). View shows old dentin, there is area of inflammatory cells infiltration (figure 4).

Four weeks duration

Microphotograph view of control group after 4 weeks of pulp capping with MTA material shows variety of

conditions of inflammation and disorganization behavior of the pulp tissue, (figures 5). After 4 weeks of laser exposure shows multiple vascular engorgement with inflammatory infiltration, increased cellular activity with remodeling fibers, new predentin formation (figure 6). Histological examination shows organized regenerated pulp tissue that is highly cellular and highly vascular with predentin formation by new differentiated odontoblasts (figures 7) for BMP group. Microphotograph view after 4 weeks of exposure to laser radiation and BMP application shows inflammatory cells and disorganization of odontoblasts, predentin layer (figure 8).

Statistical Results

According to LSD test of multiple comparison (Table 1), there is a significant difference between (C, L), (C, B), and (C, B/L) groups, while there is non-significant difference between (L, B), (L, B/L), and (B, B/L) groups in predentin thickness parameter. There is a non-significant difference between all the groups in the inflammatory cell response score parameter. As well as there is a non-significant difference between all the groups in the odontoblasts score parameter (Figures 9, 10, 11).

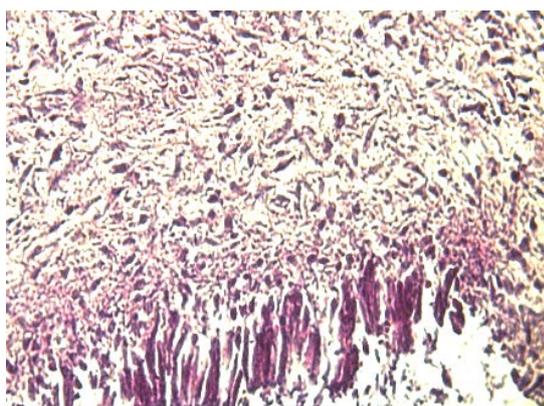


Figure 1- Magnified view of control group shows odontoblast like cells fibroblasts, inflammatory cells. **H&E x40**

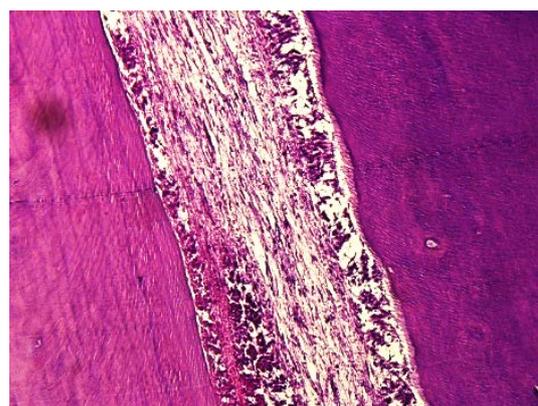


Figure 2- View of L1 group after one week shows necrotic tissue, disoriented odontoblasts and odontoblast like cells. **H&Ex20**

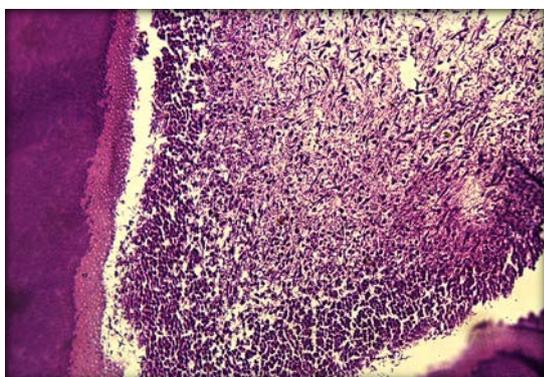


Figure 3 - View of B1 group shows extensive inflammatory reaction. **H&Ex20**

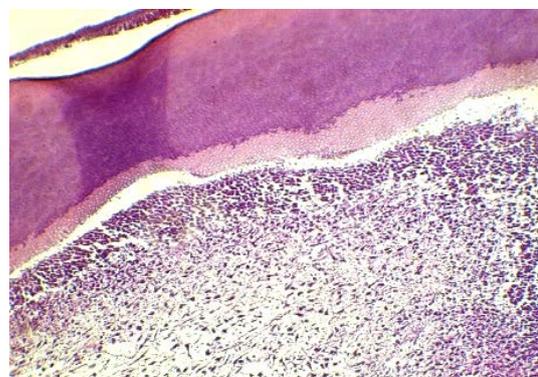


Figure 4 - View shows predentin, inflamed pulp. **H&Ex20**

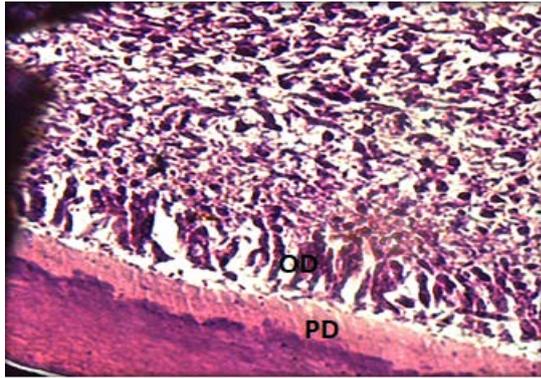


Figure 5- Shows the disorganization of odontoblast cells (OD) with inflammatory cell infiltration, and predentin(PD). **H & E X40**

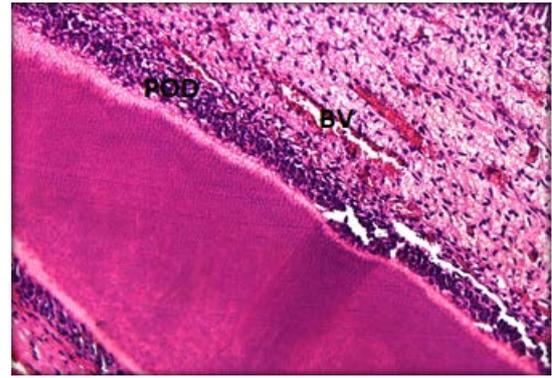


Figure 6- Shows the presence of preodontoblast (POD) cells and multiple vascular congestion (BV). **H & E X20**

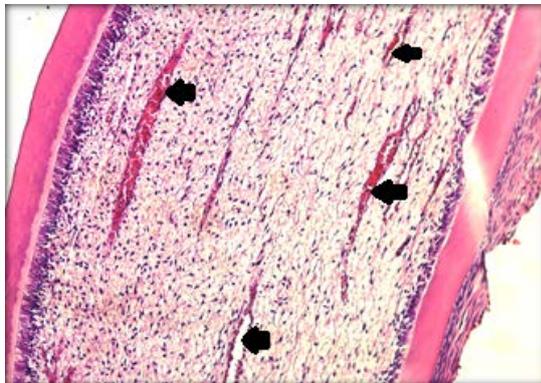


Figure 7- Shows numerous blood vessels (arrows), odontoblasts, predentin layer. **H & E X10**

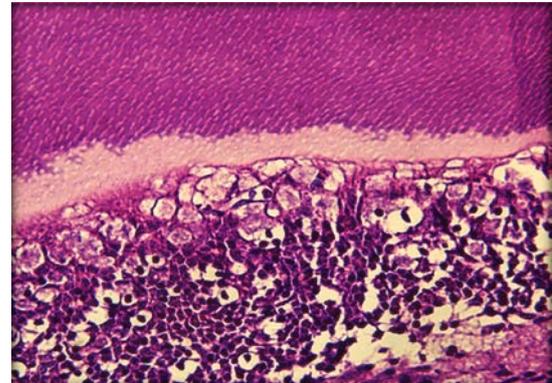


Figure 8- Magnified view shows inflammatory cells infiltration and new odontoblast cells. **H & E X40**

Table (1) LSD Multiple Comparisons

Dep. Variable	(I) GROUPS	(J) GROUPS	M. D. (I-J)	Std. Error	Sig.	
Predentin Thickness	C	L	-4.8570*	1.97095	.019	S
		B	-5.2770*	1.97095	.012	S
		B/L	-5.5570*	1.97095	.008	S
	L	B	-.4200	1.97095	.833	NS
		B/L	-.7000	1.97095	.725	NS
	B	B/L	-.2800	1.97095	.888	NS
I.C.R. Score	C	L	.5000	.40311	.224	NS
		B	.7000	.40311	.092	NS
		B/L	.7000	.40311	.092	NS
	L	B	.2000	.40311	.623	NS
		B/L	.2000	.40311	.623	NS
	B	B/L	.0000	.40311	1.000	NS
Odntoblast Score	C	L	.8000	.47697	.103	NS
		B	.8000	.47697	.103	NS
		B/L	.8000	.47697	.103	NS
	L	B	.0000	.47697	1.000	NS
		B/L	.0000	.47697	1.000	NS
	B	B/L	.0000	.47697	1.000	NS

Based on observed means. -- The error term is Mean Square(Error) = 1.138.

*. The mean difference is significant at the .05 level.

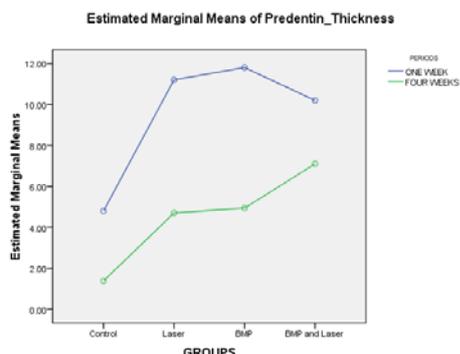


Figure 9 – Estimated marginal means of predentin thickness parameter among groups in 1 and 4 weeks periods

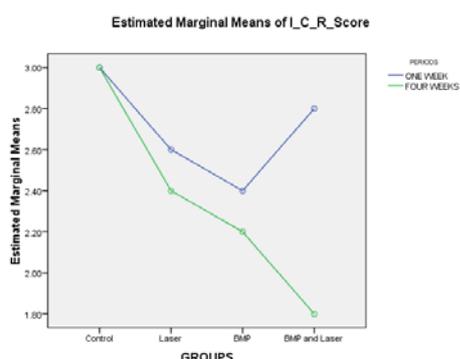


Figure 10 – Estimated marginal means of Inflammatory Cell Response Score parameter among groups in 1 and 4 weeks periods.

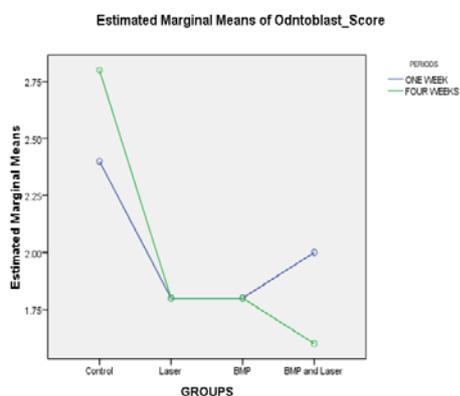


Figure 11 – Estimated marginal means of Odontoblasts Score parameter among groups in 1 and 4 weeks periods

DISCUSSION

Restorative and endodontic procedures have been developed in an attempt to preserve the vitality of dental pulp after exposure to external stimuli, such as caries infection or traumatic injury. When damage to dental pulp is reversible, pulp wound healing can proceed, whereas irreversible damage induces pathological changes in dental pulp, eventually requiring its removal [22].

During operating procedure in the present study pulp bleeding was controlled through irrigation with saline solution and the placement of sterile cotton pellets onto the pulp exposure sites. All the cases achieved hemostasis.

Other authors also describe the use of saline solution for hemostasis as being nontoxic and effective [21, 23].

Mineral trioxide aggregate (MTA) indicates decontaminative, high alkalinity that has been related to its bactericidal properties biostimulative, and hemostatic effects and these properties, altogether, could lead to a successful DPC treatment [12, 24, 25].

In this study all of this experimental groups were capped with MTA material to seal the cavity and standardized the effect of MTA material on pulp regeneration, and according to obtained findings of this study where odontoblast layer was noticed in control groups where only MTA used to seal exposed pulp.

Bone morphogenic protein (BMP-7) or Osteogenic Protein 1 (OP-1) was one of growth factors to be examined in pulp repair as induction of widespread osteodentin formation throughout the pulp was detected in monkey, including the total occlusion of the roots of treated teeth [26, 27]. An irregular reparative osteodentin was deposited in the coronal pulp of rat molars 30 days after BMP-7 treatment, and extensive reparative dentin was observed in the roots of these teeth, beneath a calico-traumatic line, with complete occlusion of the mesial root canal in many cases [28]. In agreement with present results obtained after 4 weeks where high vascularity and cellular organization and fibers remodeling were noticed. Inflammation was higher in the MTA-only group than in the MTA with BMP group, although this difference did not attain statistical significance. The addition of BMP-2 had a beneficial effect in vitro, reducing the initial cytotoxicity of freshly mixed MTA. However, the pulp reaction to a combination of MTA and BMP-2 was not significantly better than use of MTA alone [29]. The present histological results where the application of BMP-7 showed more recruitment of inflammatory cells in different durations.

The obtained results concerning B group showed that the highest mean values were recorded in 1week duration. Group comparison showed highly significant value between (C and B groups) and duration comparison showed high significant difference between (1 and 4 weeks). That may suggest that application of BMP may improve the reparative dentinogenesis process indirectly by inhibiting the inflammatory process which always occurs after pulp exposure.

Regarding effect of agent used in present study on regeneration process the present results support the findings of other studies done (although different agents used) they investigated the effects of dexamethason when directly applied on dental pulp tissue, where they found that this steroidal anti-inflammatory agent regulate the commitment of progenitors derived from dental pulp cells to form odontoblast-like cells leading to reparative dentinogenesis as frequency of abnormal histopathological features were much less than in controls [30, 31].

There are evidences that inflammation is a prerequisite for pulp healing, with series of events ahead of regeneration. Immuno-competent cells are recruited in the apical part slide along the root and migrate toward the crown, pulp cells display mild inflammation, proliferate, and increase

in number and size and initiate mineralization, pulp fibroblasts become odontoblast-like cells^[32].

Regarding healing periods in the present study the inflammatory response scoring showed highest values after 1 week but non-significant difference was obtained in duration comparison of different applied materials.

Evaluation of effect of demineralized bone matrix and dycal on pulp tissue of rats showed that after 7 days, the inflammatory reaction decreased gradually, matrix secretion could be observed in studied groups, and on 28 days, inflammatory cell infiltration was still observed^[33], in the line with statistical results of this study regarding progress of healing process showed that mean of inflammatory scores were high in 1 week period and decreased with time lowest score means noticed in 4 weeks period although different materials used for pulp capping.

It has been suggested that for the initiation of dentinogenesis, pulp and odontoblasts preservation is of primary importance, as well as the absence of infection and necrosis, but not the type of material used for pulp capping^[34]. Similar results in our study may be partially explained by the fact that the procedure was performed under aseptic conditions with minimal pulp perforation and good cavity sealing.

CONCLUSION:

From the obtained results we can conclude that BMP-7 application in direct pulp capping can enhance and accelerate pulp healing moreover the combined application of laser irradiation with BMP-7 may induce an additional effect over the application of BMP-7 itself.

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