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Effect of laser parameters and mode on pulp surgery outcome

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ABSTRACT

The objective of this study was to determine the effectiveness of localised laser pulp surgery in the canine model. Effects of laser parameters on treatment outcome were also investigated. Pulpal exposures 3mm in diameter were prepared in healthy teeth and left open to infection from the oral cavity for 72 hours. Pulpal tissue was then removed using a high speed handpiece with sterile irrigation, or a CO_2 laser. Teeth were monitored clinically, radiographically for 3 months.Results for each criterion were evaluated on a scale of 0-(-2). After sacrifice, histological assessment was made soft and hard tissue response. Results for each category were evaluated on a standard scale of 0-(-2). All evaluations were performed by 1 blinded, pre-standardised clinician. Statistical assessment using the chi-square test and Fisher's Exact Test (2-tail) associated laser treatment with a significantly better ($p<0.001$) clinical, radiographic and histological treatment outcome.
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Keywords: laser pulp surgery, CO_2 laser, pulpotomy, pulp.

1. INTRODUCTION

In mature adult teeth, conventional pulp treatment options include pulp capping or root canal therapy. The outcome of pulp capping procedures, whether direct or indirect, is unpredictable and success rates ranging from 44-97% have been reported [1]. Pulpal extirpation and root canal treatment are performed if pulp capping procedures are not indicated. Success rates of 78 - 90% have been cited for this treatment form, depending on factors such as the pathologies treated, tooth anatomy and follow-up duration [2,3].

In immature permanent teeth, endodontic treatment of choice comprises pulpotomy and subsequent dressing with calcium hydroxide. Internal resorption may occur after this type of therapy [4]. Moreover, long-term survival of teeth treated in this fashion is not assured and the main goal of this therapy is to permit complete root development prior to subsequent devitalisation and root canal treatment [4].

Conventional devitalisation and root canal treatment techniques are seldom indicated in the primary dentition [4]. Pulpotomy procedures are still widely used in conjunction with formocresol in primary teeth, despite the demonstrated irritancy, mutagenicity and toxicity of this agent [5,6]. The results obtained with this technique are unpredictable, and include strong tissue irritation and dental ankylosis [5] . Other agents including ferrous sulphate and glutaraldehyde are currently under investigation for use in conjunction with pulpotomy procedures [6].

Clinicians and patients alike would prefer to avoid unnecessary tooth devitalisation and root canal therapy procedures, yet we do not possess an adequate clinical capability for performing localised pulp surgery (eg pulpotomy) and wound dressing to allow predictable pulpal healing and long-term survival.

The preconditions for localised pulp surgery include atraumatic removal of compromised pulpal tissues [7], hemostasis and minimal clot formation [8,9], bacterial elimination [4]. These preconditions can be achieved using the CO_2 laser at appropriate parameters. Non-traumatic soft tissue surgery using this device has been documented for a wide range of clinical applications over 30 years [10,11,12]. The CO_2 laser emits an infrared beam at 10.6µm which is readily absorbed by the water in soft tissue [12]. The absorbed energy causes vaporisation of the intra- and extracellular fluid and destruction of cell membranes [10]. Tissue penetration by the laser beam is minimal and laser effects remain superficial [13]. Because the laser handpiece is used in the non-contact mode, it has no mechanical contact with the tissue, and trauma to the residual tissues is avoided [11,12]. Laser irradiation at this wavelength consistently achieves rapid and effective hemostasis and minimal clot formation in blood vessels up to 0.5mm in diameter [14,15]. Many authors report effective wound sterilization during soft tissue surgery with this laser [10,16]. CO_2 laser irradiation can achieve sterilisation and hemostasis in an open pulp wound [16]. Many authors associate CO_2 laser soft tissue surgery with reduced swelling, oedema and pain [10,11,14,15]. Pulpal exposure to CO_2 laser irradiation has been linked with reparative dentin formation [16]. Thus, the CO_2 laser is able to fulfil the prerequisites for successful localised laser pulp surgery.

The objective of this research was to determine the effects of localised laser surgery of infected pulps in dogs and to compare these results with those achieved using a sterile excavator. Further aims included an histological assessment of treatment effects 3 months post-treatment, and identification of optimal laser treatment parameters.

2. MATERIALS AND METHODS

2.1. Treatment

5 healthy adult beagle dogs approximately 12 months in age were used. The protocol was reviewed and approved by the appropriate animal care committees. Animals were premedicated with i.m. Promazine Maleate (0.1-0.25mg/kg) prior to the induction and maintenance of anesthesia using i.v. pentobarbital sodium (30mg/kg). Once full anesthesia was achieved, pre-operative standardized periapical radiographs were taken. 20 healthy teeth were included in this investigation: left and right lower third premolars and first molars in each dog. In each animal, 1 premolar and 1 molar received laser treatment, whilst contralateral teeth were used as controls. 3mm exposures were prepared from the buccal or labial aspect using the high speed dental drill and sterile saline as coolant. The cavity floor was thinned so that the underlying pulp tissue could be seen. Then, stilll under sterile saline cooling, a 1/4 round carbide bur rotating at slow speed (<5000rpm) was used to expose the pulp. These exposures were left open to contamination by the oral environment for 72 hours. Then superficial pulpal tissues were removed to an approximate depth of 2-3mm beyond the exposure site in the control teeth using a freshly sharpened, sterile spoon excavator. Sterile saline was used for debridement of the access cavity and bleeding was controlled with sterilized cotton pledgets where necessary. In the laser-treated group, pulpal tissues were ablated to an approximate depth of 1mm beyond the exposure site using the CO_2 laser, and sterilized cotton pledgets were available for control of bleeding if necessary. Immediately after pulp treatment, calcium hydroxide dressing (Dycal, Dentsply, Milford, DE) and amalgam restorations (Dispersalloy, Kerr, Emeryville, CA 94608) were placed and covered with reinforced zinc oxide-eugenol (RZOE) to prevent leakage. Postoperative radiographs were taken.

2.2. Laser device and laser parameters

A CO_2 laser emitting at 10.6µm (Premier Laser Systems, Irvine, CA) was used in the pulsed mode at the following parameters:

pulse duration: 0.01s

pulse interval: 1.0s

spot size: 0.004cm^2

energy per pulse: 276J/cm^2

These parameters were selected for 2 reasons: performance range of the laser device used, and thermal constraints. Preliminary investigations had shown that these parameters conform to the thermal safety thresholds which apply to the pulp [17].

2.3. Evaluation of treatment

Clinical and Radiographic Evaluation

At weekly intervals, treated teeth were monitored by the same, blinded, pre-standardised clinician.

a. Clinical Evaluation

Response to treatment was scored after visual and tactile examination as follows:

-2: Clinical presence of abscess formation, pus drainage, sinus tract
-1: Periapical palpation suggests the development of a pathosis
0: Unchanged from pre-treatment condition

b. Radiographic Evaluation

Standardised periapical radiographs were scored as follows:

-2: Development of periapical pathosis indicated by an area of rarefaction or internal root resorption
-1: Break in continuity of the lamina dura
0: Unchanged from pre-treatment condition

Histological Assessment

The animals were sacrificed after 3 months. Jaws underwent routine fixation and decalcification, then paraffin blocks were prepared for each tooth/jaw specimen. Blocks were serially sectioned at 6µm, mounted and stained with haematoxylin and eosin. Pulpal status was evaluated by one blinded, pre-trained assessor for hard and soft tissue response as detailed below. A sequential evaluation of all serial sections was performed for each tooth.

a. Pulpal Soft Tissue Response

As attempts at conventional evaluation of any inflammatory response are usually not constructive three months postoperatively, the following criteria were used:

-2: Definite degeneration in at least the coronal one third of the pulp.
-1: Incomplete cellular reorganization at the exposure site and adjacent to the cut dentin tubules. The remainder of the tooth pulp is normal.
0: Normal or close to normal cellular structure at the exposure site and throughout the rest of the tooth pulp.

Moreover, the following criteria for pulpal health were qualitatively documented as follows:

- Hyperemia
- String-like degenerative manifestations
- Blood infiltration
- Vacuolization
- Odontoblast displacement
- Inflammatory cells

b. Pulpal Hard Tissue Response (dentine bridge formation)

0: No evidence of mineralized tissue along the exposure site.
1: Organization of discontinuous segments of a mineralized tissue bridge.
2: Organization of a complete mineralized tissue bridge.

Moreover, the dentin bridge status was qualitatively documented as follows:

- Extent of mineralization (by color: the more basophilic, the less mineralized the tissue)
- Dentine structure (tubular vs fibrillar, osteoid or amorphous; presence of inclusions)
- Interface between dentine bridge and capping agent (smooth, spiculated; in contact with capping agent or necrotic tissue)
- Interface between dentine bridge and pulpal tissue (organization and status of odontoblasts, presence of reparative tissue)

2.4. Statistics

Statistical assessment was performed using Wilcoxon's Rank Sum Test and Fisher's Exact Test (2-tail).

3. RESULTS

Numerical scores for the treatment groups are depicted in Table 1.

Table 1: Clinical, radiographic and histological scores of teeth treated with laser- vs. conventionally-performed pulpotomies.

SCORES	LASER GROUP (Number of Teeth)	CONTROL GROUP (Number of Teeth)
HEMOSTASIS-YES	10	2
HEMOSTASIS-NO	0	8
CLIN: (-2)	1	3
CLIN: (-1)	1	2
CLIN: (0)	8	5
RADIOGR.: (-2)	1	4
RADIOGR.: (-1)	2	3
RADIOGR.: (0)	7	3
HISTOL (SOFT TISS.): (-2)	0	9
HISTOL (SOFT TISS.): (-1)	3	1
HISTOL (SOFT TISS.): (0)	7	0
HISTOL (HARD TISS.): (-2)	0	2
HISTOL (HARD TISS.): (-1)	2	2
HISTOL (HARD TISS.): (0)	8	6

3.1. Clinical and Radiographic Outcome

Rapid and effective hemostasis was achieved intra-operatively in all teath treated with the laser device. In the control group, pressure from a cotton wool pledget soaked with sterile saline was required in 8 of the 10 teeth in order to achieve hemostasis.

In the laser-treated group, 1 tooth developed a palpable lesion in the periapical area, 1 tooth developed an abscess. In 2 teeth, a break in continuity of the lamina dura was radiographically evident. In 1 tooth an area of rarefaction was radiographically apparent.

In the control group, 2 teeth developed a palpable lesion in the periapical area, 3 teeth developed abscesses. In 3 teeth, a break in continuity of the lamina dura was radiographically evident. In 4 other teeth an area of periapical rarefaction was radiographically apparent.

Statistical assessment using Wilcoxon's Rank Sum Test and Fisher's Exact Test (2-tail) associated laser treatment with a significantly better ($p<0.001$) clinical and radiographic treatment outcome.

3.2. Histological Results

Soft Tissue Response

In the laser-treated group, 70% of histological samples showed normal or close to normal cellular structure at the exposure site and throughout the rest of the tooth pulp with the presence of normal blood vessels and a regular, organized odontoblast layer. 30% of histological samples showed incomplete cellular reorganization at the exposure site and adjacent to the cut dentin tubules, whilst the remainder of the tooth pulp appeared normal. In these samples, odontoblasts were often locally displaced and/or the odontoblast layer was disrupted. Occasional patches of blood infiltration/degraded erythrocytes were observed, with some vacuolization, edema and hyperemia in small blood vessels. Areas of charring and thermal damage or bacterial infiltration of the dentin structure were also seen occasionally.

In the control group, 10% of all histological samples showed incomplete cellular reorganization at the exposure site and adjacent to the cut dentin tubules, whilst the remainder of the tooth pulp appeared normal. 90% showed definite degeneration in at least the coronal one third of the pulp; 30% of all pulp chambers were almost completely empty, exhibiting massive pulpal shrinkage. Another 30% of all pulps demonstrated large areas of string-like deterioration of pulpal structure. 20% of all pulps showed evidence of marked hyperemia. Areas of degraded erythrocytes, loss of healthy vasculature and breakdown of the odontoblast layer were frequently observed.

Hard Tissue Response (Dentin Bridge Formation)

In the laser-treated group, 80% of tooth pulps showed organization of a complete mineralized tissue bridge; in the remaining 20% of tooth pulps, discontinuous segments of a mineralized tissue bridge were observed. 80% of all tissue bridges were predominantly highly mineralized; 20% were demonstrated lesser degrees of mineralization. The peripheral margins of the bridge structure were usually more highly mineralized than the younger, central areas. Dentin bridge structures were predominantly atubular on the outer (coronal) surface, often more osteoid centrally, and tubular or occasionally fibrillar towards the margins of the exposure site. Cellular and carbonized inclusions were documented in approximately 30% of all samples. In most sections, the dentin bridge surface was smooth; in 2 cases it was locally separated by necrotic tissue and debris from the pulp capping agent and its surface appeared more spiculated.

In the control group, 60% of tooth pulps demonstrated organization of a complete mineralized tissue bridge, 20% showed discontinuous segments and 20% showed no evidence of mineralized tissue along the exposure site. All of the tissue bridge components were predominantly moderately mineralized. Dentin bridge structures were far more variable than in the laser-treated group, with atubular, tubular, osteoid and sometimes poorly defined loose fibrillar structures apparent in most samples with any evidence of bridge formation. The dentin bridge surface was rough and spiculated in all of these samples; in 50% of them it was locally separated from the capping agent by necrotic tissue and debris.

Statistical assessment using Wilcoxon's Rank Sum Test and Fisher's Exact Test (2-tail) associated laser treatment with significantly better histological (soft and hard tissue) outcome ($p<0.005$).

4. DISCUSSION

Clinical and radiographic treatment outcome was better in the laser-treated group than in the control group. These results are attributed to several laser-related factors observed in this study: Good hemostasis, minimal clot formation, and minimal surgical trauma to underlying tissues. Other factors may include wound sterilization [10,16] and reduced post-operative swelling and oedema [10,11,14].

Pulps were left open to contamination from the oral environment for 72h - a rather long timespan, dictated by our animal-sharing protocol. This factor probably played a role in the relatively low survival rate of all teeth, regardless of treatment modality: A significantly worse than normal treatment outcome has been reported for pulpotomies performed in teeth exposed to contamination from the oral environment for >48h [18]. This author observed the development of severe inflammatory changes within the pulp tisues approximately 48h after onset of contamination.

Several intra-operative events contributed to the results obtained in this study. In the control group, tissues underlying the exposure site were removed to a depth of approximately 2-3mm using a sterile excavator, until the residual surface tissues appeared healthy to the naked eye. Using the laser device, soft tissue removal was attempted to a depth of 2-3mm, but assessment of the ablation depth and of the health status of the residual tissues was problematic, due to the laser-induced changes in tissue colour and texture. Moreover, the laser parameters used in this investigation were selected from previous studies [17] to induce minimal temperature increases within the pulp, rather than for ablation efficiency, and were also dictated to a considerable extent by the laser device available to us. Several studies are currently in progress to optimise the laser parameters used and their effects in pulp tissue. By altering the laser configuration, ablation effects can be considerably improved whilst minimising collateral effects [19].

Immediate and effective intraoperative hemostasis was achieved in all laser-treated samples; subsequent blood clot formation was minimal. This observation is consistent with results published by other authors regarding blood vessels $\leq$ 5mm in diameter [14,20]. However, in the control group, hemostasis was difficult to achieve in 8 of 10 teeth, and substantial post-operative clot formation was observed. Treatment outcome was poor in all of these teeth. Several authors have attributed poor treatment outcome after pulpotomy to the presence of an extrapulpal blood clot, preventing direct contact of the calcium hydroxide capping agent with the underlying pulp tissue [7,21]. In addition to inferior treatment outcome, a spiculated secondary dentin surface resembling that observed in our investigation was described in monkey's teeth with large blood clots after pulpotomy [22].

Histological treatment outcome differed substantially between the 2 groups. In the laser-treated group, overall post-treatment pulpal health was considerably better than in the control group. A significantly greater number of pulps in the laser-treated group remained vital, maintained a healthy histological structure, and demonstrated a normal odontoblast layer than in the control group. Moreover, dentin bridge formation was significantly more frequent and successful in the laser-treated group. Areas of vacuolization, edema and hyperemia in laser-treated samples tended to be associated with the presence of carbonised and thermally-damaged tissue residues. Other authors have reported that these factors at the wound site can elicit a prolonged tissue response of the type observed in these specimens [13,23]. Recent incisional and ablational studies in soft tissue have demonstrated the capability for efficient and effective laser soft tissue ablation producing zones of collateral thermal damage measuring as little as 15μm [19]. Thus, the potential exists for substantially improving the outcome of localised laser pulp surgery by optimising the laser parameters selected.

Far more dentin bridging activity was observed in the laser-treated than in the control group. The regular development of bridging in contact with the wound dressing means that new tissue has grown in from below, which is usually consistent with non-contaminated healthy residual pulpal tissues [24,25]. Incomplete bridging, or separation of the bridge components from the capping site, indicates significantly changed initial intra-pulpal bridging conditions, rendering the pulp stump unfit to act as a proper scaffold for ingrowth of new tissue, and making it prone to nucleate pathological mineralisation [18]. Such observations have been described in human and monkey's teeth after pulpotomy with a spoon excavator [18,26]. From the results of this investigation we were unable to identify whether laser irradiation itself may have exerted a dentinogenic effect.

In conclusion, this study demonstrated that localised laser pulp surgery is feasible, and that it provides better results than conventional methods. Further investigations are currently in process to identify optimal clinical conditions, laser parameters and techniques for this promising new application.

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