Efficacy of the low level laser therapy (LLLT) on hair regrowth in dogs with non-inflammatory alopecia: a pilot study.

Lara Olivieri*, Damiano Cavina*, Giada Radicchi†, Vincenzo Miragliotta‡, Francesca Abramo‡

*Clinica Veterinaria Modena Sud, Spilamberto, Italy
†Private practitioner - Biotechnologist, Massa, Italy
‡Department of Veterinary Sciences, University of Pisa, Pisa, Italy

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Corresponding author
Vincenzo Miragliotta, DVM, PhD
Assistant Professor – University of Pisa
Dept. of Veterinary Sciences
Viale delle Piagge 2, 56124 – Pisa
Tel. +39-050-2216865
Fax +39-050-2216868
vincenzo.miragliotta@unipi.it

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Short running title: Laser therapy and canine alopecia
Abstract

**Background** – Canine non-inflammatory alopecia (CNA) is a heterogeneous group of skin disease of dogs with different underlying pathogeneses. The therapeutic approach is challenging and new options for the treatment would be desirable.

**Hypothesis/Objectives** – To test the clinical efficacy of low level laser therapy (LLLT) on hair regrowth in CNA.

**Animals** – Seven dogs of different age, breeds and genders with a clinical and histopathological diagnosis of non-inflammatory alopecia.

**Methods** – Each dog was treated twice weekly for a maximum of two months with a therapeutic laser producing three different wavelengths: 13x16mW-470nm, 4x50mW-685nm and 4x200 mW-830nm. Fluence given was 3 J/cm², with a frequency of 5 Hz, amplitude on an irradiated area of 25 cm² and application time of 1,34 minutes. A predetermined alopecic area was left untreated and served as a control. From one dog a post-treatment biopsy from treated and untreated sites was obtained to histologically evaluate the hair density and the percentage of haired and non-haired follicles.

**Results** – At the end of the study coat regrowth was highly improved in 6/7 animals and improved in 1/7. By morphometry the area occupied by hair follicles was higher in the treated sample (18%) compared to the untreated one (11%); haired follicles were (per area) 93% in the treated sample and only 9% in the control.

**Conclusions and clinical importance** – Our clinical and histological data documents promising effects of LLLT on hair regrowth in CNA. Further studies investigating the biological mechanism underlying the effect of LLLT on hair follicle cycling are warranted.
Introduction

Canine non-inflammatory alopecia (CNA) is a clinical presentation which encompasses skin diseases characterized by loss of hair for underlying dysplastic, functional (hair cycling) or endocrine disorders: follicular dysplasia, pattern alopecia, recurrent flank alopecia, hair cycle arrest, post clipping alopecia, hypercorticism or hypothyroidism are some of them. Diagnosis of these conditions relies on the history, clinical and laboratory findings and histopathology. A correct histological detection of the well known morphological features of the normal cycling follicle is indeed essential for the identification of different patterns that can be classified as CNA. Besides anagen, telogen and catagen a new phase called kenogen was recently described in dogs: this indicates hair follicles (HFs) that remained empty after losing their hair shaft and before a new anagen phase is initiated. The number of kenogen follicles was increased in all 76 patients with CNA included in a recent study where authors concluded that anagen induction was impaired due to either a lack of stimuli or a defect in the progenitor cells activation.

In spite of these hypotheses many aspects of the pathogenesis underlying the hair follicle cycling impairment is still unrevealed and the therapeutic approach is thus challenging. Off-label therapies are indeed still attempted for the treatment of CNA with either poor results or unwanted side effects. Since Albert Einstein in 1917 first described the concept of laser (Light Amplification by Stimulated Emission of Radiation), the first cutaneous applications for skin pathologies date back to the sixties. Several studies in human medicine suggest the use of low power lasers and light therapies for the treatment of some forms of non-inflammatory alopecia, particularly androgenetic alopecia and alopecia areata.

The idea for the study emerged from the clinical observation by one of the author (LO) of fast hair regrowth in a clipped coxo-femoral region of a dog receiving low level laser therapy (LLLT) as antalgic treatment. Thus, we tested the clinical efficacy of LLLT on hair regrowth in seven cases of CNA. LLLT expose cells to low levels of red and near infrared light and its energy density is low compared to other forms of laser treatments (i.e. High Level Laser Treatment-HLLT) that use laser power to induce a photothermal damage on target tissue and are used in many surgical fields for ablation and cutting.
Low levels of light is thought to induce a photochemical interaction with cellular chromophores and evidence exists that the chromophore is the mitochondrial cytochrome c oxidase (CCO). The result of the excited state of CCO is an increase in the production of ATP and ROS which ultimately act as signalling molecules which promote cell cycle progression, enzyme activation, nucleic acid and protein synthesis. Despite a detailed mechanism of action is still under investigation and its clinical application is pioneering for human as well as veterinary medicine, the authors believe LLLT would represent a new treatment option for hair regrowth in CNA.
Materials and Methods

Inclusion criteria - Dogs with one or more alopecic areas on the body were included. Patients were subjected to a pre-inclusion examination: screening exams included a complete haematology and clinical chemistry profile including thyroidal and adrenal hormones (in order to exclude hypothyroidism and Cushing syndrome), skin scraping, wood lamp examination, trichogramme and fungal cultures. Written consent was obtained from the owners of all dogs.

Histological examinations and morphometry - A pre-treatment biopsy was obtained as control specimen and to confirm the clinical diagnosis of non-inflammatory alopecia from all dogs. Biopsies underwent formalin fixation and paraffin embedding (FFPE) following the routine methods. The histopathological diagnosis was performed on Haematoxilyn & Eosin (H&E) stained 5 μm sections. From one dog (case N° 5), a post-treatment biopsy from both treated and untreated sites was obtained for the histological and morphometrical analysis of follicular units. Transverse sections at the isthmus level were obtained and stained with H&E and Mallory’s trichrome and were considered representative of the isthmus region if sebaceous glands were present. Morphometrical parameters evaluated in this single dog included percent of measured area occupied by hair follicles and percent of haired and non-haired follicles.

LLLT treatment - After inclusion, each dog was treated twice weekly for a maximum of two months with a type BTL 4000® (BTL Italia Srl, Salerno, Italy) therapeutic laser with a cluster probe producing three different wavelengths emerging simultaneously from 21 foci: 13x16mW (470nm), 4x50mW (685nm) and 4x200 mW (830nm) (Fig 1). Fluence given at each therapeutic session was 3 J/cm², with a frequency of 5 Hz, amplitude on an irradiated area of 25 cm² and application time of 1,34 minutes. The laser probe was kept at a definite distance from the alopecic area (< 2 cm) and was continuously moved back and forth on the skin surface to fully cover the entire lesion. In dogs with only one alopecic lesion a predetermined portion was left untreated. When multifocal alopecia was present, treated and control areas were pre-determined: when alopecia was bilateral, one side was treated and the contralateral served as control; in other cases
treated and non treated areas were pre-determined assuring that no irradiation was given to control areas. Neither pharmacological treatments nor food restriction was given to the patients during the study.

LLLT effect assessment – LLLT efficacy was clinically assessed in all dogs by visual examination of the animal and written recording of the status in comparison with the previous examination. Areas were photographically documented at the beginning of the study, after eight treatments (four weeks) and at the end of the study; pictures served to grade alopecic areas as: “unchanged.”, “worsened”, “improved”, “highly improved”.
Results

Seven dogs of different age and breed with clinical and histological diagnosis of CNA were included in the study. Patient data are reported in table 1.

No case showed inflammatory cell infiltrate at the histological examination. Cases n. 5 and 7 showed signs of recurrent flank alopecia (severe infundibular hyperkeratosis in enlarged infundibula, presence of kenogen follicles, and epidermal multifocal marked hyperpigmentation). Cases n. 1 and 2 were diagnosed with post clipping alopecia (haired telogen with prominent trichilemmal keratinization). Cases n. 3, 4 and 6 showed no specific histological change but the presence of small hair follicles thus corroborating the clinical diagnosis of pattern alopecia.

All enrolled dogs ended the study: 4/7 received 16 laser treatments (cases n. 1, 4, 5, 6) and 3/7 only 10 (cases n. 2, 3, 7). At the end of the study, in 6/7 animals (cases n. 1, 2, 4, 5, 6, 7) the coat quality was highly improved (Fig. 2, 3, 4) while in 1/7 (case nos. 3) it was graded as improved. On the biopsy specimen taken from case n. 5 at the end of the study, longitudinal sections showed recovery of the histological signs related to recurrent flank alopecia only in the treated side. Transverse sections allowed to distinguish the triplet assembling of canine follicle units. While the majority of hair follicles in the non-treated side of these sections were kenogen (absence of hair shaft, lumen collapsing either with or without trichilemmal keratin) a remarkable presence of haired follicles was visible in the treated sample (Fig. 5).

Histomorphometry showed changes in both considered parameters: percent of area occupied by hair follicles was higher (18%) in the treated sample compared to the untreated one (11%); haired follicles were (per area) 93% in the treated sample and only 9% in the control.
Discussion

LLLT is widely used in veterinary medicine and its efficacy is reported in various conditions\textsuperscript{10} but not alopecic disorders. LLLT has shown beneficial effects for a variety of human clinical conditions including several types of hair loss\textsuperscript{6,11}. To the author’s knowledge, this is the first study that evaluates clinical efficacy of LLLT in dogs affected with non-inflammatory alopecia. Our data indicate that LLLT is an effective and safe option for treating CNA, since a positive effect was visible in 100\% of subjects that ended the study and a dramatic improvement in the recovery of the coat was obtained in 6/7 animals (85.7\%). This might be of remarkable importance since current pharmacological treatment of CNA relies on the administration of minoxidil\textsuperscript{1}, finasteride\textsuperscript{12} or melatonin\textsuperscript{13,14}, off label therapies that might lead to unwanted side-effects\textsuperscript{14-16}. The clinical (all dogs) and morphometrical (one dog) evaluation of the untreated sites, which remained unchanged, allowed to exclude spontaneous hair regrowth as hypothesized for some of the CNA, particularly for cycling flank alopecia\textsuperscript{17}.

The phenomenon of paradoxical hypertrichosis and our observation of hair regrowth in the alopecic coxo-femoral region recalled the experience of Endre Master who, in the late 1960, improved hair regrowth on the shaved back of mice by using a low-power ruby laser while his intention was to induce carcinogenesis\textsuperscript{18}.

Since then most studies investigating the effects of LLLT on hair regrowth have used wavelengths ranging from 500 to 1100 nm (red and near-infrared spectrum) with delivered fluencies of 1-10 J/cm\textsuperscript{2} and a power density of 3-90 mW/cm\textsuperscript{2}.

Among physical parameters that can be managed when using LLLT devices, fluence (the energy dose administered on the surface unit) probably plays a major biological role\textsuperscript{19}. In our study we used a fluence of 3J/cm\textsuperscript{2} following the manufacturer instructions and we are not able to hypothesize whether lower or higher values would have influenced the clinical efficacy.

Despite in 2007 and 2011 FDA approved LLLT as a safe treatment for male and female pattern hair loss respectively\textsuperscript{20}, the exact mechanism of action of LLLT in hair growth is not known\textsuperscript{6}. Laser phototherapy is assumed to stimulate anagen phase re-activation in telogen hair follicles and increase the degree of proliferation in active anagen hair follicles together with preventing catagen development\textsuperscript{20,21}. 
Moreover it has been reported that sub-therapeutic fluences at the periphery of treated areas can induce terminal differentiation of hair growth rather than the wanted miniaturization, probably because instead of entering prolonged telogen phase, follicles are shifted towards terminal anagen hair growth\textsuperscript{22}. Transverse sections through the isthmus area have allowed, even if in only one case, to determine the presence of kenogen follicles in the alopecic areas: this would have been more difficult in longitudinal sections due to the thickness of the follicle itself. The absence of the hair inside the lumen and the collapse of the wall in the mid-portion of the follicle is unequivocal for the diagnosis of the kenogen state.

In conclusion our study documents promising effect of LLLT on hair regrowth; due to its clinical nature, our study does not allow to hypothesise any mechanism whereby LLLT can induce hair regrowth in dogs with non-inflammatory alopecia; further investigations are thus needed to establish the cellular and molecular mechanisms for the growth-promoting effect of LLLT in dogs. \textbf{Also, our study will need to be corroborated by a larger placebo-controlled trial.}


Legends for figures

Figure 1: Therapeutic laser and its cluster probe (inset).

Figure 2: Clinical photographs case no 5; Recurrent Flank Alopecia in a neutered female Lagotto; a, b) pretreatment photographs; c) untreated area after 4 treatments; d) treated area after 4 treatments; e) follow-up after 10 months from the end of study of the untreated area; f) follow-up after 10 months from the end of study of the treated area.

Figure 3: Clinical photographs case no 6; ear Pattern alopecia in a male Deutsch Kurzhaar; a & b) ears at the beginning of the study; c & d) untreated and treated ear at the end of the study, respectively.

Figure 4: Clinical photographs case no 7; Recurrent Flank Alopecia in a neutered female Boxer; a & b) alopecic areas at the beginning of the study; c & d) untreated and treated areas at the end of the study, respectively.

Figure 5: Microphotographs of transverse sections through the isthmus from case no 5 at the end of the study; a & c) low magnification of the untreated and treated areas respectively; b & d) higher magnification of the untreated and treated areas respectively; arrector pili muscle (*) and sebaceous glands (arrowhead) are shown. The majority of follicles are kenogen in the untreated area (b) while they are anagen in treated one (d). a & c: scale bar = 1 mm; b & d: scale bar = 100 µm.