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## **THE EFFECTS OF BIOPTRON LIGHT THERAPY ON WOUND HEALING IN DOGS**

### **INTRODUCTION**

Wound repair is a complex, integrated series of biochemical, cellular and physiological processes.<sup>1</sup> Almost immediately after a wound develops, the process of healing begins. Injured tissue goes through four phases: hemostasis, acute inflammation, proliferation (granulation) and remodeling. In the first phase skin wounds are filled with blood and a fibrin clot, which provides the first line of defense against infection. The inflammation phase of wound healing is fully established 24 hours after vascular injury. Neutrophils and macrophages, through phagocytosis and their degradative enzymes, breakdown and remove ("clean up") the cells debris resulting from tissue injury. The proliferation phase begins approximately 4 days after injury and can last up to 3 to 4 weeks or longer depending on the size of the wound. It is characterized by the generation of new endothelium (angiogenesis), epithelium (epithelisation) and connective tissue stroma (fibroplasia/desmoplasia) to restore normal structure and function to the injured tissue. The remodeling phase begins approximately 3 to 4 weeks following injury, but only after the inflammation and proliferation phases have been successfully completed. This phase includes remodeling of granulation tissue by immature connective tissue and the conversion of immature connective tissue to mature connective tissue through extracellular collagen formation. Remodeling can last for 2 or more years.<sup>2</sup>

The application of polarized light stimulates human immune competent cells and promotes natural defenses of an immune-repressed organism<sup>3</sup>. Visible light can penetrate epidermal and dermal layers of the skin and may directly interact with circulating lymphocytes to modulate immune function<sup>4</sup>. In vitro study of the exposure of macrophage-like cell line (U-937) to polarized light was conducted. The results suggested that polarized light stimulated the proliferative activity of macrophages by releasing growth factors that are important mediators of wound repair<sup>5,6</sup>.

Aim of this study was to investigate the influence of Bioptron light therapy on process of wound healing, by stimulation of neoangiogenesis, improving microcirculation, promoting of phagocytosis, stimulation of the specific enzymes involved in cell regeneration, activating fibroblasts and creating greater amount of collagen.

### **MATERIALS AND METHODS**

#### **Study population**

A total of 30 female dogs were used in this study. Most of the dogs (22) stayed for 10 days at the Small Animal Clinic at the University of Veterinary Medicine in Belgrade, and rest of the dogs were receiving therapy at their home. Upon arrival to the clinic, dogs were housed in an environmentally controlled facility for 2 days for acclimatization. The dogs were kept in standard cages, one in each and room temperature were maintained at 20-25°C. All

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dogs were fed with Royal Canin and water was available *ad libidum*. They were walked twice a day for 1 hour. Care was taken to avoid unnecessary stress and discomfort to the animals throughout the experimental period. The study protocol was approved by Ethical Committee of University of Veterinary Medicine, Belgrade.

Dogs were divided into two groups; 20 were treated with BLT, and 10 served as a control group

Age of the dogs ranged from 2 to 6 years of age. There were 9 different dog breeds, but most of them were mixed breed (10) and German shepherd (7).

### **Operative procedures**

Prior to operation, a complete clinical examination and blood cell count and biochemistry profile was performed for each patient. Only dogs that were clinically healthy with blood and biochemistry profile in normal range could enter the study.

The dogs were sedated with medetomidine hydrochloride (Cepetor™ CP Pharma) and Butorphanol (Alvesic® Alvetra U. Werfft GmbH), and anaesthetized with Isoflurane (Forane® Abbott). Carprofen (Rimadyl®, Pfizer Inc.) was injected to all animals once just before the operation for pre-emptive analgesia and every 24h for 3 days postoperatively. A single dose of Cefquinome (Cobactan® Intervet) was administered for antibiotic therapy preoperatively, and Amoxicillin/Clavulanic acid (Synolux®, Pfizer) P.O. for 5 days postoperatively.

Each dog was positioned in dorsal recumbency. The ventral hair was clipped with an electric razor. The skin surface was surgically prepared with povidone-iodine (Betadine®), then washed thoroughly with sterile saline and surgically draped. Ovariohysterectomy operation was performed on 30 female dogs under the same conditions. Incision was made through *Linea alba*. Extraperitonealisation of the ovaries and hysterectomy was performed in standard procedure. Closing of the abdominal wall was performed by continuous suture with Vicryl 4/0 (Ethicon) and closing the skin by simple interrupted suture with Syntofil 3/0 (B.Braun)

### **Treatment protocol**

Twenty of the dogs were treated with BLT. The treatment involved application of Oxi spray and BLT 1x daily for 6 minutes. The distance between lamp and wound was 10 cm.

### **Wound healing evaluation**

#### *Clinical examination*

During clinical examination we were looking for the presence of hyperemia, exudation and inflammation of the wound tissue. Clinical examination was performed blindly, by the same investigator.

#### *Histopathological evaluation*

Six-millimetre punch biopsy instrument was used to take skin samples from wound edges. Samples were taken on day 4 and 6 postoperatively. The specimens were fixed in 10% neutral buffered formalin and processed routinely for histopathological examination. To evaluate the progression of the healing process from inflammatory to repair stage, infiltration with neutrophils and macrophages, haemorrhage, angiogenesis, formation of granulation



tissue and epithelisation was examined. All histological sections were blindly evaluated by the same investigator.

## RESULTS

### Clinical examination

Hyperemia was observed on day 4 in both groups. In BLT group it was visible in 11 dogs (55%) and in control group in 9 dogs (90%). On day 6, hyperemia was noticeable only on the skin of one dog in BLT group (5%) and 6 dogs that didn't go under BLT (60%).

Exudation from the opening of the wound was recorded only in 6 dogs from BLT group on day 4, but was not visible on next visit. In control group various quantity of exudation was spotted in 7 dogs (70%) on day 4, and in 3 dogs on day 6.

Most of the dogs were calm and show good tolerance during this treatment, only 3 dogs (15%) were nervous and have to be hold. In the group of dogs treated with BLT just 3 (15%) licked or scratched a wound. In untreated group 7 dogs (70%) shown various degree of licking or scratching the wound.

### Histopathological evaluation

Infiltration with neutrophils and macrophages is very intensive on day 4 after the surgery and there is no statistically significant differences between two groups. (BLT 33,17; Control 33,10;  $P>0,05$ ). But, on day 6 BLT group had significantly lower number of inflammatory cells compared to control dogs (BLT 73,50; Control 43,20;  $P<0,01$ ).

In both groups we determined various degree of haemorrhages, either on day 4 or day 6 after the surgery. If we compare this results we can conclude that there is no statistically significant differences between two groups either on day 4 (BLT 51,89; Control 56,90;  $P>0,05$ ) or on day 6 (BLT 56,90; Control 66,90;  $P>0,05$ ) after the surgery.

There were no statistically significant differences between two groups in process of angiogenesis and formation of granulation tissue on day 4 (BLT 31,1; Control 33,0;  $P>0,05$ ). On day 6 situation is completely different and in BLT group intensive repair process was notable (BLT 71,9; Control 43,2;  $P<0,01$ ).

Epithelisation was noticed on day 4 with slight difference between two groups (BLT 20,16; Control 6,60;  $P<0,01$ ). This difference was more pronounced on day 6 (BLT 60,15; Control 29,70;  $P<0,01$ ).

## DISCUSSION

Bioptron light therapy can positively influence the process of wound healing by stimulation of neoangiogenesis, improving microcirculation, promoting of phagocytosis, stimulation of the specific enzymes involved in cell regeneration, activating fibroblasts and creating greater amount of collagen. Bioptron light therapy stimulates and accelerate wound healing due to stimulation and modulation of reparative and regenerative process. Also, it has anti-inflammatory action and stimulate immune system.



BLT was well tolerated, most dogs were relaxed during the therapy. In small percentage mild hyperemic reaction occurred on the application site immediately after the Bioptron light therapy, but this does not represent a side effect or a risk. It might be considered as a normal physiological reaction of the skin due to biostimulative effects of the light on skin microcirculation. In addition, active light therapy was found to be safe and well tolerated; no treatment-related adverse events were recorded.

Hyperemia and exudation were examined as a clinical sign of inflammation of the wound tissue. Inflammation is the most common complication after the surgical procedures, and it occurs due to different causes: inadequate preoperative, operative and postoperative procedures, infection, licking of the wound, suture reaction. Comparing the results from two groups on day 4 and 6 we can conclude that BLT decrease the clinically visible inflammation of wound tissue after the ovariohysterectomy operation. Also the pruritus measured through licking or scratching the wound was less present in BLT group.

Regarding the histopathological examination we can conclude that in group treated with BLT there is decrease in neutrophil count and macrophages and increase in neovascularization and epithelisation. Neovascularization is a major constituent of every healing wound. Mature vessels in deeper tissue layers give buds into the granulation wound to provide the wound area with sufficient blood supply<sup>7</sup>.

## CONCLUSION

This results suggest that application of BLT on wound prevent infection and accelerate wound healing in dogs.

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