EVALUATION OF LOW LEVEL LASER THERAPY ON OPEN WOUND HEALING OF THE TEAT IN DAIRY CATTLE

Seyed Mehdi Ghamsari¹, Haruo Yamada¹, Jezie A. Acorda¹, and Naoya Unno²

¹Department of Veterinary Surgery, School of Veterinary Medicine, and ²Department of Biosource Chemistry, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan

Open wounds (3 cm x 1 cm) on the anterior surface of teats in four dairy cattle were irradiated using low level laser therapy (LLLT). The laser used was a helium-neon (HeNe) system with an output of 8.5 mW, continuous wave at 632.8 nm. The 16 teats from 4 dairy cattle were divided into 4 groups using Latin Square design and were subjected to daily doses of LLLT: 3.64 J/cm² for 15 min (Group A); 2.42 J/cm² for 10 min (Group B); and 1.21 J/cm² for 5 min (Group C). Group D served as the control. The durations of healing were 18.5 ± 0.5 days for group A; 19 ± 0.7 days for group B; 23 ± 0.7 days for group C; and 24.5 ± 0.5 days for group D, respectively. The rate of wound healing between Groups A and C, A and D, B and C, and B and D were significantly different ($P < 0.01$). The difference in the hydroxyproline content between normal and healed specimens from Group A was significantly different from that of Group D ($P < 0.05$). The cosmetic appearance of healed Groups A and B was significantly better than those of Groups C and D.

Key words: LLLT; dairy cattle; teat; open wound healing; hydroxyproline

Introduction

Few animal tissues are exposed to as much rigorous manipulation and risk of injury as the cow's teat. In recent years, the importance and economic value of surgical treatment of teat disorders in dairy cattle has been recognized. For the injured teat to return to normal function and for prevention of subsequent adverse effects on mammary gland parenchyma, surgical treatment of teat lesions using new techniques seems to be necessary.⁽¹⁻³⁾

Among the different disorders of the teat, traumatic injuries have the highest incidence, sometimes reaching epidemiological proportion.^(4,5) In most non-penetrating open wounds of the teat wall where suturing is not possible, secondary healing utilizing different chemical agents and bandaging are employed.^(6,7)

Numerous studies have been conducted concerning the effects of low level laser therapy in different *in vitro* cell cultures,⁽⁸⁻¹⁰⁾ different *in vivo* human tissues⁽¹¹⁻¹³⁾ and various animals.⁽¹⁴⁻²⁴⁾ These reports mostly dealt with laser/tissue interactions, influence of low level laser therapy (LLLT) on healing and probable acceleration of the healing process. Although a few of these studies do not confirm the positive effect of low

level laser therapy,^(14,20,21) the advantage of the relatively shorter period of application of this physical method warrants further research for use in both human and veterinary medicine.

The purpose of this study was to assess the effect of LLLT on secondary wound healing of the teat wall in dairy cattle.

Materials and Methods

Four healthy adult Holstein-Friesian dairy cattle of variable ages (mean 4.75 ± 0.95 years), weights (mean 548.5 ± 17.69 kg) and number of calvings (mean 3.25 ± 0.95) were used in this study. The animals were placed in a chute and thoracolumbar anesthesia⁽²⁵⁾ was carried out in the standing position by a combination of 2% Xylazine HCl (Ceractal, Bayer Co., Ltd., Tokyo, Japan) at a dose rate of 0.05 mg/kg and 2% Lidocaine HCl (Xylocaine, Fujisawa Yakuhin Kogyo Co., Osaka, Japan) added to a total volume of 6 ml per cow, using a modified disposable epidural needle (Disposable Komakugaishin, Hakko Co., Tokyo, Japan) and epidural catheter (1 Perifix, Katheter, B. Bruan Melsungen AG, 0-3508 Melsungen, Germany).

Ten minutes after injection, the teats were aseptically prepared for surgery. Preoperative hemostasis was achieved using an intestinal clamp (Gillmaun

Addresssee for Correspondence:

Seyed Mehdi Ghamsari, Department of Veterinary Surgery, School of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, Japan.

Manuscript received: December, 1993

Accepted for publication: March 4, 1994

Table 1: Latin Square design (with four-way method) of different treatment groups.

Cow No.	Treatment Groups			Control Group D
	A	B	C	
1	LF	RH	RF	LH
2	RF	LF	LH	RH
3	LH	RF	RH	LF
4	RH	LH	LF	RF

RF=right front; LF=left front; RH=right hind; LH=left hind

compression forceps) at the base of the teat. A 1 x 3 cm section of the skin and intermediate layer was incised around a sterile plastic template in the anterior surface of the teat. The wounds were wrapped with a nonpressure bandage using a nonadhesive sterile silicon gauze (Torex-C, Fuji Systems Co., Tokyo, Japan) covered by a layer of surgical dressing sponge (Sof-Wick, Johnson & Johnson Med., Inc. Arlington, Texas 76004-0130, USA) and held in place by an adhesive tape (Hi-Latex, Iwatsuki Co., Tokyo, Japan).

The teats were divided into 4 groups (3 treatment and one control). 4 teats in each group, in a Latin Square design (with 4-way method), as shown in Table 1. The treatment groups were subjected to LLLT. The laser used was a helium-neon (HeNe) system (Mera HeNe Cold Laser Stimulator type PDT-A1, Senko Ika Kogyo Kobushi Co., Tokyo, Japan) delivering 8.5 mW in continuous wave at 632.8 nm in the red visible band of the spectrum, with 114 mW/cm² power density. Xylazine HCl 2% (0.08 mg/kg intramuscularly) was injected as a tranquilizer to prepare the animal for accurate focusing of the laser beam. Low level laser therapy was performed in a non-contact technique using the static method daily from the 1st to 8th day after surgery and every other day thereafter for a total of thirteen sessions. The grid pattern for laser therapy consisted of 30 circles (3 x 10) with a 0.3 cm diameter corresponding to 30 shots per session; the terminal delivery device was held at a distance of 1 cm from the wound surface. The duration of lasing, energy density and other details of treatment for each treatment group are shown in Table 2.

For the duration of the study, the cows were confined to an outdoor paddock except for two days after surgery, and fed grass hay and concentrates. In each therapy session, the bandages were removed in all groups (both treatment and control), the wounds were gently cleaned with gauze sponges soaked in physiologic saline solution and rebandaged, so that the wounds remained in clean, though not strictly sterile, condition.

For evaluation of wound healing, the wounds were photographed every other day starting from the

Table 2: Parameters used in different treatment groups.

Parameters	Treatment Group		
	A	B	C
Power density (W/cm ²)	0.114	0.114	0.114
Exposure time per shot (s)	30	20	10
Energy Density (J/cm ²)	3.642	2.428	1.214
Shots/session	30	30	30
Total exposure/session (min)	15	10	5
Sessions/course	13	13	13

day of surgery, using a camera fitted with a lens system chosen to ensure constant magnification for all wounds. The color transparencies were projected onto scaled paper and wound areas were measured planimetrically (Figure 1). Images of the left, right, upper and lower edges and unhealed portions of each wound were taken by macroscope (Macroscopic, Nihon Kohden Co., Tokyo, Japan) using the same magnification (x 50). These images were recorded through a commercially-available still video player (Still Video Rec/Player, R1000MH, Fujix, Fuji Photo Film Co., Ltd. Tokyo, Japan) and later used for assessment of epithelialization and wound bed variations and measurement of new epithelial tissue.

Cosmetic appearance evaluation of each wound, graded on a scale of 0 - 5 (0 for different from normal skin and 5 for resembling normal skin) was done by one of the researchers who was not involved in day-to-day treatment of animals and had no knowledge of the specific treatment for each wound.

Bacterial culture samples were taken two days after surgery and repeated after every four days, for a

Fig 1: Areas of wound of the teat.

Table 3: Mean values ($\pm$ standard deviation) of various wound healing measurements.

Variable	Treatment Groups			Control Group D
	A	B	C	
No. of days to heal	18.25 $\pm$ 0.50	19.00 $\pm$ 0.70	23.00 $\pm$ 0.70	24.50 $\pm$ 0.50
Maximum wound area attained (cm ²)	4.15 $\pm$ 0.03	4.18 $\pm$ 0.04	4.23 $\pm$ 0.07	4.26 $\pm$ 0.05
Daily healing rate (mm ²)	16.43 $\pm$ 0.45	15.61 $\pm$ 0.85	13.05 $\pm$ 0.46	12.25 $\pm$ 0.28
wound healing by epithelialization (%)	29.83 $\pm$ 0.03	30.32 $\pm$ 0.17	30.85 $\pm$ 0.48	30.70 $\pm$ 0.10
Daily epithelialization (mm ²)	4.90 $\pm$ 0.14	4.79 $\pm$ 0.19	4.07 $\pm$ 0.24	3.76 $\pm$ 0.08
Wound healing by contraction (%)	70.15 $\pm$ 0.11	69.67 $\pm$ 0.19	69.14 $\pm$ 0.48	69.10 $\pm$ 0.01
Daily contraction (mm ²)	11.53 $\pm$ 0.3	11.03 $\pm$ 0.5	9.12 $\pm$ 0.32	8.47 $\pm$ 0.20
Cosmetic appearance on a scale of 0 (poor) - 5 (good)	4.20 $\pm$ 0.06	4.07 $\pm$ 0.06	3.25 $\pm$ 0.04	2.92 $\pm$ 0.04

total of four collections. Samples were taken by rolling a sterile cotton swab across each wound surface and placing them in a sterile culture medium (Seedswab No.3, Eiken Kizal Co. Ltd., Tokyo, Japan). The samples were then submitted for microbiological examination.

Specimens of skin and intermediate layer of teat wall of normal tissue taken during surgery and healed wounds taken two days after closure of all wounds in each cow were prepared for analysis of hydroxyproline. Dried samples, after primary preparations, were hydrolyzed in sealed glass tubes in redistilled 6N HCl at 110°C for 24 hours under vacuum in hot block bath. After hydrolysis, the amino acid mixture was dried by rotary evaporation at 50°C. The residue was dissolved in 3 ml of 0.2N HCl and analyzed for hydroxyproline content by routine ion exchange amino acid analysis using ninhydrin detection (Amino Acids Analyzer 535, Hitachi Co., Tokyo, Japan).⁽²⁶⁾

Duncan's multiple range test was applied for comparison of the means of days to complete healing.

Fig 2: Correlation between number of postoperative days and wound area.

contraction-epithelialization ratio, daily rate of healing, contraction, epithelialization, and cosmetic appearance in the different groups. The statistical interpretations were based on a 99% confidence level for all means except for hydroxyproline content which was based on a 95% confidence level.

Results

All wounds enlarged in the first 5-7 days for all groups (Table 3) thus, wound contraction began sometime in the 5-8 days after surgery. Wounds returned to their original sizes on days 8-10, 9-11, 8-11 and 9-12 post-surgery in Groups A, B, C and D, respectively. The wounds roughly maintained their rectangular shape throughout the duration of the experiment. Wound healing curves had an initial lag phase during which little or no contraction and epithelialization occurred, followed by a period of rapid contraction during which most or all of the contraction occurred, after which almost no contraction occurred (Figure 2). The wounds finally closed by epithelialization in the absence of contraction or in the presence of a greatly reduced rate of contraction. Wounds subjected to 15 min LLLT (Group A) healed significantly faster than control (Group D) and those subjected to 5 min LLLT (Group C) ($p < 0.01$). Wounds subjected to 10 min LLLT (Group B) also healed significantly faster than Groups C and D ($p < 0.01$). Differences of the mean days to complete healing between Groups A and B and between Groups C and D were not significant ($p > 0.01$).

There were significant differences in the daily healing rate between Groups A and C, A and D, B and C, and B and D ($p < 0.01$) (Figure 3). The mean percentages of wound contraction for the different groups are shown in Table 3. In nearly all wounds of the different groups, about 70% of wound healing was due to contraction. Although there were no significant

Fig 3: Daily healing rate (in mm²) compared for the different treatment and control groups.

Fig 4: Correlation between the number of postoperative days and percentage of contraction.

Fig 5: Correlation between the number of postoperative days and percentage of epithelialization.

differences in the percentage of contraction between the different groups, the number of days to reach this percentage between Groups A and C, A and D, B and C, and B and D was significantly different ($p < 0.01$) (Figure 4). In addition, there were significant differ-

Table 4: Mean ($\pm$ standard deviation) of hydroxyproline content (mg/g dry weight) of teats in different groups.

Group	Normal Samples	Healed Samples	Difference
A (15 min LLLT)	63.43 $\pm$ 0.93	62.60 $\pm$ 0.78	0.83 $\pm$ 0.20
B (10 min LLLT)	59.73 $\pm$ 10.5	57.7 $\pm$ 8.83	2.03 $\pm$ 1.76
C (5 min LLLT)	65.43 $\pm$ 6.03	61.17 $\pm$ 5.68	4.26 $\pm$ 1.41
D (Control)	67.60 $\pm$ 2.19	56.77 $\pm$ 5.92	10.83 $\pm$ 5.86

ences in the daily contraction rate for the above-mentioned groups ($p < 0.01$) (Table 3).

Epithelialization in the wounds was evident 3-4 days earlier than contraction, but most of the epithelialization occurred after 13-14 days. Although the rate of epithelialization was not significantly different among the groups ($p > 0.01$), the wounds of Groups A and B were completely covered by epithelium in significantly fewer days than Groups C and D ($p < 0.01$) (Figure 5). The mean daily epithelialization rate was significantly different between Groups A and C, A and D, B and C, and B and D ($p < 0.01$) (Table 3).

The mean hydroxyproline contents of normal samples in all groups were higher than healed samples (Table 4). Although with the higher doses of LLLT, the hydroxyproline content of the healed samples almost reached that of the normal control sample, the differences in the hydroxyproline count between healed and normal samples were significantly different only between Groups A and D ($p < 0.05$).

Cosmetically, the treated wounds of Groups A and B had significantly better cosmetic appearance grades than those of Groups C and D ($p < 0.01$). No significant differences were detected in the other wound healing variables between front, hind, left and right teats ($p > 0.01$). In addition, no significant differences in the number and kind of microorganisms were detected between the different groups ($p > 0.01$).

Discussion

Wound healing is a complex series of cellular events that requires the recruitment of cells into the wound site, cellular proliferation, and synthesis and deposition of a new connective tissue matrix.⁽²⁷⁾ There is evidence in the literature that low level laser therapy has a beneficial effect on wound healing and in several publications a tendency towards a more rapid rate of healing (decreased number of days to healing) using LLLT has been observed.^(14,18,20,23)

During the first 5-7 days, wounds in all groups enlarged as the normal changes occurred within each

wound caused by mechanical stress from the surrounding tissue; these changes were similar in all groups. In addition, there was little variation in the commencement of healing in all groups. It seems that LLLT at the stated doses has neither positively nor negatively affected these two processes (enlargement of wound and commencement of healing).

In some reports,^(22,23) the most appropriate power for maximal biostimulation for wound healing has been mentioned as a dose of 1 J/cm² per day. In the present study, however, 1.21 J/cm²/day (group C) was not enough to accelerate healing. This may be due to differences in the characteristic of teat wall structure and skin of loose-skinned animals such as the rat or rabbit mentioned in other studies.^(22,23)

Wound contraction and collagen synthesis are myofibroblast- and fibroblast-mediated functions, whereas epithelialization is dependent on proliferation of epidermal cells. As mentioned earlier, the contraction/epithelialization ratio in all wounds was about 70/30, but the essential times to reach these percentages were significantly different between the various groups ($p < 0.01$).

Various explanations have been propounded regarding the mechanisms of LLLT on wound healing. In some works, the effects of LLLT on effective factors of contraction, such as selective stimulation of fibroblasts,⁽²⁰⁾ stimulation of fibroblast proliferation and collagen synthesis,^(9,28) and effects on the transcriptional level of procollagen gene expression⁽¹⁸⁾ have been mentioned. In other studies the effects of LLLT on epithelialization have been reported.^(22,23) Other LLLT-mediated mechanisms include an antiinflammatory effect in which edema is decreased and the local neutrophilic response is controlled,^(10,29) induced stimulation of myofibroblasts and increased leukocyte phagocytic activity.^(28,30) The results of this study show that LLLT at doses of 3.64 J/cm²/day (group A) and 2.64 J/cm²/day (group B) has an accelerative effect on both the contraction and epithelialization phenomena.

It is important to point out that although there is acceleration of the daily rate of contraction and epithelialization with LLLT, the ratio between contraction and epithelialization remained constant in all groups. It is postulated that while LLLT influences all stages of the healing process^(9,10,18,20,22,23,28-30) its effect on the cellular proliferation stage of the healing process is greater than on other stages, particularly, on the fibroblasts and epithelial cells. This suggests that LLLT accelerates both contraction and epithelialization and not either contraction or epithelialization as reported in other studies.^(9,18,20,22,23,28) Results from evaluation of cosmetic appearance, showing significant differences between Groups A and B, on one hand, and

Groups C and D on the other, tend to confirm this finding.

The synthesis of collagen is normally enhanced in any reparative process.⁽²²⁾ Its deposition is essential in the normal wound healing process⁽³¹⁾ and its synthesis can be further stimulated by light.⁽²²⁾ The concentration of hydroxyproline, an amino acid characteristic to collagen, can be used to assess the collagen content of tissue.⁽³²⁾ Hydroxyproline content is a suitable indicator of normal tissue because contents above or below the normal range can be caused by various disorders (impaired tensile strength, hypertrophic scars or keloids). In this study, the hydroxyproline content of healed samples was influenced by LLLT and the highest content (nearest the normal range) was obtained with 15 minutes of LLLT.

Microbiological examination showed that LLLT at the stated doses has neither positive nor negative effects on microbial activity in the surface of wound.

The results of the study suggest that 10-15 minutes of low level laser therapy at energy densities between 2.42-3.64 J/cm² promotes healing of open wounds; thus, laser therapy at the stated duration and doses is recommended for open wounds of the teat in dairy cattle.

References

- 1: Makady FM, Whitmore HL, Nelson DR, and Simon J (1991): Effect of tissue adhesives and suture patterns on experimentally induced teat lacerations in lactating dairy cattle. *Journal of the American Veterinary Medical Association*, **198**: 1932-1934.
- 2: Trent AM, Smith DF, Cooley AJ, Beck K, and Buffer RE (1990): Use of mucosal grafts and temporary tube implants for treatment of teat sinus mucosal injuries. **54**: 666-676.
- 3: Amstutz HE (1987): Teat surgery. *Modern Veterinary Practice*, **59**: 674-677.
- 4: Donawiek WJ (1981): New concepts for the surgical treatment of teat damage in cattle. *Proceedings of Soft-tissue Surgery: Refresher Course for Veterinarians*. University of Sydney, Australia. pp 157-160.
- 5: Koshiniemi K (1984): Observations on the incidence of teat injuries in different cowsheds. *Nordish Veterinary Medicine*, **34**: 13-19.
- 6: Morton JM, Fitzpatrick DH, Morris DC, and White MD (1987): Teat burns in dairy cattle: the prognosis and effect of treatment. *Australian Veterinary Journal*, **4**: 69-72.
- 7: Fennell C (1979): The treatment of teat lesions in milking cows with a pollen extract cream - a clinical trial. *Irish Veterinary Journal*, **35**: 151-155.
- 8: Kubasova T, Kovacs L, Somosy Z, Unk P, and Kokai A (1984): Biological effect of He-Ne laser; investigations on functional and micromorphological alterations of cell membranes *in vitro*. *Lasers in Surgery and Medicine*, **4**: 381-388.

- 9: Lam TS, Abergel RP, Dwyer RM, and Uitto J (1983): Biological effects of laser stimulation of collagen production by low energy lasers in human skin fibroblast cultures. *Lasers in Surgery and Medicine*, **3**: 189.
- 10: Meyers AD, Joyce J, and Cohen JJ (1987): Effect of low watt helium neon laser radiation on human lymphocyte cultures. *Lasers in Surgery and Medicine*, **6**: 540-542.
- 11: Kohno T, Kasai S, Kotai H, Saito T, and Mito M (1989): Analgesic effects of low power laser irradiation on clinical and experimental examinations. *Journal of Japan Society for Laser Medicine*, **10**: 85-89.
- 12: Fisher JC (1982): Interaction of laser light with living tissue. *Proceeding of American Association of Equine Practice*, **28**: 155-183.
- 13: Goldman JA (1982): Laser biostimulation and laser acupuncture. *Proceeding of American Association of Equine Practice*, **28**: 171-174.
- 14: Fretz PB, and Li Z (1992): Low energy laser irradiation treatment for second intention wound healing in horses. *Canadian Veterinary Journal*, **33**: 650-853.
- 15: Wang L, Kameya T, and Yamada H (1989): A review of clinical applications of low level laser therapy in veterinary medicine. *Laser Therapy*, **1**: 188-190.
- 16: Yamada H, Kameya T, Abe N, and Miyahara K (1989): Low level laser therapy in horses. *Laser Therapy*, **1**: 31-35.
- 17: Maeno N, Kameya T, Yamada H, and Abe N (1989): Effects of LLLT, using helium-neon laser on infectious bovine keratoconjunctivitis. *Laser Therapy*, **1**: 79-82.
- 18: Saperia D, Classberg E, Lyons RF, Abergel RP, Baneux P, Castel JC, Dwyer RM, and Uitto J (1988): Demonstration of elevated type I and type III procollagen mRNA levels in cutaneous wounds treated with helium-neon laser. *Biochemical and Biophysical Research Communications*, **138**: 1123-1128.
- 19: McKibbin LS, and Paraschak D (1984): Use of laser light to treat certain lesions in standardbreds. *Modern Veterinary Practice*, **85**: 210-213.
- 20: Hunter J, Leonard L, Wilson R, Snider G, and Dixon J (1984): Effects of low energy laser on wound healing in a porcine model. *Lasers in Surgery and Medicine*, **1**: 285-290.
- 21: Surinchak JS, Alago ML, Bellamy RF, Struck BE, and Belikin M (1983): Effects of low-level energy lasers on the healing of full thickness skin defects. *Lasers in Surgery and Medicine*, **2**: 287-274.
- 22: Kana JS, Hutschenreiter G, Haina D, and Waidelich W (1891): Effect of low-level density laser radiation on healing of open skin wounds in rats. *Archives of Surgery*, **116**: 293-298.
- 23: Mester E, Spiry T, Szende B and Tota JG (1971): Effect of laser rays on wound healing. *The American Journal of Surgery*, **122**: 832-838.
- 24: Stoffel M, Schallibaum M, Schlit W, and Gerber H (1989): Low-energy HeNe laser irradiation of the bovine mammary gland. *Journal of Veterinary Medicine*, **A36**: 898-902.
- 25: Skada TR, Muir WW, and Hubbell JAE (1989): Comparative study of continuous lumbar segmental epidural and subarachnoid analgesia in Holstein cows. *American Journal of Veterinary Research*, **50**: 89-44.
- 20: Bailey AJ, and Light ND (1989): *Connective Tissue in Meat and Meat Products*. Elsevier Applied Science, London, UK. pp 278-279.
- 27: Madison JB, Hamir AN, Ehrlich HP, Haberman J, Topkis V, and Villasin JV (1992): Effects of a proprietary topical medication on wound healing and collagen deposition to horses. *American Journal of veterinary Research*, **52**: 1128-1181.
- 28: Hardy LB, Hardy FS, Fine S, and Sokai J (1987): Effect of ruby laser radiation on mouse fibroblast culture. *Federation Proceedings*, **26**: 688.
- 29: Chegin VM, Skobelkin OK, and Brechov EL (1984): Laser surgery for soft tissue purulent diseases. *Lasers in Surgery and Medicine*, **4**: 279-282.
- 30: Mester E (1970): Stimulating effect of lower laser on biological systems. *Medical and Biological Engineering*, **8**: 430.
- 31: Prockop DJ (1981): Collagen biochemistry and the design of agents to inhibit excessive accumulation of collagen during wound repair. In: *The Surgical Wound* (eds. Dineen P and Hildick-Smith G). Lea & Febiger, Philadelphia, USA. pp. 97-109.
- 32: Madden JW, and Peacock EE (1988): Studies on the biology of collagen during wound healing. *Surgery*, **64**: 288-294.