The effect of low intensity laser irradiation of inflamed udders on the efficacy of antibiotic treatment of clinical mastitis in dairy cows

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Veterinaria Italiana 2019, **55** (3), 253-260. doi: 10.12834/VetIt.818.3989.2
Accepted: 11.03.2016 | Available on line: 30.09.2019

Keywords
Antibiotic treatment,
Clinical mastitis,
Cow,
Laser irradiation.

Summary
The aim of the study was to evaluate the effect of STP-99 laser irradiation applied locally to inflamed cow udders on the efficacy of clinical mastitis treatment with either intramammary infusions of antibiotic products or systemic injections of antibiotics. Examinations were carried out on 124 milking dairy cows suffering from clinical, bacterial mastitis. Cows with signs of local acute inflammation were treated with approved intramammary antibiotic products at labeled doses as control. The exposed cows received the same antibiotic treatment but were also subjected to irradiation of the inflamed udders with a laser for 5 consecutive days (2 minutes a day). Cows with local and systemic signs of mastitis were treated with either intramuscular injections of approved antibiotics in label doses alone (controls), or with the same intramuscular treatment protocol and laser irradiation of inflamed glands for 5 consecutive days (2 minutes a day). The recovery rate after intramammary treatment with antibiotics was 43.7%. Irradiation with laser significantly ($P < 0.05\%$) increased the recovery rate by 31.2%. The recovery rate in the cow cohort receiving systemic treatment with antibiotics was 46.7%. The laser irradiation resulted in a 16.6% increase in recovery. Supportive treatment with laser irradiation increased recovery rates by 24.2%.

Mastite cronica nei bovini da latte: effetto della radiazione laser STP-99 in associazione al trattamento antibiotico

Parole chiave
Trattamento antibiotico,
Mastite clinica,
Vacca da latte,
Radiazione laser.

Riassunto
Scopo dello studio è stato quello di valutare, ai fini del trattamento della mastite, l'effetto dell'applicazione locale di radiazioni laser STP-99 su mammelle infiammate, confrontandolo con i risultati raggiunti utilizzando le sole infiltrazioni sistemiche o intramammarie di antibiotici. Sono state esaminate 124 vacche da latte affette da mastite batterica; un gruppo con segni di infiammazione acuta locale è stato trattato con somministrazione intramammaria di antibiotici autorizzati alla posologia indicata nel foglietto illustrativo (gruppo di controllo). Ad un secondo gruppo (gruppo sperimentale), invece, il trattamento antibiotico delle mammelle infiammate è stato supportato da un trattamento radiante con tecnica laser per 5 giorni consecutivi (2 minuti al giorno). Le mucche che presentavano anche sintomi sistemici sono state trattate con iniezioni intramuscolari di antibiotici autorizzati alla posologia indicata nel foglietto illustrativo (gruppo di controllo), associando il trattamento laser in quelle oggetto di sperimentazione. Nel trattamento intramammario, il tasso di recupero funzionale è stato del 43,7%, in quello sistemico del 46,7%. Nel primo caso, la radiazione laser ha aumentato significativamente ($P < 0,05\%$) il valore (+ 32%). Sebbene non in modo significativo ($P > 0,05\%$), anche nel secondo caso il trattamento radiante ha aumentato il tasso di recupero (+ 16,6%) delle mammelle. L'aumento dei recuperi funzionali

ritorna significativo (P < 0,05%) se si confrontano i risultati ottenuti con il solo trattamento antibiotico (intrammamario + sistemico) con quelli ottenuti con il trattamento antibiotico (intrammamario + sistemico) associato al trattamento laser (+ 24,2%).

Introduction

Udder inflammations (mastitis) are still the most frequent and costly diseases affecting dairy cows across the world (Bar *et al.* 2008, Hogeveen *et al.* 2011). Therapy with antimicrobials is the primary method of combating udder infections and mastitis. The treatment is extremely difficult due to diverse types and sources of pathogens as well as lack of effective, specific methods of prophylaxis. The efficacy depends on clinical course of the disease, etiological agents, type of drugs and the methods and routes of therapy. Different authors reported recovery rates from 30% to 70% (Bradley and Green 2009, Roberson *et al.* 2004, Serieys *et al.* 2005, Taponen *et al.* 2003 a, b), however, in many cases recovery rates exceed 80% (Roberson *et al.* 2004, Serieys *et al.* 2005).

It should be emphasized that antibiotic residue in milk and meat can be harmful to human health. During last decades the efforts have been focusing on decreasing the total selective pressure for antibiotic resistance development in bacteria within the animal population as well as in humans (Sheldon 2005, Taylor 1999). Therefore, the search continues for new methods of treatment and prophylaxis that would allow a reduction in the use of antibiotics.

Promising results in the treatment of mastitis during the lactating period were obtained with the use of ginseng saponin, herbal extracts, propolis, lysosubtilin, antibacterial proteins, and most prominently lysozyme dimers (Malinowski 2002). In addition to the above mentioned compounds, other non-antibiotic methods such as frequent milk-out (Roberson *et al.* 2004), intramammary ozone infusion (Ogata and Nagahata 2000) or homeopathatic drugs injection (Hektoen *et al.* 2004, Werner *et al.* 2010) were tested. Some methods and drugs were examined to improve the efficacy of antibiotics. More or less favorable results were brought about by frequent milk-out (Roberson *et al.* 2004), injections of the lysozyme dimers (Malinowski *et al.* 2006a), oxytocin (Morin *et al.* 1998), non-steroidal anti-inflammatory drugs (Krömker *et al.* 2011, McDougall *et al.* 2007) or intramammary infusions of lactoferrin (Lacasse *et al.* 2008).

In recent years, while searching for new, more efficient and organic methods for treatment and prophylaxis, laser therapy has been widely discussed as a promising method in the treatment of bovine clinical mastitis. Low-intensity laser irradiation has been shown to affect cell metabolism, stimulate regeneration, and reduce pain and inflammation (Huang *et al.* 2009). Low level laser therapy was used clinically in many areas, including Canada, Europe, and Asia, for the treatment of various neurologic, chiropractic, dental, and dermatologic disorders in humans (Posten *et al.* 2005). Stoffel and colleagues, Hoedemaker and Hackenfort, Beneduci and colleagues examined the effect of low power laser in treatment of bovine mastitis (Stoffel *et al.* 1989, Hoedemaker and Hackenfort 2003, Beneduci *et al.* 2007). Some authors did not observe any beneficial effects of such a method of a treatment on the affected cows, but other revealed that a laser radiation treatment caused an effective beneficial response of the cows against the mastitis.

The aim of the examinations was to evaluate the effect of local STP-99 laser irradiation of inflamed cow udders on the efficacy of clinical mastitis treatment with either intramammary infusions or systemic injections of antibiotics.

Material and methods

Examinations were carried out on 124 lactating Polish Holstein-Friesian dairy cows suffering from clinical mastitis. Field trials were performed in 2 cowsheds (A - 66 cows and B - 58 cows) belonging to one dairy farm (free stall boxes, total mixed ratio - TMR) located in the north-western part of Poland. Average milk yield in sheds A and B during 305 days of lactation was 7,100 and 7,500 kg of milk, respectively.

Laboratory examinations were performed in the Department of Pathophysiology of Reproduction and Mammary Gland, National Veterinary Research Institute, in Bydgoszcz. The study was subdivided into two experiments.

Experiment 1. Effect of laser irradiation on the efficacy of intramammary treatment

Examinations were carried out on 64 cows (32 control animals and 32 exposed animals) with clinical mastitis and signs characteristic for local acute inflammation (swelling, pain, redness, hardness, and macroscopic changes in milk), without fever. Control cows (22 with one sick quarter and 10 with two sick quarters) were treated with approved intramammary antibiotic products - Synulox LC (Pfizer) in label doses (3 times every 12 hours; amoxicillinum trihydrate 200 mg/clavulanic acid 50 mg/prednisolone 10 mg). Exposed cows (24 with one sick quarter and 8 with two sick quarters) were treated with the same antibiotic product and with laser irradiation of the inflamed glands. The experimental cohort was exposed daily to one 2 minute session for 5 days consecutively with a low intensity laser device. The laser head was kept at a distance of 10 cm under the treated area and the depth of penetration of the laser beam exceeded 70 cm.

Experiment 2. The effect of laser irradiation on the efficacy of systemic treatment with antibiotic

Examinations were carried out on 60 cows (30 control and 30 exposed animals) that in addition to signs characteristic for local acute inflammation (swelling, pain, redness, hardness, macroscopic changes in milk) showed an increase in rectal temperature above 39.5 degrees centigrade. Control cows (17 with one sick quarter and 13 with two sick quarters) were treated with intramuscular injections only of approved antibiotics - Synulox (Pfizer) in label doses (once a day for 3 consecutive days; 7 mg/kg amoxycillinum trihydrate /1.75 clavulanic acid). Exposed cows (18 with one sick quarter and 12 with two sick quarters) were treated with the same antibiotic and with STP-99 laser irradiation of the inflamed glands according to the protocol described in the Experiment 1.

The antimicrobial drug was selected on the basis of bacterial sensitivity to antibiotics previously isolated in this herd.

In the experiments an STP-99 laser device (STP Company, Garshino, Nizhny Novgorod, Russia) was used. The laser has 6 unique diodes, which emit low intensity laser pulses in the near-infrared spectrum. The laser radiation wavelength was 870-970 nm. The duration of the pulse wave of variable frequency and length was 1.0 seconds. Peak radiated power was a maximum of 1.5 W. The laser devices STP-99 were certified in the EU in 2007.

All cows were examined on day zero (at the time of diagnosis) and 7 and 21 days after treatment. The clinical examination of the cows and the udders together with macroscopic evaluation of milk, California Mastitis Test (CMT) and bacteriological examination of milk samples were carried out on day 0. Clinical examinations, bacteriological tests, somatic cell count (SCC), were also performed on days 7 and 21 after treatment.

The first intramammary or systemic treatment of clinical mastitis in control and exposed cows started immediately after diagnosis. Quarter milk samples (inflamed secretion) were collected aseptically by field veterinary practitioners or by scientific personnel of the Department of Pathophysiology of Reproduction and Mammary Gland. Samples were cooled and transported to the Institute for laboratory examinations. Bacteriological examinations were performed according to commonly accepted procedures (Malinowski and Kłossowska 2002). Milk somatic cell count was determined by Fossomatic 90 (Foss, Denmark). Criteria of recovery were based on the regression of signs of clinical inflammation in udders, normal appearance of milk, decrease in the SCC and negative results of two bacteriological examinations after treatment.

The obtained results are presented as arithmetic mean (x) and standard deviation (± SD). The significance of the differences between mean values was verified using Tukey test assuming the differences to be significant if their probability was below 5%. Statistical analyses of treatment results were performed using chi-square test. All statistical analyses were performed using Statistica v. 6.0 StatSoft software.

Results

Clinical mastitis cases in each group were caused by CAMP-negative *Streptococcus* species, coliform bacteria, coagulase-negative staphylococci (CNS) and *Staphylococcus aureus* (Table I). The number of infected quarters and etiological mastitis agents were very similar in each group. There were no differences in the effectiveness of treatment between cows with a sick quarter and those with two sick quarters. Also, the location of quarters (front or rear) had not affected the outcome of treatment (data not shown). Hence, the total data of all cows were analyzed together.

The course of disappearance of bacteria (etiological agents of mastitis) in infected quarters (negative results of bacteriological examinations), independently on final efficacy of treatment in particular groups, is presented in Table II. It is shown that on days 7 and 21 after treatment less infected quarters were visible in cows treated with supportive laser irradiation compared to groups treated with antibiotics only. All cows from Experiment 1 received intramammary antibiotic products (Synulox LC) according to the label doses (3 times every 12 hours).

The efficacy of treatment is presented in Table III.

Table I. *Etiological agents of mastitis in quarters of cows treated with different methods.*

Etiological agents	Number of infected quarters in particular groups			
	Intramammary	Intramammary + laser	Systemically	Systemically + laser
Streptococcus sp.	22	23	21	22
Staphylococcus aureus	7	5	5	6
Coagulase-negative staphylococci (CNS)	7	6	9	8
Coliforms bacteria	6	6	7	5
Trueperella pyogenes	0	0	1	1
Total	**42**	**40**	**43**	**42**

Table II. *Numbers and percentages of infected quarters before and after antibiotic with or without laser treatment according to groups.*

Days	Intramammary		Intramammary + laser		Systemically		Systemically + laser	
	n	%	n	%	n	%	n	%
0	42	100[a]	40	100[a]	43	100[a]	42	100[a]
7	22	52.4[b]	10	25.0[b]	28	65.1[b]	14	33.3[b]
21	22	52.4[b]	10	25.0[b]	21	48.8[b]	12	28.6[b]

Difference statistically significant: [a,b] $p < 0.05$

Table III. *The effect of laser radiation on efficacy of clinical mastitis treatment with antibiotics.*

Treatment method	Number of treated cows	Cured cows		Unhealed cows	
		n	%	n	%
Intramammary	32	14	43,7 a	18	56.2
Intramammary + laser	32	24	74.9 b	8	25.0
Systemically	30	14	46.7	16	53.3
Systemically + laser	30	19	63.3	11	36.7
Antibiotics together	62	28	45.2 c	34	54.8
Antibiotics + laser together	62	43	69.4 d	19	30.6

Difference statistically significant: [a,b] $p < 0.05$; [c,d] $p < 0.01$.

From this table it is visible that the recovery rate after intramammary treatment with antibiotics was 43.7%. The irradiation with STP-99 laser increased the recovery rate by 31.2%. This difference was statistically significant ($p < 0.05$). The recovery rate due to systemic treatment with antibiotics was 46.7%. The laser irradiation caused an increase in recoveries by 16.6% (the difference was not statistically significant). When the results of antibiotic treatment (intramammary infusions or intramuscular injections) were taken together, supportive treatment with laser irradiation caused an increase in recovery rates by 24.2% (statistically significant).

Recovery rates in quarters according to the presence of different species of bacteria are presented in Table IV. Irradiation with laser increased recoveries in clinical mastitis caused by non-agalactiae (environmental) streptococci, coliform bacteria and coagulase-negative staphylococci.

Table V contains the somatic cell count in milk samples taken from recovered quarters of different groups of cows. At the time of the diagnosis, milk was profoundly changed in all inflamed quarters, so SCC could not be determined by the Fossomatic 90. Milk from bacteriologically negative quarters regained its normal appearance on day 7 after treatment. However, SCC in milk samples taken 7 days after starting treatment was high in all groups. Somatic cell count decreased significantly in the following 14 consecutive days in the groups of cows subjected to intramammary treatment but not significantly in cows belonging to groups subjected to systemic treatment.

Discussion

Streptococcus sp., *Escherichia coli, Staphylococcus aureus* and coagulase-negative staphylococci were the main etiological agents in cows with clinical forms of mastitis. Many authors (Bengtsson *et al.* 2009, Bradley and Green 2001, Gröhn *et al.* 2004, Malinowski *et al.* 2006b, Pitkälä *et al.* 2004, Sargeant *et al.* 1998, Tenhagen *et al.* 2009), also isolated the same microorganisms but the percentage of particular species sometimes differed significantly. All treated cows showed local or systemic clinical signs and macroscopic changes in milk typical for acute mastitis (Deluyker *et al.* 1999, Serieys *et al.* 2005, Wenz *et al.* 2006).

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Table IV. *Quarter recoveries according to etiological agents.*

Etiological agents	Number and percentage of recoveries							
	Intramammary		Intramammary + laser		Systemically		Systemically + laser	
	n/n	%	n/n	%	n/n	%	n/n	%
Streptococcus sp.	12/22	54.5[a]	20/23	86.9[b]	11/21	52.4	17/22	77.3
Staphylococcus aureus	1/7	14.3[a]	0/5	0 b	1/5	20.0	1/6	16.7
Coagulase-negative staphylococci (CNS)	5/7	71.4	5/6	83.3	6/9	66.7	6/8	75.0
Coliforms bacteria	2/6	33.3[a]	5/6	83.3[b]	4/7	57.1	4/5	80.0
Trueperella pyogenes	0	0	0	0	0/1	0 a	1/1	100[b]
Total	**20/42**	**47.6[a]**	**30/40**	**75.0[b]**	**22/43**	**51.2**	**30/42**	**71.4**

Difference statistically significant: [a,b] $p < 0.05$

Table V. *Numbers and percentages of infected quarters before and after antibiotic with or without laser treatment according to groups.*

Days	Groups			
	Intramammary	Intramammary + laser	Systemically	Systemically + laser
0	not determined - changes in milk	not determined - changes in milk	not determined - changes in milk	not determined - changes in milk
7	1,400,920[a] ± 1,102,190	1,790,682[a] ± 1,663,660	1,892,000[a] ± 2,491,270	2,390,700[a] ± 2,617,800
21	477,560[b] ± 404,950	837,591[b] ± 808,625	412,640[a] ± 400,500	629,950[a] ± 743,540

[a,b] Values in columns marked with different letters differ significantly ($p < 0.05$).

The average efficacy of treatment that ranged between 43.5% (intramammary infusions) and 46.7% (intramuscular injections) was not satisfactory. Inflammation caused by *Staph. aureus* was particularly refractory to therapy. The results of mastitis treatment with intramammary antibiotic products alone are comparable (Bradley and Green 2009, Deluyker *et al.* 1999, Malinowski *et al.* 2006a, Milne *et al.* 2005) or lower than data reported by other authors (McDougall *et al.* 2007, Roberson *et al.* 2004, Serieys *et al.* 2005, Taponen *et al.* 2003 a, b). A better effectiveness was found in cases of fresh mastitis with the use of parenteral (Barkema *et al.* 2006, McDougall *et al.* 2007, Serieys *et al.* 2005, Suojala *et al.* 2010) or traditional intramammary therapy (McDougall 2003). Other authors reported a significant increase in recovery as a result of extended (Gillespie *et al.* 2002, Oliver *et al.* 2004) or more aggressive (Hillerton and Kliem 2002) intramammary therapy or when intramammary infusions were combined with either intramuscular antibiotics (Taponen *et al.* 2003 a, b) or NSAID injections (Krömker *et al.* 2011).

Irradiation of inflamed udder quarters with the STP-99 laser increased recovery rate from mastitis treated intramammarily or intramuscularly with antibiotics by 31.2% and 16.6%, respectively. Irradiated cows showed faster regression of clinical signs such as redness, pain, hardening, faster disappearance of macroscopic changes in milk, and better elimination of intramammary infections compared to mammary glands of cows treated with antibiotics alone. The decrease in milk SCC could be comparable with data reported by Deluyker and colleagues (Deluyker *et al.* 1999) and Taponen and colleagues (Taponen *et al.* 2003a) for cases cured clinically and bacteriologically.

The supportive effect of laser irradiation in treated mammary glands is probably due to the regulatory effect on pro- and anti-inflammatory cytokines *in vivo* and *in vitro* (Zhevago *et al.* 2006), and with stimulation of the immunological system *in vivo* (Funk *et al.* 1992, Novoselova *et al.* 2006). This type of light stimulates proliferation of different kinds of cells (Pinheiro *et al.* 2003, Shanyfelt *et al.* 2008), increases growth of cells stressed by nutritional deficits *in vitro* (Eduardo *et al.* 2007), and results in inhibition of apoptosis in cells participating in the process of skin regeneration (Chyczewski *et al.* 2010). The use of low levels of visible or near infrared light for reducing pain, inflammation and oedema, promoting healing of wounds, deeper tissues, and nerves and preventing cell death and tissue damage has been reviewed most recently by Huang and colleagues (Huang *et al.* 2009). In non-steroid laser-treated rats, significant acceleration of epithelization and collagen synthesis 2 days and 6 days after surgery was observed in simulated wounds (Gál *et al.* 2009). Silveira and colleagues (Silveira *et al.* 2011) recently suggested that low-power laser irradiation of the skin accelerates wound healing due to a reduction of the extent of the inflammatory phase.

It seems that laser irradiation stimulates the phagocytic activity of milk granulocytes which then are more active in destroying the etiological agents of mastitis. The activation of the mammary gland immunological system plays a fundamental role both in prophylaxis and treatment of mastitis in cows (Burvenich *et al.* 2004, Paape *et al.* 2002). The bactericidal effect of low-intensity laser irradiation that was demonstrated *in vitro* (Žilaitis *et al.* 2008) can also be considered. In addition, Russian scientists (Makarimov *et al.* 2002) reported that laser irradiation is highly effective in the treatment of endometritis in cows as a sole therapy method or in combination with antibiotics. On the other hand, Stoffel and colleagues (Stoffel *et al.* 1989) examined the effects of low-energy laser irradiation with 25 mW on an area of 7.5 cm in diameter on the right front quarter which lasted 30 minutes daily for five consecutive days. Parameters measured included milk yield, somatic cell count, conductivity, Na/K-ratio in milk whey, and fat, protein and lactose concentrations in milk. No evidence for any stimulation of the healthy mammary gland or therapeutic effects on mastitis could be found. Contrary to this study, Hoedemaker and Hockenfort (Hoedemaker and Hockenfort 2003) reported a high percentage (84.4%) of clinical recoveries and only 25% of bacteriological recoveries as a result of therapy with the BMSD Sport-laser IR 904 nm for the duration of 5-8 min on days 1, 2, 3, 5 and 7. A significant decrease in milk somatic cell count was observed by Beneduci (Beneduci *et al.* 2007) as a result of irradiation of subclinically inflammed mammary glands with a STP-8 laser once a day for 30 sec for 5 days.

Summarizing, it is known from the literature data that the activity of the immunological system of the udder plays a role not only in prophylaxis and treatment of mastitis but also in the repair process of mammary tissue that was destroyed as a result of inflammation (Paape *et al.* 2002, Silveira *et al.* 2011). However, supportive irradiation with STP-99 laser for 5 consecutive days is also too much time consuming. The next problem is a slightly higher milk somatic cell count comparing to quarters that recovered as a result of antibiotic application without irradiation. The higher number of the SCC in cows treated with low-intensity laser therapy may be due to a bio-stimulating effect. At the tissue level, laser therapy stimulates the immune system by accelerating blood and lymph circulation. This therapy also stimulates phagocytosis and intracellular generation of active oxygen forms (Novoselova *et al.* 2006).

Further research is therefore needed to evaluate the effect of irradiation for a shorter time periods, such as 3 days versus 5 days, for example. It seems that shorter irradiation can also increase the efficacy of antibiotics but SCC could decrease in a faster manner. Further research on the mechanisms of laser action inside cow mammary glands is also necessary. It seems that this treatment method can be realized on organic dairy farming, as in an intensive dairy farming this method may cause organizational problems.

Conclusion

Two minutes of daily irradiation for 5 consecutive days with STP-99 laser of clinically inflamed mammary glands in cows treated systemically or with intramammary antibiotics increases recovery rates from clinical bacterial mastitis.

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