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Short Communication

Antimicrobial photodynamic therapy can be an effective adjuvant for surgical wound healing in cattle

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ABSTRACT

Background: Rumenostomy is a useful procedure commonly performed in cattle for medical treatment of domestic ruminants with forestomach diseases. Methylene blue (MB)-mediated antimicrobial photodynamic therapy (APDT) has been broadly investigated to treat infected wounds.
Aim: The aim of the study was to evaluate the effectiveness of MB-mediated APDT (MB-APDT) combined with chlorhexidine and zinc oxide ointment on wound healing process after rumenostomy.
Methods: Fourteen Nelore bulls were subjected to rumenostomy procedure. Animals were randomly divided into MB-APDT (MB associated with a red diode laser performed immediately after surgery and repeated on days 3, 5, 7 and 10) and control groups. Daily care included topical cleaning with chlorhexidine 2% followed by topical zinc oxide ointment. Animals were followed-up until the 28th day.
Results: Wounds presented a better post-surgical profile in MB-APDT group when it was compared with the control group. In MB-APDT group, it was also possible to observe less pain on palpation of wounds borders, less edema and inflammatory exudate. Additionally, animals from MB-APDT group were faster discharged from the cattle care facility.
Conclusion: Our results support the use of MB-APDT for the post-surgical management of rumenostomy. This pilot study ratifies the use of APDT in cattle and also suggests that it could be performed for other surgical procedures as a complementary approach or an alternative for topical administration of antibiotics.

1. Background

Rumenostomy is a surgical procedure performed in cattle to get easy access to ruminal lumen trough cannula fixation [1]. It has been commonly used for experimental procedures to evaluate ingestion, digestion, ruminal gas production, and also for rumen transfaunation from a healthy donor animal to treat a diseased animal [1,2]. Wound care is usually performed by cleaning with antiseptic solutions, however infection in surgical site may occur due to perioperative contamination [3,4].

Antimicrobial photodynamic therapy (APDT) has been widely investigated in different areas of medical science [5–10], including veterinary medicine [11]. APDT combines light, a photosensitizing agent and molecular oxygen to produce oxygen reactive species (ROS) that inactivate microbial cells [5–10]. In this regard, methylene blue (MB)-mediated APDT (MB-APDT) has been in the spotlight to treat topical infected wounds, being considered easy to perform, cost-effective and without causing adverse effects [12,13]. Indeed, MB-APDT has been successfully used to treat superficial infections in companion animals [14], exotic and wild animals [15–17] and food-producing animals [18–20].

2. Aim

The aim of the study was to evaluate the viability of MB-APDT as an adjuvant treatment for wound healing after rumenostomy.

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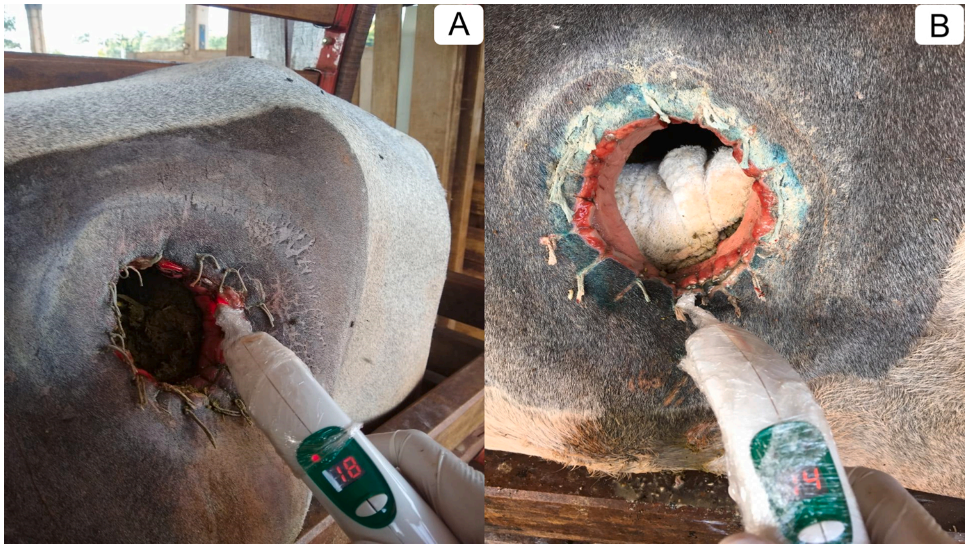

Fig. 1. Representative images of MB-APDT in the space between sutures (rumen-skin junction) (A), and around the wound borders in the postoperative period (B).

Table 1
Clinical evaluation of MB-APDT on the healing of surgical wounds of cattle submitted to ruminostomy during the experimental period. Scores ranged from 0 to 3 (absent, slight, moderate and severe). Values are presented as median and interquartile range. D: Day.

Clinical sign		D0	D3	D5	D7	D10	D14	D21	D28
Pain	Control	0 (0-1)	2 (1-3)	2 (0-2)	1 (1-2)^{A}	0 (0-2)	0 (0-1)	0 (0-0)	0 (0-0)
	MB-APDT	0 (0-1)	1 (0-1)	1 (0-1)	0 (0-1)^{B}	0 (0-0)	1 (0-1)	0 (0-1)	0 (0-0)
Edema	Control	1 (0-1)	1 (1-3)^{A}	1 (1-3)	1 (0-3)	1 (1-2)^{A}	0 (0-2)	0 (0-1)	0 (0-0)
	MB-APDT	0 (0-0)	0 (0-1)^{B}	1 (0-1)	0 (0-1)	0 (0-1)^{B}	0 (0-1)	0 (0-0)	0 (0-0)
Exudate	Control	0 (0-0)	1 (0-1)^{A}	1 (1-3)^{A}	1 (0-2)	1 (1-3)^{A}	2 (1-3)^{A}	1 (1-3)	1 (0-2)^{A}
	MB-APDT	0 (0-0)	0 (0-0)^{B}	0 (0-0)^{B}	0 (0-1)	1 (0-1)^{B}	1 (0-1)^{B}	1 (1-2)	0 (0-0)^{B}
Suture dehiscence	Control	0 (0-0)	0 (0-0)	0 (0-0)	1 (0-2)^{A}	3 (1-3)^{A}	3 (1-3)^{A}	2 (0-3)^{A}	0 (0-2)
	MB-APDT	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)^{B}	0 (0-1)^{B}	0 (0-1)^{B}	0 (0-0)^{B}	0 (0-0)

Uppercase letters represent statistically significant differences between groups following Friedman test ($p < 0.05$).

3. Methods

This study was approved by the local animal care and use committee of the School of Animal Science and Food Engineering, University of São Paulo (number: 2368040719/ID001322).

Fourteen 10 months old healthy Nelore bulls, weighing ≅ 310 kg were randomly divided in MB-APDT (n = 7) and control (n = 7) groups, kept in individual stalls, receiving corn silage and water *ad libitum* during the experimental period.

Prior to the surgery, the hair at the left-paralumbar fossa was clipped, followed by local antisepsis and local anesthesia performed with 2% lidocaine infiltrated accordingly the inverted L-block technique [21]. Rumenostomy surgery [1,22] was performed and no intercurrences have occurred. Immediately after surgery, penicillin and rifamycin were topically applied, and a silicon cannula was placed through the rumenostomy site. Daily care included topical cleaning with chlorhexidine 2% followed by topical zinc oxide ointment. All sutures were removed at day 12, and animals were followed-up until the 28th day. We also controlled external factors on wound recovery, such as fecal contamination and the influence of environmental humidity since all animals were maintained in the same conditions.

Animals in the MB-APDT group were treated with topical administration of 15 mL of MB aqueous solution at a concentration of 0.01 % (Sigma-Aldrich, USA) directly on the surgical wounds through a syringe. After 5 min, we irradiated punctually the wounds with a diode laser emitting a wavelength of 660 nm, radiant exposure of 70 J/cm^2, 2 J and 200 s per point (total of 30 points), 100 mW, spot size 0.028 cm^2 and irradiance of 3.5 W/cm^2 (TheraVet, DMC®, São Carlos, SP, Brazil) (Fig. 1A-B). The MB-APDT protocol was performed immediately after surgery and repeated on days 3, 5, 7 and 10. Clinical evaluation was made twice a week during 28 days, and was based on reduction of inflammatory signs (edema and inflammatory exudate), pain assessment (determined by manual palpation of the wound borders and observation of the animals' reactivity), and suture dehiscence. Clinical signs were scored from 0 to 3 (absent, slight, moderate and severe). Data were compared by Friedman test and were considered statistically significant when $p < 0.05$.

4. Results

Table 1 summarizes the scores of clinical signs over the experimental period. Although all wounds from both groups healed uneventfully, some aspects are noteworthy to remark and should be taken in consideration. Observe that on day 7 MB-APDT group showed a statistically lower pain score than the control group. Additionally, on day 10, MB-APDT group exhibited less pronounced exudate and edema. Interestingly, the control group presented a higher score for suture dehiscence on days 7, 10, 14 and 21 than the MB-APDT group.

Overall, in MB-APDT group, no necrotic tissue in the surgical wounds was observed during the evaluated period. On the other hand, in control group, 42.9 % (3/7) of lesions presented tissue necrosis. In the same way, while suture dehiscence occurred in 71.4 % (5/7) of wounds in the control group, only 14.3 % (1/7) was evidenced in MB-APDT group.

The color of the wounds ranged between bright pink, red bright and blackish, however there was no difference between the groups. In brief, in MB-APDT group, it was possible to observe less pain on palpation of wounds borders and animals' reactivity, less edema and inflammatory exudate. Macroscopical assessments of wounds also revealed a better

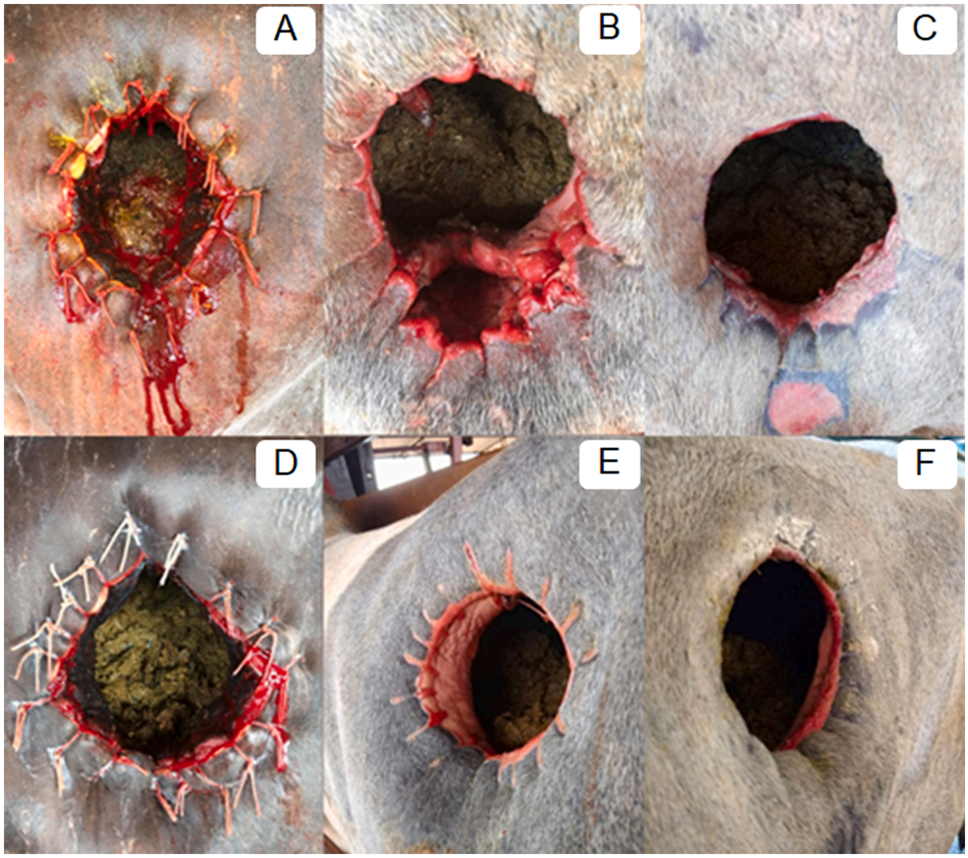

Fig. 2. Representative images of surgical wound healing in cattle. Aspect of wounds from control group immediately after surgical procedure (A), 14 days (B), and 28 days (C). MB-APDT-treated wounds immediately after surgical procedure (D), 14 days (E), and 28 days (F). MB-APDT-treated wounds showed a significant and better improvement at the end of evaluated period.

post-surgical profile in MB-APDT group when it was compared with the control group (Fig. 2A-F).

On the 28th day, 85.7 % (6/7) of animals from MB-APDT group presented complete edge healing, proper cannula fitting, and absence of rumen fluid leakage, being discharged from the cattle care facility. On the other hand, in the control group, only 42.9 % (3/7) showed suitable healing results to be discharged.

5. Discussion

In this study, MB-APDT proved to be a novel and effective adjuvant approach for surgical wound healing in cattle. The choice of MB concentration was based on previous studies that described successful outcomes in Veterinary Medicine and Dentistry [17,23,24]. However, the choice of photosensitizer concentration should consider specific aspects of each wound (bleeding, purulent exudate, etc…). Remarkably, MB-APDT-treated wounds presented better healing than the control group. In this regard, other studies have already demonstrated the ability of MB-APDT to treat food-producing animals with satisfactory results in dermatological and infectious diseases [18–20,25].

In large animal practice, surgical wounds are frequently treated with empirical administration of antibiotics [26] and topical antiseptics [22]. In this regard, a review of outcomes after rumenostomy reported the use of empirical perioperative antibiotics treatment in 92 % of the animals with no further discussion about this strategy regarding the outcomes in the cattle that received elective placement of rumen cannulas [26].

Worryingly, the use of antibiotics in these animals could contribute to the dissemination of antibiotic-resistant bacteria, which is currently considered a critical issue to global public health [27]. Although in our study all animals have received antibiotic therapy, some studies have reported that MB-APDT could promote a better outcome than antimicrobials [16,28]. Therefore, the replacement of antibiotics for non-antibiotic approaches should be strongly encouraged in contaminated surgical sites and infected wounds such as those from rumenostomy and if there are clinical indications to do so.

In conclusion, we demonstrated that APDT is an effective tool for the post-surgical management of rumenostomy. MB-APDT-treated wounds presented better wound closure, less post-surgery complications, and more animals were discharged from the cattle care facility at the same period. This pilot study ratifies the use of APDT in cattle and also may suggest that it could be performed for other surgical procedures as a complementary approach or an alternative for topical administration of antibiotics. Finally, subsequent studies are essential to clarify the potential applications of APDT in veterinary medicine as well as to assess its feasibility and effectiveness in large animal practice.

Declaration of Competing Interest

The authors report no declarations of interest.

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