

Liquid Formulation of Methylene Blue: Characterization and *In Vivo* Evaluation Against Canine Fibrosarcoma

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ABSTRACT

Due to the high prevalence of fibrosarcoma, a neoplasm that affects connective tissue in dogs, it is imperative to explore alternative therapies that offer better results and reduce adverse effects. In this context, a liquid formulation of the photosensitizer methylene blue was developed and characterized. This formulation was then evaluated in the *in vivo* treatment of fibrosarcoma in dogs. To this end, a mixed-breed female dog was subjected to photodynamic therapy protocols. The results showed a significant reduction in the tumor, indicating that the therapy not only improved the clinical condition but also contributed to the dog's well-being and quality of life.

Keywords: photodynamic therapy; methylene blue; fibrosarcoma; dog.

INTRODUCTION

Sarcomas originate from mesenchymal cells and have subtypes such as osteosarcoma, dermatofibrosarcoma protuberans, rhabdomyosarcoma, angiosarcoma, and fibrosarcoma (Demirdover et al., 2023. Radak; Ghamari; Fallahi, 2023). Among them, fibrosarcoma, observed in both human and veterinary medicine, is characterized by the uncontrollable multiplication of fibrous tissue cells, usually affecting deep soft tissues or tissues adjacent to bone (Al-Obaidy et al, 2023; Kitai et al, 2021).

In the veterinary field, the prevalence was estimated at 7,714 cases in 2021, with 80% affecting dogs and the skin being the most affected organ (50.3%), as it is a neoplasm that mainly affects connective tissue; and high treatment costs due to late diagnosis, advanced staging, expensive medications, and tests (Laki et al., 2022). Currently, surgical resection, radiotherapy, and traditional chemotherapy are used as treatment options (Machado, 2022. Daleck et al., 2007). However, there are several challenges, including low selectivity, high costs, risk of metastasis, long-term recurrence, side effects, and delayed healing. (Fernandes et al., 2023. Hu et al., 2024. Souza et al., 2023).

As a result, new strategies have been developed to reduce these therapeutic limitations, such as photodynamic therapy (PDT). This innovative tool is an efficient

and low-cost alternative for treating fibrosarcoma. PDT is characterized by the combined action of three fundamental elements: i) molecular oxygen; ii) photosensitizer (PS); iii) light source with an appropriate wavelength emission spectrum, resulting in the production of reactive oxygen species (ROS). The PDT mechanism is classified according to the type of productive oxidative species, type I: H_2O_2 (hydrogen peroxide), OH^{\cdot} (hydroxyl radical), O_2 (oxygen) and type II: 1O_2 (singlet oxygen). However, both mechanistic pathways have the ability to oxidize biomolecules present in cancer cells and consequently treat the referred disease (Algorri, 2021. Correia, 2021. Alvarez; Sevilla, 2024). Compounds acting as photosensitizers remain under extensive investigation. In oncology, which has been an area of constant discussion, they play a crucial role as light-activated agents capable of inducing the rupture of pathological cells (Taldaev et al., 2023; Belekov et al., 2020).

In this context, the present study aims to develop and characterize a liquid formulation of methylene blue and deepen the understanding of the effects of photodynamic therapy using methylene blue in canine fibrosarcomas, with the objective of evaluating its efficacy and potential as a viable alternative to conventional treatments.

METHODOLOGY

Preparation of the aqueous pharmaceutical formulation of methylene blue

An aqueous solution of methylene blue (500-1000 μ M) was prepared using commercial reagents.

***In vivo* photodynamic therapy protocol for the treatment of canine fibrosarcoma**

A female dog presented with lesions on the lower limb for 8 years, specifically in the radius and ulna region, characterized by muscle laceration, hyperemia, and destruction of muscle, dermal, epidermal, and subcutaneous tissues. The wounds had irregular edges and were accompanied by a marked inflammatory process with exudate.

Initially, an additional diagnostic test was performed, including a swab cytology smear with the help of a swab, which resulted in a confirmation for Fibrosarcoma-Type Mesenchymal Cell Neoplasm, a malignant tumor that predominantly occurs in

connective tissue. Next, a trichotomy and cleaning of the injured skin were performed, revealing inflammatory conditions, multilayer skin lesions, delayed healing, and alopecia.

The wound was cleaned and treated with a liquid pharmaceutical formulation of methylene blue, activated by an LED system according to the absorption spectrum of the chosen photosensitizer. Daily photodynamic therapy protocols were then carried out.

RESULTS AND DISCUSSION

After 30 phototherapy sessions, there was a significant improvement in tissue regeneration and healing. The lesion between the radius and ulna, which initially measured 1.5 cm, was reduced to 0.5 cm. In the central region of the metacarpal, the lesion decreased from 2 cm to 1 cm. In the distal portion of the metacarpal, the reduction was from 2.5 cm to 1.5 cm. Subsequently, a new swab cytology smear was performed, which showed the absence of neoplasia, a significant decrease in the inflammatory and infectious process, and complete interruption of the exudative process. The presence of pyogranulomatous lymphocytes and fibroblasts was also noted.

After 60 phototherapy sessions, there was even a further reduction in lesion size, accompanied by a significant improvement in the appearance of the skin layers. Healing led to the closure of the edges, and the hypodermis showed reconstitution of its adjacent structures. The dermis showed a favorable regenerative response, and the epidermis exhibited an excellent response in the reconstitution of its molecular structures and adipose tissue, with the formation of brown-to-black crusts.

In addition to improvements in the appearance of the lesions, the dog gained 2.5 kg in weight, showed increased muscle mass, greater strength and endurance, and a notable improvement in ambulation, with no signs of lameness.

CONCLUSION

A liquid pharmaceutical formulation was developed and characterized. In the context of in vivo evaluation, photodynamic therapy with the photosensitizer methylene blue demonstrated significant progress, proving to be a safe, effective, and efficient therapeutic option for the prevention of new systemic adverse effects. In addition, it

proved to be easy to handle, minimally stressful, time-saving, and economically viable. Thus, it outperformed conventional treatments, emerging as a promising photosensitizer capable of generating Reactive Oxygen Species (ROS) and acting as a potential antitumor agent. Further studies are underway.

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