The use of tigilanol tiglate (Stelfonta®) for the treatment of canine mast cell tumors

Het gebruik van tigilanol-tiglaat (Stelfonta®) ter behandeling van caniene mastceltumoren

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ABSTRACT

Mast cell tumors are by far the most common skin tumors in dogs. The traditional treatment approach is the removal of the entire tumor, typically with 2 to 3 cm lateral margins and one deep facial plane. However, due to several factors, including anatomic location, condition of the patient, and potential involvement of vital tissues, it is not always feasible to take adequate lateral or deep margins, which increases the risk of local tumor recurrence. In cases where the traditional approach cannot be used, the use of tigilanol tiglate (Stelfonta®) as local therapy may offer a potential alternative treatment. Tigilanol tiglate has recently been registered as an intratumoral medicine for non-resectable, non-metastatic mast cell tumors. The aim of this narrative review is to provide a practical overview on the use of tigilanol tiglate in canine mast cell tumors. According to the available literature, tigilanol tiglate is a valuable alternative approach when surgical excision with complete margins is not feasible or desirable. Its application is described as easy-to-administer, and therefore this therapy can be applied by general practitioners as well as in specialized veterinary practices.

SAMENVATTING

Mastceltumoren zijn veruit de meest voorkomende huidtumoren bij de hond. De traditionele behandelingsbenadering is de verwijdering van de gehele tumor, typisch met 2 tot 3 cm laterale marges en een diepe fascialaag. Door verschillende factoren, waaronder de anatomische lokalisatie, de conditie van de patiënt en mogelijke betrokkenheid van vitale weefsels, is het echter niet altijd haalbaar om adequate laterale of diepe marges te nemen, waardoor het risico op lokaal recidief verhoogt. In gevallen waarbij de gebruikelijke benadering niet kan worden toegepast, kan het gebruik van tigilanol-tiglaat (Stelfonta®) als lokale therapie een potentiële alternatieve behandeling bieden. Tigilanol-tiglaat is relatief recent geregistreerd als een intratumoraal geneesmiddel voor niet-resectabele, niet-metastatische mastceltumoren. Het doel van deze literatuurbespreking is om een praktisch overzicht te geven van het gebruik van tigilanol-tiglaat bij mastceltumoren bij de hond. Volgens de beschikbare literatuur is tigilanol-tiglaat een waardevol alternatief wanneer chirurgische excisie met volledige marges niet haalbaar of wenselijk is. De toepassing wordt beschreven als eenvoudig en daarom kan deze therapie zowel door eerstelijnsdierenartsen als in gespecialiseerde dierenartspraktijken worden gebruikt.

INTRODUCTION

A mast cell tumor (MCT) arises from the neoplastic proliferation of mast cells and represents approximately 20% of all canine skin tumors (Patnaik et al., 1984; Blackwood et al., 2012; Oliveira et al., 2020). Although most mast cell tumors (MCTs) are solitary, a significant number of dogs may be presented with multiple tumors (Patnaik et al., 1984).

There are several options for the treatment of MCTs, based on the presence or absence of adverse prognostic factors, the clinical stage of the disease, and the tumor grade (London et al., 2020). Traditionally, whenever feasible, treatment involves surgical excision of the neoplastic mass with 2-3 cm (depending on the grade) lateral margins and one fascia plane deep margin, and preferentially, also excision of the sentinel lymph node (Simpson et al., 2004; Fulcher et al., 2006; Blackwood et al., 2012; Marconato et al., 2018; London et al., 2020). Surgical removal of the primary MCT as the sole treatment is considered curative in low-grade tumors that were resected with adequate margins in the absence of lymph node metastasis. Adjuvant therapy with chemotherapy, radiation therapy or tyrosine kinase receptor inhibitors should be considered in case of histopathological confirmation of dirty margins, high-grade MCTs, and/or metastatic lymph nodes. Furthermore, adequate surgical margins cannot always be obtained, such as in case of neighboring vital tissues or impractical localization of the tumor (e.g. on the distal extremities) (Boyle et al., 2014). In those cases, other local therapies can be explored (Boyle et al., 2014; Panizza et al., 2019). A major advantage in general is that systemic toxicity is minimized, while the local accumulation of the drug allows for an effective dose to be reached (Boyle et al., 2014). Examples of local tumor treatment are intratumoral interleukin-2 therapy, intralesional corticosteroids, intraregional deionized water, hyperthermia combined with radiotherapy, photodynamic therapy, cryotherapy, electrochemotherapy, and the administration of Sendai virus with oncolytic properties (Krahwinkel, 1980; Kodre et al., 2009; Spugnini et al., 2011; Ziekman et al., 2013). In case of canine MCTs, a new treatment option that is easy to administer intratumorally and certainly practice-friendly has reached the market, namely tigilanol tiglate (TT; Stelfonta®, Virbac, the Netherlands).

Tigilanol tiglate possesses anti-tumor activity and stimulates wound healing in the region of the treated site through the activation of protein kinase C (Wiest et al., 2021)..

Within this narrative review, the published data on the use of TT as an approved novel intratumoral therapy in/for MCTs in dogs are addressed.

DISCOVERY, USE AND MODE OF ACTION OF TIGILANOL TIGLATE

Tigilanol tiglate (also known as EBC-46) is a diterpene ester isolated from the seed of the Australian rainforest plant Fontainea picrosperma (Boyle et al., 2014). This new small molecule is currently approved

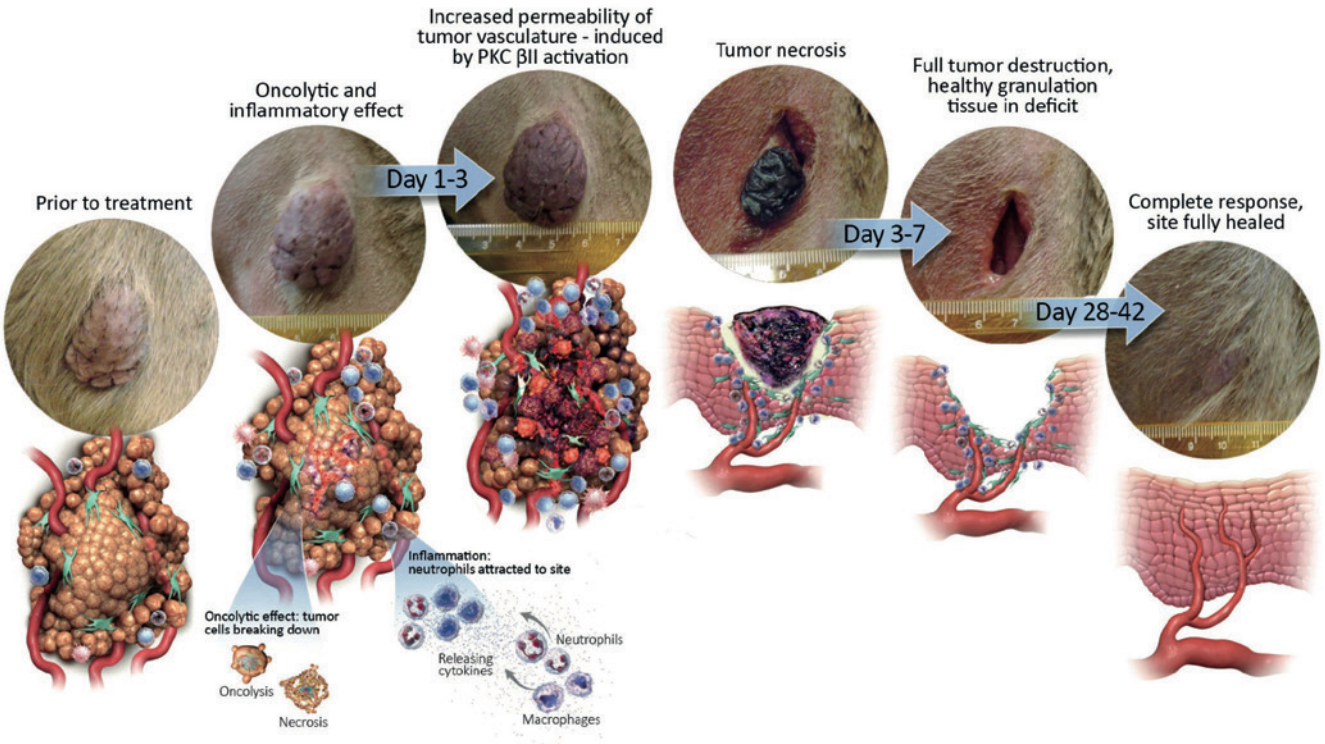

Figure 1. Chronological presentation of the multifactorial mechanism of action of tigilanol tiglate (Qbiotics, 2020).

by the European Medicine Agency (EMA) as a veterinary medicinal product, under the commercial name Stelfonta®, in Europe, the UK and the USA to treat dogs with non-metastatic, non-resectable cutaneous and subcutaneous MCTs, and all grades of non-metastatic cutaneous MCTs (Jones et al., 2021). The drug has also been evaluated in clinical studies as intratumoral treatment of various other (sub)cutaneous tumor types in dogs, horses but also in humans, showing promising results (Boyle et al., 2014; Miller et al., 2019; Panizza et al., 2019; de Ridder et al., 2021).

Tigilanol tiglate is a potent cellular signalling molecule with a multifactorial mode of action (Figure 1). This diterpene ester is a phorbol ester that activates alpha, beta I, beta II, and gamma isoforms of protein kinase C. Protein kinase C enzymes are ubiquitous serine-threonine kinases found in different cell types. After activation, they translocate to membranes and promote downregulation of enzymes. Once this cascade is initiated, processes, such as proliferation, differentiation, apoptosis and migration, are regulated (Breitkreutz et al., Newton, 2010; Boyle et al., 2014). Prolonged stimulation of certain protein kinase C isoforms can exert potent antitumor effects (Boyle et al., 2014). The effects of TT appear already a few moments after administration; a rapid, acute and highly localized inflammatory response is seen in the tumor mass and its surroundings. Then, as a response to the inflammation, the innate immune system will be triggered. At the same time, activation of the protein kinase C pathway in the tumor vascular endothelial cells will induce a loss of tumor vascular integrity, which will eventually lead to tumor cell death with swelling and subsequent necrosis, usually achieved within three to seven days post injection. After the complete destruction of the tumor, wound healing is facilitated by the induction of the innate immune response that triggers a downstream of cytokines and chemokines, important to the initiation of wound healing processes at the treatment site (Boyle et al., 2014; Campbell et al., 2014; Barnett et al. 2019; Panizza et al., 2019). Wound healing after tumor destruction by the TT-injection is typically completed/achieved within four to six weeks (de Ridder et al., 2021; Reddell et al., 2021).

TIGILANOL TIGLATE TREATMENT PROTOCOL

Indications and contra-indications

Stelfonta® is registered for the treatment of non-resectable, non-metastatic (sub)cutaneous mast cell tumors that are accessible to intratumoral injection.

Patient factors, such as age, breed or sex of the dog, are not relevant. In contrast, the size and location of the primary tumor are. The tumor volume should not exceed 8 cm^3. It is essential that the volume is determined at the day of the drug administration (EMA, 2020). Subcutaneous MCTs are excluded if they are located above the elbow or hock (e.g. on the head, neck, body) as necrotic debris from the injected tumor may accumulate in the subcutaneous space, increasing the risk of systemic adverse reactions, including death, from mast cell degranulation (Blackwood et al., 2021). This restriction does not apply to cutaneous MCTs, for which eligibility is independent of tumor location. On the other hand, caution should be taken when treating tumors in mucocutaneous and sensitive locations as irritation or subsequent necrosis might impair function. Furthermore, all grades are officially eligible for treatment with Stelfonta®. Yet, it is noteworthy to mention that tumors with an unfavorable cytological grading will less frequently have a complete response (Camus et al., 2016; Brown et al., 2021).

Diagnosis of a MCT should always be confirmed by cytological examination of a fine needle aspirate (FNA) (Camus et al., 2016). In addition, the presence of metastatic disease should be excluded. Therefore, complete staging of the dog is recommended, including bloodwork (hematology and biochemistry), assessment of the regional/sentinel lymph nodes, abdominal ultrasound with FNAs of the spleen and liver, and (arguably) thoracic radiographs (Blackwood et al., 2012). In a large study on the use of TT in veterinary medicine, the majority of dogs did not have complete staging (de Ridder et al., 2021). This reflects the situation in routine clinical practice, although staging is strongly recommended in dogs with suspected aggressive MCTs/MCTs showing aggressive clinical behavior. Since the drug has not been evaluated in dogs with systemic signs of MCTs, including vomiting, diarrhea and inappetence, such patients were also not eligible for treatment in that study (de Ridder et al., 2021). Further exclusion criteria were previous treatment and/or interventions, including: (1) radiotherapy, chemotherapy, surgery or biopsy of target tumor at any time; (2) systemic or local anticancer therapy in the last two months; (3) non-steroidal anti-inflammatory drugs (NSAIDs) in $\leq$ 7 days; (4) immunosuppressive doses of corticosteroids or anti-allergic medication (e.g. oclacitinib or cyclosporin) or canonized monoclonal antibodies within the last 14 days (de Ridder et al., 2021). It is recommended to include dogs with MCTs where the surface is intact, with the expectation of minimal product leakage from the tumor surface after the intratumoral injection. In MCTs with an ulcerated surface, part of the intratumorally injected TT might leak from the surface, potentially reducing effectiveness.

Although sedation or general anesthesia is not required for Stelfonta® treatment, in case of a frightened/aggressive dog or a MCT at a sensitive location, sedation is preferred to ensure an accurate and safe injection of the tumor site while minimizing the risk of self-injection (de Ridder et al., 2021).

Dose calculation

The administered dose of TT depends on the tumor volume (in cm^3).

Tumor volume (cm^3) = ½ x length (cm) x width (cm) x depth (cm)

The dose rate is 0.5 mL of 1 mg/mL TT per cm^3 of tumor volume, except when the calculated volume is <0.2 cm^3, in which case a minimum dose of 0.1 mL should be injected.

Dose volume (mL) = tumor volume (cm^3) x 0.5 mL (with a minimum volume of 0.1 mL)

Intratumoral injection of tigilanol tiglate

First, the area around the MCT is clipped with minimal manipulation to provide a clearly visible injection site. Extensive clipping is recommended so that any local reaction and potential adverse effects will not be masked by the coat. Secondary healing will also proceed better as there will be less risk of bacterial contamination. It is recommended to use a 23 Gauche needle on a Luer-Lock syringe for intratumoral injection. Otherwise, increased intratumoral pressure during injection may cause separation of the needle and the syringe, and lead to inadequate dosing and a risk of exposure of Stelfonta® to the administrator.

The appropriate point for inserting the needle is on the edge of the tumor. The product must be dispersed within one injection, i.e. after inserting the needle and controlling that no vessels were hit. Injection should be performed with equal pressure and a fanning manner to maximize distribution into the tumor (Figure 2); and the needle should not be immediately redrawn to prevent expulsion of the product.

Concomitant medication

The injection with TT results in an acute local inflammatory response with swelling, bruising and erythema, followed by hemorrhagic necrosis. Concomitant medications, shown in Table 1, are used to minimalize the associated side effects and discomfort. In several studies, it has been demonstrated that using concomitant drugs leads to a treatment with fewer effects of local or systemic degranulation that can occur when MCTs are disturbed (Blackwood et al., 2012; de Ridder et al., 2021; Reddell et al., 2021).

The corticosteroids (e.g. oral prednisone or prednisolone at anti-inflammatory dose) must be initiated orally at inflammatory dose two days before Stelfonta® treatment to minimize the inflammation, and must be continued for eight days after injection. During the first seven days, a dose of 0.5 mg/kg q12 hours must be administered/continued, followed by three days at a dose of 0.5 mg/kg q24 hours. The H1 receptor blocking agent (e.g. oral diphenhydramine, 2 mg/kg PO q12 hours) potentially prevents the development of gastric ulceration and must be initiated on the day of Stelfonta® treatment and be continued for a total of eight days. In addition, the H2 receptor blocking agent (e.g. oral famotidine, 0.5mg/kg q12 hours) decreases the adverse effects of histamine on the peripheral vasculature and wound healing and must be started on the day of Stelfonta® treatment and be continued for eight days.

In a randomized controlled clinical study by de Ridder et al. (2021) evaluating the efficacy and safety of TT-treatment, three types of pain medication were used post injection. Tramadol was used in more than three quarters of the cases; buprenorphine and gabapentin being less commonly used and less frequently combined with tramadol (de Ridder et al., 2021).

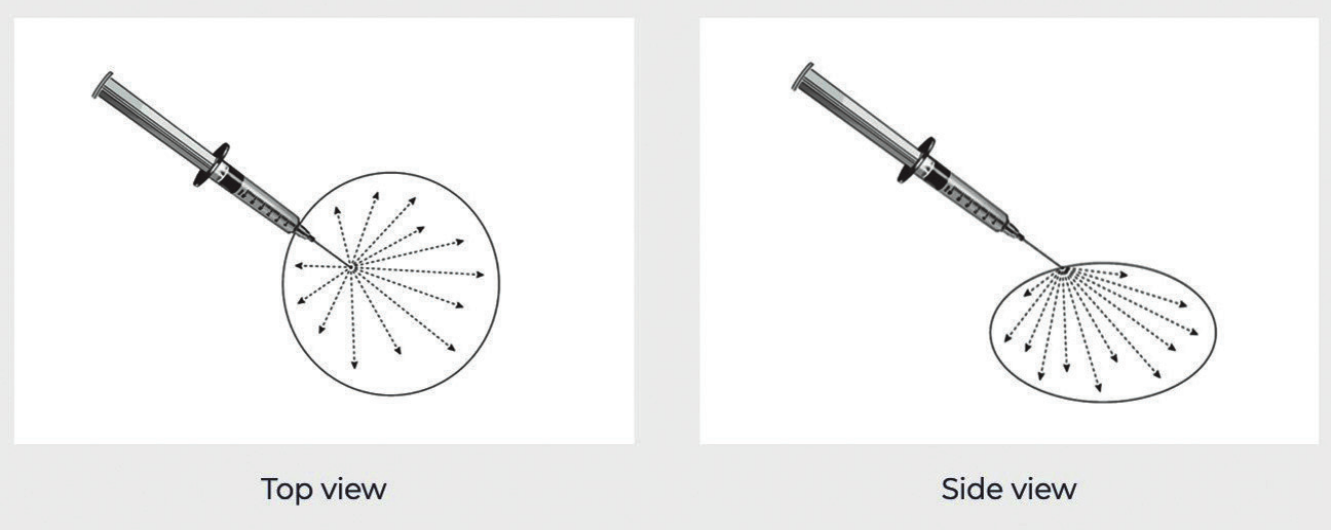

Figure 2. Simulation of the intra-tumoral injection with tigilanol tiglate in a mast cell tumor (Qbiotics, 2020).

Table 1. A general schedule of concomitant medication based on the concurrent medication, based on the dosing scheme by Virbac (2020), to minimize the risk of mast cell degranulation during treatment with tigilanol tiglate.

Concomitant medication	Day-2		Day-1		Day of injection		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
Corticosteroids (e.g. prednisolone 0.5 mg/kg po q12/24h)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x	
H1 receptor blocking agent (e.g. diphenydramine 2.0 mg/kg po q12h)					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
H2 receptor blocking agent (e.g. famotidine 0.5 mg/kg po q12h)					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Pain relief (e.g. tramadol 3-5 mg/kg po q8h)					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Follow-up and prognosis

In the literature, no mandatory rechecks have been described after injection with TT. Ideally, rechecks should be scheduled every other week after injection until complete wound healing has occurred. A subsequent control consultation may take place three months later to evaluate whether there is any recurrence. Within the first four days after injection, it is recommended to remind the owner of the importance of the concomitant medications and to monitor whetherany pain management needs to be implemented. On day seven, a check-up may be helpful to assess the size of the wound, initiate wound management if necessary and address the concerns of the owner.

The use and necessity of a bandage after the injection is variable and dependent upon the patient characteristics, tumor location, response to therapy and treatment site drainage. Dressing and bandaging are not necessary nor recommended in most of the cases. In a pivotal study on wound management and healing, the use of a bandage at any stage after the TT-injection was discouraged because of the possible interference with the resolution and drainage of local edema, followed by an increased wound size due to affection of the surrounding tissue (reddell et al., 2021).

In the large study by de Ridder et al. (2021), complete response after one treatment was achieved in 75% of cases by day 28, and an additional 12% with two injections combined in case complete response was not achieved with one single injection.

When no response or an incomplete response occurs, potential repeated dosing can be conducted, but some conditions should be considered. It is recommended to wait at least 28 days before a second injection is given. This way, it is easier to obtain a noticeable difference in treatment response and wound healing. Before re-treating a tumor that did not achieve complete response, it is crucial to get a better understanding of the possible reasons for therapy failure. There are two main reasons: underdosing and interference with the immune response due to concurrent medication or disease.

In terms of safety and tolerability, most adverse events, including wound formation, tumor site pain, injection site bruising, swelling and erythema and lameness in the treated limb are low-grade, transient and manageable. There are two essential indicators of the efficacy of TT and thereby desirable side effects, namely erythema at the injection site and the formation of tumor necrosis and skin defect (de Ridder et al., 2021).

CONCLUSION

Tigilanol tiglate is a potential candidate to treat non-metastatic, non-resectable MCTs in dogs; however, several inclusion and exclusion criteria must be considered when evaluating whether a dog with a MCT is eligible for TT treatment or not. Clinical staging should be carried out before the injection to rule out metastatic disease.

Because of the multifactorial mechanism of action

of TT that relies mainly on the response of the 'host tissues', the use of TT also offers a potential treatment for a range of different tumor types in various species.

Tigilanol tiglate is a local therapy, with little systemic toxicity. Nevertheless, it is important to closely follow the treatment protocol so that pre- and concomitant medication and the injection are carried out correctly to aim for a complete response and minimize the risk of adverse events due to MCT degranulation.

A major concern of TT treatment and thereby also a significant difference with surgical excision is the fact that no margins and biopsies are taken. As a result, the information on the histological grade and/or the presence of the satellite tumor cells remains lacking.

The price of a Stelfonta® treatment varies according to the initial tumor volume. A single Stelfonta® treatment is generally less expensive than the cost of surgical excision. An additional advantage of TT in this regard is that the procedure can be performed in basically any veterinary practice, whereas chemotherapy and/or radiation therapy should be performed in more specialized practices/centers.

It can be concluded that in selected cases, TT can be proposed to owners as a worthy alternative to surgery. It offers a new opportunity for general practitioners and specialized veterinary centers to treat cutaneous and subcutaneous MCTs below the elbow and the hock in dogs, thereby avoiding general anesthetic and invasive surgery.

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