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Literature and clinical study evaluating the efficacy of electrochemotherapy using bleomycin and cisplatin against canine mast cell tumours

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LIST OF ABBREVIATIONS

bFGF: Basic fibroblast growth factor

CCL2: Chemokine ligand 2

CDDP: Cisplatin

CI: Confidence interval

DAMP: Danger-associated molecular pattern

ECT: Electrochemotherapy

EGT: Electrogene therapy

FNA: fine needle aspiration

GMPs: Granulocyte/ monocyte progenitors

HPF: High power field

ICD: Immunogenic cell death

IFN: Interferon

IFN-γ: Interferon gamma

IL: Interleukin

MCP: Mast cell progenitor

MCT: Mast cell tumour

MHC: Major histocompatibility complex

PAMP: Pathogen-associated molecular pattern

PEF: Pulsed electric field

TLR: Toll-like receptor

TMV: Transmembrane voltage

TNF-α: Tissue necrosis factor alpha

VEGF: Vascular endothelial growth factor

1. INTRODUCTION

Mast cell tumours (MCT) are the proliferation of mast cells, also known as a mastocytoma or a mast cell sarcoma. MCTs are amongst the most diagnosed dermatological neoplasms, accounting for 7% to 21% of all skin tumours and 11% to 27% of all cutaneous malignancies in dogs (Spugnini *et al*., 2006). They primarily appear in older dogs, mean age approximately 8-9 years, but can also occur in young dogs and there is no apparent sex predilection. Most MCTs arise in mixed breeds. However, some breeds appear to be at an increased risk such as those of bulldog descent (boxer, boston terrier, English bulldog, pug), Labrador, golden retrievers, cocker spaniels, Rhodesian ridgeback and Chinese shar-pei (London and Thamm, 2020).

The first mode of treatment for low-grade MCTs is surgical removal. However, Weisse, Shofer and Sorenmo (2002) reported that MCTs have a recurrence rate of 22% to 54% after surgery when surgical margins are not wide enough. Due to the high recurrence rates with surgical excision alone, it becomes evident that alternative treatments should be studied and conducted on MCTs either solely or in conjunction with surgery.

Electroporation, or electro-permeabilization, is the phenomenon in which cell membrane permeability to ions and other non-permanent molecules is increased via exposing the cell to short high electrical field impulses. The process causes nanoscale defects or pores in the cell membrane.

A breakthrough occurred in 1991 and the term electrochemotherapy was coined. The use of reversible electroporation to facilitate the diffusion of bleomycin, an anticancer drug, into malignant cells was carried out in its first clinical trial in human patients with head and neck squamous carcinomas in France (Mir *et al*., 1991). Similarly, another anticancer drug cisplatin was first used to treat a patient in 1995 Ljubljana, Slovenia (Rudolf *et al*., 1995). Bleomycin and cisplatin are two primary drugs used today in electrochemotherapy.

The aim of this thesis is to examine current literature describing the efficacy of cisplatin and bleomycin with electrochemotherapy in canine MCTs and to underline its advantages in the future use of veterinary medicine. My goal is to aggregate the current scientific data surrounding electrochemotherapy and its possible use against canine MCTs. The thesis will include a comparative investigation into different papers and will also include some case studies from my own supervisor's clinic, Dr. Juhász Orsolya.

2. LITERATURE REVIEW

2.1 CANINE MAST CELLS

The origin of mast cells begins in bone marrow derived hematopoietic stem cells, where mast cell progenitors (MCPs) are derived from granulocyte/ monocyte progenitors (GMPs) during development, a complex network of transcription factors regulate the transformation. MCPs leave the bone marrow in an immature state and migrate in the peripheral blood. Here, they invade epithelial and connective tissue such as the gastrointestinal tract, respiratory tract, blood vessels and skin to mature (Dahlin and Hallgreen, 2015)

The diameter of canine mast cells ranges from 12-14 μm and the average size of granules is 0.47 μm. The granules contain many varying chemical mediators which are preformed or newly formed upon activation and released (Kumar and Sharma, 2006); proteases such as tryptase, proteoglycans like heparin, vasoactive amines histamine and serotonin, cytokines TNF-α and interleukins, prostaglandins, chemokines CCL2, growth factors VEGF and bFGF. Histamine is the most important mediator discharged and is involved with an allergic response (oedema, warmth, erythema, itchiness, etc.). The stimulus of H1 receptors by binding to histamine prompts the classical allergic reaction, causing increased vascular permeability and constriction of smooth muscles and mucous secretion e.g. an asthmatic reaction (London and Thamm, 2020).

Mast cells are key in the innate immune system by binding to pathogens directly or associating with pathogen-associated molecular patterns (PAMPs) on the mast cell surface. Once the pathogen has attached to the receptors on the mast cell, it causes the release of inflammatory mediators to eliminate the pathogen. TNF-α activates macrophages, endothelium and cytokines. Interleukins and colony stimulating factors express eosinophil production and chemokines activate macrophages and neutrophils. Followed by an increase in vascular permeability, increased fluid accumulation, recruitment of immune cells and the production of antibacterial products such as cathelicidans, psidins and defensins (Krystel-Whittemore, Dileepan and Wood, 2016).

Regarding adaptive immunity, mast cells process and highlight antigens via MHC-I and MHC-II. For instance, toll-like receptor (TLR)-7 is stimulated on the mast cell surface, IL-1 and TNF-α cause dendritic cells to proceed from their locality in the skin to the local lymph nodes activating cytotoxic T cells. It becomes strikingly evident the role mast cells play in both the innate and adaptive immune systems (Krystel-Whittemore, Dileepan and Wood, 2016).

Finally, mast cells are associated with enhancing angiogenesis. The cells discharge pro-angiogenic factors like VEGF, bFGF, TGF-beta, TNF-alpha and IL-8. Proteases and heparin activate a cascade system causing even more release of proangiogenic factors. Histamine is also released and induces permeability of the microvasculature promoting further angiogenesis. Evidence also describes mast cells enhancing angiogenesis in tumour growth (Norrby, 2002).

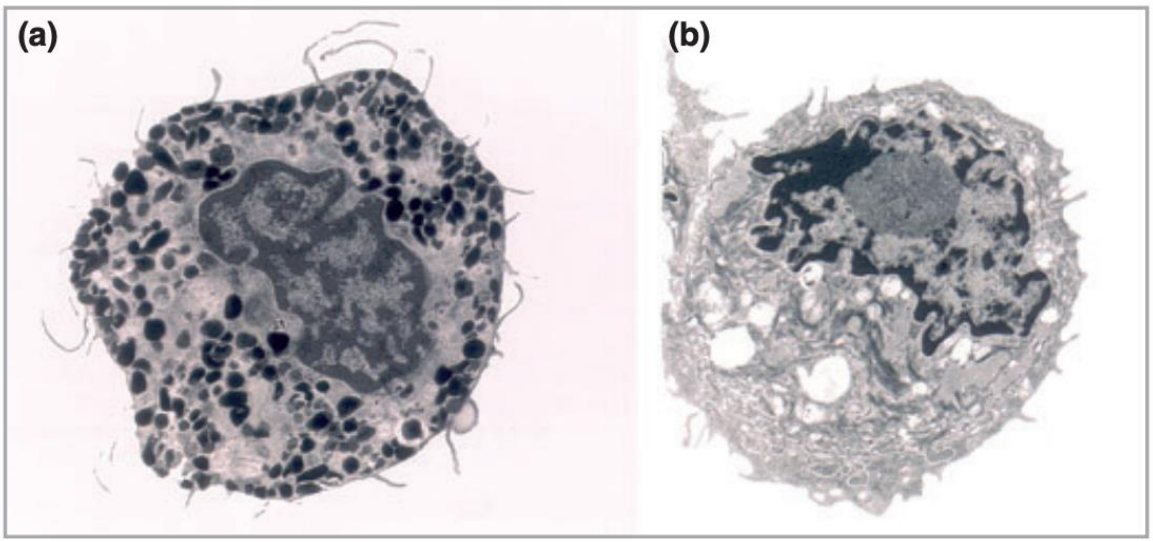

Figure 1: Electron microscopy image of an isolated canine mast cell before (a) and after (b) degranulation (De Mora, Puigdemont and Torres, 2006).

2.2 Canine Mast Cell Tumours

2.2.1 Pathogenesis of Canine Mast Cell Tumours (MCTs)

The aetiology of MCTs in dogs is for the most part unknown, but as with most neoplasms is probably multifactorial. The well documented breed predispositions like golden retrievers, Labradors, those of bulldog descent, Rhodesian ridgeback and Chinese shar-pei imply an underlying genetic component (London and Thamm, 2020).

Studies have indicated the stem cell factor receptor, KIT, in the aetiology of MCTs. KIT is normally expressed on mast cells and is a surface growth factor receptor. KIT consists of an extracellular ligand-binding domain, a transmembrane region and a cytoplasmic tail with ligand dependant tyrosine kinase activity. It is encoded by the proto-oncogene c-kit, which is a type of receptor tyrosine kinase and tumour marker. Activated KIT phosphorylates intracellular proteins commencing a signalling cascade of a wide range of biological activates including the proliferation, migration and maturation of hemopoietic stem cells such as mast cells (Welle *et al*., 2008). The ligand for KIT is the stem cell factor, KIT ligand

or mast cell growth factor. KIT expression has been demonstrated in both normal mast cells and neoplastic mast cells with a higher expression in poorly differentiated MCTs. The aberrant cytoplasmic localisation and/ or increased phosphorylation of KIT may be associated with dysregulated KIT function (London and Thamm, 2020). Other authors have expressed somatic mutation in the c-kit gene involving either the juxtamembrane domain, exons 11-12, or the extracellular domain, exons 8-9. The mutations result in an activated KIT protein in the absence of ligands generating subsequent unregulated KIT signal transduction. These c-kit mutations have been associated with 15-40% of all canine MCTs (Welle *et al*., 2008).

In more recent times, research has been undertaken to better understand copy number variation, gene expression profile and proteomic profile of canine MCTs to identify pathways contributing to aggressive cell behaviour. The studies displayed mutations in the p53 gene pathways and regions of loss in phosphatase and tensin homologs (London and Thamm, 2020).

Setting aside genetic components, some investigations concluded that chronic cutaneous inflammation may be a source of MCTs. Although only rare cases of chronic dermatitis, scar formation or the application of skin irritants have actually been documented. No decisive evidence has been encountered regarding a viral aetiology even though MCTs have been transplanted to young laboratory dogs using tumour cell tissues (Welle *et al*., 2008)

2.2.2 Appearance of Mast Cell Tumours

Dogs with cutaneous MCTs are most frequently presented for the examination of a solitary cutaneous mass and rarely for any clinical symptoms related to the release of the mediators from the granules. Approximately 50% of cutaneous MCTs arise on the perineal and trunk area, 40% on the limbs and 10% on head and neck (London and Thamm, 2020). MCTs have also been reported in other regions such as the conjunctiva, salivary gland, nasopharynx, larynx, oral cavity, ureter and even spine (Iwata *et al*., 2000).

The gross appearance of MCTs varies greatly and can mimic other cutaneous tumours and non-neoplastic conditions. Therefore, MCTs should be listed as a differential diagnosis for any type of skin nodule. There might be some correlation between MCT appearance and histological grade. Low grade well differentiated MCTs often present as slowly growing, solitary and a rubbery nodules, 1-4 cm in diameter, often present for longer than 6 months before diagnosis. May also appear alopecic but not usually ulcerated. MCTs can be subcutaneous presenting as soft and fleshy on palpation, these are often misdiagnosed as

lipomas. Undifferentiated MCTs are likely to be rapidly growing, ulcerate, irritate and give rise to smaller nodules in nearby tissues. The surrounding area may be accompanied with urticarial swelling and diffuse oedema and inflammation similar to cellulitis. In most dogs, MCTs appear usually with solitary masses, but 5%-25% present with multiple skin tumours that appear either synchronously or sequentially (Welle *et al*., 2008).

The metastatic pattern of MCTs is different from majority of other tumours in that pulmonary metastasis is relatively infrequent. The most common location of MCT metastasis is regional lymph nodes, spleen and liver. Organ enlargement is usually present and the pattern is often nodular rather than diffuse. Less frequent locations are the bone marrow, myocardium, kidney and mesentery (O'Keefe, 1990)

It's important to note that palpation of a MCT may cause degranulation, releasing histamine and other vasoactive substances, resulting in local vasodilation, oedema and erythema. This phenomenon is known as Darier's sign (Rabanal and Ferrer, 2002).

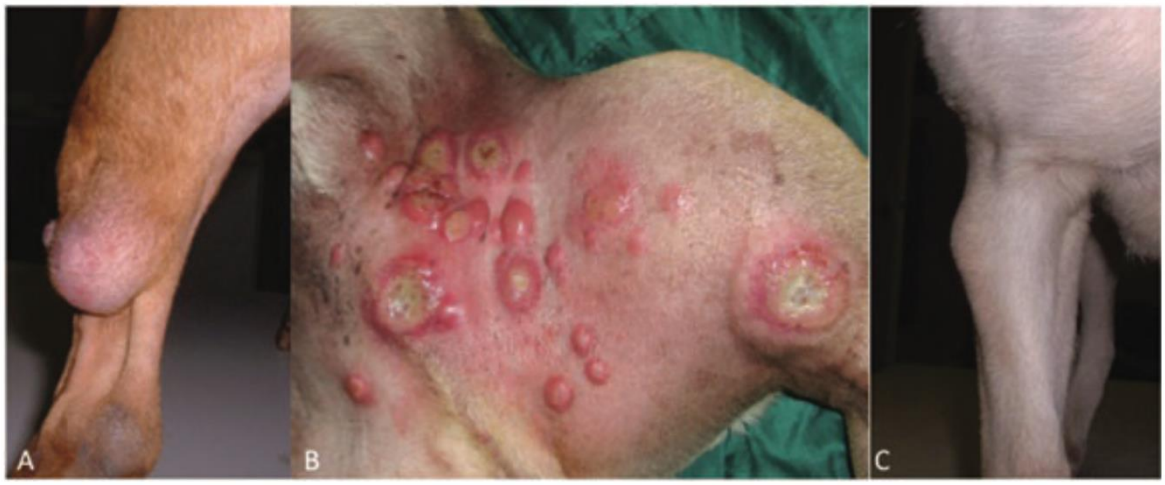

Figure 2: Example of a clinical appearance of canine MCTs. (a) Well differentiated, (b) poorly differentiated and (c) a subcutaneous MCT (Blackwood *et al*., 2012).

2.2.3 Diagnosis of Canine Mast Cell Tumours

A diagnosis investigation of a canine MCT has three goals to fulfil: (i) definitive diagnosis via cytology and/ or histopathology (ii) clinical staging and (iii) documentation of paraneoplastic clinical signs (Rogers, 1996).

First protocol is taking a fine-needle aspirate (FNA) of any dermal or subcutaneous masses, even if a MCT isn't suspected. FNA is a simple procedure and should be performed prior to any surgery because a preoperative diagnosis of a MCT will influence the type of treatment. Rapid modified Romanowsky stains (e.g. Diff-quick) can be used but sometimes granules can stain poorly. For best results, metachromatic stains like Giemsa-Wright or toluidine blue should be used which colour the cytoplasmic granules purple-to-red (Govier, 2003). Stained cytology slides from FNAs can diagnose MCTs, yielding a correct diagnosis in 92-96% of histologically confirmed MCTs (London and Thamm, 2020). Cytology presents discrete round cell population with moderate amounts of purplish cytoplasmic granules of variable number and size. They have an oval to round nucleus which can be masked by the intense granule staining. Other cells found in the cytology sample may include eosinophils and fibroblasts (Welle *et al*., 2008).

FNA plays a significant role in the ECT treatment, as we often don't have a histological grade before ECT sessions, only a cytology result because it is quicker and easier to prepare. Cell morphology and staining characteristics observed on cytological slides can provide an indication about the grade of differentiation. For example, high-grade tumours normally present with a high cellular sample and a low number of cytoplasmic granules, irregular nuclei, increased mitotic figures and pleomorphism (Welle *et al*., 2008).

Scarpa, Sabattini and Bettini (2016) discussed how cytoplasmic granules in mast cells are more easily recognisable cytologically rather than histologically due to the bigger cell size and use of metachromatic stains. The study investigated the ability to accurately grade a series of 50 cutaneous MCTs with cytological smears. The histological grade was correctly predicted in 94% of cases. Hence, it is understandable why pre-operative biopsies are sometimes not performed.

The use of conjugated avidin Pío Del Río Hortega silver carbonate, alpha-trypsin and vimentin have also been described to provide additional information for poorly differentiated MCTs (Misdorp, 2004)

A biopsy sample should be taken for histopathology grading to confirm a clear diagnosis even in conjunction with a cytological diagnosis. Further diagnostic work can be carried out after cytology and histopathology of the mass. This consists of FNA of the draining lymph nodes, even if normal in size. Abdominal ultrasound and cytology of the liver and spleen is advised along with a thoracic radiography to rule out metastasis (Welle *et al*., 2008).

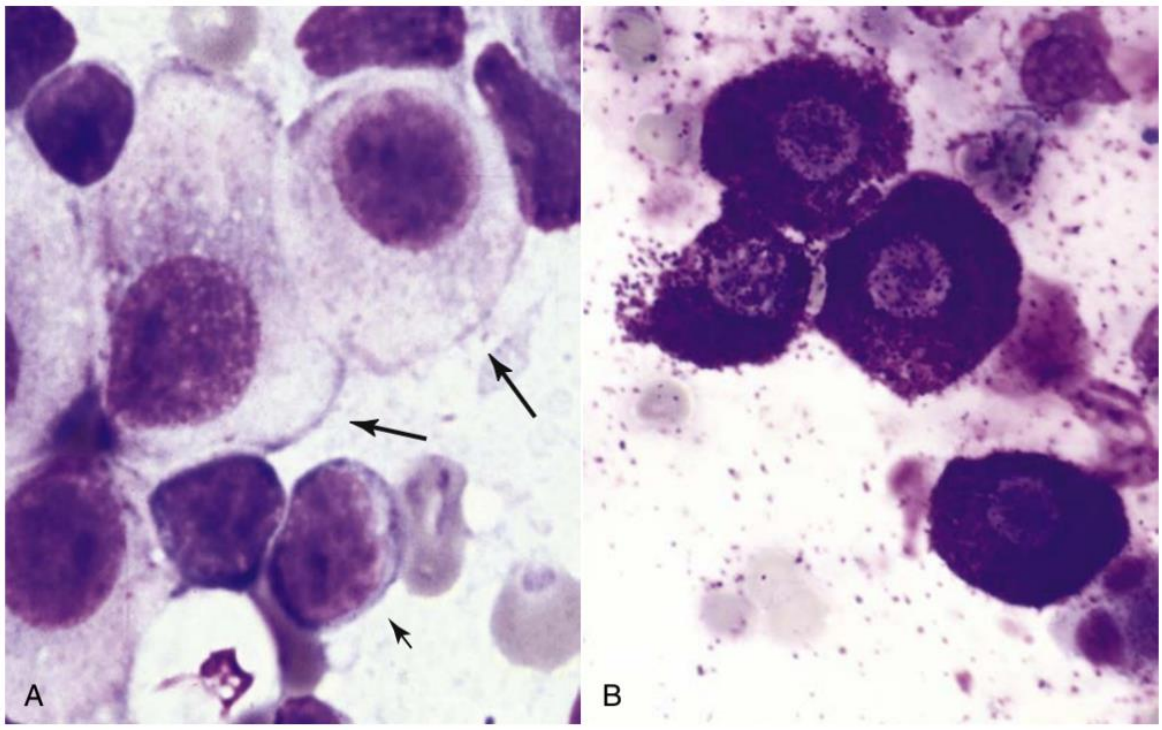

Figure 3: Fine-needle aspirate of a mast cell tumour. (A) Granules in mast cells (large arrows) fail to stain when specimen is stained with an aqueous quick stain. Small lymphocytes (small arrow) are also present. (B) Granules are prominent in a Wright-stained specimen from the same tumour (London and Thamm, 2020).

2.2.4 Histological Grading of Canine Mast Cell Tumours

There are two histological grading schemes for MCTs published, Bostock (1973) and Patnaik (1984). Both were refined using a relatively small number of retrospectively collected MCT biopsy samples (Dobson and Scase, 2007). With regard to Patnaik scheme which is more frequently used, there are numerous issues in standardising the histological grading criteria because of user subjectivity. A study carried out by Northrup *et al*. (2005) discussed how different veterinary pathologists will assign different histological grades to the same MCT.

The Patnaik system classifies MCTs into 3 grades based on histological characteristics based on depth of invasion, cellular atypia, granularity, nuclear features, mitotic count and

multinucleation. According to this classification, grade 1 MCTs are low grade, well differentiated with excellent long-term prognosis. Grade 3 MCTs are poorly differentiated, high grade, being more invasive and likely to metastasise. However, grade 2 behaviour is more difficult to predict and in combination with interobserver variation, it becomes a repeatedly cited weakness of the Patnaik grading scheme (Kiupel *et al*., 2011).

To improve consistency amongst pathologists and reduce uncertainty of the intermediate grade 2, a two-tier histologic grading system was proposed in 2011 by Kiupel and colleagues. In the Kiupel system, diagnosis of a high grade MCT is defined by; at least 7 mitotic figures in 10 high power fields (HPFs), least 3 multi-nucleated cells, 3 bizarre nuclei and karyomegaly. If these standards are not met, the tumour is considered low grade. Importantly, the study demonstrated a 96.8% consistency rate amongst pathologists employing the Kiupel scheme (Kiupel *et al*., 2011).

In conjunction with histopathological grading, various other factors play a role in prognosis. These include but not limited to clinical stage, location, cell proliferation rate, micro-vessel density, systemic signs, age and tumour size (London and Thamm, 2020).

Table 1: Histological Classification of Mast Cell Tumours in Dogs (London and Thamm, 2020)

Grade	Bostock Grading	Patnaik Grading	Microscopic Description
Anaplastic, undifferentiated (high grade)	1	3	Highly cellular, undifferentiated cytoplasmic boundaries, irregular size and shape of nuclei; frequent mitoses, sparse cytoplasmic granules
Intermediate grade	2	2	Cells closely packed with indistinct cytoplasmic boundaries; nucleus-to-cytoplasmic ratio lower than anaplastic; infrequent mitoses; more granules than anaplastic
Well differentiated (low grade)	3	1	Clearly defined cytoplasmic boundaries with regular, spherical or ovoid nuclei, mitoses rare or absent; cytoplasmic granules large, deep staining, and abundant

2.3 ELECTROCHEMOTHERAPY

2.3.1 Electroporation/ Electropermeabilization

Exposure of biological cells and tissues to short electric pulses with sufficient amplitude increases the permeability of the membrane. The phenomenon is termed electroporation or electropermeabilization, often used as synonyms. However, the former term is narrower and refers only to the contribution to the increased permeability of the membrane due to the formation of aqueous pores in the lipid bilayer. Meanwhile, the latter is a more general term and indicates the biophysical and biomechanical mechanisms (Kotnik *et al*., 2019).

At a cellular level, there is an electrical potential difference between the inner and outer side of the plasma membrane. Regulated by a system of ion pumps and channels, a resting

transmembrane voltage (TMV) is sustained ranging from -40 to -70mV. Exposure of the cell to a significantly strong pulsed electric field (PEF) can cause an induced TMV far exceeding its resting range altering the membrane structure and constituent molecules (Pucihar *et al*., 2006).

The electropermeabilization facilitates the inflow of previously impermeant molecules into the cell and the outflow of biomolecules from the cell. Figure 4 below describes the electropermeabilization molecular mechanisms occurring in the cell membrane. (a) Electroporation: electrically induced formation of aqueous pores in the lipid bilayer, shown in two stages, with water molecules first penetrating bilayer and thus forming an unstable hydrophobic pore (middle), and with adjacent lipids then reorientating with their polar headgroups towards the water molecules forming a metastable hydrophilic pore (bottom). (b) Electrically induced chemicals changes to membrane lipids, including peroxidation, deforms their tails and increases the bilayer's permeability to water, ions and small molecules. (c) Electrically induced modulation of the membranes protein's function. Arrow lengths for the electrical field (E, red) correspond to its strength (i.e. amplitude of pulse) while those for transitions between states of membrane permeability reflect the transition rate (shorter arrow = shorter rate) (Kotnik *et al*., 2019)

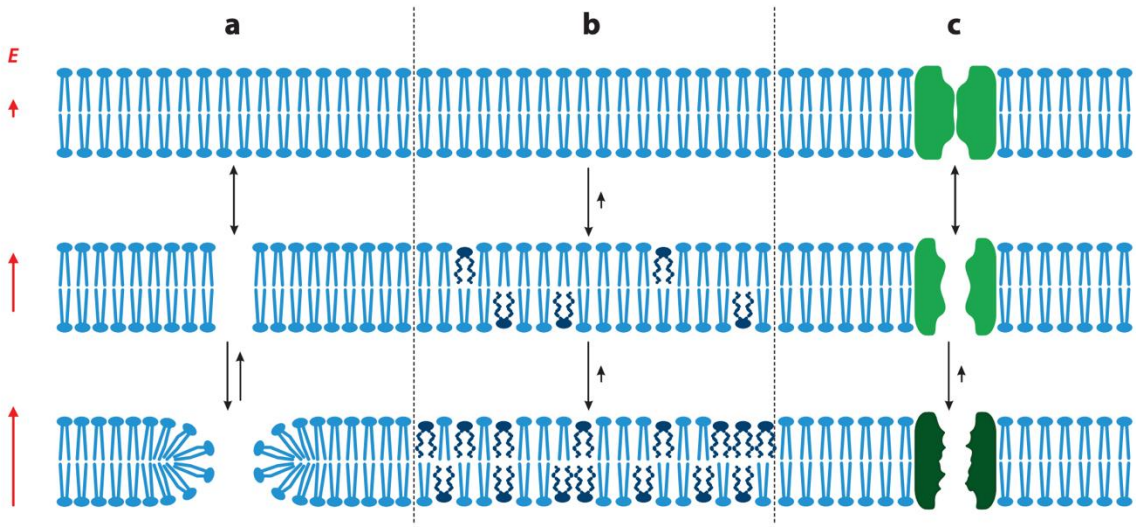

Figure 4: Conceptual Scheme of molecular level mechanisms of electropermeabilization starting from an intact membrane (Kotnik *et al.*,2019)

The kinetics of the transmembrane transport mediated by electropermeabilization has been researched extensively describing that membrane conductivity and permeability is increased within 1 microsecond after the onset of the PEF, provided the TMV reaches a certain threshold. Nevertheless, the TMV threshold to exceed is not a constant but a variable depending on other factors such as cell type, transport molecule, exposure duration, cell size, temperature and osmotic pressure (Kotnik *et al*., 2019). The PEF must also stay below values which can lead to permanent non-reversible changes in the cell membrane and ultimately cell death (Mir and Orlowski, 1999).

Studies characterise the electropermeabilization into 5 stages as shown in table 2.

Table 2*:* Stages of electropermeabilization (Kotnik *et al*., 2019)

Stage	Timescale
Initiation: Membrane electrical conductivity and permeability start increasing detectably when transmembrane voltage (TMV) exceeds a "critical" value.	Nanoseconds (conductivity) Microseconds (permeability)
Expansion: As long as TMV remains above the "critical" value, conductivity and permeability persist and/or intensify.	Until the end of the pulse (up to milliseconds)
Partial recovery: After TMV drops below the "critical" value, membrane conductivity and permeability decrease rapidly but not fully, stabilizing at a detectably increased level and still allowing transmembrane diffusion of ions and molecules.	Microseconds (conductivity) Milliseconds (permeability)
Resealing: The membrane gradually recovers its physiological level of impermeability (unless damage was irreversible and cell loses viability).	Seconds to minutes (~20–37°C) Hours (~4°C)
Memory: Even after full membrane resealing, the cell can exhibit alterations in its physiological processes and reactions to stressors before finally returning fully to its normal state.	Hours

2.3.2 Electric Pulse Parameters

Several types of electric pulse shapes have been employed to obtain cell permeabilization. Studies have shown square-wave pulses to be the most efficient because they allow the independent setting of the delivered voltage and length of every electric pulse. Voltage can be set to a predetermined constant slightly higher than the threshold. Furthermore, with square wave pulse generators the selected electrical values are maintained despite variations in volume or conductivity of samples (Mir and Orlowski, 1999). For sake of comparison, if one was to use exponentially decaying pulses, the initial electric field strength required for the minimal period of time would be much higher than the threshold required, resulting in cell death and marked burns (Weaver *et al*., 2012).

Through research, 8 electric pulses, 1300 V/cm, duration 100μs and frequency 1 Hz has proven to be most common values for electrochemotherapy (Sersa *et al*., 2008). The duration of 100μs is the best compromise to ensure pulse duration is long enough to obtain membrane modifications but not so long to create irreversible modifications and excessive heating (Kotnik *et al*., 2019). It is important to note that these electrical values change depending on the study. For example, volts per centimetre (V/cm) is 1300 V/cm using plate electrodes and 1000 V/cm with needle electrodes.

2.3.3 Active Agents Used In Electrochemotherapy Treatments

Bleomycin is a non-permeant cytotoxic drug possessing a high intrinsic cytotoxicity. Bleomycin can be transported across non-permeabilised plasma membranes by carrier proteins via the endocytotic pathway. However, this process is limited by the low number of carrier proteins exposed on the cell surface (Sersa *et al*., 2008). Electropermeabilization overcomes this barrier enabling bleomycin direct access to the cytosol and potentiating the cytotoxicity of the drug by several thousand-fold (Cemazar *et al*., 2008). Cytotoxicity is maximised when bleomycin is internalised after membrane permeability is restored (Mir, Tounekti and Orlowski, 1996). Once inside the cell, bleomycin acts on cells of proliferation. It functions as an endonuclease promoting single and double stranded DNA. At low concentration, bleomycin kills cells via mitotic death (after 3 doubling times) or in a process similar to apoptosis at higher levels of concentration (Rangel *et al*., 2019). Additionally, it must be highlighted that bleomycin is not an immunosuppressant drug, instead it has been shown to stimulate various immune responses. Bleomycin increases tumoricidal activities of macrophages, elimination of tumour specific T-suppressor activity and induction of IL-2 secretion (Mir and Orlowski, 1999). Bleomycin can be given intravenously and intratumorally. Side effects include; skin reactions, gastrointestinal tract damage and particularly pulmonary fibrosis if given too quickly (Gustafson and Bailey, 2020).

Cisplatin is also a non-permeant cytotoxic drug. Only 50% of cisplatin is carried through the plasma membrane via passive diffusion, the rest is limited by the number of carrier molecules. Again, electropermeabilization increases the accumulation of cisplatin inside the cell and, thus, the cytotoxicity by 80-fold. Cisplatin binds to DNA and inhibits its replication, but although cisplatin's cytotoxicity is significant, it is still lower than bleomycin (Sersa *et al*., 2008). Cisplatin is to be given intratumorally only, can be nephrotoxic is received

intravenously, other side effects include myelosuppression and gastrointestinal damage (Gustafson and Bailey, 2020).

Interleukin 12 is a heterodimeric pro-inflammatory cytokine which forms a link between innate resistance and adaptive immunity. IL-12 is involved in the differentiation of naïve T-cells into T-helper cells, stimulates the production of interferon-gamma (IFN-γ) and tumour necrosis factor-alpha from T cells and natural killer cells. It also reduces the production of IL-4, which suppresses IFN-γ production. Finally, IL-12 has an anti-angiogenic activity blocking the formation of new channels, thus highlighting its potential as an anti-cancer drug (Trinchieri and Scott, 1995).

Electrochemotherapy in combination with calcium has also been tested *in vitro*, *in vivo* and in clinical trial with promising results as an anticancer treatment. In ordinary conditions, calcium is a tightly regulated intracellular ion but by employing ECT, supraphysiological concentrations of calcium can be achieved. Cell necrotic death occurs due to acute and severe ATP depletion, increased activity of proteases, lipases and reactive oxygen species, and also stimulating a systemic immune response (Frandsen, Vissing and Gehl, 2020).

ECT is only a local treatment and not effective for metastatic MCTs. Therefore, we require a complex therapy plan consisting of local and systemic treatment for metastatic MCTs. Frequently used systemic chemotherapeutic drugs used include vinblastine and lomustine. Vinblastine is known to diminish microtubule dynamics causing the arrest of cell division at metaphase, induction of apoptosis, interference with tumour vascular supply (Rassnick *et al.*, 2008). Lomustine causes inter-strand and intra-strand cross-linking of DNA, which inactivates DNA synthesis and cell death (Papich, 2016).

ECT is described to have no affect for metastatic MCT treatment due to lack of evidence. However, new emerging research is questioning this hypothesis. One study describes the concept of immunogenic cell death (ICD), where ECT with bleomycin generates danger-associated molecular patterns (DAMPs) that trigger an adaptive immune response against tumours. Hence, the dying cancer cells act as a therapeutic vaccine eliciting a cytotoxic response against surviving malignant cells elsewhere in the body (Calvet *et al.*, 2014).

2.3.4 Electrochemotherapy equipment

There are various types of pulse generators and electrodes available on the market. Fundamentally, two types of electrodes exist: plate electrodes and needle electrodes. Plate electrodes are used for the treatment of skin and superficial lesions like mast cell tumours and come in differing shapes. The depth of the electrical field is small and depends on the distance between the electrodes. The greater the distance, the deeper the penetration of the electrical field into the tissue, given that a larger voltage needs to be applied between both electrodes. Needle electrodes are used in deep cavity and subcutaneous tumours requiring placement throughout the tumour tissue. Several types of needle electrodes are procurable, needles are usually positioned in two parallel rows or in a circular/ hexagonal array (Sersa *et al*., 2008)

2.4 Efficacy of Electrochemotherapy Using Bleomycin and Cisplatin Against Canine Mast Cell Tumours

Researchers Tozon, Cemazar and Sersa published the first clinical study of electrochemotherapy on canine mast cell tumours in Slovenia, 2000. The investigation was carried out on two nodules of MCTs in the one dog. Although the study sample is small, the results are quite interesting. The dog was sedated, hair clipped, tumour site cleaned, and cisplatin injected intratumorally. The dosage was 1 mg/100 mm^3 tumour volume and electric pulses were delivered 2 minutes after drug delivery to ensure pharmacokinetic peak. The results revealed 4 weeks post-treatment, a reduction in the tumour size from 1,0 cm^3 to disappearing completely. A 6 month follow up also reported the MCT tumour was still in complete response. The paper also highlighted no major or local side effects bar muscle contractions occurring at each pulse which disappeared instantaneously after. The limitations of this study were the very small sample size. However, it also highlighted the safe and effective use of cisplatin in ECT. Advantages such as simplicity, short duration of sessions, insignificant side effects and the fact the patient went home the very same day paved the path for future use of ECT in canine mast cell tumours.

The same researchers, Tozon, Cemazar and Sersa, released another paper the following year (2001) with a larger sample study. Three cats with mammary adenocarcinoma and fibrosarcoma, and seven dogs with mammary adenocarcinoma, cutaneous mast cell tumour, haemangioma, hemangiosarcoma, adenocarcinoma glandulae paranalis and neurofibroma. The study also carried out a comparison on cisplatin alone versus cisplatin in conjunction with ECT. Thus, twenty-four tumour nodules of different size were treated, 5 with cisplatin

injected intratumorally alone and 19 with ECT and intratumoral cisplatin My research is focused solely on canine mast cell tumours, therefore, I will only discuss the patients and results pertaining to the MCTs in the above study, as seen in Table 3. One patient (no. 1) was a 6 year old intact female American Staffordshire terrier which received no previous treatment. The dog had only 1 tumour nodule and was injected with cisplatin intratumorally without ECT treatment. The original tumour volume was 18 cm^3 which reduced to 4 cm^3 after 4 weeks post-injection. It is important to note that 18 cm^3 is a very large MCT and one of the largest tumours in the study, so it is quite significant to have a reduction of 12 cm^3 in only 4 weeks time. Another patient (no. 2), a 4 year old male boxer which also received no prior treatment. The dog had 2 tumour nodules approximately 1 cm^3 both in volume and the patient received ECT with cisplatin. The follow up 4 weeks later showed a complete response, 0 cm^3, to ECT treatment and this continued for the remainder of the study's observation time.

Table 3: Summary of treatment parameters and tumour response for patient no. 1 and 2. CDDP cisplatin, PR partial response, CR complete response, EP electric pulses (Tozon, Cemazar and Sersa, 2001)

Animal no.	Nodule	Treatment	Number of sessions	CDDP[1] dose (mg/nodule)	Number of EP[2]/nodule	Tumour volume (cm^3)	Tumour volume after 4 wks (cm^3)	Response[3]	Duration of response (months)	Observation time (months)
1	1	CDDP	2	4.0	-	18.00	4.00	PR	13	13
2	1	ECT[4]	1	0.5	8	1.00	0	CR	14	14
	2	ECT	1	0.5	8	1.00	0	CR	14	14

Spugnini *et al.* (2006) investigated the adjuvant potentials of ECT for the treatment of incompletely excised canine MCTs. The reasoning behind the study is MCTs have been reported to have recurrence rates ranging from 22-54% after surgical excision (Weisse, Schofer and Soremno, 2002). Adjuvant treatment applied at the time was classical chemotherapy, yielding complete and partial responses from 28% to 53% but were also short lived and accompanied with severe haematological, gastrointestinal and hepatic toxicity. Table 4 shows the study encompassed 28 dogs, all of which presented with histopathologically confirmed, incompletely excised mast cell tumours. None of the animals had distant metastases, life threatening diseases or bone marrow involvement. Unlike Tozon's and colleagues' previous research involving cisplatin, Spugnini *et al.* employed bleomycin intratumorally and the patients received two ECT sessions, one week apart. Response to treatment and local toxicity were assessed prior to the second session and every

two months thereafter. The results described 2 dogs experiencing local edema and mild erythema at the electroporation site but subsided within 30 minutes. These signs are compatible with the degranulation of residual mast cells, possibly from the manipulation of the tumour mass. Another dog showed partial wound dehiscence and delayed healing requiring a minor surgical debridement but the patient displayed a very aggressive recurrent mast cell tumour.

Table 4: Individual data and response to ECT in 28 dogs with cutaneous mast cell tumours. F female, FS female spayed, M male, MC male castrated, WHWT West Highland white terrier (Spugnini *et al*., 2006)

Age Breed	Sex	Site	Grade Outcome
3 Boxer	FS	Anus II	In remission
13 Mixed Breed	F	Leg II	In remission
11 Argentinean Dogo	M	Head II	In remission
2 Boxer	M	Trunk II	In remission
12 Dalmatian	M	Head II	Recurrence
13 Pug	M	Leg II	In remission
10 Boxer	M	Trunk III	In remission
6 Argentinean Dogo	MC	Leg III	In remission
13 Mixed Breed	M	Leg III	In remission
6 Boxer	M	Leg II	In remission
6 Schnautzer	F	Leg II	In remission
4 Mixed Breed	M	Leg II	In remission
8 Boxer	M	Leg II	In remission
10 Mixed	F	Digit II	In remission
6 Labrador	F	Leg I	In remission
8 Boxer	M	Leg II	In remission
10 Boxer	M	Anus II	In remission
3 Poodle	F	Trunk II	In remission
2 Boxer	F	Trunk II	In remission
7 Great Pyrenees	F	Digit I	In remission
10 Mixed Breed	FS	Leg I	In remission
6 Boxer	FS	Leg II	In remission
14 Yorkshire	M	Head II	Recurrence, retreated, in remission for 2 months
14 Syberian Husky	M	Leg I	Recurrence, progressive disease
7 Setter	M	Trunk III	In remission
10 Setter	M	Digit II	In remission, dead of leptospirosis
7 Setter	FS	Trunk III	Recurrence, dead of metastases
10 WHWT	FS	Trunk III	Recurrence, dead of metastases

Boxer dogs are over-represented in the study, this is most likely due to the breed predisposition to develop MCT and the breed popularity in Italy, where the study was conducted. The overall response rate was 85% with a total of 23 patients still in remission at different times from the end of therapy. The median survival was not reached at the time of publishing, but an estimated mean survival time was calculated to be 52.76 +/- 6.5 months (range 39.99 to 65.52 months, 95% CI). Moreover, two dogs with grade III MCTs on the trunk experienced metastasis and were euthanised, indicating a possible link between ECT MCT anatomical location and grade severity. Another dog experienced local recurrence of

a mucosal grade II MCT on the lower lip. The patient was retreated with surgery and ECT obtaining a remission that lasted 22+ months. Spugnini *et al.* (2006) highlighted ECT resulted in long term control without long term consequences. Rate and duration of responses can be favourably compared to chemotherapy and radiation therapy.

Spugnini *et al.* also carried out another study in 2011, evaluating the efficacy of cisplatin (CDDP) as an ECT agent in an adjuvant fashion after incomplete surgical resection of canine MCTs. Whereas bleomycin was used in the 2006 study. The sample size contained 37 dogs with histopathologically confirmed MCTs, 18 dogs presented with visible gross disease (4 of them recurrences) and 19 with only a surgical scar. There were 7 grade I, 24 grade II and 6 grade III MCTs. The animals received 2 ECT sessions 1 or 2 weeks apart based on clinical consideration. During each treatment, the tumour bed and a 1.5 cm possible margin of normal tissue surrounding the surgical scar were injected with CDDP, ECT began 5 minutes after injection. The only toxicities occurring was 32% (12/37) of dogs showing Dariers syndrome of local oedema and mild erythema which subsided within 30 mins. Significantly, 29 dogs out of 37 (78%) had no evidence of recurrence over the 6 year study period highlighting the advantages of ECT as a successful, cheaper and safer alternative for MCTs. The mean time to recurrence was 1,218 days and distant metastasis was not observed among any of the dogs enrolled. The rate and duration of disease free intervals obtained in the study compares favourably to those described after surgery and radiation therapy. However, the study included some negative results such as one dog dying of non-cancer pathology (gastric dilation volvulus) after successful ECT and another 6 dogs being euthanised at different times because of tumour recurrence. Moreover, grade II MCTs were also over-represented in the sample but also displayed the strong response ECT has on them.

Spugnini *et al.* (2006) assessed ECT as an adjuvant therapy in conjunction with surgery, whereas Kodre *et al.* (2009) evaluated the effectiveness of ECT with cisplatin and compared it to the effectiveness of standard surgical treatment of MCTs in dogs. The study comprised 25 dogs, 20 males and 5 females. Of the total 25, 16 dogs with 16 tumour nodules were operated on, using 2 cm surgical margin with a fascial plane and with complete surgical margins between 2-5mm confirmed by histology. The remaining 9 dogs with 12 tumour nodules were treated with ECT after the owners refused surgical treatment. Each tumour received cytology and/or biopsy examination confirming MCTs. However, histological examination was performed only on tumours that were excised surgically, not in tumours receiving ECT due to the owners' refusal of surgical treatment. In this study, ECT with

cisplatin was used as a single treatment; therefore it was important to treat adequate margins, as is recommended for surgical excision, to obtain a complete response with long duration. The intratumoral dose was 1 mg/cm^3 and tissue blanching, tissue whitening from drug retention, was used as an indicator of good tumour infiltration with cisplatin. If tissue blanching was not witnessed, the intratumoral injection was repeated, which happened rarely. Kodre *et al.* adopted a technique of applying the electric pulses first to the tumour margin in order to reduce blood flow to the tumour, which contributed to solution retention and can prevent release of inflammatory mediators from entering the bloodstream due to possible degranulation. If the tumour did not respond completely after the first ECT session, additional sessions were performed at 2-4 week intervals. The patients were examined 2 and 4 weeks post treatment and monthly thereafter with callipers measurements and photos. Firstly, the group of patients treated with complete surgical excision resulted in; a larger median tumour size, mean duration of clinical signs 20 days and median time of follow up was 18.3 months. The median time to local recurrence was 22.5 months in two dogs with a MCT grade I and 8.5 months for 7 dogs with MCT grade II. In 8/16 dogs, the tumours recurred after 0.7-22.5 months, with highest recurrence rate in MCT grade III. Meanwhile, the ECT group of 9 patients with 12 nodules had a smaller median tumour size prior to treatment; mean duration of clinical signs 25 days and the median time of follow up was 26 months. Importantly, the estimated median duration of local tumour control was not reached at the time of writing, even though the maximal observation time was 43 months, 13.5 months longer than the excision group. Overall, a 62.5% complete response rate was achieved, with most cases occurring after only 4-5 weeks. At 30 months post treatment, ECT resulted in 70% CR whilst surgery had a 50% CR. However, 2/9 patients did not respond to ECT, these dogs possessed big tumours, >8cm^3, and were euthanised. Statistical analysis between surgical excision and ECT groups showed no real significant difference bar the duration of local tumour control was longer in the ECT treated group. The Kaplan-Meir method, Figure 5, calculated estimated median duration of response (time to recurrence) was 31.5 months for the surgery group, while it was not yet reached for the ECT treated group.

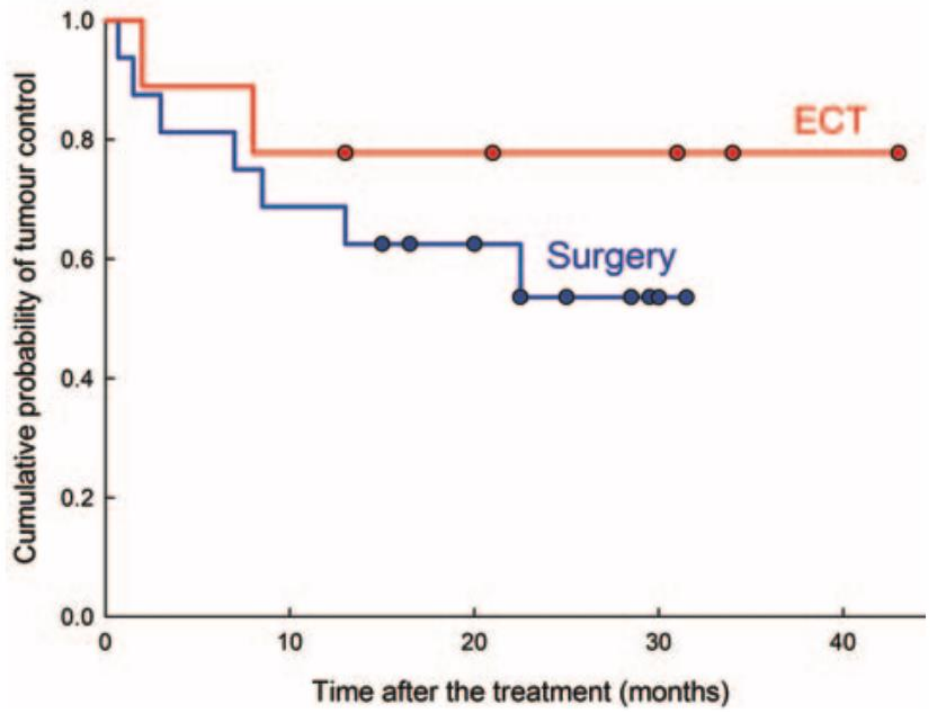

Figure 5: Kaplan-Meir survival curves for tumour regression for patients treated with surgery and ECT with cisplatin (Kodre *et al*., 2009)

Lastly, the result of Kodre *et al.* (2009) study demonstrated tumours with a higher MCT grade have a higher recurrence rate and that ECT with cisplatin as a single treatment is highly effective and can represent an alternative to surgical treatment, especially in those cases when owners do not consent to surgery.

The largest study conducted on MCTs in dogs was conducted in 2016 by Lowe *et al*, consisting of 51 dogs in total, divided into 4 separate groups. (1) ECT alone therapy performed in 15 dogs with smaller MCTs, mean size 1.39 cm. (2) ECT intra-operative carried out in 11 dogs with larger MCTs, mean size 2.71cm. The majority of the tumour was excised with no attempt at obtaining complete margins and ECT was applied to lateral and deep margins before wound closure. (3) ECT adjuvant to surgery (post op) in 14 dogs applied after a period of 2-4 weeks post-surgery. Reason for delay is to allow adequate wound strength to develop after surgery. (4) ECT performed after surgery at the recurrence of a MCT, in 11 dogs, which was macroscopically visible at the initial surgery site. For all ECT procedures, bleomycin was administered intravenously at a dose of 15,000 IU per square metre of body surface. Electric pulses were delivered after a delay of 8 mins to allow the drug to reach its pharmacokinetic peak. In general, the four groups displayed almost the same distribution in terms of breed, gender and age. However, grade 2 MCTs (Patnaik

grading) were more represented. The best results for complete response were found for *ECT post-op* and *ECT intra-op*, respectively 93% and 91%. Next was *ECT alone* with 80% CR and lastly 64% for *ECT recurrence*. Significantly, the remaining dogs scored a progressive response to treatment, which is considered at least 50% reduction in tumour size. The disease-free interval (DFI) in days with complete remission identified *ECT post-op* with the best score as evident in Figure 6.

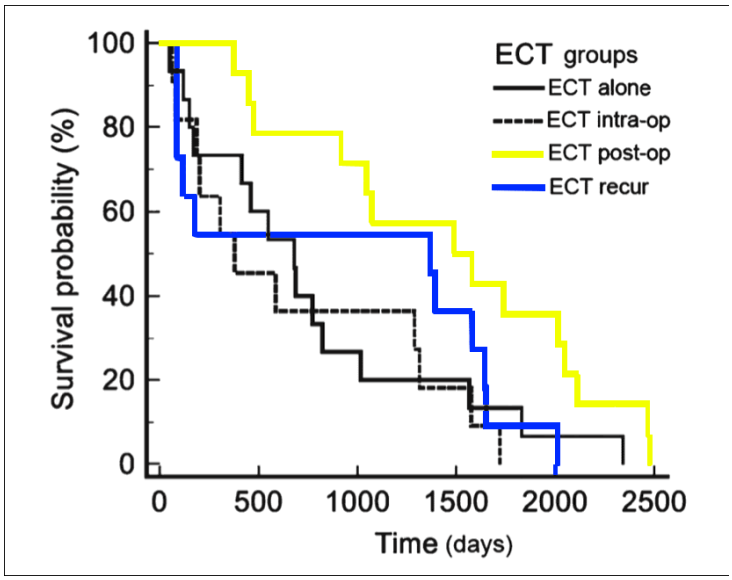

Figure 6: Kaplan-Meyer graph of the four groups of dogs with MCT showing the DFI time (Lowe *et al*., 2016)

Lowe *et al.* demonstrated, once again, the therapeutic effect of ECT on canine MCTs particularly as an adjuvant or stand-alone treatment in comparison to surgical excision. The study also described how surgery cannot guarantee an adequate resection with wide margins. To illiterate, the distal extremities would experience a loss or reduced function possibly if only surgery was carried out and represented 50% of the sample cases. Furthermore, MCTs located on the head summed to 30% where a cosmetic surgery was considered to not choose surgery. Hence, ECT can be described as a valuable tool for treatment against MCTs, depending on size and anatomical location to maintain a functional patient.

2.5 Electrochemotherapy Combined with Peritumoral Interleukin-12 Gene Electro-transfer

Similarly to ECT, electro-gene therapy can be used for intracellular delivery of other molecules such as plasmid DNA. IL-12 exhibits a range of biological activities, potentially important in the immunotherapy of cancer. These include the activation of natural killer cells, induction of IFN-γ, inhibition of angiogenesis and promoting nitric oxide production (Pavlin *et al.*, 2011).

Cemazar *et al.* (2017) suggested combining the use of electrochemotherapy and electrogene therapy (EGT) for the treatment of canine MCTs. 18 tumour nodules in 18 patients were treated with intratumoral ECT using cisplatin which was immediately followed by intradermal injection of human IL-12 plasmid injection, followed by electric pulses delivered to sites of injection. Cisplatin was the first drug of choice. In case of no or minimal antitumor response at 4 weeks, cisplatin was replaced with bleomycin intravenously. Complete response was achieved in 72% of patients with 100% CR in tumours smaller than 2 cm^3. The response rate in bigger tumours were lower, CR 60% in tumours greater than 2 cm^3. Patients presenting without metastasis before treatment (14/18), the disease did not progress despite 6 of them being higher grade tumours. Poor response occurred in 3 patients who already had metastasis and higher clinical stage of disease. However, one dog with metastatic disease was cured and remained tumour free for over 2 years. Lastly, systemic release of human IL-12 appeared in 88% of patients and an elevation in IFN-γ was detected in 70% up to 3 months after therapy. To conclude, Cemazar *et al.* study demonstrated the combination of ECT with cisplatin and IL-12 is a highly effective and safe form of MCT treatment.

Salvadori *et al.* (2017) further emphasised Cemazar *et al.*'s study by performing a histopathologic and immunohistochemical investigation on combined ECT and peritumoral IL-12 gene transfer in canine MCTs. The sample included 11 dogs with 11 tumours all diagnosed with low grade MCTs. All subjects had biopsy sampling before treatment (T_0), and at 4 (T_1) and 8 (T_2) weeks post-treatment. As seen in Figure 7, at T_0, all 11 MCT skin tumours were characterised with sheets of polygonal neoplastic cells with and abundance of metachromatic granules in the superficial and deep dermis, sometimes even extending to the deep muscular layer. Moderate to massive infiltration of eosinophils was present and a low mitotic index was witnessed. T_1 biopsies were collected from 7 dogs showing complete response from treatment. The neoplastic tissue was substituted with fibrotic tissue composed of wavy collagen fibres and inflammatory infiltrates like lymphocytes and macrophages. A

partial response was seen in 3 more patients demonstrating single or small clusters of neoplastic cells still present. At T_2, the 7 dogs in complete response were still free of neoplastic cells and consisted of a dense fibrous tissue still infiltrated by mononuclear cells. A stable disease and partial response scored in the remaining 4 animals. Immunohistochemical analysis revealed that an increase in T-lymphocyte number occurred at T_1, then slowly reduced at T_2 but macrophages were significantly higher in T_2. In biopsies collected from subjects with complete remission, the number of CD3+ lymphocytes were significantly higher than in dogs with stable or progressive disease at T_1. Meanwhile, proliferation activity of neoplastic cells was statistically reduced at T_1 and T_2. Lastly, microvessel density was drastically reduced in all sample sizes after treatment. Thus, Salvadori *et al.* (2017) affirmed that combined ECT and IL-12 gene electrotransfer effectively induced a cellular response against neoplastic cells characterised mainly by recruitment of T-lymphocytes and macrophages and a fibrotic proliferation with reduction of microvessels.

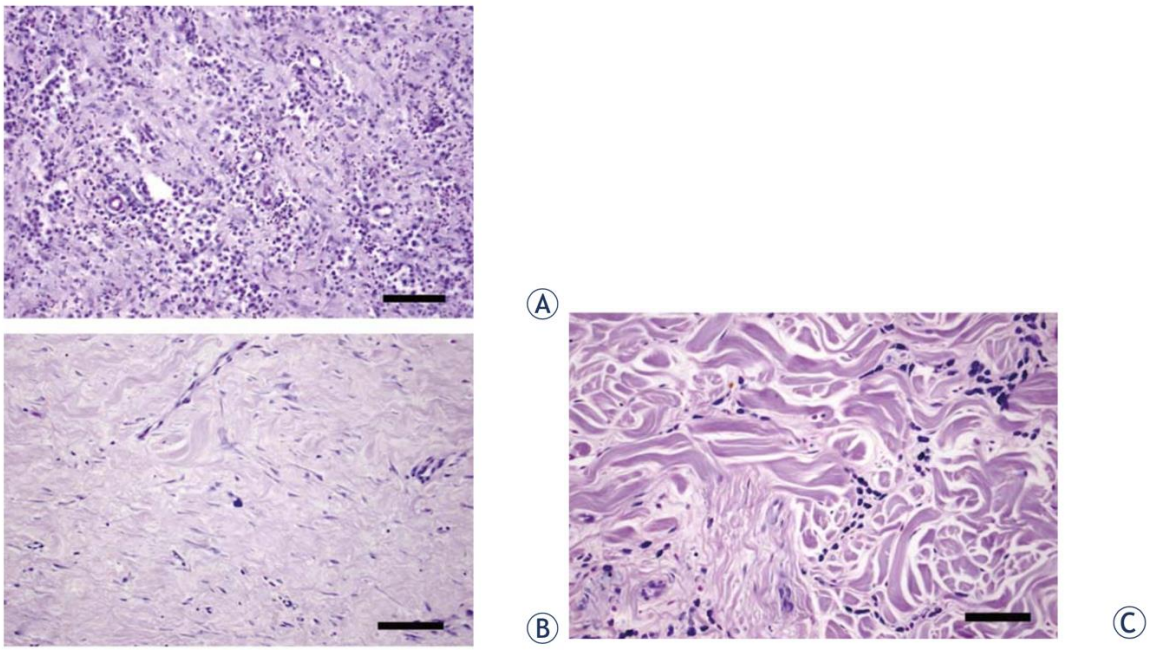

Figure 7: Histology of samples collected. (A) At T_0, low grade MCT with sheets of neoplastic cells with metachromatic granules. (B) T_1 shows neoplastic tissue replaced with fibrotic tissue. (C) T_2 in dogs with partial responses (Salvadori *et al.*, 2017)

3. CLINICAL STUDY

The first case study is an 11-year-old spayed female, cross breed, named Lady. A cytological diagnosis confirmed a MCT on the left forelimb, measured at 2,35 cm^3, as seen in Figure 8. Surgery was not an option due to location and the owner was against amputation. ECT was chosen and the first session was performed on 16/11/2020.

Treatment involved bleomycin intravenously and cisplatin intratumorally, blanching of the tissue was witnessed indicating high retention in the MCT. After bleomycin was dispensed, we waited 8 minutes and 1 minute after cisplatin to ensure a pharmacokinetic peak was reached. During ECT, we initially used small needle electrodes for a deeper penetration because the MCT was quite thick, then switched to a L-shaped electrode for superficial treatment. The clinic uses the ELECTROVET EZ machine produced by Leroy Biotech.

A second ECT session occurred on the 05/01/2021, 7 weeks later. The tumour reduced to 1,1 cm^3 in size and a second cytology sample was taken, this time indicating only dead mast cells present. The tumour reoccurred by the 14/04/21, 3 months later. Thus, a third ECT treatment was performed. At time of writing, two more cytology samples have been examined with no evidence of mast cells being present. Histology described the original MCT to be low grade/ grade II.

Figure 8: (Left) Patient presented with a large MCT on left forelimb. (Right) Reduction in tumour after two ECT sessions.

The second case is a male neutered 9-year-old Labrador, Csoki. The patient originally had a low-grade/ Grade II MCT surgically resected from the medial surface of the right thigh, approximately 1,5 cm^3 in size. However, an examination of the surgical margins revealed tumour mast cells still present. The owner opted for adjuvant ECT treatment rather than another surgery. Two ECT sessions were performed on the scar tissue 4 weeks apart, first on the 25/10/2020 and second 23/11/2020, as seen in Figure 9. Bleomycin was received intravenously and an L-shaped electrode was used.

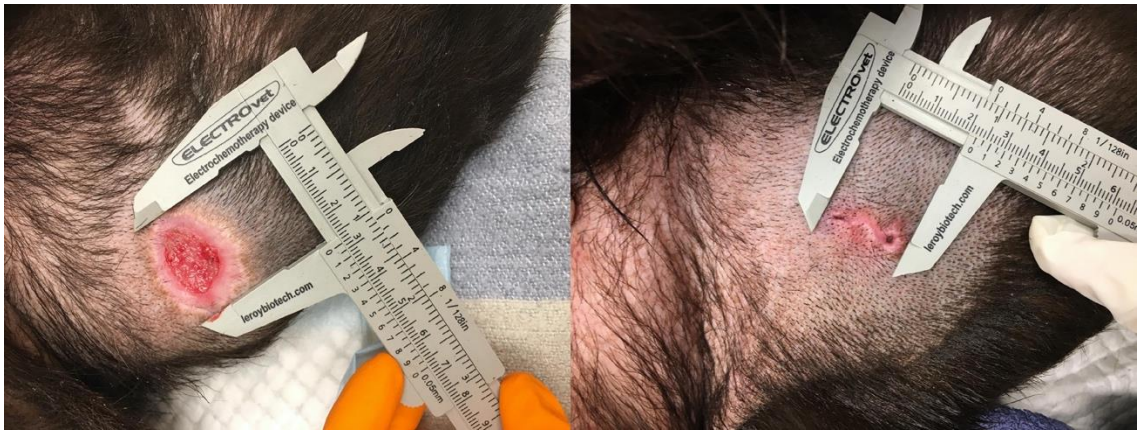

Figure 9: (Left) MCT after resection with tumour cells in margin prior to ECT. (Right) Scar tissue after two ECT sessions.

A new MCT was discovered on the left hind paw on the same Labrador, Csoki. Cytology hinted low grade and ECT was performed twice again with bleomycin intravenously as seen in Figure 10. To date, Csoki shows no signs of reoccurrence or metastasis.

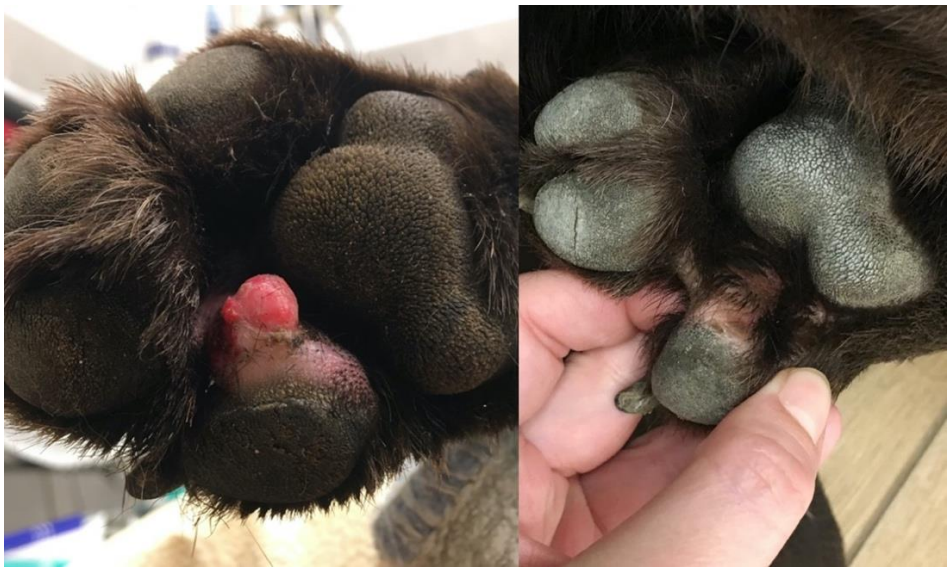

Figure 10: (Left) MCT present on left hind paw. (Right) Remnants of scar tissue after two ECT sessions with complete disappearance of the MCT.

The final case is a 10-year-old female intact vizsla, named Veby. Cytology revealed a high grade MCT lateral to the perineal area 1,8 cm^3 in size. Surgery was not feasible and the owner did not want chemotherapy treatment. ECT was chosen and a total of three treatments was performed occurring on the 05/01/2021, 05/02/21 and 19/06/21.

The vizsla received bleomycin intravenously and cisplatin intratumorally. An L-shaped electrode was employed. A cytological exam 4 weeks after the final treatment displayed no evidence of tumour cells. As seen in figure 11, a dramatic response is evident after three ECT treatments. At time of writing, the patient showed no signs of disease or metastasis.

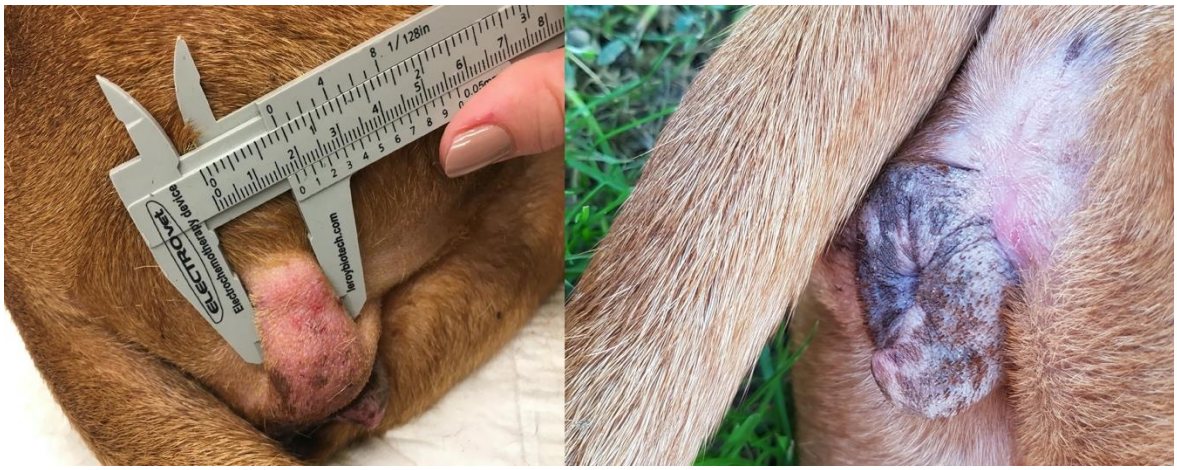

Figure 11: (Left) Large MCT presented at perineal area prior to ECT. (Right) Complete response after 3 ECT sessions.

It is important to note that due to the small sample size of the study, a separate materials/ methods and results section were kept separate in the thesis. Instead, combined under the heading clinical study.

4. DISCUSSION

MCTs have a recurrence rate of 22%-54% after surgical removal, if the margin is not wide enough (Weisse, Shofer and Sorenmo, 2002). Thus, it becomes evident that an alternative solution or adjuvant therapy is required for treatment of MCTs with better results than surgery alone. Significantly, this study has successfully highlighted electrochemotherapy as a safe and effective therapy displaying the ease of administration, the lack of toxicities to the animal, quick recovery time post-treatment and the low cost for the owner's perspective.

The literature review exhibited, in 2000, how the very first clinical study of ECT was a success in a single dog using cisplatin which paved the way for future research. The animal showed complete response to therapy even 6 months post-treatment. Most importantly, the paper revealed no major or local side effects bar muscle contractions occurring at each pulse which disappeared instantaneously after. The same researchers published another report the following year, using cisplatin intratumorally with ECT, and the success rate became consistent with the first paper. Again, no major side effects were reported.

Spugnini *et al*. is a major researcher in the field of ECT. Their 2006 study unveiled the adjuvant potentials of ECT for the treatment of incompletely excised canine MCTs with bleomycin intratumorally. Classically, adjuvant therapy at the time was chemotherapy but disadvantages included severe haematological, gastrointestinal and hepatic toxicity. Moreover, the study reported an 85% overall response, which is higher than adjuvant chemotherapy with none of the side effects. Following up in 2011, Spugnini *et al*. recorded similar positive results with cisplatin and ECT as an adjuvant therapy.

Lowe *et al*. (2016) further stressed Spugnini *et al.*'s research solidifying how ECT is the best adjuvant therapy to be combined with surgical removal of MCTs, trumping over chemotherapy and radiation therapy. ECT therapy post-surgery displayed 93% complete response in the largest ECT study to date.

Alternatively, Kodre *et al.* (2009) focused on evaluating the effectiveness of ECT with cisplatin and compared it to the effectiveness of standard surgical treatment of MCTs in dogs, instead of being used as an adjuvant therapy like Spugnini *et al* and Lowe *et al.* The paper published that at 30 months post treatment, ECT resulted in 70% CR whilst surgery had a 50% CR. A notable result emphasising the benefits of ECT over conventional surgery. The report made an interesting finding, ECT has better longstanding results with smaller MCTs, whereas larger MCTs responded better to surgical resection.

New frontier research in recent years by Cemazar *et al.* and Salvadori *et al.* is exhibiting the potential of ECT combined with electrogene-therapy with strong results. The deliverance of IL-12 intratumorally exhibits a range of biological activities, potentially important in the immunotherapy of cancer.

The results of the three patients used in the clinical study are consistent with the findings in the literature review. The owners were all offered the option of surgical removal, amputation, chemotherapy and radiation therapy. Some refused due to personal preference, financial reasons or even geographic location. After ECT, all cases showed a reduction in tumour size after first initial treatment, then a complete response after 2-3 more ECT sessions. ECT sessions typically lasted 20 minutes with the animal under general anaesthesia, easy recovering, and going home the very same day. Once more, none of the patients displayed signs of disease or metastasis.

One of the disadvantages of ECT include tumour size. Generally, tumours greater than 2 cm^3 had poorer prognosis and should often choose surgery combined with ECT rather than ECT alone. This is due to the inability of the electrodes to penetrate the tumour adequately enough, even with small needle electrodes. Similarly, ECT has more favourable results for local treatment of cutaneous and sub-cutaneous MCTs. Metastatic treatment of organs and cavities with ECT is more complex and inadequate. Nevertheless, future research and development in years to come can provide a solution to the problem. The use of bleomycin and cisplatin also have their drawbacks, including the risk of pulmonary fibrosis if bleomycin given too quickly or cisplatin being nephrotoxic intravenously. Although, the benefits far outweigh the use of chemotherapeutic drugs such as vinblastine or lomustine.

To conclude, this paper successfully underlined the advantages of ECT, with bleomycin or and/or cisplatin, whether as a stand-alone or treatment or in combination with surgery against the treatment of mast cell tumours. ECT is going to gain more ground with stronger evidence in years to come and I believe it will become more standardised in clinics around the world.

SUMMARY

Mast cell tumours (MCTs) are a prominent skin disease commonly diagnosed in dogs. The classical approach to treatment should be a complex therapy, however, the success rate can be variable. Electrochemotherapy (ECT) is a relatively new local anticancer therapy that combines the delivery of trains of appropriate electrical pulses with the administration of chemotherapy agents. The aim of this study was to evaluate the effectiveness of ECT against canine MCTs. We investigated studies describing the use of ECT; as a stand-alone therapy, in conjunction with surgery, combined with gene therapy or with differing chemotherapeutic agents. A ECT clinical study was also performed and analysed. The outcome concluded ECT is a safe and effective therapy for MCTs in dogs, with significant results. Its ease of administration, lack of toxicities and low cost make it an alternative solution or adjuvant option to standard treatments.

Kutyákban a mastocytoma kiemelkedő jelentőségű bőrdaganat. Kezelése komplex terápiát igényel, melynek sikeressége változó. Az elektrokemoterápia (ECT) viszonylag új lokális daganatellenes kezelés, amelynél elektromos impulzusokat kombinálnak kemoterápiás szerekkel. Tanulmányunk célja az elektrokemoterápia hatékonyságának vizsgálata volt kutyák mastocytomái ellen. Feldolgoztuk a nemzetközi szakirodalmat, amely az elektrokemoterápiát önálló kezelésként, műtéti eltávolítással, génterápiával kombinálva vagy különböző daganatellenes szerekkel vizsgálta. Emellett klinikai vizsgálatokat is végeztünk. Arra a következtetésre jutottunk, hogy az ECT biztonságos és hatékony terápia kutyák mastocytomái ellen. A kezelések elvégzése viszonylag egyszerű, rövid időt vesz igénybe, nem jár jelentős mellékhatásokkal, így kiváló alternatív vagy adjuváns kezelési lehetőséget kínál.

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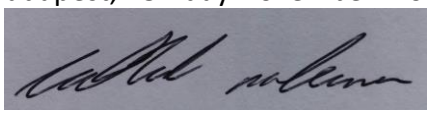

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