Electrochemotherapy with intra-tumoral cisplatin for the treatment of fibrosarcoma in a horse

M. Bharathidasan*, B. Justin William, Ravi Sundar George, A. Arunprasad and R. Sivasankar

Department of Veterinary Surgery and Radiology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 051, Tamil Nadu, India.

Received: 29-11-2017 Accepted: 15-01-2018 DOI: 10.18805/ijar.B-3532

ABSTRACT
A two years old Kathiawar stallion was reported with the history of two, pedunculated hard mass medially on the thigh and hock of the right hind limb, progressively increasing for the past two months. Fine needle aspiration cytology revealed fibrosarcoma. The tumour on the medial aspect of the thigh was injected with cisplatin intra-tumorally at a dose rate of 0.3 mg/cm³ of tumour volume and was exposed to ECT. The tumour on the medial aspect of the hock was excised incompletely to preserve skin and subcutaneous tissues around the tumour for wound opposition and treated with intra-tumoral injection of cisplatin followed by ECT. Following electrochemotherapy complete response was noticed on the 3rd and 4th week for the tumours on the thigh and hock respectively. No recurrence was noticed during the follow-up period of one year revealing ETC with cisplatin as a single treatment and also in combination with surgery is effective for the treatment of fibrosarcoma in equines.

Key words: Cisplatin, Electrochemotherapy, Equines, Fibrosarcoma.

INTRODUCTION
Electro-chemotherapy (ECT) is evolving as a new field of cancer therapy as an alternative to radiation therapy based on the location and type of tumours. Electro-chemotherapy works on the principle of electroporation in which, the living cells are subjected to interact with electrical field to create transient pores on bi-lipid layer of cell membrane and the extra-cellular substances and molecules of higher concentration gradient are internalized into the cell (Cemazar et al., 2017). Cancer cells are sensitive to electrical field than the normal cells. Exposure of cells to high trans-membrane potential difference (200 mV) using pulsed electric fields of intensity in the range of 300-3000 V/cm for durations of microseconds (μs) results in the formation of transient hydrophilic pores which are reversible (Spugnini et al., 2006). Exposure of cells to trains of low electric fields pulsed square waves in the range of 20-100 V/cm leads to efficient uptake of macromolecules with molecular weight in the range of 300-2,000,000 Da into cells (Mir et al., 2006). The uptake of macromolecules does not proceed through electroporation but through an endo-cytic-like mechanism. Therefore, electrically induced endocytosis of intra-lesionally administered chemotherapeutics results in increased influx of antineoplastic drugs into the cells of the solid tumour to induce cyto-reduction and facilitated ablation.

Fibrosarcomas accounts for 1.9 per cent of all cutaneous and musculo-cutaneous neoplasia in horses. They are generally invasive, large, firm, poorly demarcated and recurrence is common due to failure of complete excision. Post-operative adjunct therapy like radiation and local chemotherapeutics also failed in preventing recurrence as the metabolic rate is slow in fibrosarcomas (Bass et al., 2017). The present case report was on the use of ECT for the management of fibrosarcoma on the skin of a horse.

MATERIALS AND METHODS
A two years old Kathiawari stallion weighing 220 kg was brought to Department of Veterinary Surgery and Radiology, Madras Veterinary College Teaching Hospital with the history of two, pedunculated hard mass on the right hind limb. The first one was on the medial aspect of thigh (Fig 1) with the dimensions of 2x2x2 cm and the second one was on the medial aspect of the hock (Fig 2) with the dimensions of 7x5x4 cm; progressively increasing for the past two months. Routine clinical examination, haematological and blood biochemical evaluation revealed all parameters were within normal range. Fine needle aspiration cytology confirmed that the mass was fibrosacoma. It was decided to treat the tumour with ECT. Water and feed were withheld for 6 and 12 hours. Anaesthesia was induced using xylazine at the rate of 1.1 mg/kg and ketamine at the rate of 2.2 mg/kg and maintained with continuous infusion of a triple mixture (Taylor et al., 2008). The sites were prepared for aseptic surgery.

The tumour at the medial aspect of the thigh was treated by ECT alone. The volume of tumour nodules was calculated by a formula \( V = \frac{a \times b \times c \times \pi}{6} \), where \( a, b \) and \( c \) are the dimensions of the tumour.
represent the diameters of the tumour nodule (Mitrovic et al., 2017). Anticancer drug was infiltrated into the lesion and surrounding 2 cm using a syringe. The calculated volume was 4.19 cm$^3$. Cisplatin was dissolved in distilled water at a concentration of 2 mg/ml (Cisteen, Miracalus) and was injected intra-tumorally at approximately 0.3 mg/cm$^3$ of tumour volume (Mir et al., 2006). Within 5 to 15 minutes after drug injection, the electroporation treatment was performed. Square wave electric pulses of 100 microseconds, at 1300V/cm, with a frequency of 500Hz was delivered through two parallel stainless steel electrodes attached to a therapeutic electroporator machine (ELECTROvet S13, LEROY Biotech, France). Each run of electric pulses was delivered in 2 trains of 4 pulses, with 1-second interval in first series and the second series was delivered perpendicular to the first direction of electrode placement or in a hexagonal pattern (Spugnini et al., 2006). Good contact between the electrodes and the skin was assured by depilation and application of a conductive gel to the treatment area. Electrical pulses were first delivered at the tumour margin in order to reduce the blood flow to the tumour and then continued in concentric circles to the centre of the tumour nodule.

The second tumour on the medial aspect of the hock was excised with minimal margin to preserve skin and subcutaneous tissues around the tumour for wound opposition (Fig 3). The excised surface, tumour remnants and 2 cm around the tumour margin were considered for tumour volume calculation. The calculated tumour volume after excision was 18.31 cm$^3$. Intra-tumoral injection with cisplatin and electroporation procedure was similar to the previous session.

The response and clinical outcome of ECT was scored after 4 weeks, according to WHO guidelines, as Complete Response (CR - absence of any trace of tumour), Partial Response (PR - more than 50% reduction in tumour volume), No Change (NC - reduction of tumour volume less than 50%), No Enlargement (NE - enlargement less than 25%) and Progressive Disease (PD - tumour volume enlarged more than 25%) (Sersa et al., 2000).

**RESULTS AND DISCUSSION**

Following electrochemotherapy the tumour on the medial aspect of the thigh progressively reduced in size with scabs of dry tissues getting sloughed off (Fig 4) and complete response was noticed in 3rd week. The wound margin of the incompletely excised tumour on the medial aspect of the hock showed progressive healing of the wound margin and the sutures were removed on the 14th post-operative day (Fig 5) and complete response was noticed on the 4th week (Fig 6). Histopathology of excised tumour also revealed fibrosarcoma. No recurrence was noticed during the follow-up period of one year.
Kodre et al. (2009) reported that a variety of tumors of different histological origin such as fibrosarcomas, perianal tumors, sarcoids, mammary carcinoma and melanoma were treated with ECT with either cisplatin or bleomycin.

Sugita et al. (2012) demonstrated the anti-tumour effect of low-voltage electroporation on metastatic fibrosarcomas. The minimal voltage required for electroporation was > 900 V/cm. Hexagonal pattern of application of parallel electrodes were advocated for homogenous electroporation of the cells in the tumour. Further the anti-tumour effect might vary widely according to the extent to which the anti-tumour agent entered the cells. The influx of cisplatin or any anti-tumour drug depended on concentration gradient and electric potential.

The healing of sutured wound edges in the partially resected tumour site without any complications such as wound dehiscence indicated that the settings for the delivery of square wave electric pulses of 100 microseconds, at 1300V/cm, with a frequency of 500Hz was safe and had anti-tumour effect along with cisplatin (Spugnini et al., 2006) without tissue damage and thermal injury. Additionally application of electrical pulses injured the vascular endothelial cells of the tissues surrounding tumours, thus decreasing the flow of blood (vascular lock) resulting in cyto-reduction and tumour cell apoptosis (Bass et al., 2017).

Cisplatin gets activated after hydrolysis or aquation in water which is determined by the chloride concentration. The high chloride concentration (103 mM) of blood plasma prevents the hydrolysis of cisplatin outside the cell membrane. Although it was proposed that the entry of cisplatin could be facilitated via the carrier-mediated transport such as Na+, K+-ATPase and members of solute carrier; the intracellular concentration remains to be low and no change occurred in cisplatin-resistant tumour cells. Upon entering the cell, the chloride concentration drops down to 4 mM which facilitates the aquation process (Basu and Krishnamurthy, 2010).

Cisplatin hydrolyzed bio transformed products causes apoptosis by interfering with cell cycle and create inter-strand distortion of DNA. It also induces apoptosis through activation of the endoplasmic reticulum (ER) stress pathway (Rabik and Dolan, 2007). The molecular dimension of cisplatin is 110 nm (Hang et al., 2016 and Ma et al., 2015) and the transient pores created on the bi-lipid layer of cell membrane by electroporation in the therapeutic setup for electrochemotherapy varies from 5 to 20μm radius (Sengelaand Wallacea, 2016). Zhao et al. 2010 reported that the pores created by electroporation for electrochemotherapy ranging from radius of 15 μm and 25 μm is unstable and transient. Thus endocytosis of low permeable cytotoxic agent; cisplatin is facilitated. ECT also potentiates cisplatin
~80 folds (Campana et al., 2016). ECT enhanced the permeability of cisplatin into the tumor cell and potentiated the effect the effect of cisplatin on metastatic lesions of tumours by endocytic-like mechanism. The added advantage of electrochemotherapy with intra-lesional cisplatin is that the main adverse effects of cisplatin like renal toxicity, gastrointestinal toxicity, peripheral neuropathy, myelotoxicity, asthenia, otoxicity and also resistance to cisplatin (Nejdl et al., 2014) can be prevented by the ECT procedure as it does not involve systemic administration of the drug.

CONCLUSION
ECT with intra-tumoural administration of cisplatin was found to be effective in treating small solid fibrosarcoma and can be used as an adjunct therapy following surgical excision of larger size fibrosarcoma without recurrence in horses.

REFERENCES