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European Society of
Veterinary Oncology

ESVONC CONGRESS LYON, France, 20-22th April 2017

PROGRAM

European Society of Veterinary Oncology

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European Society of Veterinary Oncology

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European Society of Veterinary Oncology

Proceedings

20th – 22nd April 2017

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The organising committee wish to thank all those persons who helped review the submitted abstracts, judged the Residents' competitions, moved chairs, carried things and generally made the smooth running of the conference possible.

And finally many thanks to the printers and staff of the congress venue

Dear colleagues and friends,

Welcome to the annual ESVONC congress being held for the first time in Lyon, France!
We will have a great program with state of the art information from different fields of veterinary and comparative oncology mixing together the recent knowledge on basic sciences and clinical oncology.
The themed sessions will be a focus on immunotherapy, THE up-to-date subject in human oncology and subsequently in veterinary oncology as well ! The second themed session will be on head and neck cancers with comparative aspects in human oncology as usual !
Living in this beautifull city for many years, I wanted, with my team, to organize a great social program ! History is everywhere in this town and is revealed in the traces that time has left behind for our collective enrichment ! Do you remember that Lyon is the place where the first veterinary school was created 250 years ago by Claude Bourgelat ?
Do you know that Lyon is THE city of gastronomy and french flair ?
Don't miss it !
ESVONC Annual congress is a place for social events and sharings: the venue will be at the wonderfull and pleasant Congress City Center surrounded by a Parc and the welcome reception will be in the first veterinary school that became the National Music school… Gorgeous!
On Friday evening, we will organize our annual gala dinner with some surprise !
And we can promise you one thing : this friendly congress in Lyon will be memorable!

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Themed sessions

Head and Neck Surgery

Lymph Node Mapping Principle And Application

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ESVONC Congress Lyon 2017

The lymphatic system is a unique silent workhorse in maintaining vital operations within the body. Functions of the lymphatic system broadly includes homeostatic fluid regulation, dietary lipid absorption, and importantly lymphocyte trafficking. The lymphatic system is also an integral scavenge system or accessory route of fluid and large particulate matter accumulating in the interstitial space for return to the blood. The lymphatic system differs from the vascular system with its unidirectional flow of lipophilic-rich fluid, albumin, lymphocytes and scavenged cells from peripheral lymphatics through lymph nodes to collecting lymph ducts prior to emptying into the cranial vena cava. The lymphatic system is also a pathway for metastasis; the presence of metastasis in lymph nodes is of clinical import. Lymphatic metastasis is aided by the process of tumor-induced lymphangiogenesis, whereby a tumor induces lymphatic vessel formation around itself which increases access to the lymphatic circulation for metastasizing tumor cells. Additionally the composition of lymph is nourishing for metastasizing tumor cells and lymph flow is less turbulent, further contributing to survival of metastasizing tumor cells.

For many solid tumors, presence of lymphatic metastasis is associated with an advanced stage of cancer diagnosis and a poorer survival prognosis. Sentinel lymph node mapping is an accurate physiologic method for finding which lymph node or nodes receive draining tumor lymph. These sentinel lymph nodes are the lymph nodes most at risk for metastasis as they are the first nodal location visited by migrating tumor cells. A positive sentinel lymph node is a lymph node having presence of metastatic cells. A negative sentinel lymph node receives draining tumor lymph but does not have the presence of metastasis. The significance of a negative sentinel lymph node is that the rest of the lymphatic basin, as well as the rest of the patient, is unlikely to have advanced disease for tumors

known to spread via locoregional progression. A positive sentinel lymph node has paramount impact on determination of clinical stage of disease as well as prognosis for a patient.

There are many different tools of sentinel lymph node mapping. The most common techniques utilize sterile blue dyes injected around a tumor to stain draining lymphatic vessels and lymph nodes visibly blue. Another tool is the use of radioisotope-labeled compounds that are similarly injected around a tumor which accumulate within draining lymphatic vessels and lymph nodes. The radioactive lymphatic structures are viewed via two different instruments, a SPECT gamma camera and a handheld gamma probe. A gamma camera is used to image the body, to discover and image noninvasively which anatomic lymph node basins are affected from a big picture global perspective, whereas the handheld gamma probe is a much more sensitive tool for isolating individual radioactive lymph nodes intraoperatively via an audible signal that is generated as the probe moves over a radioactive lymph node. Other tools include fluorescein dyes with ultraviolet lamps, indocyanine green with near infrared imaging, and more recently indirect lymphography with iodized oil and water-soluble iodinated compounds. Adoption of sentinel lymph node mapping revolutionized human surgical oncology. Benefits to patients include decreased surgical morbidity, decreased postoperative pain, improved mobility, decreased occurrence of lymphedema, and decreased iatrogenic numbness. With sentinel lymph node mapping, fewer lymph nodes are removed or extirpated. This improves pathologic analysis as more intensive investigations can be made on fewer extirpated lymph nodes. Patient care has also been improved through the phenomenon of stage shift, as additional therapies are offered to more patients with identified lymph node metastasis than prior to sentinel lymph nod

Adaption of peritumoral indirect CT lymphography in dogs with head and neck neoplasia

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ESVONC Congress Lyon 2017

Identifying the sentinel lymph node (SLN) provides critical cancer staging information, affecting adjuvant therapy recommendations and patient prognosis. This study compared three SLN mapping techniques--CT lymphography, vital dye injection, and intraoperative lymphoscintigraphy. The purpose was to assess the feasibility and reliability of all three techniques in a population of dogs with naturally occurring head and neck cancers. Twenty dogs with primary head and neck cancers were prospectively enrolled. Following pre and post intravenous contrast CT imaging, iohexol was injected at 4 quadrants peritumorally or along tumor the scar with images acquired at 1, 3, and 6 minutes post injection evaluate draining sentinel lymphatic vessels and nodes. Subsequently Tc99-sulfur colloid was injected in like fashion preoperatively. Intraoperatively vital dye was injected similarly in 4 quadrants, and a handheld gamma probe was used to detect radioactively lymph nodes. Surgical approach was made to any lymph node identified as sentinel from any three techniques and extirpated. All excised lymph nodes were submitted for histopathology, as were lymph nodes called abnormal on CT. Primary tumors and scars were biopsied or excised. CT lymphography indicated draining lymphatic vessels and or SLNs in 18 of 20 dogs, lymphoscintigraphy indicated the SLN in 20/20 dogs, and vital dye in 17/19 dogs. Neither of the three SLN modalities are fool proof, and that consistent with human medicine, combination SLN mapping with at least two modalities is ideal. Additionally CT lymphography is easy to perform and able to be incorporated in many diverse practice settings unlike lymphoscintigraphy.

Intraoperative and postoperative complications of partial maxillectomy for the treatment of oral tumors in the dog: 193 cases (2000 - 2011)

Roxane H. MacLellan, Jennifer E. Rawlinson, Sangeeta Rao, Deanna R. Worley

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ESVONC Congress Lyon 2017

The objective was to describe and compare intraoperative and postoperative complications of maxillectomies in dogs. This is a retrospective case series of 193 unirradiated dogs that received a maxillectomy to excise an oral tumor. Medical records were reviewed for signalment, tumor location and size, histologic results, clinical staging, maxillectomy type, surgical approach, intraoperative and postoperative complications, additional therapeutics, and short-term outcome. Descriptive statistics were calculated using Pearson χ^2 analysis. Of 193 dogs with maxillectomies, the major intraoperative complication was excessive surgical bleeding (103/193; 53.3%) resulting in intraoperative transfusion (44/193; 22.7%). Excessive surgical bleeding and intraoperative transfusions were significantly associated with tumor size and location, maxillectomy type, and surgical approach ($P < 0.05$). The DL/IO approach had an increased incidence of excessive surgical bleeding (48/58; 82.8%) and longer mean surgical time (106 minutes) compared to the IO approach (excessive surgical bleeding 29/54; 53.7% and 77 minutes mean surgical time) for caudal tumors ($P < 0.05$). Immediate postoperative complications (< 48 hours postoperatively) were epistaxis (99/193; 51.3%), excessive facial swelling (71/193; 36.8%), facial pawing (21/193; 10.9%), and difficulty eating (22/193; 11.4%). Short-term complications (48 hours to 4 weeks postoperatively) were lip trauma (22/193; 11.4%), oronasal fistula formation (18/193; 9.3%), wound dehiscence (18/193; 9.3%), and infection (13/193; 6.7%). Intraoperative and postoperative complications of maxillectomies are varied. Certain maxillectomy complications are significantly associated with tumor size and location, maxillectomy category, and approach.

Use of preoperative indirect lymphangiography with lipid based iodated contrast and per-operative methylene blue.

Brissot Hervé.DVM, DECVS. Pride veterinary Centre, Derby, UK

Introduction

Sentinel lymph node (SLN) identification and lymph node mapping is a well known concept in human oncology. Each area in the body has a dedicated lymphatic drainage pathway which can be associated with multiple lymph nodes. The SLN is defined as the first lymph node to drain the area. Consequently, if an area is affected by a neoplastic process, the SLN will be the first one to show metastases. Based on the TNM staging system, the sentinel lymph node should be the one to be assessed first, even if it is considered normal on palpation or on conventional imaging as microscopic disease can not be ruled out.

We hypothesised that identification and sampling for subsequent histological examination of the sentinel lymph node will improve the comprehension of the disease and allow better treatment for our patients due to earlier detection of metastatic disease. Our aim was to establish a protocol where every solid tumour will have an additional radiographs for identification of the sentinel lymph node after peritumoral iodine based contrast medium injection.

Material and Method:

Client owned dogs treated at our hospital with solid tumour were recruited for SLN mapping. A lipid based contrast medium: Lipiodol Ultra-Fluid TM (Guerbet, Aulnay-sous-bois, France; 480 mg iodine per ml) was injected slow rate infiltration (2 to 4 ml, over 1 to 5 minutes). Injection was performed into the 4 quadrants encircling the tumour and in some cases within the tumour. The injection was to be done in the tissue from which the mass was coming from or directly within the mass if it was not to change further surgical approach (no modifications of the boundaries or risks of capsule damages and potential seeding). The choice of Lipiodol TM was made based on the report of slow resorption, reliable lymphatic uptake and its ability to concentrate and being retain within the lymphatic tissue for more than 24 hours.Two-view radiographs study was performed 24 hours after contrast injection.

Methylene blue (Proveblue TM (methythionium choride, 5mg/ml, Proverpharm SAS-Cenerexi, Fontenay-sous-bois, France)) was injected similarly at the time of surgery, 10 to 15 minutes before surgical incision. A total injection of 0.5 to 1 ml (5mg/ml was performed. In animals less than 10 kg, a diluted solution (1:1, in dextrose) was used.

Results:

The protocol was applied in 30 consecutive cases among these 15 dogs were affected with head and neck neoplasia.

In all cases, identification of SLN was achieved. Correct identification and matching of imaging and surgical findings were reported after gross examination of the removed lymph nodes (blue coloration or none) and subsequent radiography (iodine enhancement or not). In all cases indirect lymphangiography allowed identification of a draining lymph node. Only 4/14 cases had the lymph node palpated on clinical presentation. 13 dog had surgery, 11 showed good coloration with methylene blue-1 showed fair colouration, 1 was not found (prescapular) and one showed no coloration. SLN was mandibular LN in 10 cases, pre-scapular LN in 3, parotid in 2 (in association with ipsi-lateral mandibular in 1 case) and retropharyngeal in 2 (in association with ipsilateral mandibular in 1 case).Secondary effect or local complication associated with the injections were not reported.

Conclusion: Preoperative lymphangiography with peri-tumoural injection of LipiodolTM is feasible and allows non invasive identification of SLN (head and neck but also over the rest of the body). This was reliably associated with methylene blue uptake by lymphatics and SLN marking.

Modified Weber-Fergusson approach for caudal maxillectomy in dogs and cats

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(Nihon University, Japan)

Malignant melanoma, fibrosarcoma, squamous cell carcinoma, and acanthomatous ameloblastoma in dogs and squamous cell carcinoma and fibrosarcoma in cats are the most common oral tumors.[1] These tumors are generally locally invasive and often increase in size significantly in the more caudally positioned maxilla. Surgery is the treatment of choice for local control of these tumors. In the extensively increased caudal maxillary tumors, aggressive surgical resection is necessary for adequate surgical margins. Many studies have described that tumor-free margins are associated with a more favorable prognosis.[1,2] However, conventional intraoral approach to maxillectomy results in a narrow field of view for the resection of the more caudal tumors. Hemorrhage and hypotension are the most common intraoperative complications, particularly during aggressive caudal maxillectomy procedures.[1]

For the more caudal maxillary tumors, the combined intraoral and lateral approach (the creation of bipedicle skin flap in the lateral nasofacial area) was reported to facilitate better exposure for the resection of the tumors and postoperative reconstruction.[2] Histopathologically, clean margins were obtained in 70% of cases in the report. In addition, significant intraoperative complications appeared to be minimal. Therefore, this approach produces an improvement in outcome over the conventional intraoral approach. However, the osteotomies for maxillary or infraorbital bones in the lateral direction using a biradial oscillating saw are relatively difficult to perform with this approach.

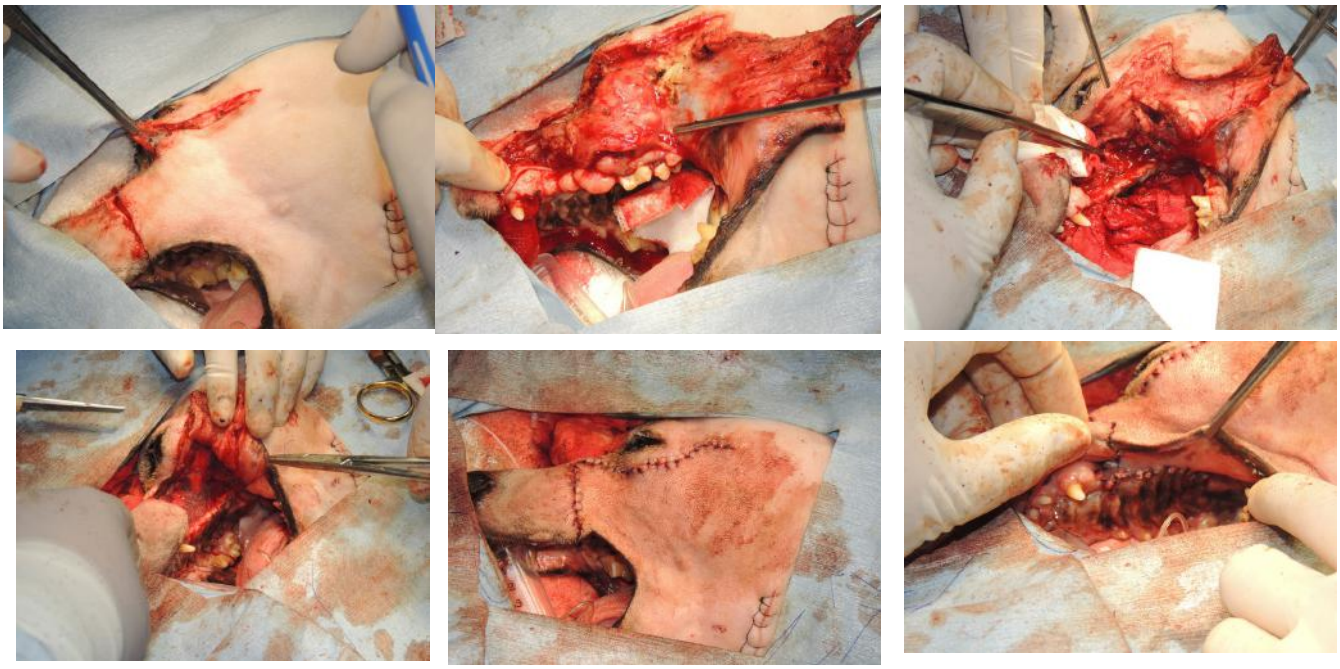
The Weber–Fergusson approach was first described by Dr. Weber in German and later modified by Dr. Fergusson in English.[3,4] It is one of the most commonly used transfacial approaches to the midface for the resection of maxillary and infraorbital tumors in humans. As far as radical maxillectomy is concerned, the Weber-Fergusson approach has the advantage of excellent exposure and minimal scarring as the incision follows the natural skin crease. Excellent exposure in the Weber-Fergusson approach may be associated with tumor-free surgical margins and a more favorable prognosis. However, the original Weber-Fergusson approach is not available for canine or feline radical maxillectomy because the morphology of human skull is completely different from that of small animal's skull. Therefore, the procedure

should be modified for clinical application of the Weber-Fergusson approach in small animal maxillectomy.

The Weber-Fergusson approach modified for dogs and cats is described briefly as follows: the intended skin incision line is shown in Fig 1. The dorsal incision is first created through the skin, just lateral to midline of the dorsal aspect of the nasal cavity. The rostral end of the incision is extended up to the rostral line of scheduled maxillary tumor resection. A second incision is continued through the subcutaneous tissues and muscles, and down to bone. The incision is made from the rostral end perpendicular to the lip. Beneath the skin incision, the subcutaneous tissues, orbicularis oris muscles, buccinator muscles, and buccal mucosa are cut by Mayo scissors and electrocautery. In addition, the incision is continued in the gingiva on the rostral scheduled line. A third incision is made in the buccal mucosa immediately dorsal to the gingiva from the second incision in a rostrocaudal direction, but dictated by the adequate margins of the tumor. The third incision in the gingiva is continued to the caudal line of scheduled tumor resection. The fourth incision is carried out caudally by sweeping down below the eye, following the midline of the zygomatic arch. Next, the cheek flap is torn and rolled over for exposure of the tumor (Fig 2).

The scheduled resection line and 1-cm caudal line of zygomatic arch are incised by a blade and electrocautery, and 1-cm block resection is made by the saw. The infraorbital vessels and nerve are cut by the vessel sealing system via the window made after the block resection of zygomatic arch. The following osteotomies for the tumor excision are routinely done using the saw. After the excision, the hemorrhage is controlled by electrocautery, vessel sealing system, and local hemostatic agents (Fig 3).

For the reconstruction of the caudal maxilla, buccal mucosa is dissected bluntly in the cheek flap. The buccal mucosa flap is made adequately to cover the defect after the tumor excision. Closure is firstly initiated from the caudal aspect (Fig 4). The buccal mucosa flap and mucosa of the hard palate are apposed with a simple interrupted suture pattern (3-0 or 4-0 mid-term or long-term absorbable monofilament suture material). The cranial and caudal stumps of lip are also apposed by interrupted sutures. The remaining tissues are closed routinely (Fig 5ab).



Figures from left to right, top to bottom: 1;2;3;4;5a ;5b

The modified Weber-Fergusson approach facilitates better exposure of more caudal maxillary tumors and provides a wider field of view for the resection of the tumor compared to the other approaches in our hospital. In addition, the remaining defect after tumor resection is more easily and cosmetically reconstructed using the modified Weber-Fergusson approach. Specific intraoperative and postoperative complications associated with this approach are not recognized historically in our hospital. Therefore, the modified Weber-Fergusson approach is suggested to provide better outcomes for canine and feline caudal radical maxillectomy.

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ELECTROPORATION IN HEAD AND NECK CANCER: CLINICAL APPLICATIONS AND FUTURE PERSPECTIVES

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INTRODUCTION

Electroporation is a technique that aims at ablating tumor cells through the direct application of current (irreversible electroporation or IRE) or at increasing the delivery of drugs through the cell membrane by applying proper permeabilizing pulses. Chemotherapy drugs are effective provided that they reach their site of action. The primary obstacle for chemotherapy agents is the cytoplasmic membrane, especially for lipophobic agents such as bleomycin. Electrochemotherapy (ECT) couples the administration of an anticancer agent (usually bleomycin or cisplatin), to the delivery of electric pulses having appropriate waveforms. The application of permeabilizing pulses leads to perturbation of the cell membrane, thus resulting in increased uptake of chemotherapeutic drugs, ultimately leading to cell apoptotic death.[3]Clinical trials evaluating humans treated with ECT for cutaneous neoplasms were first reported in the early 90s, and have been followed by many trials that confirm its potential for the treatment or palliation of dermatological tumors.[3-5] Recently, a standard operating procedure has been proposed.[6] At the moment, several trials are ongoing worldwide to implement the use of this therapy in different cancer conditions.[7]

ELECTROCHEMOTHERAPY EQUIPMENTS

Different waveforms have been adopted by investigators: exponentially decaying, square, rectangular or biphasic. In general the number of pulses delivered is set in 8 single pulses applied per cm of tumor area at a voltage of 1300 V/cm (800 V/cm for intraoperative use), with a duration of 100 µs and a frequency of 1 Hz. Treatments are repeated until the whole tumor area is covered. We recently developed a shortened protocol with decreased morbidity and maintained efficacy.[8]

CHEMOTHERAPY AGENTS ADOPTED IN ELECTROCHEMOTHERAPY

This technique increases the efficacy of all the drugs that enter the cell through membrane carriers (i.e. bleomycin, methotrexate etc) or agents that enter the cell by means of passive diffusion (i.e. cisplatin or mitoxantrone). Agents such as bleomycin are potentiated by a 300-700 factor, cisplatin is potentiated by a 4 to 8 factor.[8]

EVALUATION OF BIPHASIC PULSES BASED ECT IN PETS WITH DIFFERENT NEOPLASMS INCLUDING HEAD AND NECK CANCER

Experimental design

Our group investigated in the past 15 years the feasibility of electrochemotherapy in companion animals affected by different neoplasms. We adopted trains of biphasic pulses delivered as a burst instead of single impulses. The electric waveform were generated by an equipment developed by our group. This device is built up by a toroidal core transformer generating a roughly rectangular pulse which is split in two halves that are sequentially driven to obtain a biphasic pulse. Pulses are not singularly produced but are created in bursts. The equipment allows choosing electric field intensity, number of pulses and pulse duration. The standard wave train used for this experiment was set to 8 pulses of 50 + 50 μs (biphasic pulse), and the electric field intensity was 1250 V/cm. The pulse repetition frequency is 1 Hz while the frequency of burst repetition is 1 kHz, resulting in a total burst duration of 7.1 ms. Bleomycin was locally injected at the tumor site and in the tumor's surrounding tissues. Five minutes after the injection, trains of biphasic pulses were administered through caliper electrodes as above described. Adherence of the caliper electrodes to the lesions was maximized using an electrophoresis gel. The treatment was repeated after 1 week.[12] More recently, a shortened protocol involving 8 pulses with 10μs interpulse interval and a total duration of 3.2 ms has been devised.[7] The adoptions of a dedicated software for the impulse stabilization and optimization resulted in improved control and minimal side effects.[7]

RESULTS

In the first trial, ECT was used to directly attack neoplasms.[9] Overall response rate was 80%, with 40% long lasting remissions (in excess of 1 year). This phase I/II study led to the development of several electrodes specific for the different body districts.[10] On the basis of the preliminary studies, cohorts of dogs and cats affected by melanoma, soft tissue sarcoma mast cell tumor and nasal squamous cell carcinoma were enrolled in phase II studies.

Melanoma Ten patients with malignant melanoma (MM) of the oral cavity were enrolled in a phase II study. The overall response rate was 80% (median time to recurrence 6 months), with a 50% of patients in remission after 1 year.[11] Of interest, most of the long term responders experienced a vitiligo like discoloration at the treatment site, potentially suggestive of recruitment of the immune system after uncovering of deep antigens.

Soft tissue sarcoma A total of 72 cats affected by soft tissue sarcoma were assigned to three different groups: 1) surgery alone, 2) surgery coupled with intraoperative ECT, 3) surgery coupled with post-operative ECT.[12] The median control times were respectively 4, 12 and 19 months. In this study were identified some prognostic factors such as previous treatment and tumor size. A cat with a bilateral rhabdomyosarcoma of the head had a remarkable long term control after surgery and ECT.[13] The only side effects were tumor dehiscence in 2 patients and focal inflammation in 2 patients. Systemic side effects were not reported among the enrolled patients.

In another study, adjuvant ECT was adopted to increase local control in a group of 22 dogs, obtaining a mean time to recurrence of 730 days, with 50% of the dogs still desease free at the time of

writing.[14] More recently, ECT has been exploited to reintroduce potentially toxic drugs in the oncology protocols. Specifically, a cohort of 64 cats with incompletely excised soft tissue sarcoma have been enrolled in a study involving the use of cisplatin as ECT agent and matched with a control cohort of 14 cats treated with surgery. All the cats tolerated the treated very well, despite the high reported toxicity of the drug when administered systemically. In terms of tumor control mean time to recurrence was 666 days and 180 days, respectively. [15]

Mast cell tumor Adjuvant bleomycin-based ECT was used in 28 dogs with incompletely excised mast cell tumors. Overall response rate was 85%. The median time to recurrence was not reached. Estimated time to recurrence was 52.76±6.5 months.[16] One dog had wound dehiscence. In 2009 Kodre et coll. compared square pulses based ECT to surgery alone in the treatment of mast cell tumors, using intralesional cisplatin, and claiming a longer control with ECT.[17] Another study, enrolling 37 dogs, evaluated the efficacy of cisplatin for the treatment of incompletely excised canine mast cell tumors obtaining a mean disease-free interval of 1218 days with 78% of the patients experiencing local control at different times.[18]

Squamous cell carcinoma This is the head and neck tumor where the larger number of studies has been performed and also the neoplasm with the larger number of treated patient. A preliminary study enrolled a small cohort of 9 cats with sun induced squamous cell carcinoma of the nasal planum that were treated with intralesional bleomycin coupled with ECT with a rate of complete responses of 77%.[19] A more recent article describes the use of square pulses based ECT for superficial squamous cell carcinoma in cats, reporting a 81% of complete responders.[20] A recently published article reports an high percentage of responders in cats with advanced head and neck carcinoma using biphasic pulses with minimal side effects.[21]

CURRENT PROTOCOLS

Electrochemotherapy is mostly used as a adjuvant therapy, locally injecting the chemotherapy drugs bleomycin or cisplatin and then following with the application of permeabilizing pulses. For larger tumors drugs can be administered locally or systemically, mostly on the basis of tumor volume, firmness or resistance to insertional electrodes.[22] Caution should be exerted when using electrochemotherapy in patients previously treated with radiation therapy due to the risk of radiation recall.[23]

ELECTROCHEMOTHERAPY IN OTHER SPECIES

ECT has been used successfully in horse to treat sarcoids using intralesional bleomycin and to treat melanomas using cisplatin as chemotherapy agent, either directly or combined with surgery. The treatment has been well tolerated and resulted in high percentage and durable responses. [24-28] Currently our group is building up a larger caseload to publish the first study on solid tumors in horses treated with ECT. A significant percentage of these lesions were located in the head and neck of the patients.

ECT has been used to treat tumors in exotic animal as well. Our group described the usefulness of adjuvant ECT in the treatment of advanced cutaneous tumors in rabbits and in a turtle with a squamous cell carcinoma of the neck.[7,29] Several exotic animals have been successfully palliated or cured with ECT.

ELECTROGENETHERAPY

The first report of electrochemogene therapy in veterinary cancer patients has been presented by Draghia and colleagues in 2002. The authors investigated the possibility to palliate several cancer induced complications such as cachexia, anemia, anorexia, and decreased activity levels that many veterinary patients experienced by delivering a plasmid encoding for insulin-like growth factor-I.[30] More recently, electrochemogene therapy using plasmids encoding for IL-12 has been successfully investigated in small cohorts of dogs with a variety of spontaneous neoplasms.[31] Finally a recent prospective study on canine mast cell tumor recruited eight dogs for a total of 11 lesions that received from one to four sessions of electrochemogene therapy with IL-12.[32] The treatment was well tolerated but the tumor response was extremely variable.

IRREVERSIBLE ELECTROPORATION

This technique aims at inducing focal tumor destruction by using direct tumor ablation through the delivery of electric pulses at higher voltage than RE (used in ECT), alone or combined with chemotherapy. Two reports have been published so far describing the successful treatment of a canine glioma patient and of a canine patient with a large histiocytic sarcoma. [33,34]These two reports are very promising and warrant further investigation to confirm the efficacy of this approach.

CONCLUSION

Electroporation in its different applications (irreversible and reversible) is a safe and efficacious approach to veterinary. Its low cost and ease of administration makes it a valuable addition to the currently available oncological therapies. One advantage of this technique is the possibility of repeated treatments in case of local recurrence and the recruitment of the patients' immune system, as suggested by the low incidence of metastatic disease among most treated patients and by the tumor selection observed among some pets with recurring disease.

FUTURE DIRECTIONS

Ongoing studies are evaluating the efficacy of ECT when other drugs than bleomycin are used, in particular anthracyclines and their analogues (mitoxantrone) and platinum compounds such as CDDP and carboplatin. Studies are being performed to characterize the membrane alterations induced by

trains of biphasic pulses in order to improve the efficacy of the technique.[35] Novel electrochemotherapy equipments adopting different electrical parameters are currently being developed.[36]

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State of the art in human head and neck oncologic surgery: from the minimal invasive surgery to the open invasive and reconstructive surgery

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Surgery actually remains one of the best options to treat head and neck carcinomas in humans, it can be proposed alone for small tumors or can be integrate in a multimodal treatment for locally advanced tumors. This surgery is challenging, the primary objective is to remove the tumors with clear margins, but also, if possible, avoid loss of function or to restore this functions as best as possible, and the surgeon also must decrease as possible the disfigurement. The complexity for the choice of the best treatment cannot be taken by only one physician, actually it is mandatory in France but also in other country to take the decision in a multi disciplinary tumor board. The author will review the different surgical technique actually use to treat the carcinomas of the mouth, the pharynx and the larynx, he will describe the principles of the trans oral surgery (laser/robot) of the sentinel lymph node and the options in free flaps used in reconstructive surgery with the preoperative modelisation used for the bone free flaps.

Immunotherapy

The reason to consider immunotherapy in veterinary oncology ?

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At present, current therapeutic procedures used in veterinary oncology include surgery, dose-dense chemotherapy and radiotherapy. Nowadays, the use of small molecules as the tyrosine-kinase inhibitors has become an every day practice, for either their specific anti-tumor or anti-angiogenic effect (often within a metronomic multidrug chemoterapeutic regimen). For high grade tumors, also characterized by a high metastatic rate (canine hemangiosarcoma, appendicular osteosarcoma, malignant melanoma, grade III soft tissue sarcoma, etc.), little improvement has been made in the last 5-10 years with regard to adjuvant treatment (based mainly on dose-dense chemotherapy), and prognosis. Hence, the need for new therapeutic strategies, which should already be effective on their own, to be potentially combined with standard treatment in an effort to improve survival and consequently prognosis. With regard to this, immunotherapy is becoming one of the most promising therapeutic modalities in oncology

State-Of-The-Art of Anti-Cancer Vaccination in Pet Animals

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www.petcancerinformation.com

This presentation will focus on the available immunotherapies for treating cancer in our pets. They include various targets such as HER2, tumor indications such as osteosarcoma and lymphoma, delivery systems such as the Vet Jet and electroporation and published or anecdotal data currently available.

Experience on anti-CSPG4 vaccination in canine oral malignant melanoma

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Reported post-surgery 1-year survival rate for oral canine malignant melanoma (cMM) is around 30%; novel treatments are needed as the role of adjuvant chemotherapy is unclear. This prospective study regards adjuvant electrovaccination with human chondroitin sulfate proteoglycan-4 (hCSPG4)-encoded plasmid in 23 dogs with resected II/III-staged CSPG4-positive oral cMM compared with 19 dogs with resected only II/III-staged CSPG4-positive oral cMM. Vaccination resulted in 6-, 12-, 18- and 24-month survival rate of 95.6, 73.9, 47.8 and 30.4%, respectively [median survival time (MST) of 684 days, range 78 – 1694, 8 of 23 dogs alive] and 6-, 12-, 18- and 24-month disease-free interval (DFI) rate of 82.6, 47.8, 26.1 and 17.4%, respectively (DFI 477 days, range 50 – 1694). Non-vaccinated dogs showed 6-, 12-, 18- and 24-month survival rate of 63.2, 26.3, 15.8 and 5.3%, respectively (MST 200 days, range 75 – 1507, 1 of 19 dogs alive) and 6-, 12-, 18- and 24-month DFI rate of 52.6, 26.3, 10.5 and 5.3%, respectively (DFI 180 days, range 38 – 1250). Overall survival and DFI of vaccinated dogs was longer in those <20 kg. In vaccinated and non-vaccinated dogs local recurrence rate was 34.8 and 42%, respectively while lung metastatic rate was 39 and 79%, respectively.

Refreshed results (January 31 2017) for dogs adjuvantly vaccinated with Hu-CSPG4 were: median survival time (MST) of 659 days, range 78 – 2090, 3 of 23 dogs alive at respectively 1169, 1294 and 2090 days] and median DFI of 477 days, range 50 – 1749.

From January 1st 2015, further 11 dogs with locally controlled (surgery and/or radiotherapy) CSPG4-positive oral cMM initially staged as II or III were enrolled (minimun follow-up of 6 months). The protocol varied as the monthly check for metastasis was performed by CT scan instead of radiography, and adjuvant vaccination was performed with HuDo-CSPG4 (chimera). The higher accuracy of CT *vs.* radiology in detecting systemic metastasis (stage IV oral cMM) emphasized even more the efficacy of the treatment; in case of suspected lung/other sites metastasis within the vaccination period, metronomic therapy was often added. Results of adjuvant vaccination with HuDo-CSPG4 (combined or not combined with metronomic chemotherapy) were comparable with results obtained with adjuvant vaccination with Hu-CSPG4 plasmid only. Also some of the latter patients received metronomic chemotherapy when systemic metastasis was suspected.

Results for dogs adjuvantly vaccinated with HuDo-CSPG4 on January 31 2017 were: 6-, 12-, 18- and 24-month survival rate of 100, 45.4, 27.3 and 9.1%, respectively [median survival time (MST) of 580 days, range 225 – 760, but 7 of 11 dogs are still alive, range 225-760 days, 1 dog dead for unrelated

causes at 685 days]; and 6-, 12-, 18- and 24-month disease-free interval (DFI) rate of 54.5, 27.3, 18.2 and 9.1%, respectively (DFI 313 days, range 0 – 760).

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Translational Cancer Immunotherapy

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ITEOS THERAPEUTICS - Gosselies, Belgium *Since 2015*

Head, Tumor Immunology

Cancer immunotherapy has moved to the forefront in the treatment of patients with cancer, providing a unique opportunity to achieve dramatic and lasting anti-tumor responses in a variety of tumor types. Major clinical breakthroughs in human cancer immunotherapy include the use of checkpoint inhibitors and engineered T cells. Many challenges still remain, including determining the sub-populations of patients who will respond, the sub-populations of patients who might experience significant toxicities and the selection of therapeutic combinations of interest.

Although advances in cancer immunotherapy depend on preclinical testing, most in-vivo testing currently relies on genetically identical inbred mouse models which, while offering critical insights regarding efficacy and mechanism of action, also vastly underrepresent the heterogeneity and complex interplay of human immune cells and cancers. Additionally, laboratory mice uncommonly develop spontaneous tumors, are housed under specific-pathogen free conditions which markedly impacts immune development, and incompletely model key aspects of the tumor/immune microenvironment. The canine model represents a powerful tool in cancer immunotherapy research as an important link between murine models and human clinical studies. Dogs represent an attractive outbred combination of companion animals that experience spontaneous cancer development in the setting of an intact immune system. This allows for study of complex immune interactions during treatment while also directly addressing long-term efficacy and toxicity of cancer immunotherapies.

As in human, the canine PD-1/PD-L1 pathway is also associated with T cell exhaustion in canine tumors and its blockade with antibody could be a new therapeutic strategy for canine tumors. However, immune dissection requires access to robust and validated immune assays and reagents as well as appropriate numbers for statistical evaluation. Canine studies will need further optimization of these important mechanistic tools for this model to fulfill its promise as a model for immunotherapy.

This presentation aims

i/ to present the history of cancer immunotherapy in human,

ii/ to discuss the most recent data in the field of cancer immunotherapy,

iii/ to illustrate the translation of preclinical to clinical findings and the reverse translation from clinical to preclinical models to further understand the mechanism of action of new therapies.

Finally (iv/) spontaneously occurring canine cancers will be discussed in the context of existing preclinical cancer immunotherapy models to evaluate both their advantages and limitations, as well as highlighting their growth as a powerful tool in the burgeoning field of both human and veterinary immunotherapy.

Principles of active immunotherapy against cancer

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Neoplastic progression is marked by the accumulation of genetic and epigenetic alterations in the cells of the tumor microenvironment that result in the anomalous expression of specific cell constituents and/or products [1]. Cells undergoing transformation and cells in their environment display changes in the expression of both membrane components and peptides expressed on the cell surface in association with the MHC glycoproteins. These changes are perceived as tumor-associated antigens (TAA) by the immune system that mounts an adaptive response mediated by both B and T cells. This reaction rarely suppresses a tumor, whereas it usually gets rid of the cells that primarily express the specific antigen [2]. The result of this culling is the survival of less antigenic cells that are better suited to escape immune attacks, an event known as immunoediting [3, 4].

Established, growing tumors are strongly immune-suppressive and give the organism little chance to induce effective and long lasting immunity against self-tolerated molecules such as TAA [5]. Several inhibitory pathways that contribute to tumor-induced immunosuppression have been identified, including cytokines, expansion of suppressive cell populations, amino acid-catabolizing enzymes production and ligation of inhibitory receptors (the so-called checkpoints of the immune system) on activated T cells [5].

The immune response elicited by therapeutic vaccinations against TAA is thus faced with immunoedited tumor cells, a diffuse tumor burden [6, 7] and a negative setting of the immunoregulatory mechanisms [2]. The reaction elicited by effective vaccines can lead to tumor shrinkage. However, remission frequently ends with the tumor recurrence. The effective elimination of a tumor requires coordinated immune mechanisms involving both the activation of immune effector cells and the removal of suppressor mechanisms; this could be obtained by combining cancer vaccines with checkpoint inhibitors [5, 8]. By contrast, cancer vaccines still hold promise if positioned appropriately in the minimal residual disease setting, where the tumor-associated inhibiting mechanisms are limited or inexistent and vaccine-induced immunity may be much more effective [9].

A key issue for the effectiveness of a cancer vaccine is the choice of an immunobiologically relevant target antigen. TAA that are unrelated to the growth and spread of cancer cells will easily be lost, or down-modulated, in the presence of an immune response. We coined the term "oncoantigens" to

distinguish persistent TAA that are directly or indirectly related to the survival, growth and spread of tumor cells [10].

Oncoantigens can be unique antigens derived from protein-altering “driver” mutations (neoantigens) or can be overexpressed normal, non-mutated proteins (conserved TAA) that participate in the oncogenic process. Despite the high immunogenicity and the low risk of autoimmunity, targeting of neoantigens poses economic and regulatory challenges since they are not readily identifiable and are generally patient-specific. Instead, the tumour contains a preponderance of conserved TAA, which, even if theoretically less immunogenic, are readily recognized by T cells and antibodies from cancer patients.

Oncoantigens expressed on the cell membrane are theoretically the most promising for vaccination, since they can be the targets of both cell-mediated and antibody-mediated immune responses. Antibodies do not require MHC glycoproteins to recognize and bind to their target, so oncoantigens accessible to antibodies are the target of direct and indirect antibody-mediated reactions and not impaired by the down-regulation of MHC molecules on the surface of tumor cells [1].

Finally, if expressed by cancer stem cells (CSC) oncoantigens are even more appealing as cancer-vaccination targets [11]. Most tumors show intra-tumor heterogeneity resulting from an intrinsic cell hierarchy, with CSC at the apex. CSC have the unique biological properties necessary for maintenance and spreading of the tumor and through asymmetric division, can differentiate into cancer cells that compose the tumor bulk [12]. Due to their resistance to traditional radio- and chemo-therapies [13, 14], which act by preferentially killing differentiated cancer cells [14], CSC represent a reservoir for the relapse, metastatic evolution and progression of the disease after treatment, representing a major barrier towards effective cancer eradication. Consequently, a key challenge in anticancer therapy is the development of treatments that are able to both shrink a tumor and kill CSC. However, the identification of ideal CSC-associated targets is particularly a tough task, since CSC appear to be “moving targets” that switch between different cell states during cancer progression. Hence, oncoantigens that are overexpressed in CSC and also present in more differentiated cancer cells would appear to be outstanding candidates. Vaccines targeting these oncoantigens may thus represent ideal tools in the adjuvant setting.

The chondroitin sulfate proteoglycan (CSPG)4 is an attractive oncoantigen [15]. It is an early melanoma cell surface progression marker involved in tumor cell proliferation, migration and invasion. It has a restricted distribution in normal tissues, but it is overexpressed by many tumor histotypes, including about 85 and 57% of human [16] and canine [17] malignant melanomas, respectively, where it is expressed by differentiated cells as well as by CSC [18, 19]. The development of an effective anti-CSPG4 therapeutic strategy could therefore be of tremendous significance to improve clinical

management of melanoma patients, potentially extendable to the treatment of the wide population of patients with other CSPG4+ tumors.

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Comparative Oncology and Clinical Trials

Clinical Trials in veterinary medicine for the benefit of both companion animals and their people

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Companion animals share many aspects of life with their human caretakers. In addition to a shared environment, they can develop diseases including cancer that are similar to the same diagnosis in people. These cancers sometimes have the same or similar genetic abnormalities, clinical presentation, challenges to treatment, response to treatment, and prognosis. While genetically engineering rodents or cell culture experiments can allow an unfettered manipulation of a cellular or tissue process, the artificial circumstances present challenges in translating findings to people and many drugs that are successful in a laboratory are not successful in human subjects. Because of the accelerated aging and shortened life span of dogs and cats relative to people, the results of intervention can be measured in a much shorter period of time compare to trials in people, and this can accelerate the development of novel anticancer diagnostic technology and treatments. Companion animals are genetically diverse and have intact immune systems, which are both important for the accurate evaluation of cancer progression in an animal with spontaneous neoplasia. This allows correlations with human health to better predict the potential challenges to successful drug development. The One Health movement embraces the genetic, environmental, and practical similarities among species that share this earth and the bidirectional translation of findings across species will provide the most efficient path to managing cancer in all species.

Oral Presentations

European Society of
Veterinary Oncology

Feline large granular lymphocyte lymphoma: an Italian Society of Veterinary Oncology (SIONCOV) retrospective study

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Introduction

Feline large granular lymphocyte (LGL) lymphoma is characterized by grave prognosis and poor response to chemotherapy. Aim of this study was to gather broader clinico-pathological information on LGL lymphoma, to better define prognosis and improve the treatment decision process.

Results

109 cats met the inclusion criteria. LGL lymphoma was localized within the gastrointestinal tract with or without extra-intestinal involvement in 91.7% of cases, and at extra-gastrointestinal sites in 8.3%. Symptoms were frequent. Anemia (31.2%) and neutrophilia (26.6%) were commonly observed, and 14 (12.8%) cats had neoplastic circulating cells. Elevated ALT (39.4%) and hypoalbuminemia (28.4%) were common. Twenty (54.1%) of 37 cats had elevated serum LDH. Treatment included surgery (11%), chemotherapy (23%), corticosteroids (38.5%), and no treatment (27.5%). Median time to progression was 5 days, and median survival time (MST) 21 days. MST was significantly shorter in the case of substage b, circulating neoplastic cells, lack of chemotherapy administration, and lack of treatment response. A small subset of cats (7.3%) survived >6 months, suggesting that a more favorable clinical course can be found among LGL lymphoma patients.

Materials and methods

Cats with newly-diagnosed LGL lymphoma that underwent initial staging (hematology, serum biochemistry, thoracic radiographs and abdominal ultrasound), and followed-up were retrospectively evaluated.

Conclusions

The high mortality was significantly associated with the aggressive disease nature and to the poor cats' condition due to the presence of severe symptoms. Further negative prognostic factors included circulating neoplastic cells, high LDH levels, lack of chemotherapy administration, and lack of treatment response. Chemotherapy may improve disease control and survival.

Keywords: *Lymphoma, Feline, large granular lymphocyte lymphoma, LGL*

Determination of urokinase plasminogen activator serum levels among healthy cats and oncologic feline patients

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Introduction

The urokinase plasminogen activator system (uPA) has been proven to be a relevant system in human oncology, its expression being related to the prognosis of several cancers. However, the serum values of uPA in feline oncology have not been determined.

Results

The average serum concentrations of uPA in cancer patients (0,54 ng/mL) and healthy cats (1,10 ng/mL) were not significantly different and not influenced by breed, gender or reproductive status. Animals with cutaneous neoplasms demonstrated a small increase of uPA when tumour-induced inflammation was identified. Animals with mammary carcinomas revealed a 43% higher mean serum concentration than those with fibrosarcomas and other adenocarcinomas. The presence of metastases did not influence uPA concentrations.

Materials and methods

In order to study the potential value of circulating uPA as a tumour marker, we collected serum samples from 19 healthy and 18 cats with spontaneous malignant neoplasms. uPA concentrations were measured using a ELISA kit specific for feline species and its relationships with intrinsic factors such as age, breed, gender and reproductive state, and clinico-pathological parameters such as tumour type, size, grade, the presence of inflammation and metastasis were analysed.

Conclusions

Our preliminary results do not support serum uPA as valuable to identify cats with cancer, but the study of larger groups are warranted to understand the role of this enzyme in feline tumours of specific types.

Keywords: *Feline, urokinase plasminogen activator, malignant neoplasms, tumor marker*

Macroscopic Feline Mammary Tumours Treated With Docetaxel A Prospective Study

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Introduction

Feline mammary tumors are highly malignant neoplasms. The standard of care is aggressive surgical resection. The role of chemotherapy in the macroscopic setting has been described while further studies need to validate its benefit in the adjuvant setting. Docetaxel, commonly used in human breast cancer has also been safely administered in tumour bearing cats. This prospective study evaluates the efficacy and safety of docetaxel in cats with measurable mammary tumors.

Results

Fifteen cats were included. Diagnosis was histopathological in eleven cats and cytological in four that represented local recurrence of previously excised adenocarcinomas. Six cats had adverse reactions to docetaxel. Two cats developed grade 1 allergic reaction, another a grade 3 allergic reaction, two grade 2 neutropaenia and one grade 3 neutropaenia. There was one CR, three PR, and five SD. Total response rate was 26.6%, clinical benefit 59.9% and median PFS 84 days.

Materials and methods

Inclusion criteria included a cytological/histopathological diagnosis of a measurable mammary adenocarcinoma. Initial assessment included physical exam, tumor measurements, CBC, biochemistry and UA, thoracic radiographs and abdominal ultrasound. Docetaxel (2.25mg/kg/1hCRI) with dexamethasone and diphenhydramine premedication was administered every 3 weeks A CBC and physical exam were performed at day 7 and before each infusion, and response evaluation, following RECIST criteria, 42 days after initiating the protocol and monthly thereafter. Adverse events were recorded and evaluated following VCOG-CTCAE.

Conclusions

This study supports that docetaxel exhibits biological efficacy in feline macroscopic mammary carcinomas with low incidence of adverse events. Further studies assessing its role in the adjuvant setting should be conducted.

Keywords: *Feline, mammary tumour, docetaxel, chemotherapy,*

Survival analysis of dogs with advanced primary pulmonary carcinoma treated by metronomic chemotherapy – a multicenter retrospective case-control study

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Introduction

Advanced (unresectable or metastatic) primary pulmonary carcinoma (PPC) represents a therapeutic challenge. Surgery may be contraindicated and the therapeutic role of maximum-tolerated dose (MTD) chemotherapy remains uncertain. This study was undertaken to explore the impact of metronomic chemotherapy (MC) in dogs with advanced PPC.

Results

107 dogs were included: 24 received MC, 35 were treated with surgery, 11 with MTD chemotherapy, and 37 received no treatment. QoL was improved in dogs receiving MC. TTP was significantly longer in patients receiving MC (105 days) than patients undergoing surgery (87 days), receiving MTD chemotherapy (22 days), or no oncologic treatment (13 days). Overall ST was similarly longer in patients receiving MC (139 days) than those undergoing surgery (92 days), MTD chemotherapy (61 days) and no oncologic treatment (35 days).

Materials and methods

Medical records were retrospectively reviewed. Previously-untreated dogs with advanced (T3 or N1 or M1) PPC, with complete staging work-up and follow-up data, receiving MC (comprising low-dose cyclophosphamide, piroxicam and thalidomide), surgery, MTD chemotherapy or no oncologic treatment were eligible for enrolment. Quality-of-life (QoL), time to progression (TTP) and survival time (ST) were evaluated. To assess QoL, owners of dogs receiving MC were asked to complete a questionnaire before and during treatment.

Conclusions

In dogs with advanced PPC, MC achieved a measurable clinical benefit without significant risk or toxicity. The lower risk, the ease of oral administration, QoL benefits and the statistically significantly prolonged TTP and ST may render MC an attractive treatment option for dogs with advanced PPC.

Keywords: *Canine, Lung Cancer, Metronomic Chemotherapy*

Mechanism of action of an autovaccine against cancer cells associating autologous tumor proteins to hydroxylapatite micro/nanoparticles

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Introduction

An autovaccine composed of specific proteins of tumor cells and hydroxylapatite (HA) microparticles has proven to improve overall survival and time to progression of dogs with aggressive and indolent lymphoma when used in combination with chemotherapy. The vaccine cross-primed the CD8-cells, and the vaccine antigens electrophoresis revealed heat shock proteins (HSP) bands, whereas dot blot identified gp96 and HSP70. Under stress condition, the chaperone proteins gp96 and HSP70 stabilize the structure of their associated proteins. They are also responsible for their associated peptide presentation through fixation on the CD91 receptors of the antigen presenting cells (APCs). The aim of this study was to document whether the vaccine antigens are ligands of CD91, thereby cross-priming the CD8-cells through HSP-dependant presentation.

Results

The cells were labelled by peroxidase and labelling was inhibited by the presence of CD91 antibodies, thereby indicating that the tumor HSPs interacted with the APCs membrane through CD91.

Materials and methods

The intracytoplasmic proteins were extracted from tumor biopsies after tissue grinding, then precipitated and passed through a column of HA particles. The particles loaded with proteins constituted the vaccine, whereas HA served as adjuvant. The desorbed proteins from the particles were labelled by a peroxidase. A CD91-positive cell line was grown with the labelled proteins with or without the presence of anti-CD91. The immobilization of the proteins on the cell membrane was then evidenced by immunocytochemistry.

Conclusions

CD8 cross-priming is due to the presentation of the chaperoned tumoral peptides by the HSPs vaccine.

Keywords: *CD91, antigen presentation, cancer vaccine*

Rapid Evaporative Ionization Mass Spectrometry (REIMS) - Identification of Canine and Feline Primary Tumors and Metastases

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Introduction

The success of tumor treatments depend on complete excision, however it runs into difficulties due to non-identifiable margins of tumors. The aim of our study was to identify the molecular fingerprint of different canine and feline tumors with REIMS.

Results

A total of 492 database entries were created from 12 different malignant, 5 benign tumors and 5 metastases. The cross-validation resulted in 98.88% correct classification, 99.2% specificity and 99.4% sensitivity. The MSMS identification of different peaks showed the presence of mainly phospholipids, plasmalogens, sphingolipids, ceramides, triglycerides and fatty acids. The ratio of these molecules was different in different samples but the lipid fingerprint of the metastatic tissues was identical with primary tumors.

Materials and methods

55dogs and cats suffering from spontaneous tumors were recruited to this study. Primary and metastatic cancer tissue from every affected organ was collected. The aerosol generated by the thermal ablation of the native samples was introduced into a Xevo G2-XS mass spectrometer and acquisition was performed in the 50-1200 mass/charge range. MSMS was carried out on different peaks of different samples for species identification. A multivariate statistical algorithm was used for the calculation of cancer-specific classification models, and tested on a separate validation set. Using the full metabolic profile and the specific peak data, we identified the tissue type or the primary cancer in each sample.

Conclusions

The results demonstrate that the metabolic profiles can be recorded in a few seconds and the profiles show tumor specificity and the tumor metastasis is also comparable to the primary cancer.

Keywords: *Canine, Feline, free margin, detection, differentiation*

Detection and prognostic relevance of circulating and disseminated tumor cells in dogs with metastatic mammary carcinoma: a pilot study

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Introduction

In human breast cancer, circulating tumor cells (CTCs) in peripheral blood (PB) are an independent prognosticator for progression-free interval (PFI) and survival time (ST). Aim of this prospective single-center study was to enumerate PB CTCs and bone marrow (BM) disseminated tumor cells (DTCs) in dogs with metastatic mammary carcinoma (MMC) before and after chemotherapy, and to determine their prognostic significance.

Results

Eighteen dogs were enrolled: 1 had stage III disease (with histologically-confirmed neoplastic emboli), 5 had stage IV, 12 had stage V. At baseline, PB and BM were collected in 18 and 7 dogs, respectively. 17/18 PB and 6/7 BM gave informative results, respectively. We found 7/17 (41.2%) CTC-positive and 4/6 (66.7%) DTC-positive dogs. The median value of CTCs and DTCs was 2 cells (range, 1-5), and 17 cells (range, 2-24), respectively. After treatment, PB was obtained from 8 dogs, while the remaining 10 dogs died before the first follow-up visit. The CTC levels negativized in 3/3 (100%) CTC-positive dogs. Median PFI and ST were 89 and 104 days, respectively. Median PFI was significantly longer in CTC/DTC-negative dogs (325 vs 77 days; p=0.01). Similarly, median ST was significantly longer in CTC/DTC-negative dogs (325 vs 91 days; p=0.01).

Materials and methods

Dogs with completely staged, histologically-confirmed, measurable MMC were tested for CTCs/DTCs both at diagnosis and at the first follow-up visit. The CellSearch system was used to enumerate CTCs/DTCs as EpCAM+, CK8/18/19+, DAPI+, CD45- cells.

Conclusions

CellSearch can detect CTCs/DTCs in canine PB and BM. CTC/DTC-negative dogs may have a better outcome compared to CTC/DTC-positive dogs.

Keywords: *Canine, mammary cancer, circulating tumor cells, disseminated tumor cells, bone marrow, CellSearch*

EGFR (Epidermal Growth Factor Receptor) Expression In Feline Invasive Triple-Negative Mammary Carcinomas

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Introduction

In human breast cancer, Epidermal Growth Factor Receptor (EGFR) is an unfavorable prognostic factor; both HER1 (the EGFR gene) amplifications and deletions have been described. Feline invasive mammary carcinomas (FMCs) are spontaneous animal models of triple-negative breast cancer (TNBC), characterized by negativity to Estrogen Receptor alpha (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor type 2 (HER2). Here we investigated the prognostic significance of EGFR expression in triple-negative FMCs, using two monoclonal antibodies, which recognize the extracellular (clone 111.6) and the intracellular (clone 5B7) domains of EGFR.

Results

In triple-negative FMCs, positivity to the EGFR extracellular domain (111.6+) was observed in 89/187 cases (48%), and positivity to the EGFR intracellular domain (5B7+) in 87/187 cases (47%); both correlated positively with CK5/6 expression. The 111.6-5B7+ phenotype, suggestive of EGFR truncation, observed in 27/187 (14%) triple-negative FMCs, was associated with shorter overall survival (Hazard Ratio HR=1.55) by multivariate analysis, with the pathologic tumor size (T

Materials and methods

279 FMCs from female cats treated with surgery alone were subjected to automated immunohistochemistry with the following antibodies (clones): ER (C311), PR (10A9), Ki-67 (MIB1), HER2 (4B5), basal cytokeratins CK5/6 (D5/16B4), and EGFR (111.6 and 5B7). Of the 279 FMCs, 187 (67%) were triple-negative.

Conclusions

A subset of aggressive triple-negative FMCs expresses the intracellular (kinase) domain of EGFR but not the extracellular (ligand-binding) domain, which suggests EGFR truncation, and possibly HER1 deletion, in feline invasive mammary carcinomas.

Keywords: *Feline, mammary carcinoma, immunohistochemistry, EGFR*

Pre-clinical surgery trial data on isotopic imaging-guided surgery based on 18F-fluorodeoxyglucose in spontaneous canine and feline oncological patients

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Introduction

As complete removal of cancer still remains a challenge for surgeons worldwide, the CLIO consortium goaled to develop a reliable intraoperative method to facilitate oncological operations. The method consists of imaging direct positrons from the tumour uptake of 18F-FDG using 2 equipments (Betascope, and ex vivo Specimen Analyser).

Results

No animal patient showed local recurrance or regional metastases at RECIST evaluations. Intraoperative, ex vivo images matched with all pathological margin-opinions. Surgeons received the highest doses within the staff but even for them the limiting finger doses allow 600 operations per year.

Materials and methods

Twenty one oncological patients were included into study between 2014-2016. One hour before surgical procedure dogs and cats received 5 MBq 18F-FDG/body weight kg intravenously, then surgical wound planning, checking the removed tissues and excision margins was assisted by the use of the intraoperative detector imager (Betascope) and all the removed tissues were imaged ex vivo using the ex vivo Specimen Analyser as well before pathological investigations. Internal dosimetry data (finger dose, eye dose, whole body dose) of the whole surgery staff were recorded by Nanodot TM dosimeters. Three months after surgery complete RECIST examinations were performed for each patient.

Conclusions

Both the intraoperative imaging device (Betascope) and the ex vivo Specimen Analyser was found to be useful and accurate in identifying the ”free margins”. Based on these promising preclinical veterinary data human clinical trials have been started, too. The work leading to these results has received funding from the European Comission under contract number EU-FP7 No:606614 CLI (www.clioproject.eu).

Keywords: *Canine, Feline, intraoperative, free margin, radioactive detection*

Impact Of Surgical Treatment On Survival In Miniature Dachshunds With Prostate Adenocarcinoma: 13 Cases (2011-2016).

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Introduction

Surgery is generally considered to be palliative for canine prostate adenocarcinomas (PAC). However, we have experienced good prognosis after TP or total prostatocystectomy (TPC) in some patients, especially small-breeds. Miniature Dachshunds are predisposed to PAC in Japan. The purpose of this retrospective study was to compare the outcome among Miniature Dachshunds with PAC undergoing medical and surgical treatment including TP and TPC. We hypothesized that surgical treatment would have more superior clinical benefits compared with medical management.

Results

The median survival time from the initial evaluation in the surgical group (709 days [96 – 950+ days]) was significantly longer than that in the non-surgical group (134 days [2 – 850 days]). Regarding the postoperative complications, the TP group showed mild urinary incontinence, whereas the TPC group showed severe.

Materials and methods

The medical records of 13 Miniature Dachshunds diagnosed with PAC were reviewed. The patients were divided into 2 groups: the non-surgical group (n=7) and the surgical group (n=6), which was subdivided into the TP group (n=4) and the TPC group (n=2). In all dogs, the diagnosis was made based on the histopathology of the prostate samples aspirated from the urinary catheter and/or the ultrasound-guided FNA cytology.

Conclusions

The surgical treatment of canine PACs is suggested to have the survival benefit compared with the medical management including NSAIDs. Especially, TP might be recommended for the improvement of survival time and quality of life in canine PACs prior to the tumor infiltration into the surrounding tissues including bladder.

Keywords: *Canine, Prostate adenocarcinoma*

Possibilities To Inhibit The Development Of Canine Chemotherapy Resistance

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Introduction

Although, recent advances in tumor therapies have revealed significant impact on patient survival, treatment of malignancies is still a major challenge in human and canine cancer. The efficacy of drugs is often hampered by the emergence of multidrug resistance (MDR). One of the main contributors of MDR is the overexpression P-glycoprotein (Pgp). Recently, an alternative mechanism has been proposed with an emphasis on epigenetic regulation.

Results

A rapid increase of P-gp expression was observed in vivo and in vitro as the initial MAF value has been changed from 0,04 to 0,6 after 4 rounds of doxorubicin treatment. Contrarily, using the combined therapy for 9 rounds of treatment the MAF was 0,16 with the combination of doxorubicin+Temozolomide and doxorubicin+Celecoxib, with the doxorubicin+SAHA combination we measured 0,2 MAF value after 102 days. The MAF has been changed from 0 to 0,14 with doxorubicin+Trichostatin-A combination in 70 days.

Materials and methods

To study mechanisms underlying the rapid emergence of drug resistance among canine lymphoma patients, we performed in vitro experiments using the P388 cell line as a model system. We applied flow cytometry assay to measure Pgp-activity and calculated Multidrug Resistance Activity Factor (MAF) to determine the resistance value. In particular, we examined the effect of epigenetic inhibitors (Temozolomide, Trichostatin-A, SAHA) and COX2 inhibition (Celecoxib) on Pgp expression by combining specific inhibitors with long term doxorubicin treatment.

Conclusions

Our data suggest that combination therapy using doxorubicin and epigenetic inhibitors or COX2 inhibitor may be an effective means to prevent the emergence of MDR.

Keywords: *Lymphoma, Canine*

Expression signature of grey horse melanoma

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Introduction

Malignant melanoma constitutes a significant health problem in vertebrates. Equine melanoma represents an interesting natural animal model: tumours are highly malignant in non-grey horses yet often quiescent in grey individuals, thus demonstrating the entire spectrum of disease from benign lesions to metastatic cancer in its individual biological complexity.

Results

More than 1000 genes were significantly (P ? 0.04) overexpressed in melanoma compared to normal skin of the same individuals. Functional categorization revealed 37 KEGG pathways to be multiply affected by upregulated gene transcription. Overexpression involved pathways confirmedly associated with tumour growth and metastasis e.g. cell migration (e.g. EGFR, LAMA5, ITGA6, EDNRA high), angiogenesis (e.g. ERBB2, VEGFR, FGFR, CXCR4, IL8 high) or NOTCH signalling (e.g. NOTCH2/3, DLL1/4, JAG2, PSEN2 high). In addition, candidate genes possibly acting as tumour promoters were identified. Overexpression of selected genes was confirmed in equine, and importantly, also canine melanoma cells.

Materials and methods

We established a differential gene expression profile of grey horse melanoma (n=4) versus normal skin (n=4). To this aim, validated mRNA extracted from tumour tissue or normal skin was subjected to RNA-seq analysis. Obtained data was processed using DAVID and KEGG bioinformatics. Selected genes showing pronounced overexpression were subjected to in vitro analyses using primary equine and canine melanoma cell lines.

Conclusions

We determined expression signatures of grey horse melanoma and identified various genes significantly overexpressed in tumour tissue. Transcription of 53 of these genes is also up-regulated in human malignant melanoma. In vitro studies point to selected genes being likewise overexpressed in canine oral melanoma.

Keywords: *Melanoma, gene expression profile, horse, dog*

Stereotactic irradiation of canine ACTH-secreting pituitary macro-tumors: endocrine and MRI time-related patterns of response.

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Introduction

The aim of this study was to evaluate time-related clinical and magnetic resonance imaging (MRI) response in dogs suffering from pituitary hyperadrenocorticism with a measurable mass at MRI treated with stereotactic radiotherapy (RT).

Results

Median pituitary tumor volume was 1.0 cmc (range 0.5 - 8.5 cmc). Mean basal and stimulated cortisol at the diagnosis were 7.8 mcg/dL and 36.1 mcg/dL. Mean initial dose of trilostane was 2.0 mg/kg/24h. Complete regression of the tumor was observed in 10/14 dogs at 6-18 months (median 8 months), partial regression in 4/14 at the end of the study. No progressions were observed. Normalization of the thickness of adrenal glands was observed in all dogs at 8-18 months (median 12 months). Normal serum cortisol level was obtained in all except 1 dogs. Trilostane was progressively tapered and withdrawn at 14-18 months (median 16.5 months). No radio toxicity were encountered.

Materials and methods

Dogs naive of any endocrine treatment suffering from pituitary hyperadrenocorticism diagnosed by standard laboratory and imaging tests were irradiated with 3.8 Gy x 10 fractions delivered by frameless stereotactic Volumetric Modulated Arc Therapy. Concomitant administration of trilostane were started and the dose recorded. Tumor and endocrine response were evaluated by repeated MRI of pituitary and adrenal glands and serial measurement of cortisol. 14 dogs were enrolled.

Conclusions

The RT schedule used in this study allowed optimal tumor and endocrine control. A gradual progression in the response was observed, starting with a volumetric response of the pituitary tumor, than a volumetric response of the adrenal glands delayed of several months after the treatment and ultimately a reduction of the serum cortisol levels was achieved. This gradual pattern of response must be taken in consideration during follow up evaluation to adjust the dose of trilostane and to establish accepted evaluation criteria.

Keywords:

Keeping up with technical advancement – A novel radiation protocol in a pilot cohort of 44 dogs with symptomatic intracranial neoplasia

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Introduction

Technical advances make it possible to deliver radiotherapy to canine intracranial neoplasia in fewer fractions. However, increasing fraction size without compromising treatment safety remains a challenge that can be partially solved with increased treatment accuracy. We compared two pilot cohorts treated with mathematically equally toxic protocols. The objectives were twofold: First, to compare occurrence of radiation toxicity. Second, to quantify outcome.

Results

Forty-four dogs were assigned to protocols of 20x2.5Gy (n=26) or 10x4Gy (n=18). No dog showed acute side effects, 1 in each group could be attributed to early delayed reactions, in 1 dog late radiation toxicity could not be ruled out. Median OS and PFI were 610 (95%CI:504-721) and 600 days (95%CI:487-699) for the 20x2.5Gy group, and were not attained in the 10x4Gy group: mean OS:566 days (95%CI:370-763), mean PFI:756 days (95%CI:625-888). Gross tumor volume as percentage of brain volume was comparable between the groups with 3.4% and 3.3% (p=0.504). Increased planning target volume size was prognostic for shorter survival (HR:13).

Materials and methods

Dogs with an imaging diagnosis of intracranial neoplasia displaying neurologic signs were treated with 3DCRT and either 20x2.5Gy or 10x4Gy. Patients were monitored for acute, early delayed and late radiation toxicity; furthermore, progression-free interval (PFI), overall survival (OS) and possible prognostic factors were evaluated.

Conclusions

As expected, clinically relevant radiation toxicity did not increase with 10x4Gy. Surprisingly, no significant difference in OS and PFI was seen. This protocol seems to provide safe treatment if applied with appropriate technical radiation therapy standard and might allow for dose escalation in the future.

Keywords: *Canine, radiation, intracranial neoplasia, radiation damage*

Tolerability of simultaneously integrated boost technique for canine sinonasal tumors using image-guided intensity-modulated radiation therapy

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Introduction

In order to overcome the common local failure of canine sinonasal tumors, integrated boost techniques were tried in the cobalt/orthovoltage era, but dismissed due to unacceptable acute toxicity. A recent calculation study of a simultaneously integrated boost (SIB) technique for sinonasal irradiation using intensity-modulated radiation therapy (IMRT) promised theoretical feasibility. The goal of this pilot study was to apply a regular protocol of 10x4.2Gy to the planning target volume (PTV) with a 20% SIB dose to the gross tumor volume (GTV). Our hypothesis expected this dose escalation to be clinically tolerable if applied with IMRT.

Results

Seven patients were included. Acute toxicity was evaluated in all at week 1, 2, 3, 8, and 12 after RT. Three patients (43%) had intermediate acute toxicity (grade III mucositis), which were easily managed medically. No patient developed ulcerations/necrosis of skin/mucosa. Only very mild ophthalmologic complications (grade I) were found.

Materials and methods

Dogs diagnosed with sinonasal tumors and without local/distant metastases were included. For treatment planning, organs at risk were contoured according to strict anatomical guidelines. Planning volume extensions (GTV/CTV/PTV) adhered to strict rules to minimize interplanner variability. Treatments were applied with rigid patient positioning and verified daily with image guidance (kiloVolt (kV)-kV-imaging or kV-cone-beam computed tomography). After radiation therapy (RT), focus was set on acute ophthalmologic/neurologic complications as well as skin/mucosal toxicity.

Conclusions

The SIB protocol applied with image-guided IMRT to treat canine sinonasal tumors led to clinically acceptable side effects. The used dose escalation of 20% can be expected to yield an increased tumor control probability.

Keywords: *Canine, Radiation therapy, nasal tumors, boost technique*

Radiation versus combined radiation and chemotherapy in the treatment of canine histiocytic sarcoma in the gross disease setting

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Introduction

Appendicular histiocytic sarcomas are often painful and aggressive tumours and associated with short survival times meaning some clients are reluctant to consider amputation. The aim of this study was to compare the progression free interval and survival time of dogs with appendicular histiocytic sarcoma (HS), treated with radiotherapy vs dogs treated with radiotherapy and chemotherapy (combination group).

Results

34 patients were treated: 26 flatcoated retrievers and 8 other breeds. Overall clinical benefit was seen in all cases (100%) that received combined treatment (n = 16) vs 87.5% receiving radiotherapy only (n = 16). Median time to disease progression was 130.5 days in the combination group vs 87 days for radiotherapy only. Median survival time was 173.5 days (range = 51-578 days) in combination group vs 101 days (range 23-1388 days) for radiotherapy only.

Materials and methods

Clinical records of dogs presented with appendicular histiocytic sarcoma to a single referral institution from 2003-2016 were reviewed. Dogs with gross disease treated with radiotherapy +/- chemotherapy which survived >21 days were included. Chemotherapeutic agents included lomustine, chlorambucil and toceranib phosphate. We assessed clinical benefit (stable disease, partial or complete response or an improvement in lameness), time to disease progression and survival time. Analgesic medication was provided for all patients.

Conclusions

This study demonstrates that radiotherapy offers effective palliation for appendicular HS and suggests that outcomes improve when RT and chemotherapy are combined. This therapeutic combination offers improved survival times over single agent chemotherapy alone and is comparable with those reported for multi-drug chemotherapy protocols.

Keywords: *Canine, Histiocytic sarcoma, Radiotherapy*

Outcome and prognostic factors in 67 medically treated canine prostatic carcinomas: a multi-institutional study

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Introduction

Literature describing medical treatment of canine prostatic carcinomas is sparse. The aims of this study were to assess outcomes of dogs with prostatic carcinoma treated with non-steroidal anti-inflammatory drugs (NSAIDs) and/or chemotherapy, and to identify prognostic factors.

Results

Sixty-seven dogs were included. Median weight was 23.2 kg and median age 9.5 years. Presenting signs were urinary (40), gastrointestinal (31) and systemic (10); 9/27 dogs had positive urine cultures. Metastases were identified in 26 dogs to nodes (20), lungs (10), bone (2) and liver (1). Treatment included NSAIDs and chemotherapy (32), NSAIDs alone (31) and chemotherapy alone (4). NSAIDs prescribed were meloxicam (46), firocoxib (13), piroxicam (6), carprofen (2). Chemotherapy included carboplatin (13), mitoxantrone (10), cyclophosphamide (10), toceranib (5), chlorambucil (4), epirubicin (1) and vinblastine (1). MST was 82 days (range 9-752) and median TTP 63 days (range 9-752). Dogs receiving NSAIDs combined with chemotherapy had a significantly longer ST (106 vs 51 days; p=0.035) and TTP (89 vs 51 days; p=0.043) compared to dogs receiving NSAIDs only. Metastatic disease was associated with significantly shorter MST (49 vs. 109 days, p=0.037) and intact dogs had a significantly shorter MST (31 vs. 90 days, p=0.018) and TTP (25 vs. 64 days, p=0.0005).

Materials and methods

Medical records from 8 institutions were searched for dogs with cytologically/histologically confirmed prostatic carcinomas without bladder involvement. Time to progression (TTP) and median survival time (MST) were assessed.

Conclusions

Dogs receiving NSAIDs/chemotherapy in combination had improved outcome, although MST and TTP were short. Metastatic disease and intact status negatively influenced the prognosis.

Keywords: *Canine, Prostatic tumours, carcinoma, chemotherapy, NSAIDs, prognostic factors*

A Flow cytometric approach to Mast Cell Tumors in dogs

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Introduction

Cytology is useful to diagnose and stage canine mast cell tumors (cMCTs). Aims of this prospective study were to 1.) Evaluate the ability of flow cytometry (FC) in identifying mast cells (MCs) and characterizing cMCT immunophenotype, 2.) Detect MCs in the draining lymph nodes (LNs).

Results

32 primary cMCTs and 10 draining LNs were evaluated. 94% of cMCTs samples had adequate cellularity for FC. Distinct populations attributable to MCs and eosinophils were recognized based on scatters and CD117/IgE positivity (confirmed by sorting and cytospin evaluation). MCs were IgE+hi, CD117+, CD11b+dim/neg, while eosinophils were IgE+dim/neg, CD117neg, CD11b+hi. Both cell types expressed CD18, CD45, CD44, but with different intensity. Four cases expressed CD34 and/or CD25. Based on the results, a single tube (IgE/CD117/CD11b/CD21) was designed to identify and quantify MCs in LNs, showing a high correlation between cytology and FC (r=0.94).

Materials and methods

Primary cMCTs and their draining LNs were sampled through fine-needle aspiration and suspended in RPMI for FC analysis. The diagnosis was confirmed by cytology and/or histology. Flow-cytometric expression of surface CD117, IgE, CD11b, CD18, CD44, CD34, CD25 and CD45 was evaluated. The LN percentage of MCs obtained by cytology and FC was correlated by Spearman correlation coefficient.

Conclusions

FC allows MC identification both in primary cMCTs and in the draining LNs.
Further studies are needed to evaluate the prognostic significance of specific MCs immunophenotypes and MCs percentages in tissues, thereby providing a non-invasive technique to diagnose metastatic disease.

Keywords: *MCT, Canine*

The Occurrence of proteinuria in dogs during the first twelve weeks of toceranib treatment for malignant neoplasms

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Introduction

Evaluate the frequency, timing of occurrence, and severity of proteinuria in toceranib-treated dogs.

Results

Seventy eight cases were identified, 31 without pre-treatment UP:C or follow-up results and 15 with pre-existing proteinuria were excluded.
Twenty one of the remaining 32 dogs had UP:C performed 1-2 weeks post-treatment. Two had developed proteinuria (UP:C 0.52 and 0.64). After 3-4 weeks, 28 previously non-proteinuric dogs remained on treatment and 2/18 that had UP:C checked were proteinuric (UP:C 0.91 and 0.53). After 5-8 weeks, 22 previously non-proteinuric dogs remained on treatment and 2/14 that had UP:C checked were proteinuric (UP:C 1.41, 3.57). After 9-12 weeks, 15 previously non-proteinuric dogs remained on treatment and 1/11 that had UP:C performed was proteinuric (UP:C 2.92).
Overall, 7/32 (22%) cases developed proteinuria within 3 months of starting toceranib. Four dogs remained on the same dose of toceranib following the development of proteinuria (UP:C 0.53, 0.91, 2.92, 3.57). The UP:C had increased by 9, 55, 25 and 34% after a further 15, 24, 26 and 5 days, respectively. In the least severe case, the UP:C subsequently normalised despite continued treatment. In the most severe case, the UP:C improved but proteinuria remained when toceranib was stopped for 14 days.

Materials and methods

Clinical records of dogs treated with toceranib from 2010-2016 were reviewed. The incidence of proteinuria (urine protein:creatinine ratio (UP:C) >0.5) was recorded before and for 3 months following treatment commencement.

Conclusions

One in five dogs developed proteinuria on toceranib treatment. This developed after varying lengths of treatment. The magnitude was mild in most cases.

Keywords: *Canine, toceranib, proteinuria*

Canine primary melanoma cells overexpress MMP2 and exhibit phagocytic activity

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Introduction

Malignant melanoma is the most common tumour type of the canine oral cavity. Gene expression profiling of grey horse melanoma revealed intralesional overexpression of matrix metalloproteinases 2 and 9 (MMP2, MMP9), macrophage marker CD68, and melanoma cell adhesion molecule CD146 (MCAM/MelCAM/Muc18). We hypothesized that MMP2, MMP9, CD68 and CD146 may be likewise overexpressed in canine oral melanoma and have a pathogenic role in disease.

Results

All tumour sections revealed MMP2 and MMP9 expression. All melanoma and metastasis cell lines digested gelatine via MMP2, especially upon activation with PMA. Surprisingly, 73 to 82% of canine melanoma cells per cell line ingested GFP-labelled latex beads, whilst only 7 and 16% of cells per metastasis cell line had this ability. CD146 expression by cell lines ranged between 16 and 46%. Preliminary data point to CD146 expression promoting metastatic events as assessed for CD146-depleted versus enriched tumour cells via cell migration and invasion assay.

Materials and methods

MMP2 and MMP9 expression was assessed by IHC of melanoma sections (n=3) and zymography of primary canine oral melanoma and metastasis cell lines (n=7). CD68 expression was assessed by IHC of tumour sections. Tumour cell lines were used to address a possible association of CD68 with phagocytic activity by phagocytosis assay, and CD146 expression by FACS.

Conclusions

Herein presented findings point to MMP2 and CD146 having a role in canine melanoma dissemination. Furthermore, canine melanoma cells showed a high degree of phagocytic activity in contrast to metastases, and irrespective of CD68 expression. The significance of this interesting finding is currently investigated.

Keywords: *Canine, oral melanoma; MMP; CD68; phagocytosis; CD146*

Inhibition of the Notch pathway targets Cancer Stem Cells in canine and human insulinoma

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Introduction

Insulinoma (INS) is the most commonly diagnosed pancreatic neuroendocrine tumour in dogs and humans. Despite current treatment modalities, malignant canine and human INS have a grave prognosis. We hyphothesise that the aggressive behaviour of malignant INS is driven by cancer stem cells (CSCs). CSCs represent a subpopulation of cancer cells characterised by inherent drug resistance and increased tumourigenicity. Our aim was to isolate INS CSCs and to identify targets for novel therapies.

Results

Putative canine and human INS CSCs expressed stem cell markers including OCT4, SOX9, CD133 and CD34. INS CSC-like cells exhibited greater resistance to chemotherapeutics and formed more substantial and disseminated tumours in the in vivo CAM model compared to non-CSCs. Notch pathway components were found to be upregulated in INS CSCs. Inhibition of the Notch pathway significantly decreased the viability of the INS CSC population in vitro and reduced INS CSC clonogenicity when used in combination with chemotherapeutics.

Materials and methods

Putative CSCs within human (CM) and canine (Nielson) INS cell lines were isolated using spheroid culture. CSCs and non-CSCs were characterised by qPCR, western blotting, chemosensitivity, colony formation, and chick embryo chorioallantoic membrane (CAM) assays. Additionally, the Notch pathway in INS CSCs was inhibited by ?-secretase inhibitors.

Conclusions

We have successfully isolated and characterised novel INS CSC populations. The Notch pathway has been identified as a key regulator of INS CSC survival and of their resistance to chemotherapeutics. We conclude that this pathway has high potential for the development of targeted INS therapies, which may improve the prognosis of INS patients.

Keywords: *Canine, human; insulinoma; CSCs; pancreatic cancer; comparative oncology; notch; targeted therapy*

Evaluation of a multi-agent chemotherapy protocol combining lomustine, procarbazine and prednisolone (LPP) for the treatment of relapsed canine lymphoma.

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Introduction

Canine lymphoma is mainly treated with a first-line chemotherapy protocol containing prednisone, cyclophosphamide, vincristine and doxorubicin (CHOP/CEOP). Combined lomustine, vincristine, procarbazine, and prednisone (LOPP) has been evaluated as a rescue, with encouraging results; however, resistance to vincristine is likely in patients relapsing on CHOP, and this agent may enhance LOPP toxicity without improving efficacy. The aim of this study was to evaluate a modified LOPP protocol that does not include vincristine (LPP) and is administered on a 21-day cycle.

Results

Thirty-seven dogs were included. Twenty-two dogs (59%) responded to LPP: 10 complete responses (CR) and 12 partial responses (PR). Responders had a significantly longer TTD .

Materials and methods

Medical records from 2012 to 2017 were reviewed. Dogs with relapsed lymphoma that received LPP as a rescue protocol were enrolled. Response, time from initiation to discontinuation (TTD) and toxicity (VCOG criteria) of LPP were assessed.

Conclusions

The LPP protocol is safe, shows similar efficacy and toxicity-profile to other rescue protocols but minimises in-hospital procedures. In a palliative setting, the use of oral chemotherapeutics likely increases owners' compliance.

Keywords: *MCT*

Targeting cCD22 in spontaneous diffuse large B-cell lymphoma (DLBCL)-bearing dogs: quantitative imaging (immuno-SPECT) and dosimetric approach in preparation of radioimmunotherapy (RIT)

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Introduction

DLBCL is a lymphoma subtype with a poor prognosis in human. Although clinical trials of radioimmunotherapy targeting CD22 (epratuzumab, Immunomedics) showed promising results no trial has explored the efficacy of such treatment as first-line therapy. As canine DLBCL is a spontaneous model for human DLBCL, we designed a preclinical trial of first-line RIT on DLBCL-bearing dogs preceded by a dosimetric study with serial SPECT-CT images.

Results

The pharmacokinetic study showed a significant accumulation of activity in the liver and kidneys and slight in the lymphoid organs. Injection of 185 MBq/m2 was correlated with mean absorbed doses of 2.3±0.1 Gy to the liver, 0.3±0.03 Gy to kidneys, and 0.1±0.02 Gy to the spleen. In the DLBCL-bearing dog, MAb infusion was well tolerated and tumor targeting was validated.

Materials and methods

A murine monoclonal antibody (MAb, clone 10C6) targeting cCD22 has been produced with good affinity and ability to stain cCD22 in canine normal tissues and DLBCLs, by immunohistochemistry and flow cytometry has been evaluated. Three healthy dogs were injected with 10C6 coupled to indium-111 and SPECT-CT acquisitions were performed 1, 24, 48, 72 and 148 hours post-infusion for pharmacokinetics evaluation. Images were quantified to determine projected absorbed dose to the normal tissues in the event of a RIT with the same MAb coupled with yttrium-90. Phenotypic images were also performed on a DLBCL-bearing dog.

Conclusions

This preliminary study allows us to consider the initiation of a safe preclinical trial of quantitative imaging combined with RIT in first line in spontaneously DLBCL-bearing dogs.

Keywords: *Lymphoma, Canine, Radioimmunotherapy, phenotypic imaging, dosimetry, canine CD22*

Oncolytic virotherapy of canine cell lines and canine mammary tumor explants using a replication-selective oncolytic vaccinia virus (TG6002)

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Introduction

In human medicine, oncolytic vaccinia viruses (VV) have shown promising results on tumor explants. This biotechnology is underused in veterinary oncology. First objective was to investigate the capacity of canine cell lines to support VV infection and replication. Second objective was to assess susceptibility and oncolytic potency of TG6002, an armed oncolytic VV, on canine mammary tumor explants.

Results

Canine cell lines were susceptible to infection with VV. A replication factor of 10^6 to 10^7 was determined 4 days post infection.
Evaluation of expression of green fluorescent protein confirmed the susceptibility of three canine mammary adenocarcinomas to VV infection. Histological analyses assessed a lysis of 90% of tubular cells 6 days post infection. Conversion of more than 50% of 5-FC to 5-FU was observed in the culture medium of infected explants 6 days post infection.

Materials and methods

Oncolytic VV expressing green fluorescent protein (VV-GFP) or FCU1 protein (TG6002), which converts 5-?uorocytosine (5-FC) to 5-?uorouracil (5-FU), were designed.
Tumoral (A72) and non tumoral (DKE1) canine cell lines were used. Three canine mammary adenocarcinomas explants were infected.
Susceptibility and replication were evaluated after infection with VV-GFP at different multiplicities of infection. Oncolytic potency on tumor explants was assessed, after infection with TG6002 in presence of 5-FC, by blinded reading of histological samples. Concentrations of 5-FC and 5-FU were monitored.

Conclusions

This study shows that TG6002 is able to replicate in canine cell lines and exert an ex-vivo oncolytic potency on canine mammary tumors. These promising results need further validation in a larger cohort of canine tumors.

Keywords: *Canine, oncolytic virotherapy, vaccinia virus*

Posters Presentations

European Society of Veterinary Oncology

Pericardial Pacth for unresectable cardiac tumors in three dogs

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Introduction

Cardiac tumors are uncommon in the canine and feline population Common types include haemangiosarcoma aortic body tumors and lymphoma. These neoplasms can cause mild to severe, life threatening clinical signs that are independent of the histological type and may be related to altered cardiovascular function or local haemorrhage/effusion into pericardial space. The aim of this report is to describe the palliative use of pericardial patches for unresectable cardiac tumors in order to prevent pleural effusion.

Results

There were no major intraoperative complications. Surgical times were 40, 50 and 45 minutes respectively. No patient developed pleural effusion in the short and long term follow up .

Materials and methods

Three dogs affected by unresectable cardiac tumors were enrolled in the study. Common clinical signs were exercise intolerance, jugular vein distension, weight loss and muffled heart sounds. Thoracic radiographs showed a cardiac silhouette consistent with a heart tumor or pericardial effusion. No metastatic pattern was seen. Echocardiography identified two dogs with right atrial mass and one with aortic body mass. Complete staging was done. Through a right thoracotomy, subtotal pericardiectomy was performed, pericardial sac was opened. The incision was prolonged cranially and caudally and a pericardial patch was applied on the mass. Incisional biopsies of the masses were performed as well. Doxorubicin-based chemotherapy protocol followed the surgical procedure. The histopathology report was diagnostic for haemangiosarcoma for the first two dogs and chemodectoma for the third dog.

Conclusions

Pericardial patch represents a good choice as a palliative treatment for unresectable cardiac tumors.

Keywords: *Hemangiosarcoma, Pericardial, pleural effusion*

Volumetric Modulated Arc Radiotherapy Of Canine Trigeminal Nerve Tumours

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Introduction

The aim of this study was to evaluate the feasibility and effectiveness of curative high dose hypofractionated (HDH) frameless volumetric modulated arc radiotherapy (VMAT) in trigeminal peripheral nerve sheath tumours (PNST).

Results

MRI follow-up examinations revealed complete response in one dog, partial response in four dogs, and stable disease in two dogs. Median overall survival was 952 days with a 95% confidence interval (CI) of [543÷1361] days.

Materials and methods

A prospective clinical trial was conducted from February 2010 to December 2013 on client-owned dogs with presumptive imaging-based diagnosis of trigeminal PNST. Seven dogs were enrolled and treated with 37 Gy in 5 fx using a 6 MV linear accelerator equipped with an external micro-multileaf beam collimator. The plans were computed using a Monte Carlo algorithm. Overall survival was estimated using a Kaplan-Meier curve analysis.

Conclusions

VMAT appears to be feasible and effective and offers the best median survival time in dogs suffering from trigeminal PNST compared with other radiotherapy treatment modalities, such as linac cone based radiotherapy or surgery.

Keywords: *Canine, Canine, Radiotherapy, Trigeminal Nerve Tumours*

Evaluation of long-term outcome and prognostic factors of feline cutaneous carcinomas treated with photodynamic therapy using liposomal m-THPC

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Introduction

Photodynamic therapy (PDT) has been demonstrated an effective and safe treatment option for UV induced carcinomas in cats. The aim of this retrospective study was to evaluate the efficacy of PDT on treatment naive and recurrent facial carcinomas and to identify potential prognostic factors.

Results

In total, 60 lesions in 39 cats underwent treatment with ?10J/cm (n=24) and 20J/cm2 (n=36). Overall response rate was 87% (CR 67%, PR 20%) with a mean PFI of 32mo (median not reached) and a median OST of 46mo (95%CI:36, 56). In regard to tumour stage, invasiveness yielded a highly significant worse outcome (p

Materials and methods

Histologically verified head & neck skin tumours were treated with PDT after intravenous injection of liposomal m-THPC and 652nm light delivered by a diode laser. One group received ?10J/cm2, the other 20J/cm2. Tumour response and duration were analysed with stage, tumour diameter and treatment intensity as prognostic factors.

Conclusions

PDT using systemic photosensitizer leads to excellent long-term tumour control in the majority of cats. However, invasive and large tumours had a clear inferior outcome, even if treated with the higher dose intensity. This suggests that advanced lesions are not indications for photodynamic therapy.

Keywords: *Feline, PDT, cutaneous carcinomas*

Short-term evaluation of bleomycin-based electrochemotherapy as a single-modality treatment of a cobblestone colorectal carcinoma in a dog

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Introduction

Electrochemotherapy (ECT) is a local treatment for solid tumors that combines systemic or local administration of cytotoxic drugs with the application of electric pulses to the tumors. This technique enhances intracellular drug delivery and ultimately leads to cancer cells death. The aim of this work is to evaluate the efficacy of ECT in the treatment of a canine colorectal carcinoma. Surgical excision is the current treatment of choice for this neoplasia, with a postsurgical mean survival time of 12 months.

Results

Hematochezia immediately subsided after the first ECT treatment. It recurred 15 days later but completely resolved following the second treatment. Transient tenesmus was reported after the second treatment. At 6 months follow-up no recurrence of clinical signs was reported. Visual inspection and a second CT scan failed to detect recurrence; the affected area was not resampled for histopathology.

Materials and methods

A 7-year-old, neutered male dog was referred because of hematochezia of 8 months duration. Colorectal carcinoma was diagnosed by means of histopathologic examination of endoscopic biopsies of a colorectal cobblestone mass. Clinical staging, including abdominal ultrasound, cytology of medial iliac lymph nodes and a total body CT scan, revealed a stage 1 disease (T1N0M0). The dog underwent two ECT treatments under general inhalational anaesthesia 3 weeks apart. After prolapsing the mass through the anus, bleomycin was administered intravenously and electrical pulses were locally applied 8 minutes later.

Conclusions

Electrochemotherapy can be an effective, safe and well tolerated treatment of canine colorectal carcinoma, and could represent an alternative to surgery.

Keywords: *Electrochemotherapy; Bleomycin; Colorectal; Carcinoma; Dog.*

Long term complete remission of a primary frontal sinus squamous cell carcinoma in a dog treated with toceranib, meloxicam and metronomic cyclophosphamide

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Introduction

Primary frontal sinus squamous cell carcinoma (pFS-SCC) is a rare tumour originating from the frontal sinus mucosa. SCCs are locally invasive with low metastastic potential. The recommended treatment for nasal SCC is radiation therapy and surgery. Canine pFS-SCC has only been described once as a unique entity. The objective of this case report is to describe the clinical outcome in a dog with pFS-SCC, receiving only medical therapy.

Results

A significant tumour reduction was observed after two weeks of treatment. Two weeks later the dog was in complete remission (CR). As the same clinical stage persisted, toceranib was discontinued 14 months later. Meloxicam and cyclophosphamide were advised to be continued for the remainder of the dog's life, but 6 months later treatment was discontinued due to financial restrictions. No side effects were observed and the dog is still alive, 27 months after initiation of treatment, and in CR.

Materials and methods

A 7-year-old, male, castrated, crossbreed dog was presented for a progressively growing left facial mass, causing frontal sinus and eye deformity, breathing difficulties and nasal discharge. Radiologically, bone destruction was observed in frontal sinus area. Diagnosis of pFS-SCC was confirmed histologically. Treatment was initiated with toceranib (2,5 mg/kg Mon/Wed/Fri po), meloxicam (0,1 mg/kg sid po) and metronomic cyclophosphamide (20 mg/m2 sid po).

Conclusions

In our patient, canine pFS-SCC was successfully treated with toceranib, meloxicam and metronomic cyclophosphamide with excellent tolerance. However, further studies are needed, since no definitive conclusions can be drawn from one single patient.

Keywords: *Canine, pFS-SCC, Toceranib*

MRI evaluation of the periphery of sarcomas and correlation with histopathology.

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Introduction

Canine soft tissue sarcomas are frequently infiltrative and thus present challenges for the surgeon attempting to achieve cure by surgical resection. In humans, evaluation of the peripheral tumour growth pattern on pre-operative Magnetic Resonance Imaging (MRI) sequences has identified different prognostic patterns. Thus the purpose of this study was to evaluate the ability of MRI to identify tumour extension into peripheral normal tissue and to determine the significance of coexisting peri-tumoural oedema.

Results

Preliminary results from three dogs indicate that MRI evaluation of hyperintensity correlates well with extent of neoplastic infiltration at the proximal extent of the tumour.

Materials and methods

Dogs with limb sarcomas where amputation was deemed the treatment of choice were identified prospectively. In each case, immediately following surgical amputation, the palpable tumour edge was marked with oil capsules. Fat suppression (Short TI Inversion Recovery=STIR) and 3 dimensional sequences (3DHyce) MRI sequences were performed, and hyperintense tissue distant to the main tumour was identified. During routine histopathological processing, sections were prepared from the main mass and at appropriate levels identified by relating the hyperintensities observed on MRI to the position of the markers. In addition to routine histopathological evaluation, sections were examined for presence of neoplastic tissue at the various levels identified on MRI.

Conclusions

These data suggest that evaluation of MRI sequences may be valuable in assessing extent of soft tissue sarcoma invasion prior to planning treatment. Future work will focus on assessment of further cases and will also encompass cases of osteosarcoma.

Keywords: *Canine, sarcoma, magnetic resonance imaging, histopathology.*

Can a tumors database improve animal cancer knowledge? The Italian Network of Laboratories for Veterinary Oncology (NILOV): a national effort to collect data on animal tumors

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Introduction

NILOV, a project by the National Reference Center for Veterinary and Comparative Oncology (CEROVEC), collects diagnosis of cancer in dogs and cats at national level since 2013. Aim of our work is to describe the data collected in 2013-2015.

Results

To date 11985 cases, 88% dog and 12% cat were collected. Mean age at diagnosis were 9.2 years for dogs and 10.7 for cats. Epithelial tumors predominate in female (benign: dog 50%, cat 69%; malignant: dog 60%, cat 56%) as well as in male (benign: dog 55%; cat 65%). The most frequent diagnosis among malignant were neural tumours in male dog (22%) and mesenchymal tumours in male cat (38%). In dog, PMR by sex and topography, although not statistically significant, suggested a higher risk to skin and to eye, brain and meninges malignancies in male dogs compared to females, whereas in cat PMR suggested an increased risk in females for skin malignancies. Comparing different territories PMR evidenced for Lazio and Tuscany a significant increased risk for dog tumors in endocrine glands (8.4, 95% CI 2.7-26.6), in respiratory and intrathoracic (3.8, 95% CI 2.6-5.5), soft tissue (3.4, 95% CI 3.1-3.8) and skin (1.08, 95% CI 1.03-1.13).

Materials and methods

A descriptive analysis of data submitted by participants was performed, considering age at diagnosis, sex, morphology and topography (those latter according to ICD-O coding). Proportional Morbidity Ratio (PMR) was also calculated to compare risk of malignant tumours.

Conclusions

Data collected by NILOV showed their potential in improving cancer knowledge, although with the limitation of registries not population based.

Keywords: *MCT, Canine, Feline, cancer diagnosis database*

Evaluation of Ki67 expression in feline non-ocular melanocytic tumours

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Introduction

Melanomas are rare in cats. The eye is the most commonly involved site, whereas few data are available about extra-ocular melanomas. Ki67 thresholds with prognostic relevance have been established for canine melanomas, but not in cats. This study was undertaken to explore the utility of Ki67 immunohistochemistry in feline non-ocular melanocytic tumours.

Results

Twenty-six tumours located in skin (n=13), digits (n=2), mucocutaneous junction (n=6) and mucosae (n=5) were included. All amelanotic tumours (n=13) were S100-positive; 7 of them (53.8%) also expressed Melan-A. Most achromic tumours were mucosal (n=5) or mucocutaneous (n=5; P=0.001) and showed spindle cell morphology (P=0.041). MC and Ki67-index were correlated (P=0.0009); median values were 20 (range, 0-153) and 27.9% (range, 0.5%-77.2%), respectively. There was no significant difference in MC and Ki67-index according to location or degree of pigmentation. MC was higher in spindle cell melanomas (P=0.0234). Follow-up information was available in 18 cases. Cats with amelanotic (P=0.0487) and mucosal tumours (P

Materials and methods

Histological samples were retrospectively reviewed. Evaluated parameters included morphologic classification, prevalent cell type, degree of pigmentation, mitotic count (MC), S100 and Melan-A immunohistochemistry and Ki67-index. MC was defined as the number of mitotic figures in a 2.37 mm2 area. Ki67-index was calculated as the mean percentage of labelled neoplastic cells in 5 HPFs. Pigmented tumours were bleached before evaluation.

Conclusions

Contrarily to the canine counterpart, this preliminary report suggests that MC and Ki67-index are of limited prognostic value in feline non-ocular melanocytic tumours.

Keywords: *Feline, melanoma, Ki67-index, mitotic activity, proliferation*

Immunohistochemical analyses of P-glycoprotein expression of feline injection-site sarcomas - in ovo studies

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Introduction

To enlarge the knowledge on multidrug resistance of feline injection-site sarcomas P-glycoprotein (PGP) expression was assessed on tumors growth on chick embryo chorioallantoic membrane from feline fibrosarcoma cell lines and correlated with PGP activity.

Results

All tumours from each tested cell line were positively stained for PGP. There was no significant differences in IRS scores as well as expression of positive stained cells between tumors from FFS1, FFS3 and FFS5 cell lines. The intensity of staining was significantly lower (p=0.002) for tumors from FFS5 cell line than for tumors from FFS1 and FFS3 cell lines. A moderate correlation between intensity of PGP staining and PGP activity has been shown (r=0,34).

Materials and methods

3 feline fibrosarcoma cell lines: FFS1, FFS3 and FFS5 with previously determined PGP activity: high, moderate, none, respectively were used. On the 6th day of chick embryo incubation 70 chick embryos were divided randomly into 3 groups and 5 x 10^6 fibrosarcoma cells were injected. After 10 days 60 tumors were collected. PGP expression was determined using mouse monoclonal antibody against human PGP (clone C494, diluted 1:100). Quantification of PGP immunolabeling was performed using immunoreactive score (IRS). IRS = staining intensity x percentage of positive cells. Statistical analyses were performed using ANOVA and Spearman correlation test (GraphPad Prism 5.0, USA).

Conclusions

The PGP activity of FISS does not correlate with PGP immunoreactive score, what suggests that PGP immunostaining should not be used as a method to assess multidrug resistance in cats with FISS. We acknowledge the support of National Science Centre (UMO-2015/17/D/NZ5/04241)

Keywords: *Feline, PGP, immunohistochemistry, in ovo model, feline injection-site sarcoma, multidrug resistance*

Implementation and preliminary results of an Animal Cancer Registry in Portugal (RCA-PT) in partnership with RCA-SP.

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Introduction

Cancer registration is a key tool for cancer control and prevention strategies, both in human and veterinary medicine and provides valuable information for the development of Comparative Oncology. The Portuguese Animal Cancer Registry (RCA-PT) was born as an international partnership between the Universities of Porto/Portugal and São Paulo/Brazil. Both RCA-SP and RCA-PT aim to provide comparative and standardized comprehensive data of spontaneous malignant neoplasms in animals, including location, morphology, diagnosis, staging, treatment, survival, geography and epidemiology.

Results

A total of 196 animals were registered, the majority being dogs (81%) and the remaining 19% cats. The most prevalent neoplasm in dogs was mammary tumor (19%), followed by mast cell tumor (15%) and lymphoma (11%). In cats, the most frequent was lymphoma (33%) followed by mammary tumor (22%) and fibrosarcoma (8%). In dogs, mongrels were more representative (34%), followed by Labradors (18.7%), Boxers (11.8%) and Golden Retrievers (5%).

Materials and methods

After adjustments, adaptations, validation and tests, RCA-PT was used by two veterinary hospitals of Porto, UPVET1 and CHV2 in 2016.

Conclusions

The strengths of RCA-PT are its foundation on a tested and implemented software and standardization with human registry systems. Its major weakness is some complexity that may compromise practitioners' compliance. We aim to cooperate with similar registries in other countries to promote global information networks on animal and comparative oncology.

Keywords: *Canine, Feline, Animal Cancer Registry, Portugal, Brazil, Epidemiology.*

Histopathological classification and immunophenotyping of canine lymphomas in Porto – Portugal: a survey.

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Introduction

Lymphomas are the most prevalent hematopoietic neoplasms in dogs. The aim of this study was to perform a survey of canine lymphomas diagnosed at the Veterinary Pathology Services of ICBAS-University of Porto, from 2005 to 2016.

Results

Mixed breed dogs were most common (28%), followed by Cocker spaniel (12%), Boxer (9%) and Labrador Retriever (6%). The mean age was 9.2 years (10.8y. for small; 8.9y. for medium and large and 6.6 years for giant breed dogs). There was no significant association between sex and lymphoma. B-cell were more prevalent (57%) than T-cell lymphomas (37%) and 6% were classified as non-B/non-T cell lymphomas. Large and giant breed dogs presented a 4-fold risk of developing T-lymphomas than small and medium breed dogs (OR:4.2; CI 95% 1.2;14). With respect to the anatomical location of the lesions, 49% of the cases presented a multicentric distribution followed by splenic (22%), cutaneous (12%), alimentary (12%) and extranodal (4%). The most common B-cell subtypes were diffuse large B-cell lymphoma (DLBCL) (35%) and marginal zone (16%) while T-zone (18%) and intestinal (18%) were the most frequent T-cell lymphomas.

Materials and methods

A total of 75 canine lymphomas from the Porto district were enrolled. All cases were immunophenotyped by CD3 and PAX5 and classified according to the current WHO classification.

Conclusions

Our study shows that the District of Porto followed the international trend for higher prevalence of B-cell lymphomas in dogs. Large and giant breed dogs showed increased risk of developing T-cell lymphomas.

Keywords: *Lymphoma, Canine, Porto, Portugal*

CSPG4 expression in canine osteosarcoma: preliminary results for a potential target in immunotherapy

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Introduction

Osteosarcoma is the most common primary bone tumor in dogs. Despite aggressive surgical treatments and adjuvant therapies, this tumor has still a poor prognosis. Chondroitin sulphate proteoglycan 4 (CSPG4) is a membrane-bound chondroitin sulfate proteoglycan highly expressed in melanoma cells, and it is nowadays considered a suitable target for specific immunotherapy in canine melanomas. The aim of the present research was to evaluate the expression of CSPG4 in canine osteosarcoma to identify suitable targets for novel therapeutical approaches.

Results

This preliminary study revealed a strong immuno-expression of CSPG4 positivity in 79.3% (23/29) of analyzed osteosarcomas. No statistically significant differences were seen comparing immunohistochemical results and clinical or survival data, even though grade I osteosarcoma were mostly negative (66.7%) whereas grade II and III tumors tended to highly express this antigen.

Materials and methods

CSPG4 immunohistochemical expression was evaluated on 29 tumor specimens from dogs surgically treated for appendicular osteosarcoma followed by chemotherapy and followed-up for at least 24 months.

Conclusions

These preliminary results, if confirmed in a greater number of cases, suggest that CSPG4 could represent an attractive candidate tumor antigen for immunotherapy in canine patients affected by OSA overexpressing CSPG4.

Keywords: *Osteosarcoma, CSPG4, canine osteosarcoma, immunotherapy*

Immunodetection and significance of stem cells markers CD44/CD24, BDNF and its receptors in canine mammary tumours

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Introduction

In a hierarchical model of carcinogenesis, cancer stem cells (CSC) would be the only cells able to regenerate and give birth to a tumour. They would be resistant to conventional therapies and responsible for recurrences. Two studies proved that CSC could be detected in canine mammary tumors (CMT) by immunohistochemistry (IHC) using the same phenotype as in woman: CD44+/CD24-/low. Accumulating data highlight the role of neurotrophins in the regulation of CSC, but no data is available in CMT. The aim of this study was to investigate expression of CSC markers, neurotrophins and their receptors in CMT, and to correlate them to histological diagnosis (type, grade) and clinical data (survival, recurrences).

Results

Our results indicate that all of them can be detected by IHC in CMT. Colocalization of BDNF and its receptors were observed. As in woman, p75NTR seemed to be a marker of myoepithelial cells. CD44+ expression was observed in 81% of tumours, CD24+ expression in 56%, and CD44+/CD24-/low phenotype in 61-72%. Different patterns of staining (tubulopapillary, myoepithelial or diffuse) were observed, and a diffuse CD24+/CD44- phenotype seemed correlated with higher grade.

Materials and methods

96 mammary neoplasms samples were selected from Oncovet Clinical Research biobank. IHC was performed on serial sections of each tumour with antibodies against CD44, CD24, neurotrophin BDNF (Brain Derived Neurotrophic Factor), its receptors TrkB and p75NTR.

Conclusions

CD24 expression was associated with a more aggressive phenotype. The high frequency of tumours positive for CD44+/CD24-/low phenotype suggests that these markers are not restricted to CSC in canine model.

Keywords: *Canine, Mammary tumors , Cancer stem cells*

Treatment of a mast cell tumor with autologous adaptive immunotherapy. A case report.

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Introduction

Adaptive immunotherapy using autologous tumor antigens associated to a phosphocalcic adjuvant already showed efficacy against B lymphomas in a doubled blind study. The tumor antigens were extracted from a tumor biopsy and associated to hydroxylapatite microparticular adjuvant. These proteins contained heat shock proteins which are chaperone molecules synthesized in high amount by cancer cells. They have an important role in the presentation of the peptide they chaperone to the CD8 by the antigen presenting cells. We used this therapy to treat a grade II mast cell tumors in two different locations.

Results

This dog received a complete cycle of autologous adaptive immunotherapy. The peri- umbilical lesion shrank from three weeks after the first injection to six weeks when it disappears. He died 835 days after diagnostic without recurrence (natural death).

Materials and methods

A 11 years old dog (Labrador) suffering from a grade II mast cell tumor in 2 locations: periumbilical region (1x1,5cm) and neck (1,5x2cm). A surgery was performed on the neck lesion while the peri-umbilical tumor was not operated. The dosis were prepared from the removed tumor using an Apavac kit. Very briefly, the intracytoplasmic proteins from a tumor biopsy are extracted after tissue grinding, precipitated then passed through a column of hydroxylapatite- particles. The particles loaded with proteins constituted the vaccine. 8 dosis are injected into subcutaneous tissue once a week for four weeks and once a month for four months.

Conclusions

Adaptive immunotherapy could be a solution for mast cell tumor when surgery could not be performed.

Keywords: *adaptive immunotherapy, heat shock proteins, mast cell tumor*

Immunocytochemical study of canine lymphomas: a practical and reliable tool in classification, proliferation assessment, prognosis and search for risk factors.

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Introduction

Unlike human lymphomas, and mainly due to financial constraints, cytology plays an important role in the diagnostic work-up of canine lymphomas, showing a good agreement with histologic classification for most subtypes. The Ki67 index can further help to differentiate low from high grade lymphomas and to detect transformation process. The aim of this study was to contribute to improvement of the cytologic diagnosis of canine lymphoma, providing morphologic and immunophenotypic classification, proliferation assessment and thus important information in the search for possible risk factors.

Results

According to the updated Kiel classification, 65% were B-cell lymphomas - three low grade (LG) and 12 high grade (HG) - and 35% were T-cell - two LG and six HG. Regarding Ki67 index, eight were high index (>60%) (7HG, 1LG), eight were medium (20-59%) (5HG, 3LG) and two low index (

Materials and methods

Carefully performed fine-needle aspirations (FNA) were obtained from at least two enlarged lymph nodes of 23 dogs. The smears were air-dried, stained with Hemacolor for diagnosis, and fixed with cold acetone to proceed to immunocytochemistry using CD3, PAX5 and Ki-67. The owners were asked to complete an epidemiologic questionnaire.

Conclusions

Smear quality was decisive for an effective immunomarking. Dissimilar Ki67 indexes within LG and HG tumours could point to the prognostic and predictive value of Ki67 immunocytochemistry. Furthermore, it could be helpful to investigate possible risk factors.

Keywords: *Lymphoma, Canine, immunocytochemistry, cytology, Ki67, risk factors*

Grade 4/5 local toxicity with treatment with standard elechtrochemotherapy protocol of labial squamous cell carcinoma in 2 cats.

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Introduction

Electrochemotherapy with bleomycin is a safe and effective treatment option for control of local disease in many tumor types including squamous cell carcinoma (SCC). Reported adverse events for superficial lesions are mild and generally manageable with non-steroidal anti-inflammatory. The aim of this case report is to present two cases of severe local toxicity not previously described.

Results

The first signs of local inflammation, pain, erythema and necrosis were observed starting from the first week after initiation of the therapy. These adverse events did not disappear after 4 weeks like previously reported but increased and progressed affecting the quality of life of both cases. Local treatment was introduced with chlorhexidine solutions and with systemic anti-inflammatory and antibiotic medication. After 7th week of treatment both cats were euthanized due to deteriorating quality of life. Histopathology of the margins confirmed residual disease.

Materials and methods

Two cats were histologicaly diagnosed with labial SCC. Previously published protocol was used with intravenous injection of bleomycin and subsequent delivery of 8 electric pulses with amplitude of 1300V for 100 microseconds (each 2 milliseconds). The procedure was performed under general anesthesia and non-steroidal anti-inflammatory was administered to prevent local inflammation.

Conclusions

Treatment of larger tumors with standard electrochemotherapy protocol could increase a risk of local adverse events and decrease the efficacy. Future studies to adjust the approach to advanced stage tumors are justified.

Keywords: *Feline, electrochemotherapy, feline squamous cell carcinoma, adverse events*

An in vitro and in vivo characterization of the cadherin-catenin adhesion complex in a feline mammary carcinoma cell line

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Introduction

Feline mammary cancers have high recurrence and metastatic potential. Abnormal expression or function of major molecules of the cadherin-catenin adhesion complex have been related to breast cancer development and associated to cell migration, invasion and metastatic dissemination. In feline mammary tumours, cadherins and catenins´ role is still poorly known. Therefore, we seek for suitable in vitro and in vivo model systems to study the leading role of P-cadherin and the molecules of the cadherin-catenin complex (CCC) in feline mammary carcinogenesis.

Results

The FMCm cell line expressed E-, P-cadherin and catenins. Moreover, E-cadherin was showed to interact with each one of the catenins, revealing a putative complete CCC. The cells had E- and P-cadherin co-expression and a close proximity between these two molecules. The FMCm cell line revealed to be highly tumourigenic and metastatic leading to the formation of primary and metastatic lesions in all animals that express all the molecules from the CCC studied.

Materials and methods

Major molecules from the CCC (E- and P-cadherin, ?-, ?- and p120-catenin) were evaluated in a feline mammary carcinoma cell line (FMCm), by Western blot analysis, immunofluorescence, imunoprecipitation and in situ proximity ligation assay. The FMCm cell line tumourigenic and metastatic capacity was assessed by orthotopically inoculation of a cell suspension in the mammary fat pad of athymic nude mice (N:NIH(S)II-nu/nu). Mice xenografts and metastatic lesions were evaluated by immunohistochemical and immunofluorescence techniques for cadherins and catenins expression.

Conclusions

FMCm cell line represents a useful model for in vitro and in vivo studies of feline mammary carcinoma progression.

Keywords: *Feline, mammary carcinoma, cadherin, catenins*

NEOPLASTIC EFFUSIONS IN DOGS AND CATS: A SIX YEARS RETROSPECTIVE STUDY 2010-2016

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Introduction

Effusions analysis, including cytologic evaluation and classification, is a quick and relatively safe way to obtain useful information for diagnosis of neoplasias causing fluid accumulations.

Results

In canine effusions, 10% were neoplastic, whereas in cats neoplastic effusions represented 14,7%. Concerning canine abdominal samples 4.1% (n=10) were neoplastic: 60% (n=6) of epithelial origin and 40% (n=4) lymphomas. Regarding thoracic effusions, 22.1% (n=33) were neoplastic being 63.6% (n=21) epithelial, 30.3% (n=10) lymphoma and 6.1% (n=21) other neoplasms. Only 9.2% (n=6) of pericardial samples were neoplastic, being 66.6% (n=4), haemangiosarcoma and chemodectoma. In cats, abdominal effusions 3.7% (n=7) were neoplastic: 28.5% (n=2) epithelial, 42.8% (n=3) lymphoma and 28.5% (n=2) other neoplasia. 21.5% (n=67) of thoracic were neoplastic: 28.4% (n=19) epithelial and 71.6% (n=48) lymphoma.

Materials and methods

460 canine and 503 feline effusions were reviewed, in a six-year period (2010-2016). In dogs 53.5% (n=246) were abdominal, 32.4% (n=149) thoracic and 14.1% (n=65) pericardial. In cats 38% (n=191) were abdominal and 62% (n=312) thoracic.

Conclusions

Neoplasia is an uncommon cause of effusions in dogs and cats, accounting for 10% and 14.7% of all effusions, respectively. In dogs, epithelial neoplasms were the most predominant, both in abdominal and thoracic effusions. Whereas in cats, lymphoma is the most common neoplasm, both effusions too. Neoplastic pericardial effusions were observed only in dogs, namely hemangiosarcomas and chemodectomas.

Keywords: *Canine, Feline, Neoplastic Effusions*

VEGFR2 immunoexpression and its association with clinicopathological features and overall survival in malignant canine mammary tumors.

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Introduction

In humans there is accumulating evidence that vascular endothelial growth factor receptor 2 (VEGFR2) pathways are tightly connected with malignant transformation. In canine mammary tumors (CMT) this topic is not well-documented yet. This study aimed to explore the correlation between VEGFR2, clinicopathological features and prognosis in CMT.

Results

The high levels of VEGFR2 were associated with tumor histological classification ($p = 0.004$), skin ulceration ($p = 0.025$), tumor necrosis ($p = 0.012$), high mitotic grade ($p < 0.001$), more marked nuclear pleomorphism ($p = 0.001$), poor differentiation of tumors ($p = 0.018$), high histological grade of malignancy (HGM) ($p < 0.001$), presence of neoplastic intravascular emboli ($p = 0.001$) and presence of lymph node metastasis ($p < 0.001$). Additionally tumors with abundant VEGFR2 were associated with shorter overall survival (OS) time ($p < 0.001$ Kaplan-Meier curves).

Materials and methods

Were included 66 malignant CMT and studied, by immunohistochemistry, the VEGFR2 expression together with several clinicopathological characteristics.

Conclusions

Results from present study suggest that VEGFR2 might play a role in CMT progression and contribute to clinical aggressiveness in these tumors. The association of VEGFR2 expression with shorter OS prove its usefulness as a potential prognostic marker.

Keywords: *Canine, Feline, Canine mammary tumors; Prognosis; VEGFR2*

Effects of Sodium Dichloroacetate on canine and human neoplastic and non-neoplastic mammary epithelial cell cultures.

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Introduction

Sodium dichloroacetate (DCA) modifies the metabolism of cancerous cells, interfering with the Warburg effect. Upon changing cell mitochondrial metabolism, there is increase in oxygen reactive species that could lead cells to death. This study aimed to investigate the effect of DCA on canine neoplastic and non-neoplastic mammary epithelial cell cultures. Human mammary cell lines were also tested, for comparison.

Results

DCA significantly reduced the number of viable canine mammary neoplastic cells and MCF7 cells starting from concentration of 20mM and more. Canine mammary normal epithelial cells presented significant decrease in viability at 100mM only. On the other hand, MCF10A showed reduction in viable cells starting at concentration of 5 mM and higher.

Materials and methods

Two canine mammary neoplastic epithelial and one canine mammary normal epithelial cell primary cultures were established at the LECO, USP. Cells were cultivated on advanced DMEM (Gibco), with supplements. MCF10 (human normal epithelial cell line) and MCF7 (human neoplastic epithelial cell line) were cultivated on enriched DMEM/F12 (Gibco). DCA was applied at 1, 5, 10, 20, 50 and 100 mM for 24, 48 and 72h.The MTT assay was used and read at 620nm. Results were statistically analysed trough ANOVA ($p<0,05$).

Conclusions

Our studies have shown that canine and human mammary neoplastic epithelial cells were equally susceptible to DCA inhibitory concentration. However, canine and human normal mammary epithelial cells differ in susceptibility to DCA. Further studies are necessary to verify the efficacy of DCA for treating mammary cancers in vivo.

Keywords: *Canine, sodium dichloroacetate, DCA, mammary tumor, MCF7, MCF10*

Slow release, gel based local chemotherapy for canine nasal tumors

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Introduction

Nasosinal tumours of the dog are rare, but locally invasive and progrediate fast. Radiation therapy is the best choice of treatment for them, but unfortunately is not available at many places. Our aim was to find an alternative option, therefore we applied slow release gel (0.7 % porcine gelatin) formulated carboplatin for local treatment of these tumors to gain better survival results than surgery and/or chemotherapy.

Results

Medial survival time was higher in group III (374.8 days) than group I (102.8 days) (p=0.0228); and in group III than group II (121.5 days, p=0.017) and Stage I+II+III differed from Stage IV (184.1; 84.5 days, respectively) (p=0.0263). During local therapy the patients quality of life was acceptable (grade I-II): vomiting (n=3), anorexia (n=5) and local swelling (n=4).

Materials and methods

After a cytoreductive surgery carboplatin gel was administered into nasal cavities using Foley catheters and into the frontal sinus by trepanation of the skull, monthly for 4-6 times, Dogs (n=65) diagnosed with intranasal tumors were enrolled. Staging was performed by Adams system using CT (Stage I, II, III, IV; n=13, 13, 11, 14, respectively). Available histopathology results with grading were simplex-, adenocarcinoma (n=46) and fibro-, chondrosarcoma (n=19). Among others following groups were established: I. cytoreductive surgery (n=9), only; II. chemotherapy, only (n=15); III. surgery plus local gel based chemotherapy (n=11).

Conclusions

This method is a possible therapeutic option for the treatment of nasal tumors, where radiation therapy is not available. Further investigation is needed if this method is suitable for radiosensitization and for combination therapy with radiotherapy.

Keywords: *Canine, nasal tumor, local chemotherapy*

Effects of methylene blue mediated photodynamic therapy on solid Ehrlich tumor and on second Ehrlich tumor implant

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Introduction

Photodynamic therapy (PDT) is based on interaction of light, a photosensitizing agent and production of reactive oxygen species, leading to cell death. PDT activates the immune system against tumor cells. Methylene blue (MB) is used for PDT due to photochemical properties. Here we aimed to investigate the effects of MB-PDT on subcutaneous Ehrlich tumor and second Ehrlich tumor implant growth.

Results

Group 1 mice showed size reduction and necrosis after MB-PDT. Second Ehrlich tumor growth curve was not significantly different between Group 1 and Group 2. However, the morphometric analysis of the Group 1 second Ehrlich tumor showed significantly lower volume fraction of tumor cells, higher inflammatory infiltrate and necrosis when compared to Group 2. Relative spleen weight was higher in Group 1 mice with white pulp hyperplasia.

Materials and methods

For MB-PDT, MB at 1% and diode laser were used. Swiss male mice received dorsal subcutaneous inoculation of Ehrlich tumor cells and 9 days after the tumor mass was treated with MB-PDT (Group 1) or surgically removed (Group 2). One day after treatment, Groups 1 and 2 received a second Ehrlich tumor implant on the left footpad, measured for 17 days. After euthanasia, the spleen, lymph nodes and tumor mass were weighed and processed for histopathology.

Conclusions

Per these results, MB-PDT not only reduced primary Ehrlich tumor growth but impacted the growth of a second tumor. These results show that the local therapy with MB-PDT may stimulate the immune system. We look forward investigating if MB-PDT could also impact on micrometastatic growth.

Keywords: *Canine, PDT, methylene blue, Ehrlich tumor*

Canine Sino-Nasal Tumours Treated With Mitoxantrone And Nsaid: A Retrospective Study

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Introduction

Radiotherapy is the current standard treatment for canine sino-nasal tumours. Little information is available regarding the response of these tumours to chemotherapy. The aim of the study was to evaluate side effects and survival data in patients treated with a combination of mitoxantrone and a NSAID.

Results

Fifteen cases were included, seven females and six males, age range was 6 to 15 years. Most common breed was mixed breed, with a median weight of 14kg. Nasal discharge, epistaxis and facial deformity were the most common signs. Ten dogs presented clinical signs for 90 days. Twelve dogs were diagnosed with carcinoma, two with adenocarcinoma and one with SCC. A total of 65 mitoxantrone treatments (median 4) were administered with a median dose of 5mg/m2. All dogs were treated concurrently with a NSAID. Five dogs had one of each of the following adverse reactions to the combination: grade 2 vomiting, grade 2 gastric ulceration, grade 2 colitis, and grade 2 and 3 neutropaenia. 78.6% of the patients had improvement of their clinical signs. Mean and median progression-free-survival were 182 and 83 days. Mean and median survival times were 297 and 176 days. Dogs with facial deformity (6 patients) lived significantly shorter (median 95 vs 394 days, p=0.011).

Materials and methods

Medical records of dogs with sino-nasal tumours treated with mitoxantrone chemotherapy between 2007-2016 in three institutions (Spain) were reviewed. Data regarding signalment, clinical presentation, tumour type, treatment and outcome were evaluated.

Conclusions

The combination of mitoxantrone and a NSAID was well tolerated and most of the patients improved clinically, it might be considered as an option when radiotherapy is not available or when signs recur after radiotherapy or other medical treatment.

Keywords: *Canine, Nasal tumour, Mitoxantrone*