NUCLEAR MEDICINE IN ONCOLOGY

Nucleaire geneeskunde in de oncologie

K. Peremans[1], V. Kersemans[2], T. Bosmans[3], H. van Bree[1], C. Van de Wiele[4]

[1]Department of Medical Imaging, Faculty of Veterinary Medicine,
Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
[2]Laboratory of Radiopharmacy, Faculty of Pharmaceutical Sciences, Ghent University,
Harelbekestraat 72, B-9000 Gent, Belgium
[3]Department of Medicine and Clinical Biology of Small Animals, Faculty of Veterinary Medicine,
Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
[4]Division of Nuclear Medicine, Ghent University Hospital,
De Pintelaan 185, B-9000 Gent, Belgium
Kathelijne.peremans@UGent.be

SAMENVATTING

In dit overzichtsartikel worden de mogelijkheden beschreven van de scintigrafie in de diagnostiek, het metastase-onderzoek en het vaststellen van het resultaat van de behandeling van tumoren bij mens en dier.

De huidige stand van zaken in de humane geneeskunde met betrekking tot deze toepassing wordt beschreven en er wordt een vergelijking gemaakt met de onderzoeken die tot op heden werden uitgevoerd in de diergeneeskunde. Er wordt een overzicht gegeven van de verschillende routinematig gebruikte radiofarmaceutica en hun werkingsmechanisme met de nadruk op het specifieke gebruik bij bepaalde klinische vraagstellingen. Er wordt ook aandacht besteed aan de nieuwere generaties radiofarmaceutica die in volle ontwikkeling zijn en potentieel een grotere specificiteit en sensitiviteit bieden. Naast de sterke punten van dit type onderzoek worden ook de limitaties aangekaart die vooral het gevolg zijn van de beperkte spatiale resolutie van het systeem en het tekort aan specificiteit van de meeste huidig gebruikte radiotracers. Deze beeldvormende techniek vult in belangrijke mate de klassieke beeldvormende modaliteiten aan in het onderzoek van de oncologische patiënt.

ABSTRACT

This review focuses on the possibilities of scintigraphy in the diagnosis, staging and therapy outcome of tumors in animals and man. A comparison is made with the current state of the art in human oncology and veterinary medicine. An overview is given concerning the working mechanism of the different commercially available radiopharmaceuticals, with emphasis on their specific use, depending on the clinical requests. Recent developments concerning new generations of radiopharmaceuticals which have the potential to offer more specificity and sensitivity, especially in the domain of therapy prediction and outcome, are mentioned.

Despite its limitations, due for the most part to the limited system resolution and the lack of specificity of the currently available radiotracers, this modality offers additional diagnostic information compared to the conventional anatomical imaging techniques.

INTRODUCTION

Veterinary oncology is a rapidly growing field because more and more owners are prepared to go all the way to give their pets the maximal care, including interventions with radiation and chemotherapy. As a consequence, pre-therapeutic evaluation of the patient is becoming more important in efforts to obtain a complete picture of the disease, to predict survival chances and to select the most appropriate therapeutic protocol. Advances in histological procedures and the determination of blood tumor markers, along with the availability of more sophisticated imaging moda-

lities, are increasing the capability to detect and monitor tumor spread.

The degree of malignancy (grading) is determined on the basis of specific histological features of biopsy specimens. In several tumors, grading correlates well with median survival time and may serve as an important prognostic indicator (Powers, 2003).

When the diagnosis is established, the presence of loco-regional or distant metastases (staging) is obligatory for establishing the prognosis for survival and for directing therapy strategies.

Several imaging techniques may be involved in both diagnosis and staging of the disease, each with competing or complementary diagnostic power. At the time of presentation, most tumors can be recognized clinically, and the use of conventional structural imaging modalities will be most relevant for delineating the tumor. Morphological imaging modalities offer structural information with excellent capability to delineate the location and size of the tumor growth. Nuclear imaging modalities have the capacity to evaluate the viability and metabolism of the tissue and to offer the possibility of imaging the entire body in search of metastases in a relative short acquisition time. Pre-therapeutically, this technique is therefore used for staging malignancies and guiding treatment possibilities. In recent years, this modality has been utilized to monitor disease in the post-treatment period and to predict recurrence of the tumor at an early stage (Podoloff, 1995). Although computed tomography (CT) and magnetic resonance imaging (MRI) have superior spatial resolution (the capacity of the system to recognize two small objects in the image as separate entities), they are not very useful for the identification of residual mass immediately after therapy. Differentiation of residual tumor or tumor recurrence and post-therapy alterations is often not possible and reduction in size (a discriminator for anatomical imaging to predict therapy response) does not correlate well with the response of the tumor (Lawrence *et al.*, 1993; Erlemann *et al.*, 1990; Holscher *et al.*, 1992; Front *et al.*, 2001). Metabolic tumor response precedes changes in size because radiation and chemotherapy influence in the first instance the metabolism of the tumor and it is only at a later stage, that tumor size alterations will become evident (Bombardieri *et al.*, 2004).

In contrast with radiography and CT, nuclear imaging is based on the detection of radiation emitted by the patient, in other words: the patient is the radioactive source. Depending on the decay mode of the injected radionuclide (positron emitting or gamma photon emitting radioisotopes), single or multiple head gamma cameras or PET (positron emitting tomography) cameras are used to detect photons emitted from the patient. Although the spatial resolution obtained with PET cameras is superior to gamma cameras, the main disadvantage of PET examinations is that only a limited number of veterinary centers have access to PET modalities and that the short half-life of some of the PET tracers necessitates a cyclotron in the near vicinity.

Several acquisition modes can be used: planar (stationary image of a local region), total body (survey of the whole body in one acquisition) and tomographic imaging.

Especially in oncologic investigations, the quality (described by "target to background" or "signal to noise ratio") of the generated images must be high, in order to detect lesions that are as small as possible. Lesion detection will depend on spatial resolution and sensitivity. In general, the lesion should be twice the resolution capacity of the system in order to be detected. Noise (or background activity) is introduced in the image by scattered photons or non-specific bound radiotracer (tracer accumulated in other tissue than the targeted tumor). Tomographic acquisitions will improve lesion detection as background radioactivity from over- and underlying tissues is removed. Higher doses of injected radioactivity and the use of highly selective radiopharmaceuticals may improve sensitivity. Unfortunately, augmentation of the dose is restricted from a radioprotective point of view and the commonly used radiopharmaceuticals are not necessarily tumor specific.

A specific class of radionuclides, which decay with the emission of particle radiation (Auger or conversion electrons, alpha and beta particle emission), is used for local therapy of the tumor. Their strength lies in the fact that they deposit high amounts of energy in a small tissue range with radiobiological damage mainly confirmed to the target cells (i.e. tumor cells), thereby sparing normal tissue. Radionuclides and radiopharmaceuticals are used which will accumulate in or bind specifically with the tumor cells (the target). In contrast with conventional radiotherapy, where a specific region of the body is irradiated, targeted radionuclide therapy has the potential to treat widely disseminated malignancies and micrometastases.

This review will focus on the working mechanism and common indications of conventional radiopharmaceuticals used in human and veterinary oncology. Ongoing research concerning the development of new generations of radiotracers will be mentioned.

GENERAL MECHANISMS OF RADIOTRACER ACCUMULATION

Uptake and accumulation of radiopharmaceuticals depend on several general physiological processes. First, perfusion has to be intact to give the tracer access to certain organs. Once the substance is on the spot, its uptake by the cells will depend on simple or facilitated diffusion, active transport (via the ATP-ase dependent Na/K pumps or utilization of an electrochemical gradient) or binding to antigens or receptors. When the radiopharmaceutical is inside the cell, accumulation may occur through binding with intracellular organelles, incorporation in vesicles or cellular molecules, or by enzymatic conversion to an irreversibly trapped compound. Uptake and retention of the radiopharmaceutical in tumor cells is altered compared to normal cells due to altered perfusion within the tumor, altered metabolism, altered membrane transport, expression of specific receptors or specific tumor antigens and increased rate of proliferation.

Tumor perfusion and hypoxia

To grow beyond a certain size, tumors develop their own blood supply as a result of increased hypoxic conditions and imbalance of the pro-angiogenic and anti-angiogenic factors, the so-called "angiogenic switch". This process of angiogenesis also enables tumor cells to migrate into surrounding and distant sites (Brack *et al.*, 2004). The tumor vessels are structurally and functionally different from normal vessels, with high flow alternating with low or no flow. Because of the abnormal blood flow, uptake of radiotracers may be heterogeneous and may decrease as the tumor grows. On the other hand, radiotracers may "wash out" fast in regions with high perfusion (Pauwels *et al.*, 1998). Several radioligands have been developed as markers for vascular antigens, which are preferentially expressed on the endothelial cell membrane of tumor vessels (Brack *et al.*, 2004). The merits of this tumor imaging strategy have not yet been elucidated because only a few radiotracers of this class have been tested in clinical trials (Brack *et al.*, 2004).

As the tumor grows, the central portions will become hypoxic as a result of compromise of the regional vasculature, or due to insufficient oxygen diffusion (Vallabhajosula., 2001). Hypoxia severely compromises the success of radiation and chemotherapy. The evaluation of tumor hypoxia is therefore important to determine the radiosensitivity of the tumor and attempts are being made to develop radiolabelled markers to demonstrate hypoxia non-invasively. The group of imidazole derivatives has been introduced for this purpose. These compounds are trapped by enzymatic conversion in the hypoxic cell but can freely diffuse in and out the cell in normal oxygenated tissue. Unfortunately, the imaging characteristics of these radiopharmaceuticals are currently far from ideal, and suboptimal signal to noise ratios preclude their clinical use (Vallabhajosula, 2001).

99mTc-dimercaptosuccinic acid (DMSA), traditionally used as a renal imaging agent, shows similarity to phosphate molecules, which are accumulated in tumor cells due to increased protein metabolism. The uptake mechanism is considered to be pH dependent and increases in the acidic conditions reached in malignant cells (Horiuchi *et al.*, 1998). This radiopharmaceutical is used in medullary thyroid carcinoma and squamous cell carcinoma of head and neck in humans (Biersack *et al.*, 1992). It has been successfully used for imaging and staging a schwannoma in a dog and decreased uptake after radiotherapy was observed (Balogh *et al.*, 2001).

Cell proliferation and tumor metabolism

Quantitative assessment of tumor proliferation through non-invasive methods can be an important tool for evaluating therapy response in a very early stage. Proliferating cells have a high glucose turnover and a high mitotic rate. As a result of proliferation, oxygen and nutrient consumption increases, leading to a tumor microenvironment characterized by low oxygen tension, low glucose levels and an acidic pH. In response to low oxygen tension, a widespread activation of hypoxia-inducible transcription factors (HIF) is observed in a wide variety of malignant tumors. The HIF system induces adaptive responses including angiogenesis, pH regulation and glycolysis, thus conferring increased resistance to the hostile tumor microenvironment. As glycolysis is an inefficient pathway for ATP provision, glucose turnover has to increase tremendously in order to provide the tumor cells with sufficient energy (Raghunand *et al.*, 2003). This enhanced glucose consumption can be assessed by means of *2-^{18}F-fluoro-2-deoxy-D-glucose (FDG)*.

^{18}F-FDG decays with positron emission and imaging is performed with dedicated positron emission tomography (PET) cameras. At the cell membrane level, increased uptake of FDG is the consequence of overexpressed glucose transporters (GLUT) (activation of mainly GluT1 and GluT3) and/or an alteration in the function of the cell membrane (Pauwels *et al.*, 1998; Bomanji *et al.*, 2001). Once within the cell, the highly active hexokinase will convert FDG to DG-6-phosphate, which cannot be further metabolized and

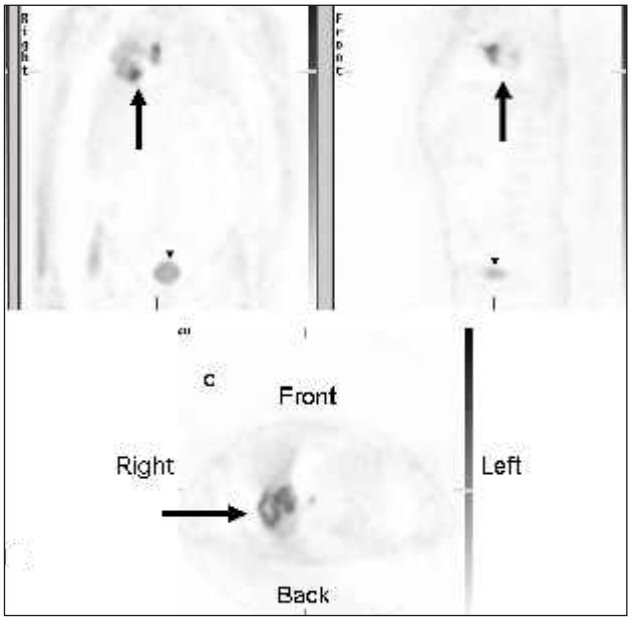

Fig. 1. An example of a FDG PET study in a man suffering from a lung tumor. Coronal (a), sagittal (b) and transversal slices (c) demonstrate an area of increased activity (large arrow) in the thoracic region. Note the physiological excretion of FDG in the bladder (small arrow).

is effectively trapped intracellularly (Pauwels *et al.*, 1998).

FDG is at this moment probably the most all-round performing radiopharmaceutical in oncology, especially for the staging of a variety of tumors in man, due to the higher resolution obtained with PET cameras compared to gamma cameras (Fig. 1). Since metabolic alterations occur before structural changes are evident, this imaging modality is basically more sensitive than conventional morphologic imaging techniques. FDG-PET is useful in the staging, follow-up and early detection of recurrence of tumors. However, due to high uptake by macrophages, which are involved in the repair processes following therapy, the diagnostic value of FDG immediately following radiation or chemotherapy is limited. Also, uptake in bone marrow after radiation or chemotherapy is high, rendering evaluation of therapy response of bone tumors and bone marrow involvement in lymphoma unreliable in the immediate post-therapy period (Bomanji *et al.*, 2001).

Up till now, no optimal protocol has been established for therapy response control in man but serial FDG examinations may provide a means to differentiate between healing processes and residual or recurrent tumors (Bomanji *et al.*, 2001; Waxman *et al.*, 2001). Despite these limitations, FDG PET has a high negative predictive value, as lack of uptake is associated with a very high chance of cure (Bomanji *et al.*, 2001).

Imaging and measuring proliferation in vivo offers the possibility to differentiate between benign and malignant cells and allows evaluation of early therapy response. *Radiolabeled nucleosides* have been developed to quantify DNA synthesis, which will be increased in cells with high proliferation rates. Radiolabeled thymidine and its analogs are taken up by cells and trapped intracellularly via phosphorylation by thymidine kinase 1 (TK1=an enzyme with 3-4 times higher activity in malignant cells than in benign cells) (Been *et al.*, 2004). Thymidine, labeled with ^{11}C, is taken up by a variety of human tumors. Due to the short half-life of ^{11}C and the fast catabolism of thymidine, its use in routine clinical settings is impractical and alternative radiotracers which are more resistant to catabolism are sought (Van de *et al.*, 2003). A radiolabeled thymidine analoge is [^{18}F]-fluoro-3'-deoxy-3'-L-fluorothymidine ([^{18}F]FLT). This tracer reflects only the first steps in the DNA synthetic pathway rather than the incorporation into DNA. Compared to [^{18}F]FDG, this tracer has lower sensitivity in general due to its lower uptake in tumor cells, but it is more specific for malignancy. Changes in uptake after therapy seem to be variable and depend on the type of treatment. Different intracellular changes are induced by different agents and the use of chemotherapy combinations complicates matters even more (Been *et al.*, 2004). The kinetics of [^{18}F]FLT were investigated in 3 normal and 2 tumor bearing dogs (one with malignant lymphoma and one with a soft tissue sarcoma). Normal uptake was seen in kidneys and bladder, reflecting the biological elimination pathway, and also in bone marrow, a known site for high proliferation activity. Little uptake was detected in the brain. Contrary to man, no uptake was seen in the liver, reflecting a different metabolic pathway. High uptake was seen in the affected lymph nodes in the dog with lymphoma and in the periphery of the sarcoma, with lack of uptake in the central area of necrosis in the dog with the soft tissue sarcoma (Shields *et al.*, 2002).

In general, firm proof that the degree of uptake of radiolabeled thymidine and its analogs always relates to alterations in proliferation rate has not been provided. Besides, not all tumor cells show a TK1 dependence of proliferation and some tumors rely on de novo synthesis of DNA precursors, which may influence the uptake of thymidine and its analogs (Schwartz *et al.*, 2003; Schwartz *et al.*, 2003).

During the last decades, the potential diagnostic use of several naturally occurring *labeled amino acids* as markers for tumor proliferation and increased metabolism has been investigated (Vallabhajosula., 2001). In this regard, ^{11}C labeled methionine (MET) and ^{18}F labeled tyrosine (TYR) have been studied. Preliminary results with labeled phenylalanine in neuroendocrine tumors have been favorable and tu-

mor lesions could be demonstrated that were not visualized with the classical radioligands (Hoegerle *et al.*, 2001). Increased uptake of ^{123}I labeled phenylalanine in a tarsal synovial cell sarcoma was demonstrated in a dog (KP pers comm).

This group of radiopharmaceuticals has the potential to be suitable for early therapy outcome prediction. Additionally, differentiation between inflammatory reaction and residual tumor cells is possible since inflammatory cells have a low protein metabolism and will not accumulate these radiolabeled compounds to the same extent as FDG.

However, clinical validation of these radiotracers at this moment is insufficient to justify their routine use (Van de Wiele *et al.*, 2003).

Membrane transport

Radionuclides and radiopharmaceuticals can be transported across cellular membranes by active or passive transport mechanisms.

Pertechnetate and radioiodide accumulate in normal thyroid tissue by an active transport mechanism. However, only radioiodide will be organified (incorporation into tyrosine). In addition to thyroid tissue, the salivary glands, stomach and intestines show uptake of both radioiodide and pertechnetate, which has its repercussions concerning radioprotective issues.

Pertechnetate has several advantages over radioiodide, including lower cost, availability, lower radiation dose to personnel and patient, and optimal physical characteristics for gamma camera imaging. Pertechnetate imaging is extensively used to diagnose thyroid adenoma in the cat, with the additional advantage that ectopic thyroid tissue can be detected (Brawner *et al.*, 1996). In dogs this modality is used in the diagnostic workup of hypothyroidism and in the evaluation of thyroid tumors (Brawner *et al.*, 1996). The degree of pertechnetate or radioiodine uptake will depend on the functional status of the tumor (Fig. 2 a,b). Although no association could be made between intensity of uptake and histological grading of the tumor, poorly circumscribed, heterogeneous uptake was associated with capsular invasion in the dog (N=29) (Marks *et al.*, 1994). Concerning tumor staging, pertechnetate is relatively insensitive compared to radioiodine for the detection of metastases in man and dogs (Marks *et al.*, 1994; Broome *et al.*, 1992; Campbell *et al.*, 1990).

Radioiodide, ^{123}I (gamma emitter) and ^{131}I (gamma and beta particle emitter) can evaluate thyroid organification and is more reliable than pertechnetate to evaluate thyroid uptake pre-therapeutically and to detect metastases. Unfortunately, ^{123}I is very expensive

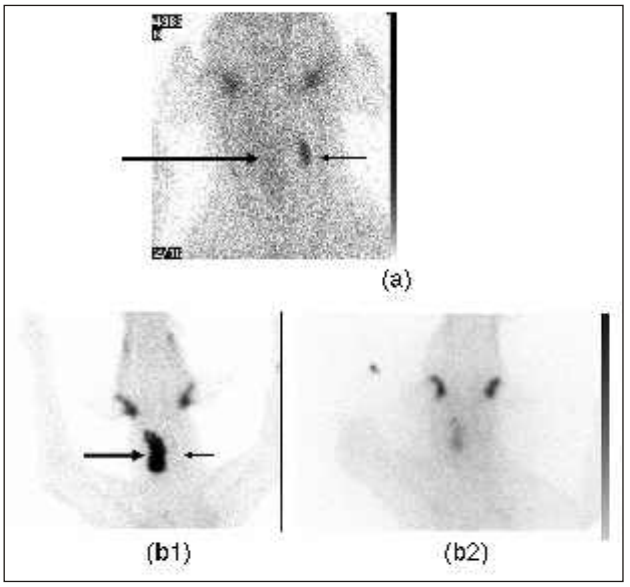

Fig. 2. (a) Lack of pertechntetate uptake in an undifferentiated thyroid carcinoma of the right thyroid lobe (thick arrow) in a dog. Note the normally sized left thyroid lobe with normal pertechnetate uptake (small arrow).
(b1) High uptake in the right thyroid lobe affected with a functional thyroid carcinoma in another dog with symptoms of hyperthyroidism (thick arrow). The left lobe is suppressed and is therefore not visualized (small arrow).
(b2) The same dog, 4 weeks after radioiodine treatment. Uptake in the affected lobe is only faintly visible. The clinical condition of the dog had improved substantially.

and is not commonly used in veterinary medicine, despite its favorable imaging characteristics and relatively short half-life (T1/2=13h). ^{131}I has a long half-life (8.1d), delivers a high dose to the thyroid (resulting from the beta particle emission), has high-energy photons (364keV) and is less than optimal for routine imaging with conventional collimated gamma cameras. Therefore, ^{131}I is mainly used for therapy of thyroid cancer in both man and animals.

Concerning thyroid carcinoma, apart from anaplastic and medullary carcinomas that do not concentrate iodine, radioiodine can be used either as a primary treatment modality without thyroidectomy when the tumor is not resectable or is recurrent, or it can be used to treat distant metastases (Fig. 3 a,b,c). It can also be used following surgery to destroy all residual thyroid tissue (thyroid ablation) in combination with thyroid hormone supplementation. The use of ablative ^{131}I after total bilateral or single complete or near complete thyroidectomy resulted in a decreased tumor related mortality compared to surgery alone or combined surgery and thyroid suppression therapy in man (Mazzaferri *et al.*, 1994). In a small study (N=7) in dogs with stage III (size of tumor >5cm, with or without regional lymph node involvement and without distant metastases) and stage IV thyroid carcinoma

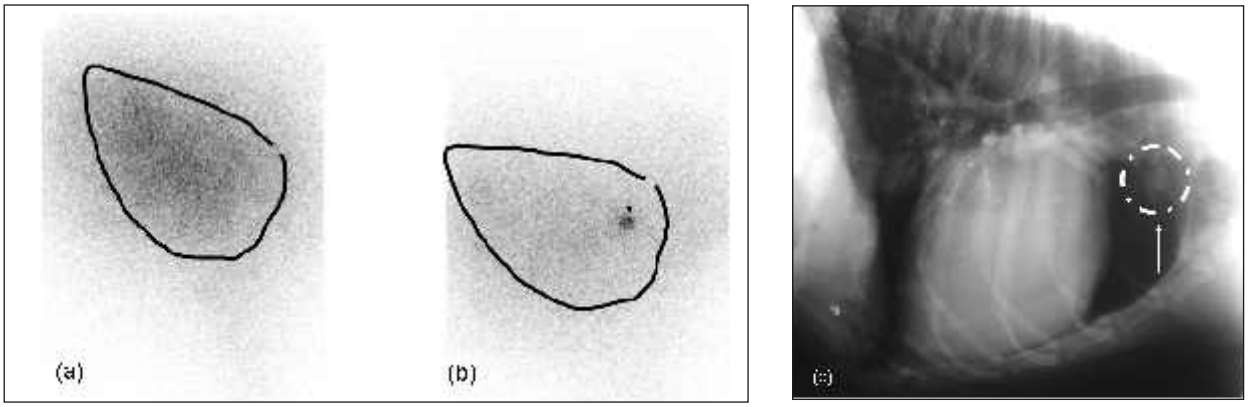

Fig. 3 (a) Left lateral static images of the left pulmonary region (delineated area) of a dog suffering from a non-resectable differentiated thyroid carcinoma with lung metastases. Diffuse uptake is noted in the lungs 24 hours after treatment with radioiodine.
(b) The same dog 48 hours after the injection of radioiodine. A focus of increased uptake is noted (arrow), which corresponds to a metastasis seen as a round opacity (interrupted circle) in the apical lung lobe on the radiographs of the lung (c). At the current time (though this will be solved in the near future) we do not have access to high-energy collimators, and therefore we have to use medium-energy collimators to image ^{131}I uptake, which means that the resolution of the system is less than optimal. This may account for the fact that not more metastases are visualized.

(any thyroid tumor with distant metastases) based on the WHO system (1980), the results of ^{131}I treatment were documented (Adams *et al.*, 1995). Mean survival times for all dogs were 25 months (range 4-48 months), with the longest survival times noted in the three dogs that received radioiodine combined with surgical excision.

The main drawback of the radioiodine therapy is the radioprotection issue. High amounts of radioactivity are used and animals have to be hospitalized. Stimulating the radioiodine uptake may decrease the ablative dose. Recent studies in man on the use of recombinant human TSH (rhTSH) to enhance radioiodine uptake in differentiated thyroid tumors and nontoxic nodular goiter have reported promising results (Nieuwlaat *et al.*, 2003; Bombardieri *et al.*, 2003; Woodmansee *et al.*, 2004; de Keizer *et al.*, 2003).

Since the effect of rhTSH on thyroid stimulation has been investigated in both normal cats and dogs (Sauve *et al.*, 2000; Stegeman *et al.*, 2003), it would be worthwhile to investigate whether it may enhance radioiodine uptake in less avid thyroid tumors and whether dose reduction is possible.

Thallous (^{201}Tl) chloride is actively transported in the cell (mainly by the ATPase-dependent Na^+-K^+ pump) in proportion to the blood supply (Vallabhajosula., 2001). This radionuclide has been used extensively in cardiology to detect myocardial infarction and/or ischemia. It also concentrates in viable tumor cells, less in inflamed connective tissue and minimally in necrotic tissue (Podoloff, 1995). ^{201}Tl has a significant affinity for bone and soft tissue sarcomas (Podoloff, 1995). The typical "donut" uptake pattern (central lucent area surrounded by a rim of intense uptake) seen in high grade tumors due to compromised vascularity in the center of fast growing tumors can be a sign to differentiate high grade from low grade tumors (Waxman *et al.*, 2001).

Concerning therapy prediction in bone tumors, this radionuclide performs better than the classical bone scan and ^{67}Gallium (Ga), because its uptake is not associated with bone healing but rather with the viability of the tumor cells (Ramanna *et al.*, 1990).

The main disadvantage is its long physical (T1/2: 73h) and biological (T1/2: 10d in man) half-life, which results in high patient dose and has an impact on radioprotective measures. The image contrast is not ideal due to dose restriction and a less than optimal energy spectrum for currently available gamma cameras (Podoloff, 1995). These factors limit the use of the compound in veterinary medicine.

Simple and facilitated diffusion involves the movement or translocation of compounds from a higher to a lower concentration gradient through the cell membrane, without expenditure of energy.

Several radiopharmaceuticals are transported intracellularly by these mechanisms.

Sestamibi and tetrofosmine, both lipophilic, cationic compounds labeled with ^{99m}Tc, are traditionally used for myocardial imaging. Accumulation of sestamibi in lung tumors in man was serendipitously observed during cardiac imaging (Vallabhajosula, 2001). The uptake of sestamibi is related to its lipophilicity and its charge. It is retained intracellularly by

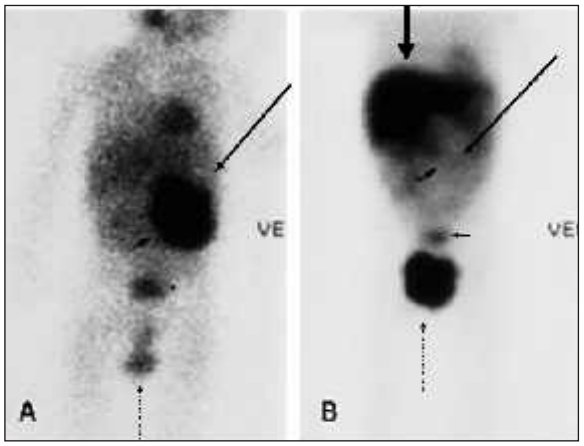

Fig. 4. The left image (A) demonstrates increased uptake of ^{123}I-MIBG in a neuroblastoma of the adrenal gland of a young child (A) (arrow). The right image (B) is of the same patient, imaged with ^{99m}Tc-MIBI. No uptake is seen in this chemoresistent tumor (arrow). The high activity between the legs corresponds to urine activity of the diapers (interrupted arrow). The outline of the bladder is seen proximal to this focus of radioactivity (short arrow). Note the increased visualization of the liver on the MIBI images due to physiological elimination of the radiotracer by this organ (thick arrow).

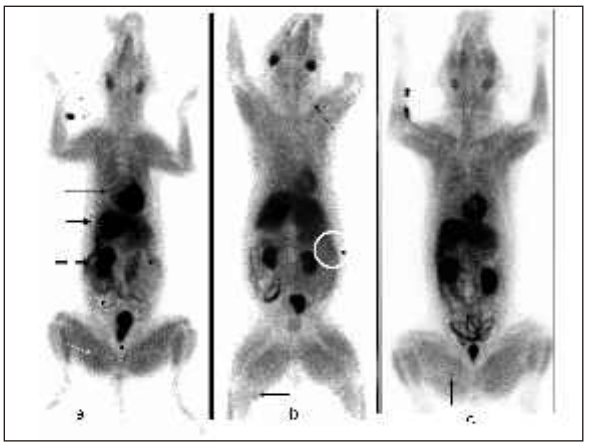

Fig. 5. Total body, ventral images, with ^{99m}Tc sestamibi in 3 dogs. Image of dog 1 (3a) illustrates the normal uptake pattern of this radiopharmaceutical. High physiologic uptake is seen in the heart (small interrupted arrow), liver (large arrow), kidneys (large interrupted arrow), bladder (vertical arrow), intestines (small arrow) and muscle (white arrow). Note the absence of the left kidney. Dog 2 (3b) was suffering from a lymphoma that had been treated with chemotherapy. The scan was performed to check therapy outcome. Lymph nodes (arrows) are clearly visible in the neck and popliteal region, by way of contrast to dog 1 and dog 3. The spleen is also visualized in dog 2 (circle with arrow). On ultrasound, the spleen showed diffuse involvement. Dog 3 (3c) suffered from a hemangiosarcoma in the right upper leg, which had been removed previously. Relapse was suspected. A small focus of increased radioactivity is seen in the right upper leg region (arrow).

the mitochondria. The uptake mechanism of tetrofosmine has not been completely elucidated though it seems to resemble that of sestamibi, with the difference that at least part of the uptake might be mediated by active transport (Vallabhajosula, 2001; Pauwels *et al.*, 1998). Both tracers only accumulate in viable tissue and may therefore be suitable for prediction of therapy outcome (Pauwels *et al.*, 1998; Podoloff, 1995). These tracers are also substrates for the P-glycoprotein pumps (Pgp), which are overexpressed in multidrug-resistant (MDR) tumors. Pgp pumps, also found in several normal cell types as a sort of self-protective mechanism against harmful agents, will pump out certain chemotherapeutic agents and will therefore render therapy with these compounds futile (Fig. 4). Sestamibi, and tetrosfosmine to a lesser extent, may therefore be valuable tools for determining *in vivo* which tumor will be drug resistant and which patients might benefit from Pgp inhibitors (Vallabhajosula, 2001). Several reports have described uptake of sestamibi and tetrofosmine in thyroid, parathyroid, brain, lung, bone and soft tissue sarcoma and breast tumors (Pauwels *et al.*, 1998; Dadparvar *et al.*, 1995). The normal distribution of sestamibi in dogs has been described with visualization of the heart, liver, gallbladder, small intestine, kidneys, urinary bladder and salivary glands (Steyn *et al.*, 1995b). Reports on the application of sestamibi in veterinary oncology have been limited to the staging of malignant lymphoma in dogs (Steyn *et al.*, 1995a; Balogh *et al.*, 2001). In one study involving 13 dogs with WHO stage III, IV and V, uptake was seen in several lymph nodes, spleen, liver, kidney and bone marrow on planar images (Steyn *et al.*, 1995a). Histology and/or cytology confirmed the presence of tumor cells in a representative number of lymph nodes with increased radiotracer uptake. Nevertheless, not all affected lymph nodes showed increased uptake. A possible reason for these false negative results may be that planar acquisitions were used which limit the visualization of smaller lymph nodes due to superposition of over- and underlying activity from neighboring regions. SPECT imaging techniques should improve lesion detection. Uptake of sestamibi was seen in organs in which no radiotracer uptake was observed in normal dogs (Fig. 5). Uptake in bone marrow was seen in 3 dogs and the uptake in one affected liver was more heterogeneous compared to normal livers, due to the in-

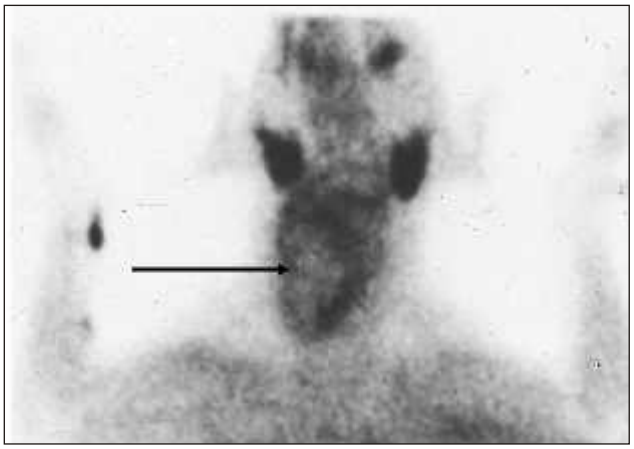

Fig. 6. Heterogeneous uptake of 99mTc-sestamibi in the enlarged right thyroid lobe (arrow). Areas of increased and decreased (necrotic) uptake are seen.

filtrative nature of hepatic lymphoma. No uptake was seen in one affected kidney, as opposed to the high uptake in normal kidneys.

Increased uptake in thyroid carcinoma, synovial cell sarcoma and hemangiosarcoma has also been observed (KP personal comm) (Fig. 5, 6).

Various theories exist concerning the exact mechanism of localization of *Gallium (^{67}Ga) citrate* in tumors. Following injection, it is bound to plasma transferrin and it is transported as a ^{67}Ga-transferrin complex to normal and tumor tissue. Two different mechanisms have been proposed for the transport of ^{67}Ga across the cell membrane. Simple diffusion of the unbound ^{67}Ga across the cell membrane may be augmented due to increased permeability of the capillary bed of the tumor. The second possible pathway is transferrin receptor mediated endocytosis (increased expression in tumor cells due to increased need of iron). Once in the cell, free ^{67}Ga will bind to various molecules, such as lactoferrin and ferritin, which prevents back diffusion of the radionuclide. The acidic pH environment of the tumor cell increases the affinity of ferritin and lactoferrin for iron and gallium. The main disadvantage is that high uptake also occurs in inflammatory lesions (Pauwels *et al.*, 1998). However, this is not much of a problem when the clinical probability is high for tumor. Its normal biodistribution to liver, bowel and kidneys limits its diagnostic power in the abdominal area. This radiotracer is still useful in the staging and follow-up of lymphoma and melanoma in human medicine, especially when PET facilities are not available. Because ^{67}Ga is only taken up by viable cells and not by necrotic or fibrotic tissue, it plays an important role in the evaluation of tumor response early post therapy and of disease recurrence (Front *et al.*, 1993; Front *et al.*, 1999; Front *et al.*, 2000; Weeks *et al.*, 1991; Schuster *et al.*, 2002). Due to radioprotection issues (T1/2=78h) and due to the time interval necessary between injection and acquisition (72h), this radionuclide has not been used frequently in veterinary medicine and no reports concerning its use are available. Nevertheless, we used ^{67}Ga to determine residual disease in a dog with an oral melanoma after treatment with surgery and radiation therapy. High uptake in a lymph node metastasis was observed, which was not evident clinically (Peremans personal observation).

Receptors, transporters and tumor antigens

Communication between cells is established by receptors and neurotransmitters or hormones. The binding of specific neurotransmitters or hormones to their receptors, located on the cell membrane or intracellularly, will generate a signal that will be passed by first and second messengers and that eventually will lead to specific cellular processes such as contraction, secretion, metabolism and growth (Pauwels *et al.*, 1998).

In tumor cells the expression of some receptors is altered, and this phenomenon can be used to image certain tumors.

Somatostatin has many, predominantly inhibitory functions throughout the nervous system and the gastrointestinal tract. Somatostatin has an antineoplastic effect, including inhibition of growth factors and hormones, angiogenesis and stimulation of apoptosis (Pauwels *et al.*, 1998; Warner *et al.*, 2002). Five receptor subtypes are found (STR1-5), with different functions and specific distributions. These receptors are distributed in various quantities over many tissues with a distribution pattern differing among species (Pauwels *et al.*, 1998; Robben *et al.*, 2003). In tumor tissue originating from tissue that normally contains the STR, the expression of the receptor is increased and, depending on the tumor, different STR subtypes may predominate.

Because the activity of native somatostatin is very short, longer acting synthetic analogues have been created for therapeutic and imaging purposes, albeit with different affinities for different receptor types. These radioligands are bound to one or several STR types, and then internalized and transported to the perinuclear and nuclear region where they can remain for relatively long periods, i.e. long enough to be useful for imaging and therapy (Warner *et al.*, 2002).

Somatostatin receptor scintigraphy (SRS) has been the preferred technique for localizing neuroendocrine tumors and tumors derived from the central nervous system in man (Warner *et al.*, 2002; Pauwels *et al.*, 1998) (Fig. 7). A comparison of SRS effective-

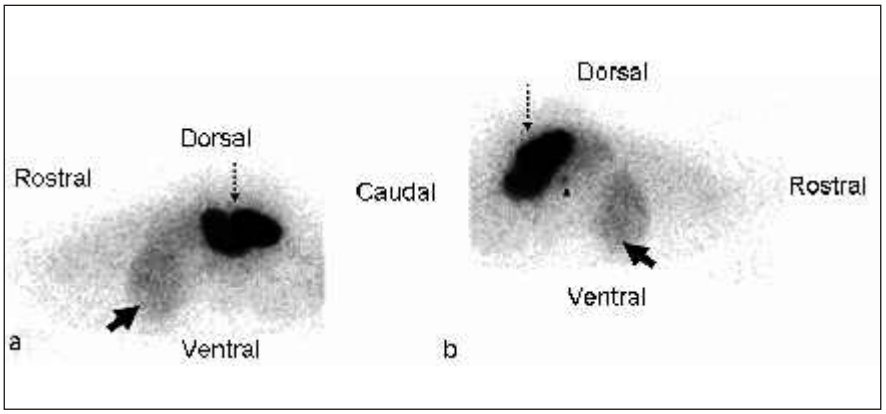

Fig. 7. Images of a dog suspected of an insulinoma. Right (a) and left (b) lateral images were taken of the cranial abdomen. Focal increased uptake of ^{111}In-octreotide is seen cranial to the left kidney (small arrow). Normal uptake is seen in the liver (short arrow) and the kidneys (interrupted arrow).

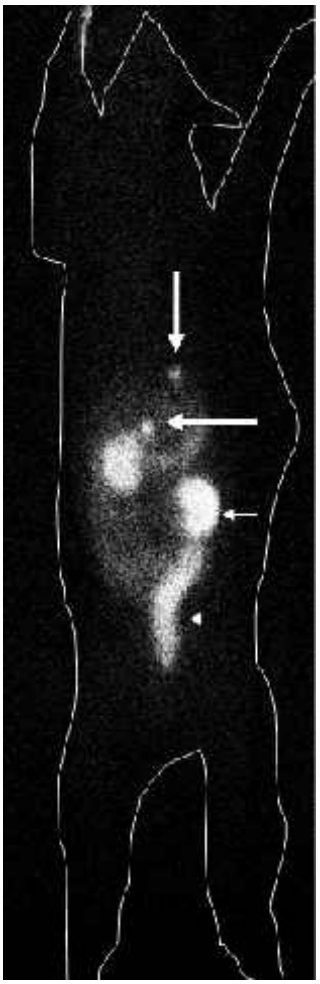

Fig. 8. A total body scan in a dog with an insulinoma with ^{111}In-octreotide. This dog had been treated with surgery and cold octreotide. Two metastatic foci are visualized in the cranial abdomen (arrows). Note the physiologically high uptake in the kidneys (small arrow) and intestines (interrupted arrow).

ness with conventional imaging methods demonstrated that SRS is superior by 50% in humans (Slooter *et al.*, 2001). SRS is also useful in monitoring the progression or regression of the tumor post-therapy (Fig. 8) and in evaluating the completeness of surgical resection or ablation after radiotherapy.

However, the occurrence of SSTR subtypes in some tumors with low affinity for the synthetic somatostatin analogue may render false negative results (Lamberts *et al.*, 1991). Promising research is being performed on gene transfer techniques to increase the expression of specific receptor subtypes in order to improve the detection capacity (Rogers *et al.*, 2000).

In veterinary medicine, the normal distribution of radiolabeled octreotide (^{111}In-DTPA-D-Phe^{1}-octreotide) was investigated in dogs with SPECT and compared with autoradiography (Robben *et al.*, 2003). This radiopharmaceutical has high affinity for somatostatin receptor (ssrt) 2 and, to a lesser extent, for ssrt5. High uptake was seen in the kidneys, gallbladder, liver, gastric fundus and intestines. The entire registered radioactivity in these organs did not account for a normal clearance pattern of the radiotracer since somatostatin receptors were found in the kidneys, gastric mucosa, and intestinal wall with in vitro autoradiography with ^{125}I-[Tyr^{3}]-octreotide. No receptors were found in vitro in the liver and the radioactivity registered in this organ is probably due to non-receptor dependent delivery to the hepatocytes. Compared to rats, the accumulation of radioactivity in the pancreas was less in the dog, and compared to man, accumulation was not found in the spleen. This interspecies difference is related to differences in expression of the receptor and the occurrence of different subtypes of the receptors in the organs (Robben *et al.*, 2003). Therefore, the extrapolation of results from one species to another has to be handled with caution.

The same group used this radiopharmaceutical successfully to stage insulinoma in 6 dogs (Robben *et al.*, 1997). In 5 dogs, metastases were visualized on the SPECT images. Nevertheless, one dog had multiple small (<3mm) metastases in the liver which were not recognized on the images, probably due to resolution limitations. The primary tumor was visualized in all but one dog, which was presented for hypoglycemia 1.5yr after surgical removal of the pancreas. On autoradiography with [^{125}I-Tyr^{3}]-octreotide and

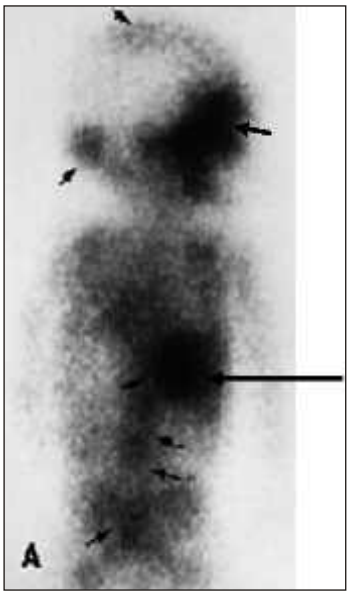

Fig. 9. Increased uptake is seen in the primary adrenal gland tumor in a young child (large arrow) imaged with ^{123}I-MIBG. Several metastases are seen in the skull (small arrows) and in the abdomen (small arrows).

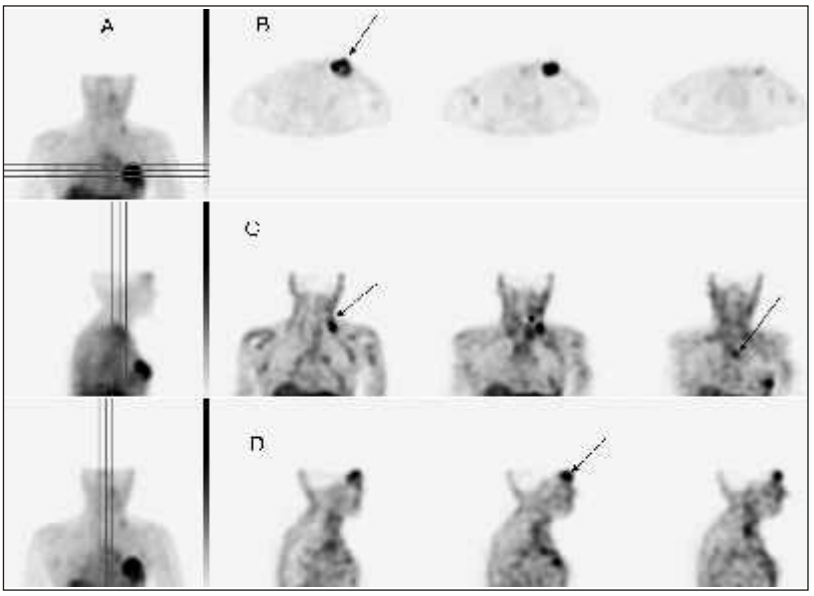

Fig. 10. A SPECT study of a mamma tumor in a woman. The radioligand used is ^{99m}Tc-labelled bombesin, a marker for gastrin releasing peptide receptors. The left images show the orientation of the slices (A). The upper row illustrates increased uptake in the affected breast on transversal slices (B) (arrow). The middle row demonstrates uptake in affected (retrosternal) lymphe nodes on coronal slices (C) (arrows). The bottom row demonstrates metastasis in the skull on sagital slices (D) (arrow). Similar to PET, the advantage of SPECT is that the foci of increased uptake are more easily recognized as the over- and underlying superimposing radioactivity is removed from the image.

[^{125}I-Tyr^{11}]-somatostatine-14, all primary tumors and their metastases demonstrated affinity for both tracers.

Uptake in the primary tumor and its metastases was demonstrated with ^{111}In-pentetreotide in a dog suspected of gastrinoma (Altschul *et al.*, 1997). Multiple regions of increased uptake in the abdomen were consistent with masses in the pancreas and liver at laparotomy. Autoradiography with ^{125}I-[Tyr^{3}]-octreotide on biopsy material confirmed the presence of somatostatin receptors in the tumor tissue (Altschul *et al.*, 1997).

Metaiodobenzylguanidine (MIBG) is structurally similar to the neurotransmitter norepinephrine and the adrenergic neuron blocker guanethidine (Connolly *et al.*, 2001). This compound is transferred by a transporter system in the pre-synaptic cell, where it accumulates in catecholamine storing granules. It has low affinity for the post-synaptic receptors (Pauwels *et al.*, 1998; Connolly *et al.*, 2001). This radiopharmaceutical is mainly used in the imaging of neuroblastoma, phaeochromocytoma, thyroid medullary carcinoma and other tumors arising from the neural crest for the identification of the primary tumor, for staging and for therapy prediction (Pauwels *et al.*, 1998; Connolly *et al.*, 2001) (Fig. 9). ^{131}I-MIBG can also be used for therapy of MIBG avid tumors. The uptake of the tracer may be influenced by certain drugs such as calcium channel blockers, as well as by various antipsychotic and sympathomimetic drugs (Pauwels *et al.*, 1998).

Both ^{123}I and ^{18}F labeled guanidine derivatives correctly identified pheochromocytoma in dogs with adrenal masses, respectively in one and two dogs (Berry *et al.*, 2002; Berry *et al.*, 1993). Intense uptake in the area of the adrenal gland was seen in all the dogs. This radiopharmaceutical's use in veterinary medicine is limited by its high cost.

The receptors for *vasoactive peptide (VIP)* are abundantly present in certain tumor cells, particularly in intestinal adenocarcinomas and various endocrine tumors (Pauwels *et al.*, 1998). VIP receptors are uniformly distributed in a larger variety of tumors compared to somatostatin receptors, but unfortunately also in normal tissue. This results in less optimal target/background ratios and decreases the power to detect tumor lesions (Pauwels *et al.*, 1998).

Other labeled neuropeptides, including substance P, bombesin, gastrin, cholecystokinin and others, have been developed, but clinical experience is still limited. Some promising results have been reported with

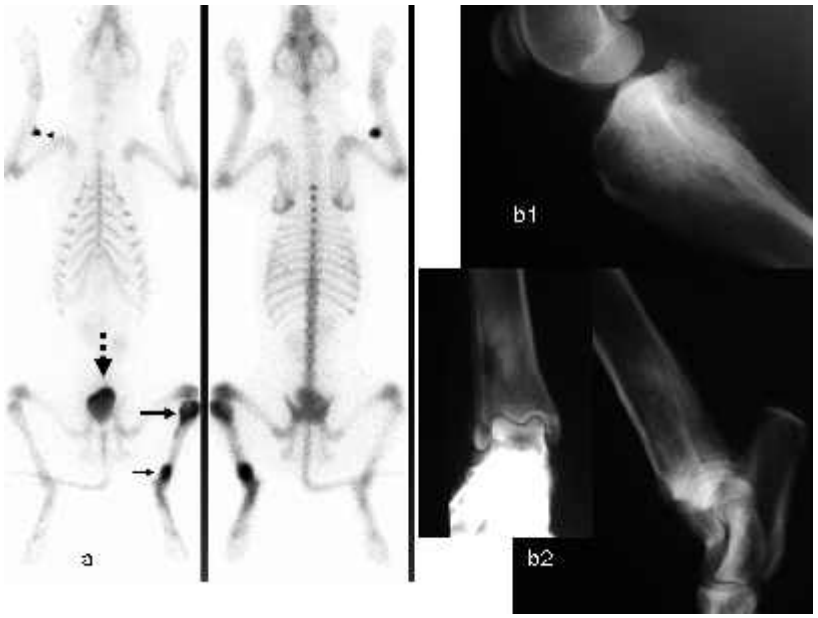

Fig. 11. (a) Total body scan with the radiotracer ^{99m}Tc-methyl diphosphonate (MDP) of a dog with a known osteosarcoma of the proximal left hind tibia (see radiograph (b1)). High uptake is present in the proximal (large arrow) and in the distal tibia (small arrow). Radiographs (b2) confirmed the presence of bone destruction and osteosclerosis in this region. Note the activity on the left at the injection site (interrupted arrow) and the high amount of physiological radioactivity in the bladder (large interrupted arrow; the radiopharmaceutical has been eliminated by the urinary system).

estrogen receptor imaging in breast cancers in man (Pauwels *et al.*, 1998) (Fig 10).

Labeled antibodies are used for tumor imaging and for therapeutic purposes. These compounds have the ability to specifically target tumor cell related antigens and improve specificity significantly. The use of SPECT imaging and fusion with CT and MRI enable better localization of malignant tissue. Sensitivity is dependent on high affinity and avidity for tumor antigens, as well as on fast clearance from the blood pool (Pauwels *et al.*, 1998). To overcome immunogenicity of the murine derived antibodies and to improve blood clearance and target/background ratios, labeled antibody fragments were introduced. Unfortunately, these compounds demonstrate a lower affinity for the antigen (Pauwels *et al.*, 1998).

Recent research has been focused on genetic manipulation of the immunoglobulin molecule to decrease immunogenecity, to improve blood clearance and binding, and to decrease catabolism.

Apoptosis

As opposed to necrosis, apoptosis is the programmed death of cells suffering from insults to their DNA, but without the inflammatory reactions typical of necrosis. When murine cells are successfully irradiated, the number of apoptotic cells increases within a very short time (peak between 3-6h post-radiation) (Van de Wiele *et al.*, 2003). Studies in man show more controversial results and more evidence will have to be provided to prove the relationship between the level of apoptosis and therapy outcome (Van de Wiele *et al.*, 2003). Histological determination of the degree of apoptosis after radiation is one possibility. Another less invasive strategy is the use of radiolabeled markers for apoptosis. In this regard, the potential use of ^{99m}Tc- annexin has been investigated as studies on human cardiac disease demonstrated that this radiotracer could be used to assess cell death dynamics and the effectiveness of therapy (Van de Wiele *et al.*, 2003). This radiotracer binds to membrane bound phosphatidyl serine, normally restricted to the inner side of the cell membrane but exposed extracellularly under apoptotic conditions. Currently, ^{99m}Tc-annexin is assessed in human tumors, but, to our knowledge, no animal studies have been conducted, except for pre-clinical studies on laboratory animals.

Bone scan

The occurrence of multiple skeletal sites of primary and secondary neoplasia has been well documented in man and animals. Radiographic surveys for skeletal evaluation of patients with primary bone tumors have limited diagnostic and prognostic value since 30-50% of bone mineral must be lost before lesions become visible. Bone scintigraphy is considered a very sensitive modality which provides the opportunity to screen the whole body (Fig. 11). ^{99m}Technetium labeled diphosphonates, which are incorporated in bone, are related to perfusion and the rate of bone turnover. Its place in oncology is mainly to detect skeletal and extraskeletal metastatic disease in man and dogs (Hahn *et al.*, 1990a; Peremans *et al.*, 2003; Forrest *et al.*, 1994; Daniel *et al.*, 1996; Parchman *et al.*, 1989; Schajowicz, 1983). Since bone scintigraphy is not specific (benign lesions may also show increased up-

take), it has to be combined with other ancillary diagnostic procedures to rule out benign disease or to distinguish among various types of tumors (Berg *et al.*, 1990; Forrest *et al.*, 1994; Hahn *et al.*, 1990b; Parchman *et al.*, 1989). The relationship between intensity of uptake and time to metastasis in dogs was demonstrated in a study including 25 dogs with osteosarcoma. The results in this study suggested that high pre-treatment uptake signifies aggressive tumors and early metastasis (Forrest *et al.*, 1992). False negative results may occur when osteoblastic activity is insufficient, such as in myeloma, and the ability to detect pulmonary metastases in dogs is poor and unreliable (Berg *et al.*, 1990; Lamb *et al.*, 1990).

The high uptake in reactive bone limits the use of bone scintigraphy in the determination of therapy outcome. Nevertheless, it may be valuable in the post-chemotherapy period for detecting new regions of increased bone uptake, because in these patients (man and dog) increased susceptibility to the development of bone metastases is seen with chemotherapy when compared with surgery alone (Berg *et al.*, 1990).

When limb sparing surgery is considered, bone scintigraphy may be used to delineate the extent of the primary bone tumor in dogs (Lamb *et al.*, 1990; Berg *et al.*, 1990; Parchman *et al.*, 1989). Bone scan images tend to overestimate tumor involvement compared to radiography, and may therefore provide more safe resection guidelines (Lamb *et al.*, 1990). Caution should be taken that this overestimation does not cause the surgeon to believe that the patient is not a suitable limb salvage candidate (Leibman *et al.*, 2001).

Sentinel node scintigraphy

^{99m}Technetium labeled human serum albumin colloid has been used for some decades in human medicine to determine the lymphatic spread of several tumors (melanoma, breast carcinoma). This radiocolloid is injected at the primary tumor site and its lymphatic drainage to regional lymph nodes is determined. Per-operative identification with hand-held gamma probes of these lymph node basins allows histological determination of the metastatic involvement of the lymph nodes at risk. The advantage is that true nodal drainage can be evaluated as these basins often do not correspond with the anatomically expected drainage basins (Berman *et al.*, 2001). In dogs the method has been evaluated in mamma carcinoma with preoperative gamma camera imaging and per-operative radioguidance with a hand-held gamma detector, and compared with blue dye injection and histology (Balogh *et al.*, 2002). On histology, 35 lymph nodes contained tumor cells, 34 (97%) were detected during radioguided surgery, and only 27 (77%) were stained blue. Of the 34 detected per-operatively with the probe, 31 (89%) were detected on the gamma camera images prior to surgery.

CONCLUSION

It is clear that nuclear medicine may help in diagnosis, staging and therapy prediction, albeit with different sensitivities depending on the type of tumor and with the restriction that small metastases may be overlooked due to resolution limitations. Extrapolating data from human oncology to veterinary oncology is not always evident. Knowledge of species and tumor dependent issues concerning the distribution and binding characteristics of the radiopharmaceuticals is therefore important. This is especially true in receptor imaging, where species and tumor type variations in the expression of receptors may occur. In this regard, biodistribution studies have been conducted for SRS and sestamibi in the dog. Abundant information is present concerning the use of nuclear imaging and radionuclide therapy in thyroid disease. Reports in the veterinary literature on other radiotracers are scarce and they are mainly limited to determining the extent of the disease. The reasons for the small number of studies are probably the limited access to scintigraphic modalities, radioprotection issues and financial restrictions concerning the use of more expensive radiopharmaceuticals. Despite these limitations, nuclear imaging may provide important information about the extent of the disease and, as a consequence, the prognosis of the patient. In addition, therapy strategies may also be influenced by the results of these imaging studies, thus preventing unnecessary treatment which would only be a physical burden for the animal and an emotional and financial burden for the owner.

REFERENCES

The reference list can be obtained from the first author.