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Review

Controlled Drug Delivery Vehicles in Veterinary Oncology: State-of-the-Art and Future Directions

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Abstract: Controlled drug delivery systems can be used to carry several anticancer agents, including classical chemotherapeutic agents such as doxorubicin, paclitaxel or cisplatin, and are also used for the encapsulation of tyrosine kinase inhibitors and monoclonal antibodies. Usually, the controlled systems are used to decrease drug toxicity, increase local drug concentration or target specific organs or systems. In dogs, liposomal doxorubicin is the most known controlled drug delivery vehicle in veterinary medicine. However, several antitumor drugs can be encapsulated within these systems. Since the delivery vehicles are a relatively new topic in veterinary oncology, this review aims to discuss the current knowledge regarding the controlled drug delivery vehicles and discuss the current challenges and future direction of its use in veterinary oncology.

Keywords: dogs; doxorubicin; nanoparticles; nanotechnology

1. Introduction

In the past years, veterinary medicine has been experiencing an increased life expectancy associated with the appearance of several aging-related diseases in pets [1]. Among these diseases, cancer is one of the most prevalent in older dogs [1]. The treatment options in veterinary oncology include surgical procedure [2], radiation therapy [3], conventional chemotherapy [4], target therapies [5], electrochemotherapy [6] or a combination of these modalities. Although all these therapies have been used in veterinary oncology, we still have poor prognosis when compared with human patients. For this reason, new antitumor therapies are required. Different from humans, cytoreductive chemotherapy is poorly explored for solid tumors in veterinary oncology and tumors as prostatic carcinomas [7], soft tissue sarcoma [8], osteosarcomas [9], hemangiosarcomas [10] and mammary gland tumors [1] show poor antitumor response. While conventional chemotherapy has been used in veterinary oncology, some drawbacks of chemotherapy are low therapeutic indices, lack of targets predicting antitumor response, development of drug resistance and low specificity for neoplastic cells.

Performing a critical review of the manuscripts published on PubMed about drug delivery systems in dogs, we identified 2338 publications and most of them, were performed in healthy dogs to evaluated pharmacological properties. Therefore, the current knowledge on drug delivery system in veterinary medicine is focused on the understanding of drug pharmacokinetics and pharmacodynamics, mainly focused on the human health [11–15]. Regarding the canine tumors, a high number of articles were on brain tumors [16–20]. The use of dogs as models for human brain tumors has been increasing in

the last years and these studies usually use controlled drug delivered vehicles in the experimental approach [16–20]. Although these studies have the human health as a primary focus, positive antitumor response can benefit dogs and humans. Different studies have used different drug delivered vehicles, including gold particles, liposomes and polymer-based nanoparticles [16–21].

There are a high number of studies evaluating drug delivery vehicles in healthy dogs, aiming to increase drug concentration in specific organs [22], drug bioavailability [13,21] or decrease drug toxicity [13,14]. Although a high number of studies have investigated pharmacokinetics of different drug delivery vehicles in healthy dogs, a limited number of studies have investigated drugs with anticancer properties in healthy dogs [11–14,21–23]. More intriguing, the translation rate of the studies performed in healthy dogs to dogs with cancer is very low. Most likely, because these studies in its majority aim to stablish drug pharmacokinetics with focus on human diseases [11,12]. Among the studies aiming to decrease drug toxicity through drug encapsulation, cisplatin [11,23], paclitaxel [14] and doxorubicin [12,24] were the most studied. Since the use of drug delivery vehicles is a relatively new topic in veterinary oncology, this review aimed to discuss the current knowledge regarding the controlled drug delivery vehicles and discuss the current challenges and future direction of its use in veterinary oncology.

2. Paclitaxel

Paclitaxel it is widely used in human medicine for treating different cancer subtypes, including metastatic breast cancer in the lungs [25,26]. Paclitaxel is an insoluble drug and should be combined with dehydrated alcohol and polyoxyethylated castor oil [27]. Unfortunately, this combination administrated intravenously have proved to induce severe and acute hypersensitivity in dogs and cats [28]. Due to its high hypersensitivity reaction during intravenous administration, Silva et al. [28] evaluated the paclitaxel subcutaneous administration expecting to find a lower rate of side effects. The results showed that even using subcutaneous administration, dogs presented several side effects and a low number of patients received more than one paclitaxel injection. Therefore, authors were not able to establish maximum tolerated dosage and no further studies have used this protocol.

Since one of the side effects of paclitaxel is associated to the hypersensitivity induced by the drug adjuvant, paclitaxel encapsulation in different controlled drug delivery vehicles were previously tested [14,29–31]. Axiak et al. [14] evaluated the safety of paclitaxel nanoparticles (CTI 52010) administration in healthy dogs. These authors showed that paclitaxel nanoparticles (CTI 52010), with a starting dosage of 80 mg/m^2, was well tolerated after intravenous administration and presented liver, kidney and spleen toxicity (evaluated by histopathology). On the other hand, Zhao et al. [30] evaluated paclitaxel liposomes for a lung target delivered system. Their liposomes were composed of Tween-80/HSPC/cholesterol (0.03:3.84:3.84, mol/mol), containing paclitaxel and lipids (1:40, mol/mol) [30]. These authors evaluated the pharmacokinetics of their preparation in 25 healthy dogs and demonstrated high lung concentration of the paclitaxel liposomes [30]. However, authors did not describe side effects of this administration.

Based on preliminary studies on paclitaxel nanoparticles (CTI 52010) [29], Selting et al. [29] evaluated the paclitaxel nanoparticles (CTI 52010) in tumor bearing dogs. In their study, paclitaxel nanoparticles (CTI 52010) was used in an increasing dosage raging to 80 mg/m^2 up to 136 mg/m^2. Fifteen dogs with different tumor subtypes were included and the maximum tolerated dosage could not be determined due the highly variable toxicity among all fifteen dogs [29]. Although it presents some preliminary results, the paclitaxel nanoparticles (CTI 52010) pharmacokinetics was similar in both health (N = 3) and tumor-bearing dogs (N = 15) and this formulation did not induce hypersensitivity. Thus, could be a promising treatment option.

3. Doxorubicin

Doxorubicin is an anthracycline antitumor drug originated as a product from *Streptomyces* classified as a chemotherapeutic from the antibiotic class [32]. It is widely used in veterinary medicine for

dogs with lymphoma [33], osteosarcoma [34], hemangiosarcoma [35] and mammary gland tumors [1]. However, in dogs [36] and in cats, relevant clinical cardiotoxicity can be highly nephrotoxic [37]. Therefore, new strategies to decrease doxorubicin toxicity has been studied [12]. In this scenario, doxorubicin liposomal encapsulation has been providing promising results [38–40]. Using domestic pigs as an experimental model to evaluate the potential of liposomal doxorubicin to induce cardiotoxicity, it was demonstrated a cardiotoxicity attenuation via induction of interferon-related DNA damage resistance [39]. Since the first description of liposomal doxorubicin, several manuscripts were published showing its efficacy in the clinical practice [12,41–48]. In a previous randomized controlled study evaluating both efficacy and toxicity of encapsulated doxorubicin into pegylated liposome compared to free doxorubicin, there was no statistical difference of overall survival in patients treated with free doxorubicin versus liposomal doxorubicin [42]. Besides that, in the studied group no patients developed cardiotoxicity (even treated with free doxorubicin) [42]. In their study, two dogs treated with liposomal doxorubicin experienced desquamating dermatitis like palmar-plantar erythrodysesthesia and other three presented anaphylactic reactions [42].

After the first studies, liposome-encapsulated doxorubicin has proved to decrease toxicity; however, the clinical efficacy has showed no improvement or only a modest improvement [12,41–48]. Thus, increased the search for different approaches aiming to increase antitumor response of liposome encapsulated chemotherapy [45]. Hauck et al. [45] evaluated the safety of a low temperature sensitive liposome-encapsulated doxorubicin related with local hyperthermia in dogs with sarcomas or carcinomas. The protocol was well tolerated with acceptable side effects and with favorable antitumor response [45]. Recently, Bredlau et al. [12] evaluated the pharmacokinetics of temperature sensitive liposomes containing doxorubicin associated with hyperthermia across the canine blood–brain barrier [12]. Their protocol was effective and showed high concentration temperature sensitive liposomes in the central nervous system and the normal tissue presented a very low toxicity [12]. Therefore, this therapy could be promising treating patients with primary brain tumors.

4. Cisplatin

Cisplatin is a well-known platinum-based anti-cancer chemotherapy drug used to treat different cancer subtypes [49]. Usually show high nephrotoxicity and should be administrated with a diuresis protocol [49]. However, a newer platin-derived drug was developed with similar mechanism of action and lower nephrotoxicity [50]. Thus, since carboplatin is less toxic than cisplatin and do not need a diuretic protocol, it is a Food and Drug Administration (FDA) approved treatment [51].

Currently, carboplatin is a widely used chemotherapeutic drug, including in the treatment of ovarian, bladder, breast and esophageal cancers [50]. When compared to antitumor effects of carboplatin and cisplatin, for some tumor subtypes, cisplatin still shows a better antitumor response than carboplatin. As a result, new strategies for the cisplatin safety use was required [52]. Aiming to reduce cisplatin toxicity and increase the drug concentration, cisplatin encapsulation in a liposomal formulation (SPI-77) was previously evaluated [14,53]. The cisplatin liposomal encapsulation allows delivered drug concentration five times more the maximum tolerated dosage when compared to free cisplatin [14,53]. The same research group published the evaluation of SPI-77 cisplatin formulation in healthy dogs [14] and dogs with osteosarcoma [53]. First, this research group evaluated SPI-77 formulation in liposomes containing a pegylated lipid [N-(carbamoyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero3-phosphoethanolamine sodium salt, MPEG-DSPE] in osteosarcoma-affected dogs [53]. In their previous study, dogs were treated with SPI-77 formulation containing cisplatin (STEALTH) versus dogs treated with maximum tolerated dosage of carboplatin and they demonstrated no increased toxicity of STEALTH formulation and identified five times higher concentration of drug delivered when compared to free cisplatin. However, their study did not show the difference in overall survival between both treatments [53].

Then, this research group published a manuscript evaluating the efficacy of the liposome encapsulate cisplatin in healthy dogs [14]. The liposome formulation was composed by

dipalmitoyl phosphatidyl glycerol, soy phosphatidyl choline, cholesterol, and methoxy-polyethylene glycol-distearoyl phosphatidylethanolamine. Four different dosages were tested, including 70, 100, 125, and 150 mg/m^2 in a small group of dogs (N = 4). As expected, the side effects were more frequently in the group treated with higher dosage; however, being acceptable. Thus, authors concluded that the dosage of 150 mg/m^2 can be used without association of hydration protocols [53]. However, no further studies evaluated this formulation in tumor-bearing dogs.

Based on the systemic toxicity using free-cisplatin, Venable et al. [54] used a natural polysaccharide (Hyaluronan) nanocarrier to conjugate with cisplatin and treat dogs with soft tissue sarcomas. After hyaluronan metabolization, the lymphatic system is responsible for its metabolites elimination via lysosomal and endocytosis degradation [54]. Thus, can be promising in intratumoral formulations. These authors tested their hyaluronan-cisplatin nanoconjugate intratumorally in five client-owned dogs and found no local reaction related to drug administration. Besides that, authors found a higher concentration of cisplatin (1000×) intratumorally compared to serum concentrations. Since it was the first manuscript using this formulation in dogs with soft tissue sarcomas, they did not focus on antitumor response. Therefore, in 2016 a phase I/II clinical trial in dogs with spontaneous cancers treated with Hyaluronan-Cisplatin Nanoconjugate was performed [52]. In this clinical trial, 16 dogs with different tumors subtyped were used, including anal sac carcinoma, oral squamous cell carcinoma, oral melanoma, nasal carcinoma and digital squamous cell carcinoma. A complete response was observed in three dogs (3/16), one experienced partial response (1/16) and other one stable disease (1/16). Thus, the formulation failed in show antitumor response in 69% of the patients (11/16). Interestingly, three patients with complete response had carcinomas from head and neck (oral or nasal carcinomas). Consequently, this formulation could be promising for carcinomas in this location. However, a new clinical trial should be performed to clarify if this formulation can benefit dogs with head and neck carcinomas. Overall, the current information does not support the use of Hyaluronan-Cisplatin Nanoconjugate in tumor-bearing dogs.

5. Small-Molecule Inhibitors and Monoclonal Antibodies

The delivered systems can be used to carrier several anticancer agents, including tyrosine kinase inhibitors and monoclonal antibodies (Figure 1) [55]. Among the small-molecule inhibitors tested in dogs, masitinib [56,57], toceranib [58,59] and sorafenib [5,60] has been most studied *in vitro* and *in vivo*. Although these drugs can be promising in the treatment of dogs with cancer, one of the most important limitation of these drugs is the high toxicity. Toceranib [58,59], one of the most studied small-molecule inhibitors, usually cannot be used at the maximum tolerated dosage (3.25 mg/kg) due to its high toxicity. In this scenario, the controlled drug vehicles can be used to minimize toxicity and increase tissue concentration.

Sorafenib is an important tyrosine kinase inhibitor in human medicine, usually used to patients with hepatocellular carcinoma [21]. This tyrosine kinase inhibitor has been studied recently in veterinary medicine [5,60], with sorafenib safety in dogs evaluated by Foskett et al. [60] and its *in vitro* efficacy against canine mammary gland tumors demonstrated by Prado et al. [5]. Due to sorafenib low aqueous solubility, Park et al. [21] loaded sorafenib in nanoparticles containing fat and supercritical fluid (NUFS™) to improve sorafenib absorption. Among the evaluated studies in this review, this is the only one using nanoparticles in a tablet formulation for oral administration [21]. The *in vivo* experiments using Beagle dogs (N = 21) demonstrated sorafenib optimization, exhibiting higher serum profiles. Thus, indicating that this formulation increased sorafenib solubilization. Since it is a recently published paper, no further studies have addressed antitumor effect.

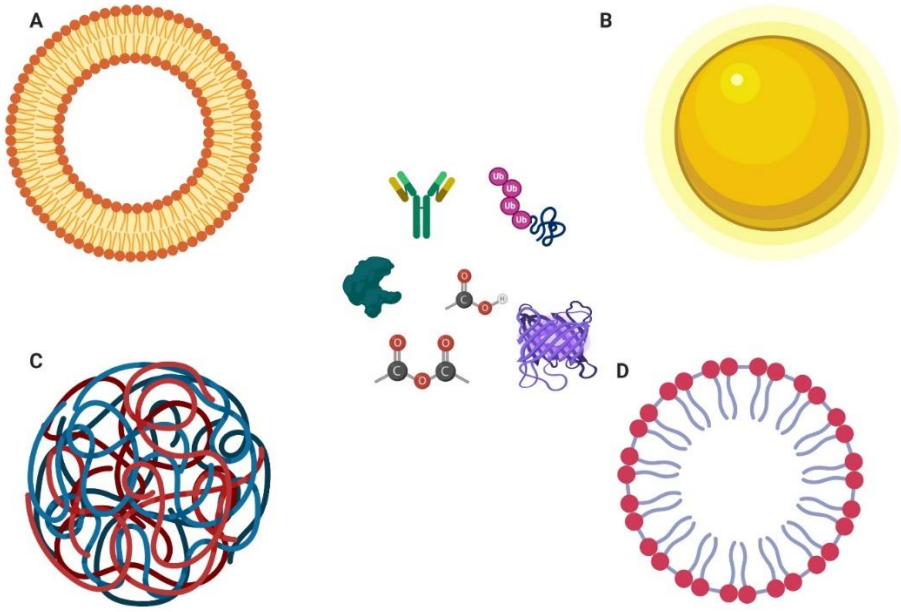

Figure 1. Representation of some controlled drug delivery vehicles, including liposome (**A**), gold Nanoparticles (**B**), polymeric particle (**C**) and micelle (**D**). Using the different systems, it is possible to encapsulate classical antitumor agents, small-molecule inhibitors, monoclonal antibodies, gene target therapies and RNA interference. Created by Biorender (https://app.biorender.com/).

Cetuximab is a human-mouse chimeric monoclonal antibody, primarily used to treat patients with non-small cell lung carcinoma and other tumors with EGFR overexpression [61]. Since cetuximab is a humanized monoclonal antibody, it is predicted to be specific to human proteins. However, Singer et al. [62] demonstrated a homology over than 90% among EGFR proteins family and demonstrated that cetuximab binds canine mammary carcinoma cells. Therefore, cetuximab demonstrated antitumor activity through inhibition canine mammary gland tumor cell proliferation, being a promising option in canine tumors [62]. Freeman et al. [19] developed a cetuximab conjugated iron-oxide nanoparticles (cetuximab-IONPs) aiming to increase cetuximab concentration in central nervous system. These authors performed a pilot study in dogs with gliomas (N = 8) implanting two FDA-approved catheters (Medtronic intrathecal catheters Indura 8709SC and Ascenda 8780) after surgery in the remaining tumor region. These authors confirmed that IONPs target tumor area through resonance magnetic image magnetic resonance imaging and is safe and effective against canine gliomas.

6. Tumor Microenvironment and Drug-Delivered Systems

Tumor microenvironment (TME) is widely studied in human oncology, and it is well accepted that TME is pivotal to determine tumor aggressiveness, prognosis and response to antitumor therapies [63,64]. TME consists in the interaction among extracellular matrix components, such as collagen, stromal fibroblasts, macrophages, lymphocytes and endothelial cells [63]. These cellular and non-cellular components are remodeled according to each tumor characteristics providing a network's formation benefiting cancer cells. In this scenario, TME can directly modify antitumor response against different drugs and delivered systems [63]. In relation to dogs, TME is poorly understood and few studies have investigated interaction between microenvironment and tumor cells [65–68]. In canine prostate cancer, higher expression of elastic fibers associated with decreased Col-IV expression were previously associated with high tumor grade [67]. The lack of Col-IV is strongly correlated with tumor invasion through stroma and the high expression of elastic fibers, can be related with a stroma reaction against the invasive cells. This interaction between tumor cells and stroma reinforce the impact of TME in therapeutic strategies. In this concept, nano drug delivery systems targeting TME has been studied [69].

The preexisting tumor stroma can represent a physical barrier for proper drug delivery and the use of different systems can induce therapeutic resistance due TME [69]. Among the cellular components, few studies have investigated TME canine tumors. The lymphocytes, macrophages, fibroblast and T-regulatory cells were previously investigated, mainly in mammary gland tumors [66,68,70]. Overall, these studies demonstrated similarities between canine mammary gland tumors and human breast cancer, with tumor associated macrophages and lymphocytes presenting a correlation with patients' overall survival. Besides these cellular components, Ettlin et al. [71] performed a comparative analysis of Col-1, αSMA, Cavelolin-1, MMP2 and other matrix components of canine mammary gland tumors and human breast cancer. In their comprehensive analysis, they identified similarities in TME, proposing dogs as a model for the breast cancer TME study.

7. Development of Controlled Drug Delivery Systems and Future Perspectives

In veterinary oncology, we lack standardization of the drug delivered systems with studies testing the different systems in small group of animals. Liposomes and nanoparticles are the most used carriers in veterinary oncology [72–75] and more recently, gold nanoparticles have been introduced *in vitro* [76–78]. In dogs, liposomal doxorubicin is commercially available (Doxil/Caelyx), presenting an effective antitumor response and lack of cardiotoxicity [72]. However, its clinical use is limited due the commercial price. Thus, conventional doxorrubicina still remains the most used in veterinary oncology. However, since dogs are pharmacokinetics model to human drugs, the tested nano-carriers canine preclinical models represent an opportunity to benefit dogs. Since the liposomes are the most studied nanocarrier in both humans and dogs represents a promising method for cytostatic drug encapsulation [72]. Liposomes are artificial and spherical nanocarrier with size varying of 30 nm until micrometers [73]. Thus, based on the previous results using liposomal doxorubicin, liposomal vehicles can be considered promising in veterinary oncology.

Zabielska-Koczywas and Lechowski [72] reviewed liposomes and nanoparticles as drug delivery systems to improve cancer treatment in dogs and cats in 2017. Based on their description, we performed a Table showing all studies describing use of drug delivery vehicles in dogs (Table 1). At that time, these authors identified six clinical trials registered at American Veterinary Medical Association (AVMA) involving drug delivery vehicles in veterinary oncology. Currently, three clinical studies are registered at AVMA website in oncology section being one study for the use of iron nanoparticles for the investigation of metastatic lymph nodes (AAHSD004735), one pilot study of AuroLase® Therapy for the Treatment of Solid Tumors in Canine and Feline Patients (AAHSD000007) and one study in phase I/II, evaluating Cisplatin Hyaluronate Nanoparticles in Tumor-bearing Dogs (AAHSD000024) (search performed on 12th March 2020).

Table 1. Studies describing use of drug delivery vehicles in dogs depending on the chosen substance.

Substance	Nanomaterial	Tumour Subtype	Study Results	Outcome	Reference
-	Silica- coated gold nanoparticles (GNPs)	Brain tumors	GNPs were more frequently found in tissue areas closer to blood vessel walls and had heterogeneous extravasation into spontaneous brain tumors.	Nanoparticles' EPR and its variations foreshadow clinical applications of nanomedicine in management of brain tumors	Arami et al. [16]
-	Colloidal solution of Gold nanorods (Nanopartz)	Prostate	Optical detection of Au NPs via the proportional absorption of the product (NP concentration and the individual absorption cross-section).	Gold nanoparticles can be used for *ex vivo* systematic study of canine and, potentially, human prostate; comprehensive diagnostic markers in determining the state of the prostate health	Grabtchak et al. [22]
Plant-based virus-like nanoparticle (VLP)	Magnetic iron oxide nanoparticle (mNPH)	Oral melanoma	Immunological reaction in the tumor correlated with the clinical response and significant increase in immune cell infiltration of tumors receiving radiotherapy with VLP treatment.	The study successfully demonstrates the feasibility, safety and promising efficacy of VLP + radiotherapy treatment in a highly translatable spontaneous preclinical model	Hoopes et al. [74]
Carmustine	Lipid nanoemulsion (LDE)	Lymphoma	LDE-carmustine showed non-hematologic toxicity or hepatic function commitment; LDE- carmustine and commercial carmustine were equivalent in terms of toxicity, tumor remission and survival time.	LDE- carmustine is safe for administration in a combined chemotherapeutic protocol with vincristine and prednisone	Lucas et al. [75]
Cetuximab	Iron-oxide nanoparticles (cetuximab-IONPs)	Spontaneous intracranial gliomas	Volume of distribution was proportional to infusion volume and dispersion of the cetuximab-IONPs and infusion can be delivered in awake dogs safely and effectively over 3 days.	Cetuximab-IONP CED is a safe and effective adjuvant therapy for spontaneous canine glioma patients at the time of their initial tumor surgery.	Freeman et al. [19]
Cisplatin	Hyaluronan-cisplatin nanoconjugate (HA-Pt)	Spontaneous cancers	Cisplatin and HA-Pt inhibited cell growth over 80% compared to control and cisplatin treatment showed similar levels of creatinine excretion. HA-Pt did not cause nephrotoxicity.	The HA-Pt formulation demonstrated positive response in spontaneous canine squamous cell carcinomas	Cai et al. [52]
Cisplatin	Platin-M nano- particles (T-Platin-M-NPs)	Canine J3TBG glioma and SDT3G glioblastoma cell lines	T-Platin-M-NPs can be effective in glial cell canine tumors and its activity is better than cisplatin and carboplatin which are currently used as chemotherapeutic agents.	There is potential to use T-Platin-M-NPs as an effective injectable chemotherapeutic agent in dogs	Feldhaeusser et al. [23]
Cisplatin	Liposome-encapsulated	Healthy animals	Toxic effects commonly associated with unencapsulated cisplatin, were not observed in dogs treated with liposome-encapsulated cisplatin at dosages equivalent to twice the known maximally tolerated dose of unencapsulated cisplatin.	Liposome- encapsulated cisplatin can be safely administered to clinically normal dogs at dosages of up to 150 mg/m^2 without the need for concurrent hydration protocols	Marr et al. [14]

Table 1. *Cont.*

Substance	Nanomaterial	Tumour Subtype	Study Results	Outcome	Reference
Cisplatin	STEALH Liposome-encapsulated cisplatin (SPI-77)	Osteosarcoma	The 11-month overall median survival found for SPI-77-treated dogs was nearly identical to that reported using native cisplatin. The systemic delivery of these escalated doses did not translate into enhanced efficacy.	Liposome encapsulation of cisplatin allows the safe and repeated delivery of doses up to five times the maximally tolerated dose of native cisplatin in tumor bearing dogs	Vail et al. [53]
Cisplatin	Hyaluronan nanocarrier	Soft tissue sarcomas	No tissue reactions were detected after hyaluronan-cisplatin injection; intratumoral administration of hyaluronan-cisplatin resulted in higher concentrations in the tumor and sentinel lymph nodes than in plasma or serum.	Intratumoral injection of the hyaluronan-cisplatin nanoconjugate was well tolerated in treated dogs and may be a safe and effective method for the administration of maintenance chemotherapy	Venable et al. [54]
Co(II)-NanoTS265 and Zn(II)-NanoTS262 compounds	Gold nanoparticles (AuNPs)	Mammary tumours (FR37-CMT) cells	Both compounds induced a reduction of viable cell. AuNPs can act as promising carriers for drug delivery; increased cytotoxic activity; metal compounds displayed lower IC_{50} than cisplatin and doxorubicin against cells.	NanoTS262 and NanoTS265 are promising chemotherapeutic formulations for mammary carcinomas and targeting anti-bodies or peptides, may further improve efficacy	Raposo et al. [76]
Cu-64	PEGylated liposomes with copper-64	Spontaneous solid tumors	New and highly efficient method for loading copper-64 PET isotopes into liposomes; moderate to high nanocarrier tumor accumulation levels were achieved in spontaneous carcinomas based on the EPR-effect.	Radiolabeled liposomes may serve as theragnostic imaging agent guiding both diagnostic and therapeutic intervention for several malignancies in future clinical practice	Hansen et al. [77]
Doxorrubicin	PEGylated liposomes with Copper-64	Splenic Haemangiosarcoma	Intraperitoneal treated dogs had fewer serosal, mesenteric, and omental metastases than historical controls treated with systemic doxorubicin. Pegylated liposomal doxorubicin was absorbed relatively quickly from the abdominal cavity.	Intraperitoneal pegylated liposomal encapsulated doxorubicin administration did not prevent intraabdominal recurrence of HSA in dogs	Sorenmo et al. [41]
Doxorrubicin	PL-DOX (Doxil®, Sequus Pharmaceuticals, Menlo Park, CA, USA)	Splenic Haemangiosarcoma	The median disease-free period for dogs treated with PL-DOX and free doxorubicin was equivalent. No significant differences in toxicity between PL-DOX and free doxorubicin were noticed.	PL-DOX was easily administered to dogs and did not lead to significant toxicities and significant difference in survival was not observed	Teske et al. [42]
Doxorrubicin	PL-DOX (Doxil®, Sequus Pharmaceuticals, Menlo Park, CA, USA)	Different tumors type	The dose-limiting toxicities are different for Doxil when compared to free doxorubicin and o significant myelosuppression or cardiotoxicity was noted in Doxil treatment.	Although the results of this study must be evaluated with care owing to the small number in each tumor group, it appears that Doxil as a single agent may have a broad spectrum of activity	Vail et al. [38]

Table 1. *Cont.*

Substance	Nanomaterial	Tumour Subtype	Study Results	Outcome	Reference
Doxorrubicin	Colloid gold nanoparticles	Feline fibrossarcoma	A higher cytotoxic effect of Au-GSH-Dox than that of free doxorubicin has been observed. GSH coated Au NPs are good doxorubicin nanocarriers for feline fibrosarcoma cell lines with high P-gp activity.	Au-GSH-Dox may be a potent new therapeutic agent to increase the efficacy of the drug by overcoming the resistance to doxorubicin in feline fibrosarcoma cell lines	Wójcik et al. [43]
Doxorrubicin	Polylactide nanoparticles (NPs) loaded with doxorubicin (Doxo) and coated with bone-seeking pamidronate (Pam) Pam-Doxo-NPs	Osteosarcoma	Enhanced bone tumor accumulation and prolonged retention compared with nontargeted NPs. The therapeutic was well tolerated without toxicities and attenuated localized osteosarcoma progression compared with nontargeted Doxo-NPs.	Pam-Doxo-NPs were capable of minimizing systemic off target toxicities and enhance localized antitumor activities in a preclinical murine tumor model and canine model	Yin et al. [44]
Doxorubicin	Temperature sensitive liposomes (TSL)	High-grade gliomas	Higher temperatures resulted in increased area of cerebral damage and TSL combined with hyperthermia allows potentially therapeutic doses of Dox across the blood–brain barrier.	The study demonstrates that localized doxorubicin delivery to the brain can be facilitated by TSL-Dox with localized hyperthermia with no significant neurological deficits	Bredlau et al. [12]
Doxorubicin	Low temperature sensitive liposome (LTSL-doxorubicin)	Solid tumors (carcinomas or sarcomas)	The dose of LTSL doxorubicin was suboptimal in many patients and had a 30% response rate and cutaneous toxicity typically observed with LTSL-doxorubicin.	LTSL-doxorubicin offers a novel approach to improving drug delivery to solid tumors; it was well tolerated and resulted in favorable response profiles in these patients	Hauck et al. [45]
Doxorubicin	Liposome-encapsulated Doxorubicin (L-DOX)	Multiple Myeloma	Liposome encapsulation of doxorubicin decreases its cardiotoxicity; the remission induced with L-DOX was complete and durable; L-DOX has a decreased toxicity compared with free doxorubicin even with a high dose	L-DOX has greater efficacy in the treatment of some tumors and decreased toxicity compared with free doxorubicin, without cardiotoxicity	Kisseberth et al. [46]
Doxorubicin	Pegylated Liposomal Doxorubicin	Feline soft tissues sarcomas	Median overall survival was 324 days and all cats developed some degree of leukotrichia. The liposomal doxorubicin and daily fractionated palliative radiotherapy were well tolerated by the cats.	The administration of pegylated liposomal doxorubicin in combination with daily palliative radiotherapy was feasible, generally well tolerate	Kleiter et al. [47]
Ferumoxytol	Ultra-small superparamagnetic iron oxide nanoparticle (USPIOs) lymphotropic nanoparticle enhanced MRI (LNMRI)	Metastatic lymph nodes in Canine Head and Neck Tumors	There were no negative side effects to the USPIOs noted and LNMRI was successful in identifying metastatic lymph nodes with 100% sensitivity and an 88% specificity.	LNMRI has the potential to be a sensitive and specific method of diagnosing lymph node metastasis	Griffin et al. [48]

Table 1. *Cont.*

Substance	Nanomaterial	Tumour Subtype	Study Results	Outcome	Reference
Gold	Gum arabic-coated 198AuNP (GA-198AuNP)	Prostate cancer	GA-198AuNPs have more homogenous dose distribution and higher emission of energy compared to current brachytherapy seeds used to treat prostate cancer and the gum arabic glycoprotein provided a nontoxic coating on NPs and is highly stable *in vivo*	This study provides evidence that intralesional injection of GA-198AuNP is safe with minimal short-term systemic toxicity in the naturally occurring large animal model of prostatic cancer	Axiak-Bechte et al. [78]
High affinity histidine×6-tagged EGFR-binding Z domain (heptameric ZEGFR domain).	Lipid-based oil-filled nanoparticles (NPs) with a high concentration of surface-chelated nickel (Ni-NPs)	A431 epidermoid carcinoma cells	Superior cell uptake achieved in EGFR overexpressing cells with these Ni-NPs with ZEGFR target; successfully target EGFR overexpressing cancer cells; targeting efficiency of the novel heptameric ZEGFR domain was also demonstrated *in vivo*	Ni-NPs could be a very useful tool for targeting and drug delivery to a wide range of EGFR positive cancers	Benhabbour et al. [79]
Nerve growth factor (NGF)	Encapsulated chitosan nanoparticles (CNPs)	Mesenchymal Stem Cells	NGF had 61% efficiency *in vitro* and these nanoparticles were found to be cytocompatible to Mesenchymal Stem Cells; NGF- CNPs were able to transdifferentiate cBM-MSCs without any chemical based reinduction.	NGF-CNPs are capable of releasing bioactive NGF with the ability to transdifferentiate mesenchymal stem cells into neurons	Mili et al. [80]
Paclitaxel	Nanoparticule paclitaxel (CTI 52010)	Healthy animals. focused on spontaneously occurring tumors	No evidence of hypersensitivity or gastrointestinal toxicity. The dose-limiting toxicity was grade 4 neutropenia and the maximum tolerated dosage was 120 mg/m^2.	CTI 52010 was well tolerated when administered intravenously to normal dogs	Axiak et al. [11]
Paclitaxel	Nanoparticulate paclitaxel (nPX) formulation (CTI52010, Crititax®)	Prostate cancer cell lines (human and dogs)	nPX was as effective as PX in decreasing cell viability, increasing apoptosis, inhibiting clonogenic potential, and modifying microtubule dynamics; it could be an effective alternate for PX	Nanoparticulate paclitaxel is as effective as paclitaxel in both human and canine castration-resistant prostate cancer	Axiak-Bechtel et al. [31]
Paclitaxel	Nanoparticulate, excipient-free formulation of paclitaxel (CTI52010)	Different tumors type	The lack of systemic absorption after subcutaneous administration; side effects were well tolerated at dosages up to 118 mg/m^2; no unique toxicity or hypersensitivity was noted	CTI52010 was administered safely to tumor-bearing dogs and is an attractive chemotherapeutic to be considered in intratumoral administration	Selting et al. [29]
Paclitaxel	Liposomes composed of Tween-80/HSPC/cholesterol	Healthy animals	The paclitaxel liposomes showed excellent lung targeting properties in comparison with paclitaxel injection; liposome carrier was associated with a lung-targeting effect; didn't cause hemolysis	The liposomes are a promising carrier for a lung-targeting drug delivery system for the treatment of lung diseases, such as lung cancer	Zhao et al. [30]

Table 1. *Cont.*

Substance	Nanomaterial	Tumour Subtype	Study Results	Outcome	Reference
Sorafenib	Nanoparticulation using fat and supercritical fluid (NUFs)	Healthy animals	Encapsulated sorafenib exhibited higher blood drug profiles indicating better absorption; concentration of PVP should be kept at low level to achieve the maximum absorption of sorafenib	Nanoparticle formulation could enhance the bioavailability and therapeutic efficacy of sorafenib	Park et al. [21]
Temozolomide (TMZ)	Polymeric magnetite nanoparticles (PMNPs)	Intracranial tumors	In 70% of the cases, the infusion accurately targeted the tumor mass was determined by the presence of PMNP signal in the tumor on immediate postoperative MRI; PMNP was enough to induce an observable decrease in tumor volume	T convection-based drug delivery using PMNPs can be safely performed in a canine model of glioma	Young et al. [18]
Vincristine	Liposomes	Healthy animals	Encapsulated vincristine showed a higher plasmatic concentration then un-encapsulated vincristine after intravenous injection of both forms of vincristine; enhanced anti-tumor activity and lower toxicity of liposome	The increased therapeutic index of encapsulated vincristine is demonstrated by the pharmacokinetic features	Zhong et al. [81]

One limitation for the use of controlled drug delivery systems in veterinary medicine, is the final price of the treatment for the owner. Overall, drugs using the different systems show a higher price compared to free chemotherapeutic agents. However, since the use of small-molecule inhibitors and monoclonal antibodies has growing in the past years, the encapsulation of these drugs can decrease toxicity of small-molecule inhibitors and enhance drug concentration in specific sites.

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