Chemotherapy in Small Animal Oncology

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Chemotherapy is a major treatment modality in veterinary oncology either used as sole treatment, for conditions such as lymphoma, or in the adjuvant setting for many other tumour types. There are a variety of chemotherapeutic classes each with specific mechanisms of action and clinical uses. Understanding the differences between each drug, their indications for use, and knowing possible adverse effects and their management is essential to help select the most appropriate treatment course for an individual patient.

PRINCIPLES OF CHEMOTHERAPY

At a cellular level, the aim of chemotherapy is to kill rapidly dividing (neoplastic) cells and slow/delay the growth and spread of the tumour. For the patient, chemotherapy aims to reduce morbidity and mortality secondary to their cancer and, most importantly, improve quality of life.

An understanding of the cell cycle is important when considering how various chemotherapy agents work. The cell cycle is divided into four main stages (Figure 1): G1 (growth) phase is where cell contents (excluding chromosomes) are duplicated; S-phase is where DNA replication occurs; G2 (growth) phase is where replicated DNA is checked; and finally, mitosis takes place in M-phase.

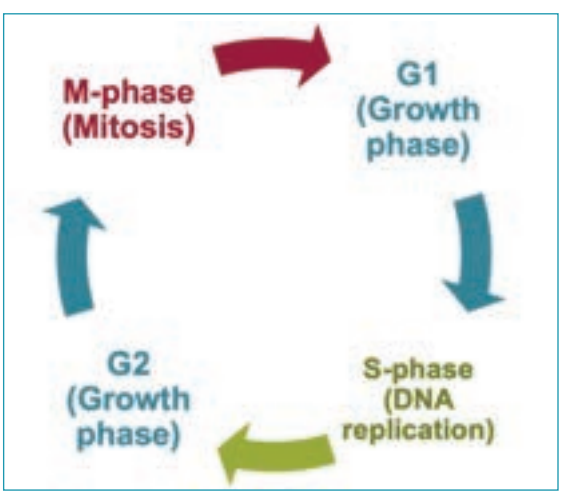

Figure 1: A summary of the cell cycle, demonstrating the four main stages.

M-phase is then divided into further stages: prophase (chromosome condensation); metaphase (nuclear envelop breaks down, microtubules attach to chromosomes, chromosomes align on metaphase plate); anaphase (sister chromatids pulled to opposite ends of the cell, cell starts to split); telophase (chromosome de-condense, nuclear envelopes re-form, spindle dismantled). As will be discussed later in the article, chemotherapy agents target specific phases of the cell cycle depending on their mechanism of action. As tumour cells are rapidly dividing, chemotherapy selectively targets these cells compared to 'normal' cells. There are some exceptions as other cells, such as those within the gastrointestinal epithelium and bone marrow progenitor cells, are also rapidly dividing and therefore targeted by chemotherapy; this explains why gastrointestinal toxicity and myelosuppression are among the most common adverse effects seen with chemotherapy.

There are terminology and concepts specific to oncology which are also useful to understand:

- **"Maximum tolerated dose (MTD)"** – this describes the maximum dose of a chemotherapeutic that can be administered, that avoids unacceptable adverse effects. Most chemotherapy protocols are designed to administer the MTD of each chemotherapy agent, with the intent to kill neoplastic cells.
- **"Metronomic chemotherapy (MC)"** – this describes the concept of lower-dose, daily, oral chemotherapy that is administered longer term. Rather than killing neoplastic cells, the intent of MC is to delay or slow disease progression by inhibition of angiogenesis and modulation of the immune-response to the tumour (by downregulating T-regulatory cells).
- **"Targeted therapy"** – targeted therapies are those designed to target specific receptors, signalling pathways and antigens. These include monoclonal antibodies and kinase inhibitors (e.g. toceranib and masitinib).
- **"Cell-cycle specific"** – certain chemotherapy agents act during specific parts of the cell cycle and therefore may require actively proliferating cells in order to work, depending on the stage of the cell cycle they target.
- **"Cell-cycle non-specific"** – this term describes chemotherapy agents that act throughout all phases of the cell cycle.
- **Body surface area (BSA; m^2)** – most chemotherapy agents are dosed by BSA rather than bodyweight, as relevant pharmacokinetic factors (cardiac output, creatinine clearance, body fat) are related to body size rather than weight.

HEALTH AND SAFETY CONSIDERATIONS

It is important to consider the potential risks to humans associated with handling and administering cytotoxic drugs; in the most severe forms these can include abortion, foetal deformities and carcinogenesis. Routes of exposure can include skin contact, skin absorption, aerosol/drug particle inhalation, ingestion and needle-stick injuries.

A fume hood is essential when preparing chemotherapy to limit exposure to hazardous fumes or vapours. A closed,

Figure 2: Administration of intracavitary chemotherapy to a patient with mesothelioma. Staff handling the patients are wearing the appropriate waterproof full-length gowns and chemotherapy resistant gloves.

needle-free system should also be used to reduce the risk of drug spillage (e.g., BD PhaSeal™, Equashield®, ChemoLock™). Personal protective equipment must include gloves (chemotherapy/cytotoxic resistant) and full-length waterproof gown; a mask and visor may also be required for certain drugs during preparation or administration (e.g., L-asparaginase). If these facilities or equipment are not available within your practice, there are companies that can send chemotherapy dose-by-dose, ready to administer with the required PPE negating the need to prepare the drugs in practice.

Chemotherapy should be administered in a dedicated chemotherapy room or in a room that is quiet, has minimal through-traffic, and can be cleaned after chemotherapy administration. All staff involved with chemotherapy administration, including those restraining the patient, should wear the appropriate PPE (Figure 2). The intravenous catheter must be placed 'first-stick' as extravasation of certain chemotherapeutics can cause potentially severe tissue damage; a closed needle-free system, as previously described, should be used when administering the chemotherapy.

The patient must be adequately restrained to avoid sudden movements during intravenous catheter placement or chemotherapy administration, especially during longer infusions. The use of topical anaesthetic creams or cold sprays can be useful for catheter placement, and the use of drugs such as butorphanol can be helpful to facilitate chemotherapy administration. When giving oral chemotherapy it is important to never split, crush or open capsules/tablets; gloves must also be worn when handling them.

Following chemotherapy administration, patients and patient waste must be handled appropriately; this is important for both the veterinary team and patient owners. Residual chemotherapy/metabolites are found in the urine, faeces, saliva and sweat following chemotherapy administration albeit at low levels. The length of time of excretion varies depending on the specific drug but can vary from just two days for chlorambucil, up to eight days following carboplatin administration. During this period, the owners should wear gloves when handling faeces, rinse down any areas in the garden where the patient urinates, and avoid excessive contact with saliva (e.g., licking).

DRUG CLASSES, MECHANISMS OF ACTION AND INDICATIONS

The main chemotherapy classes include alkylating agents, platinum agents, antitumour antibiotics, antimetabolites and antimicrotubule agents. Targeted therapies that will also be discussed include tyrosine kinase inhibitors (TKIs). A summary of the chemotherapy classes, their mechanism of action, most clinically relevant individual drugs and their uses is presented in Table 1 (page 534).

Alkylating agents

Alkylating agents are classified into nitrogen mustards (melphalan, cyclophosphamide, chlorambucil), nitrosureas (lomustine, streptozotocin) and 'other' (dacarbazine, procarbazine, temozolomide). After drug administration there

(continued on page 535)

Drug Class	Mechanism of Action	Specific Drugs	Clinical Uses
Alkylating agent	Binding of alkyl group to DNA, causing DNA cross-link formation and cell death	Chlorambucil	Lymphoma (low-grade), chronic lymphocytic leukaemia, metronomic chemotherapy
		Cyclophosphamide	Lymphoma, haemangiosarcoma, metronomic chemotherapy
		Lomustine	Lymphoma, mast cell tumours, histiocytic sarcoma
		Melphalan	Multiple myeloma, plasma cell tumours
Platinum agents	Binding of platinum agent to DNA, causing DNA cross-link formation and cell death	Cisplatin	Osteosarcoma, mesothelioma, but rarely used due to nephrotoxicity. Must not use in cats (causes fatal pulmonary vasculitis and oedema)
		Carboplatin	Osteosarcoma, mesothelioma, variety of carcinomas. Can be used as a radiosensitiser during radiotherapy.
Antitumour antibiotics	Doxorubicin mainly inhibits topoisomerase-II, causing DNA breakage and cell death. Also acts by DNA intercalation and free radical formation.	Doxorubicin/epirubicin	Lymphoma, haemangiosarcoma, other (sarcoma, carcinoma, histiocytic sarcoma)
		Mitoxantrone	Lymphoma, haemangiosarcoma, other (mesothelioma, canine transitional cell carcinoma, anal sac adenocarcinoma)
Antimetabolites	Inhibit the use of cellular metabolites used in the course of cell growth and division	Cytarabine (pyrimidine antagonist)	Lymphoma (CNS involvement), leukaemia
		Rabacfosadine	Lymphoma (B-cell)
Antimicrotubule agents	Inhibit with polymerisation (vinca alkaloids) or depolymerisation (taxanes) of the microtubules that play important roles in cell function and division	Vincristine (vinca alkaloid)	Lymphoma, transmissible venereal tumour
		Vinblastine (vinca alkaloid)	Mast cell tumour, transitional cell carcinoma
		Paclitaxel and docetaxel (taxanes)	Little clinical use due to high risk of acute hypersensitivity reactions, due to excipients
Tyrosine kinase inhibitors	Inhibition of various receptor tyrosine kinases such as KIT, PDGFR, VEGFR	Toceranib	Mast cell tumours, anal sac adenocarcinoma, gastrointestinal stromal tumours, other (carcinomas, sarcomas, neuroendocrine tumours)
		Masitinib	Mast cell tumours
L-asparaginase	Deprives neoplastic cells of asparagine, disrupting protein synthesis leading to cell death	L-asparaginase	Lymphoma, acute lymphoid leukaemia

Table 1: A summary of the main chemotherapy classes, their mechanisms of action, most used individual drugs within each class, and clinical use.

is covalent binding of an alkyl group to the DNA, usually onto a guanine base, causing inter- or intra-strand cross-link formation preventing DNA uncoiling and leading to cell death. Alkylating agents are cell-cycle non-specific, meaning they bind to DNA regardless of the phase of the cell cycle. The three most commonly used drugs within the alkylating class are cyclophosphamide, chlorambucil and lomustine. Cyclophosphamide is one of the main chemotherapeutics used to treat high-grade lymphoma in both cats and dogs as part of COP or CHOP protocols, but is also the primary agent used in metronomic chemotherapy protocols against a wide variety of neoplasms. Chlorambucil is typically used for the treatment of low-grade lymphoma in cats, chronic lymphocytic leukaemia, and is also used in the metronomic setting in cases where cyclophosphamide cannot be used (e.g., previous sterile haemorrhagic cystitis or urinary tract infections). Lomustine is mainly used in protocols for lymphoma (either as a rescue treatment, or as part of a LOPP protocol for T-cell lymphomas) and is also effective against mast cell tumours.

Platinum Agents

The platinum agents include cisplatin and carboplatin. Their mechanism of action is similar to the alkylating agents although the platinum agent, rather than an alkyl group, binds to the DNA leading to DNA cross-link formation and eventual cell death. Platinum agents are also cell-cycle non-specific. Cisplatin has historically been used for a variety of cancers although, due to moderate nephrotoxicity, is rarely used any more in veterinary medicine. It is also contraindicated in cats as it causes fatal pulmonary vasculitis and oedema. Carboplatin is now used in place of cisplatin, mainly for the treatment of osteosarcoma, various carcinomas and mesothelioma. It can also be used alongside radiation therapy as a radiosensitiser.

Antitumour Antibiotics

Antitumour antibiotics are natural products derived from microbial fermentation (fungus *Streptomyces*), although some are now synthetic. Specific drugs include the anthracyclines (doxorubicin, epirubicin, mitoxantrone), the chromomycins (dactinomycin) and other drugs such as bleomycin. The most common antitumour antibiotic is doxorubicin which inhibits the topoisomerase II enzyme, preventing resealing of topoisomerase II-induced DNA cleavage, leading to DNA breakage and cell death. It also works by DNA intercalation (insertion of the drug between base pairs) and free radical (superoxide) formation. Epirubicin and mitoxantrone have similar mechanisms of action, although mitoxantrone does not cause cellular damage by oxidative damage. Antitumour antibiotics are cell-cycle specific. Doxorubicin is one of the most effective chemotherapeutics against canine lymphoma and is a key drug within the CHOP protocol; it is also important for the treatment of canine haemangiosarcoma, and used for treating various other carcinomas, sarcomas and histiocytic sarcoma. Epirubicin and mitoxantrone are used for similar tumour types as doxorubicin but are both considered less cardiotoxic, so may be used in place of doxorubicin where required.

Antimetabolites

The antimetabolites are agents that inhibit the use of cellular metabolites used during cell growth and division. They are cell-cycle specific. They include folic acid inhibitors (methotrexate), pyrimidine antagonists (5-fluorouracil, cytarabine, gemcitabine), and rabacfosadine (Tanovea®). Cytarabine, used mainly for the management of leukaemia and certain lymphomas, is metabolised to ara-CTP which inhibits DNA polymerase α preventing DNA replication and ultimately leading to cell death. Rabacfosadine is a recently licensed treatment for naïve and relapsed canine lymphoma, demonstrating highest efficacy in B-cell immunophenotypes. It is a prodrug resulting in intracellular generation of PMEG (a nucleotide analogue), which intercalates with the DNA causing DNA chain termination and cell death.

Antimicrotubule Agents

These are agents that interfere with polymerisation or depolymerisation of the microtubules that play important roles in cell function and division; they are cell-cycle specific (M-phase). Within the antimicrotubule agents are the vinca alkaloids (vincristine, vinblastine, vinorelbine), derived from the periwinkle plant, and the taxanes (paclitaxel, docetaxel). The vinca alkaloids bind to tubulin and inhibit microtubule assembly, whereas the taxanes stabilise the microtubules and prevent their reorganisation; both actions result in cell death. The vinca alkaloids are among some of the most used chemotherapy drugs in veterinary oncology. Vincristine is one of the main chemotherapeutics for canine and feline lymphoma and is also the treatment of choice for transmissible venereal tumours. Vinblastine is mostly used for the medical management of mast cell tumours and transitional cell carcinomas. Vinorelbine is semi-synthetic and reaches much higher pulmonary concentrations compared to vincristine and vinblastine, so is often used in the management of pulmonary carcinomas. The taxanes, paclitaxel and docetaxel, have little clinical use due to a high risk of acute hypersensitivity reactions (due to the excipients used).

L-asparaginase

L-asparaginase is a non-cytotoxic drug that deprives neoplastic cells (specifically lymphocytes) of the amino acid asparagine, by degrading extracellular asparagine. Healthy non-neoplastic cells can produce their own asparagine via asparagine synthetase, but neoplastic lymphocytes lack this enzyme and thus rely solely on exogenous supplies of asparagine. A lack of asparagine results in disruption of protein synthesis and cell death. As it does not affect normal cells within the body, there are minimal/no adverse effects, making it useful for the management of lymphoma or lymphoid leukaemia patients who are already systemically unwell at the time of presentation. As it is a foreign protein, hypersensitivity reactions can develop, although this is more likely with repeated administration; one study demonstrated the risk to be <2 per cent with each dose of L-asparaginase.

Tyrosine Kinase Inhibitors (TKIs)

These are defined as targeted therapies, rather than

chemotherapy. TKIs inhibit receptor tyrosine kinases (RTKs), which are cell surface receptors for growth factors, cytokines and hormones. Normally, activation of RTKs leads to intracellular signalling cascades resulting in cell growth, proliferation, differentiation, survival and metabolism. In many cancers there is constitutive activation of these receptors (e.g., VEGFR and PDGFR), or increased ligand binding, promoting cell proliferation and survival. An example of the genetic mutations that drive these abnormalities is the c-Kit mutation found in some canine mast cell tumours, resulting in constitutive activation of the RTK KIT in absence of ligand binding. The two TKIs licensed in veterinary medicine are toceranib phosphate (Palladia™), which targets PDGFR-ß, KIT, VEGFR-2 and FLT-3, and masitinib mesylate (Masivet®) which targets PDGFR-α, PDGFR-ß and KIT. Both are only licensed for the management of canine mast cell tumours. However, due to a wider range of targets, toceranib is commonly used for a variety of other tumour types mainly including anal sac adenocarcinomas, gastrointestinal stromal cell tumours (many express KIT) and neuroendocrine tumours.

ADVERSE EFFECTS AND THEIR MANAGEMENT

As outlined earlier the adverse effects of chemotherapy mainly relate to damage of rapidly proliferating healthy tissues; the most common adverse effects are therefore gastrointestinal toxicity and myelosuppression. There are also specific toxicities and considerations with certain individual drugs, which will be discussed later in this section.

Acute Adverse Effects

Extravasation is an acute event that occurs at the time of chemotherapy administration, although clinical signs are often delayed (< seven days). Extravasation often results from inadequate IV catheter placement or movement of the patient during treatment administration and can be serious, even leading to amputation in the most severe cases. The two main chemotherapeutic groups known to cause extravasation reactions are the vinca alkaloids and doxorubicin, although the platinum agents have also been reported. Management of doxorubicin extravasation should involve administration of dexrazoxane (a direct 'antidote' to doxorubicin, reducing superoxide radical formation) within six hours of the event, and cooling the tissue with ice packs to limit local dispersion of the drug within tissues. For vinca alkaloid extravasation, dispersion of the drug through tissue is beneficial as it is then metabolised; therefore, treatment consists of tissue warming and local subcutaneous administration of hyaluronidase. Tumour lysis syndrome is another potential consequence of chemotherapy administration, mainly in lymphoma patients. Although overall it is a rare event, it should always be discussed with owners especially in cases with a large tumour burden. Tumour lysis syndrome is caused by sudden lysis of tumour cells and subsequent release of cell contents into circulation. It is characterised by hyperkalaemia, hyperphosphataemia, hypocalcaemia and azotaemia. There is no specific treatment, instead management revolves around intensive supportive care to correct the metabolic derangements.

Common Adverse Effects

Gastrointestinal toxicity is a more common adverse effect of chemotherapy, although generally chemotherapy is well tolerated in most patients (especially cats). Certain chemotherapy drugs such as doxorubicin and carboplatin are more likely to cause gastrointestinal upset compared to other chemotherapeutics. Most of the time, the clinical signs are delayed in onset, occurring approximately three to five days post-chemotherapy. Management involves supportive care tailored to the severity of clinical signs. Mild adverse effects may only require a short course of maropitant and/or pro-biotic or kaolin pastes, whereas additional medications such as ondansetron, mirtazapine and metronidazole may be required in more moderate cases. Hospitalisation for intravenous medications and fluid therapy is indicated in severe cases. Prophylactic administration of antiemetics is often not required. However, in patients receiving more emetogenic drugs, such as doxorubicin and carboplatin, it should be provided routinely (prior to administration, and then for three to five days afterwards).

Myelosuppression also occurs relatively frequently. Neutropenia is most common, but thrombocytopenia and anaemia can also be observed. For most chemotherapeutics, the neutrophil nadir occurs seven days post-administration, although for some drugs, such as carboplatin and lomustine, this can be delayed and unpredictable. The degree of neutropenia is graded according to the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events (VCOG CTCAE), as outlined in Table 2; the VCOG CTCAE also has grading systems for all other forms of adverse effects including gastrointestinal signs. Often mild neutropenia is tolerated, and chemotherapy can continue to be administered if the neutrophil count is >1.5 – 2.0K/µL (clinician dependant). Prophylactic antibiotics (amoxicillin-clavulanate or TMPS) are only indicated when a neutrophil count <0.75K/µL is encountered; haematology should be rechecked frequently, and antibiotics stopped when the neutrophil count exceeds the 0.75K/µL threshold. Antibiotics can be administered orally if the patient is clinically well and afebrile. In cases where a febrile neutropenia occurs, regardless of the neutropenia grade, antibiotics should be administered intravenously initially until the pyrexia resolves and the patient improves. Amoxicillin-clavulanate is still an acceptable first-line antibiotic in this situation; however, should the pyrexia not improve initially, fluoroquinolones should be used instead.

Grade	Neutrophil Count
1	1.5K/µL – <LLN
2	1.0 – 1.49K/µL
3	0.5 – 0.99K/µL
4	<0.5K/µL
5	Death

Table 2: The grading system for recording neutropenia, according to the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events (VCOG CTCAE). LLN = lower limit of normal.

Specific Adverse Effects

Some individual chemotherapy drugs have specific potential adverse effects that are important to be aware of. In summary, these include:

- **Lomustine** – Lomustine can cause cumulative hepatopathy in dogs, observed as an increase in ALT. Therefore, baseline biochemistry and ALT should be checked prior to each lomustine administration. If ALT increases >300U/l then dose reductions or treatment delays should be considered. Concurrent administration of SAMe (Denamarin®) significantly reduces the risk of lomustine-induced hepatopathy. Hepatotoxicity is not commonly observed in cats.
- **Doxorubicin** – Doxorubicin is cardiotoxic and this risk is increased with multiple doses. The observed effects of doxorubicin-induced cardiotoxicity include arrhythmias and reduced systolic function. The risk of clinical cardiotoxicity is approximately four per cent but is increased to around 15 per cent in high-risk breeds such as Boxers, Cocker Spaniels, Dobermanns and Great Danes. Echocardiography should be performed in high-risk breeds and any dog with a heart murmur/other cardiac abnormality prior to starting chemotherapy with doxorubicin. The concurrent administration of dexrazoxane can be considered to reduce the risk of cardiotoxicity in certain cases, but alternative chemotherapeutics such as epirubicin or mitoxantrone could be considered instead of doxorubicin. Doxorubicin is not cardiotoxic in cats, but can be nephrotoxic.
- **Cyclophosphamide** – Sterile haemorrhagic cystitis (SHC) can be seen with chronic use of cyclophosphamide (i.e., in the metronomic setting) in dogs only, due to direct irritation of the bladder epithelium by the metabolite acrolein. The risk of SHC is reduced to around 10 per cent by concurrent furosemide administration.
- **TKIs (toceranib and masitinib)–** As well as gastrointestinal toxicity and myelosuppression, other adverse effects including hepatopathy, hypertension, proteinuria and lameness can be observed. Therefore, full haematology, biochemistry, blood pressure and urinalysis (including UPCR) should be monitored every four weeks for patients receiving TKIs.

References available on request.

READER QUESTIONS AND ANSWERS

Q1. WHICH OF THE FOLLOWING CHEMOTHERAPY DRUGS IS AN ALKYLATING AGENT?
- **A.** Doxorubicin
- **B.** Paclitaxel
- **C.** Lomustine
- **D** Vincristine

Q2. THE FOLLOWING DESCRIPTION DESCRIBES THE MECHANISM OF ACTION OF WHICH DRUG? "DRUG BINDING TO TUBULIN CAUSES INHIBITION OF MICROTUBULE ASSEMBLY, LEADING TO CELL DEATH"
- **A.** Carboplatin
- **B.** Vinblastine
- **C.** Chlorambucil
- **D.** L-asparaginase

Q3. HYPERTENSION IS A POSSIBLE ADVERSE EFFECT OF WHICH DRUG?
- **A.** Cisplatin
- **B.** Toceranib
- **C.** Rabacfosadine
- **D.** Cytarabine

Q4. WHICH ELECTROLYTE ABNORMALITY IS OFTEN OBSERVED IN TUMOUR LYSIS SYNDROME?
- **A.** Hyperkalaemia
- **B.** Hypophosphataemia
- **C.** Hypercalcaemia
- **D.** Hyponatraemia

Q5. THE RISK OF STERILE HAEMORRHAGIC CYSTITIS WITH METRONOMIC CYCLOPHOSPHAMIDE CAN BE REDUCED TO APPROXIMATELY ___ PER CENT WITH CONCURRENT ADMINISTRATION OF FUROSEMIDE?
- **A.** 5 per cent
- **B.** 10 per cent
- **C.** 15 per cent
- **D.** 20 per cent

ANSWERS: 1C; 2B; 3B; 4A; 5B.