Comparative aspects of pulmonary toxicity induced by cytotoxic agents with emphasis on lomustine, and a veterinary case report

Vergelijkende aspecten van longtoxiciteit door cytostatica met de nadruk op lomustine en een diergeneeskundig casereport

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ABSTRACT

In veterinary oncology the use of the nitrosourea compound lomustine is increasing. Veterinary oncologists need to be aware of the pulmonary toxicity of this drug.

Because of the lack of veterinary publications on this subject, the incidence and pathophysiology in human cancer patients of pulmonary toxicity induced by cytotoxic agents in general and by nitrosoureas in particular are discussed. Three clinical syndromes can be recognized, the most devastating of which is interstitial pneumonitis resulting in pulmonary fibrosis. Disturbances in the homeostatic mechanisms of the oxidant/antioxidant-, immunologic-, matrix repair-, proteolytic-, and central nervous systems are some of the major mechanisms of pulmonary injury in human medicine. Risk factors such as cumulative dose, age, radiation, oxygen administration and multi-drug regimens are recognized.

For the first time in veterinary medicine, a case report of a dog with pulmonary fibrosis, probably caused by chronic lomustine administration, is presented.

SAMENVATTING

Het gebruik van lomustine, behorende tot de nitrosourea's, neemt toe in de veterinaire oncologie. Dierenartsen dienen zich bewust te zijn van de longtoxiciteit van dit medicijn.

Door het gebrek aan diergeneeskundige bronnen worden het voorkomen en de pathofysiologie van de longtoxiciteit bij humane kankerpatiënten, veroorzaakt door cytostatica in het algemeen en door nitrosourea's in het bijzonder, besproken. Drie klinische syndromen worden beschreven, waarvan interstitiële pneumonie resulterend in longfibrose de meeste consequenties voor de patiënt heeft. De verstoring van de homeostase van de oxidant/antioxidant-, immunologische, 'matrix repair'- en proteolytische systemen en het centrale zenuwstelsel zijn verantwoordelijk voor de longschade bij humane patiënten. Risicofactoren, zoals cumulatieve dosis, leeftijd, bestraling, zuurstoftoediening en multidrugprotocollen worden vermeld.

Voor het eerst in de diergeneeskunde wordt een case report van een hond met longfibrose, die waarschijnlijk veroorzaakt is door een langdurig lomustinegebruik, beschreven.

INTRODUCTION

In veterinary oncology the use of the cytotoxic drug 1-(2-chloro-ethyl)-3-cyclohexyl-1-nitrosourea (lomustine) is on the rise. In human oncology, the major indications for the use of lomustine are primary and metastatic brain tumors in patients who have already received appropriate surgery and/or radiotherapy or as a palliative treatment (Parney and Chang, 2003). Lomustine is indicated as secondary treatment in combination with other approved drugs in patients with relapsed or primary therapy resistant Hodgkin's or non-Hodgkin lymphoma (Stuart *et al.,* 2001; Musolino *et al.,* 2005; Alexandrescu *et al.,* 2006) and in cases of melanoma skin metastasis (Lens and Eisen, 2003). The major indication in veterinary medicine for the use of this drug is the treatment of canine lymphoma patients, either during the protocols used to achieve a first remission in the different subtypes of T-cell lymphomas or as a rescue agent in relapsed T-and B cell lymphoma (Risbon *et al.*, 2006; Williams *et al.*, 2006; Saba *et al.*, 2007; Sauerbrey *et al.,* 2007). Also in dogs with mast cell tumors (Rassnick *et al.*, 1999), brain tumors (Van Meervenne *et al.*, 2007) and histiocytic proliferative

lesions (Skorupski *et al.*, 2007), lomustine shows promising results.

The side effects of lomustine in human oncology are transient leucopenia, cumulative thrombocytopenia, hepatic and renal toxicity and pulmonary toxicity resulting in lung fibrosis (Schacht *et al.*, 1981; Vats *et al.*, 1982; Wakui, 1982; Stone and Richardson, 1987; Marquette *et al.*, 1992; Stonich and Bressler, 1998). In veterinary medicine, especially leucopenia, cumulative thrombocytopenia and hepatic toxicity are reported (Rassnick *et al.*, 1999; Fan and Kitchell, 2000; Kristal *et al.*, 2004; Sauerbrey *et al.,* 2007). Since little is known about the pulmonary toxic side effects of lomustine in veterinary oncology, the purpose of this article is to discuss comparative aspects, and to present a case report of lung fibrosis in a dog, most likely induced by lomustine. Awareness of, and alertness to this toxicity and its relative risk is important for veterinary clinical oncologists. Early recognition of this problem and withdrawal of the injurious agent is the best treatment. With an improved understanding of how agents like lomustine damage lung tissue, veterinary clinicians may be able to administer these drugs to their patients, while still preventing or delaying pulmonary toxicity.

GENERAL COMPARATIVE AND PHARMACOLOGICAL ASPECTS

One of the side effects of medical treatment of neoplastic disease is damage to the vulnerable tissues of the pulmonary system. Diffuse pulmonary diseases seen in human patients receiving chemotherapy have a wide variety of etiologies, including infection, involvement with the underlying disease, injury from diagnostic agents or radiation, and toxicity from chemotherapeutic drugs (Klein and Wilds, 1983; Cooper *et al.*, 1986; Dweik and Ahmad, 1998; Camus *et al.*, 2001; Foucher and Camus, 2001; Ozkan *et al.*, 2001; Evans and Limper, 2006).

The chemotherapeutic agents currently known to cause pulmonary toxic side effects in humans, usually manifested as interstitial pneumonitis, alveolitis and ultimately pulmonary fibrosis, are considerably greater in number than may be generally appreciated by clinicians. Bleomycin, busulfan, methotrexate, carmustine, lomustine, semustine, zinostatin, mitomycin, procarbazin and chlorambucil are agents known to cause these problems. Methotrexate, lomustine, procarbazine and chlorambucil are also used in veterinary medicine in cumulative dosages (Weiss and Muggia, 1980; Weiss *et al.*, 1981; Klein and Wilds, 1983; Cooper *et al.*, 1986; Twohig and Matthay, 1990; Dweik and Ahmad, 1998; Camus *et al.*, 2001; Ozkan *et al.*, 2001; Evans and Limper, 2006). The appearance of pulmonary fibrosis secondary to antineoplastic therapy may be insidious in onset and progress to a fatal outcome (Klein and Wilds, 1983).

Clinical features are similar for most categories of cytotoxic agents. However, treatment and outcome vary with each particular agent (Klein and Wilds, 1983; Cooper *et al.*, 1986).

Interaction between these drugs and thoracic radiation or high oxygen fractions in inspired air has produced pneumonitis at doses lower than when a drug is used alone. Synergism between the drugs themselves, when given concurrently, is believed to produce pulmonary toxicity at lower doses (Weiss and Muggia, 1980; Klein and Wilds, 1983; Stonich and Bressler, 1998; Camus *et al.*, 2001; Evans and Limper, 2006).

Within the group of pulmonary toxicities induced by cytotoxic drugs in general, lung injury by nitrosoureas has emerged as a significant problem in human oncology (Hundley and Lukens, 1979; Weiss *et al.*, 1981; Smith, 1989; Massin *et al.*, 1992; O'Driscoll, 2000). Nitrosoureas belong to the group of alkylating agents and include carmustine (BCNU), lomustine (CCNU) and semustine (methyl-CCNU). Nitrosoureas are highly lipophilic compounds that undergo hydrolysis in the liver by the hepatic microsomal enzyme oxidation system to form reactive metabolites. These metabolites cause alkylation and cross-linking of DNA. Other biologic effects include inhibition of DNA synthesis and some cell cycle phase specificity. Nitrosoureas generally lack cross-resistance with other alkylating agents. While it is difficult to ascertain the exact prevalence of nitrosoureas induced lung pathologies, it is now clear that a direct relationship exists between cumulated exposure to a nitrosourea and the likelihood of developing pulmonary toxicity (Schabel, 1976; Wakui, 1982).

Carmustine is the most widely used nitrosourea compound in human oncology and is associated with a high risk of pulmonary toxicity. Symptoms may begin as early as one month and as late as one year after initiation of treatment (Klein and Wilds, 1983). Up to 25% of patients receiving carmustine develop early-onset pulmonary fibrosis within 36 months after start of therapy. Lung fibrosis is especially likely to occur with cumulative doses of carmustine over 1500 mg/m^2 (Ozkan *et al*., 2001). However, one study of 94 Hodgkin's lymphoma patients receiving carmustine reported early-onset interstitial pneumonitis in up to 47% of the patients whose cumulative doses were more than 535 mg/m^2 and 26% of these patients died, whereas 15% developed toxicity at doses lower than 475 mg/m^2. Statistical analysis revealed that the only independent variables associated with lung disease were total dose of carmustine and female sex (Phillips and Reece, 1986). Carmustine may also cause a late-presenting form of lung fibrosis, which has been reported in survivors of childhood brain tumors. In one study, after 16 to 20 years of follow-up, 8 of 17 patients died of pulmonary fibrosis. Some patients may be asymptomatic for many years and become symptomatic at any time (O'Driscoll, 2000; Mertens *et al.*, 2002).

Semustine and lomustine are less frequently reported to be toxic to the lungs. Onset of toxicity after use of semustine and lomustine has occurred after an interval of 6 months or longer from the start of therapy. Usually toxicity occurs with a cumulative dose of lomustine over 1100 mg/m^2 (Lee *et al.*, 1978; Dent, 1982 ; Vats *et al.*, 1982 ; Cordonnier *et al.*, 1983 ;

Tucci *et al.*, 1986 ; Stone and Richardson, 1987; Block *et al.*, 1990 ; Marquette *et al.*, 1992). There is one report of pulmonary toxicity after semustine use at a cumulative dose of only 600 mg/m^2 (Block *et al.*, 1990). At present, only lomustine is of significance for clinical use in veterinary oncology. Carmustine has been used in veterinary medicine for the treatment of brain tumors and lymphoma, though with several disadvantages. The drug is not easily available, it is expensive and it has to be slowly administered intravenously. Gastro-intestinal complaints, delayed and cumulative bone marrow suppression and lethargy were frequently encountered side effects (Merker *et al.,* 1975; Lucas *et al.,* 2004).

To the author's knowledge, no case of pulmonary fibrosis associated with lomustine treatment has been reported.

CLINICAL SYNDROMES IN HUMAN CANCER PATIENTS

While much of the pathophysiology of toxic lung injury from specific agents is unknown, three common clinical-pathologic syndromes have been associated with chemotherapy induced lung injury: interstitial pneumonitis followed by fibrosis, hypersensitivity pneumonitis, and an acute pneumonitis with non-cardiogenic pulmonary edema. Drug-induced interstitial pneumonitis can lead to permanent damage through fibrosis, whereas hypersensitivity pneumonitis and non-cardiogenic pulmonary edema are usually reversible. Some human patients will present with symptoms from more than one syndrome, and some drugs can cause more than one type of toxicity (Klein and Wilds, 1983; Stonich and Bressler, 1998; Ozkan *et al.*, 2001; Evans and Limper, 2006).

Interstitial pneumonitis progressing to pulmonary fibrosis has a clinical picture which is remarkably consistent for pulmonary toxicity attributable to the spectrum of antineoplastic agents. However, it should be noted that it is rarely associated with the antimetabolites, such as methotrexate. The patients experience slow, progressive (i.e. weeks to months) dyspnea on physical exertion, a non-productive cough, fever, weight loss and fatigue. The development of these symptoms generally precedes the appearance of radiographic changes. The radiographic findings are reticular infiltrates starting in the sub-pleural region of the lung bases and progressing to include the entire lung. Based on clinical utility and assessment of severity, the antineoplastic drug may be discontinued in an attempt to manage this toxicity. Steroids have been administered to enhance resolution of this syndrome, but documentation of their efficacy is anecdotal. Usually, the outcome is poor, with most of the patients dying. Lung transplant offers the best hope of long-term survival (Klein and Wilds, 1983; Massin *et al.*, 1992; Stonich and Bressler, 1998; Ozkan *et al.*, 2001).

Hypersensitivity pneumonitis is commonly associated with bleomycin, methotrexate and procarbazine, and is manifested as an acute syndrome (i.e. hours to days) consisting of dyspnea, fever and non-productive cough. Peripheral and/or pulmonary eosinophilia can be observed. Chest radiographs show localized or bilateral alveolar infiltrates. Standard treatment of these hypersensitivity reactions includes drug discontinuation and steroid administration, resulting in a good prognosis for the affected patient (Klein and Wilds, 1983; Stonich and Bressler, 1998; Ozkan *et al.*, 2001; Evans and Limper, 2006).

Non-cardiogenic pulmonary edema has a pattern of pulmonary toxicity which is very rare. It is reported as an acute complication in association with the use of cytarabine, methotrexate and cyclophosphamide. Patients are presented in acute respiratory distress occurring over several hours. Chest radiographs show diffuse ill-defined acinar infiltrates and normal heart size. The prognosis is variable and no validated therapy is available for this group of patients (Klein and Wilds, 1983; Stonich and Bressler, 1998; Ozkan *et al.*, 2001; Evans and Limper, 2006).

MECHANISMS OF PULMONARY INJURY

Pulmonary toxicity secondary to antineoplastic drugs may be due to a variety of mechanisms. There are five major postulated mechanisms in human medicine to explain the development of this toxicity, each of which can cause pulmonary injury either as a single mechanism or in combination with any of the other four. Generally it is thought that antineoplastic agents induce pulmonary injury by disturbing homeostatic mechanisms. An imbalance between inflammatory reactions that may cause pulmonary damage and protective detoxification reactions can occur for the following systems:

Oxidant/antioxidant system

Lung tissue and the alveolar epithelial lining fluid contain high levels of antioxidants that presumably protect against oxidants and/or free radicals. Cytotoxic drugs can trigger the formation of reactive oxygen metabolites such as superoxide anions, hydrogen peroxide, and hydroxyl- and carbonate radicals. These oxidant molecules, formed within phagocytic cells such as monocytes, macrophages and neutrophils, can result in direct injury to the lungs. They can also initiate a metabolic cascade that produces immunoreactive substances like prostaglandins and other cytokines, which in turn lead to inflammation and lung damage. The toxic effects of the free radicals may compromise the production of lung surfactant and damage the delicate alveolar structures, resulting in histopathologic changes that may progress to fibrosis. They also may participate in redox reactions resulting in fatty acid oxidation that can lead to membrane instability and perhaps autologous cytotoxicity (Klein and Wilds, 1983; Stonich and Bressler, 1998; Abushamaa *et al.*, 2002).

Normally, antioxidant defense mechanisms such as the production of superoxide dismutase, glutathione peroxidase, and alpha-tocopherol provide the neces-

sary balance to compensate for the noxious oxidant effects. When antineoplastic drugs are administered, there may be a disturbance of this homeostasis resulting in pulmonary injury (Klein and Wilds, 1983; Stonich and Bressler, 1998; Abushamaa *et al.*, 2002).

Theoretically, the production of these highly oxidizing radicals might be increased by the inspiration of high concentrations of oxygen, for example delivered during oxygen therapy and anesthesia protocols. Also, ionizing thoracic radiotherapy may cause additive toxic effects by the additional production of free radicals (Klein and Wilds, 1983; Stonich and Bressler, 1998).

Immunologic system

Even in the healthy state, pulmonary host cells can exaggerate toxic reactions caused by exposure to substances that initiate or activate the immunologic system. Pulmonary cells release mediators (e.g. cytokines) that attract and activate inflammatory cell types like eosinophils, monocytes and neutrophils. To counterbalance the amplified effects of the immunologic system that may result in pulmonary tissue damage, tolerant cells exist, such as lymphocytes and alveolar macrophages. It is postulated that when cytotoxic drugs are administered, there is a disturbance of this homeostasis of toxic reaction and tolerant suppressor cells. This may be a result of different kinds of immunologic mechanisms, such as hypersensitivity reactions, leading to pulmonary injury and, in some patients, finally to pulmonary fibrosis. Eosinophilia has been reported with procarbazine- and methotrexate-induced pulmonary toxicity. Steroid responsiveness suggests the possibility of this immunologic mechanism (Klein and Wilds, 1983; Stonich and Bressler, 1998).

Matrix repair system

Normally, the proliferation of fibroblasts leading to collagen deposition is helpful in repairing or limiting tissue injury. However, excessive deposition of collagen can result in organ structure impairment. Cytotoxic drugs can alter this balance between collagen formation and collagenolysis, resulting in fibrosis through modulation of fibroblast proliferation and/or excessive collagen deposition (Stonich and Bressler, 1998).

Proteolytic system

Inflammatory cells produce a number of proteolytic enzymes that are associated with many pulmonary disturbances. Proteolytic enzymes are normally controlled or inactivated by protease inhibitors that are mediated by oxidant molecules. It is thought that although antineoplastic drug parent molecules do not affect this homeostasis, their oxidant radicals may inactivate protease inhibitors, thereby allowing proteolytic enzymes to function unopposed (Stonich and Bressler, 1998).

Central nervous system

The central nervous system provides some control over pulmonary capillary permeability, for example by influencing the isoprenoid pathway. Dysfunction of this pathway, through an increase of endogenous digoxine in the hypothalamus, contributes to the pathogenesis of idiopathic pulmonary fibrosis in humans (Kurup and Kurup, 2003). It has been postulated that cytotoxic drugs may influence the hypothalamus and medulla in such a way that pulmonary capillary permeability is increased (Stonich and Bressler, 1998). However, a direct relation between the dysfunction of this pathway and the chronic use of cytotoxic agents has not yet been proven.

RISK FACTORS FOR THE DEVELOPMENT OF PULMONARY TOXICITY IN HUMANS

As has been discussed, antineoplastic drugs do induce pulmonary toxicity. However, it should be considered that not all patients receiving these agents experience this toxicity. To identify those patients at risk, five major predisposing factors for the development of pulmonary toxicity are noted, together with some predisposing factors of minor importance.

Cumulative dose

Cytotoxic agents that are directly toxic to the lungs generally exhibit increasing toxicity with increasing dose. This is believed to be a result of drug accumulation in the lung itself. Two patterns of dose related pulmonary toxicity have been clinically observed (Stonich and Bressler, 1998).

A threshold effect, where there is a marked increase in pulmonary toxicity beyond a specific amount of drug received.

A linear effect, where there is a constantly increasing risk for the development of pulmonary toxicity, directly related to the amount of drug administered (e.g. lomustine) (Massin *et al.*, 1992).

Age

A normal physiologic phenomenon that has been observed with aging is a decrease in the effectiveness of the antioxidant defense system. Accordingly, as they age, patients become more susceptible to pulmonary toxicity induced by cytotoxic drugs. However, increased age has been shown to be a risk factor only for the development of bleomycin-induced pulmonary disease (Stonich and Bressler, 1998).

Radiation

Radiation therapy results in the production of oxidant products that may lead to pulmonary damage. When antineoplastic agents, which also affect the oxidant/antioxidant homeostasis, are administered, there may be synergistic pulmonary toxicity (Klein and Wilds, 1983; Stonich and Bressler, 1998).

Oxygen administration

Reactive oxidant metabolites are produced when high concentrations of oxygen are administered, such as during oxygen therapy. Synergistic toxicity may also be possible between high concentrations of oxygen and drugs that can disrupt the normal oxidant/antioxidant homeostasis. This appears to be the case with bleomycin, cyclophosphamide and mitomycin (Klein and Wilds, 1983; Stonich and Bressler, 1998).

Multidrug regimens

Although not clearly defined, the incidence and severity of pulmonary toxicity may increase with multi-drug regimens. Typically, these chemotherapy regimens include bleomycin, mitomycin, cyclophosphamide, methotrexate or carmustine. It has not been determined whether a single drug is the causative agent or if the interaction of these antineoplastic drugs results in enhanced toxicity (Stonich and Bressler, 1998).

Miscellaneous

Other risk factors include smoking, pre-existing lung disease, quantitative and qualitative differences among individuals in activation and detoxification pathways of drugs and chemicals, and also ethnicity, as some ethnic groups may be at increased risk of developing adverse pulmonary reactions to drugs (Klein and Wilds, 1983; Camus *et al.*, 2001; Foucher and Camus, 2001).

DIAGNOSIS OF PULMONARY FIBROSIS IN HUMAN AND VETERINARY PATIENTS

The earliest detection of pulmonary fibrosis may be achieved through serial evaluations of pulmonary function. However, the definitive diagnosis of cytotoxic drug induced pulmonary toxicity is difficult since a detailed history of drug administration, in the absence of other situations that may lead to pulmonary damage, is required. Patients receiving antineoplastic therapy may have coincidental parenchymal and airway diseases, complicating the clinical picture. Presentation may therefore be highly variable, contingent upon individual drug regimen, incidental disease, and coincidental pathology (Camus *et al.*, 2001).

Chest X-rays may show changes consistent with progressive pulmonary fibrosis. Radiographic changes typically appear as bilateral diffuse interstitial infiltrates with patchy areas of consolidation. Alveolar and interstitial reaction and pleural effusion may be found. However, a patient may have a normal pulmonary X-ray pattern, even when histological evidence of pulmonary damage is present. Once radiographic signs are noted, the process has often progressed irreversibly (Camus *et al.*, 2001).

Sequential carbon monoxide diffusion capacity tests may indicate the presence of occult pulmonary changes. During pulmonary function tests, typical abnormalities for a restrictive process are seen, with a reduced diffusion capacity, decreased total lung capacity, and other ventilatory defects, while arterial blood gases will show hypoxia with hypocapnia.

Pulmonary histo- or cytopathologic examination of lung biopsies or tracheo-bronchial washes may reveal endothelial cell damage, fibroblast proliferation, and epithelial abnormalities (Camus *et al.*, 2001).

Canine idiopathic pulmonary fibrosis is presented as a chronic, progressively worsening lung disease characterized by clinical signs of coughing, exercise intolerance and variable dyspnoea and tachypnoea. This disease is especially recognized in West Highland White Terriers, although it has been diagnosed in other breeds as well (Corcoran *et al.*, 1999; Lobetti *et al.*, 2001; Webb and Armstrong, 2002; Norris *et al.*, 2005). The most prominent feature of chest auscultation are inspiratory crackles. The main thoracic radiographic change is a mild to severe, diffuse, interstitial pulmonary pattern, with varying degrees of bronchial involvement. Bronchoscopy sometimes reveals a mild airway mucoid reaction. A definitive diagnosis can only be made by histopathological examination of lung tissue, which displays thickening of the alveolar septa, interstitial fibrosis and pneumocyte hyperplasia (Corcoran *et al.*, 1999; Lobetti *et al.*, 2001; Webb and Armstrong, 2002).

CASE REPORT

A 10-year old neutered female Basset Fauve de Bretagne, with a body weight of 18 kg and a body surface of 0.7 m^2, was diagnosed with malignant lymphoma in De Ottenhorst Clinic on 9 September 2002. On clinical examination, the dog was found to have generally enlarged peripheral lymph nodes and profuse hepato- and splenomegaly. Definite diagnosis was made through cytologic exams of fine-needle aspiration biopsies of peripheral lymph nodes, liver and spleen. No T – B cell differentiation on cytological smears was available at that time and the owner declined the option of histologic biopsies. Thoracic X-rays showed no abnormalities. A complete blood count (CBC) revealed an increased leukocyte count of 17.0 x 10^9/l (reference range 3.0–12.0 x 10^9/l), caused by a neutrophil count of 14.3 x 10^9/l (reference range 3.6-13 x 10^9/l), but without further abnormalities. Biochemistry revealed no significant changes. The initial treatment consisted of two L-asparaginase (Paronal, Nycomed BV) injections (400 IU/kg) intramuscularly with a one week interval. One week after the second L-asparaginase injection, methylprednisolon (Moderin, Pfizer Animal Health BV) was started orally in a dose of 1 mg/kg twice daily and tapered over 4 weeks to an alternating dose of 0.5 mg/kg twice daily. In combination with the start of methylprednisolon, vincristin (Vincristin sulphate, Pharmachemie BV) was administered, in a dose of 0.65 mg/m^2 intravenously. One week after the vincristin infusion, cyclophosphamide (Endoxan, Baxter BV) was given to the dog orally on two consecutive days, in a dose of 125 mg/m^2 per day, and vincristin was repeated one week later. After the

second dose of vincristin, the dog became lethargic. At that time, the peripheral lymph nodes were still moderately enlarged, and hepato- and splenomegaly still existed. The owner declined further chemotherapy according to the planned protocol with a combination of cyclophosphamide, doxorubicin, vincristin, and prednisolon (short Wisconsin-Madison protocol). The administration of methylprednisolon was stopped as well.

On 25 October 2002, therapy was started with lomustine (CeCeNU, Medac) orally in a single dose of 50 mg, which equals 70 mg/m^2, once every three weeks. Amoxycillin-clavulanic acid (Synulox, Pfizer Animal Health BV) therapy, to prevent septicemia from lomustine induced myelosuppression, was started two days after each lomustine administration, for eight consecutive days. One week after the first lomustine administration, all lymph node swelling and abdominal enlargement had disappeared. Immediately before each dose of lomustine, a CBC was performed to determine possible contra-indications for treatment due to myelosuppression.

In July 2003 a cumulative dose of 570 mg/m^2 was reached with the lymphoma still in complete remission. The lomustine dosing interval was increased to once every five weeks and in September 2003 to once every six weeks. Because of the potential increased risk of renal toxicity, from July 2003 blood urea nitrogen and serum creatinine determination was performed before each treatment, together with routine urine examinations.

The final dose of lomustine was delivered to the dog on 7 November 2003, and at that time the total dose received was 720 mg lomustine, which equals a cumulative dose of 1029 mg/m^2. The urine examination at the end of October showed a significant proteinuria with a normal blood creatinine level of 102 µmol/l (reference range 65-139 µmol/l). Therapy was started with the angiotensin-converting-enzyme (ACE) inhibitor ramipril (Vasotop, Intervet Nederland BV) in a dose of 2.5mg once daily. The results of recent studies suggest that when markedly proteinuric dogs are treated with ACE inhibitors, a reduction in the magnitude of proteinuria and renoprotective effects are observed during treatment (Lees *et al.,* 2005)

In January 2004 the dog started coughing and had moderately labored breathing. Thoracic auscultation revealed bilateral crackled lung sounds over the complete thoracic wall. On routine biochemistry there was an increase of the blood urea nitrogen 29.2 mmol/l (reference range 2.3-9.1 mmol/l), creatinine 143 µmol/l (reference range 65-139 µmol/l) and alkaline phosphatase 335 IU/l (reference range < 130 IU/l). On thoracic radiographs, mild cardiomegaly was visible, without any abnormal pulmonary pattern. On cardiac ultrasound, the left atrial dimension was slightly enlarged, without further abnormalities. The dog received 100 mg enrofloxacin (Baytril, Bayer Animal Health Division BV) orally once daily for 10 consecutive days, which resulted in a partial remission of the pulmonary signs. The owners stopped ramipril at the end of January 2004, because in their opinion this seemed to aggravate coughing. The coughing completely stopped.

In February and March 2004, the biochemistry was regularly repeated. No worsening of kidney function was noticed. Because of an increase in ALP, the bile acids and urinary cortisol/creatinine ratio were monitored. Bile acids 7 µmol/l (reference range < 10 µmol/l), ALP 232 IU/l (reference range < 73 IU/l), ALP (65°) 183 IU/l (reference range < 73 IU/l) and urinary cortisol/creatinine ratio of 1.3x10^6 made the presence of liver damage or Cushing disease unlikely. In April 2004 coughing started again, together with a dyspnea, and the dog was referred to the Clinic of Small Animal Medicine, Faculty of Veterinary Medicine, Ghent University in Belgium. On clinical examination, moderate inspiratory and expiratory dyspnea was present and mild crackles could be heard at the level of the nasal plane. The mucous membranes were pink and the capillary refilling time was within the normal range. Thoracic auscultation revealed moderate crackles bilaterally. On cardiac auscultation, the heart frequency was 120 beats per minute with a regular rhythm, and no murmur was present. On thoracic radiographs, a severe diffuse interstitial to alveolar pattern was found, mostly in the caudal lung lobes. Right-sided cardiomegaly was present (Vertebral Heart Size = 11 vertebrae). The pulmonic arteries towards the right cranial lung lobe and both caudal lung lobes were thickened and tortuous. The caudal aspect of the trachea at the level of the heart base seemed mildly dilated. Echocardiography revealed a severe left ventricular hypertrophy with a mildly decreased left ventricular diameter. The left atrial diameter was at the higher end of the normal range. The fractional shortening was 63%, which is high. The right ventricular wall thickness could not clearly be discerned, but the right ventricular diameter was at the higher end of the normal range. The right atrium was mildly dilated. On Doppler echocardiography, no regurgitations were found and all blood flow velocities were within the normal range. Systolic blood pressure, measured with a Parks Doppler device, was 130 to 140 mmHg. Differential diagnosis included chronic bronchitis, interstitial pulmonary fibrosis and pulmonary infection. Chronic bronchitis could be excluded because of the absence of any pulmonary abnormalities on the previous radiographs. Pulmonary infection was less likely because of the atypical thoracic auscultation of crackles and lack of explanation for the right sided cardiomegaly. In conclusion, a most likely diagnosis of diffuse interstitial pulmonary fibrosis with secondary right heart enlargement was made. As the owners did not agree to give oral corticosteroids, treatment was started with theophyllin (Theolair, 3M) 125mg twice daily, fluticason (FlixotideGlaxo-Smith-Kline) inhaler 50µg/dose 2 doses twice daily for 2 weeks followed by 1 dose twice daily, and salbutamol (Ventolin, Glaxo-Smith-Kline) inhaler 100µg/dose when needed. As no significant heart failure was present, no cardiac therapy was started at this moment.

Despite treatment, the dog's pulmonary signs worsened and most of the time she was cyanotic. Eu-

thanasia was performed at the owners' request on 25 May 2004. The lymphoma was still in complete remission at the time of death. Post-mortem examination was not permitted by the owners.

CONCLUSION

Survival time for an increasing number of veterinary oncology patients is improving, for example through a better knowledge of tumor subtype-specific treatment protocols with cytotoxic drugs, and individualized dose variations adapted to the response to these drugs. In the near future, dogs treated with lomustine-incorporated protocols will be part of this group of long-time survivors. There are no indications that the 1100 mg/m^2 cumulative dose of lomustine that leads to an increased risk of developing pulmonary fibrosis in human cancer patients will be different for canine patients. Veterinary oncologists need to be aware of this late side effect. In lomustine incorporated treatment protocols, this maximum cumulative dose of 1100 mg/m^2 should be reached at a point as late as possible in the treatment period of dogs with lomustine-sensitive tumors, or else the therapy should be finished before this cumulative dose has been reached. Since the advanced pulmonary function tests used in human medicine are not available on a routine basis for dogs, accurate pulmonary auscultation and thoracic X-rays for early detection of lung fibrosis are recommended during the course of lomustine treatment.

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