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EFFECT OF ATORVASTATIN IN A CASE OF FELINE MULTICENTRIC LYMPHOMA – CASE REPORT

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A case of feline multicentric lymphoma is reported in an 8-year-old male cat weighing 4.7 kg. At the time of the clinical consultation the animal presented weight loss, anorexia and generalised lymphadenomegaly. After careful clinical observation and a detailed laboratory workup, the diagnosis of small cleaved cell lymphoma was established. It was classified as a stage III b multicentric lymphoma. Chemotherapy was initiated according to a classical COP protocol to which atorvastatin was added. After 34 months, the cat continues to enjoy an excellent quality of life with no clinical or haematological signs of lymphoma. This is the first report in clinical veterinary medicine about a new effective adjuvant therapy in feline multicentric lymphoma. Further studies are needed to confirm that the addition of atorvastatin can provide a regular, safe and improved treatment in feline lymphoma cases.

Key words: Multicentric lymphoma, cat, statin, COP, adjuvant therapy

In cats, lymphoma represents 50–90% of all haematopoietic neoplasia cases with an incidence of 200 out of 100,000 cases (Essex and Francis, 1976). The incidence of lymphoma is higher in cats than in dogs or humans. Feline lymphoma is much more common in cats infected with feline leukaemia virus (FeLV) or feline immunodeficiency virus (FIV). The type C retrovirus of FeLV is the main cause of this disease in cats. About 70% of the cases are positive for the p27 viral antigen. However, the demographics of feline lymphoma has changed substantially in the last 15 years due to the declining prevalence of FeLV in Europe and the USA; thus, at present lymphoma most commonly occurs in older FeLV-negative cats (Louwerens et al., 2005).

Without treatment, a cat with lymphoma can survive for 6–8 weeks only. Using corticoids as the sole treatment, the survival time can be extended by approximately 3 months, and with chemotherapy it may increase by 6 to 9 months (Mooney et al., 1989). There are many protocols for the treatment of this disease but, irrespective of which treatment is used, approximately 65–75% of feline

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multicentric lymphoma cases reach remission and remain in that condition for 6–9 months in average. Once a relapse has occurred, prognosis worsens, with only 50% of the cases responding positively to the rescue treatment. Lymphoma is a very radiosensitive tumour and thus, in theory, radiotherapy should be extremely successful in the treatment of this disease. However, total or partial irradiation is associated with too many side effects in animals, and therefore this type of treatment is used only in cases of nasal lymphoma (Mooney et al., 1989).

In this work, a standard chemotherapy protocol combined with the administration of atorvastatin, a molecule belonging to the statin family, was used in a case of feline multicentric lymphoma.

Statins are used in the treatment of hypercholesterolaemia as competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the major regulatory enzyme of *de novo* cholesterol synthesis (Graaf et al., 2004). This enzyme catalyses the formation of mevalonate, which is also the precursor of isoprenoid moieties that are incorporated into several molecules essential for cell signalling and replication. Among other actions, inhibition of the cholesterol biosynthetic pathway disrupts protein glycosylation via inhibition of dolichol production and blocks prenylation of signalling proteins, such as Ras, Rho and other heterotrimeric G proteins and small G proteins involved in cancer (Farina et al., 2002).

Based on the aforementioned scientific findings, we suggested that a standard chemotherapy protocol could be improved by adding a drug which may regulate cell proliferation and the apoptotic process on tumour cells. Therefore, with studies demonstrating safety in other species and following the relevant ethical standards, we proceeded to evaluate atorvastatin as an adjuvant treatment, with a special focus on its safety and toxicity.

Case report

An 8-year-old male cat (body weight: 4.7 kg) was presented with weight loss, anorexia and generalised lymphadenomegaly, without signs indicative of liver or spleen involvement. The study was conducted in accordance with the relevant international recommendations, and a consent form was signed by the owner.

A blood sample was taken from the cat to carry out a complete blood count (CBC with differential cell count), a platelet count, a serum chemistry profile and a serological test for FIV–FeLV–Feline heartworm (SNAP® Triple™ Test, IDEXX). Urinalysis and cytological examination of the lymph nodes were also performed.

The first CBC showed normocytocis, normochromasia, leukocytosis (20.2 $\times$ 10^9/l) and a left shift of the neutrophils without mature neutrophilia and lymphocytosis.

Table 1

Laboratory results

Haematology	Units	%	Value
Packed cell volume	%		33
Haemoglobin	g/dl		11.4
Red blood cell (RBC) count	value × 10^{12}/l		6.83
White blood cell (WBC) count	value × 10^9/l		20.2
Band neutrophils	value × 10^9/l	3	0.606
Segmented neutrophils	value × 10^9/l	54	10.908
Eosinophils	value × 10^9/l	6	1.212
Basophils	value × 10^9/l	0	0
Lymphocytes	value × 10^9/l	25	5.050
Reactive lymphocytes	value × 10^9/l	10	2.020
Monocytes	value × 10^9/l	2	0.404

Chemistry profile	Units	Value
Urea	mg/dl	50
Creatinine	mg/dl	0.99
Serum total protein	g/dl	8.00
Albumin	g/dl	2.60
Total globulins	g/dl	6.00
Albumin/Globulin ratio		0.4
Alanine transaminase (ALT)	U/l	30
Alkaline phosphatase (ALP)	U/l	198
Total calcium	mg/dl	10
Inorganic phosphate	mg/dl	4.61

Urinalysis				
Physical		Sediment		
Aspect	Limpid	Epithelial cells	0	/field
Colour	Amber	Leukocytes	0	/field
Foam	Colourless	Pus cells	0	/field
Specific gravity	1040	Red cells	0	/field
pH	6.5	Germs	No	
Chemistry		Mucus	No	
		Sperm	No	
Protein (mg/dl)	No	Cylinders	No	
Glucose (mg/dl)	No	Crystals	No	
Bilirubin	No			
Ketones	No			
Haemoglobin	No			

Serum chemistry profile and urinalysis showed no data of clinical interest (see Table 1). The serological test was positive for FIV and FeLV, and negative for feline heartworm. Cytological analysis of the lymph nodes demonstrated an

abundant quantity of big lymphoid cells with fragmented nuclei. Chromatin was seen as thin cords with one or several evident nucleoli. Two mitotic figures per 10 oil immersion fields (OIF) were found, and 93% of the lymphoid cells had an evident nucleolus, with occasional neutrophils. Many red cells were observed at the bottom of the preparation. This cytological image, together with the laboratory and clinical data, was compatible with lymphoma, which was clinically classified as a multicentric lymphoma of at least stage III b (Mooney and Hayes, 1986).

Chemotherapy was initiated using a classical COP protocol (Teske et al., 2002) to which atorvastatin was added (Table 2). A CBC analysis was performed prior to the application of chemotherapeutic agents. In brief: at the 6th cycle a maintenance phase was started, in which classical chemotherapy drugs were replaced by atorvastatin for 5 months. During that phase a blood test was also made. On days 621 and 711 after beginning the treatment, the pet owner agreed to have a sample taken from the cat for reconfirmation of the diagnosis. Two incisional biopsies were made from the same popliteal lymph node to assess the effect of atorvastatin. Small cleaved cell lymphoma was confirmed by histopathology on both occasions. Unfortunately, due to the lack of specific antibodies proper immunophenotyping was not possible.

The effect of atorvastatin was explored again in the rescue phase, but in that case it was studied as a monodrug without the chemotherapy complex. Atorvastatin was eventually prescribed as a lifelong treatment.

During the induction period, a complete remission was observed 42 days after the beginning of treatment. After the fourth cycle of chemotherapy, the cat started to suffer from diarrhoea, anorexia and hypothermia (37.3 °C). TRhe animal was admitted for 5 days for symptomatic treatment, and the chemotherapy doses were lowered in the following cycle, except for atorvastatin, which was continued to be administered at the same dose. A new consultation was made 621 days after the beginning of treatment, and generalised lymphadenomegaly could be observed with overall decline and difficulties when breathing. A popliteal lymph node biopsy was made in which the histopathological diagnosis of diffuse small cleaved cell lymphoma was clearly described by the pathologist. In the rescue phase, after 711 days, we could see that the lymph nodes had become considerably reduced in size. The general state of the animal improved and dyspnoea was no longer observed.

At the second histopathological examination we found a diffuse small cleaved cell lymphoma again, now likely to form lymphoid follicles in some areas. The animal's clinical status and haematological values were satisfactory and normal.

In routine haemograms, we did not find results beyond the expected range in the CBC made either prior to the application of the COP chemotherapy protocol or during the maintenance phase.

At present, after 34 months, the cat continues to enjoy an excellent quality of life with no clinical or haematological signs of lymphoma. Furthermore, atorvastatin seems to be safe and non-toxic at the doses used.

Table 2

Standard chemotherapy protocol plus atorvastatin in a case of feline multicentric lymphoma

Treatment	Drugs	Doses	Time	No. of cycles	Treatment
Induction	Cyclophosphamide	50 mg/m^2 BSA, O	4 days a week, on alternate days		
	Vincristine	0.75 mg/m^2 BSA, IV	once a week		
	Prednisolone	2 mg/kg	once a day, nightly		
	Atorvastatin	1 mg/kg	once a day for 6 weeks	6	Induction
Maintenance	Atorvastatin	1 mg/kg	once a day for 5 months	1	Maintenance
Rescue	Atorvastatin	10 mg/kg	once a day lifelong, steady treatment	1	Rescue

BSA: Body Surface Area; O: Orally; IV: Intravenously

Discussion

Lymphoma is the most common malignancy diagnosed in cats. In young cats, it occurs most frequently following infection with FeLV or, to a lesser degree, with FIV. Such cats tend to have involvement of the lymph nodes, spine, or mediastinum. Cats with FeLV are 62 times, while those with both FeLV and FIV are 77 times, more likely to develop lymphoma. In contrast to FeLV, which plays a direct role in tumorigenesis, FIV appears to have an indirect role, likely secondary to the immunosuppressive effects of the virus. Coinfection by FIV and FeLV further potentiates the development of lymphoproliferative disorders. (Gleich et al., 2009).

In this report, we evaluate the case of a FeLV-positive and FIV-positive cat that developed a diffuse small cleaved cell lymphoma and was subjected to a chemotherapy regimen using the classical COP protocol to which atorvastatin was added. An important feature of malignant transformation is the loss of the cholesterol feedback inhibition mechanism that regulates cholesterol synthesis. Cancer cells seem to require an increase in the concentrations of cholesterol and its precursors. Therefore, a reasonable assumption is that prevention of tumour cell growth can be achieved by restricting either cholesterol availability or cholesterol synthesis (Graaf et al., 2004). In fact, *in vivo* and cell culture experiments have shown that lowering the plasma cholesterol concentration or intervening in the mevalonate pathway with HMG-CoA reductase inhibitors can decrease tumour growth (De Noyelle et al., 2001; Shachaf et al., 2007).

One of the most investigated oncogenes is Ras, which has been associated with some cases of canine, feline and bovine tumours (Mayr et al., 2002). Normally, proteins of the Ras family are located on the internal surface of the plasmatic membrane, having lipid modifications at their carboxyl termini which are responsible for attachment to the cell membrane. By inhibiting the synthesis of mevalonate, statins block the formation of downstream isoprenoids, farnesyl pyrophosphate and geranylgeranyl pyrophosphate. In this sense, inhibition of geranylgeranylation of Rho proteins seems to be a major antitumour mechanism of statins. Among other features, statins also inhibit the proteasome degradation machinery, leading to apoptosis and inhibition of proliferation (Graaf et al., 2004).

The reduced expression of p27 kip1, an inhibitor of cyclin-dependent-kinase (Cdki), has been reported in felines with lymphoma (Madewell et al., 2001). Some statins have been proven to control the G1/S transition of the cell cycle through Cdki overexpression, which includes P27 kip1 as P21 Cip1/Waf1/Cap20 and P16 lnk4 (Hengst and Reed, 1996; Wachtershauser and Stein, 2001). Defects of the G1-S cell cycle are considered critical in tumour progression. The loss of p27 kip1 does not seem to be the first cause of cancer, but the low levels of these inhibitors may probably accelerate the disease (Okuda et al., 1997).

The overexpression of certain genes such as Bcl-2/Bcl-xL, as well as those observed in tumour cell lines of feline leukaemia, suppress apoptosis (Sano et al., 2005). These data show that statins can produce a type of disruption in the balance between proteins of the proapoptotic and the antiapoptotic family.

The MDR-1 gene produces P-glycoprotein which, working as a pump, rapidly expels all the hydrophobic chemical components from the cell and is associated with inherent resistance to chemotherapy. Calcium channel blockers such as verapamil, amiodarone, quinidine, cyclosporine, phenothiazines and other agents such as statins have been studied because of their ability to revert or block the effects of P170 (Wang et al., 2001).

The expression of P-glycoprotein has been documented on feline lymphoma cell lines (Okai et al., 2000). Many statins such as lovastatin, atorvastatin and simvastatin have been reported to inhibit the action of P-glycoprotein by the inhibition of glycosylation. In conclusion, statins could increase the activity of chemotherapy drugs in tumour cells (Wang et al., 2001).

Since the antitumour effect of atorvastatin has been demonstrated in mice (Ajith et al., 2008), and cats have been reported to share the same possible antitumour targets affected by atorvastatin, this drug seems a rational option as an adjuvant treatment. We do not know yet what antitumour mechanism was effectively involved, but we could find a quick tumour regression leading to complete remission as a result of combining atorvastatin with the COP protocol. The regression was observed when only atorvastatin was used in the rescue phase. There were neither clinical nor haematological side effects. A fast tumour regression within 42 days and a long-term disease-free survival time (579 days) since

the first remission stand out in comparison with some other published protocols, such as the 'University of Wisconsin-Madison Chemotherapy Protocol', in which the duration of the first remission was only 87 to 316 days (Milner et al., 2005). In view of these results, combination therapy should also be considered. Statins plus chemotherapeutic agents may increase the arrest of tumour cells in different phases of the cell cycle and induce apoptosis to generate an improved antitumour effect, as has been demonstrated previously (Ajith et al., 2008; Rozados et al., 2008).

Although the use of statins in neoplastic disorders is still a subject of controversy (Fortuny et al., 2006; Burke et al., 2008; Nowakowski et al., 2010), the results obtained in this case confirm that the addition of atorvastatin could provide a regular, safe and improved treatment in feline lymphoma cases. Further studies should be carried out to confirm the possibility of a promising treatment using a safe, non-toxic drug which has known antitumour mechanisms of action at the molecular level.

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