Clinics in Oncology

Research Article
Published: 03 Apr, 2017

Evaluation of Adverse Events in Dogs with Adenoviral Therapy by Intralymphonodal Administration in Canine Spontaneous Multicentric Lymphosarcoma

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Abstract

Background: Therapy administration in cancer is mainly performed by intravenous, oral, and in situ routes. Adverse Events (AE) are a significant limitation of adenoviral vectors during somatic gene therapy. There are some partial evaluations of AE in canine cancer research. The objective of this study was to evaluate AE in adenoviral vector-mediated gene therapy administered by Intralymphonodal Route (ILNR) in canine lymphosarcoma.

Methods: AE were determined in five dogs with spontaneous multicentric lymphosarcoma. A non replicative recombinant adenovirus vector with a LacZ reporter gene was administered once by ILNR at a starting dose of 1.35 X 10^{10} pfu/kg, with a dose-escalation model to 1.25 X 10^{12} pfu/kg considered under these conditions, as the Maximum Tolerated Dose (MTD). AE was evaluated by a canine scale for attribution of AE based in selected clinical findings, hemogram, biochemistry, and urinalysis.

Results: No significant AE were observed during the study, therefore, no Dose-Limiting Toxicity (DLT) and MTD were found in any dog.

Conclusion: Administration of adenovirus vector exhibited no clinical, nor laboratory significant AE in this canine ILNR clinical trial. This suggests that adenoviral gene therapy by ILNR is safe for use in dogs with lymphosarcoma and a potential model of administration in animals and human beings with metastasis to lymph nodes.

Keywords: Adverse events; Dogs; Lymph node; Intralymphonodal administration; Adenoviral vector; Gene therapy; Lymphosarcoma; Lymphoma; Hemogram; Clinical biochemistry

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Received Date: *27 Jan 2017*
Accepted Date: *31 Mar 2017*
Published Date: *03 Apr 2017*

Citation:
Núñez-Ochoa L, Madrid-Marina V, Gutiérrez-López A. Evaluation of Adverse Events in Dogs with Adenoviral Therapy by Intralymphonodal Administration in Canine Spontaneous Multicentric Lymphosarcoma. Clin Oncol. 2017; 2: 1258.

Introduction

The treatment of Lymphosarcoma (LSA) in human and canine patients has predominantly been based on conventional chemotherapy and radiotherapy [1-3]. Although an improvement in response rates and survival has been obtained with these therapies over the years, a significant number of patients do not respond or relapse. In addition, conventional chemotherapy is often associated with morbidity, toxicity [4] and chemoresistance [5,6]. During the last decade, advancements brought about by the introduction of new biotechnological therapies have entered into the human oncology. These targeted therapies are dominated by the monoclonal antibodies, which have emerged as important therapeutic agents in the treatment of several malignancies including non-Hodgkin lymphosarcoma (NHL) [7,8]. Viral vectors have frequently been applied in somatic gene therapy with the final goal of treating various genetic diseases in the areas of neurology, metabolic disease, hemostasis and cancer. Vectors have been engineered based on AAV, adenoviruses, alphaviruses, herpes simplex viruses, lentiviruses, and retroviruses [9,10]. One of the challenges of current gene therapy vector development, concerns targeting a therapeutic gene to diseased cells with the aim of achieving sufficient gene expression in the affected tissue, while minimizing toxicity and expression in other tissues. Human adenovirus serotype 5 of subgroup C (Ad5) vectors are very popular in somatic gene therapy because the most is known about the structure and biology, it can be easily modified and there are convenient biological reagents available to produce recombinant Ad5 vectors in large quantities without modifying their ability to infect cells, which are not oncogenic

Table 1: Selected mesurands for adverse events evaluation in liver, kidneys, pancreas, muscle, hearth, general cellular integrity and acid-base.

Organ/Tissue	Mesurands
Liver	Glucose, urea, cholesterol, triglycerides, total bilirubin, conjugate and unconjugate bilirubin, total proteins, albumin, ALT, AST, ALP, and LDH.
Kidneys	Urea, creatinine, albumin, calcium, inorganic phosphates, potassium and LDH.
Pancreas	Glucose, ALP, and amylase.
Muscle, heart and cellular integrity	CK, AST and LDH.
Acid-base balance	K^+, Na^+, Cl^-, HCO^-_3, clinical strong ion difference (SID_{clin}), and non volatile acids (NVA)

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; LDH: Lactate Deshydrogenase; CK: Creatine Kinase; K^+: Potassium; Na^+: Sodium; Cl^-: Chloride; HCO^-_3: bicarbonate.

in humans [11,12] and remain an attractive tool for gene therapy approaches because of their nonintegrating nature and the ability to infect dividing and non-dividing cells [13]. Dogs have been used as animal models of many human diseases, and several gene therapy approaches, such as strategies for hemophilia A [14], hemophilia B [15], cancer [16-18], hematopoietic growth factors [19], lesions in the avascular portion of the meniscus [20], biological pacemaker activity of heart [21], diabetic canine pancreas [22], cerebral vasospasm [23], canine muscular dystrophy [24], and hereditary retinal degeneration [25] have been assayed.

It is now well accepted that there is a dose-dependent toxicity associated with systemic delivery of adenoviral vectors, the risk of hepatotoxicity is a major concern. Vectors that can target specific tissues following systemic or minimally invasive administration would enhance their therapeutic potential and expand their application [26]. LSA is the most frequently occurring hematological malignant neoplasm in the dog. The multicentric form of LSA is most common, with varying degrees of involvement of lymph nodes, liver, spleen, blood, and bone marrow [27]. During clinical trials one important feature is the drug safety assessment monitoring of possible drug-induced organ injury. The evaluation of Adverse Events (AE) considered as any unfavorable and unintended sign including abnormal laboratory values, symptoms, or disease findings temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses [28]. There are some partial or organ specific recommendations for assessment of AE as the one proposed by the Regulatory Affairs Committee of the American Society for Veterinary Clinical Pathology for the selection and interpretation of clinical pathology of liver-specific analytes data for a consistent and rigorous approach to the use in the identification and assessment of the potential for drug-induced hepatic injury in animals and the potential for hepatic injury in humans [29].

Objective

The present study was conducted to assess the clinical, hematological, biochemical and urinary AE associated with a single intralymphonodal administration of adenovirus vector in dogs with spontaneous multicentric lymphosarcoma.

Materials and Methods

The study was carried out at the Small Animal Teaching Hospital of UNAM, Rehabilitation National Institute and Experto Sur Veterinary Clinical Pathology Laboratory in Mexico City. Dogs were evaluated before and monitored for selected clinical and laboratory AE throughout the trial in the clinic on the 12, 24, 48, and 72 h after receiving intralymphonodal (ILN) adenoviral vector in D-MEM/F-12 vehicle (11039-021 GIBCO™ Invitrogen Co. USA).

Animals

Five adult dogs (1 male and 4 females) with spontaneous multicentric lymphosarcoma were used and housed individually in raised metal cages during this study. Prior to each sampling dogs were fasted for 12 hours and water retired 4 hours before sampling. Design of the study was approved by the local ethic committee at College of Veterinary Medicine of UNAM.

Lymphosarcoma diagnosis

All cases had a clinical, radiological, hematological and cytological diagnosis of WHO's clinical stage III a to V b multicentric lymphosarcoma [30].

Adenovirus

The non-replicative recombinant Ad5 vector (E1 and E3 regions deleted) with a *E. coli lacZ* reporter gene (provided by Dr. Curiel, Gene Therapy Center Alabama University), which expresses the enzyme beta-galactosidase (Ad5B-gal) was amplified and tittered (AdEasy™ Vector System Quantum Technologies. Application Manual. Version 1.2) and purified (Adeno-X™ Virus Mini Purificator kit. Clontech Laboratories, Inc).

Adenovirus infectivity test

Prior the ILN administration Ad5B-gal infectivity was demonstrated in Hela cell line (VCA-1001, Amaxa, Inc. USA) using the detection of β-galactosidase by overnight X-Gal staining at 37°C (AdEasy™ Vector System Quantum Technologies. Application Manual. Version 1.2. Montreal, Qc. Canada).

Clinical signs

The clinical examination included anorexia, body temperature, cardiac frequency, respiratory frequency, vomit, dehydration, diarrhea and edema. A complete laboratory panel was used for AE research. The animals were observed every two hours during the 72 hours of study.

Clinical Pathology

A complete clinical pathology profile was established to evaluate hematological (hematocrit, hemoglobin, erythrocytes, MCV, MCHC, reticulocytes, nucleated and abnormal erythrocytic morphology, leukocytes, neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, platelets, total solids, and fibrinogen), hepatic, renal, pancreatic, muscular, cardiac, acid-base balance changes, and general cellular integrity (Table 1) [31].

Experimental design

A total of five Ad5B-gal ILN dose escalation levels were explored (Table 2), one dose by dog (following the AE guide of Table 3). The attribution grade of adverse events was applied according to

Table 2: Experimental design.

Dog	Dose by intralymphonodal route of Ad5B-gal in single administration	Evaluation of adverse events
1	Sterile DMEM/F-12 (vehicle)	Before 1 2 24 48 72 h
2	1.35 X 10^{10}/VP/kg	Before 1 2 24 48 72 h
3	2.53 X 10^{10}/VPkg	Before 1 2 24 48 72 h
4	6.10 X 10^{10}/VP/kg	Before 1 2 24 48 72 h
5	18.38 X 10^{10}/VP/kg	Before 1 2 24 48 72 h
6	153.85 X 10^{10}/VP/kg	Before 1 2 24 48 72 h

Table 3: Adverse events guide for dogs.

Adverse event	0	1	2	3	4
Anorexia	No changes	< 1 day	1-3 days	4-5 days	>5 days
Temperature	38-39 ºC	39.1-39.5	39.6-40.0	40.1-40.5	>40.5
Cardiac frequency (adults)	70-120/min	121-140	141-160	161-180	>180
Respiratory frequency	11-30/min	31-40	41-50	51-60	>60
Vomit	absent	nausea	sporadic	< 5/day, <2 days	>5/day, >2 days
Dehydration	absent	5%	8%	10%	>10%
Diarrhea (watery)	absent	Soft feces	<4/day <2 days	4-7/day or >2 days	>7/day or bloody
Edema	absent	light	moderate	elevated	severe extremities and facial
Hematocrit (L/L)	0.37-0.55	0.30-0.36	0.22-0.29	0.15-0.21	<0.15
Hemoglobin (g/L)	120-180	97-119	74-96	50-73	<50
Erythrocytes (X10^{12}/L)	5.5-8.5	4.5-5.49	3.5-4.49	2.5-3.49	<2.5
Platelets (X10^{9}/L) L	200-600	100-199	50-99	20-49	<20
Platelets (X10^{9}/L) H		601-800	801-1000	1001-1200	>1200
Leukocytes (X 10^{9}/L) L	6.0-17.0	5.0-5.9	4.0-4.9	3.0-3.9	<3.0
Leukocytes (X 10^{9}/L) H		17.1-30.0	30.1-40.0	40.1-50.0	>50
Neutrophils (X 10^{9}/L) L	3.0-11.5	1.5-3.0	1.0-1.4	0.5-0.9	<0.5
Neutrophils (X 10^{9}/L) H		11.6-20.0	20.1-30.0	30.1-40.0	>40.0
Band Neutrophils (X 10^{9}/L)	0.0-0.3	0.4-0.8	0.9-1.8	1.9-3.8	>3.8
Lymphocytes (X 10^{9}/L)	1.0-4.8	0.9-0.7	0.6-0.4	0.3-0.1	0.0
Monocytes (X 10^{9}/L)	0-1.4	1.5-2.0	2.1-2.5	2.6-3.0	>3.0
Eosinophils (X 10^{9}/L)	0-0.9	1-1.5	1.6-2.0	2.1-2.5	>2.5
Total solids (g/L)	60-75	55-59	50-54	45-49	<45
Metarubricytes/100 WBC	Negative	1-2	3-5	6-10	>10
Fibrinogen (g/L)	1-2	2.1-3.0	3.1-4.0	4.1-5.0	>5.0
Glucose (mmol/L)	3.38-6.88	3.00-3.37	2.50-2.99	2.0-2.49	<2.0
Urea (mmol/L) L	2.09-7.91	1.7-2.08	1.3-1.69	1.00-1.29	<1.00
Urea (mmol/L) H		7.92-8.50	8.51-9.50	9.51-10.50	>10.5
Creatinine (μmol/L)	<126	133-265	266-354	355-442	>442 or > 4 weeks
Bilirubin (total) (μmol/L)	<5.16	5.16-15.0	15.1-25.0	25.1-35.0	>35.0
Bil. (conjugate) (μmol/L)	<5.1	5.1-10.0	10.1-15.0	15.1-20	>20.0
Bil. (unconjugate) (μmol/L)	<1.0	1-5	5.1-10.0	10.1-15.0	>15.0
Cholesterol (mmol/L) L	2.85-7.76	2.35-2.84	1.85-2.34	1.35-1.84	<1.35
Cholesterol (mmol/L) H		7.77-9.77	9.78-10.76	10.77-11.76	>11.76
Triglycerides (mmol/L)	0.57-1.14	1.15-2.28	2.29-5.70	5.71-11.4	>11.4
ALT (UI/L)	<70	70-140	141-280	281-420	>420
AST (UI/L)	<55	56-110	111-220	221-330	>330
ALP (UI/L)	<190	190-473	474-945	946-3780	>3780

GGT (UI/L)	<7	7-15	16-30	31-45	>45
CK (UI/L)	<213	213-532	533-1065	1066-2130	>2130
Amylase (UI/L)	<1100	1101-1650	1651-2200	2201-5500	>5500
LDH (UI/L)	<150	150-250	251-350	351-450	>450
Total Proteins (g/L)	56.6-74.8	53.6-56.5	51.6-53.5	49.6-51.5	<49.6
Albumin (g/L)	29.1-39.7	25.0-29.0	20.0-24.9	15.0-19.9	<15.0
Globulins (g/L) L	23.5-39.1	21.5-23.4	19.5-21.4	17.5-19.4	<17.5
Globulins (g/L) H		39.2-42.0	42.1-44.0	44.1-46.0	>46.0
A/G	0.78-1.46	0.70-0.77	0.60-0.69	0.50-0.59	<0.50
Calcium (mmol/L) L	2.27-2.91	2.01-2.26	1.75-2.00	1.50-1.74	<1.50
Calcium (mmol/L) H		2.92-3.12	3-13-3.32	3.33-3.42	>3.42
I. phosphate (mmol/L) L	0.75-1.70	0.65-0.74	0.55-0.64	0.45-0.54	<0.54
I. phosphate (mmol/L) H		1.71-3.00	3.10-4.50	4.51-6.00	>6.00
Potassium (mmol/L) L	3.82-5.34	3.32-3.81	2.82-3.31	2.32-2.81	<2.32
Potassium (mmol/L) H		5.35-6.00	6.01-6.66	6.67-7.32	>7.32
Sodium (mmol/L) L	141-153	135-140	130-134	125-129	<125
Sodium (mmol/L) H		154-158	159-163	164-168	>168
Chloride (mmol/L) L	108.0-117.0	103.0-107.9	98.0-102.9	93.0-97.9	<93.0
Chloride (mmol/L) H		117.1-122.0	122.1-127	127.1-132.0	>132
Bicarbonate (mmol/L)	17.0-25.0	14.0-16.9	11.0-13.9	8.0-10.9	<8.0
Non volatile acids (mmol/L)	12-24	25-27	28-31	32-35	>35
SID_{clin} (mmol/L) L	30.0-40.0	25.0-29.9	20.0-24.9	15.0-19.9	<15.0
SID_{clin} (mmol/L) H		40.1-45.0	45.1-55.0	55.1-65.0	>65.0
Albuminuria (g/L)	Negative	0.1-0.3	0.4-1.0	2.0-5.0	>5.0
Glucosuria (mmol/L)	Negative	0.1-2.7	2.8-5.55	5.6-16.7	16.7-55.0
Bilirrubinuria (μmol/L)	Negative	0.1-8.55	8.56-17.1	17.2-34.2	>34.2
Hematuria (erythrocytes/field/400x)	Negative	5-10	11-50	51-250	>250
Proteinuria (g/L)	Negative	0.3	1.0	5.0	>5.0
Glucose (mmol/L)	Negative	2.8	5.5	17	55
Casts (field/100x)	Negative	0-1	0-2	1-2	>2
Renal cells (field/400x)	Negative	0-1	0-2	1-2	>2

Bil.= Bilirubin, ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, ALP= Alkaline phosphatase, GGT= Gamma glutamyltransferase, CK= Creatine kinase, LDH= Lactate deshydrogenase, I. phosphate= inorganic phosphate, SID_{clin}= Strong ion difference (clinical).

Table 4: Grading scale for attribution of adverse events.

Descriptor	**Unrelated**	**Unlikely**	**Possible**	**Probable**	**Definite**
Grades	0	1	2	3	4
Definition	No adverse event or within reference limits	Mild adverse event	Moderate adverse event	Severe and undesirable adverse event	Life-threatening or disabling adverse event

Table 4 from which the adjustment value was subtracted from the Table 5 indications, to ensure that treatment-related conditions are distinguished from disease-related conditions to obtain the real grade (modified from National Cancer Institute NCI CTEP version 4.03 2010).

Dose-limiting toxicity

The dose-limiting toxicity was defined as a grade 3 or grater for clinical or laboratory AE.

Laboratory analysis

Blood was collected for serum biochemistry and hemogram evaluation. The biochemistry analytes were determined by Cobas Mira® Chemistry Analyzer (Roche Diagnostic Systems, Inc. New Jersey, USA), and EasyLyte Plus $Na^+/K^+/Cl^-$ (Medica Corporation MA USA), the hemogram, was evaluated by the Coulter Counter® T-540 (Coulter Electronics, Inc. Florida, USA). Urine voided samples was collected and chemistry was evaluated with Combur[10] Test® M (Roche, Germany).

Data analysis

Data were classified in accordance with our adverse events guide for dogs (Table 3) and the grading adjustment values related with the attribution of adverse events (Table 5). Statistical significance of experimental results was analyzed by two-tailed Student's *t*-test (SPSS) to compare paired data in dogs with LSA. Differences were

Table 5: Grading adjustment values related with the attribution of adverse events.

Adjustment values	4	3	2	1	0
Definition	The adverse event is *clearly not related* to the investigational agent(s)	The adverse event is *doubtfully related* to the investigational agent(s)	The adverse event *may be related* to the investigational agent(s)	The adverse event is *likely related* to the investigational agent(s)	The adverse event is *clearly related* to the investigational agent(s)

Modified from The NCI Common Terminology Criteria for Adverse Events. [Version 4.03], 2010

Table 6: Descriptive statistics and grades of clinical adverse events in 5 dogs with spontaneous multicentric lymphosarcoma. Grade adjustment after Ad5B-gal intralymphonodal administration.

Time (h)	0		12		24		48		72	
	$\overline{X}$ ± (SD)	EA	$\overline{X}$ ± (SD)	EA	$\overline{X}$ ± (SD)	EA	$\overline{X}$ ± (SD)	EA	$\overline{X}$ ± (SD)	EA
Anorexia	2	2	1	0	Neg	0	Neg	0	Neg	0
Temperature (ºC)	2	2	1	0	Neg	0	Neg	0	Neg	0
Heart rate (min)	39.26 (0.87)	1	39.04 (0.54)	0	38.84 (0.39)	0	39.04 (0.54)	0	38.82 (0.49)	0
Respiratory rate (min)	132.6 (16.5)	1	126.0 (15.1)	1	120.0 (12.8)	0	137 (9.3)	1	136.0 (22.6)	1
Vomit	46.0 (19.3)	1	39.6 (17.1)	0	44.8 (14.5)	0	44.8 (19.6)	0	45.2 (15.6)	0
Dehydration	Neg	0	1	1	Neg	0	Neg	0	Neg	0
Diarrhea	Neg	0	Neg	0	Neg	0	Neg	0	Neg	0
Edema	2	2	2	0	2	0	2	0	2	0

$\overline{X}$ ± (SD)= Mean ± standard deviation, AE= Adverse events. Neg= Negative.
The clinical elements according to the sampling stages, on the table with an exponential number indicate a statistically significant difference (P <0.5). [1](0 - 12 h), [2](0 –24 h), [3](0 – 48 h), [4](0 - 72 h), [5](12 –24 h), [6](12 – 48 h), [7](12 – 72 h), [8](24 – 48 h), [9](24 – 72 h), [10](48 – 72 h)

Table 7: Descriptive statistics and grades of hematological adverse events in 5 dogs with spontaneous multicentric lymphosarcoma. Grade adjustment after Ad5B-gal intralymphonodal administration.

Time (h)	0		12		24		48		72	
	$\overline{X}$ ± (SD)	EA	$\overline{X}$ ± (SD)	EA	$\overline{X}$ ± (SD)	EA	$\overline{X}$ ± (SD)	EA	$\overline{X}$ ± (SD)	EA
Hematocrit (L/L)	0.358 (0.052)	1	0.364 (0.058)	0	0.350 (0.051)	0	0.344 (0.057)	0	0.330 (0.054)	0
Leukocytes (x10^9/L)	16.00 (8.12)	0	19.54 (6.88)	1	17.67 (7.35)	1	19.92 (12.03)	1	21.40 (13.20)	1
Neutrophils (x10^9/L)	13.04 (7.00)	1	16.28 (6.71)	0	13.84 (5.21)	0	15.31 (9.19)	0	17.46 (11.33)	0
Band (x10^9/L)	0.028 (0.06)	0	0.152 (0.17)	0	0.082 (0.12)	0	0.08 (0.13)	0	0.10 (0.22)	0
Lymphocytes (x10^9/L)	1.96 (0.92)	0	1.98 (1.00)	0	2.89 (1.80)	0	3.09 (2.41)	0	2.60 (2.10)	0
Monocytes (x10^9/L)	0.75 (0.55)	0	0.82 (0.58)	0	0.65 (0.43)	0	1.23 (0.98)	0	1.13 (0.75)	0
Eosinophils (x10^9/L)	0.23 (0.21)	0	0.11 (0.11)	0	0.13 (0.22)	0	0.16 (0.18)	0	0.21 (0.25)	0
Total solids[5,6] g/L	63.8 (5.93)	0	64.8 (7.01)	0	62.0 (6.96)	0	62.2 (7.88)	0	60.4 (4.77)	0
Fibrinogen g/L	2.0 (1.22)	0	1.6 (0.55)	0	1.8 (0.45)	0	2.0 (0.00)	0	2.2 (1.10)	0
NE/100 leukocytes	1.4 (3.13)	1	1.0 (2.24)	0	1.4 (2.61)	0	0.0 (0.00)	0	1.6 (3.58)	0

$\overline{X}$ ± (SD) = Mean ± standard deviation, AE= Adverse events, NE = Nucleated erythrocytes.
The hematological mesurands according to the sampling stages, on the table with an exponential number indicate a statistically significant difference (P <0.5). [1](0 - 12 h), [2](0 –24 h), [3](0 – 48 h), [4](0 - 72 h), [5](12 –24 h), [6](12 – 48 h), [7](12 – 72 h), [8](24 – 48 h), [9](24 – 72 h), [10](48 – 72 h)

Table 8: Descriptive statistics and grades of hepatic adverse events in 5 dogs with spontaneous multicentric lymphosarcoma. Grade adjustment after Ad5B-gal intralymphonodal administration.

Time (h)	0		12		24		48		72	
	$\bar{X}$ ± (SD)	EA	$\bar{X}$ ± (SD)	EA	$\bar{X}$ ± (SD)	EA	$\bar{X}$ ± (SD)	EA	$\bar{X}$ ± (SD)	EA
Glucose[5] (mmol/L)	4.44 (1.38)	0	4.70 (1.16)	0	4.14 (0.91)	0	4.12 (0.85)	0	4.16 (1.06)	0
Cholesterol (mmol/L)	5.85 (2.46)	0	6.04 (2.49)	0	6.21 (3.00)	0	6.41 (3.23)	0	6.12 (3.49)	0
Triglycerids[3,10] (mmol/L)	0.644 (0.07)	0	0.712 (0.24)	0	0.798 (0.38)	0	0.798 (0.15)	0	0.508 (0.24)	0
Bilirubin (µmol/L)	6.80 (4.93)	1	7.24 (4.96)	0	7.22 (5.44)	0	7.65 (4.83)	0	7.10 (4.36)	0
Conjugated Bil. (µmol/L)	3.9 (2.32)	0	4.77 (3.42)	0	4.88 (3.32)	0	4.30 (2.28)	0	3.75 (2.72)	0
Unconjugated Bil. (µmol/L)	2.9 (2.65)	1	2.47 (2.13)	0	2.34 (3.02)	0	3.35 (2.55)	0	3.36 (1.90)	0
ALT (U/L)	213 (298)	2	220 (294)	0	223 (290)	0	215 (279)	0	176 (217)	0
AST (U/L)	90.0 (74.9)	1	114.0 (69.8)	0	114.8 (59.7)	0	76.4 (43.6)	0	68.0 (27.4)	0
ALP (U/L)	871 (1374)	2	922 (1393)	0	993 (1418)	0	1046 (1548)	0	1242 (1826)	0
T. Proteins[4] (g/L)	62.6 (10.16)	0	60.2 (7.12)	0	60.2 (8.64)	0	60.6 (8.79)	0	57.0 (6.78)	0
Albumin[3,4] (g/L)	26.6 (3.44)	1	26.2 (3.96)	0	25.8 (2.78)	0	25.2 (3.27)	0	24.4 (2.70)	0

$\bar{X}$ ± (SD) = Mean ± standard deviation, AE= Adverse events. Bil= Bilirubin, ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, ALP= Alkaline phosphatase. T. Proteins= Total Proteins.

The hepatic mesurands according to the sampling stages, on the table with an exponential number indicate a statistically significant difference (P <0.5). [1](0 - 12 h), [2](0

considered significant if P was <0.05.

Results

None of dogs that received Ad5B-gal died during the course of the study.

No DLT was observed in all patients, and therefore, the MTD was not established.

Clinical evaluation

All doses of Ad5B-gal were well tolerated; no significant AE was related to the administration of vector (Table 6). Anorexia was from grade 2 to 0 in 24 h after intralymphonodal treatment indicating a favorable event. In dogs receiving higher doses (18.38 X 10^{10}/VP/kg and 153.85 X 10^{10}/VP/kg) a small single vomit was present in the first 12 h post adenoviral administration. Cardiac frequency was elevated during most evaluations. Respiratory frequency was statistically different in 12-24 h but not dose-dependent. Four dogs had pre-dose submandibular or limb edema, therefore these results were not considered secondary to adenoviral vector. Diarrhea and fever changes were clearly related to disease (Table 6).

Hematological evaluation

The gradual decrease from pre-dose to 72 h (9.2%) of hematocrit was found. Two dogs manifested anemia at 0 h and another at 72 h. Leukocytosis and neutrophilia was present during the 5 sampling times in two dogs, one dog only at 12 hours (high dose dog). The progressive decrease of total solids was statistically significant at 12-24 h and 12-48 h comparison (Table 7).

Liver evaluation

The levels of glucose and triglycerides were statistically different but within the reference values. The high values of ALT, AST and AP enzymes were similar pre and post-dose. Even if the decrease of proteins was significant between 0-72 h, the results are within reference values. This was associated with gradual decrease of albumin statistically different at 0-48 h and 0-72 h (Table 8).

Acid-base evaluation

No observable changes were seen with K^+ and SID_{clin}. Electrolytes (Na^+, Cl^- and HCO_3^-) decreased at 12 h post-dose and were different at 0 and 24 h. There was a slight increase at grade 1 in the concentration of NVA after 72 h of dosing.

Cell integrity

Total LDH was found increased at 12 and 24 h post-dose at grade 1 but not statistically significant.

No significant variations were seen in kidneys, urinalysis, pancreas, muscle and heart evaluations.

Discussion

To our knowledge, there are no reports in the literature about ILNR oncolytic adenovirus administration. The different routes utilized for adenovector-mediated gene transfer administration *in vivo* have had different tolerance and limitations. Major disadvantages of human adenovirus vectors in gene therapy include preexisting or induced immune responses, and possible coreplication of recombinant Ad in the presence of wild-type Ads [32]. One of these limitations is the low gene transfer rate into organs other than the liver after systemic intravenous vector injection [33]. In a single-dose intravenous injection of 6X10^{12} viral particles in dogs with hemophilia A for human factor VIII transfer [14], 2X10^{12} into hepatic artery [17], in situ administration into primary gastric cancer [18], 1X10^{12} of intraprostatic injection [34], doses of 8.57X10^{11}/VP/kg in dogs with hemophilia B injected intravenously have had no significant AE [13]. Transient hepatic integrity (ALT, AST, ALP), muscular (CK), and primary hemostasis (platelet counts) abnormalities were found after administration of high (3X10^{12}/VP/kg) [35] and low dose (6X10^{11}/VP/kg) [36]. A high i.v. dose of vector (>10^{13} VP) has been leading to a systemic cytokine shock and may be resulted in the death [37]. AE was also observed in some studies after Ad administration in situ, not for Ad but for their proteins expressed [18]. The route of administration and the vector affect the level and duration of expression [38]. In situ

route is best for transferring the therapeutic gene into cancer cells [18]. The rationale for this approach is to increase the dose effects in a specific tissue, improving antineoplasic efficacy, and reducing or even eliminating the immunosuppression period and other critical AE. Therefore, the neoplasic tissue receives higher quantity of therapeutic product, and its systemic distribution to other sensible healthy organs is reduced. The mild decrease of hematocrit is associated with an evolution of anemia in natural lymphosarcoma cases [39-43] and not related to adenoviral administration, because anemia is one of the most common paraneoplastic syndrome seen in veterinary and human oncology [44]. Cardiac frequency was elevated secondary to anemia which also happens in other cancer patients [45]. Neutrophilic leucocytosis corresponded in our study, with findings reported in dogs with lymphosarcoma [43]. The gradual decrease of total solids, proteins and albumin was none related to treatment. Frequently, hypoproteinemia and hypoalbuminemia are considered as secondary toxic responses to experimental drugs [46,47]. As in this work, these changes are normally present in lymphosarcoma [48]. The decrease of proteins is associated to constant decrease of albumin. In this case the hypoalbuminemia is caused by its decreased synthesis, since it is a negative acute-phase protein [31]. The significant difference in total solids samples was related to mild hemoconcentration found at 12 h. The evaluation of hepatic integrity was similar all times and was dose-independent as opposed to published paper [36]. The difference of electrolyte concentrations among 12 h and 0 h or 24 h was related to the degree of animal hydration, because their appetite and water consumption improved from 12 h post-dose to the end of study. The marginal AE of NVA evaluation at 72 h is consistent with a mild pseudometabolic acidosis or spurious decrease of HCO_3^- due to *in vitro* loss because the serum sample was analyzed 12 hours after the sampling [49].

Most of clinical and laboratory changes were considered not treatment-related, but disease-related conditions.

Conclusions

To the author's knowledge, this is the first report about intralymphonodal administration of adenovirus for gene therapy. The administration of Ad5 vector in canine spontaneous multicentric lymphosarcoma at high dose exhibited no clinical, nor laboratory significant adverse events. This suggests that Ad5B-gal is a safe vector for use in lymphosarcoma gene therapy. This data provides the basis to consider the lymphonodal route as an appropriate way for therapy administration without significant adverse events in canine lymphosarcoma and a potential model for applied therapy research in lymphonodal metastasis in animals and human beings.

Acknowledgments

This project was supported by National Council for Science and Technology of Mexico and National Autonomous University of Mexico and funded by grant from Experto Sur Veterinary Laboratory, Mexico city (LE01-0206).

References

1. Mayer MN, Larue SM. Radiation therapy in the treatment of canine lymphoma. Can Vet J. 2005;46(9):842-4.
2. Williams LE, Johnson JL, Hauck ML, Ruslander DM, Price GS, Thrall DE. Chemotherapy followed by half-body radiation therapy for canine lymphoma. J Vet Intern Med. 2004;18(5): 703-709.
3. Escobar C, Grindem C, Neel JA, Suter SE. Hematologic changes after total body Irradiation and autologous transplantation of hematopoietic peripheral blood progenitor cells in dogs with lymphoma. Vet Pathol. 2012;49(2):341-3.
4. Kisseberth WC, MacEwen EG. Complications of Cancer and Its Treatment. In: Withrow SJ and MacEwen EG (Eds.), Small Animal Clinical Oncology. W.B. Saunders Co., Philadelphia. 2001. pp. 198-219.
5. Frimberger AE, Moore AS, Rassnick KM, Cotter SM, O'Sullivan JL, Quesenberry PJ. A combination chemotherapy protocol with dose intensification and autologous bone marrow transplant (VELCAP-HDC) for canine lymphoma. J Vet Intern Med. 2006;20(2):355-364.
6. MacDonald V. Chemotherapy: managing side effects and safe handling. Can Vet J. 2009;50(6):665-8.
7. Impellizeri JA, Howell K, McKeever KP, Crow SE. The role of rituximab in the treatment of canine lymphoma: an ex vivo evaluation. Vet J. 2006;171(3):556-8.
8. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-64.
9. Lundstrom K. Gene therapy applications of viral vectors. Technol Cancer Res Treat. 2004;3(5):467-77.
10. Miest TS, Cattaneo R. New viruses for cancer therapy: meeting clinical needs. Nat Rev Microbiol. 2014;12(1):23-34.
11. Hackett N, Crystal R. Adenovirus Vectors for Gene Therapy. In: Templeton N.S. and Lasic D.D. (Eds.) Gene Therapy. Therapeutic Mechanisms and Strategies. Marcel Dekker, Inc., New York. 2000; pp. 17-40.
12. Larson C, Oronsky B, Scicinski J, Fanger GR, Stirn M, Oronsky A, et al. Going viral: a review of replication-selective oncolytic Adenoviruses. Oncotarget. 2015; 6(24):19976-89.
13. Ehrhardt A, Xu H, Dillow AM, Bellinger DA, Nichols TC, Kay MA. A gene-deleted adenoviral vector results in phenotypic correction of canine hemophilia B without liver toxicity or thrombocytopenia. Blood. 2003;102(7):2403-2411.
14. Zhang WW, Josephs SF, Zhou J, Fang X, Alemany R, Balagué C, et al. Development and application of a minimal-adenoviral vector system for gene therapy of hemophilia A. Thrombosis and Haemostasis. 1999;82(2):562-71.
15. Herzog RW, Yang EY, Couto LB, Hagstrom JN, Elwell D, Fields PA, et al. Long-term correction of canine hemophilia B by gene transfer of blood coagulation factor IX mediated by adeno-associated viral vector. Nature Medicine. 1999;5(1):56-63.
16. Barton KN, Tyson D, Stricker H, Lew YS, Heisey G, Koul S, et al. GENIS: gene expression of sodium iodide symporter for noninvasive imaging of gene therapy vectors and quantification of gene expression *in vivo*. Molecular Therapy. 2003;8(3):508-18.
17. Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nature Medicine. 2006;12(3);342-47.
18. Matsukura N, Hoshino A, Igarashi T, Hasegawa H, Okino T, Onda M, et al. In situ gene transfer and suicide gene therapy of gastric cancer induced by N-ethyl-N'-nitro-N-nitrosoguanidine in dogs. Jpn J Cancer Res. 1999;90(9):1039-49.
19. Foley R, Ellis R, Walker I, Wan Y, Carter R, Boyle M, et al. Intramarrow cytokine gene transfer by adenoviral vectors in dogs. Hum Gene Ther. 1997;8(5):545-53.
20. Goto H1, Shuler FD, Lamsam C, Moller HD, Niyibizi C, Fu FH, et al. Transfer of lacZ marker gene to the meniscus. J Bone Joint Surg Am. 1999;81(7):918-25.
21. Plotnikov AN, Sosunov EA, Qu J, Shlapakova IN, Anyukhovsky EP, Liu

L, et al. Biological pacemaker implanted in canine left bundle branch provides ventricular escape rhythms that have physiologically acceptable rates. Circulation. 2004;109(4):506-12.

22. Ayuso E, Chillón M, García F, Agudo J, Andaluz A, Carretero A, et al. *In vivo* gene transfer to healthy and diabetic canine pancreas. Molecular Therapy. 2006;13(4):747-755.

23. Khurana VG, Smith LA, Baker TA, Eguchi D, O'Brien T, Katusic ZS. Protective vasomotor effects of *in vivo* recombinant endothelial nitric oxide synthase gene expression in a canine model of cerebral vasospasm. Stroke. 2002;33(3):782-789.

24. Cerletti M, Negri T, Cozzi F, Colpo R, Andreetta F, Croci D, et al. Dystrophic phenotype of canine X-linked muscular dystrophy is mitigated by adenovirus-mediated utrophin gene transfer. Gene Therapy. 2003;10(9):750-757.

25. Acland GM, Aguirre GD, Ray J, Zhang Q, Aleman TS, Cideciyan AV, et al. Gene therapy restores vision in a canine model of childhood blindness. Nature Genetics. 2001;28(1):92-95.

26. O'Riordan CR Song A, Lanciotti J. Strategies to Adapt Adenoviral Vectors to targeted delivery. In: Machida, C.A. (Ed.), Viral vectors for Gene Therapy. Methods and Protocols. Humana Press, Totowa, New Jersey. 2003. pp. 89-112.

27. Vonderhaar MA, Morrison WB. Lymphosarcoma. In: Morrison WB (Ed.), Cancer in Dogs and Cats. Medical and Surgical Management. Williams and Wilkins, Baltimore. 1998. pp. 667-695.

28. National Cancer Institute. The NCI Common Terminology Criteria for Adverse Events.

29. Boone L, Meyer D, Cusick P, Ennulat D, Bolliger AP, Everds N, et al. Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies. Veterinary Clinical Pathology. 2005;34(3):182-88.

30. Vail DM. Hematopoietic tumors. In: Ettinger SJ, Feldman EC (editors). Textbook of Veterinary Internal Medicine: Diseases of the Cat and Dog, 7th ed. Saunders, St. Louis. 2010. 2148-2158.

31. Latimer KS. Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, Fifth ed. Blackwell Publishing. Iowa State Press. 2011.

32. Rasmussen UB, Benchaibi M, Meyer V, Schlesinger Y, Schughart K. Novel human gene transfer vectors: evaluation of wild-type and recombinant animal adenoviruses in human-derived cells. Human Gene Therapy. 1999;10(16):2587-99.

33. Fechner H, Haack A, Wang H, Wang X, Eizema K, Pauschinger M, et al. Expression of coxsackie adenovirus receptor and alphav-integrin does not correlate with adenovector targeting *in vivo* indicating anatomical vector barriers. Gene Therapy. 1999;6(9):1520-35.

34. Dwyer RM, Schatz SM, Bergert ER, Myers RM, Harvey ME, Classic KL, et al. A preclinical large animal model of adenovirus-mediated expression of the sodium-iodide symporter for radioiodide imaging and therapy of locally recurrent prostate cancer. Molecular Therapy. 2005;12(5):835-41.

35. Brunetti-Pierri N, Nichols TC, McCorquodale S, Merricks E, Palmer DJ, Beaudet AL, et al. Sustained phenotypic correction of canine hemophilia B after systemic administration of helper-dependent adenoviral vector. Human Gene Therapy. 2005;16(7):811-20.

36. Gallo-Penn AM, Shirley PS, Andrews JL, Tinlin S, Webster S, Cameron C, et al. Systemic delivery of an adenoviral vector encoding canine factor VIII results in short-term phenotypic correction, inhibitor development, and biphasic liver toxicity in hemophilia A dogs. Blood. 2001;97(1):107-13.

37. Andrawiss M, Maron A, Beltran W, Opolon P, Connault E, Griscelli F, et al. Adenovirus-mediated gene transfer in canine eyes: a preclinical study for gene therapy of human uveal melanoma. J Gene Med. 2001;3(3):228-39.

38. Liles D, Landen CN, Monroe DM, Lindley CM, Read MS, Roberts HR, et al. Extravascular administration of factor IX: potential for replacement therapy of canine and human hemophilia B. Thrombosis and Haemostasis. 1997;77(5):944-48.

39. Caldin M, Carli E, Furlanello T, Solano-Gallego L, Tasca S, Patron C, et al. A retrospective study of 60 cases of eccentrocytosis in the dog. Vet Clin Pathol. 2005;34(3):224-31.

40. Fukuoka A, Nakayama H, Nakayama Y, Yasoshima A, Uetsuka K, Fujino Y, et al. Thymoma in a dog with a part of granular cell proliferation and concurrent lymphoma cells. J Vet Med Sci. 2004;66(6):713-15.

41. Tecles F, Spiranelli E, Bonfanti U, Cerón JJ, Paltrinieri S. Preliminary studies of serum acute-phase protein concentrations in hematologic and neoplastic diseases of the dog. J Vet Intern Med. 2005;19(6):865-870.

42. Weiss DJ. Bone marrow necrosis in dogs: 34 cases (1996-2004). J Am Vet Med Assoc. 2005;227(2):263-7.

43. Mortier F, Daminet S, Vandenabeele S, Van de Maele I. Canine lymphoma: a retrospective study (2009 – 2010). Vlaams Diergeneeskundig Tijdschrift 2012 ; 81(6):341-351.

44. Childress MO. Hematologic Abnormalities in the Small Animal Cancer Patient. Veterinary Clinics of North America Small Animal Practice. 2012; 42(1):123–155.

45. Pronzato P. Cancer-related anaemia management in the 21st century. Cancer Treat Rev. 2006;32:S1-3.

46. Kreitman RJ, Squires DR, Stetler-Stevenson M, Noel P, FitzGerald DJ, Wilson WH, et al. Phase I trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with B-cell malignancies. J Clin Oncol. 2005;23(27):6719-6729.

47. Robertson MJ, Mier JW, Logan T, Atkins M, Koon H, Koch KM, et al. Clinical and biological effects of recombinant human interleukin-18 administered by intravenous infusion to patients with advanced cancer. Clinical Cancer Research. 2006; 12(14 Pt 1):4265-73.

48. Price GS, Page RL, Fischer BM, Levine JF, Gerig TM. Efficacy and toxicity of doxorubicin/cyclophosphamide maintenance therapy in dogs with multicentric lymphosarcoma. J Vet Inter Med. 1991;5(5):259-62.

49. Stockham SL, Scott MA. Fundamentals of Veterinary Clinical Pathology. 2nd edition Iowa State Press. Blackwell Publishing Co., Ames, Iowa. 2008.