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Evaluation of ions and metals in the blood of GRMD dogs submitted to hASCs therapy by NAA and XRF techniques

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HIGHLIGHTS

- Multielement analysis of blood samples of Golden Retriever animal model, affected by Duchenne Muscular Dystrophy (DMD) and submitted to human adipose stromal cells (hASCs) transplantation therapy.
- During treatment, a significant increase was observed in K blood levels. There were also variations in blood levels of Br, Cr, Fe, K, Rb, S and Zn.
- These results contribute to the detailed knowledge of the DMD inorganic blood elements behavior.
- The EDXRF and INAA analytical techniques were combined for these blood tests: they are accurate and complementary.

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ABSTRACT

The elements Br, Ca, Cl, Cr, Fe, K, Mg, Na, P, Rb, S, and Zn were investigated in the whole blood samples of Golden Retriever dogs submitted to cell therapy (hASCs). These analyses were performed over 2 years using Neutron Activation Analysis and X-Ray Fluorescence techniques. The results were compared with control and untreated dog's. A significant increase was observed in K blood levels. There was also variation in blood levels of Br, Cr, Fe, Rb, S, and Zn.

1. Introduction

Progressive muscular dystrophies (PMDs) are characterized by presenting irreversible, progressive skeletal muscle degeneration. The Duchenne Muscular Dystrophy (DMD) is the most aggressive and typical of them. It cannot be reversed and has no cure (Zatz, 2005; Associação Brasileira de Distrofia Muscular ABDIM, 2010; Bushby et al., 2010; Leiden University Medical Center, 2004; Angelini, 2013). The dystrophin gene mutation located in humans on the X chromosome cause the DMD disorder. Unlike most genes, which come in pairs in both sexes and stay active throughout life, in males there is only one X chromosome, for this reason, this disorder usually affected much more boys than girls (1/3500 boys) (Bushby et al., 2010; Angelini, 2013; Emery, 2002). This gene has 79 exons (the largest of our body) fitted together like pieces of a jigsaw puzzle and form the genetic code for the dystrophin protein (Muscular Dystrophy Campaign, 2009). Specifically, the absence of exon 50 that interferes in the sequencing of amino acids of dystrophin protein leads to skeletal muscle damage. Currently, no effective treatment is available, and the DMD research progress depends on animal models, faithful to human clinical pathology, to conduct pre-clinical tests to verify the effectiveness and safety of new therapies before be performed in humans Muscular Dystrophy Campaign (2009). In the last years, the Spectroscopy Radiation Laboratory (IPEN, CNEN/SP, São Paulo – SP, Brazil) has used Neutron Activation Analysis (NAA) and X-ray Fluorescence (XRF) techniques for the investigation of ions and metals in blood of dystrophic mice strains: Dmd^{mdx}/J, A/J and SJL/J (Metairon et al., 2017, 2012, 2011; Redígolo et al., 2016; Zamboni et al., 2009, 2008). These investigations were done focusing on the evidences of similarities with human blood, to establish possible indices (reference values) for clinical research of new PMDs treatments.

Recently, the possibilities of a new treatment for DMD with cell therapy performed in dystrophic animal models have been investigated at the Human Genome Research Center (University of São Paulo, São Paulo - SP, Brazil). Vieira et al. (2010, 2008) showed that human adipose-derived stromal cells (hASCs) when systemically injected without

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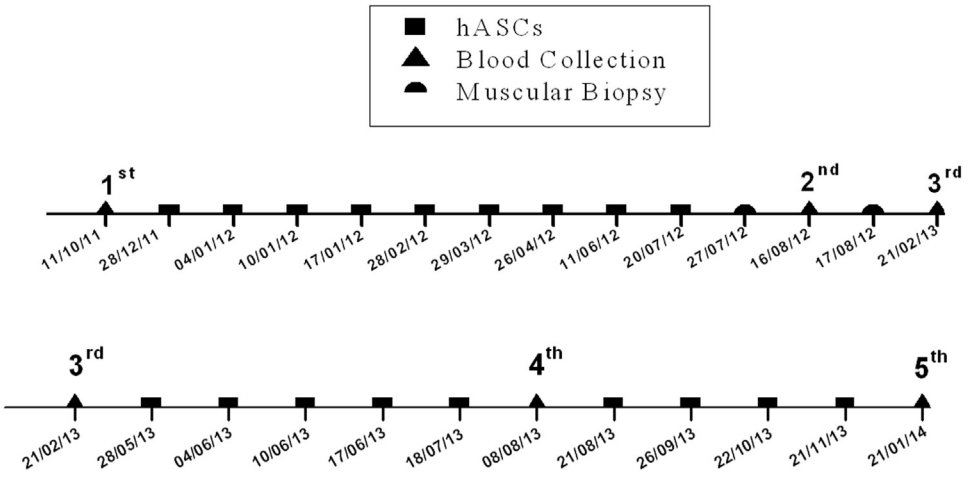

Fig. 1. Whole blood collection (▲); hASCs treatment (▬) and muscle biopsies (◠).

Table 1
Certified reference material NIST-1573b data.

Elements	Reference material Mean ± 1SD	Present study Mean ± 1SD	RSD, %	|Z-score|
Br, μg g^{-1}	*9.7	12.0 ± 1.0[a]	9.8[a]	–
Ca, μg g^{-1}	116 ± 4	112 ± 7[a]	6.3[a]	1.0[a]
		114 ± 9[b]	7.9[b]	0.5[b]
Cl, g kg^{-1}	2.87 ± 0.06	2.76 ± 0.09[a]	3.3[a]	0.3[a]
		2.83 ± 0.09[b]	3.2[b]	0.8[b]
Fe, μg g^{-1}	184 ± 15	180 ± 13[a]	7.2[a]	0.3[a]
		177 ± 10[b]	5.6[b]	0.5[b]
K, g kg^{-1}	9.94 ± 0.02	9.93 ± 0.07[a]	0.7[a]	0.5[a]
		9.96 ± 0.26[b]	2.6[b]	1.0[b]
Mg, μg g^{-1}	601 ± 28	610 ± 54[a]	8.9[a]	0.3[a]
Mn, μg g^{-1}	10.5 ± 1.7	11.8 ± 0.9[a]	7.8[a]	0.8[a]
Na, g kg^{-1}	2.42 ± 0.06	2.38 ± 0.15[a]	6.3[a]	0.7[a]
P, g kg^{-1}	11.0 ± 0.3	11.2 ± 0.3[a]	2.7[a]	0.7[a]
		10.9 ± 0.7[b]	6.4[b]	0.3[b]
Rb, μg g^{-1}	13.7 ± 1.1	14.2 ± 1.2[a]	8.4[a]	0.5[a]
S, g kg^{-1}	7.85 ± 0.06	7.80 ± 0.17[a]	2.2[a]	0.8[a]
Zn, μg g^{-1}	127 ± 16	118 ± 12[a]	10.2[a]	0.6[a]

SD Standard deviation.
RSD relative standard deviation.
* Noncertified value.
Number of repetition: 3 for both techniques.
[a] NAA.
[b] XRF.

immunosuppression in SJL/J mice strain were able to express improvements in the skeletal muscle performance of these animals. Likewise, this treatment also showed to be promising in experiments with the Golden Retriever animal model affected by DMD (GRMD), after some transplantations (Vieira et al., 2012; Metairon et al., 2016a), which motivated the continuity of treatment in this animal model. Now, in this investigation, the purpose was to investigate GRMD (Golden Retriever Muscular Dystrophy) dogs blood submitted to hASCs treatment, using Instrumental Neutron Activation Analysis (INAA) and Energy Dispersive X-Ray Fluorescence (ED-XRF) analytical techniques. These analytic procedures are complementary and appropriate for blood analysis (Redígolo et al., 2016, 2013; Metairon et al., 2016b). We intend to improve the understanding of this treatment regarding to ions and metals (excess and deficiency) during hASCs transplantations. These analyses were performed over 2 years at regular intervals (every 6 months).

Table 2
Compositional variations of the rations for CG and DMD dog's.

Elements	MV	± 1SD	Min	Max
Br, mg kg^{-1}				
CG	1.2	0.4	0.9	1.7
DMD	1.8	0.6	1.0	2.2
Ca, g kg^{-1}				
CG	8.48	0.29	6.05	8.14
DMD	9.11	0.70	7.21	9.91
Cl, g kg^{-1}				
CG	6.52	0.12	4.16	6.62
DMD	4.81	0.11	3.97	4.98
Fe, mg kg^{-1}				
CG	180	12	131	227
DMD	153	8	114	205
K, g kg^{-1}				
CG	13.3	3.5	9.3	16.0
DMD	15.1	0.7	14.8	16.5
Mg, g kg^{-1}				
CG	0.75	0.24	0.55	0.93
DMD	0.70	0.38	0.51	1.10
Mn, mg kg^{-1}				
CG	5.0	1.8	2.4	5.8
DMD	2.5	1.2	1.7	3.9
Na, mg kg^{-1}				
CG	2.4	0.8	1.6	2.9
DMD	4.4	2.5	1.1	7.6
P, mg kg^{-1}				
CG	41	16	22	60
DMD	54	22	39	72
Rb, mg kg^{-1}				
CG	3.9	1.2	1.7	4.1
DMD	2.0	0.9	1.0	2.8
Zn, mg kg^{-1}				
CG	99	48	105	207
DMD	65	34	111	166

MV: Mean Value.
SD: Standard Deviation.
Min: Minimum value.
Max: Maximum value.

2. Material and methods

2.1. Collection and sample preparation

To perform this investigation, blood samples from 12 Golden Retriever male dogs (5 control and 7 affected) at the average age of 7.1 ± 1.3 years old and weight of 28 ± 5 kg were collected. Two of the affected dogs were submitted to hASCs treatment. For sample preparation, about 2.0 mL of blood was collected from the cephalic vein in a syringe and transfer to a plastic microtube (without anticoagulant).

Table 3
Whole blood element concentrations.

Elements	Samples collections	Control (CG)	Affected treated (T_n)		Affected untreated (UT)
		MV ± 1SD [a][range]	T_1 MV ± 1SD	T_2 MV ± 1SD	MV ± 1SD
Br, mg L^{-1}	1st	3.5 ± 0.6 [2.3–4.7]	3.3 ± 0.4	3.9 ± 0.3	3.6 ± 1.4
	2nd		5.9 ± 0.6	5.2 ± 0.6	8.4 ± 1.8
	3rd		5.2 ± 0.4	6.7 ± 0.6	7.4 ± 2.1
	4th		7.7 ± 0.7	6.1 ± 0.5	6.0 ± 0.7
	5th		4.8 ± 0.4	6.7 ± 0.6	8.8 ± 2.5
Ca, g L^{-1}	1st	0.079 ± 0.033 [0.013–0.145]	0.169 ± 0.011	0.110 ± 0.007	0.121 ± 0.039
	2nd		0.081 ± 0.005	0.071 ± 0.005	0.088 ± 0.019
	3rd		0.508 ± 0.003	0.612 ± 0.004	0.356 ± 0.012
	4th		0.215 ± 0.001	0.508 ± 0.003	0.347 ± 0.048
	5th		0.176 ± 0.012	0.155 ± 0.010	0.181 ± 0.020
Cl, g L^{-1}	1st	2.76 ± 0.49 [1.78–3.74]	3.26 ± 0.15	3.33 ± 0.15	2.71 ± 0.77
	2nd		3.35 ± 0.15	3.26 ± 0.15	3.91 ± 0.34
	3rd		2.99 ± 0.13	4.09 ± 0.18	3.81 ± 0.77
	4th		2.97 ± 0.13	3.28 ± 0.15	3.23 ± 0.13
	5th		3.79 ± 0.18	3.76 ± 0.17	3.69 ± 0.20
Cr, µg L^{-1}	1st	0.538 ± 0.039 [0.460–0.616]	0.498 ± 0.031	0.543 ± 0.034	0.433 ± 0.104
	2nd		0.523 ± 0.033	0.485 ± 0.030	0.463 ± 0.071
	3rd		0.545 ± 0.034	0.563 ± 0.035	0.547 ± 0.031
	4th		0.367 ± 0.023	0.397 ± 0.025	0.395 ± 0.016
	5th		0.610 ± 0.038	0.675 ± 0.043	0.487 ± 0.077
Fe, g L^{-1}	1st	0.581 ± 0.030 [0.521–0.641]	0.483 ± 0.015	0.434 ± 0.032	0.626 ± 0.072
	2nd		0.433 ± 0.018	0.400 ± 0.017	0.529 ± 0.056
	3rd		0.484 ± 0.020	0.769 ± 0.032	0.520 ± 0.042
	4th		0.523 ± 0.021	0.574 ± 0.024	0.664 ± 0.101
	5th		0.487 ± 0.020	0.447 ± 0.018	0.593 ± 0.048
K, g L^{-1}	1st	0.127 ± 0.023 [0.081–0.173]	0.102 ± 0.013	0.216 ± 0.014	0.160 ± 0.023
	2nd		0.109 ± 0.015	0.214 ± 0.015	0.117 ± 0.019
	3rd		0.279 ± 0.037	0.225 ± 0.036	0.178 ± 0.033
	4th		0.174 ± 0.023	0.290 ± 0.023	0.135 ± 0.015
	5th		0.317 ± 0.044	0.496 ± 0.042	0.263 ± 0.117
Mg, g L^{-1}	1st	0.029 ± 0.013 [0.003–0.055]	0.027 ± 0.004	0.027 ± 0.004	0.020 ± 0.010
	2nd		0.049 ± 0.007	0.047 ± 0.007	0.064 ± 0.015
	3rd		0.012 ± 0.002	0.015 ± 0.002	0.061 ± 0.041
	4th		0.014 ± 0.002	0.012 ± 0.002	0.015 ± 0.001
	5th		0.011 ± 0.002	0.010 ± 0.001	0.013 ± 0.004
Na, g L^{-1}	1st	2.73 ± 0.49 [1.75–3.71]	2.64 ± 0.10	3.24 ± 0.12	3.11 ± 0.42
	2nd		3.21 ± 0.12	3.05 ± 0.12	3.54 ± 0.19
	3rd		3.38 ± 0.13	4.67 ± 0.18	4.16 ± 0.48
	4th		2.97 ± 0.11	3.28 ± 0.12	3.59 ± 0.07
	5th		3.79 ± 0.14	3.76 ± 0.11	3.96 ± 0.22
P, mg L^{-1}	1st	595 ± 120 [355–835]	466 ± 28	970 ± 58	493 ± 205
	2nd		293 ± 18	241 ± 15	343 ± 57
	3rd		510 ± 31	691 ± 42	501 ± 162
	4th		740 ± 44	554 ± 33	696 ± 124
	5th		863 ± 43	769 ± 46	759 ± 213
Rb, mg L^{-1}	1st	0.69 ± 0.42 [< 1.5]	0.41 ± 0.05	0.81 ± 0.09	0.42 ± 0.08
	2nd		0.18 ± 0.02	*nd*	0.25 ± 0.10
	3rd		*nd*	*nd*	0.26 ± 0.11
	4th		0.40 ± 0.05	0.22 ± 0.03	0.24 ± 0.06
	5th		0.48 ± 0.05	0.93 ± 0.10	0.51 ± 0.03
S, g L^{-1}	1st	0.67 ± 0.31 [0.05–1.29]	0.82 ± 0.09	0.77 ± 0.08	0.90 ± 0.16
	2nd		0.75 ± 0.08	0.82 ± 0.09	0.83 ± 0.23
	3rd		0.63 ± 0.07	1.03 ± 0.11	0.91 ± 0.10
	4th		0.56 ± 0.06	1.24 ± 0.13	0.90 ± 0.47
	5th		1.00 ± 0.11	1.03 ± 0.11	0.77 ± 0.11
Zn, mg L^{-1}	1st	5.5 ± 1.1 [3.3–7.7]	5.1 ± 0.5	5.2 ± 0.4	5.9 ± 1.2
	2nd		*nd*	*nd*	*nd*
	3rd		*nd*	*nd*	*nd*
	4th		2.4 ± 0.2	1.6 ± 0.1	2.3 ± 0.9
	5th		4.0 ± 0.4	2.7 ± 0.2	2.4 ± 0.7

T_n: blood samples of GRMD affected treated dogs (n = 1, 2).
MV: Mean Value.
± 1SD: Standard Deviation.
nd: not determined (below the detection limit).
[a] Control Group range for a confidence interval of 95%.

Immediately after the collection, before the blood coagulation, pre-established aliquot of 100 µL was transferred to the Whatman (no 41) filter paper (2.2 cm^2 - deposit area) and 500 µL was transferred to a polyethylene cylinder. Each sample deposit in filter paper (100 µL) was dried for few seconds using an infrared lamp and transferred to appropriate container without refrigeration and stored at room temperature until use. These samples were measured by using INAA and ED-XRF (non-destructive methods). The 500 µL samples were frozen,

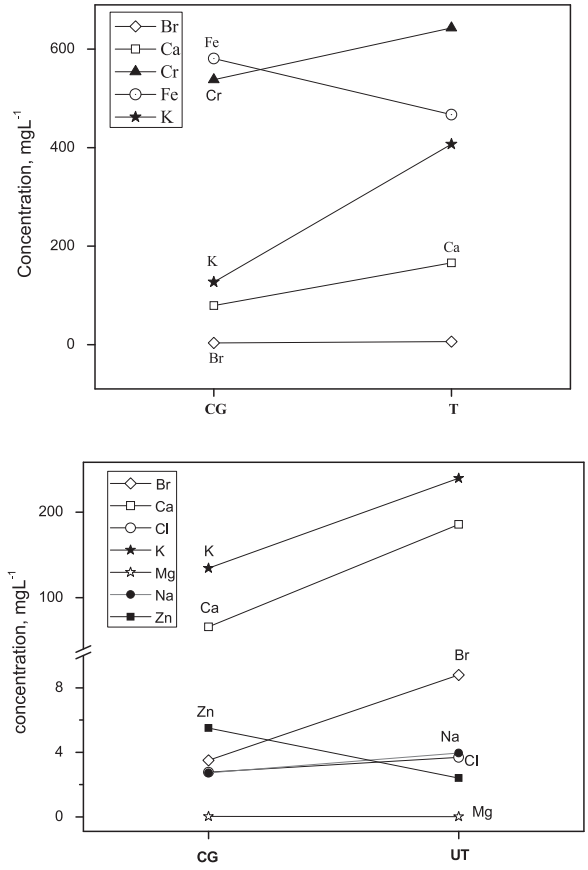

Fig. 2. *Student t-distribution* between Control (CG), Affected Treated (T) and Affected Untreated (UT) dogs at the end of the treatment.

lyophilized and stored at room temperature until they were used. The INAA technique was used to measure these samples. All the blood samples were prepared in duplicate and were collected at Human Genome and Stem Cell Research Center in Institute of Biosciences of University of Sao Paulo (São Paulo - SP, Brazil), in agreement with the rules approved by Animal Research Ethics Committee. The analyses took place from October 2011 to January 2014. During this period, several cell transplantations, biopsies and collections were carried out as shown in Fig. 1.

The dogs were fed twice daily (250–370 g daily intake) with water *ad libtum*. Animal nutrition was differentiated for control and DMD (treated and untreated) and the variations in the rations composition were systematically verified by using NAA and XRF techniques. For these analyses, pellets weighing 200 mg of each purchased batch, were used.

2.2. Methods

2.2.1. Human adipose-derived stromal cells (hASCs) systemic transplantations

Human adipose tissue was obtained from elective liposuction procedures. Cells isolation and expansion process were previous described by Vieira et al. (2012). Each treated dog received a single injection of 5×10^7 cells kg^{-1} in 0.1 mL of Hank's buffered salt solution (HBSS) in the cephalic vein. The cells were injected right after their preparation.

2.2.2. Instrumental Neutron Activation Analysis (INAA)

Each blood samples was irradiated together with standard (blood IAEA-A-13) in the IEA-R1 nuclear reactor at IPEN (IEA-R1, 4.5 MW, pool type). Two different irradiation procedures were taken for the whole blood elements determination. For Br, Ca, Cl, K, Mg, Na and S a short irradiation time of 4 min followed by 1 min decay and 15 min counting time was performed, using samples prepared in filter paper. For Fe, Rb and Zn determination, lyophilized samples were irradiated for 4–8 h and after decay time of several days they were gamma counted for hours to days. All the measurements were carried out using high-purity Germanium detector (ORTEC GEM – 60195). The concentration of each element (Br, Ca, Cl, Fe, K, Mg, Na, Rb, S and Zn) in each blood sample was obtained by using in-house software (Medeiros et al., 2005).

2.2.3. Energy dispersive X-ray fluorescence (ED-XRF)

The analysis was performed using portable X-ray spectrometer (model X123 SDD Amptek) with Ag X-ray tube (without need of vacuum). The characteristic X-ray fluorescent intensity from K_α line was measured with a Si Drift detector (25 mm^2 × 500 μm) with Be window (12.5 μm). A sensitivity curve was obtained using the certified reference material, NIST SRM 1577b-Bovine liver. This methodology have been applied for the determination of inorganic elements in whole blood samples in previous studies (Redígolo et al., 2016, 2013). The samples prepared in filter paper (the same used in INAA measurements) were irradiated for 600 s using 30 kV and 5 μA excitation. This procedure was applied to quantified Cr and P. The analysis was performed using WinQxas software (QXAS). Although the elements Ca, Cl, Fe, K and S were also determined, the INAA results were adopted in function of a better statistic analysis.

3. Results and discussion

The methods were evaluated using the certified reference material NIST-SRM 1577b-Bovine liver. The Table 1 shown the obtained values for: Mean Value (± 1 SD), relative standard deviation (RSD %) and Z-score. According to this table, the repeatability of the methods, by both techniques, presented satisfactory values (RSD values: 0.7–10.2% for NAA and 2.6–7.9% for X-rays, analysis) as well as with an excellent accuracy of the methods ($-1 < Z < 1$ values). The compositional variations of the rations are shown in Table 2 and emphasize significant difference for Mn and Na. However, in both rations the concentrations are low. In Table 3 the whole blood results are presented. The elements Br, Ca, Cl, Cr, Fe, K, Mg, Na, P, Rb, S and Zn in the whole blood samples of Golden Retriever dogs were investigated during 2 years. Specifically, As and Cd elements in blood samples were not possible to quantify; they should be below the detection limit (0.003 μg/L for As and 0.019 μg/L for Cd). In Table 3, the results (Mean Value ± 1 SD) are presented in function of the samples collections. The results for the two treated animals (referred as T_1 and T_2) are individually presented in this Table. The mean value and the range for the control group, for a confidence interval of 95%, were also included for comparison. The *Student t-test* was applied to compare the difference among control and affected dogs (statistical differences considering $p < 0.05$). These comparisons, at the end of treatment, are presented in Fig. 2.

Related to the data presented in Table 3, during the treatment, the Ca, Mg and K blood levels showed significant increase in Ca and K and decrease in Mg blood levels. Only Ca levels returned to the normal range (near to the upper limit) at the end of treatment. Fig. 3 was proposed to visualize the elements behavior that have concentration levels changed ($p < 0.05$) during the treatment (Br, Ca, Cr, Fe, K and Mg). In this figure the whole blood average concentrations (mean value and ± 1 standard deviation), of these elements for the control group (CG) were included for comparison. In these comparisons, the control group confidence interval used was 95% which usually is adopted for clinical practice. According to this figure, between 1st and 2nd collections, an increase in Mg (85%) and Br (79%) and a reduction in Ca (52%) and Fe (10%) blood levels was observed, which may be related to the higher number of transplantations (n = 9, Fig. 1). In the case of Br levels, these variations were above of the tolerable upper limit (4.7 mg L^{-1}) throughout treatment (from 2nd, 3rd and 4th collections)

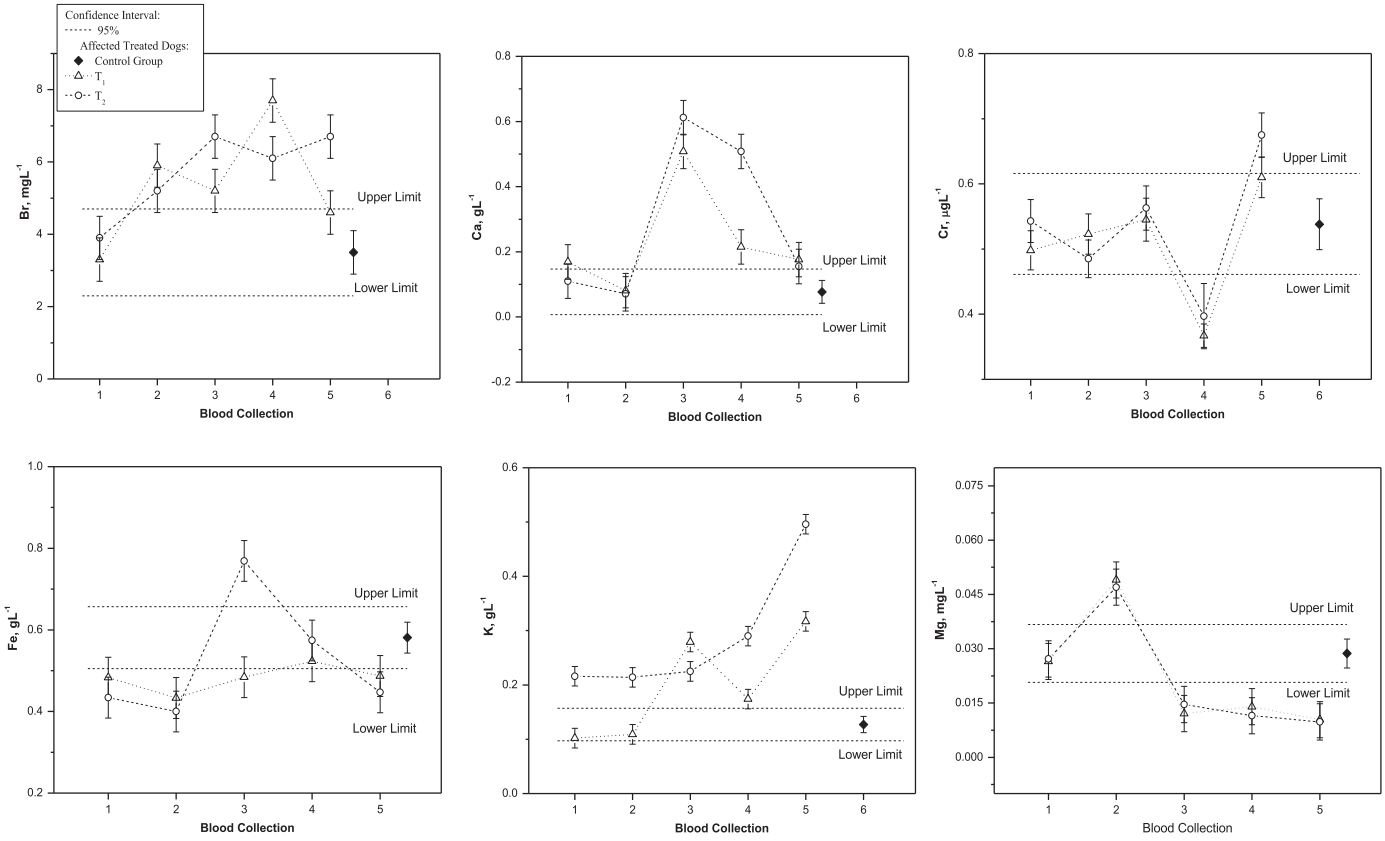

Fig. 3. Br, Ca, Cr, Fe, K and Mg whole blood concentrations for GRMD Affected Treated (T_1 and T_2) dogs and Control Group (CG). The mean value and the range for the Control Group, considering ± 2 SD was presented for comparison.

Table 4
Whole blood concentrations ratio (in percentage) between Control (CG), affected Treated (T) and affected Untreated (UT) groups of dogs for elements from blood of the 5th collection.

Elements	T/CG (%)	UT/CG (%)	T/UT (%)
Br	> 66	> 151	< 34
Ca	> 110	> 129	< 8
Cl	> 37	> 34	> 2
Cr	> 39	> 5	> 32
Fe	< 20	> 2	< 21
K	> 220	> 107	> 55
Mg	< 65	< 54	< 24
Na	> 38	> 45	< 5
P	> 37	> 28	> 8
Rb	> 3	< 26	> 39
S	> 51	> 15	> 31
Zn	< 38	< 56	> 42

CG is adopted 100%.
> increased ratio.
< decreased ratio.

and only one of the treated animals (T_1) returns to the normal level (2.3 – 4.7 mg L^{-1}) at the end of treatment, i.e., after the 5th collection (T_1: 4.8 ± 0.4 mg L^{-1}). Related to Fe blood levels the results had shown another situation: it was the only element in which the concentrations are below to the lower limit (0.521 gL^{-1}), before started the treatment (1st collection, T_1 = 0.483 ± 0.015 g L^{-1} and T_2 = 0.434 ± 0.032 g L^{-1}), although the clinical status evaluation did not evidence any pathological changes (dysfunction). Besides that, mainly in the period when most of transplantations occurred (between 1st and 2nd collections) the Fe levels suffer a decrease. Although the behavior of T_2 showed abrupt oscillations (above the tolerance in the 3rd collection, 0.769 ± 0.032 g L^{-1}), at the end of the treatment (5th collection, 0.447 ± 0.018 g L-1) it is again below of the lower limit (0.521 g L^{-1}). This behavior suggests that these variations may be associated with the treatment and also with nutritional needs. Another element that had a behavior altered, due probably to the treatment was Cr, i.e., between 3nd and 4th collections there was a decrease followed by a significant increase in the end of the treatment. For Zn and Rb concentrations, between 2nd and 3rd collections, were not possible to quantify (detection limit 0.5 mg L^{-1} for Zn and 0.09 mg L^{-1} for Rb). This may be an indication that these elements have been also affected by the hASCs therapy. We also performed a concentration ratio comparison between T/CG, UT/CG and T/UT, for all elements, at the end of the treatment (5th collection). These ratios (express in percentage) are presented in Table 4 and they are shown in Fig. 4. Specifically, for the untreated group (UT), the data were considered only for four dogs, since one died before the last collection (5th collection).

3.1. Treated and control

Regarding to T/CG ratios, except for Rb (> 3%), all elements showed significant variations, an increase for Br (66%), Ca (110%), Cl (37%), Cr (39%), K (220%), Na (38%), P (37%) and S (51%) and a decrease in Fe (20%), Mg (65%) and Zn (38%).

3.2. Untreated and control

Regarding to UT/CG ratios a quite significant increase mainly for Br (151%), Ca (129%), K (107%) and Na (45%) and a decrease in Mg (54%) and Zn (56%), was observed in the affected untreated group. Only for Cr and Fe no significant variation were observed. Yet, according to T/CG and UT/CG ratios, the behavior for most of the elements is quite similar (increase for Br, Ca, K, Cl, Na and P and a decrease for Mg and Zn). Exception for Fe and Rb: while the iron levels in

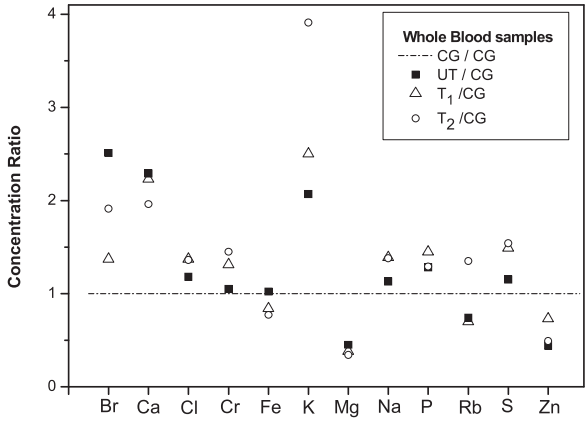

Fig. 4. Whole blood concentration ratio between Affected Treated (T_1 and T_2) and Untreated (UT) dogs in function of Control Group (CG) for elements from blood of the 5th collection.

the treated animals showed deficiency (decrease of 20%), in the untreated animals there was evidence of 2% increment; and for Rb this situation is reversed: decrease in untreated (26%) and a slight increase in treated (3%) dogs.

3.3. Treated and untreated

Regarding to T/UT ratios the increase in Cr (32%), K (55%), Rb (39%) and Zn (42%) and the decrease of Br (34%) and Fe (20%) blood levels in the treated dogs seem to be due to the treatment. The analysis showed that this cellular therapy leads to a significant variations of ions and metals in blood and emphasize that besides the conventional analyses (Ca, Cl, Fe, K, Mg and Na), the monitoring of Br, Cr, S and Zn, although not usual in clinical practice, are recommended.

4. Conclusion

In this study concentration of ions and metals in blood samples of Golden Retriever animal model, affected by Duchenne Muscular Dystrophy and subjected to human adipose-derived stromal cells (hASCs) transplantations, were investigated using INAA and EDXRF techniques. During the treatment period (over two years), there was significant increase of K blood levels suggest that regular clinical assessment is recommended for the use of this cell therapy in humans. Furthermore, the Br, Cr, Fe, Rb, S and Zn blood levels variation seem to be due to the treatment and should be monitored. Related to Mg, a decrease in blood levels is observed regardless of treatment and therefore also need continuous evaluation. These results may contribute to the detailed knowledge of the DMD inorganic blood elements behavior when hASCs cell therapy is used. In addition to that, the data from the control animals may help researchers to evaluate and compare the advantages and disadvantages of different treatments performed in muscular dystrophy when this animal model is employed, aiding in predicting the efficacy of new therapeutic procedures before being used in patient with DMD.

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