PROCEEDINGS OF SCIENCE

Investigation of Ca and Mg in blood of dystrophic animal model using NAA

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In recent years, the Spectroscopy and Spectrometry Radiation Laboratory at IPEN – CNEN/SP (Brasil) has performed measurements related to the determination of inorganic elements in body fluids of experimental animals (rats, rabbits, mice) applying Neutron Activation Analysis technique (NAA). The success in several investigations of clinical disease that have high prevalence in Brazilian population has motivated us to study in more details the anomalies caused by Duchenne Muscular Dystrophy (DMD). In this investigation Ca and Mg were determined in blood samples from Golden Retriever Muscular Dystrophy dogs (GRMD) (Control, Carrier and Affected) using NAA. The samples were obtained from Biosciences Institute/USP (São Paulo, Brasil) and they were irradiated in the IEA - R1 nuclear reactor at IPEN-CNEN/SP (Brasil). This data may help to evaluate the efficiency of new treatments as well as to show in more details the alterations of this disease, which may permit to compare the advantages of different treatment schedules before performing tests in patients with DMD.

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1. Introduction

The Duchenne Muscular Dystrophy (DMD) is the most severe illness of hereditary character and prevalent type of muscular dystrophy [1]. Muscle weakness, premature death, and instability of the membrane that involves the muscle fibers - causing functional/structural abnormalities and cell death - are main characteristics of this genetic disease. Previously considered rare, currently at least thirty different types have been identified. Nowadays, many therapeutic strategies have been developed in animal models with DMD. However, despite the scientific efforts, some forms of muscular dystrophy cannot be prevented or reversed.

The DMD is the most common of them and affects approximately 1 in every 3,600 to 6,000 live male births in the world [2]. It is caused by a mutation of the dystrophin gene (the largest gene of the human genome with 79 exons [3]) and consequently a truncated dystrophin protein in muscles are expressed [4,5]. The most common mutation is called deletion when one or more exons are missing from the gene [6]. Specifically, the DMD disorder is caused by a mutation in the dystrophin gene, located in humans, mice and dogs on the X chromosome. Unlike most genes, which come in pairs in both sexes and stay active throughout life, X chromosome genes come in pairs in females but unique in males (only one X chromosome), for this reason this disorder usually affected much more boys than girls [2,6,7].

Considering that the blood is responsible for transportation, regulation and protection of the body, the functions performed by its circulation in biological tissues are essential to the proper functioning of the body and act as an indicator in case of malfunctions [8,9]. Based on this, in the last years we have investigated blood of dystrophic mice (spontaneous mutation) such as: A/J, Dmd^{mdx}/J and SJL/J [1,10-15], using Neutron Activation Analysis technique (NAA). Considering the relevance of Ca and Mg in blood levels in DMD mice, now we investigated these elements in Golden Retriever Muscular Dystrophy dogs (GRMD), an animal model which has a phenotype similar to patients with DMD. In this animal model, muscle degeneration and fibrosis are predominating, leading to a progressive loss of structure and muscle function, as in humans (once they have a comparable muscle mass to that of human beings).

Recently, the Human Genome Research Center (Biosciences Institute in Brasil) has shown that human adipose derived from stromal cells (hASCs) and injected systemically into GRMD dog cephalic vein paw are able to reach, engraft, and express human dystrophin in the host GRMD dystrophic muscle up to 6 months after transplantation, which improves the functional performance of injected animals without any immunosuppressant [16].

In this study, the elements Ca and Mg were investigated in blood due to the relevance for evaluation of the transplantation process. In normal muscle, sarcolemma injuries lead to accumulation of dystrophin (a protein present in muscles) and the resealing of the membrane in the presence of Calcium [4,8]. Nonetheless, in subjects with DMD this protein is altered causing critical muscular disease in several body functions, such as: calcium homeostasis and

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dysfunction in the mechanisms of membrane permeability control performed by magnesium [4,8] causing a degeneration of the membrane that involves the muscular cell, leading to its death.

To perform this investigation, blood samples from nineteen GRMD (4 Control, 7 Carrier and 8 Affected), that were breeding at Biosciences Institute/USP (São Paulo, Brasil), were irradiated in the IEA - R1 nuclear reactor at IPEN - CNEN/SP (Brasil). The Ca e Mg concentrations were determined using NAA technique. The blood collection was performed before start the treatment (1^{st} collection) and after the beginning of the treatment (2^{nd} collection). The advantage in using blood is relative to the fact that the NAA procedure needs small quantity of blood (0.1 mL is enough) when compared with the conventional analyses (0.5 to 1.0 mL using serum) and it also permits the simultaneous quantification of these elements. Moreover, the NAA technique is not destructive, i. e., the samples can be irradiated with neutrons [17] many times (if necessary) resulting in an efficient procedure for clinical practice.

2. Experimental

The GRMD dogs were from 2 to 10 years old, Control Group (2 males and 2 females), Carrier (7 females) and Affected (7 males and 1 female), were bred at Biosciences Institute/USP (São Paulo, Brasil).

The first step was to collect the blood samples: about 100 μL (duplicated) was withdrawn from a cephalic left vein paw and transferred to ~2.4 cm^2 pieces of Whatman filter paper using a calibrated micro pipette. Each sample was dried for a few minutes using an infrared lamp and stored (separately) in plastic bags at room temperature. Standard solutions were prepared following the same procedure (transferred to filter paper). After that, each sample and standard were irradiated together in the nuclear reactor with neutrons at IPEN/SP (IEA-R1, 4.5MW, pool type), for four minutes, resulting in the production of radioactive isotopes and then gamma rays emitted were analyzed by gamma-ray spectrometry [18]. The IAEA-A13 Blood Animal and the Bovine Muscle Powder (NIST 1557c) were used for analytical quality control.

The measurements of the gamma induced activity of the samples and standard were carried out using a 60% efficiency high-purity Germanium detector (GEM-60195, ORTEC Model) and an amplifier (ORTEC- 671) coupled to a MCA (ORTEC- 919E) connected to a PC. The concentration of each element in each blood sample was obtained by using in-house software [19].

3. Results and Discussion

In Table 1 the concentration results for Ca and Mg for 1^{st} and 2^{nd} blood collection are presented as the mean value from duplicate analyses and standard deviation (±1SD) and the

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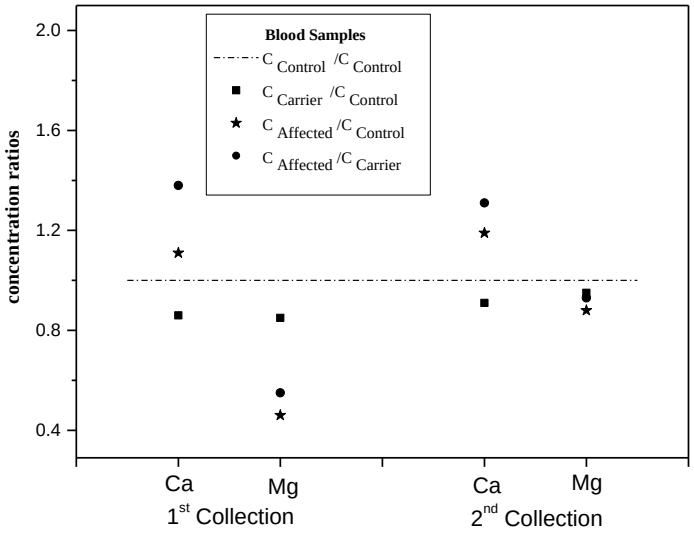
range for control were also presented for comparison. The concentration ratios between mean value of Ca and Mg, between dog groups, for both blood collections are presented in Figure 1.

Table 1. The Ca and Mg concentration for 1st and 2nd blood collections

Elements	Blood Collection	Control Mean ± 1 SD	Carrier Mean ± 1 SD	Affected Mean ± 1 SD
Ca, mgL^{-1}				
	1st	0.117 ± 0.022	0.101 ± 0.094	0.130 ± 0.040
	2nd	0.108 ± 0.021	0.098 ± 0.014	0.128 ± 0.011
Mg, mgL^{-1}				
	1st	0.032 ± 0.013	0.023 ± 0.013	0.018 ± 0.012
	2nd	0.028 ± 0.003	0.026 ± 0.010	0.024 ± 0.003

Figure 1: Mean ratios between mean value of $C_{carrier}/C_{control}$, $C_{affected}/C_{Control}$ and $C_{affected}/C_{Carrier}$ for 1st and 2nd blood collection



The comparison between concentration ratios, before start the treatment (1st collection) and after the beginning of the treatment (2nd collection) showed that the Mg blood levels of carrier and affected dogs were lower, nearby the control, then showing an improvement, while for Ca the ratios were higher.

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4. Conclusion

These data are the first results for comparing the progress of hASCs treatment in GRMD animal model.

The knowledge of Ca and Mg levels in blood of GRMD dogs may help to evaluate the progress of hASCs treatment in this animal model, before tests being performed on humans with DMD. We intend to continue the investigation of these elements every six months over the next years (2011 – 2016).

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References

[1] S. Metairon, C. B. Zamboni, M. F. Suzuki, C. R. Bueno Jr., O. A. Sant'Anna, *Elemental analysis of biological tissues of Dmd^{mdx}/J and C57BL/6J mice strains investigated by neutron activation analysis, J Radioanal Nucl Chem*, **296**: 579-584, (2013)

[2] K. Bushby (2010), *Duchenne Muscular Dystrophy*, http://www.treat-nmd.eu/home.php. Accessed 10 Aug 2010

[3] Muscular Dystrophy Campaign (2009) http://www.Muscular-dystrophy.org/ Accessed 5 Jun 2009

[4] R. Han, K.P. Campbell, *Dysferlin and Muscle Membrane Repair*, *Curr Opin Cell Biol* **19**:409-416 (2007)

[5] D.J. Blake, A. Weir, S.E. Newey, K.E. Davies, *Function and genetics of dystrophin and dystrophin-related proteins in muscle*, *Physiol Rev* **82**:291-329 (2002)

[6] M.Matsuo, *Duchenne muscular dystrophy*, *Southeast Asian J. Trop. Med. Public Health*, **26**: 166 -171, (1995)

[7] A.E. Emery, *The dystrophy of Duchenne*, *The Lancet* **359**(9307):687-695 (2002)

[8] M. W. Berchtold, H. Brinkmeier, M. Muntener, *Calcium ion in skeletal muscle: Its crucial role for muscle function, plasticity and disease, Physiol. Rev.* **80**, 1215-1266, (2008)

[9] G. Meola, V.Sansone, K.; SALVUCCI, J. *Quantitative myotonia assessment: an experimental protocol*, ***Neurol. Sci.*** **21** (5), S971-974, (2000).

[10] S. Metairon; C.B. Zamboni; M.F Suzuki.; C.R. Jr Bueno; O. A. Sant'Anna, *Elemental analysis of biological tissues of animal models in muscular dystrophies investigation, J Radioanal Nucl Chem* **291**:373-378, (2012)

[11]C.B. Zamboni, M.F.Suzuki, S. Metairon, M.F.D. Carvalho, O.A. Sant'Anna *Investigation of whole blood of SJL/J mice using neutron activation analysis, J Radioanal Nucl Chem* **281**:97-99, (2009)

[12]C.B.Zamboni, G.S. Zahn, O.A. Sant'Anna, *Trace elements at whole blood of distinct mouse lines by NAA,* VI Latin American Symposium on Nuclear Physics and Applications, A J Kreiner et al., in *AIP Conference Proceedings*, **884**:507-509, (2007)

[13]C.B. Zamboni, M.F. Suzuki, O.A. Sant'Anna, *Simultaneous determination of five elements in whole blood of dystrophin deficient mdx mouse by NAA, J Radioanal Nucl Chem* **278**:585-589, (2008)

[14]C.B. Zamboni, S. Metairon, M.F. Suzuki, M.F. Furtado, O.A. Sant'Anna, D.V. Tambourgi, *Quantitative evaluation of blood elements by neutron activation analysis in mice immunized with Bothrops snake venoms , J Radioanal Nucl Chem* **282**:37-39, (2009)

[15]S. Metairon *Elements determination of clinical relevance in biological tissues of* Dmd^{mdx}*/J dystrophic mice strains investigated by NAA, PhD in Nuclear Technology. Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN-SP) / Universidade São Paulo (USP), São Paulo, SP, Brasil* (2012)

[16]N.M. Vieira, M. Valadares, E. Zucconi, M. Secco, C.R. Bueno Junior, V. Brandalise, A. Assoni, J. Gomes, L V. Landini, T. Andrade, H.V. Caetano, M. Vainzof, M. Zatz, *Human Adipose-Derived Mesenchymal Stromal Cells Injected Systemically Into GRMD Dogs Without Immunosuppression Are Able to Reach the Host Muscle and Express Human Dystrophin, Cell Transplant* **21**(7):1407-17, (2012)

[17]J. A. G. Medeiros, C. B. Zamboni, A. L. Lapolli, G. Kenchian, M. T. F. Da Cruz, *Decay of Ga-72 Appl Radiat Isot* **54**:245-259, (2001)

[18]L. C. Oliveira, C. B. Zamboni, F. A. Genezini, A. M. G. Figueiredo, G. S. Zahn (2005), *Use of thermal neutrons to perform clinical analyses in blood and urine samples, J Radioanal Nucl Chem* **263**(3):783-786

[19]J. A. G. Medeiros, C. B. Zamboni, G. S. Zanh, L. C. Oliveira, L. Dalaqua Jr, *Software para realização de análises hematológicas utilizando processo radioanalítico, Proceedings of 39º Congresso Brasileiro de Patologia Clínica*, 19 oct, São Paulo, Brasil, CD ROM, (2005)

