Comparison of Reference Values in Whole Blood of DMD^{mdx}/J and C57BL/6J Mice Using Neutron Activation Analysis

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Abstract. The Br, Ca, Cl, K, Na and S concentrations in whole blood of DMD^{mdx}/J and C57BL/6J mice were determined using Neutron Activation Analysis technique. Reference values obtained from twenty one whole blood samples of these strains were analyzed in the IEA - nuclear reactor at IPEN (São Paulo, Brasil). These data contribute for applications in veterinary medicine related to biochemistry analyses using whole blood as well as to evaluate the performance of treatments in muscular dystrophy.
R1

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INTRODUCTION

In health area animal models are very used in medical investigation of new treatment before to be applied in human being. As a routine these animals are submitted to biochemistry analyses performed using serum (0.5 to 1.0 mL). However, when small size animal model is involved the biological material can be scarce and confine its collects. The viability to perform these clinical analyses using NAA can help to simplify the investigations due some advantages: it can be done using small quantity of whole blood (10 to 100 µL) and also permits the simultaneous quantifications of several elements relevant for clinical practices. But, to use whole blood it is essential establish the reference value of the elements of clinical relevance in blood for the species or for the animal models. Base on this, in the last years the NAA technique has been successfully applied for investigation of several elements in whole blood of small sized animals resulting in an efficiency procedure for clinical practice [1-4]. The obtained data for several mouse strains have indicated a different behavior for some elements, mainly when compared with human whole blood

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estimation, suggesting that specific measurement must be done for each strain in order to allow the choice of a better mice strain for experimental model.

To study Duchenne Muscular Dystrophy (DMD) some mice strains with spontaneous mutation such as: DMD^{mdx}/J, A/J and SJL/J are used. Nowadays, this muscular dystrophy (DMD) is being investigated at IPEN, Biosciences Institute and Butantan Institute (Research Centers, Brazil) using these animals models.

The muscular dystrophy is an illness of hereditary character. Its main characteristic is the degeneration of the membrane that involves the muscular cell, causing its death. Previously considered rare, currently at least thirty different types have been identified. All the forms of muscular dystrophy are genetic; they can not be prevented or reverted. The Duchenne Muscular Dystrophy (DMD) is the most common of them [5] where that affects approximately 1 in every 3,600 to 6,000 live male births in the world [6]. In general, only males are afflicted and approximately 10% of female carriers show some disease manifestations that might include or even exclusively affect cognitive and/or cardiac function though females can be carriers. Although the disorder in affected girls is usually much milder than in boys, a few cases do have disease severity similar to that seen in affected boys. Many promising therapeutic strategies have since been developed in animal models [7]. Particularly in Brazil, the DMD^{mdx}/J strain mouse is relevant as animal model once it has been widely used as a model for progressive muscular dystrophy investigation. However, there is no biological whole blood characterization for this strain. Then, the determination of metal concentrations in their biological whole blood may help to evaluate the efficiency of the new treatments as well as to compare the advantages of different treatment schedules before performing tests in patients with muscular dystrophy.

Duchenne muscular dystrophy is caused by a mutation of the dystrophin gene. The absence of dystrophin (a protein present in muscles) permits the excess of calcium to penetrate sarcolemma (cell membrane) [8]. However, in the subject with DMD this protein is altered and causing a critical muscular dysfunction in several bodily functions [9-11].

In this study we intend to determine the range of Br, Ca, Cl, K, Mg, Na and S in blood samples of DMD^{mdx}/J and C57BL/6J (control group) male mice strain, using NAA technique. The knowledge of the elements concentration in whole blood from these species can be used for biochemistry analyses and contributing in diagnosis and treatments for studying in more details the anomalies caused by DMD. Moreover, the knowledge of the elemental composition in these strains may also help to identify the physiologic difference among them.

EXPERIMENTAL

To perform this investigation, the whole blood samples of two-month-old adult males, DMD^{mdx}/J (n = 9) and C57BL/6J (n = 12), were used. They were originally obtained from the Jackson Laboratory (Maine, USA) and further inbred at IPEN – CNEN/SP (São Paulo, Brasil). About 0.3 mL of whole blood was collected from the frontal belly. A 100μL (duplicate) was aliquots in the filter paper (~2.2 cm^2 pieces of Whatman filter paper) and then was dried for few minutes using an infrared lamp. Each biological sample was sealed into a polyethylene bag and irradiated in the IEA-

R1, in a power of 3.5 MW in the nuclear reactor at IPEN. For the elements determination, a 4 min irradiation followed by 1 min decay and 15 min counting time was used. The IAEA - A13 Blood Animal was prepared in a same manner as the samples were for the analytical quality control.

The measurements of the gamma induced activity of the samples were carried out using an ORTEC Model, GEM-60195 detector and an ORTEC 671 amplifier, in pile up rejection mode, coupled to a MCA ORTEC 919E connected to a PC. The background radiation as well as the escape peaks was reduced by employing the iron shield described by Medeiros et al. [12]. The source-detector distance in this experimental apparatus is 12 cm. The concentration of each element was obtained by using in-house software [13].

RESULTS AND DISCUSSION

In Table 1, the elements concentrations in whole blood for the Dmd^{mdx}/J strain are presented as: the mean from duplicate analyses, the standard deviation, minimum and maximum values. The control group (C57BL/6J strain) data were also included for comparison.

TABLE 1. The Br, Ca, Cl, K, Na and S concentrations (gL^{-1}) in whole blood of Dmd^{mdx}/J and C57B/6J male mice.

Elements	Mean	± 1 SD	Minimum Value	Maximum Value	Reference Interval (95%) *
Br	0.0052 ^{CG}	0.0009	0.0042	0.0062	0.0034 – 0.0070
	0.0039 ^{D}	0.0008	0.0029	0.0048	
Ca	0.186 ^{CG}	0.037	0.145	0.218	0.112 – 0.260
	0.184 ^{D}	0.059	0.127	0.296	
Cl	3.39 ^{CG}	0.28	2.90	3.93	2.83 – 3.95
	2.94 ^{D}	0.20	1.85	2.06	
K	2.49 ^{CG}	0.37	2.07	3.00	1.75 – 3.23
	1.91 ^{D}	0.39	1.31	2.55	
Na	2.06 ^{CG}	0.24	1.49	2.28	1.58 – 2.54
	2.44 ^{D}	0.24	2.08	2.73	
S	1.15 ^{CG}	0.28	0.95	1.34	0.59 – 1.71
	1.22 ^{D}	0.26	1.06	1.62	

^{CG}*: Control Group* [C57BL/6J]

^{D}*: Dystrophic Group* [DMD^{mdx}/J]

**interval usually adopted as reference of normal range for clinical practice*

According to this table the results of NAA technique for both strains were compatible considering a confidence interval of 95% adopted for clinical practice. This nuclear procedure has been applied due the possibility of simultaneous evaluation of several elements using small quantities of blood.

CONCLUSION

The concentrations of elements in whole blood of Dmd^{mdx}/J and C57B/6J mice were evaluated by NAA. They are the first indicative interval for reference values in whole blood and they could be used for checking the clinical status of them, when animal model is used. Moreover, these data could be used to perform biochemistry analyses using whole blood where can help to evaluate the different performance of treatment schedules in muscular dystrophy.

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