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CORRELATION ANALYSIS OF INORGANIC ELEMENTS IN BIOLOGICAL TISSUES OF DMD^{mdx}/J MICE USING INAA

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ABSTRACT

Instrumental neutron activation analysis technique (INAA) has been used to determine Br, Ca, Cl, K, Mg, Na and S concentrations in bone and other organs samples from DMD^{mdx}/J dystrophic mice as well as C57BL/6J control group mice. The DMD^{mdx}/J mouse strain is relevant as an experimental model for Duchenne Muscular Dystrophy (DMD), which is the most severe and prevalent type of muscular dystrophy. 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. To show in more details the alterations that this disease may cause in bones (tibiae) and organs (quadriceps and heart), correlations matrixes were generated for both strains permitting a comparison between these groups. A significant change was observed in the analysis of the heart of dystrophic mice suggesting that this dysfunction affects severely the heart muscle. The results emphasize physiologic differences for Na, Ca and Mg and suggest that Br and S results are altered, emphasizing a constant monitoring needs. Other than that, these results may help the researchers to evaluate the efficiency of new treatments and to compare the advantages of different treatment approaches before performing tests in patients with muscular dystrophy.

1. INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is the most severe and prevalent type of muscular dystrophy. 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. DMD is a dysfunction that affects approximately 1 in every 3500 - 6000 newborn boys in the world [1,2]. In general, males are affected and only

approximately 10% of female carriers can show some disease manifestations. The worldwide incidence of genetic disorders among newborns in United States and in Europe is 3%. In Brazil, according to the latest census conducted by the IBGE (Instituto Brasileiro de Geografia e Estatística), this percentage means more than 5 million people will be affected, and in São Paulo State, the number will be about 1 million [3]. While there is no cure for DMD, substantial strategic research activities along with a search for new therapies are currently being tested in clinical trials and animal models [4].

Particularly in Brazil, the Dmd^{mdx}/J strain mouse has been widely used as an animal model in progressive muscular dystrophy investigations. However there is no elemental characterization for whole blood, bone and organs for this strain. Consequently, the determination of element concentrations in whole blood and biological tissues may help to evaluate the efficacy of the new treatments, as well as compare the advantages of different types of treatment schedules before performing tests in patients with muscular dystrophy. Moreover, a profound knowledge of all pathological characteristics of the animal models is crucial for its reliable use.

Nowadays, the DMD dystrophy is been investigated at IPEN, Instituto de Biociências and Instituto Butantan (Research Centers, from Brazil), using mice genetically modified such as: A/J, Dmd^{mdx}/J and SJL/J. Recently the elemental composition of blood in A/J, Dmd^{mdx}/J and SJL/J mouse lines [5-10] were analyzed and a comparison with human blood revealed physiologic differences among them.

In this study the correlation matrixes were generated with the concentrations values [5] obtained from bones and organs of Dmd^{mdx}/J (dystrophic male group) and C57BL/6J (control male group) mice, using the instrumental neutron activation analysis (INAA) technique, permitting a comparison between these groups. These elements were selected due to the clinical relevance for evaluation of electrolyte disorders and nutritional relevance [11,12].

2. EXPERIMENTAL PROCEDURE

INAA was applied to conduct this investigation. The animal models, Dmd^{mdx}/J male (n = 9) and C57BL/6J male (n = 12), were obtained from Jackson Laboratory [13] (Maine, USA) and later inbred at IPEN – CNEN/SP (São Paulo, Brazil).

Twenty one mice (4 month-old) were sacrificed and the biological materials were collected. The bones (tibiae) and organs (hearts and quadriceps) were removed, weighed and immediately frozen until they were used. For irradiation each biological sample was weighed and sealed into a polyethylene capsule and irradiated in the IEA-R1 nuclear reactor at IPEN-CNEN/SP (3.5 MW, pool type). The thermal neutron flux utilized ranged from $8.45\ 10^{11}$ $cm^{-2}\cdot s^{-1}$ to $1.19\cdot 10^{12}$ $cm^{-2}\cdot s^{-1}$. For organs and bones the experimental conditions are presented in Table 1. After neutron irradiation, the organs and bone samples were transferred to a clean container before counting. Each sample (tibia, quadriceps and heart) was analyzed two times at least. Bovine Liver Powder (NIST 1557b) and Bone Powder (NIST 1486) were used as standards and for analytical quality control.

Table 1. Experimental Conditions

Biological Material	MV (± 0.1%)	Mass (min)	Mass (max)	Element/ Ti: Td: Tc
Tibia	0.0644 ^{CG} 0.0749 ^{m}	0.0571 ^{CG} 0.0601 ^{m}	0.0734 ^{CG} 0.0930 ^{m}	Ca/ 30s: 60s: 120s Cl, Mg, Na/ 30s: 60s: 900s
Quadriceps	0.2118 ^{CG} 0.3592 ^{m}	0.1552 ^{CG} 0.3321 ^{m}	0.2546 ^{CG} 0.4309 ^{m}	Ca/ 240s: 60s: 120s Br,Cl, K, Mg, Na/ 240s: 60s: 900s
Heart	0.1004 ^{CG} 0.1393 ^{m}	0.0893 ^{CG} 0.1217 ^{m}	0.1135 ^{CG} 0.1691 ^{m}	Br,Ca, Cl, K, Mg, Na, S/ 240s:60s:900s

MV: Mean Value (arithmetic mean; wet mass in g)
mass (min): smaller mass (wet mass in g)
mass (max): largest mass (wet mass in g)
Ti: Irradiation Time
Td: Decay Time
Tc: Counting Time
CG: Control Group C57BL/6J (males)
m: Dystrophic - Dmd^{mdx}/J (males)

The measurements of the neutron 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 was reduced by employing the iron shield described by Medeiros et al. [14]. The source-detector distance in this experimental apparatus is 12.5 cm. The concentration of each element in each biological sample was obtained by using in-house software [15].

3. RESULTS AND DISCUSSION

After established the biological samples concentrations [5], it is possible to construct the Pearson's correlation matrix [16,17], which provides correlation coefficients for each element in tibia, quadriceps and heart. The correlation matrices in Tables 2, 3 and 4 are shown for C57BL/6J (control male group) and for Tables 5, 6 and 7, are shown for Dmd^{mdx}/J (dystrophic male group). The main correlation coefficients are highlighted.

Table 2 – Correlation coefficients of Mg, Na, Ca and Cl, (gkg^{-1}) in bone (tibia) of C57BL/6J mouse strain

C57BL/6J	Mg	Na	Ca	Cl
Mg	1	0.50	0.51	-0.21
Na		1	0.54	0.09
Ca			1	0.57
Cl				1

Table 3 – Correlation coefficients of Mg, Na, Ca, Cl, Br, K and S (gkg^{-1}) in quadriceps of C57BL/6J mouse strain

C57BL/6J	Mg	Na	Ca	Cl	Br	K	S
Mg	1	-0.19	-0.19	-0.20	-0.16	-0.16	0.10
Na		1	0.18	**0.88**	**0.90**	0.66	-0.13
Ca			1	0.09	0.05	0.21	0.49
Cl				1	**0.86**	0.65	-0.10
Br					1	0.62	-0.16
K						1	0.53
S							1

Table 4 – Correlation coefficients of Mg, Na, Ca, Cl, Br, K and S (gkg^{-1}) in heart of C57BL/6J mouse strain

C57BL/6J	Mg	Na	Ca	Cl	Br	K	S
Mg	1	-0.73	-0.43	-0.68	-0.74	-0.46	-0.26
Na		1	0.78	**0.99**	0.37	**0.94**	**0.85**
Ca			1	**0.81**	-0.22	**0.87**	0.74
Cl				1	0.30	**0.96**	**0.88**
Br					1	0.04	-0.02
K						1	**0.96**
S							1

Table 5 – Correlation coefficients of Mg, Na, Ca and Cl, (gkg^{-1}) in bone (tibia) of Dmd^{mdx}/J mouse strain

Dmd^{mdx}/J	Mg	Na	Ca	Cl
Mg	1	**-0.96**	**-0.62**	0.16
Na		1	**0.73**	0.10
Ca			1	0.07
Cl				1

Table 6 – Correlation coefficients of Mg, Na, Ca, Cl, Br, K and S (gkg^{-1}) in quadriceps of Dmd^{mdx}/J mouse strain

Dmd^{mdx}/J	Mg	Na	Ca	Cl	Br	K	S
Mg	1	0.49	0.52	-0.46	0.31	0.26	-0.27
Na		1	**0.98**	-0.36	-0.04	**0.96**	0.49
Ca			1	-0.21	0.14	**0.91**	0.57
Cl				1	0.70	-0.38	0.57
Br					1	-0.24	0.35
K						1	0.54
S							1

Table 7 – Correlation coefficients of Mg, Na, Ca, Cl, Br, K and S (gkg^{-1}) in heart of Dmd^{mdx}/J mouse strain

Dmd^{mdx}/J	Mg	Na	Ca	Cl	Br	K	S
Mg	1	0.62	0.27	0.19	-0.19	-0.46	**0.82**
Na		1	**0.91**	0.22	-0.37	-0.25	**0.93**
Ca			1	0.00	-0.52	-0.63	0.77
Cl				1	**0.83**	0.54	0.03
Br					1	0.65	-0.51
K						1	-0.10
S							1

For tibia, Mg:Na, Mg:Ca, Na:Ca and Ca:Cl are the predominant correlations between the groups in Tables 2 and 5, respectively, however the correlations between Mg:Na (-0.96) and Mg:Ca (-0.62) for dystrophic group (Table 5) show an accentuated and inverted coefficients.

Predominant correlations for control group muscle (Table 3) are expressed by Na:Cl (0.88), Na:Br (0.90) and Cl:Br (0.86) which are not kept in the dystrophic group - a strong dependence between Ca:Na (0.98), Ca:K (0.91) and Na:K (0.96) (Table 6).

Related to the heart for DMD group (Table 7) the correlations were substantially altered. The only strong correlation that is kept in DMD group is between Na:Ca (0.78 for the control group (Table 4) and 0.91 for DMD group. Although Br and S are not usually monitored in the conventional clinic, the strong correlations between Br:Cl (0.83), S:Mg (0.82) and S:Na (0.93) in DMD group suggest that Br and S should also be monitored in this dysfunction.

3. CONCLUSIONS

In this study the correlation coefficients of Mg, Na, Ca, Cl, Br, K and S, in quadriceps and heart, and Ca, Cl, Mg and Na in tibia for C57BL/6J and Dmd^{mdx}/J mice strain were obtained. These results may help the researchers to evaluate the efficiency of new treatments and to compare the advantages of different treatment approaches before performing tests in patients with muscular dystrophy. Besides, the alteration in some correlation coefficients data among the elements in the health status and in the diseased status indicates a connection between these elements in tibia, quadriceps and heart.

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