

Investigation of whole blood of SJL/J mice using neutron activation analysis

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Abstract The Br ($0.0022 \pm 0.0006 \text{ gL}^{-1}$), Ca ($0.113 \pm 0.012 \text{ gL}^{-1}$), Cl ($3.07 \pm 0.36 \text{ gL}^{-1}$), K ($2.63 \pm 0.14 \text{ gL}^{-1}$), Mg ($0.045 \pm 0.002 \text{ gL}^{-1}$) and Na ($2.09 \pm 0.10 \text{ gL}^{-1}$) concentrations were determined in whole blood of SJL/J mice using the Neutron Activation Analysis (NAA) technique. Eleven whole blood samples were analyzed in the IEA-R1 nuclear reactor at IPEN (São Paulo, Brazil). These data contribute for applications in veterinary medicine related to biochemistry analyses using whole blood. Moreover, the correlation with human blood estimation allows to checking the similarities for studying muscular dystrophy using this model animal.

Keywords Whole blood · SJL/J mice · NAA ·
References values · Biological material · DMD

Introduction

In recent years, the Nuclear Structure Laboratory at IPEN (SP, Brazil) has performed measurements related to the determination of metals and ions in whole blood in the inhabitants from Brazil as well as in several animals used for experimentation (animal model) using NAA [1–7]. This

analytical procedure has been chosen because it presents advantages when compared with the conventional methods for biochemistry analyzes mainly for investigation of small sized animal [1]. Until now, the whole blood of nine mice strains, namely NZB, B10.RIII, BALB/c, A/J, Dmd^{mdx}/J, H_{III}, L_{III}, AIR_{MAX} and AIR_{MIN}, have been investigated by us using NAA technique [6, 7].

The data obtained in those investigations have indicated a different behavior for some elements, mainly when compared with human whole blood estimation (control group) [2, 4], suggesting that specific measurement must be done for each strain in order to allow the choice of a better mouse strain for experimental model: while H_{III}, L_{III}, NZB, B10.RIII and BALB/c strains can be used for studying the antibody responsiveness, AIR_{MAX} and AIR_{MIN} strains are usually selected for investigations related to inflammatory reactivity. To study muscular dystrophy [8–11] some mouse strains with spontaneous mutation such as: A/J, Dmd^{mdx}/J and SJL/J have been used.

Muscular dystrophy is being investigated at IPEN and Butantan (Research Centers, Brazil), using these mice as an animal model. Recently, we analyzed the elemental composition of blood from A/J and Dmd^{mdx}/J mouse lines [6, 7], and a comparison with human blood estimation revealed physiological differences among them. Now we intend to investigate the blood of SJL/J mouse strain using NAA.

In this study, we determined the Br, Ca, Cl, K, Mg and Na concentrations in whole blood of SJL/J strain using NAA. The knowledge of the elemental composition of its blood can be used for clinical chemistry investigation with advantages when compared with the conventional procedure [12] (which is performed using serum, a limitation when the biological material is scarce) and it also permits to check the similarities with other strains (A/J and Dmd^{mdx}/J) as well as with human whole blood estimation

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values (control group) for studying in more details the anomalies caused by DMD.

Experimental

To perform this investigation the whole blood samples of two-month-old adult females ($n = 7$) and males ($n = 4$) that was originally obtained from the Jackson Laboratory (Maine, USA) and further inbred at IPEN-CNEN/SP (São Paulo, Brazil) were obtained. About 0.3 ml of whole blood was collected by the retro-orbital venous plexus and aliquots of 100 μL (duplicate) was then transferred to the filter paper ($\sim 2.2 \text{ cm}^2$ pieces of Whatman filter paper) and dried for few minutes using an infrared lamp. Standard solutions obtained from high purity metals and salts were prepared following the same procedure.

Samples and standards were sealed into a polyethylene bag and irradiated in the IEA-R1, 2–4 MW nuclear reactor at IPEN. For the elements determination a 5 min irradiation followed by 1 min decay and 15 min counting time was used. The NIST 8414 Bovine Muscle Powder was analyzed for 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. [13]. The source-detector distance in this experimental apparatus is 12 cm. The concentration of each element was obtained by using in-house software.

Results and discussion

The results for the reference material are presented in Table 1. The Z-score values [14] obtained indicated that our results are satisfactory considering the range of certified data at the 95% confidence level.

The concentration values for Br, Ca, Cl, K, Mg and Na in whole blood of SJL/J mice are presented in Table 2. The range from human blood (control group) [2, 4] were included, considering one standard deviation, for checking the similarities. Although male and female blood samples have been analyzed no significant difference was observed.

The concentration values in whole blood of SJL/J mice can also be compared to other dystrophic strains, namely the, A/J⁶ and Dmd^{mdx}/J⁷ as well as with the human being whole blood estimation [2, 4].

For Na, the mean value in for Dmd^{mdx}/J strain ($1.54 \pm 0.08 \text{ gL}^{-1}$) is in agreement with the upper limit

Table 1 Element concentrations obtained in the analysis of NIST 8414 Bovine Muscle Powder standard reference material

Element	Mean \pm SD	RSD, %	Er, %	Z score
Br, mg kg ⁻¹	1.4 \pm 0.4	28.6	27.3	0.6
	1.1 \pm 0.5*			
Ca, mg kg ⁻¹	164 \pm 15	9.1	13.1	1
	145 \pm 20*			
Cl, %	0.191 \pm 0.023	12	1.6	0.2
	0.188 \pm 0.015*			
K, %	1.528 \pm 0.083	5.4	0.7	0.3
	1.517 \pm 0.037*			
Mg, mg·kg ⁻¹	935 \pm 49	5.2	-2.6	-0.3
	960 \pm 95*			
Na, %	0.211 \pm 0.010	4.7	0.5	0.13
	0.210 \pm 0.008*			

* Certified values

Table 2 The Br, Ca, Cl, K, Mg and Na concentrations (g L^{-1}) in blood of SJL/L mouse

Element	Mean	SD	Min.	Max.	Range
Br	0.0022	0.0009	0.0011	0.0038	0.0013–0.0031 0.0024–0.0096 ^a
Ca	0.113	0.065	0.030	0.230	0.048–0.178 0.150–0.316 ^b
Cl	3.07	0.61	2.23	4.01	2.46–3.68 2.34–3.00 ^a
K	2.63	0.57	2.10	3.56	2.06–3.20 1.09–1.53 ^a
Mg	0.045	0.018	0.016	0.087	0.027–0.063 0.040–0.0074 ^b
Na	2.09	0.37	1.71	2.78	1.72–2.46 1.24–1.60 ^a

^a Human values from reference [4]

^b Human value from reference [2]

for human being estimation (1.60 gL^{-1}) considering one standard deviation; for A/J strain the mean value ($1.77 \pm 0.08 \text{ gL}^{-1}$) is in agreement with the upper limit for human being whole blood estimation (1.78 gL^{-1}) considering a confidence interval of 95%, usually adopted as reference for clinical practice, but for SJL/J ($2.09 \pm 0.10 \text{ gL}^{-1}$) strain, since considering a confidence interval of 99% for human blood estimation (1.96 gL^{-1}) it is yet out of the upper limit.

For Br, all the three genetic modified strains ($0.0022 \pm 0.0006 \text{ gL}^{-1}$ for SJL/J; $0.0037 \pm 0.0008 \text{ gL}^{-1}$ for A/J and $0.0020 \pm 0.0007 \text{ gL}^{-1}$ for Dmd^{mdx}/J, respectively) are near the lower limit for human being whole blood estimation (0.0024 gL^{-1}) while for K this comparison shows

that the mean values for all strains ($2.63 \pm 0.14 \text{ gL}^{-1}$ for SJL/J; $2.12 \pm 0.14 \text{ gL}^{-1}$ for A/J and $2.86 \pm 0.13 \text{ gL}^{-1}$ for Dmd^{mdx}/J, respectively) are significantly higher even when compared to the upper limit (1.97 gL^{-1}) for human being whole blood estimation for a confidence interval of 99%.

Considering that variations in K levels can be associated to dysfunction in muscle (as cramps and/or weakness and/or paralysis) the increase observed in the K concentration for all strains could be related to muscular dystrophy. On the other side, for Br the proximity with the lower limit for human being whole blood estimation in all strains can be related to many factors which can contribute for its increase in human blood, such as: magnesium bromide found in seawater so present in fish and sea food; the presence of Br in water as the result of chlorination and ozonation disinfection processes, which are essential to water treatment in order to maintain microbiological safety suitable for human consumption and its presence in food stuffs (CH₃ Br) as pesticide residue with designated tolerance of 0.1 ppm to 10 ppm (USDA) [15] as well as the use of medicines by humans, mainly antidepressants. For Ca ($0.113 \pm 0.012 \text{ gL}^{-1}$ for SJL/J; $0.141 \pm 0.014 \text{ gL}^{-1}$ for A/J and $0.260 \pm 0.023 \text{ gL}^{-1}$ for Dmd^{mdx}/J, respectively) and for Cl ($3.07 \pm 0.36 \text{ gL}^{-1}$ for SJL/J; $3.07 \pm 0.36 \text{ gL}^{-1}$ for A/J and $2.86 \pm 0.30 \text{ gL}^{-1}$ for Dmd^{mdx}/J) the results are in agreement with themselves as well as with the human being whole blood estimation considering one standard deviation. For Mg this comparison is only possible for SJL/L mouse and human being and they are in agreement (see Table 2).

Conclusions

The determination of elements relevant for clinical chemistry in whole blood of SJL/J mouse was evaluated by NAA. These data may help to evaluate and compare the advantages of different treatment schedules in muscular dystrophy when this model animal is used. Furthermore the comparisons of the three dystrophic strains (A/J, Dmd^{mdx}/J and SJL/J) with human blood values (control group) suggest that K and Na levels in these strains are affected by muscular dystrophy suggesting that these elements must be constantly evaluated during medical investigations

using these animals as model for diagnostic and therapeutic procedures.

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