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Simultaneous determination of five elements in whole blood of dystrophin-deficient mdx mouse by NAA

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Concentrations of Br, Ca, Cl, K and Na in whole blood of dystrophin-deficient mouse [the Dmd^{mdx} line] were determined using NAA, resulting in reference values that are relevant for clinical blood investigation. The comparison with human being whole blood values was also performed in order to establish possible indexes and similarities among the experimental and clinical applications.

Introduction

Several laboratory animals, mainly rats and mice, are currently used in the medical area on investigations for new medicines, vaccines or antibiotics, for example before being tested in humans. It is usually very important in these researches to check the health status of the animals performing biochemistry analyses mainly in serum and/or urine. Particularly for these analyses the NAA technique can be used with advantages towards the conventional methods because it uses small quantities of biological material and allows the simultaneous evaluation of several element concentrations,[1,2] something not always possible in the conventional clinical analysis.[3] These simplifications are fundamental when the biological material is restricted, for example when small sized animals are involved. Based on these facts, in the last years the Nuclear Structure Laboratory at IPEN has investigated different biological materials using NAA aiming to use these data for diagnostic applications.[4–6] One important contribution extracted from these studies is the use of whole blood for clinical practice in mouse, an animal model used in medical researches.

The interest of studying this animal is due to the fact that it represents an efficient choice in immunobiological field investigations, due to the low cost and easy handling,[7] moreover, the knowledge of the elemental composition of its blood may reveals physiologic differences among distinct mouse strains which might be important in applications in health areas, including reference values for clinical investigations.

In this context, initially, we analyzed five inbred strains (NZB, B10.RIII, BALB/c and A/J and SLJ/L) as well as the genetically selected lines for High or Low antibody responsiveness (H_{III} and L_{III}) and also two selected for the maximal or minimal acute inflammatory reactivity (AIR_{MAX} and AIR_{MIN}) using NAA technique.[8] The elements Cl, K and Na were investigated in the whole blood of these mouse strains because this body fluid represents the most important biological referential of circulatory system conditions and alterations in these major elements in blood represent an important tool for checking electrolyte diseases. The bromide level has also been evaluated in some strains because it is present in high quantities in some medicines, mainly in anti depressants that have large consumption by the Brazilian population.

In this study we intend to determine the concentrations of Br, Ca, Cl, K and Na in whole blood of the mutated Dmd^{mdx} mouse strain, an animal model for Duchenne muscular dystrophy, since comparison with the corresponding background line (C57Bl/10) shows expressive changes in ions and metals concentrations in blood, organs and muscles.[9–13] Furthermore, we also intend to check the similarities between this mouse strain and the human whole blood estimation values for studying in more detail the anomalies caused by the muscular dystrophy as well as the diagnostic and treatment to be applied.

Experimental

To perform the analysis in whole blood of the inbred Dmd^{mdx} line, fourteen animals were assayed in order to estimate the level of environmental factors that influence the general character. These animals came from IPEN facilities (São Paulo, Brazil). They were housed in cages at controlled room temperature and fed daily with a standard chow.

For sample preparation, about 0.3 ml of the whole blood was collected by the retro-orbital venous plexus from fourteen male adult mice (3–4 months old) and immediately after the collection, before the blood coagulation, exactly 100 μL of blood were transferred to the filter paper (~2.2 cm^2 pieces of Whatman filter paper) and dried for a few minutes using an infrared lamp. The samples were prepared in duplicate.

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Each biological sample was sealed into a polyethylene bag and irradiated in the IEA-R1, 2–4 MW nuclear reactor at IPEN. For the determination of the elements a 5 minute irradiation followed by 1 minute decay and 10 minute counting time was used. Au was irradiated together with the sample for neutron flux monitor,[1] the thermal neutron flux utilized ranged from $4.55 \cdot 10^{11}$ to $8.92 \cdot 10^{12}$ n·cm^{-2}·s^{-1}. The precision and accuracy of the results were checked by analyzing NIST 8414 Bovine Muscle Powder standard reference material. It was also analyzed using the same procedure (it was shaped in the same manner as the biological sample) for analytical quality control.

The measurements of the gamma-induced activity of the samples were carried out using an ORTEC Model GEM-60195 hyperpure Ge detector and an ORTEC 671 amplifier, in pile-up rejection mode, coupled to a MCA ORTEC Model 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.[14] The source–detector distance in this experimental apparatus is 15.2 cm. The concentration of each element was obtained by using in-house software.[6] Following this experimental procedure it was possible to quantify simultaneously the following radioactive nuclides: ^{80}Br ($T_{1/2}$ = 16 min, E_γ = 616 keV), ^{49}Ca ($T_{1/2}$ = 8 min, E_γ = 3084 keV), ^{38}Cl ($T_{1/2}$ = 37 min, E_γ = 1642 keV and 2176 keV), ^{42}K ($T_{1/2}$ = 12 h, E_γ = 1525 keV) and ^{24}Na ($T_{1/2}$ = 15 h, E_γ = 1368 keV), in about 30 minutes or less, making this nuclear procedure very agile.

Results and discussion

The presence of impurities in the filter paper was examined first. The elements Na and Cl were found but they could be considered negligible in comparison with the amounts present in the samples.

The precision and the accuracy of the results by analysis of the reference material are presented in Table 1. The *Z*-score values[15] indicate that our results are satisfactory and are within the ranges of certified data at the 95% confidence interval.

For each blood samples the concentration value was established by the mean of two replicate samples. The results of Br, Ca, Cl, K and Na in whole blood of dystrophin-deficient mdx mouse are shown in Table 2 as well as the range for the reference values considering one standard deviation (SD).

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

Element	Certified values	This work mean ± SD	RSD, %	Er, %	*Z*-score
Br, mg·kg^{-1}	1.1 ± 0.5	1.3 ± 0.3	23.1	18.2	0.4
Ca, mg·kg^{-1}	145 ± 20	132 ± 14	10.6	–9	–0.5
Cl, %	0.188 ± 0.015	0.202 ± 0.013	6.4	7.4	0.7
K, %	1.517 ± 0.037	1.476 ± 0.104	7.1	–2.7	–0.4
Na, %	0.210 ± 0.008	0.211 ± 0.013	6.2	0.5	0.1

RSD: Relative standard deviation.
Er: Relative error.

Table 2. Element concentrations (in g·L^{-1}) in whole blood samples of Dmd^{mdx} mouse

Element	Mean	Minimum value	Maximum value	Range
Br (n = 12)	0.0020 ± 0.0013	0.0010	0.041	0.007–0.033 [0.0024–0.0096][a]
Ca (n = 12)	0.26 ± 0.12	0.14	0.47	0.14–0.38 [0.14–0.32][b]
Cl (n = 14)	2.86 ± 0.80	2.40	3.78	2.06–3.66 [2.34–3.00][a]
K (n = 14)	2.97 ± 0.10	2.49	3.26	2.87–3.07 [1.09–1.53][a]
Na (n = 14)	1.54 ± 0.41	1.19	1.98	1.13–1.95 [1.24–1.60][a]

[a] Human reference values from Ref. 16.
[b] Human reference value from Ref. 17.
n: Number of samples analyzed.

The result for Br concentration was compared to five strains,[8] i.e., NZB, B10.RIII, SLJ/L, H_{III} and AIR_{MAX} and also with human being whole blood estimation,[15] for Cl, K and Na these comparisons were also performed and extended to other strains[8] (BALB/c, A/J, L_{III} and AIR_{MIN}). All these comparisons can be seen in Figs 1, 2, 3 and 4, where the intervals from human reference[16,17] were included, considering one standard deviation (SD), for checking the similarities. As for Ca, although it had not been evaluated in the whole blood for the other strains, its determination in this mouse line is important for clinical practice based on the fact that muscular dystrophy is related to significant changes in Ca concentration in muscles as well as in blood.[10] It could be compared with the human estimation (0.233 ± 0.083 g·L^{-1})[17] showing to be in agreement. For chlorine and sodium, levels in Dmd^{mdx} mouse strain are also in agreement with whole blood human estimation, but for Br and K, however, the levels are altered: while for Br the mean value (0.0020 ± 0.0013 g·L^{-1}) in Dmd^{mdx} mouse is near the lower limit for human being (0.0024 g·L^{-1})[16] for K this comparison shows that the mean value (2.97 ± 0.10 g·L^{-1}) in Dmd^{mdx} mouse is significant higher even when compared to the upper limit (1.75 g·L^{-1})[16] in whole blood estimation for human being, for a confidence interval of 95% usually adopted as reference for clinical practice.

Related to the behavior of the Br, Cl, K and Na concentrations (Figs 1, 2, 3 and 4), the continuous distribution of them in whole blood samples of the distinct mouse lines are suggestive of the polygenic control for the constitutive and/or absorption and/or metabolism of these elements. There is an association between the immune adaptive (antibody responsiveness) or innate immune function (inflammation) and the distinct element concentrations as indicated by the distribution of the genetically selected mouse lines. Besides, the comparative study involving the humans and the all lines (MDX, NZB, B10.RIII, BALB/c, A/J, SLJ/L, H_{III}, L_{III}, AIR_{MAX} and AIR_{MIN}) show differences for some components, mainly K for all lines (Fig. 3) and Na for L_{III}, B10.RIII, AIR_{MIN} and SLJ/L strains (Fig. 4), indicating the necessity to establish specific measurement in a mouse strain to allow the better choice of reference for clinical marker.

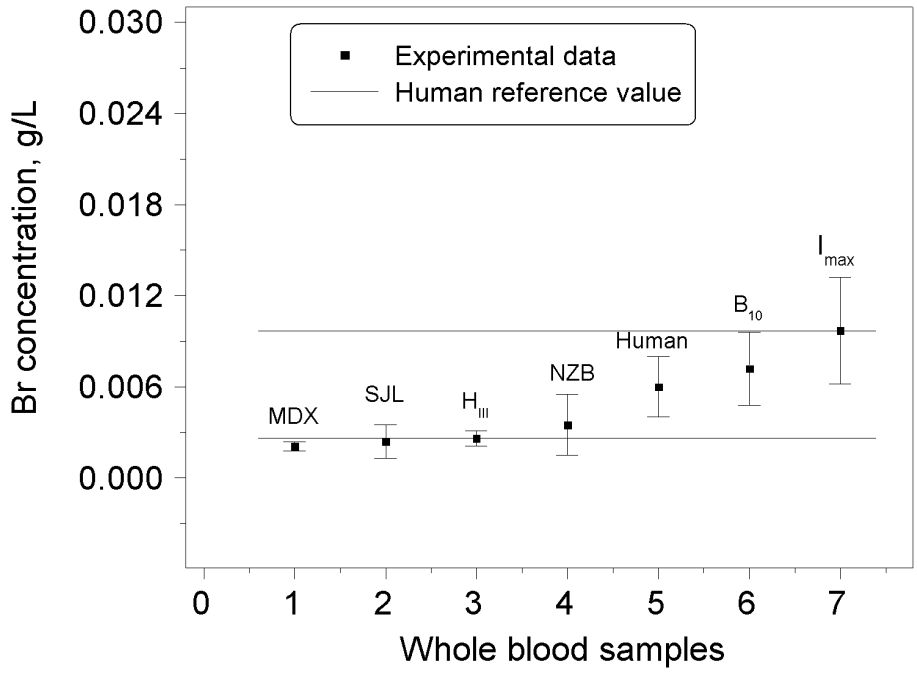

Fig. 1. Concentration of Br in whole blood samples of Dmd^{mdx} mouse compared to other strains with well-established values SJL, H_{III}, NZB, B10, AIR_{MAX}[8] as well as with human being whole blood estimation[16]

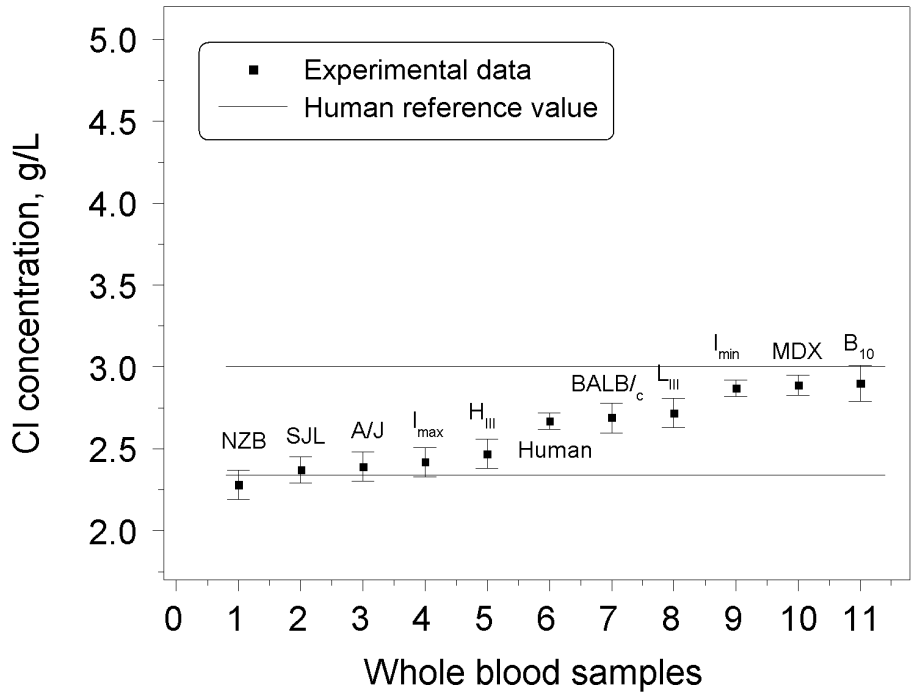

Fig. 2. Concentration of Cl in whole blood samples of Dmd^{mdx} mouse compared to other strains with well-established values in NZB, SJL/J, A/J, AIR_{MAX}, H_{III}, BALB/c, L_{III}, AIR_{MIN} and B10.RIII[8] as well as with human being whole blood estimation[16]

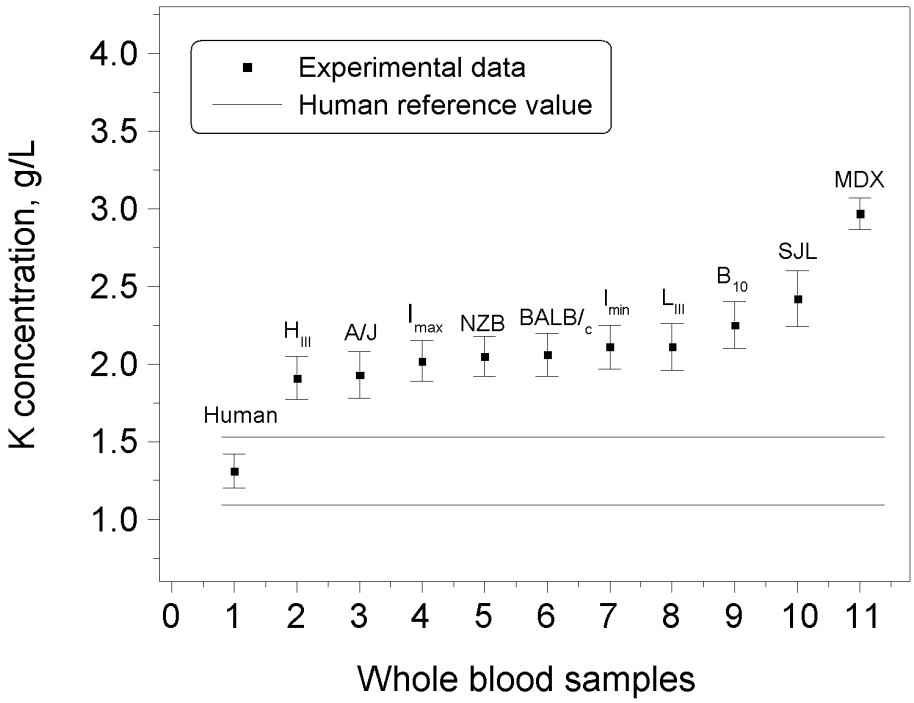

Fig. 3. Concentration of K in whole blood samples of Dmd^{mdx} mouse compared to other strains with well-established values in H_{III}, A/J, AIR_{MAX}, NZB, BALB/c, AIR_{MIN}, L_{III}, B10.RIII and SLJ/L[8] as well as with human being whole blood estimation[16]

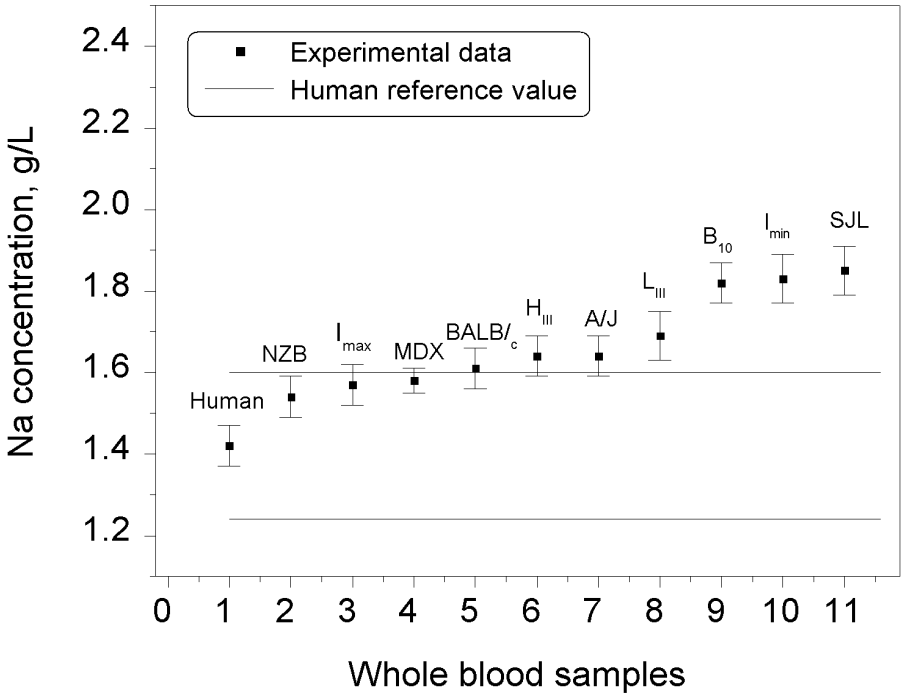

Fig. 4. Concentration of Na in whole blood samples of Dmd^{mdx} mouse compared to other strains with well-established values NZB, AIR_{MAX}, BALB/c, H_{III}, A/J, L_{III}, B10.RIII, AIR_{MIN} and SLJ/L^{8} strain as well as with human being whole blood estimation[16]

Conclusions

The Dmd^{mdx} mouse line seems to be of interest to better understand the anomalies caused by the muscular dystrophy in the body organs. The comparison analysis performed with the human being shows that Ca, Cl and Na determination is in good agreement with human data; however high K levels suggest that this element must be constantly evaluated during investigations using this animal model for diagnostic and therapeutic procedures of this anomaly.

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