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Determination of elements in kidney, serum and urine of Wistar rats with Acute Renal Insufficiency using NAA

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Abstract In this investigation ions in serum, urine and kidney of Wistar rats (control group) and Wistar with Acute Renal Insufficiency (ARI) were quantified using instrumental neutron activation analysis. The measurements in serum and urine were performed before, during and after ischemia-induced ARI. The measurements in kidney were performed for the control and ARI groups. Also, a comparative analysis between the concentration ratios before, during and after ARI was performed in urine and serum samples for both groups. The variations results for Cu in serum and I in urine, before and after ischemia-induced ARI, suggest that these elements must be also investigated in renal dysfunction.

Keywords Kidney · Serum · Urine · ARI · INAA

Introduction

The elementary constituents of blood, especially the ions of Ca, Cl, K, Mg, and Na, although in small quantities (<2%) are of great importance for the maintenance of body organs functions. The kidney plays a major role in regulating levels of these minerals in the blood and removes toxic waste products, excess of water and blood salts [1]. Several clinical conditions such as myocardial infarction, intestinal and cerebral ischemia (restriction of oxygen and nutrients in tissues) [2–8] can lead to kidney damage and several diseases like Acute Renal Insufficiency (ARI).

The ARI is defined as an abrupt decline in renal filtration function caused by the ischemia and reperfusion (I/R). This disease includes a complex interaction between the tubular injury, inflammation and alterations in the renal homodynamic [1, 9]. In most of the cases it is irreversible and the mortality in the world has increased in the last few decades [10]. In Brazil, ARI has an incidence of up to 5% of hospitalized patients due mainly to septic shock and cardiac failure [10–12]. Basically, the treatment involves: a control of the hydro-electrolytic balance, performed by clinical analyses of serum (Na, Ca and Mg); dialysis (peritoneal and hemodialysis) and balanced diet with low concentration of salt (Na). Tissue damage observed during the ischemia is mainly due to ionic alterations of calcium and proteolysis [13]. Moreover, considering that kidneys are responsible for excretion of 90% of ingested K, in patients with ARI the K serum levels must also be controlled because changes (rapid increase) can be lethal. However, when a blood reperfusion is restored, the oxygen flux returns to tissues in high proportions, normalizing renal functions [14–19].

According to the last census performed in Brazil by Sociedade Brasileira de Nefrologia [10] the number of patients with renal dysfunction has increased and the incidence of the mortality caused by ARI, already reaching 8%, has become a public health problem. Due to the severity of this disease there is intense research related to development of complementary or alternative clinical procedures to prevent and give a better quality of live for these patients.

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In this investigation, ions in serum, urine and kidney of Wistar rats (control group) and Wistar rats with ARI were quantified using Instrumental Neutron Activation Analysis. The elements Ca, Cl, K, Mg and Na were determined for evaluation of electrolyte disorders; because of the frequent presence of Br in drugs highly consumed by patients with renal disease and due to nutritional relevance of S (Brazilian diet is rich in sulfur [20]) these two elements were also determined. The measurements in urine and serum were performed before, during and after the ischemia-induced ARI. The measurements in kidney were performed for the control and ARI groups. The quantitative knowledge of these elements in these biological materials allows for evaluation of the functions that regulate the kidneys behavior during the decline in renal filtration function.

Experimental

The biological samples (urine, serum and kidney) were collected from 12 adult Wistar rats (males) raised in a vivarium at UNIFESP (Federal University from São Paulo, Brasil). The urine and serum collections were obtained from rats previously anesthetized. The kidneys were obtained after the sacrifice of rats. The clinical procedure for ischemia- and reperfusion induced (I/R) was performed in the Laboratório de Nefrologia (UNIFESP, SP, Brazil). For irradiation each kidney sample was weighed (wet mass) and sealed into a polyethylene capsule.

The urine samples were collected with a catheter attached to their bladder and aliquots of 50 ± (0.5%) μL and 200 ± (0.5%) μL were transferred to filter paper (Whatman no. 42). For serum sample about 1 mL of whole blood was collected directly from the heart (intracardiac) and packed in a plastic tube. The collected blood was centrifuged and aliquots of serum (100 ± 0.5% μL and 200 ± 0.5% μL) were then transferred to filter paper (Whatman no. 42). For ARI group the urine and serum samples were collected before, during and after ARI. All the samples were prepared in duplicate.

To determine the concentration of the elements, each biological sample was sealed into individual polyethylene bag and irradiated under a thermal neutron flux of 3×10^{12} cm^{-2} s^{-1} in the IEA-R1 nuclear reactor (3.5 MW, pool type) at IPEN. The irradiation time of 3 min and the counting time of 15 min were used. Each kidney sample was transferred to a clean container before counting and each one was measured at least twice.

The measurements were performed using an ORTEC Model GEM-60195 detector coupled to an ORTEC 671 amplifier (in pile-up rejection mode) and to an ORTEC Model 919E MCA. The data was analyzed using in-house software. Reference material (IAEA-A13 Blood Animal) was analyzed to verify the accuracy and precision of the results.

Results and discussion

The results for the quality control (Z-score values <2) indicate the adequacy of the method for all determined elements. The Br, Ca, Cl, Cu, K, Mg, Na and S

Table 1 Element concentration in kidney of Wistar rats

Elements, g Kg^{-1}	MV	1SD	Reference interval, (95%)	DL (3σ)
Control group mass: 0.170 ± 0.1% mg				
Br	0.0024	0.0006	0.0012–0.0036	0.0008
Ca	0.098	0.022	0.054–0.142	0.004
Cl	0.042	0.004	0.034–0.050	0.020
Cu	0.0074	0.0021	0.0032–0.0116	0.0029
K	1.29	0.15	0.99–1.59	0.55
Mg	0.142	0.010	0.122–0.162	0.089
Na	2.44	0.23	1.98–2.90	0.14
S	4.29	1.40	1.49–7.09	0.72
ARI Group mass: 0.140 ± 0.1% mg				
Br	0.0019	0.0004	0.0011–0.0027	–
Ca	0.114	0.008	0.098–0.130	–
Cl	0.033	0.002	0.029–0.037	–
Cu	0.0121	0.0018	0.0085–0.0157	–
K	0.50	0.08	0.34–0.66	–
Mg	0.244	0.050	0.144–0.344	–
Na	4.31	0.40	3.51–5.11	–
S	2.37	0.39	1.59–3.15	–

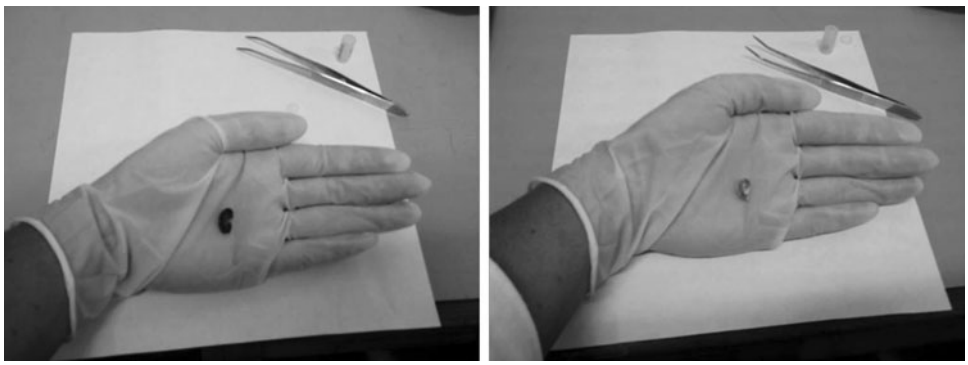

Fig. 1 Dimension of kidney samples (*left*: control group; *right*: ARI)

concentrations determined in kidney samples of ARI and control group are presented in Table 1. The mean value (MV), the standard deviation (1SD), the reference interval (95%), the detection limit (DL) and the wet mass (mean value) are also presented. To view, Fig. 1 shows the sample size.

The Br, Ca, Cl, Cu, K, Mg, Na and S concentrations determined in serum samples of the control group are presented in Table 2. The mean value (MV), the standard deviation (1SD), the reference interval (95%) and the detection limit (DL) are also presented.

The Br, Ca, Cl, I, K, Mg, Na and S concentrations determined in urine samples of the control group are presented in Table 3. The mean value (MV), the standard deviation (1SD), the reference interval (95%) and the detection limit (DL) are also presented.

The element concentrations determined in serum samples of ARI group are presented in Fig. 2 and for urine in Fig. 3. In these figures the concentration result for each element (Before, During and After) was normalized in relation to the concentration value obtained before the renal ischemia (where: CB is the concentration result before I/R, in g L^{-1}; CD is the concentration result during I/R, in g L^{-1} and CA is the concentration result after I/R, in g L^{-1}).

Table 2 Element concentration in serum of Wistar rats (control group)

Elements	MV	1SD	Reference interval, (95%)	DL (3σ)
Br, mg L^{-1}	6.4	0.4	5.6–7.2	1.1
Ca, mg L^{-1}	260	66	128–392	72
Cl, g L^{-1}	3.58	0.45	2.68–4.48	0.014
Cu, mg L^{-1}	0.15	0.06	0.03–0.27	0.013
K, g L^{-1}	0.22	0.08	0.06–0.38	0.03
Mg, mg L^{-1}	51	22	7–95	3.2
Na, g L^{-1}	3.49	0.25	2.99–3.99	0.07
S, g L^{-1}	1.63	0.47	0.69–2.57	0.29

Table 3 Element concentration in urine of Wistar rats (control group)

Elements	MV	1SD	Reference interval, (95%)	DL (3σ)
Br, mg L^{-1}	2.6	0.5	1.4–3.8	0.5
Ca, mg L^{-1}	263	82	99–427	46
Cl, g L^{-1}	2.85	0.84	1.17–4.53	0.24
I, mg L^{-1}	1.2	0.3	0.6–1.8	0.3
K, g L^{-1}	6.48	2.48	1.52–11.44	0.63
Mg, mg L^{-1}	47	17	13–81	4.1
Na, g L^{-1}	1.26	0.48	0.30–2.22	0.62
S, g L^{-1}	1.65	0.29	1.07–2.23	0.54

In the comparative analysis performed in serum (Fig. 2) and urine (Fig. 3) there is an increase for all elements during the renal ischemia (CD/CB). For Cl, Na and S, in serum, the ratios after the renal ischemia (CA/CB) were kept: (1.0; 1.2; 0.9), (1.0; 1.2; 1.0) and (1.0; 1.5; 1.1) respectively, considering the associated uncertainty, but for Ca (1.0; 1.8; 1.4), Cu (1.0; 1.9; 1.5), K (1.0; 1.9; 1.6) and Mg (1.0; 2.9; 1.7) there is a significant increase while a

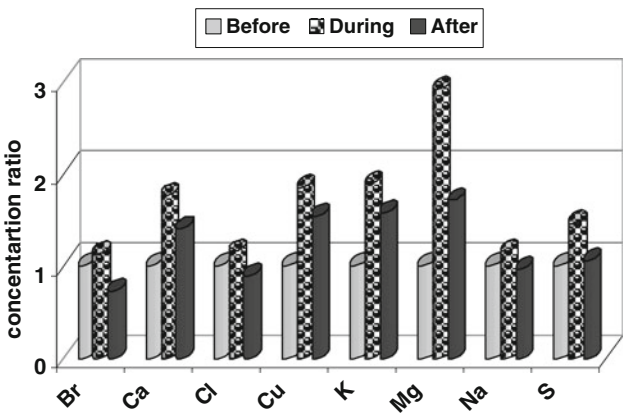

Fig. 2 The behavior of the element concentration ratio in serum during (CD/CB) and after (CA/CB) the ARI induced. The data were normalized in relation of CB (concentration result before I/R) for each element

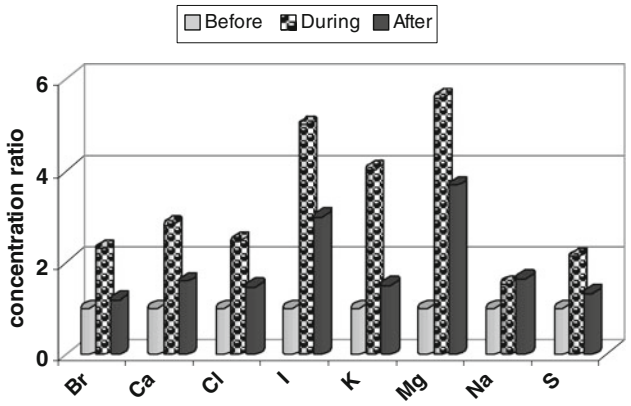

Fig. 3 The behavior of the element concentration ratio in urine during (CD/CB) and after (CA/CB) the ARI induced. The data were normalized in relation of CB (concentration result before I/R) for each element

small decrease is observed only for Br (1.0; 1.1; 0.7). Considering that the efficiency of renal treatment is checked by monitoring the levels of Ca, K, Mg and Na in serum (before and after the dialysis) [21], following the recommendations from national Health Surveillance Agency (ANVISA) Resolution—RDC N154-15 June (2004) [22], the behavior of these elements (Ca, K, Mg and Na) confirm their use as biomarkers for renal diseases and also suggest that Cu may be consider as a potential biomonitor in serum.

The comparative analysis performed in urine (Fig. 3) emphasizes a significant increase of I and Mg after the renal ischemia (1; 5; 3) and (1.0; 5.6; 3.7) respectively. For Br, K and S this ratio returns to the control ratio considering the associated uncertainty; but for Ca (1.0; 2.8; 1.6), Cl (1.0; 2.5; 1.5) and Na (1.0; 1.5; 1.7) there is an increase in their levels after the renal ischemia. Although urine is not used in the conventional renal treatment (clinical analysis is performed using serum), the data from this study suggest that it can also be used as an alternative to check renal dysfunction, performing Ca, Cl, Mg, Na and I evaluation before and after the renal treatment.

Conclusion

In this investigation the behavior of some ions in serum and urine during and after the renal ischemia were studied in detail. The comparative analyses between the concentration ratios before, during and after ARI in these biological materials suggest that Cu in serum and I in urine can also be useful in renal treatment. However, this experiment should be repeated on a larger scale to confirm the elements behavior.

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