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Effects of furosemide administration on the concentration of essential and toxic elements in Wistar rats by inductively coupled plasma optical emission spectrometry

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ABSTRACT

Furosemide can interfere with the metabolism of chemical elements, changing their levels in several tissues, thus causing imbalance. In this study, inductively coupled plasma optical emission spectrometry (ICP OES) was used for multi-element analysis (Cd, Cu, Fe, Mg, Pb, Se and Zn) after microwave-assisted digestion, to evaluate the effect of furosemide (loop diuretic) on the composition of these essential and toxic elements in biological samples (liver, kidney, heart, lung and serum) of Wistar rats. Male and female Wistar rats (n = 40, 180–350 g) were randomly divided into 2 groups (n = 20/group). The results were expressed as μg/g dry weight. The mean tissue concentrations (minimum-maximum in μg/g) of Cu, Fe, Mg and Zn in the biological samples ranged between 5.2 and 1023.5. The levels of Cd, Pb and Se were below the detection limit of the ICP OES. Accuracy was assessed by microwave-assisted digestion and recovery values of 83–116% were obtained. Liver had significantly higher trace element concentrations in most of the analyzed samples. Mg showed a significant reduction (for males and females) in its levels in the heart. In both genders, there was similarity in the Cu concentration reduction (around 16%) for all tissues. The highest iron losses were found for serum (52% and 12%) for male and female rats, respectively. Reductions in Zn occurred between 0.3 and 18.0%, mainly for kidneys and heart, respectively. This study demonstrated that furosemide altered the concentration of some elements in rats.

1. Introduction

Monitoring essential and toxic elements in biological samples is extremely important, since variations in concentration can cause diseases and metabolic disorders [1–5]. The obtention of values related to elemental concentrations in organs and tissues of different species is necessary as a source of reference data for nutritional, environmental, pharmacological, toxicological and other evaluations in animals and humans [6–12]. However, the number of elements determined in specific organs of animals has been limited in previous studies, especially those using small rodents.

The use of diuretics may alter levels of essential elements and, consequently, lead to disorders [13]. Some drugs can interfere with the metabolism of chemical elements, changing their levels, thus causing imbalance [14–17]. Furosemide is a loop diuretic, widely used in clinical practice to treat cardiovascular diseases, which causes the elimination of many elements from the body [18]. In addition, the elimination of other essential elements from the organism can cause disturbances, which requires the evaluation of the availability of these metals in organisms that use furosemide in the long term [19]. However, the current literature does not provide much information on the physiological and qualitative changes of essential elements and contaminants in the composition of animal tissues.

Analytical determinations from biological tissue samples require sample digestion [20–22] and sensitive techniques, with lower limits of detection, higher analytical speed, low cost and relative lack of analytical interference. The microwave acid digestion method is a fast and reproducible extraction procedure for samples of biological importance, such as tissues and human blood [6,23]. Inductively coupled plasma optical emission spectrometry (ICP OES) has the advantages described above, in addition to good quantitative multi-element capability, wide linear dynamic ranges and reasonable cost [24–26].

The aim of this study was to evaluate the effect of furosemide on the composition of Cd, Cu, Fe, Mg, Pb, Se and Zn in biological samples (liver, kidney, lung and serum) of Wistar rats, by axial view ICP OES, after microwave-assisted digestion.

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2. Material and methods

2.1. Animals and exposure

Forty adult (male and female), 8-week-old Wistar rats weighing 180–350 g, were randomly divided into 2 groups (n = 20/group). The rats were exposed to standard conditions (22 ± 1 °C, 50 ± 10% relative humidity and 12 h light/dark cycle) and kept individually under standard conditions, according to European Council Directive 2010/63. The study was in accordance with the Declaration of Helsinki guidelines and was approved by the local animal ethics committee (008/07).

The control group (10 males and 10 females) was placed in conventional stainless steel cages and received a standard diet and 0.9% NaCl (m/v) at 15 mL/kg body weight p.o. for 8 days The second group (10 males and 10 females) received the same standard diet and furosemide (30 mg/kg/day), intraperitoneally, for 8 days and were sacrificed for sample collection. The rats were offered diet and water *ad libitum* for 8 days [27].

The rats were sacrificed by displacement of the cervical spine [28] for collection of blood (by intracardiac puncture) and tissues (liver, kidney, lung and heart), with dry weights ranging, respectively, as follows: 10.0; 2.0; 0.8 and 2.1 g for males and 2.8; 0.5; 0.3 and 0.5 g for females. The tissues were dissected and the remaining carcasses were frozen for subsequent appropriate disposal. The unused biological samples were washed in physiological cold saline solution (0.9% (w/v) NaCl) immediately after sampling, dried on filter paper, weighed and stored at −80 °C for further lyophilization [29].

2.2. Reagents and solutions

All glassware and plastics used in sample collection and analysis were decontaminated in a 10% (v/v) HNO_3 bath for at least 24 h and then washed with ultrapure distilled water using a Milli-Q purification system (Millipore − Bedford, MA, USA).

Chemicals and reagents were purchased from Merck® (Darmstadt, DA, Germany), except when indicated. Sample digests were obtained using the following reagents: 65% nitric acid and 30% hydrogen peroxide (m/v). The multi-element solutions containing 20.0 mg/L of Cd, Cu, Fe, Se, Pb and Zn and 60.0 mg/L Mg were prepared by diluting a 2.0 mol/L solution of HNO_3 in water, from the stock solutions of each of the elements at 1000.0 mg/L, from Qhemis® (São Paulo, SP, Brazil). For the administration of the drug, 2 mL ampoules containing 40 mg of Furosemide (Lasix®) from Sanofi Aventis (Rio de Janeiro, RJ, Brazil) were used.

2.3. Instrumentation

A Terroni Fauvel LT 1000/8 (São Carlos, SP, Brazil) lyophilizer coupled to a vacuum pump was used to process the biological samples (liver, kidney, heart, lung and serum) of Wistar rats. For the total acid digestion of samples, wet digestion with a mixture of mineral acids (microwave-assisted digestion procedure) was investigated. A commercial high-pressure laboratory microwave oven (Milestone Ethos 1600 Microwave Labstation – Sorisole, LM, Italy) was used at a frequency of 2450 Hz and energy output of 900 W. This microwave digestion system was equipped with ten 100-mL tetrafluoromethoxy vessels and a ceramic vessel jacket. The maximum operating temperature and pressure were 180 °C, 1000 W and 100 bar, respectively, in 30 min.

A Vista simultaneous inductively coupled plasma optical emission spectrometer (Varian® – Mulgrave, Australia), with axial viewing and a Charge Coupled Device (CCD) solid state detector that allows measurements (167–785 nm), was used to determine the analytes. The optical ICP OES system was calibrated with multielementary stock solution whereas, for the optical alignment, a solution containing 5.0 mg/L Mn was used. The spectral lines were selected according to the absence of spectral interferences and adequate sensitivity for the determination of elements at low and high concentrations, by studying emission lines of the elements to be investigated. The instrumental parameters used for multi-element determination are shown in Table 1.

Table 1
Experimental conditions used in ICP OES equipment with axial configuration.

Instrumental parameter	ICP optimum condition
RF generator power (kW)	1.3
Nebulizer gas rate (mL/min)	0.7
Auxiliary gas rate (mL/min)	1,5
Plasma gas rate (mL/min)	15
Signal integration time (s)	1,0
Stabilization time (min)	15
Reading time (min)	1
Replicates	3
Nebulizer	V-Groove
Nebulization Chamber	Sturman – master
Spectral Lines (nm)	Cd (II) 226.502; Fe (II) 238.203; Cu (I) 327.398; Mg (II) 280.267; Pb (II) 220.354; Se (II) 196.026; Zn (I) 213.858

(I) atomic emission line/(II) ionic emission line.

2.4. Digestion procedure

For the sample digestion procedure, aliquots of about 0.50 g of each tissue sample were transferred to polytetrafluoroethylene (PTFE) vials. Volumes of 3.5 mL of 65% HNO_3 (m/m) + 3.5 mL of H_2O (Milli QR type) + 1.0 mL of 30% H_2O_2 (v/v) were added. The flasks were then closed and placed on the cavity microwave carousel. The heating program used is described in Table 2. After completion of the heating program, the samples were opened, and the digests were transferred to polypropylene vials and boosted with deionized water to the volume of 20 mL. Serum sample processing and a 0.1 mL aliquot of plasma samples was diluted 100-fold with 0.01% Triton X-100® (v/v) to a volume 10 mL and stored in a refrigerator prior to analysis [30].

2.5. Validation studies

After defining the sample preparation procedure, the parameters of precision, limits of detection (LOD), limit of quantification (LOQ), matrix effect and accuracy were used to validate the proposed method. The purpose of the validation process is to ensure that the proposed analytical procedure produces reproducible and reliable results that are in accordance with the purposes for which they were prepared.

The accuracy of the measurements was assessed using bovine liver certified reference material (CRM 1577b) from NIST. In addition, the accuracy of the methods was evaluated by addition and recovery tests, conducted in biological tissue samples. The precision of the method was determined in terms of the percentage of the variation coefficient. LOD and LOQ were determined by following the 3σ and 10σ criteria, respectively, using the standard deviation (σ) of the 10-blank

Table 2
Heating program of the microwave oven with cavity.

Stage	Time (min)	Maximum power (W)	Temperature (°C)	Pressure (bar)
1	6	750	90	35
2	4	750	90	35
3	8	1000	180	35
4	15	1000	180	35
Ventilation	20	–	–	–