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ANALYSIS OF BRAZILIAN SNAKE VENOMS BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Instrumental neutron activation analysis (INAA) has been applied to multielemental determinations of Brazilian snake venoms from the species: Bothrops jararacussu, Crotalus durissus terrificus and Bothrops jararaca.

Concentrations of Br, Ca, Cl, Cs, K, Mg, Na, Rb, Sb, Se and Zn have been determined in lyophilized venoms by using short and long irradiations in the IEA-R1 nuclear reactor under a thermal neutron flux of 10^{11} to 10^{13} n $cm^{-2}s^{-1}$. The reference materials NIST Bovine Liver 1577 and IUPAC Bowen's Kale were also analysed simultaneously with the venoms to evaluate the accuracy and the reproducibility of the method.

The concentrations of the elements found in snake venoms from different species were compared. The Crotalus durissus terrificus venoms presented high concentration of Se but low concentrations of Zn when these results are compared with those obtained from genera Bothrops venoms.

INTRODUCTION

Accidents with snakes are still a serious public health problem mainly in rural areas. There are about 2500 species of snakes distributed throughout the world and approximately fifteen percent of them are dangerous to man.

In Brazil snakebites are still common and an average of 70000 accidents are estimated per year. At present, treatment of these snakebites are made by administration of antivenin serum.

The studies on immunization and treatment of venom toxicity from several species of snakes were started in our country by Vital Brazil[1] who founded the Instituto Butantan of São Paulo, which is one of the most important centers for study of venoms in the world.

Although the snake venoms have been extensively studied,there are few papers about systematic analysis of metal contents in snake venom as well as the study of the correlation between the presence of metal and biological activity.

In recent years, the most systematic study of metal composition is that of Friedrich and Tu[2] who examined the distributions of metals in venoms from 17 snake species using the method of atomic absorption.

Bjarnason and Fox[3] presented a review about hemorrhagic toxins from snake venoms and they pointed out that the biological role of each element in not clear. Anyhow, some of them are important in the stabilization of the structure of certain proteins and the metals are also involved in the mechanism of catalysis for some enzymatic reactions.

Moreover the determination of trace elements in venoms from different species of snakes is relevant to choose a specific trace element that could be used as radioactive tracer for studying the mechanism of the spreading of venoms through the body of the victim or of the experimental animal.

Neutron activation analysis is highly suitable to be applied to this kind of study, but very few papers can be found in the literature about the use of this method in the characterization of snake venoms[4].

The purpose of this work was to apply the method of instrumental neutron activation analysis (INAA) to determine trace elements in Brazilian snake venoms for further contribution to the knowledge of the role of metals in the pharmaco-

logical action of the venoms and in the biochemical characterization of snake species.

The method of INAA was applied to the determination of elements in venoms from three different species of Brazilian snakes: Bothrops jararacussu, Crotalus durissus terrificus and Bothrops jararaca.

EXPERIMENTAL

Samples of Snake Venoms

Venoms from three species of Brazilian snakes: Bothrops jararacussu, Crotalus durissus terrificus (Rattlesnake) and Bothrops jararaca were analysed. These samples were provided by the Instituto Butantan of São Paulo in a lyophilized form.

Standard and Sample Preparations for Irradiation

Synthetic standards were prepared in our laboratory using standard solutions of the elements to be determined.Stock solutions of elements were prepared by dissolving high purity metal, oxide or salt of elements with adequate pure reagents and then diluting using distilled water in quartz apparatus.Multielement standard solutions were prepared by combining appropriate amounts of the stock solutions. These solutions were pipetted in a small sheet of Whatman nº 42 filter paper and dried in a dessicator at room temperature. The amounts of elements in these standards were about ten times higher than those found in the sample. The sheets of filter paper were inserted in plastic bags made using a heat sealer and plastic sheet that was previously washed with dilute nitric acid solution.

Dried snake venoms (50-100 mg) were weighed and heat-sealed in clean plastic bags for irradiation together with synthetic standards. During weighing, special care was taken to avoid contamination of the samples before irradiation and

mainly not to breathe the venoms or to allow them to be in contact with the skin.

Irradiations

Irradiations were performed in the IEA-R1 nuclear reactor. The samples, standards and blanks (empty plastic bags) were placed into a second bag and the packed in special containers for irradiations. Short irradiations of 6 min under a thermal neutron flux of 3.7×10^{11} $ncm^{-2}s^{-1}$ were carried out for the determinations of Cl, K, Mg and Na and long irradiations of 8 h and neutron flux of $10^{13} ncm^{-2}s^{-1}$ for the determinations of Br, Ca, Cs, Na, Rb, Sb, Se and Zn.

Counting

After irradiation, the outer bag was removed and the standards and samples were counted using an ENERTEC hyperpure Ge detector coupled to an EG & G ORTEC 4096 channel pulse height analyzer connected to a Monydata PC 200 Plus microcomputer. The counting system had a resolution (FWHM) of 2.45 keV for the 1332 keV gamma ray of ^{60}Co and 1.15 keV for the 122 keV gamma ray of ^{57}Co. The samples and standards were counted twice after irradiation to optimize conditions to count radioisotopes with different half lives. The gamma ray spectra were processed by using the modified version of FALA[5] program, written in Pascal language. This program locates peak positions and calculates gamma ray energies and net areas. The area under the photopeaks corresponding to the gamma-rays of ^{82}Br at 776 keV, ^{47}Ca at 1296 keV, ^{38}Cl at 1642 keV, ^{134}Cs at 795 keV, ^{60}Co at 1173 keV, ^{42}K at 1525 keV, ^{27}Mg at 1014 keV, ^{24}Na at 1368 keV, ^{86}Rb at 1076 keV, ^{122}Sb at 564 keV, ^{124}Sb at 1691 keV, ^{75}Se at 264 keV and ^{65}Zn at 1115 keV was used.

Analysis of Plastic Bags and of Reference Materials

Since the samples and standards were measured in the plastic bags used in

their irradiations, the presence of impurities in these involucres was examined. The elements Br, Cr and Na were found but they could be considered negligible in comparison with the amounts of elements present in the sample.

Two certified reference materials: Bowen's Kale provided by International Union of Pure and Applied Chemistry (IUPAC) and Bovine Liver (1577) from National Institute of Standards and Technology (NIST) were analysed in this work for the evaluation of the accuracy of the method. These reference materials were dried as recommended in ref.(6,7). With the drying process there was a weight loss of 5.5% for Bovine Liver and of 11.6% for Bowen's Kale. These values were used to calculate the weights of materials analysed on dried basis. A mass of about 100mg was used in the reference materials.

RESULTS AND DISCUSSION

Table 1 shows the results of the INAA of the Brazilian snake venoms.As shown in Table 1, four samples of venoms from Bothrops jararacussu, two of Crotalus durissus terrificus and two of Bothrops jararaca were analysed. Each sample consisted of a pool of venoms obtained from a certain number of individual snakes in a determined period. The reported values are the averages and standard deviations of at least two determinations. Acceptable precisions, with relative standard deviations lower than 10%, were obtained for most of the elements analysed.

TABLE 1

The less precise results were obtained for antimony. The induced activity of ^{122}Sb or of ^{124}Sb was not high due to the small concentration of this element in the samples (< 0.5 ppm). In some samples this element was not detected. Also magnesium was not detected in venoms from genera Bothrops because the 1014 keV gamma ray photopeak of ^{27}Mg was not very pronounced, resulting in poor counting statistic. For Mg the quantitative determination limit was evaluated according to Currie[8].

The elements Br, Cl, Na, Rb, Se and Zn were found in all the venoms. Na

and K were found in the highest concentrations. Zn concentrations in Bothrops genera venoms were about fivefold higher than those found in venoms of Crotalus durissus terrificus. On the other hand the concentrations of Se in Bothrops venoms were slightly low. Also, the Rb concentrations in Bothrops jararacussu venoms were higher than those found in venoms from other species analysed in this paper.

This work will be now extended for venom analysis from snakes originating from different regions of Brazil with different ages (newborn and elder snakes) as well as venoms from snakes from captivity and natural habitat. These determinations are important in the extraction of venoms for antivenim serum production since the biological effect of snake poisoning depends on the age, size, habitat and eating habits of snakes.

The accuracy of the method was checked by analysing Bowen's Kale and Bovine Liver reference materials. Table 2 shows the results obtained in these analyses together with literature values. We have determined 12 elements and most of them are in reasonably good agreement with their certified values.

TABLE 2

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REFERENCES

1. E.E.BUCKLEY, N. PORGES, Venoms. Publication nº 44. American Association for the Advancement of Science. Washington DC, 1956.

2. C. FRIEDERICH, A.T. TU, Biochem. Pharm., 20 (1971) 1549.

3. J.B. BJARNASON, J.W. FOX, J. Toxicol Toxin Reviews, 7(2) (1988-89) 121.

4. B. MOAV, S. GITTER, Y. WELLWART, S. AMIEL, Radiochemical Methods of Analysis, Proceedings of a Symposium, Salzburg, Austria, 1 (1964) 205.

5. F.W. LIMA, L.T. ATALLA, J. Radioanal. Chem., 20 (1974) 769.

6. H.J.M. BOWEN, Atomic Energy Review, IEA 13 (3) (1975) 451.

7. NATIONAL BUREAU OF STANDARDS, Certificate of Analysis Standard Reference Material 1577 a, Bovine Liver, 1982.

8. L.A. CURRIE, Anal. Chem., 40 (3) (1968) 586.

9. E.C. TORO, R.M. PARR, S.A. CLEMENTS. Biological and Environmental Reference Materials for Trace Elements, Nuclides and Organic Microcontaminantes. A Survey. Report IAEA/RL/128 (Rev.1), Vienna, Austria, 1990.

TABLE 1 - Results of Brazilian Snake Venom Analysis by Instrumental Neutron Activation Method

Element \ Sample	Bothrops jararacussu Nº 1	Bothrops jararacussu Nº 2	Bothrops jararacussu Nº 3	Bothrops jararacussu Nº 4	Crotalus durissus terrificus Nº 1	Crotalus durissus terrificus Nº 2	Bothrops jararaca Nº 1	Bothrops jararaca Nº 2
Br (ppm)	4.24 ± 0.15	12.29 ± 0.59	7.51 ± 0.36	19.45 ± 1.80	6.33 ± 0.09	2.15 ± 0.09	3.08 ± 0.29	13.65 ± 0.19
Ca (ppm)	625 ± 45	655 ± 70		1661 ± 14		569 ± 201	1054 ± 166	1523 ± 266
Cl (ppm)	1184 ± 79	2966 ± 117	1968 ± 100	3906 ± 153	2064 ± 275	1305 ± 80	1339 ± 113	2092 ± 126
Cs (ppb)	101 ± 8	297 ± 9		319 ± 11	16 ± 4	23 ± 6		
K (%)	1.05 ± 0.08	1.14 ± 0.08	1.25 ± 0.16	1.00 ± 0.07	⩽ 0.3	⩽ 0.3	2.62 ± 0.33	⩽ 0.5
Mg (ppm)	⩽ 970	⩽ 970	⩽ 970	⩽ 970	3689 ± 136	2943 ± 287	⩽ 970	⩽ 970
Na (%)	0.832±0.022	0.763 ± 0.028	0.925±0.094	0.947 ± 0.020	2.20 ± 0.27	2.53 ± 0.31	1.89 ± 0.11	2.31 ± 0.40
Rb (ppm)	56.2 ± 1.4	82.1 ± 8.2	71.0 ± 9.1	69.6 ± 2.9	6.8 ± 0.2	10.1 ± 0.8	12.1 ± 0.4	26.3 ± 0.4
Sb (ppb)	472 ± 167	91 ± 12		179 ± 15	66 ± 11		2296 ± 198	805 ± 62
Se (ppb)	3134 ± 178	3554 ± 34	1660 ± 430	2055 ± 447	7335 ± 453	6270 ± 771	3566 ± 48	2167 ± 462
Zn (ppm)	557 ± 40	678 ± 74	537 ± 56	654 ± 38	142 ± 19	96 ± 4	798 ± 10	803 ± 10

TABLE 2 - Concentrations of Elements in Bowen's Kale (IUPAC) and in Bovine Liver 1577 a (NIST) Reference Materials Obtained by INAA

Element	Bowen's Kale		Bovine Liver 1577 a	
	This work	Ref (9)	This work	Ref (9)
Br (ppm)	26.32 ± 0.23	24.9 ± 2.4	8.10 ± 0.10	9
Ca (ppm)	37959 ± 1840	41060 ± 2217		120 ± 7
Cl (ppm)	4140 ± 424	3560 ± 427	2979 ± 230	2800 ± 98
Cs (ppb)	83.7 ± 5.9	76.3 ± 5.9		
Co (ppb)	77.3 ± 5.5	63.2 ± 10.7	154 ± 19	210 ± 50
K (%)	2.636 ± 0.275	2.4370 ± 0.1462	0.974 ± 0.02	0.9960 ± 0.0069
Mg (ppm)	1459 ± 308	1605 ± 176		600 ± 15
Na (ppm)	2330 ± 167	2366 ± 279	2400 ± 200	2430 ± 129
Rb (ppm)	56.7 ± 2.9	53.4 ± 5.3	12.7 ± 0.6	12.5 ± 0.1
Sb (ppb)	53 ± 13	68.5 ± 14.4	1.6 ± 0.5	3
Se (ppb)	142 ± 6	134 ± 20	749 ± 49	710 ± 70
Zn (ppm)	34.9 ± 4.2	32.29 ± 2.74	123.7 ± 1.9	123 ± 8

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Assinatura	Nº	Data	Visto