Letter to the Editor

Effect of Antiretroviral Therapy Medications for Acquired Immune Deficiency Syndrome on Serum Elemental Concentrations

ZHANG Peng[1,&,#], CHEN Cheng[1], and LIU Li Ying[2]

Trace elements in the human body participate in the synthesis of hormones and vitamins and interact with various biomolecules, such as nucleic acids and protein macromolecules. Such elements are key essential elements of larger molecular compounds, such as metalloproteins. Thus, disorders of metal ion metabolism in the body could lead to the disruption of normal physiological functions and cause a variety of diseases. To provide resonable suggestions for the development and clinical application of acquired immune deficiency syndrome (AIDS) medicines, it's essential to determinate Serum Elemental Concentrations of AIDS patients.

Most medicines contain a certain amount of elements as an active ingredient. Synthetic anticancer medicines, for example, contain Pt complexes and As compounds; antidepressants contain Li; antimicrobial agents for severe burns contain Ag; and anti-syphilis and *Helicobacter pylori* medicines contain Bi. However, long-term use of medicines could lead to the accumulation of metal ions in the body and cause adverse reactions[1-2].

AIDS patients must take medication daily to achieve the expected antiviral effects. China, like many low- and middle-income countries, has implemented the unified distribution of government funds for anti-AIDS medicines. AIDS patients take medication for years to decades. Medication taken nationwide are roughly the same. This study selected anti-AIDS medicines to initially assess the effects of long-term drug use on human serum metal accumulation.

Current research focuses on differences in trace elements and CD4 cells before and after HIV infection. Reports on differences in serum trace elements after taking some medicines, such as artemisinin, have been published[3-4]. Unfortunately, the content of elements in medicines and the serum of patients taking such medicines are not the main indicators of pharmaceutical safety evaluations; thus, few studies on the subject are available.

In this experiment, inductively coupled plasma–mass spectrometry (ICP-MS) was used to determine the contents of metal elements in the serum of AIDS patients and analyze differences between groups. The aims of this work are to evaluate the potential risk of long-term drug use and provide reasonable suggestions for the development and clinical application of AIDS medicines[5].

An ICP-MS spectrometer (Agilent, 7700x, USA) and ultrapure water system (Millipore, Milli-Q Integral, USA) were used in this work. Standard stock solutions of Al, As, Ba, Cd, Cr, Cu, Hg, Li, Mn, Pb, Sb, Se, Tl, and V (100 mg/L) were purchased from China National Nonferrous Metals and Electronic Materials Assay and Testing Center. Mg (1,000 mg/L), Fe (1,000 mg/L), and Zn (1,000 mg/L) were also purchased from China National Nonferrous Metals and Electronic Materials Assay and Testing Center. Tuning solutions of Ce, Co, Li, Y, and Tl (1 μg/L) were purchased from Agilent. Internal standard solutions of Bi, Ge, In, Lu, Rh, Sc, and Tb (100 mg/L) were purchased from Agilent and diluted to 1.00 mg/L. HNO_3 (GR) was obtained from Thermo Fisher.

Three medicines (A, B, and C) used to treat HIV patients in a district in Chongqing were selected for this work. The types and dosages of the medicines are shown in Table 1.

Medicines A, B, and C were crushed to powders. Approximately 0.5 g of each powder was placed in a microwave digestion tank and added with 5.00 mL of HNO_3, 2.00 mL of H_2O_2, and 2.00 mL of pure water. The samples were mixed well, soaked for 30 min, placed in a microwave digestion apparatus, and then digested at 180 °C for 20 min. Thereafter, the volume of the solutions was adjusted to 100.0 mL using ultrapure water, and the samples were mixed well for analysis.

doi: 10.3967/bes2020.073

1. Jiulongpo District Center for Disease Control and Prevention, Chongqing 400032, China; 2. Nan'an District Center for Disease Control and Prevention, Chongqing 400030, China

Inclusion criteria: AIDS patients living in Chongqing, taking medicine for more than 3 months, and between the ages of 18 and 65 years.

Exclusion criteria: AIDS patients suffering from other major diseases, with a long history of taking medicines other than those detailed in Table 1, and reporting changes in habitation, dietary habits, or smoking status during medication.

This research did not assign a placebo group because the patients needed to take their medication regularly and implementing a placebo could delay their treatment.

Self-comparison was used in this research to eliminate the interference of eating habits, age, gender, environment, smoking habits, and HIV virulence, among others, and improve the credibility of the results. This study was approved by the ethics committee of Jiulongpo District Center for Disease Control and Prevention.

The pre-medication group comprised blood samples obtained before medication, and the post-medication group comprised blood samples obtained after medication.

Approximately 0.5 mL of venous blood serum was obtained before and after medication. The serum samples were diluted to 5 mL with 2% HNO_3 solution for testing. Serum was separated from samples obtained before medication and stored at −80 °C. Elements in serum do not degrade over time. This fact provides the appropriate conditions for self-comparison.

Because the components of the samples differ, the pretreatment methods for serum and the medicines also differ. The main component of tablet-form medications is starch, which must be digested prior to elemental analysis of the medicine. By contrast, the main component of serum is water. Therefore, addition of a low concentration of HNO_3 to the serum samples is adequate for analysis[6].

Selection of Analytical Methods and Quality Control

Exactly 0.5 mL of each sample solution was obtained, and the metal ion contents of this solution were detected by ICP-MS under the following conditions: atomizing chamber: dual path Gothic type; central channel: quartz; power: 1,550 W; carrier airflow speed: 0.80 L/min; number of repeat samples: 3; online internal standard: Sc, In, Bi; nebulizer type: concentric; torch tube material: quartz; sampling cone material: Ni; sampling depth: 8.0 mm; quantitative analysis mode: He mode; and points time: 0.09 s.

In this research, recovery rate and precision were selected as quality control measures. Mg (500 mg/kg), Fe (20 mg/kg), and Zn (3 mg/kg) were added to the medicines to be tested on the basis of the results of the pre-experiment. The recoveries obtained were in the range of 85.11%–101.32%, and RSDs were less than 4.97% (n = 6). Mg (7 mg/L), Fe (1 mg/L), and Zn (0.4 mg/kg) were also added to the serum samples. The recoveries obtained were in the range of 97.26%–103.02%, and RSDs were less than 3.27% (n = 6).

Calculation of Sample Number and Selection of Statistical Methods The sample size was calculated according to the paired-samples Wilcoxon test.

$$n = (u_\alpha + u_\beta)^2 / \left[3(p' - 0.5)^2\right] \quad (1)$$

where $p' = Sin^2(0.067897 + 1.433858p)$ and $p = P$. According to the data of previous experiments (α = 0.05, β = 0.10), 30 samples were calculated.

Median and interquartile spacings was calculated for all variables. The paired-samples Wilcoxon test was used to compared the differences of two groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Table 2 shows that the Fe and Zn content of medicines A–C were between 15.6 and 65.8 mg/kg and between 1.7 and 3.2 mg/kg, respectively; moreover, Se and As contents were less than 0.1 mg/kg. Thus, the contents of elements in these medicines is much lower than the recommended intake specified in *The Survey of Dietary Nutrition of Residents*.

Compared with Pre-medication group, the Fe and Zn was significantly higher in post-medication group, while the Se and As was lower (Table 3).

Table 1. Types and dosages of medicines A–C

Code name	Product name	Manufacturer	Dosage (tablet/d)	Lot number
A	Efavirenz	Desano	1	1251C19125
B	Lamivudine	Desano	1	AZ19087
C	Tenofovir disoproxil fumarate	Brilliant	1	9E1172DF6

Although Fe and Zn are elements found at high levels in the human body, prolonged accumulation of these metals increases the risk of developing other diseases. Long-term increases in serum Fe levels could increase risks of developing diabetes, joint disease, cardiac complications, gastrointestinal bleeding, and bacterial peritonitis. Long-term increases in blood Zn can lead to electrolyte imbalance and kidney damage. Finally, long-term increases in blood Pb can lead to anemia, dizziness, digestive ulcers, and other symptoms.

The serum content of Se after medication was lower than that observed before medication. Low Se causes poor immunity and increased risk of developing nervous system-related diseases. Moreover, the risk of infection with viral diseases increases significantly, and the pathogenicity of viruses, including HIV, may be enhanced. No report on the effect of As reduction on human body is available in the literature[7].

Most organisms show significant differences in the ways they absorb, transport, and metabolize different forms of metals, and the level of difficulty of absorption is related to the level of toxicity[8]. Metal forms in medicine and food differ; they are mainly ionic in medicine and easily absorbed into the bloodstream[9]. Humans mainly consume food in the forms of vegetables, meat, and cereals. Inorganic acids in vegetables form complexes and could reduce the absorption and utilization of metals. Many organic ligands in meat and grains can be combined with heavy metals. These heavy metals are also used in the formation of complexes and amino acids. Metals readily form complexes with ligands, and these ligands exist in a state of dynamic equilibrium. When a ligand with greater affinity to a metal ion is available, the metal may form a more stable complex. The absorption rate and efficiency of complexed metals in the body is lower than those of ionic-state metals. Therefore, a small amount of medicine intake may cause a large change in the metal content in the blood[10].

While they are major elements, Fe and Zn show low utilization in muscle and bone; thus, they can easily enter the blood. Se and As present several valence forms and can easily form stable complexes. Thus, the content of these elements in blood may easily be reduced.

Table 2. Elemental concentrations of medicines A–C (mg/kg)

Element	Medicine A	Medicine B	Medicine C
Mg	509.7	655.7	1020.1
Al	34.2	16.2	23.5
Cr	0.2	0.3	0.3
Mn	0.4	0.7	0.6
Fe	65.8	17.9	15.6
Cu	0.8	0.5	0.4
Zn	3.2	2.4	1.7
As	< 0.1	< 0.1	< 0.1
Se	< 0.1	< 0.1	< 0.1
Cd	< 0.1	< 0.1	< 0.1
Hg	< 0.1	< 0.1	< 0.1
Pb	0.5	0.2	0.2

Table 3. Serum test results (μg/L)

Element	Pre-medication group M (P25, P75)	Post-medication group M (P25, P75)	*S*	*P*
Mg	9919.46 (6803.3, 12274.48)	9568.97 (8188.01, 11581.33)	−27.5	0.5804
AL	414.10 (339.25, 634.83)	425.47 (365.66, 495.06)	−33.5	0.5001
Cr	15.90 (12.57, 129.75)	14.88 (12.48, 18.53)	−78.5	0.1074
Mn	15.13 (11.92, 163.21)	17.64 (11.50, 21.05)	−54.5	0.2694
Fe	1282.01 (1084.56, 1541.3)	1814.59 (1433.79, 3075.42)	184.5	< 0.0001
Cu	393.68 (300.65, 485.57)	389.08 (283.33, 585.32)	−17.5	0.7254
Zn	409.22 (303.17, 545.68)	658.10 (489.84, 998.37)	215.5	< 0.0001
As	1.69 (1.11, 3.42)	1.18 (0.88, 1.62)	−141.5	0.0021
Se	12.54 (9.94, 16.65)	9.35 (8.20, 10.56)	−139.5	0.0025
Cd	0.59 (0.32, 0.97)	0.67 (0.51, 1.03)	15.5	0.7558
Hg	17.41 (0.49, 58.46)	1.06 (0.87, 3.92)	−48.5	0.3268
Pb	28.11 (17.13, 45.20)	28.29 (18.5, 33.86)	−10.5	0.8332

We wish to thank the timely help given by ZOU Lei in analyzing the large number of samples used in this work.

#Correspondence should be addressed to ZHANG Peng, Master, Tel: 86-23-68190359, E-mail: zhpeng80@163.com

Biographical note of the first author: ZHANG Peng, male, born in 1980, MD, majoring in public health testing technology.

Received: November 1, 2019;
Accepted: January 8, 2020

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