Research on the Metabolism of Nitrogenous Free Radicals in Ducklings with Selenium poisoning

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Abstract: The ducklings (n=100) were divided into control group and experimental group randomly, and selenium poisoning of ducklings was artificially caused by feeding ration containing 8 mg kg^{-1} Se every day. The dynamic changes of No content and NOS activity in the serum and tissues were determined by means of the method of nitric acid reductase. The results showed that the NO content and NOS activity in serum and tissues in experimental group increased significantly ($P<0.05$) and they were time-dependent. It was suggested that the high level of selenium in bodies could increase the NOS activity and NO content as it destroyed the metabolism of material and energy as well as structure and function of tissues and cells. These changes of NO content and NOS activity might be involved in the metabolism of selenium poisoning. [The Journal of American Science. 2005;1(2):77-81].

Key words: Selenium poisoning; Duckling; Serum; Tissue; Nitric oxide; Nitric oxide synthase

Introduction

Selenium, one kind of chemical element with atomic number 34 and atomic weight 78.96, was first found by Sweden chemist Berzelius in 1817, and existed widely in the nature with four kinds of oxidation states such as –2,0,4 and 6. It, one of essential trace elements animal required, owned chemical property between nonmetal and metal nature and existed in the cell of animals and plants[1]. Owing to its different levels and forms in soil and plants, it often caused selenium deficiency and poisoning in farm animals. In the selenium-deficient areas, selenium poisoning often occurred because of supplement of excessive selenium in the rations to prevent or treat selenium deficiency. At present, there were many research reports on selenium poisoning of farm animals at home and abroad, mainly on two aspects, selenium distribution and change of path-morphology in tissues[2,3], but there was no report on the effect of selenium poisoning on the level of nitrogen oxide (NO) and Nitric Oxide Synthase (NOS) activity. Healthy duckling were selected to study the effect of selenium poisoning on the level of NO and NOS activity in the experiment, and our aim was to elucidate the molecular mechanism of selenium poisoning and to set up the basis for preventing and treating clinical selenium poisoning.

1 Materials and Methods

1.1 Materials

1.1.1 Animals and rations

One-day-old healthy Jinding ducklings (female n=50 ,male n=50) were purchased from Zhengli duck farm in Harbin. The rations containing 0.15±0.023 mg kg^{-1} selenium were bought from Heilongjiang Dasheng Feed Corporation.

1.1.2 Drugs and instruments

Sodium selenate（$Na_2SeO_3 \cdot 5H_2O$）, produced by Second Reagent Factory in Shenyang; Nitrogen Oxide (NO) reagent box, Nitric Oxide Synthase (NOS) reagent box and Komasilianglan protein reagent box, produced by Jiancheng Bioengineering Research Institute in Nanjing; 7230G visible light spectrometer, made in Shanghai Analyses Instrument Factory; DF200A complete automatic analysis scales, made in Changshu Weighing Apparatus Factory in Jiangsu; Avanti™30 low-temperature hypervelocity centrifuge, made in Becman Corporation, Germany.

1.2 Methods

1.2.1 Grouping and feeding of ducklings

One-day-old healthy ducklings (n=100) were ad libitum fed with ration and water, and randomly grouped into two (n=50, respectively) after two weeks. The number of female and male ducklings in each group was equal. The ducklings in control group (Group I)

were fed with full ration, but the ducklings in poisoning group (Group II) were fed with full ration containing 8 mg kg^{-1} selenium. The ducklings in two groups were slaughtered and collected at day 15,30,60 and 90 (n=10, respectively).

1.2.2 Collection and determination of samples [4]

1 ml heart blood was collected and disposed for a while in room temperature, and serum was finally separated. Meanwhile, the ducklings were slaughtered and their heart, liver, kidney, spleen, cerebrum, testis and muscle were collected, weighed and placed in cold saline (1:9 W/V), and quickly grinded into 10% homogenate in ice-water by glass homogenizer, Homogenate was centrifuged at 3000-4000 rpm, then NO content and NOS activity in serum and tissues and protein content in upper liquid of homogenate were measured, respectively. NO content (μ mol l^{-1} in serum, μ mol g^{-1} Pr in tissue)was measured with nitric acid reductase; NOS activity (U mg^{-1} ·Pr) by spectrometer and protein content in homogenate by Komasilianglan method.

1.2.3 Analysis of data

Data in the experiment were analyzed by SAS software.

2 Results

2.1 Model replication of selenium poisoning

The ducklings in Group Ⅱ had no significant sign in the early period of experiment, and there was no significant weight difference between two groups at day 15 (P>0.05), but the weight of ducklings in Group Ⅰ was significantly higher than that in Group Ⅱ at day 30 (P<0.05), and after day 60 (P<0.01). The ducklings in Group II had clinical signs: listlessness, disorderly and deciduous hair, crying and braying, white and black manure, in later period paralysis, death owing to loss of deglutition. There were no marked typical changes with eyes in the early period, but the chronic inflammation occurred in the tissues, especially in liver and kidney. In addition, there appeared hyperemia, hemorrhage and edema in cerebrum, cerebral membrane and alimentary tracts, and even extensive ulceration or necrosis in severe patients. The results showed that the model replication of selenium poisoning in the experiment was successful because there existed 2.5 mg L^{-1}selenium in blood and 12.34 mg kg^{-1}selenium in feather.

2.2 NO content and NOS activity in the serum and tissues of ducklings with selenium poisoning

The results in Table 1-2 showed that NO content in serum of ducklings in Group Ⅱ was higher or significantly higher than that in control group at day 15 (P<0.01), at day 30, 60 and 90 (P<0.05); NO content in heart and testis of ducklings in Group Ⅱ was significantly higher than that in control group at day 15,30,60 and 90 (P<0.01); NO content in liver of ducklings in Group Ⅱ was significantly higher than that in control group at day 15 (P<0.05), 30,60 AND 90 (P<0.01); NO content in kidney of ducklings in Group Ⅱ was significantly higher than that in control group at day 15 and 30 (P<0.05),60 and 90 (P<0.01); NO content in spleen and cerebrum of ducklings in Group Ⅱ was significantly higher than that in control group at day 15 and 30, 60 and 90 (P<0.05); NO content in muscle in Group Ⅱ was significantly higher than that in control group at day 15, 60 and 90 (P<0.05).

The results in Table 3-4 showed that there was no significant NOS activity difference in serum of ducklings between Group Ⅱ and control group at day 15,30,60 and 90 (P>0.05); NOS activity in heart of ducklings in Group Ⅱ was significantly higher than that in control group at day 30,60 and 90 (P<0.05); NOS activity in liver spleen, cerebrum, testis and muscle of ducklings in Group Ⅱ was significantly higher than that in control group at day 15,30,60 and 90 (P<0.01); NOS activity in kidney of ducklings in Group Ⅱ was higher than that in control group at day 15 and 30 (P<0.05), at day 60 and 90 (P<0.01).

Table 1. The contents of NO in serum and tissue-homogenate in each group of 15,30-day old[1)]

Samples	n	Control group		Poisoning group	
		15d	30d	15d	30d
Serum	10	40.78±5.621	36.66±4.502	89.51±10.018**	56.84±6.400*
Heart	10	30.52±4.020	32.65±4.301	49.55±5.201**	58.00±6.834**

Liver	10	42.30±6.021	46.02±6.210	55.06±5.238*	79.25±8.366**
Kidney	10	16.23±2.305	18.56±2.539	20.65±3.201*	32.56±4.158*
Spleen	10	35.55±4.201	32.14±4.820	46.10±4.302*	44.96±5.362*
Cerebrum	10	60.25±7.666	56.12±6.585	74.32±8.654*	79.56±9.015*
Testis	10	22.82±3.256	25.16±4.252	49.26±5.685**	59.20±7.018**
Muscle	10	62.31±7.567	71.02±8.659	81.66±9.987*	76.26±8.564

1) * mean $P<0.05$ on the same day，** mean $P<0.01$ on the same day，The same as below.

Table 2. The contents of NO in serum and tissue-homogenate in each group of 60,90-day old

Samples	n	Control group		Poisoning group	
		60d	90d	60d	90d
Serum	10	35.68±4.216	37.91±4.010	45.29±5.000*	49.45±5.641*
Heart	10	30.87±4.235	36.21±5.012	50.45±6.982**	61.25±8.010**
Liver	10	43.55±5.890	40.26±5.642	84.23±9.260**	97.56±10.203**
Kidney	10	15.32±2.301	17.50±2.540	43.21±5.298**	50.21±6.325**
Spleen	10	36.48±5.031	38.21±4.005	50.36±6.301*	68.25±8.300*
Cerebrum	10	59.23±6.201	58.02±6.250	83.25±9.563*	94.58±10.256*
Testis	10	21.56±3.560	25.36±3.598	77.26±9.265**	95.00±10.230**
Muscle	10	68.12±7.530	68.25±7.465	90.21±10.255*	97.26±11.247*

Table 3. The activity of NOS in serum and tissue-homogenate in each group of 15, 30-day old

Samples	n	Control group		Poisoning group	
		15d	30d	15d	30d
Serum	10	0.148±0.021	0.123±0.020	0.168±0.025	0.153±0.024
Heart	10	4.254±0.478	4.613±0.510	5.642±0.612	6.720±0.710*
Liver	10	3.210±0.410	4.265±0.251	5.689±0.621**	10.256±1.023**
Kidney	10	3.031±0.321	2.998±0.301	4.325±0.421*	4.326±0.510*
Spleen	10	0.235±0.012	0.254±0.035	1.320±0.210**	1.541±0.164**

Cerebrum	10	1.432±0.140	0.895±0.094	2.654±0.021**	2.651±0.026**
Testis	10	0.651±0.054	0.215±0.031	1.958±0.203**	0.684±0.053**
Muscle	10	1.458±0.165	1.911±0.216	2.351±0.210**	4.650±0.521**

Table 4. The activity of NOS in serum and tissue-homogenate in each group of 60, 90-day old

Samples	n	Control group		Poisoning group	
		60d	90d	60d	90d
Serum	10	0.153±0.025	0.195±0.021	0.185±0.021	0.234±0.029
Heart	10	4.025±0.510	5.021±0.684	6.352±0.710*	7.984±0.752*
Liver	10	3.684±0.358	5.021±0.510	11.356±0.900**	14.250±2.013**
Kidney	10	4.612±0.320	3.265±0.402	7.024±0.510**	7.952±0.640**
Spleen	10	0.541±0.042	0.421±0.051	1.654±0.098**	1.589±0.087**
Cerebrum	10	1.265±0.021	1.695±0.026	3.259±0.362**	3.295±0.042**
Testis	10	0.589±0.062	0.774±0.081	2.056±0.214**	2.950±0.032**
Muscle	10	1.537±0.210	1.234±0.201	7.578±0.654**	8.258±0.400**

3 Analysis and Discussion

NO is one kind of small molecule gas and typical free radical with low solubility in water and high solubility in lipid, which can diffuse easily through the cell membrane .Its chemical reaction is very strong as it possesses extra electron. Its biological half-life span is 3 to 5s. NO is one of products from which L-Arg is decompound into L-Cit under the catalysis of NOS with the help of NADPH, haemochrome and calmodulin [5,6]. NOS is the rate-limiting enzyme of the reaction, and it can be divided into three types, neuron NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS), according to its source and effect property[6]. NO in body has double-side effect. It has important physiological effects when NO metabolism is normal and its concentration is low ($pmoL^{-1}$ or $fmolP^{-1}$), but it has important pathological effects when NO metabolism is disordered and its concentration is high ($nmolL^{-1}$). It can damage cells and destroy information transmission[7,8].

The results in the experiment showed that NOS activity in serum rose slightly, but NOS activity in tissues rose markedly in poisoning group as compared with ones in control group, which suggested that the high level of selenium was able to induce enhancement in NOS activity in serum, heart, liver, kidney, spleen, cerebrum, testis and muscle, among which the change in liver was the most notable , next in cerebrum, spleen, testis, muscle, kidney and heart, in spite of insignificant difference in NOS activity in serum at day 15,30,60 and 90,and in heart at day 15,and it was also higher as compared with one in control group. The results showed that excess of selenium could cause enhancement in NOS activity in serum and tissues, and then increase in NO content. Because NOS was the major rate-limiting factor, the change in NO content could be consistent with change in NOS activity.

It was analyzed that the changes in NOS activity and NO content resulted probably from two factors. Fist, selenium poisoning resulted in damage to DNA by active oxygen induced by complex reaction of selenium compound[9]. As mRNA was one important adjustment location in NOS activity, it was able to cause the change in NOS activity and damage to DNA, which led to gene mutation and cancer. It was reported that single chain in DNA in human TK6 mature lymphocyte broke after it contacted NO ($5.5\text{-}22mmolL^{-1}$), and the condition became more severe with increase of dosage and

lengthening of time[10]. Second, the selenium poisoning led to disorder of normal biochemical function of cell, and among which the calcium metabolic disorder was most important because Ca^{2+} was essential to keep NOS activity. When animal was in the condition of low calcium, iNOS tightly combined with calmodulin, causing the opening of calcium channel and transmission of biological electrical current from flavoprotein to protoheme which resulted in the production of NOS, and then production of a large number of NO. The excess of NO content in tissues had pathological effect on all organs such as digestive system, cardiovascular system, nervous system and urogenital system etc, resulting in damage to cell structure and function.

The unstable measurement of NOS activity in control group resulted probably from the fault in the experiment. The change of NOS activity in liver was most apparent, therefore, the pathological damage in liver was the severest because the liver was one of major target organs damaged by the selenium poisoning. Moreover, the change of NOS activity and NO content in cerebrum suggested that selenium poisoning influenced the function of central nervous system. It was reported that NO in central nervous system promoted the release of some materials, including the acetylcholine and ɣ-amino butyrate from dopamine etc, in the superoxgnitrite form, and these transmitters had important influence on the function the central nervous system[11]. Therefore, it was inferred that the change of NOS activity and NO content in the cerebrum resulted in functional abnormality of acetylcholine nerve owing probably to its effect on the release of acetylcholine nerve transmitters, and finally led to the clinical typical nerve symptom.

As mentioned above, toxicological mechanism of selenium announced by way of research of NOS activity and NO content was complete new research interest. It had an important part in the prevention and treatment of the disease, and in the new development of medicine. The molecular biology research of NOS, from now on , was involved in localization, extraction purification, classification, analysis of gene alignment, determination of NOS mRNA, and cloning of NOS gene, and transmission of gene in cell by way of the carrier, whose aim was to cure some disease etc.

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