Bull. Fac. Life Env. Sci. Shimane Univ., 17：3－7, September 30, 2012

Bioavailability of rumen protozoan selenium fed to mice using tissue uptake technique

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Abstract In the present study, the utilization of protozoan selenium (Se) synthesized in the rumen of sheep was investigated in mice using the rate of Se incorporation into the blood, organs and tissues as an index. Protozoan Se was prepared from rumen content of sheep supplemented Na selenite (0.4 mg/kg DM of diet). Twenty female mice (over 8-week-age) were divided two groups of 10 each, and ten of them fed the diet containing rumen protozoa matter (rpm: 100μg Se/kg DM) during consecutive 7 days, and another 10 mice were dealt with as control group. Then, all the animals were slaughtered to collect whole blood, kidney, liver, pancreas, spleen, lung, heart, brain and muscles for analyzing Se. The Se contents in protozoa and bacteria in the rumen of sheep supplemented Na selenite (0.4 mg/ Kg DM) was 0.45mg /Kg DM and 0.20 mg/ Kg DM, respectively. In the concentration of Se in blood, organs and tissues, the values tended to higher in mice fed rpm diet than in control group. The Se contents in blood, organs and muscles also tended to high in the group fed rpm diet compared with that in control group. From these findings, it can be obviously concluded that the protozoan Se incorporated in the rumen is efficiently utilized in animals.

Introduction

Selenium (Se) is one of the essential mineral (trace element) for animals, although it is only 0.00002% in the soil (Sakurai, 1996). Before indicating the physiological importance of Se, it has been regarded as rather poisonous substance than nutrient (Naya, 1995). After understanding Se as essential element, its metabolism in animal body is gradually made clear (Raymond, 1989). For animals, it is obvious that the extent of a proper quantity in Se intake is very narrow, and then they will show toxic symptoms after an excess intake. On the other hand, if the intake of it is below their requirement, they will show easily a deficiency disease, such as white muscle symptom, heart attack, difficulty in walking and some disorders in reproduction (NRC, 1983).

There are two types of selenium, i. e., inorganic type (metallic Se, selenate and selenite) and organic type (seleno-cystein and seleno-methionine). In general, it has been reported that organic type Se absorbs more than inorganic type Se in animals (Nicholson et al., 1991). Although the dietary Se is mainly organic type, the type Se used in supplement is mostly inorganic type. An inorganic Se supplemented utilizes as organic Se after combined with amino acid in animal body. It has been also reported that the difference of chemical type of inorganic Se; selenate or selenite, did not significantly effect on Se utilization, when they were supplemented to meet the requirement of sheep (Serra et al., 1994a). Furthermore, it has been also clarified that the rumen microbes incorporate efficiently when inorganic Se was fed to ruminants (Hudman and Glenn, 1984). On the other hand, there will be possibility that the rumen microbes change an inorganic Se to insoluble types, i. e., it was demonstrated that more than 50 % of total Se in the rumen will be insoluble (Serra et al., 1994b).

In general, Se absorption in ruminants is obviously lesser than that in mono-gastric animals, and this is thought to be effect that the ingested Se is incorporated by microbes in the rumen. However, there is little information about utilization of microbial Se in ruminants. According to Serra

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et al. (Serra et al., 1997), the bacterial Se isolated from rumen fluid was fully utilized in mice used as model animal when it supplemented to purified diet, although deposited amount of Se in the body was a little lesser than that of Na selenite. There was little information about utilization of Se incorporated into protozoa after engulfing bacteria in animals. Then, in the present study, the utilization efficiency of protozoan Se in mice used as a model animal was investigated using the blood concentration and incorporation to body tissues of Se as an index.

Materials and Methods

Preparation of protozoan Se

The diet consisted mixed hay and concentrate (8∶2/DM base) with supplemented Na selenite (0.4mg/kg DM) was fed to 2 sheep (male, BW∶45.8 kg and female, BW∶52.6 kg) fistulated with rumen cannula at a level of 1.3 kg/d per head. At 3 hours after morning feed, the rumen content was collected through the rumen fistula of each sheep, and strained through the 5 layers-gauze. The filtrate diluted 1.25 times with physiological salt solution, and transferred into a separately funnel. The funnel was left in a water bath controlled at 39℃ for 2 hours, and the 3 layers; feed debris, bacteria and protozoa were separated. The protozoa layer was dried in air-forced drier at 45℃ for 24 hours (Nakashima, 1998). The dried rumen content (rumen protozoan matter: rpm) was ground in a mortar, and stored at 4 ℃ until preparation of diet for mice.

Animals and diet

Twenty 8-week-age mice (average BW 19.5±1.0 g) were divided into 2 groups of the treated group (10 heads) and control group (10 heads). They were kept in a plastic cages in the controlled room at 22℃. In the treated group, animals were fed the diet supplemented with 100 μg rpm per Kg DM (see Table 1). The constituent of the diet was made based on NRC standard for mice (NRC, 1978).

Experimental

Sample collection: After 7 days feeding all the animals was slaughtered using ethyl ether for anesthesia. The blood was collected into a heparinized test tube, and centrifuged to separate plasma for 20 minutes at 1,630g (Smith and Mangkoewidjojo, 1987). The internal organs and the tissues of liver, kidney, pancreas, spleen, brain, heart, lung and muscle were collected, and the sample of them for Se analysis weighed after washing with cooled physiological salt solution.

Analysis of Se: An analysis for Se content in the diet and all the samples of organs and tissues was done using the fluorometric detection of 2,3-diaminonaphthalene (DAN) method described by Watkinson (Watkinson, 1996).

Statistical analysis: The statistical analysis of the data was made by *t*-test.

Results and Discussion

In the present experiment, to obtain the protozoan Se produced in the rumen Na selenite amount of Se added to the diet of sheep was 0.4 mg Se/kg DM diet (Table 1), and this value of Se in the diet was mostly maximum, which will be allowable limit of Se in sheep. The Se content in obtained rpm was 0.41 mg/kg DM, and this is about more than two times compared to that (0.20 mg/kg DM) in rumen bacterial matter (rbm) of sheep reported Serra et al. (Serra et al., 1997). This could be thought to that the Se would accumulate in protozoa after engulfing bacteria in the rumen.

Table 1. Composition of the diet

Ingredients	Control[1]	RPM diet[2]
	---------%---------	
Protozoan Se[3]	—	24.0
Casein	20.0	14.9
DL-methionin	0.3	0.3
Corn starch	15.0	15.0
Sucrose	50.0	31.1
Cellulose	5.0	5.0
Corn oil	5.0	5.0
Vitamin mixture	1.0	1.0
Colin-acid-tartar acid	0.2	0.2
Mineral mixture (-Se)	3.5	3.5

[1] Based on AIN-76 (NRC, 12)
[2] 100μg Se/kg diet
[3] Contained 21% of CP and 0.41mgSe/Kg DM.

Table 2 shows the Se concentrations (ng/g wet tissue) in the blood and tissues in mice after fed the diet supplemented protozoan Se during 7 days, and slaughtered. In the table the data with bacterial Se (rpm) is shown as a reference, which obtained before by other workers (Serra

Table 2. Se concentrations in blood, muscle and organs of mice fed the diet with orwithout RPM.

Items	Control Diet	RPM diet	RBM diet[1]
	----------ng/g wet tissue----------		
Kidney	556.9±150.2[2]	705.0± 64.0	261.7± 6.6
Liver	728.9± 36.3	938.8±139.1	90.2± 1.9
Pancreas	153.3± 56.3	192.1± 95.3	92.0± 4.0
Spleen	214.9± 53.9	159.3± 31.1	95.7± 8.3
Lung	133.1± 27.7	157.2± 47.2	86.3± 5.8
Heart	108.2± 25.8	134.7± 39.0	91.0± 6.4
Brain	95.8± 23.6	106.9± 43.3	77.7± 8.1
Muscle	137.7± 9.8[a]	175.8± 6.3[b]	82.0± 3.3
Whole blood	258.6± 54.5[a]	541.2± 77.1[b]	172.5±17.5
Plasma	201.5± 38.9	99.4± 45.3	141.0± 6.0

[1] See Serra et al., 1997.
[2] Mean ± S.E. of 10 animals.
[a,b] Values in the same row with different superscripts differ significantly (P<0.01).

et al., 1997). Se concentration in blood and body tissues except spleen and plasma of mice fed diet with rpm was higher than that of mice fed control diet, and the difference in whole blood and muscle was statistically significant (P <0.01). In comparison of all the data in mice fed rpm diet with those in mice fed rbm (Serra et al., 1997), all the values except value of plasma was higher, and then it can be conclude that the protozoan Se tended to incorporate more efficiently than that of rbm.

The Se contents in blood and all the organs are shown in Table 3. The volumes of total blood and total muscles were calculated by body weight × 6.37% and body weight × 43.85%, respectively. Se contents of blood and tissues except lung, spleen and liver in the mice fed diet with rpm were higher than that in control mice, and the difference in figures of whole blood (P<0.01) and muscles (P<0.05) was statistically significant. Total Se contents in blood, organs and tissue of mice fed the diet with rpm was 1.1−1.8 times more than those of control mice. This clearly indicate that the Se intake increased after feeding of rpm diet than control diet, although the number of organs/tissues indicated higher Se value in mice fed rpm diet than in mice fed control diet as compared with Se level of blood, organs and tissues. This discrepancy in concentration and contents of Se of blood, organs and tissue would be due to difference in weight of organs and tissue, and also in body weight of animals in both groups. In comparison with those values in mice fed bacterial Se as a reference (Serra et al., 1997), the values except spleen in mice fed protozoan Se tended to high. From this, there could be a tendency that protozoan Se clearly incorporates efficiently into the body as compared with bacterial Se in mice.

From these findings above, Se retention tended to high in mice fed rpm diet than in mice fed control diet, and this clearly indicate that Se in rpm diet is utilizable in mice. Furthermore, the values in the concentrations and contents of Se in blood, organs and tissue tended to higher in mice fed rpm diet than in the mice fed rbm diet as a reference (Serra et al., 1997), and it could be concluded that proto-

Table 3. Total Se content in blood, muscles and organs of mice fed the diet with or without RPM.

Items	Control Diet	RPM diet	RBM diet[1]
	----------ng Se/ organ, etc----------		
Kidney	174.1± 44.3[2]	224.0±29.0	90.5± 2.3
Liver	785.4±113.7	784.4±98.6	443.5± 6.5
Pancreas	27.2± 15.2	42.4±22.8	33.8± 1.5
Spleen	16.1± 3.4	9.6± 1.9	11.9± 1.0
Lung	24.3± 6.2	23.2± 6.3	19.7± 1.3
Heart	12.2± 2.9	15.1± 4.7	11.1± 0.8
Brain	39.0± 10.3	40.9±18.4	21.4± 2.2
Muscle	1193.2± 76.0[a]	1376.0±49.9[b]	1095.9±44.7
Whole blood	328.0± 71.6[a]	595.6±74.2[b]	341.4±20.2

[1] See Serra et al., 1997.
[2] Mean ± S. E. of 10 animals.
[a, b, c, d]: Values in the same row with different superscripts differ significantly (P<0.05 &P<0.01).

Table 4. Se balance in mice and utilizability of protozoan Se

	Protozoan Se	Control
Se balance		
	---------- ng----------	
Intake	858.10± 1.41[1]	−
Fecal excretion	190.49± 50.56	25.21± 3.40
Urinary excretion	750.28±135.89	444.25±92.81
Se Utilizability (% of intake)	----------%----------	
Rate of excretion		
Fecal	22.2± 5.9	
Urinary	87.4±15.7	
Rate of absorption	77.8± 5.9	
Rate of retention	−9.6±16.9	
Bioavailability	−15.7±21.9	

[1] Mean ± S. E. of 6 (5×2 cage ×3days).

zoan Se utilizes more efficiently than bacterial Se.

It has been reported that a growing lambs with Se deficiency recover after maturing would be mainly due to a role of Se accumulation in rumen microbes (Church et al., 1971). However, there is little information about utilizability of Se incorporated by protozoa, and then, the result obtained in this study could be valuable information to resolve Se deficiency in ruminants in future.

In the present experiment, it has been shown that the Se incorporated into protozoa in the rumen of sheep was more utilized in mice than that incorporated into bacteria. However, there is no information about Se absorption in mice after feeding rpm diet and control diet. In Table 4, Se balance and ratios of excreted, absorbed and retained to total intake of protozoan Se in mice are shown. The urinary excretion of Se in mice after feeding rpm diet was extremely high and also varied quite large extent as compared with control group. This would be due to relatively high water intake in mice fed rpm diet as compared with that in control group. As indicated in preparation of protozoan Se, for separating rpm from rumen contents physiological salt solution was used, and then the final materials should be relatively salty taste. So as a result, all the mice fed with rpm diet drunk relatively more to excrete NaCl out of body than in mice fed control diet. There was also big variance in urinary Se excretion of mice fed rpm diet, and it could be due to individuality in ability to tolerate feeding salty diet.

Conclusion

From the results obtained in the present experiment, it can be obviously concluded that the Se supplemented to the diet of sheep will be more efficiently incorporated into protozoa than into bacteria, and then, it is efficiently utilized in mice as a model animal.

Acknowledgement

Authors are very grateful to Ms S. Fuji for her assistance during the course of the experiment.

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