Absorption and Distribution for Subtoxic Level of Selenite by Vascularly Perfused Small Intestine in Rats

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Intestinally absorptive and distributive aspects of the subtoxic level of selenite in rats were investigated using a double perfusion system. The double-perfusion technique is an *in situ, in vitro* preparation in which the intestinal lumen and its vasculature are perfused simultaneously. In the previous study, the subtoxic level of sodium selenite was determined to be 1.2 mM through inhibition of 3-0-methyl glucose (3MG) absorption. Thus, the selenite used to identify the intestinally absorptive mechanism of selenite was perfused at a luminal concentration of 1, 10, 50, 100 and 200 μM. Appearance of radiolabeled-Selenium (Se) was identified in three compartments: luminal perfusate, small intestine and vascular perfusate. Dose-response curves for Se in the three compartments indicate that selenite is absorbed by non-mediated passive diffusion. Regarding the distributive aspect, 21.02±3.92% of the total amount of selenite in the lumen was transported into the blood vessels across the small intestine. However, 4.75±1.75% of the total amount of selenite in the lumen is retained by the small intestine. Therefore, a total of 25.67±4.46% of the test dose was taken up from the luminal perfusate.

Key words : Selenite, subtoxic, absorption & distribution, intestine, perfusion

Introduction

Selenium (Se) has been recognized as a toxic element as well as an essential nutrient. As an essential element, Se is a component of glutathione peroxidase [1,2,11]. Acute human selenium toxicosis has been reported from industrial and other accidental exposures [2]. However, it has been known that there is a relatively narrow range between levels of selenium intake resulting in deficiency and those causing toxicity, resulting in a very narrow margin of safety [4]. Indeed, several reports show that the prooxidant and toxic effects induced by selenium compounds occur at lower doses in malignant cells compared to benign and non neoplastic cells and thereby giving a large therapeutic potential in cancer treatment [6,8]. Because of these potent biological effects, emphasis has been on the aspect of subtoxic level for Se absorption and distribution.

It has been known that the valence state of Se affects it's toxicity and bioavailability [4]. Selenium is a nonmetallic element chemically related to sulfur. It occurs naturally in four oxidation states: selenide (-2), elemental selenium (0), selenite (+4) and selenate (+6) states. In a view of Se toxicity, selenite is most potent cytotoxic agent among inorganic forms of selenium [9]. However, the mechanism and aspect responsible for intestinal absorption and distribution of selenite are not clear. It was reported that sodium selenite is not absorbed against a concentration gradient in everted gut sacs, indicating a passive absorption process [7]. A more specific transport process probably involving reactions with cellular and/or intracellular glutathione and cysteine has been reported [1,13,15]. In addition, this interaction of selenite with cellular materials has been known to affect the distribution of selenite in intestinal mucosa. Thus, it is suggested that absorption and distribution of selenite are dependent on the presence of either interacting substances in the diet as well as selenite itself.

However, the absorption and distribution of selenite in a view of subtoxic level have not been studied even if Se has a very narrow margin of safety. Thus, it is necessary to make clear the mechanism or aspect responsible for intestinal absorption and distribution of selenite in a view of subtoxic level. In our previous study, the subtoxic level of selenite for intestinal absorption in rats was identified by using double-perfusion system, which is an *in situ*, in vitro preparation in which the intestinal lumen and its vasculature are perfused simultaneously [10]. Under consideration of these subtoxic level of selenite by a double-perfusion system,

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the absorption and distribution characteristics of selenite by the rat small intestine was investigated by the quantification of three components of the absorptive and distributive process: 1) selenite uptake from luminal perfusate, 2) selenite retention by intestine and 3) selenite transport into vascular perfusate. Also, dose-response curves were obtained when selenite transport, retention and uptake were plotted against the luminal selenite concentration. In a view of the distributive aspect, total amount of selenite taken by the small intestine was observed in blood vessel and mucosa.

Materials and Methods

Animals and diets

Sprague-Dawley rats with a body weight of 250±10 g were used for all experiments. The animals were fed a commercial rat chow.

Perfusion solution

The perfusion solutions for lumen and vasculature were similar to that described by the previous study [3]. The basic vascular perfusate consisted of a standard Krebs-Ringer bicarbonate buffer (KRB) containing 5% horse serum, 6% high molecular weight (70,000-90,000 Da) dextran, 0.1% glucose, and 0.15% dexamethasone. Dextran was used as a substitute for plasma albumin in order to maintain the colloidal osmotic pressure of the vascular perfusate. The hormone, 0.15% dexamethasone, was added in physiological concentrations directly to the KRB solution. However, 0.2 μM norepinephrine was infused via a syringe pump into the vascular circuit and the arterial pressure was maintained within the physiological range of 60-80 mmHg to prevent intestinal hypermotility. For 1 hr prior to and throughout the perfusion, the vascular perfusate was gassed with a mixture of 95% O_2 : 5% CO_2, warmed to 37°C and stirred to ensure complete saturation with oxygen. Just before the start of surgery, the solution was titrated to pH of 7.4. The lumen of the intestine was perfused with a solution containing 154 mM NaCl, 5 mM HEPES, 5 mM glucose, and 6 mM glutamine at pH 7.0. Inorganic sodium selenite was added to the luminal perfusate to obtain the desired selenite concentrations. In experiments involving radioactivity, 2-5 μCi of carrier free ^{75}Se as selenite was used.

Surgical method and measurement

As shown in Fig. 1, the perfusion apparatus consisted of two circuits, one supplying the vascular bed and the other supplying the intestinal lumen. The surgical procedure was a modification of those described by the previous study [3]. Rat were anaesthetized with sodium pentobarbital (80 mg/kg body weight) by intramuscular injection. The abdomen was opened, and the bulk of the intestinal tract was gently exteriorized and covered with gauze soaked in saline (0.9% NaCl) at 37°C. The celiac artery was isolated and ligated, approximately 1.5 mm from its junction with abdominal aorta. The right and middle colic arteries and veins were isolated and ligated. Loose ligatures were placed around the abdominal aorta, both cranial and caudal to the origin of the superior mesenteric artery. In addition, a loose tie was paced around the superior mesenteric artery 1 to 3 mm from the abdominal aorta. After the cranial tie was secured, a slit was made in the wall of the abdominal aorta and the cannula inserted where it was secured. Flow of the vascular perfusate was immediately begun at a rate of 7.5 ml/min and the caudal ligature secured. The portal vein was cannulated for collection of the venous effluent. The apparatus, perfusion solutions and intestine were maintained at 37°C during the entire isolation and perfusion procedure. A 95% O_2 : 5% CO_2 mixture was used to force the vascular perfusate from the reservoir through a sponge rubber mesh filter into a peristaltic pump. The pulsed perfusate was then passed through a bubble trap. Arterial pressure was measured at the block with an pressure gauge, and was corrected for the resistance

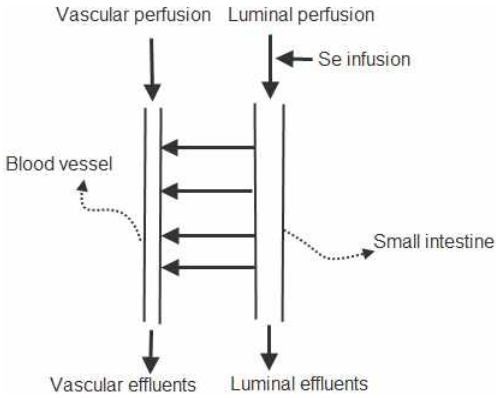

Fig. 1. Diagram of rat small intestine double-perfusion. The perfusion apparatus consisted of two circuits, one supplying the vascular bed and the other supplying the intestinal lumen. The absorption and distribution characteristics of selenite by the rat small intestine was investigated by the quantification of three components of the absorptive and distributive process: 1) selenite uptake from luminal perfusate, 2) selenite retention by intestine and 3) selenite transport into vascular perfusate.

of the tubing between the gauge and tip of the arterial cannula. Arterial pressure was maintained at 40 to 100 mmHg and venous pressure at 150 to 160 mmHg. Both the superior mesenteric artery and the portal vein were cannulated with polyethlene tubing (0.186 mm i.d., 1.27 mm o.d.). The vascular perfusate was collected in fractions (approximately 60 ml) from the venous cannulation at 2 min intervals and was not recirculated. The distal ileum, proximal to the ileocolic valve was secured by a ligature, and a second loose tie was placed around the ileum approximately 5 mm from the first tie. Then an incision was cannulated through the sphincter and secured 1 to 3 mm into the duodenum. The intestine was also cannulated proximal to the ileocolic valve to facilitate the recovery of the luminal perfusate. Upon completion of the surgical isolation of the organ, 8.0 ml of luminal perfusate was rapidly infused into the lumen to fill the intestine. Thereafter, perfusate containing radioactivity was infused by a syringe pump at a rate of 0.39 ml/min for 20 min followed by a rate of 0.10 ml/min for the remainder of the experiment. Perfusion of the vasculature that supplies the small intestine was completed with an inflow cannula at the superior mesenteric artery and an outflow cannula at the portal vein. Through a small abdominal incision, the portal vein was isolated and two loose ties placed around it. Through two slits in the portal vein, cannulations were made resulting in a 2 to 3 mm bypass. The free ends of the tubing were connected to a valve in such a way that sample vascular perfusate could be drawn from the portal cannula just after it had passed through the intestinal mesentery. Using this procedure samples (6 ml) were taken for 60 min and the appearance of ^{75}Se in the vascular perfusate was measured. At the time radioactive intestinal perfusate entered the duodenum was considered the initial time. Beginning at this time, all of the vascular effluent from the portal vein cannula was collected continuously at 2-min intervals into glass tubes (30 fractions) for 60 min. The lumen of the intestine was perfused with the radioactive intestinal perfusate for 60 min followed by rinsing with nonradiactive luminal perfusate plus air to remove ^{75}Se from the luminal perfusate. When both perfusions were stopped, the intestine was quickly removed, and dried at 100°C for 24 hr. Luminal effluents and washing solutions were also collected. One hour before the start of perfusion, selenite was added to the intestinal perfusate, which contained 2.5 μCi of ^{75}Se (obtained from the University of Missouri). Absorption and distribution using various concentrations of selenite in the intestinal perfusate was studied in a series of trials. ^{75}Se activity in the samples of vascular perfusate and the dried intestinal segments was measured with a Beckman Gamma 8000 γ-counter.

Subtoxic level and concentrations of selenite

In our previous study [10,14], the toxicity of sodium selenite was determined by inhibition of 3-O-methyl glucose (3MG) absorption and by histological examination. When 0.5, 1.0, and 2.0 mM of sodium selenite were added, the EC_{50} of selenite on 3MG transport inhibition is approximately 1.2 mM. However, it was identified that the concentration of selenite that the histological examination revealed no signs of edema, necrosis, or structural damage as well as no inhibition of 3MG absorption was less than 200 μM. Thus, the selenite to identify the intestinally absorptive and distributive mechanism of selenite was perfused at a luminal concentration of 1, 10, 50, 100 and 200 μM defined as subtoxic level of selenite. Three rats were perfused for each concentration of selenite selected.

Results and Discussion

The experimental design of this study was based on a chromium absorption studies by the double perfusion system [3,8,12]. We used the criterion of saturability to examine mechanisms of intestinal absorption of selenite. Saturation is recognized as a decreased percentage of absorption with increasing luminal selenite concentration. Thus, dependence of selenite absorption on a carrier-mediated transport system of passive diffusion in the rat small intestine was determined by the slope values of the dose-response study presented as percentages. In order to identify the absorptive mechanism, selenite transport was measured at one time point, 4 min in this study, taken as early as possible in order to approximate the true initial rate conditions. This might reduce the error due to saturation of the transport system, and the unexpected effect on the membrane by different concentrations of selenite.

The results presented in Fig. 2A and 2B show the does-response curves for selenite transport at 4 min for the initial rate of selenite absorption. The graphs depict the total amount of Se transported in nmol Se/min/g dry weight (Fig. 2A) and percentages (Fig. 2B) into the vascular perfusate (response) with the various luminal Se concentrations tested (dose). There is a significant positive correlation

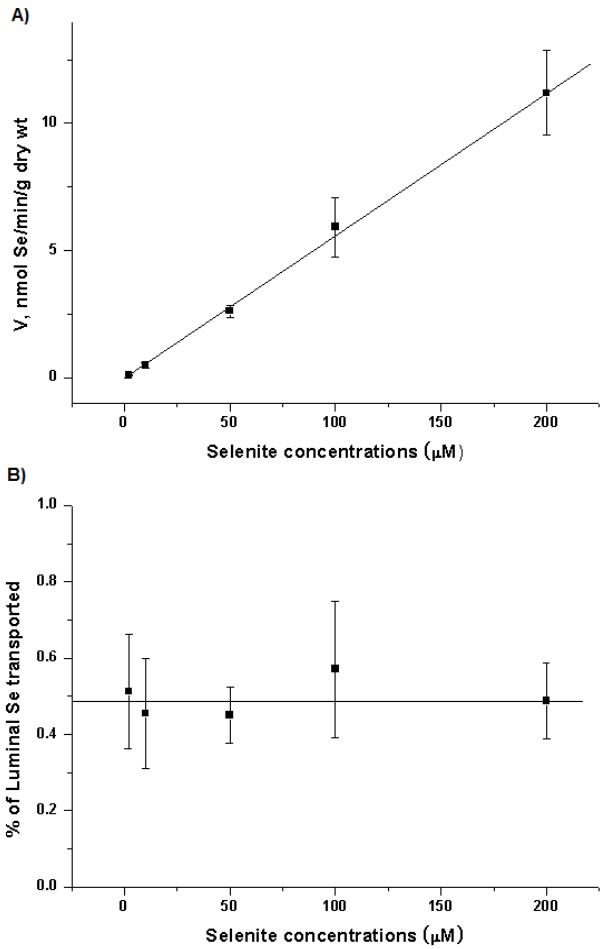

Fig. 2. The dose-response curves for selenite transport. The rate of Se transport into the vascular effluent was measured at 4 min. The dose-response curve of slenite transport presented as nmoles/hr (A) was transformed to precentage of luminal selenite (B). Each point is mean±SD for triplicate preparations. In (B), there is no evidence of a negative slope.

(r^2=0.97, $p<0.0001$) between nmol Se/min/g dry wt transported and luminal Se concentration (slope=0.057±0.002%). In Fig. 2B, there is no slope and the percentage of luminal Se transported is equal to 0.49±0.12%. The statistical parameters, r^2=0.94 and p=0.73, indicate that the percentage transported is not affected by concentration of selenite.

According to the study using initial rate of transport, the rapid saturability of selenite uptake was observed in brush border membrane vesicles from chicks [5]. In our study, initial rate of selenite transport at time interval of 2-4 min showed a linear dependence on concentration in luminal perfusate. When the Se absorption data were transposed from nmol Se/g dry wt to percentage of the luminal selenite concentration, the slope was not negative with the concentration of selenite used, indicating that saturability was not reached in our studies. In a view of initial transport rates, this indicates that the absorption of selenite by the rat small intestine was by a non-carrier mediated process at subtoxic level of selenite. In addition, the selenite absorption identified by using intestinal double-perfusion is consistent with the result from using everted gut sacs of rat, showing that sodium selenite is not absorbed against a concentration gradient of selenite [7].

In order to confirm the absorptive mechanism of selenite at one time point, 4 min, the total amount of the vascular effluents collected during 60 min (30 fractions) was analyzed at various luminal concentrations of selenite. In addition, this total amount was analyzed for the distributive aspect of selenite. The dose-response curves for three components of the absorptive and distributive aspect were classified as transport, retention and uptake. Se uptake from intestinal perfusate is the sum of the Se transported into the vascular effluent and the Se retained by the small intestine.

In Fig. 3A, the slope of the line is 6.31±0.2, and there is a very strong correlation (r^2=0.98, $p<0.00001$) between luminal Se concentration and Se nmole transported/g dry wt. In Fig. 3B, the slope of the line is -0.013±0.014, but this slightly negative slope was insignificant (r^2=0.06, $p<0.39$). The average percentage of Se transport into the vascular effluent is 21.02±3.92% of the luminal selenite concentration. Se retained by the entire small intestine is shown in Fig. 4A Retained Se refers to the Se that remained in the perfused small intestine after rinsing. The slope of the line is 0.86±0.06. A correlation (r^2=0.94, $p<0.0001$) is evident between the luminal Se concentration and the amount of retained Se. Se uptake from the intestinal perfusate is the sum of the Se transported into the vascular effluent and the Se retained by the small intestine. In Fig. 5A, Se uptake (nmol/g dry wt) is equal to the slope (7.17±0.2) multiplied by the luminal Se concentration. A very strong correlation (r^2=0.99, $p<0.00001$) exists between Se uptake and the luminal selenite concentration. There were statistically significant linear correlations between Se transport, retention and uptake, and the luminal selenite concentration when the dose-response curves (Fig. 3A, 4A, and 5A) were expressed as the total amount. Compared to the result from the initial transport rates at one time point, the result form these dose-response curves for three components were not different, indicating that selenite is absorbed by a passive diffusion system in the rat small intestine.

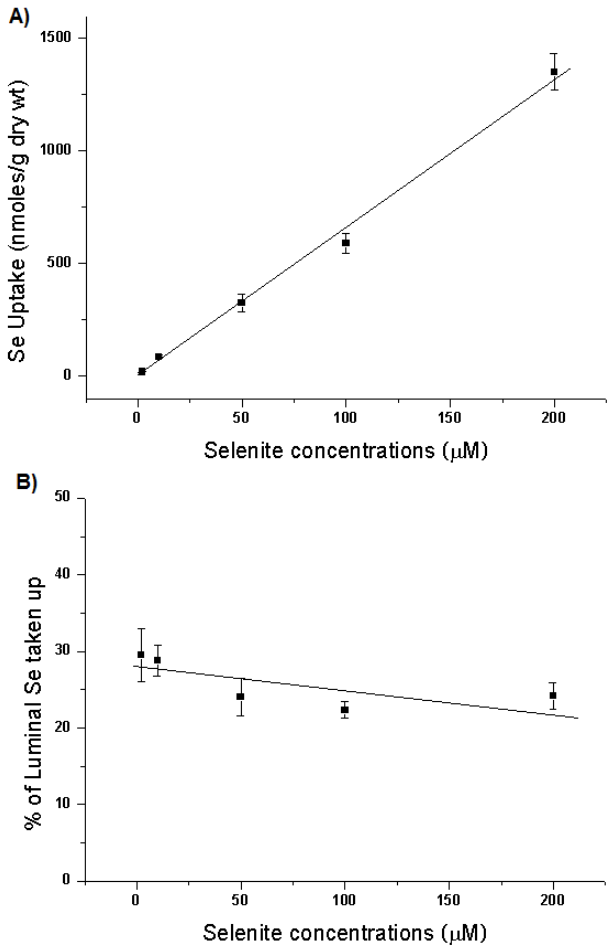

Fig. 3. The dose-response curves for selenite transport into the vascular perfusate. The total Se transported after 1 hr perfusion was expressed as nmoles/hr (A) and transformed to precentage of luminal selenite (B). Each point is mean±SD for triplicate preparations. In (B), the slope of the line is zero.

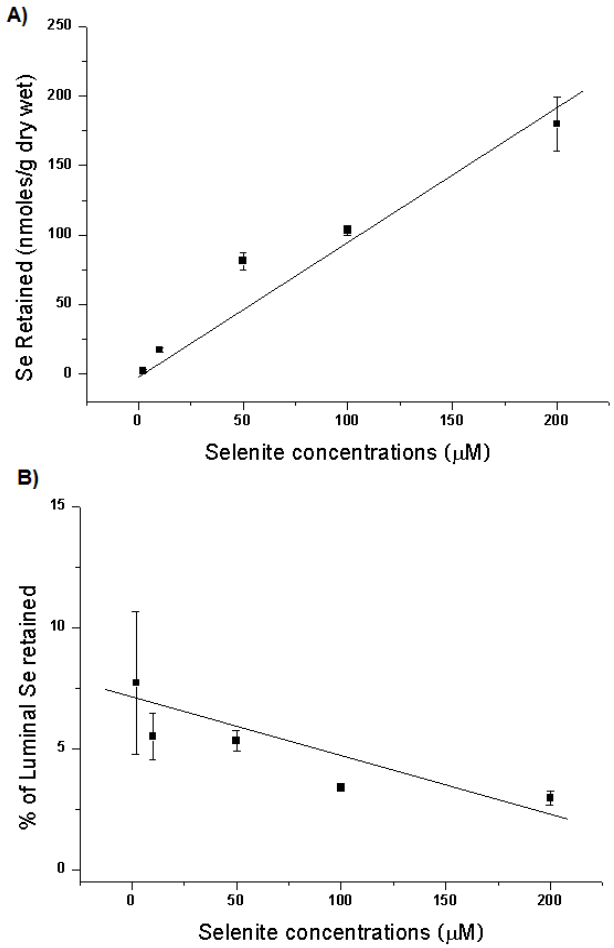

Fig. 4. The dose-response curves for selenite retained by small intestine. Twice-washed intestine with 0.9% saline was dried at 100°C, and its Se content was expressed as nmoles/hr (A) and precentage of luminal selenite (B). Each point is mean±SD for triplicate preparations. In (B), the slope of the line is negative.

In order to identify the distributive aspect of selenite in three compartments, the total amounts of ^{75}Se for these compartment were expressed as percentage. In Fig. 3B, the average percentage of Se transport into the vascular effluent is 21.02±3.92% of the luminal selenite concentration. The slope of the line is -0.013±0.014, but this slightly negative slope was insignificant (r^2=0.06, $p < 0.39$). In Fig. 4B, the average percentage of Se retained by small intestine is 4.75±1.75% of the luminal selenite concentration. The slope of the line is negative, -0.011±0.004. In addition, an r^2 of 0.34 (p=0.022) suggests that there is a slightly negative slope between the percentage of Se retained and the luminal concentrations. Thus, the percentage of Se retained by the intestine is dependent on the luminal selenite concentration. In Fig. 5B, a total of 25.67±4.46% of the test dose was taken up from the luminal perfusate. The slope is slightly negative, -0.024±0.014, but a r^2 of 0.19 (p=0.1) indicates that there is no correlation between Se uptake (%) and the luminal selenite concentration.

It is interpreted that 21.02±3.92% of a total amount of selenite in the lumen is transported into blood vessel across the small intestine. However, 4.75±1.75% of a total amount of selenite in the lumen is retained by small intestine. Therefore, a total of 25.67±4.46% of the test dose was taken up from the luminal perfusate. In a view of the slops for the dose-response curve expressed as percentage, none of the slopes was negative except the dose-response curve for intestinal retention. This data indicated that Se retention by the mucosa reached saturation, but Se transport into the vascular perfusate did not reach saturation

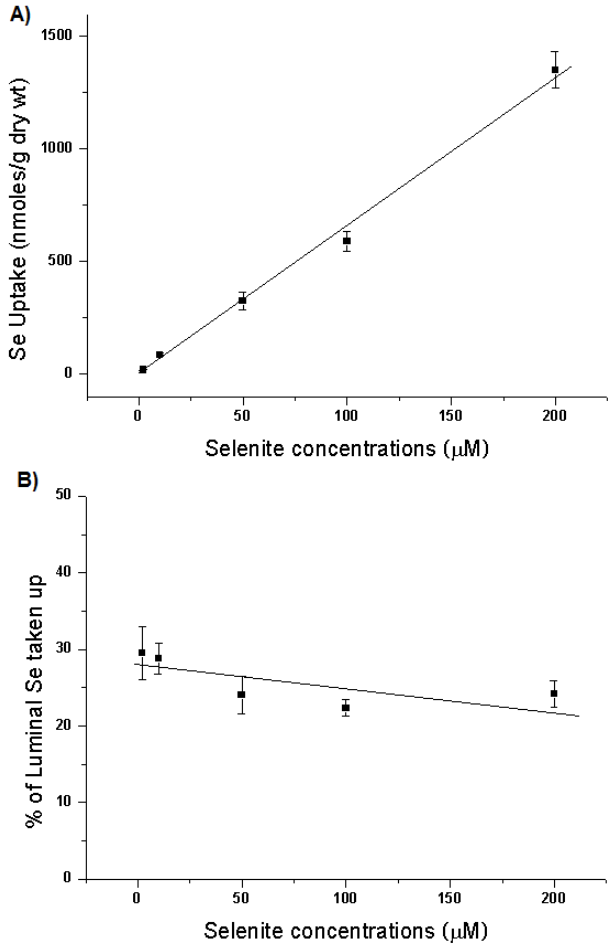

Fig. 5. The dose-response curves for selenite uptake from luminal perfusate. Selenite uptake from luminal perfusate indicates the sum of Se transported into the luminal perfusate and Se retained by small intestine. Data is expressed as nmoles/hr (A) and transformed to precentage of luminal selenite (B).

with the concentrations used. Even though there was a slight decrease (on a % basis) at high concentrations (statistically insignificant), Se transported into the vascular perfusate was not saturated by any selenite concentration of the luminal perfusate. The percentage of Se taken up from the intestinal perfusate did not reach saturation. Thus, the saturation of percentage retention of Se in small intestine appears to have no affect on the total percentage of Se absorbed on the vascular perfusate. This could be explained by the inter and intracellular formation of selenotrisulfides which produce a high concentration gradient of the intact selenite between compartments, and by the active transport of selenorisulfide [1]. It was reported that increasing the stable selenite concentration slightly reduced the percentage of ^{75}Se-selenite transported from the intestinal lumen to the body [5]. Their explanations for these findings focused on the dependency of selenite on a carrier transport system, and the toxic effects to the intestine at high concentrations of selenite. In the present experiments, there was a slight decrease in percentage of Se uptake by the small intestine at high concentrations of selenite in the lumen (from 28.1 to 24.3%), but it was not statistically significant. Our data indicated that the slight decrease could be the result of depletion of the intracellular SH groups and glutathione.

In summary, in spite of the usage of selenite as a nutrient and as a toxicant, there has surprisingly been little work of its absorption by the intestine. Recently, a few studies have reported on the factors affecting intestinal absorption of selenite. We conclude that sodium selenite is absorbed by passive diffusion in perfused rat small intestine. However, it is assumed that the saturation of Se retention would be cased by limitation of glutathione, cysteinyl residues of proteins and/or membranes. In addition, it is assumed that selenite metabolism affect its absorption by maintaining a concentration gradient of selenite in the intestine. In a view of the distributive aspect, 21.02±3.92% of a total amount of selenite in the lumen is transported into blood vessel across the small intestine. However, 4.75±1.75% of a total amount of selenite in the lumen is retained by small intestine. Therefore, a total of 25.67±4.46% of the test dose was taken up from the luminal perfusate.

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초록 : 랫드의 소장-혈관의 이중 관류를 통한 저독성 농도의 selenite 흡수와 분포

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Selenite는 필수미량원소인 동시에 독성을 유발한다. 본 연구에서 랫드의 소장-혈관의 이중 관류법을 통해 저독성인 selenite인 1, 10, 50, 100 and 200 μM 등의 농도를 이용하여 소장에서의 흡수 및 장 내, 소장 조직 내 그리고 혈관에서의 분포를 확인하였다. Selenite에 대한 저독성 기준은 3-0-methyl glucose (3MG)의 장애 흡수를 저해하는 1.2 mM 이하 농도에서 설정되었다. 장내, 소장 조직 내 그리고 혈액에서의 용량-반응 관계를 통해 selenite가 비-매개 수동확산(non-mediated passive diffusion)을 통해 장에 의해 흡수되는 것이 확인되었다. 또한 장에서의 분포는 관류된 selenite 농도의 21.02±3.92%가 소장을 통과하여 혈관에서 확인되었으며 4.75±1.75%는 소장 조직에서 확인되었다. 따라서 관류된 selenite의 전체 농도는 25.67±4.46%으로 나머지는 소장 내에 분포하는 것으로 추정된다.