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The influence of zinc status on the kinetics of zinc uptake into cultured endothelial cells

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To better understand cellular zinc homeostasis and characterize the zinc transport process, a mammalian cell culture model was utilized to investigate the influence of zinc status on the kinetics of zinc uptake. Culturing conditions were optimized to induce moderate zinc deficiency and zinc excess while still sustaining the general health of the cells. Cells were grown in (1) control medium of 10% fetal bovine serum (FBS) in minimum essential medium (MEM; 5.0 μmol zinc/L), (2) low zinc medium (10% dialyzed FBS in MEM; 1.5 μmol zinc/L), or (3) zinc back medium (10% dialyzed FBS in MEM with zinc added as $ZnCl_2$; 5.0 μmol zinc/L). Bovine pulmonary artery endothelial cells (BPAEC), porcine aortic endothelial cells (PAEC), and porcine venous endothelial cells (PVEC) were evaluated as to their responsiveness to our zinc-deficient conditions. Zinc uptake was faster ($P < 0.001$) in all three cell types when they were grown in low zinc medium compared with controls; the increases were 32% in PAEC, 37% in PVEC, and 66% in BPAEC. Further kinetic analysis with BPAEC demonstrated a 31% increase ($P < 0.05$) in the maximum rate of zinc uptake (Jmax) grown in low zinc medium compared with controls, but no difference ($P > 0.05$) between the low zinc group and the control group in the concentration at which uptake was half-maximal (K). Zinc uptake into BPAEC grown in excess zinc conditions was not different ($P > 0.05$) unless the medium contained greater than 50 μmol zinc/L. In conclusion, BPAEC increased their ability for zinc uptake in response to moderate zinc deficiency, but did not change their kinetics of zinc uptake during moderate zinc excess. (J. Nutr. Biochem. 10:484–489, 1999)

Keywords: zinc transport; zinc deficiency; zinc toxicity; endothelial cells

Introduction

Zinc is a trace element that is essential to life. It functions as a catalyst in over 300 enzymes, including those responsible for DNA replication, RNA and protein synthesis, and gene transcription.[1] The clinical manifestations of improper zinc nutriture are severe. Zinc deficiency has been associated with growth retardation, impaired sexual development, and neurologic dysfunction. Following the initial discovery of nutritional zinc deficiency by Prasad et al.,[2] zinc deficiency syndromes have been reported in several populations. Hambidge et al.[3] reported zinc deficiency in children, characterized by low zinc concentrations in hair and plasma, in an average socioeconomic population. Zinc deficiency syndromes characterized by low zinc concentrations in plasma have been reported in infants, women, and the elderly as well.[4,5] Although less common, conditions of zinc toxicity also have been reported. Excessive concentrations of zinc in specific locations, including the brain, have been implicated in the pathology of disease states, including Alzheimer's disease.[6] Not withstanding zinc's essential importance, the mechanism of zinc transport across membranes remains to be elucidated.[7]

To better understand cellular zinc homeostasis and characterize the zinc transport process, a mammalian cell culture model was used to investigate the influence of zinc status on the kinetics of zinc uptake. Culturing conditions were optimized to induce moderate zinc deficiency and zinc excess while sustaining the general health of the cells. We chose to evaluate endothelial cells as a potential model system because they are in direct contact with the plasma and form a barrier through which all nutrients, including zinc, must pass prior to entry into peripheral tissues and organs. Due to the location and function of endothelial cells,

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they may be involved in the homeostatic regulation of zinc exchange between the plasma and the periphery, and can be a valuable model for investigations of nutrient transport.

Blood plasma in rats,[8] pigs,[9] and humans[10] is sensitive to changes in zinc nutriture. We examined the effect that changes in the extracellular environment might exert on zinc uptake into cultured endothelial cells. We evaluated three types of endothelial cells for their suitability as an in vitro model of zinc deficiency, and refined procedures that use bovine pulmonary artery endothelial cells (BPAEC). We believe the results presented here demonstrate a regulatable mechanism of zinc uptake into endothelial cells that responds to moderate zinc deficiency, but not zinc excess.

Methods and materials

Cell cultures

Three types of endothelial cells were evaluated in this research: (1) porcine aortic endothelial cells (PAEC), (2) porcine venous endothelial cells (PVEC), and (3) BPAEC. PVEC and PAEC were obtained from primary culture; BPAEC were obtained from American Type Culture Collection (#CCL-209; Rockville, MD USA). Culturing conditions were slightly different for each line of cells, depending on their needs.

PAEC and PVEC were isolated from the aorta and inferior vena cava of Yucatan miniature swine (*sus scrofa*) by standard procedures for isolating endothelial cells from large vessels and in accordance with guidelines set forth by the National Institutes of Health. Briefly, the freshly isolated blood vessels were washed with collection medium containing minimum essential medium Eagle with Earle's salts, L-glutamine, and 25 mmol HEPES/L plus 2% fetal bovine serum (FBS; Summit Biotechnology, Ft. Collins, CO USA), 50 mg gentamicin/L, and 5 mg amphotericin B/L. The lumen was exposed and bathed in 0.1% collagenase (Type IA, 380 units/mg) in a 37°C incubator with 5% carbon dioxide for 15 minutes. Collagenase was then removed and the lumen was gently brushed with a sterile cotton swab moistened with collection medium. The swab was then agitated in 10 mL of collection medium, which was centrifuged (Damon, Centra 8R, Needham, MA USA) at 500 × g for 10 minutes. The supernatant was removed and cells were resuspended in 2.5 mL of growth medium. Growth medium contained minimum essential medium Eagle with Earle's salts and L-glutamine (MEM) plus 10% FBS, 100 mg heparin/L, and 50 mg endothelial cell growth supplement (ECGS)/L. Heparin and ECGS were deemed necessary for the proper growth of PVEC and PAEC, but not BPAEC. ECGS was isolated from bovine hypothalamus by the methods of Maciag et al.[11] Cells were seeded into fibronectin coated (2 $\mu g/cm^2$) flasks and grew to confluence in approximately 3 to 5 days, when they were passaged at a ratio of 1:3. Cells for experiments were at passages 4 through 6 and were seeded into T-25 flasks. Cells in experimental flasks reached confluence in 3 days and zinc uptake studies were performed on day 5.

BPAEC were obtained at passage 16 and experiments were conducted with cells from passages 18 through 22. Cells were seeded into T-25 flasks at a density of 15,000 cells/cm^2 and supported in growth medium containing MEM plus 10% FBS. Cells in experimental flasks reached confluence in 5 days, when they contained approximately 3 million cells and 0.6 mg of cell protein. Zinc uptake studies were performed on day 8.

All endothelial cells that were used in this research grew into confluent monolayers, their growth was density-inhibited, and they exhibited morphologies typical of endothelial cells. All cultures tested positive for the factor VIII antigen[12,13] and uptake of acetylated low density lipoprotein,[14] which are established criteria for endothelial cell characterization.

Induction of altered zinc status

The induction of an in vitro zinc deficiency involved the use of low zinc growth medium containing 10% FBS that had been stripped of zinc by a dialysis procedure similar to that of Pilz et al.,[15] with modifications. Dialysis bags (1,000 MW, 20 mm diameter; Spectrum, Houston, TX USA) containing FBS were bathed for 10 days in sterile 4°C dialysis solution (pH 7.4), at a solution to serum ratio of 50:1. On days 1 through 3, dialysis solution was composed of 10 mmol Na_2EDTA/L, 8 mmol Na_2HPO_4/L, 1.4 mmol KH_2PO_4/L, and 130 mmol NaCl/L. On days 4 through 6, dialysis solution was composed of 100 mmol NaBr/L, 8 mmol Na_2HPO_4/L, and 1.4 mmol KH_2PO_4/L. On days 6 through 10, dialysis solution was composed of 8 mmol Na_2HPO_4/L, 1.4 mmol KH_2PO4/L, and 141 mmol NaCl/L. On day 11, dialysis bags were removed from sterile solution and FBS was harvested. Upon mineral analysis, dialyzed FBS had a zinc concentration of 15 μmol/L, compared with approximately 50 μmol/L in undialyzed FBS. Protein concentration was at least 85% of that found in undialyzed FBS.

Cells were grown in treatment media that were designated (1) control (zinc normal), (2) low zinc, (3) zinc back, and (4) excess zinc. With BPAEC, fresh treatment medium was applied on days 1, 4, and 7; zinc uptake was measured on day 8. With PAEC and PVEC, fresh treatment medium was applied on days 1 and 4; zinc uptake was measured on day 5.

Control (zinc normal) medium applied to BPAEC was composed of MEM with 10% FBS. Control medium applied to PAEC and PVEC was the same plus 100 mg heparin/L and 50 mg ECGS/L. Heparin and ECGS contributed no zinc to the medium, by analysis. The zinc concentration of control medium was 5 μmol/L, by analysis.

Low zinc medium applied to BPAEC was composed of MEM with 10% dialyzed FBS. PAEC and PVEC received the same plus 100 mg heparin/L and 50 mg ECGS/L. The zinc concentration was 1.5 μmol/L, by analysis.

Zinc back medium applied to BPAEC was composed of MEM with 10% dialyzed FBS plus 3.5 μmol $ZnCl_2$/L. PAEC and PVEC received the same,plus 100 mg heparin/L and 50 mg ECGS/L. The zinc concentration was 5 μmol/L, by analysis.

Excess zinc media was composed of MEM with 10% FBS plus 20, 45, and 70 μmol $ZnCl_2$/L. The resulting zinc concentrations of excess zinc media were 25, 50, and 75 μmol/L, respectively.

The cells that were used in these experiments appeared healthy by all the criteria that we evaluated. They had the same amounts of protein and DNA across all treatment groups, as has been more thoroughly described in a previous publication.[16] We were unable to detect any significant differences in cell morphology or growth rates. The only differences we could detect were their rates of zinc transport.

Analysis of zinc uptake

The rate of zinc uptake into endothelial cells was measured using the procedures of Bobilya et al.[17] On the appropriate day, treatment medium was removed from confluent cells. Cells were washed twice with 37°C HEPES buffer (10 mmol HEPES/L, 140 mmol NaCl/L, 7 mmol KCl/L, 5.6 mmol glucose/L, pH 7.4), and 3.0 mL of incubation medium was placed in the flask. Incubation medium was composed of 10% FBS in MEM (5 μmol zinc/L). Incubation medium included 10% serum in MEM to approximate the extracellular environment that these cells are exposed to in vivo.[18] Carrier-free ^{65}Zn (Amersham, Arlington Heights, IL USA) was added to incubation media 24 hours prior to experiments at a concentration of 9.25×10^6 Bq/L for equilibration of ^{65}Zn with all

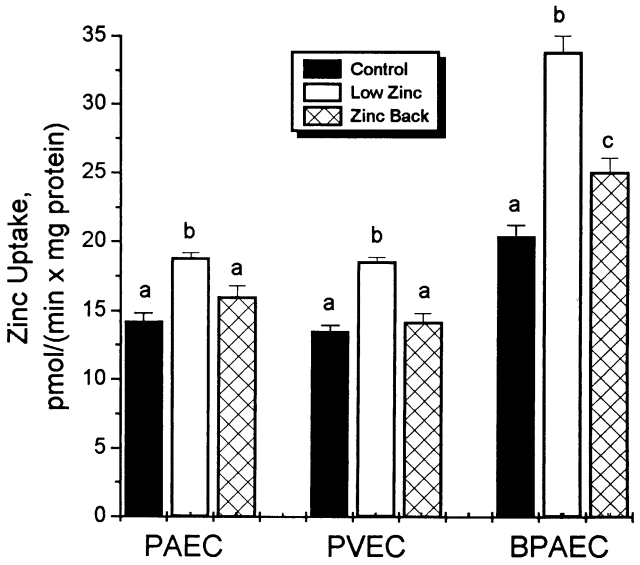

Figure 1 Influence of zinc deficiency on the rate of zinc uptake into three lines of endothelial cells: porcine aortic endothelial cells (PAEC), porcine venous endothelial cells (PVEC), and bovine pulmonary artery cells (BPAEC). Cells were grown in control medium (5 μmol zinc/L), low zinc medium (1.5 μmol zinc/L), or zinc back medium (5.0 μmol zinc/L). Zinc uptake was measured with ^{65}Zn-labeled control medium. Results are presented as means ± SE, $n = 10$ (five replicates per treatment in two independent experiments per cell type). Bars with different letters within a cell line are different, $P < 0.05$. Direct comparisons between the different cell types is not appropriate because the growing conditions varied depending on the specific requirements of each cell line.

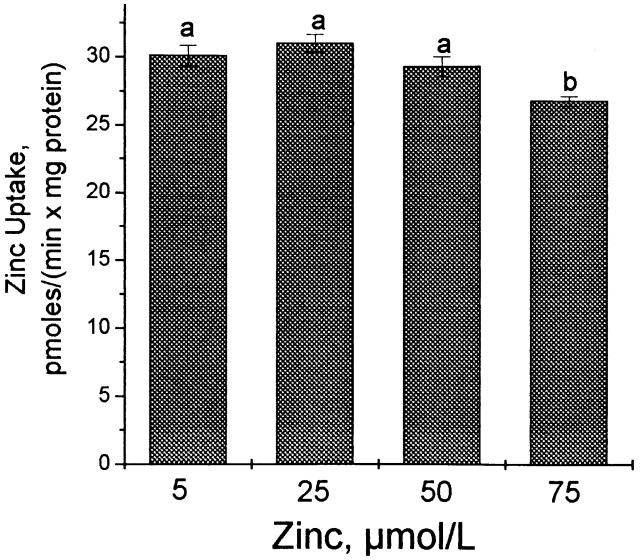

Figure 2 The influence of zinc excess on the rate of zinc uptake into bovine pulmonary artery cells (BPAEC) was measured after growing the cells in medium containing 5, 25, 50, or 75 μmol zinc/L for 7 days. Zinc uptake was measured by incubating the cells for 10 minutes with ^{65}Zn-labeled control medium (5 μmol zinc/L). Results are presented as means ± SE, $n = 15$ (five replicates per treatment in three independent experiments). Bars with different letters are different, $P < 0.05$.

labile zinc pools in the media. Cells were incubated on an orbital shaker (Innova 2000, New Brunswick Scientific, Edison, NJ USA) at 30 rpm inside a 37°C incubator for 10 minutes. This length of incubation was chosen to measure initial velocity because zinc uptake into BPAEC under these conditions is linear for the first 15 minutes.[17,19] Incubation medium was removed and cells were briefly (6–8 seconds) washed in 4°C HEPES buffer with 10 mmol EDTA/L. Cells were then washed twice with 4°C HEPES buffer. Following the washes, the monolayer was solubilized with 2 mL of 0.01% sodium dodecyl sulfate (SDS) in 0.2 mol/L NaOH. Solubilized cells were analyzed for radioactivity using a gamma emissions detector (#1282 CompuGamma, Universal Gamma Counter, LKB Nuclear Inc., Gaithersburg, MD USA). Protein concentrations of the samples were analyzed by the method of Bradford.[20] Rate of zinc uptake was calculated as pmol zinc/(min × mg cellular protein) based on the specific activity of the transport media (typically 1.1×10^{14} dpm/mol zinc).

In studies investigating the concentration-dependent kinetics of zinc uptake into BPAEC, zinc status was modified as described above. The kinetics of zinc uptake were measured on day 8 by measuring the rate of zinc uptake from incubation medium containing five different concentrations of zinc. Incubation media were composed of MEM with 10% dialyzed FBS and zinc added as $ZnCl_2$. Zinc concentrations were within the physiologic range (1.5–18 μmol zinc/L) that is most appropriate for evaluating the saturation kinetics of zinc transport by endothelial cells.[17,21] The kinetic parameters of zinc transport—K and Jmax—were calculated using nonlinear regression analysis of the data fit to a rectangular hyperbola.

Other assays

Zinc concentrations were measured using flame atomic absorption spectrophotometry (Smith Hieftje 12, Thermo Jarrel Ash Co., Franklin, MA USA). Reference standards were prepared in 157 mmol HNO_3/L of deionized water from Fisher Scientific certified standards (Pittsburgh, PA USA). Unless stated otherwise, all reagents were obtained through Sigma Chemical Co. (St. Louis, MO USA).

Statistical analyses

The data were analyzed as a randomized complete block with experiments as blocks. Data from replications of the same experimental design were pooled when they passed the test of homogeneity. Analysis of variance was performed with the Crunch Statistical Package (Version 4, Crunch Software Corp., Oakland, CA USA). Fisher's protected least significant difference (LSD) test was used for pair-wise comparisons of multiple groups. Dunnett's test was used for comparison of multiple groups with a control group. Differences were considered significant if the *P*-value was less than 0.05. Nonlinear regression was performed using Systat computer software (Version 7.0.1, Chicago, IL USA). Comparisons of the regression curves were performed as described by Zarr.[22]

Results

The influence of zinc status on the rate of zinc uptake

The influence of zinc deficiency on the rate of zinc uptake into three types of endothelial cells appears in *Figure 1*. In PAEC and PVEC, the rate of zinc uptake into cells treated with low zinc medium was faster ($P < 0.001$) than into cells treated with control medium by 32% and 37%, respectively. In BPAEC, the rate of zinc uptake into cells treated with low zinc medium was 66% faster ($P < 0.0001$) than into cells treated with control medium, and 19% faster ($P < 0.0001$) than into cells treated with zinc back medium.

The influence of zinc excess on the rate of zinc uptake was examined in BPAEC (*Figure 2*). The rates of zinc

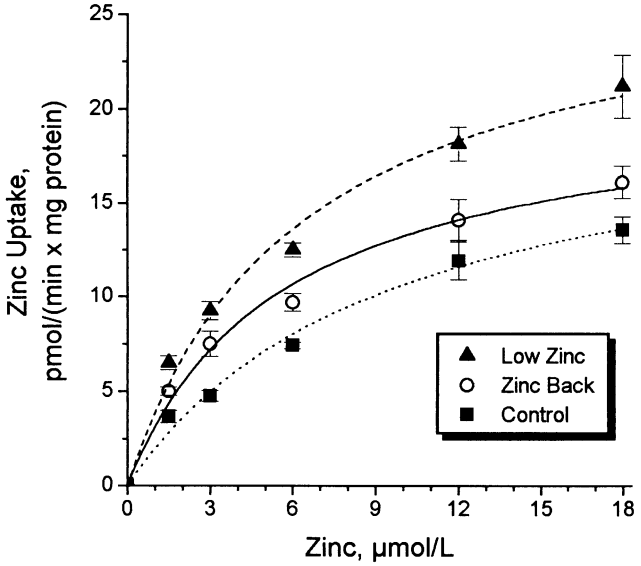

Figure 3 The influence of zinc deficiency on the kinetic parameters of zinc uptake into bovine pulmonary artery cells (BPAEC). Cells were grown for 7 days in control medium (5 μmol zinc/L), low zinc medium (1.5 μmol zinc/L), or zinc back medium (5.0 μmol zinc/L). The rate of zinc uptake was measured by incubating the cells with ^{65}Zn-labeled medium containing 1.5, 3.0, 6.0, 12, and 18 μmol zinc/L. Results are presented as means ± SE, $n = 4$. The results of one experiment are shown, but two other replications of the same experimental design yielded the same response. Variation between the cells of the three experiments was too great to permit their combination into one figure. The curves were generated by fitting the data to a rectangular hyperbola using nonlinear regression techniques.

uptake by cells grown in 25 or 50 μmol zinc/L were not different ($P > 0.05$) from the control group (5 μmol zinc/L). Cells grown in 75 μmol zinc/L had a zinc uptake rate that was 14% less ($P < 0.01$) than the control group.

The influence of zinc deficiency on the kinetic parameters of zinc uptake

Because BPAEC were more sensitive than PAEC and PVEC to our in vitro zinc deficiency conditions, they were chosen as a model to investigate the influence of zinc deficiency on the kinetic parameters of zinc uptake (*Figure 3*). The maximum rates of zinc uptake (Jmax) and the zinc concentrations at which uptake is half-maximal (K) for each treatment group appear in *Table 1*. The Jmax values for the control and zinc back treatments were not different ($P > 0.05$), but the zinc-deficient cells had a 31% greater ($P < 0.05$) Jmax than the controls and a 34% greater ($P < 0.05$) Jmax than cells grown in the zinc back treatment. The zinc concentration at which zinc uptake was half-maximal (K) in BPAEC grown in low zinc medium was not different ($P < 0.05$) than BPAEC grown in control medium or zinc back medium. The zinc back group had a lower ($P < 0.05$) K than the control group.

Discussion

This is the first report of any mammalian cells (other than intestinal cells) that have been shown to respond to zinc restriction by increasing their rate of zinc uptake. The endothelial cells in this research were able to grow and proliferate under our in vitro conditions of zinc deficiency and zinc excess. This demonstrates an ability to maintain cellular zinc homeostasis under changing zinc environments. Investigating the influences of changing zinc status is made difficult by the absence of reliable zinc status indicators in cells or organisms. Our goal was to influence zinc status without inflicting mortal injury on the cells. We were unable to detect any significant differences in cell morphology, cell protein, DNA, or metallothionein content of BPAEC cultures grown under identical circumstances.[16] Instead, the most sensitive change that was detected was an increase in the rate of zinc uptake into the zinc-deficient cells. This would appear to be a useful indicator of zinc deficiency, at least under the conditions we developed for use in this in vitro research model.

Table 1 Influence of zinc deficiency on the kinetic parameters of zinc uptake into bovine pulmonary artery endothelial cells (BPAEC)*†

Medium	Jmax pmol/(min × mg protein)	K μmol/L
Control	22.1 ± 2.6[a]	11.1 ± 2.6[a]
Low zinc	29.0 ± 2.3[b]	7.2 ± 1.3[a,b]
Zinc back	21.6 ± 1.8[a]	6.5 ± 1.4[b]

*Cells were grown for 7 days in control medium (5 μmol zinc/L), low zinc medium (1.5 μmol zinc/L), or zinc back medium (5.0 μmol zinc/L).
†Values are means ±SE, based on four measurements at each of five zinc concentrations (1.5, 3.0, 6.0, 12, and 18 μmol zinc/L) for each treatment. Calculations were performed by fitting the data in *Figure 3* to a rectangular hyperbola using nonlinear regression statistics. Values in a column with different superscripts are different ($P < 0.05$).
[a]Jmax–the maximum rate of zinc uptake. K–the zinc concentration at which uptake is half maximal.

Three different types of endothelial cells were evaluated. We were searching for cells that serve as a suitable cell culture model of zinc deficiency. Our selection criteria included that the cells remain healthy under the zinc-deficient conditions, that they have a relatively high rate of zinc uptake, and that they respond to zinc deficiency by increasing their rate of zinc uptake. All cells were healthy under the conditions we ultimately used to induce the zinc deficiency, although this required considerable testing. We estimated that the cells were at an optimal state of moderate zinc deficiency when they were grown in the low zinc medium for 3 to 4 generations (5–7 days under these culturing conditions). During preliminary studies, we observed that less time than this in the low zinc medium did not yield a significant response to zinc deficiency, as determined by zinc uptake. The 3 to 4 generations of growth in the low zinc medium appears to have been necessary for depletion of the intracellular zinc stores during the moderate zinc deficiency. When grown for longer periods of time, the cells began to develop physiologic abnormalities such as enlarged cytoplasm and irregularly shaped plasma membranes. The physiologic abnormalities indicated to us that the cells were approaching senility and death due to zinc deficiency and would be unsuitable for experimentation.

Direct comparisons between the three cell types should

be done cautiously, because the origin and history of each cell type is unique. The donor animal and tissue of origin are unique. The feeding and subculturing regimens differed. In addition, the PAEC and PVEC required heparin and ECGS in their growth media, but BPAEC did not. All three cell lines responded to our in vitro zinc-deficient environment. Under these conditions, BPAEC had the highest rate of zinc uptake and were most responsive to zinc deficiency; therefore, these cells were used to examine specific changes in the kinetics of zinc uptake.

Measuring the in vitro response of endothelial cells to altered zinc status required the selective addition or removal of zinc from cell culture systems. Serum is the principal endogenous source of zinc in cell culture media. In the present investigation, a procedure involving the dialysis of FBS with EDTA was used. When 10% dialyzed FBS was used in conjunction with MEM, the zinc concentration of the growth medium was reduced to 1.5 μmol/L, compared with 5 μmol/L in medium containing undialyzed FBS. Concentrations of other essential nutrients in the serum removed by dialysis, including calcium and magnesium, were present in adequate concentrations in MEM and were little changed in the complete media through this protocol on the serum. The observation that the cells grew and proliferated in media containing the dialyzed serum demonstrates that this protocol was effective. We found that thoroughly dialyzing the serum for 7 days in buffer after the EDTA was removed from the dialyzing solution was important for the dialyzed serum to support optimal cell growth. Significant increases in the rate of zinc uptake into all three cell types were observed after treatment with low zinc medium for 5 to 7 days. Cells treated with zinc back medium yielded rates of zinc uptake similar to cells treated with control medium, confirming that zinc was the most important factor influencing zinc uptake removed by serum dialysis.

The mechanisms by which zinc enters mammalian cells have been investigated in several cell systems, but remain to be adequately elucidated.[7] The importance of examining the mechanism of zinc uptake into endothelial cells is highlighted by their role in the delivery of nutrients to peripheral tissues. The mechanism of zinc uptake into endothelial cells has been described as biphasic.[17] Zinc uptake demonstrated saturable kinetics and was indicative of a carrier-mediated mechanism in the range of zinc concentrations likely to exist in vivo.

Our hypothesis was that, in a state of zinc deficiency, the quantity or affinity of zinc transporters on the endothelial cells would be increased, thus enabling the cells to maintain zinc homeostasis. A significant increase in the relative rate of zinc uptake was observed in cells grown in low zinc medium. Subsequent kinetic analysis determined that the maximum rate of zinc uptake (Jmax) was increased, most likely due to an increase in zinc transporter number, in response to zinc deficiency. Similar increases in zinc uptake in response to zinc deficiency have been demonstrated in intestinal cells responsible for absorption. In vivo studies have demonstrated increases in the rate of zinc absorption into the enterocyte in rats fed zinc-deficient diets.[23,24] In vascularly perfused rat intestine, a saturable mechanism of zinc uptake was stimulated by zinc depletion, and the apparent maximal transport rate increased threefold.[25,26] No zinc uptake transporter has ever been demonstrated in intestinal cells, although a zinc exporter has been reported.[27,28] Hepatocytes grown in zinc-deficient medium did not change their rate of zinc uptake.[29] Instead, they maintained zinc homeostasis primarily by increasing their zinc efflux.

Apparent affinity (K) was not affected by the zinc-deficient conditions, relative to either the control group or the zinc back group. This would indicate that the transport mechanism remains the same, whereas the number (Jmax) of transporters changes in response to zinc deficiency. K appeared to increase in cells grown in the zinc back medium compared with the control group. The incubation solutions for measuring zinc uptake at each zinc concentration were identical across zinc status groups; therefore, the difference was most likely in the cells and not the uptake solutions. We cannot explain this apparent zinc-independent change in the affinity of zinc for its uptake mechanism in the zinc back group. The estimated K values were all within the range of zinc concentrations likely to exist in vivo.[8–10]

Zinc uptake by endothelial cells was less responsive to excess zinc conditions than to low zinc conditions. After growing the cells in different zinc concentrations, zinc uptake was measured in all treatments at the same zinc concentration (5 μmol zinc/L). The rate of uptake was not affected by growing the cells in 5 or 10 times the zinc concentration in the control group, indicating that the number of zinc transporters was not reduced by these excessively high zinc environments. A 15-fold increase in the zinc concentration of the medium only reduced the zinc uptake rate by 14%. First, this demonstrates that zinc uptake was relatively adequate in cells grown in the control medium with 5 μmol zinc/L. Second, the endothelial cells might not be able to restrict their uptake of zinc, possibly because zinc concentrations are not often elevated in the plasma. More importantly, because the cells grew well in the high zinc environment without restricting their rate of zinc uptake, they must have relied on other mechanisms to maintain zinc homeostasis. Under similar high zinc conditions, we found that endothelial cells did not increase their metallothionein content.[16] This leads us to speculate that the endothelial cells probably increased their zinc export rate, which was not measured in these studies. This suggests that endothelial cells rely on a different mechanism for zinc uptake than for zinc export. Zinc export proteins have been identified in rat kidney cells,[30,31] and they appear to have similar homology and function to proteins present in some mammalian cells.[27,28] The presence of similar zinc export proteins in endothelial cells remains to be elucidated.

Our kinetic analysis demonstrated that zinc homeostasis in endothelial cells is mediated by more than one mechanism. In response to zinc excess, zinc uptake is not altered, indicating that increased zinc export is probably the principal response. During zinc deficiency, the cells increased their zinc uptake potential. The increased Jmax indicates that this was accomplished by increasing the quantity of zinc uptake transporters. Zinc uptake proteins have been identified in yeast cells,[32,33] but not yet in any mammalian cells. In the future, this model of an in vitro zinc deficiency could be utilized to identify a zinc uptake mechanism.

Acknowledgments

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