Published in final edited form as:

Toxicol Appl Pharmacol. 2007 February 15; 219(1): 33–41. doi:10.1016/j.taap.2006.11.035.

Iron supplement prevents lead-induced disruption of the blood–brain barrier during rat development

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Abstract

Children are known to be vulnerable to lead (Pb) toxicity. The blood–brain barrier (BBB) in immature brain is particularly vulnerable to Pb insults. This study was designed to test the hypothesis that Pb exposure damaged the integrity of the BBB in young animals and iron (Fe) supplement may prevent against Pb-induced BBB disruption. Male weanling Sprague–Dawley rats were divided into four groups. Three groups of rats were exposed to Pb in drinking water containing 342 µg Pb/mL as Pb acetate, among which two groups were concurrently administered by oral gavage once every other day with 7 mg Fe/kg and 14 mg Fe/kg as FeSO₄ solution as the low and high Fe treatment group, respectively, for 6 weeks. The control group received sodium acetate in drinking water. Pb exposure significantly increased Pb concentrations in blood by 6.6-folds ($p < 0.05$) and brain tissues by 1.5–2.0-folds ($p < 0.05$) as compared to controls. Under the electron microscope, Pb exposure in young animals caused an extensive extravascular staining of lanthanum nitrate in brain parenchyma, suggesting a leakage of cerebral vasculature. Western blot showed that Pb treatment led to 29–68% reduction ($p < 0.05$) in the expression of occludin as compared to the controls. Fe supplement among Pb-exposed rats maintained the normal ultra-structure of the BBB and restored the expression of occludin to normal levels. Moreover, the low dose Fe supplement significantly reduced Pb levels in blood and brain tissues. These data suggest that Pb exposure disrupts the structure of the BBB in young animals. The increased BBB permeability may facilitate the accumulation of Pb. Fe supplement appears to protect the integrity of the BBB against Pb insults, a beneficial effect that may have significant clinical implications.

Keywords

Lead; Iron; Blood–brain barrier; Tight junction; Occludin

Introduction

Environmental lead (Pb) intoxication has been known to cause irreversible neurological disturbances by mechanisms remaining to be identified. Children are particularly vulnerable to Pb toxicities for several reasons (Leggett, 1993; Cory-Slechta and Schaumburg, 2000):

they absorb the ingested Pb from the gastrointestinal tract better than do the adults; a greater proportion of systemically circulating Pb gains access to the brain more easily in children, especially those of 5 years old or younger, than in adults; and the developing nervous system in children is far more sensitive to Pb toxicity than the mature adult brains. Evidence in literature also suggests that at low concentrations, Pb disrupts normal blood–brain barrier (BBB) function, resulting in regionally specific increases in permeability to plasma proteins (Dyatlov et al., 1998; Moorhouse et al., 1988; Struzynska et al., 1997; Sundstrom et al., 1985).

The homeostasis of brain microenvironment, which is essential for its normal function, is maintained by the BBB. The BBB is formed by highly specialized endothelial cells whose tight junctions between adjacent cells restrict the paracellular diffusion of hydrophilic molecules. The tight junctions are the intricate combination of transmembrane and cytoplasmic proteins linked to an actin-based cytoskeleton system. Typical tight junction proteins include claudin-1, occludin, and ZO-1 (Huber et al., 2001a; Matter and Balda, 2003). Claudins constitute the backbone of tight junction strands by forming dimers and binding homotypically to claudins on adjacent cells to produce the primary seal of the tight junctions. Occludin, a 65 kDa protein existing in various phosphorylated forms, functions as a dynamic regulatory protein, whose presence in the membrane is correlated with decreased paracellular permeability. The cytoplasmic proteins interacting with transmembrane strands include zonula occludens proteins (ZO-1 and ZO-2), cingulin, AF6, and 7H6 antigen. It is noteworthy that the structure of occludin consists of two extracellular loops, which project into the paracellular space and interact with claudins, and one cytoplasmic domain, which interacts with ZO-1. The multiple functions of occludin render it indispensable for the tightness of the BBB and more vulnerable to insults of toxicants. A previous study (Balda et al., 1996) has shown that occludin presence increases electrical resistance across the junction. The development of the specialized intercellular junctions seems to depend on the appearance of high levels of the occludin and intracellular signaling processes (Rubin and Staddon, 1999). Furthermore, occludin is known to be much more highly expressed in brain endothelial cells than in endothelial cells of nonneural tissue (Hirase et al., 1997). Since occludin expression is down-regulated in various brain disorders accompanied by TJ disruption (Huber et al., 2001b; Davies, 2002), the expression level of occludin is important for TJ maintenance at the mature BBB. Therefore, analyzing the changes of the expression of occludin will provide deeper insight into the maintenance and disruption of the TJ properties at the BBB. To the best of our knowledge, there has been no report in literature investigating the effect of Pb exposure on the function of occludin.

Iron (Fe) is an essential trace element in mammals. Researches in the past have demonstrated that there is an intimate relationship between Pb exposure and Fe metabolism in biological systems. For example, Fe deficiency can lead to an augment in Pb absorption (Bradman et al., 2001; Wright et al., 1999); vice versa, Fe supplement can reduce the Pb absorption from the intestine (Choi and Kim, 2003; Hammad et al., 1996; Kim et al., 2003). However, the questions as to whether or not Fe supplement may protect against Pb toxicity on the BBB have never been investigated.

The hypothesis tested in this report was that Fe supplementation may prevent against Pb-induced disruption of the BBB permeability during rat development. To test this hypothesis, we exposed the weanling rats to Pb in drinking water and supplemented Fe by oral gavage. The integrity of the BBB was assessed by nitric acid lanthanum exclusion test by using transmission electronic microscope (TEM). Moreover, the effect of Pb exposure on the expression of typical tight junction protein occludin was analyzed by the Western blotting technique in order to reveal the mechanism of Pb toxicity on the BBB.

Materials and methods

Materials

SDS, acrylamide, and bisacrylamide were purchased from Bio-Rad Laboratories (Richmond, CA, USA), goat anti rat occludin antiserum from Santa Cruze Biotechnology (Santa Cruz, CA, USA), rabbit anti-goat secondary antibody conjugated with horseradish peroxidase from Amersham Pharmacia Biotech (Piscataway, NJ, USA), standards for iron (1000 µg Fe/mL) and lead (1000 µg Pb/mL) for atomic absorption spectrophotometry (AAS) from Alpha Products (Danvers, MA), and molecular weight standards for Western blotting from Amersham Pharmacia Biotech (Piscataway, NJ, USA). All other chemicals were purchased from Tianjin Kermel Chemical Reagent Development Center. Electronic microscopic studies were conducted at the Electronic Microscope Center of the Fourth Military Medical University using JEM-100SX electronic microscope (Hitachi, Tokyo, Japan).

Animal exposure

Male Sprague–Dawley rats (littermates, Fourth Military Medical University, Xi'an, China) aged 20–22 days weighing 30–50 g upon arrival, were assigned to four groups ($n=12$) such that the group mean body weights were comparable. The animals were housed in stainless-steel cages in a temperature-controlled, 12/12 light/dark room, and allowed to have free access to pelleted semi-purified rat chow (solid, Vital Keao Feed Co., Beijing, China) as well as pre-prepared drinking water. On the third day (age of 22–24 days) after arrival, the animals started to receive Pb in drinking water and/or Fe by oral gavage. The rat chow is a purified, synthetic diet providing essential nutrients for maintenance, growth, gestation, and lactation of laboratory mice and rats. The ingredient of the diet has been consistent and well controlled with proteins (20.60%), fat (4.16%), fibers (4.92%), and carbohydrates (61%). For essential minerals, the diet contains 1.50% calcium, 0.81% phosphorus, 0.40% potassium, 0.07% magnesium, 0.21% sodium, 0.90 ppm lead, 76 ppm iron, 20 ppm zinc, 65 ppm manganese, 15 ppm copper, 3.2 ppm cobalt, 0.6 ppm iodine, 3.0 ppm chromium, and 0.2 ppm selenium.

The Pb exposure paradigm was chosen based on that of Cory-Slechta, since this was known to be associated with subtle developmental deficits (Cory-Slechta et al., 1983; Zhao et al., 1998; Zheng et al., 1996). Pb concentration (342 µg Pb/mL) in drinking water was chosen because Pb-induced brain hemorrhage was seen at high doses (Holtzman et al., 1984; Press, 1977). The drinking water was prepared by dissolving Pb acetate in distilled, deionized water (342 µg Pb/mL). Pb concentrations were verified by an electrothermal atomization AAS. Three groups of rats took the drinking water containing 342 µg Pb/mL as Pb acetate *ad libitum*, among which two of the groups were concurrently given 7 mg Fe/kg and 14 mg Fe/kg as FeSO₄ solution as the low and high Fe group, respectively, for 6 weeks. The Fe doses were administered orally by gavage once every other day according to animal body weight. For the control group, Na acetate with an acetate concentration equivalent to the high dose of Pb acetate was prepared in the same manner. All procedures involving animal studies were in accordance with guidelines of and therefore approved by the local Animal Care and Use Committee.

Collection of blood and tissue samples

At the end of the study, eight animals from each group were decapitated. Blood samples were collected into heparinized syringes and aliquots were taken immediately for the determination of hemoglobin (Hb) concentration from the whole blood by the cyanmethemoglobin method (Azim et al., 2002). The serum samples were analyzed for serum iron, total iron-binding capacity (TIBC), and transferrin saturation (serum iron/TIBC). The whole

blood samples were used for determination of blood Pb by AAS. The serum samples were used for determination of Fe by a flame AAS. The TIBC was determined using a commercially available assay kit (Jiancheng Bioengineering Institute, Nanjing, China). Brain tissues were excised and weighed. The hippocampus, cerebellum, and cerebral cortex were quickly dissected and stored at -80°C for bioassays described below.

TEM study of blood–brain barrier permeability

At the end of the study, four animals from each group were anesthetized with 40 mg/kg sodium pentobarbital, i.p. The heart was exposed and the left ventricles were perfused with 0.9% saline via a catheter through the artery, followed by perfusion with the fixative consisting of one part 4% lanthanum nitrate and two parts 6% glutaraldehyde–0.1 M sodium cacodylate (pH 7.40–7.50) for 2 h. At the end of brain perfusion, brains were excised; the brains were isolated and cut into 1 mm³ pieces and laid on a glass slice. The isolated tissues were immersed in 4% glutaraldehyde for 2 h, and then washed in two changes of PBS. The tissues were immersed in 1% osmium tetroxide for 2 h, and then washed with PBS for 5 min. The specimens were dehydrated with 50%, 75%, 90%, and 100% acetone for 10 min each. After being embedded for 2 h, the specimens were heated at 60°C for 48 h. The sections were dyed with acetic acid uranium and lead, and then observed under TEM.

Western blot analysis of occludin

Hippocampus, cerebellum, and cerebral cortex were homogenized in RIPA buffer (1 g:10 mL) consisting of 50 mM Tris, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.1% SDS, 1% NP-40, 1% deoxycholate, 1% Triton X-100, 10 mM PMSF, and 0.1% protease inhibitors cocktail (Roche, Switzerland). After centrifugation at $12,000\times g$ for 15 min at 4°C , the supernatant was collected and the protein concentration was subsequently assayed by using Bradford method (Bradford, 1976). Equal amounts of total proteins (10 μg per lane) were loaded on 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel, electrophoresized, and finally transferred onto PVDF membrane overnight at 4°C . The molecular weight standards (Amersham Pharmacia Biotech, Piscataway, NJ, USA) were run in parallel. Nonspecific binding sites on the membrane were blocked by incubation with 5% defatted milk powder in a TBS-T solution consisting of 20 mM Tris–Cl, pH 7.6, 137 mM NaCl, and 0.1% Tween-20 for 2 h at room temperature, followed by incubation with antibody against occludin (1:500) in TBS-T with 5% milk overnight at 4°C . The blots were washed and incubated in anti-goat secondary antibody conjugated with horseradish peroxidase (1:500) in TBS-T with 5% milk for 1 h at 37°C . The blots were visualized using West Pico Chemiluminescent kit (Pierce, Rockford, IL, USA), and the density of protein bands was quantified by transmittance densitometry using volume integration with LumiAnalyst Image Analysis software.

Reverse transcription (RT)-PCR amplification of occludin mRNA sequences

Total RNA was prepared from hippocampus, cerebellum, and cerebral cortex using trizol (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Total RNA (1 μg) was RT in a 20 μL reaction using Advantage RT-for-PCR kit (Clontech, Palo Alto, CA, USA) with oligo dT primers according to the manufacturers instructions. The forward and reverse primers for occludin cDNA were 5'-TTGGGACAGAGGCTATGG-3' and 5'-ACCCACTCTTCAA-CATTGGG-3', 622 bp. Amplification was performed with initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 45 s, 53°C for 45 s, and 72°C for 2 min, and a single final extension at 72°C for 7 min. The reaction mixture lacking RT was used as a negative control and β -actin cDNA (5'-GGTCACC-CACACTGTG CCCATCTA-3' and antisense primer 5'-GACCGTCAGG-CAGCTCACATAGCTCT-3', 353 bp) was amplified simultaneously as the internal control. The PCR products were analyzed on a 0.9% agarose gel using LumiAnalyst Image Analysis

software (Roche, Mannheim, Germany). Gene expression values were normalized for β -actin expression and expressed in units relative to the controls.

Statistical analysis

The results were expressed as mean $\pm$ SD. Difference between means was determined by one-way ANOVA followed by a least-significant-difference test for multiple comparisons. A probability value of $p < 0.05$ was regarded to be statistically significant.

Results

Blood and brain Pb level following Pb exposure

Chronic exposure to Pb in drinking water under the current dose regimen resulted in a 6.6-fold increase in blood Pb (BPb) as compared to control rats ($p < 0.05$) (Fig. 1). Concomitant supplement with low dose Fe (7 mg Fe/kg) by oral gavage significantly reduced BPb in Pb-exposed rats; but it did not completely restore BPb to the normal level seen in control rats (Fig. 1). Interestingly, the high dose of Fe (14 mg Fe/kg) did not reduce, but instead increased BPb in Pb-exposed rats (Fig. 1).

Similar to changes in BPb, Pb exposure resulted in 1.5–2.0-fold increases in Pb concentrations among brain tissues examined, with the hippocampus having the highest Pb concentration (Fig. 2). The low-dose Fe supplement significantly reduced Pb levels in all three brain regions examined as compared to the Pb-only group; brain Pb levels after low-dose Fe treatment were not statistically significant different from those in control rats (Fig. 2). The high-dose Fe supplement had no significant effect on brain Pb levels (Fig. 2).

Pb exposure and Fe status in blood and brain

Chronic exposure to Pb among young animals caused about 16%, yet significant reduction of the hemoglobin (Table 1); the results are consistent with reports in the literature (Corpas et al., 2002; Iavicoli et al., 2003). Fe supplement restored the hemoglobin to the normal level. Pb exposure, however, did not alter the levels of serum Fe, TIBC, and transferrin saturation to any statistically significant extent, as compared to those of controls. Interestingly, both low and high doses of Fe supplemental treatment resulted in serum TIBC significantly lower than those of rats treated Pb only or even control rats (Table 1).

While chronic Pb exposure did not alter serum Fe level, it did cause a significant decrease in brain Fe concentrations in all tested regions (Table 1). Fe supplement at both dose levels restored the brain Fe to the normal levels as compared to control rats (Table 1).

Pb toxicity to BBB permeability by TEM

Lanthanum nitrate has been proven lacking the ability to penetrate the BBB and thus widely used as a marker to examine the integrity of BBB by TEM (Bradbury and Deane, 1993; Sundstrom et al., 1985). In control rat brain, the lanthanum stains were exclusively located in cerebral capillary (Fig. 3A). Pb exposure in young animals resulted in the leakage of capillary lanthanum stain to the surrounding brain parenchyma (Fig. 3B); some even invaded into the deep parenchyma area with nerve fibers (Fig. 3C). In Fe supplemented rat brain, lanthanum stains were mainly restricted within the cerebral capillary (Figs. 3D, E). Thus, Fe supplemental treatment appeared to effectively protect the BBB from the damage induced by Pb exposure.

Pb toxicity on tight junction protein occludin

Pb-induced BBB leakage could be due to Pb effect on the tight junction proteins. To test this hypothesis, we used Western blot technique to quantify the expression of occludin in selected brain tissues, including hippocampus, cerebellum, and cerebral cortex. Exposure of young animals to Pb led to a significant decrease in the expression of occludin as compared to the controls (Figs. 4A, B). Fe supplemental treatment at both dose levels restored the expression of occludin to the normal level (Figs. 4A, B).

Pb toxicity on occludin mRNA

To further confirm the changes of occludin protein in hippocampus, cerebellum, and cerebral cortex after experiment, we extended the analysis to the quantity of occludin mRNA. We performed RT-PCR for occludin mRNA. The results demonstrated consistent and significant decrease of occludin mRNA in hippocampus, cerebellum, and cerebral cortex in only Pb-treated (Figs. 5A, B). Fe supplemental treatment at both doses also restored the levels of occludin mRNA to the controls (Figs. 5A, B). These findings are consistent with the results of occludin expression.

Discussion

To the best of our knowledge, this is the first study to demonstrate that Fe supplement can protect against Pb toxicity to the BBB in young animals, a beneficial effect that may have significant clinical implications in humans.

Brain barrier systems including BBB and blood-cerebrospinal (CSF) barrier constitute the first defense line against harmful insults from the blood circulation (Zheng et al., 1991, 2003). Conceivably, any alterations in barriers' structural or functional integrity may impair the normal brain function. Pb exposure is known to produce irreversible neurotoxicity (Muldoon et al., 1996; Schwartz et al., 2000; Stollery, 1996), particularly in young children (Bellinger, 2004; Bressler et al., 1999; Cory-Slechta and Schaumburg, 2000). The high vulnerability of children's brain to Pb toxicity could be partly attributed to the less well-developed brain barrier system, or a possibly high sensitivity of their BBB to Pb insults. Evidence has suggested that Pb can directly act on the barrier structure to increase the permeability of the BBB (Bradbury and Deane, 1993; Dyatlov et al., 1998; Kerper and Hinkle, 1997; Press, 1985; Struzynska et al., 1997; Sundstrom et al., 1985). Others have suggested that the growing capillaries may be the primary target to Pb action. The endothelial bud appears to be particularly sensitive to Pb toxicity, which leads to Pb encephalopathy probably resulting from the death of many of these buds (Press, 1977). By using lanthanum nitrate, a known marker for BBB integrity in TEM studies (Bradbury and Deane, 1993; Sundstrom et al., 1985), we have demonstrated that Pb exposure via drinking water altered the permeability of the BBB, as evidenced by extensive extra-vascular staining not only in areas near the cerebral vasculature, but also in brain parenchyma predominated with neurons and neuroglia. While we did not quantify the extent to which the cerebral microvessels were damaged by Pb treatment, the leakage of BBB appears to be the main reason for elevated Pb concentrations in selected brain regions, especially in hippocampus.

The mechanism by which Pb alters BBB permeability remains elusive. Since the tight junctional protein occludin is closely involved in maintaining endothelial barriers (Kevil et al., 1998a, 1998b; Trocha et al., 1999), alteration of this protein may lead to the opening of inter-endothelial tight junctions. By Western blot analyses, we have clearly shown a decreased expression of occludin in brain areas examined. Since we did not directly determine the occludin expression in enriched brain endothelial fractions in this study, we cannot exclude the possibility that this observation may be partly due to Pb inhibition of

occludin in neuronal cell types. However, our data appeared to suggest that the level of Pb accumulated in brain areas was inversely associated with Pb effect on the expression of occludin protein. Our data are consistent with the reports in literature. Ruan and his colleagues (1999) have observed that accumulation of Pb on the plasmalemma of brain endothelial surface can damage the tight junctions between endothelial cells. Thus, a reduction of occludin protein by Pb treatment may, at least in part, be responsible for an increased BBB permeability following Pb exposure.

At present, the interaction between Pb and Fe in the biological system has been well documented. The increased absorption of ingested Pb in Fe-deficient rats was demonstrated by Six and Goyer (1972) and has been corroborated by others (Angle et al., 1977; Ragan, 1977; Robertson and Worwood, 1978; Flanagan et al., 1979; Wright et al., 1998). Consistently, Fe supplement reduces Pb body burden (Choi and Kim, 2003; Hammad et al., 1996; Kim et al., 2003). Specific cellular importers for Pb are unlikely as the metal serves no nutritional requirement. It is more likely that Pb is inadvertently uptaken through pathways intended for Fe (Bannon et al., 2002). The intestinal mucosa represents a potential site of entry of toxic substances into the blood circulation. Studies suggest that the divalent metal transporter 1 (DMT1) is a transporter for Fe and Pb in the small intestine and regulated in accord with Fe status. This protein is higher in iron deficiency and lower in iron overload in villous enterocytes (Canonne-Hergaux et al., 1999; Fleming et al., 1997; Gunshin et al., 1997; Oates et al., 2000; Trinder et al., 2000; Zoller et al., 2001). Therefore a plausible system could exist where variations of Fe stores within the normal range do not cause a significant increase in Pb absorption, but during periods of Fe deficiency, the level of DMT1 becomes high enough to allow for increased Pb absorption. Adequate Fe intake may serve a dual function in preventing the absorption of Pb (Bannon et al., 2002). First, intake of Fe lowers the number of Pb transporters in the gut since DMT1 regulation in the duodenum is sensitive to levels of Fe uptake (Tallkvist et al., 2000; Morgan and Oates, 2002). Second, since DMT1 has a much higher affinity for Fe over Pb, the presence of Fe in the gut can competitively inhibit the uptake of Pb. Fe has been shown capable of completely inhibiting Pb uptake by DMT1 (Bannon et al., 2002). The mentioned biological mechanisms appear in accord with our studies suggesting the protective effects of Fe supplementation against Pb toxicity. Our results indicated that this protective effect was primarily due to an inhibited Pb absorption from the gastrointestinal track; a decreased Pb absorption further lowered the total body burden of Pb. In the brain, the disposition, especially cellular uptake, of Pb has been addressed sparingly. We do not clearly know how Pb crosses the BBB, nor do we understand the mechanism by which Pb is taken up by cells in the brain. Our results indicated that a concurrent supplement of Fe (7 mg Fe/kg) in Pb treatment animals significantly reduced blood levels of Pb. Consequently, the Pb concentrations in brain regions examined were also significantly reduced. The reduced brain Pb level could be also due to Fe competition for Pb uptake at brain barriers. At the normal condition, Fe deficiency states do not seem primarily to affect the brain, suggesting that it is capable of possibly reusing iron or mobilizing it from other sources to maintain normal physiological functions. In addition, high levels of serum Fe, as occur in hemochromatosis, do not lead to Fe accumulation in the CNS (Anderson and Powell, 2002) possibly indicating a unique homeostatic mechanism at either the level of the endothelial cells in the CNS or in cells that surround CNS capillaries, namely astrocytes. In our studies, we interestingly found that Fe supplement protected the integrity of cerebral vasculature from Pb insults. Courtois et al. (2000) reported that treatment with 50–400 μ M Fe (II)-ascorbate on tight junction permeability in Caco-2 cells for 24 h did not alter TEER values. The localizations and expressions of ZO-1, occludin, and E-cadherin appeared unaffected by treatment with 50 and 100 μ M FeSO₄ (Ferruzza et al., 2002). These results suggest that Fe itself does not induce the expression of occluding protein. We also found that Fe supplement restored the expression of brain occludin to the normal level. Within the context of an overall reduction

of Pb concentrations in blood following Fe supplement, it is highly possible that the protective effect of Fe on brain barrier integrity is a result of reduced blood Pb. However, other mechanisms, such as the competition of Fe for Pb cellular targets (transporters, binding ligands, or enzymes) on the barrier structure and possible repair of Pb-induced damage by Fe, cannot be excluded and deserve further investigation.

It should be noted that the high dose of Fe supplement (14 mg Fe/kg) did not lower blood Pb, nor did it reduce brain Pb concentrations, although it did maintain the structural integrity of cerebral vasculature and restore the expression of occludin in Pb-treated animals. This discrepancy could be due to the differential sensitivity of brain parenchyma and brain endothelia to Fe treatment. It is possible that the expression of occludin may be sensitive to the Fe concentration in brain endothelial cells. Alternatively, Pb exposure may primarily target at the formation of the tight junction during the developmental stage, but may cause less damage to developmentally complete, mature BBB. The initial breakdown of BBB at the early stage due to Pb exposure may lead to the accumulation of Pb in brain tissues. This accumulation may persist and not be reversed throughout the entire experiment, even with a restored BBB structure by high dose of Fe supplement as shown in our TEM data in Fig. 3E. Thus, the caution must be taken that Pb-induced neurotoxicity seems unlikely to be solely associated with body Fe homeostasis.

In conclusion, our data demonstrate that Pb exposure in young animals disrupts the structure of the BBB, possibly due to Pb inhibition of the expression of tight junctional protein occludin. The increased BBB permeability may facilitate the accumulation of Pb in brain parenchyma. Low-dose Fe supplement reduces Pb concentrations in the blood and brain tissues. Moreover, we found that Fe supplement protects the integrity of the BBB against Pb insults. These observations shall assist our understanding of the mechanism of Pb toxicity in children for a better design of the therapeutic strategy for children with lead poisoning.

Acknowledgments

The authors gratefully acknowledge Mr. Gao SB and Mr. Xu DL for their technical assistance. This research was supported in part by National Natural Science Foundation of China Grant # 30371228 (JC) and USA-National Institute of Environmental Health Sciences Grant RO1-ES08146 (WZ).

Abbreviations

CNS	central nervous system
Hb	hemoglobin
TIBC	total iron-binding capacity
TJ	tight junction
NTBI	non-transferrin bound iron
BBB	blood–brain barrier

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Fig. 1.

Pb concentrations in blood following Pb exposure and concomitant Fe supplement treatment. Weanling male Sprague–Dawley rats were exposed to Pb as Pb acetate in drinking water (342 µg Pb/mL) daily and concomitantly administered orally by gavage once every other day with 7 mg Fe/kg (as the Low Fe Supplement group) or 14 mg Fe/kg (as the High Fe Supplement group) as FeSO₄ solution, for 6 weeks. Data represent mean±SD (*n* =8), *: *p* <0.05 compared to controls; #: *p* <0.05 compared to Pb-alone group.

Fig. 2.

Pb concentrations in brain tissues following Pb exposure and concomitant Fe supplement treatment. Animal dose regimen has been described in the legend to Fig. 1. Data represent mean $\pm$ SD ($n=8$), *: $p<0.05$ compared to controls; #: $p<0.05$ compared to Pb-alone group.

Fig. 3. Iron supplement prevents against Pb-induced BBB disruption. Animal dose regimen has been described in the legend to Fig. 1. Rat brains were perfused with lanthanum nitrate prior to tissue preparation for TEM examination. (A). Control rat brain ($\times 800$). Lanthanum granules were closely aligned along the endothelial membrane of cerebral capillaries and not found anywhere in brain parenchyma. (B). Pb-exposed brain ($\times 600$). Lanthanum granules were found in brain parenchyma nearby the capillary vessel. The arrow indicates that lanthanum granules escaped from the cerebral capillary. (C). Pb-exposed brain ($\times 800$). The arrows indicate lanthanum granules presented in the gaps between myelinated nerve fibers. (D). Brain of Pb-exposed rat concomitantly treated with low dose of Fe ($\times 600$). Lanthanum granules were not found in brain parenchyma. (E) Brain of Pb-exposed rat concomitantly treated high dose of Fe ($\times 600$). Lanthanum granules were not found in brain parenchyma.

Fig. 4.

Western blot analyses of occludin expression in rat brain. Animal dose regimen has been described in the legend to Fig. 1. Cytosolic fractions were prepared for Western blot. (A). Representative blots of occludin proteins in brain cortex, cerebellum and hippocampus. (B). Occludin protein levels determined by densitometry were initially normalized to those of β -actin. The changes were then expressed as the percentage of controls. Data represent Mean $\pm$ SD ($n=3$), *: $p<0.05$ compared to controls; #: $p<0.05$ compared to Pb-alone group.

Fig. 5. Reverse transcription (RT)-PCR amplification of occludin mRNA sequences in rat brain. Animal dose regimen has been described in the legend to Fig. 1. Total RNA was isolated from brains and occludin mRNA sequences were analyzed by RT-PCR. (A). Representative blots of occludin mRNA in brain cortex, cerebellum, and hippocampus. (B). Expressions of occludin mRNA were quantified by normalized to those of β -actin mRNA. The changes were then expressed as the percentage of controls. Data represent mean $\pm$ SD ($n=3$), *: $p<0.05$ compared to controls; #: $p<0.05$ compared to Pb-alone group.

Table 1

Effect of Pb exposure on hematological parameters and brain Fe concentration

Parameters	Control	Pb treated		
		Pb alone	Low Fe	High Fe
Serum Fe (μg/dL)	(18.4±6.4)×10 ³	(15.1±4.0)×10 ³	(18.2±7.2)×10 ³	(21.7±8.2)×10 ³ #
Hemoglobin (g/dL)	16.1±2.29	13.6±1.29**	17.1±2.12##	15.4±3.26#
Fe% saturation (%)	39.3±15.2	34.5±12.2	48.5±27.1	58.0±19.9#
TIBC (μg/dL)	(47.5±4.3)×10 ³	(45.4±6.7)×10 ³	(32.9±6.6)×10 ³ **##	(37.6±7.4)×10 ³ **##
Brain Fe (μg/g)				
Cortex	16.4±1.77	12.7±2.87*	18.1±1.43##	16.2±2.54#
Hippocampus	21.9±3.74	16.9±3.89*	23.1±5.42#	24.1±3.11##
Cerebellum	19.7±3.31	14.5±4.28*	20.4±6.48#	25.6±1.79*##

Rats were exposed to Pb in drinking water (342 mg Pb/L as Pb acetate) for 6 weeks. Some animals received concomitant Fe treatment as Fe sulfate by oral gavage once every other day throughout experimentation. Data represent mean±SD, *n*=8.

* *p*<0.05,

** *p*<0.01 as compared to values in control rats;

p<0.05,

p<0.01 as compared to values in the Pb-alone group.

Low-Fe: rats received 7 mg Fe/kg; High-Fe: rats received 14 mg Fe/kg.