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Toxicology of Choroid Plexus: Special Reference to Metal-Induced Neurotoxicities

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Abstract

The chemical stability in the brain underlies normal human thinking, learning, and behavior. Compelling evidence demonstrates a definite capacity of the choroid plexus in sequestering toxic heavy metal and metalloid ions. As the integrity of blood-brain and blood-CSF barriers, both structurally and functionally, is essential to brain chemical stability, the role of the choroid plexus in metal-induced neurotoxicities has become an important, yet under-investigated research area in neurotoxicology. Metals acting on the choroid plexus can be categorized into three major groups. A general choroid plexus toxicant can directly damage the choroid plexus structure such as mercury and cadmium. A selective choroid plexus toxicant may impair specific plexus regulatory pathways that are critical to brain development and function, rather than induce massive pathological alteration. The typical examples in this category include lead-induced alteration in transthyretin production and secretion as well as manganese interaction with iron in the choroid plexus. Furthermore, a sequestered choroid plexus toxicant, such as iron, silver, or gold, may be sequestered by the choroid plexus as an essential CNS defense mechanism. Our current knowledge on the toxicological aspect of choroid plexus research is still incomplete. Thus, the future research needs have been suggested to focus on the role of choroid plexus in early CNS development as affected by metal sequestration in this tissue, to explore how metal accumulation alters the capacity of the choroid plexus in regulation of certain essential elements involved in the etiology of neurodegenerative diseases, and to better understand the blood-CSF barrier as a defense mechanism in overall CNS function.

Keywords

choroid plexus; neurotoxicity; lead; manganese; mercury; cadmium; arsenic; copper; iron; silver; zinc; gold; transthyretin; thyroxine; transferrin receptor; aconitase; protein kinase C; Alzheimer's disease; Parkinsonism; glutathione; metallothionein

INTRODUCTION

The choroid plexus constitutes the blood-cerebrospinal fluid (CSF) barrier. One side of this barrier confronts the systemic compartment, which, by blood circulation, embraces most tissues and organs except brain. The other side of the blood-CSF barrier faces the cerebral compartment whose extracellular circulation is completely secluded from blood stream. Thus, the structural and functional integrity of this barrier is crucial to the homeostasis of the internal milieu of the central nervous system (CNS). The choroid plexus regulates the chemical composition of the cerebral compartment by rigorously restricting access of substances from the blood to the CSF, by producing and secreting essential materials toward the CNS, and by transporting substances bidirectionally between the blood and CSF. The impairment of this barrier has been reportedly associated with certain clinical encephalopathies (Jorgensen, 1988; Levine, 1987; Ormerod and Venkatesan, 1970; Philip et al., 1994; Rudin, 1981).

Owing to its unique position between the blood and cerebral compartment, the choroid plexus is destined to be the target for toxic organic and inorganic chemicals. Certain heavy metals and metalloids such as lead, mercury, cadmium, and arsenic, by as yet undefined mechanisms, tend to accumulate in the choroid plexus at concentrations much greater than those found in the CSF and elsewhere in brain tissues. These metals may either cause morphological damage to the barrier's structure, or induce the subtle alterations in the barrier's function. Nonetheless, whether and how the dysfunction of the choroid plexus ultimately leads to neurological and neurobehavioral disorders has received little attention.

The present article reviews the current understanding of the toxicological aspect of the choroid plexus with a particular reference to metal-induced neurotoxicities. The anatomical and physiological bases on which the choroid plexus becomes the target of xenobiotics will be first examined. This will be followed by the classification of metals acting on the choroid plexus according to their mechanisms of action. Much of this article will then focus on the effect of toxic metals on the choroid plexus upon unwanted exposure, and the possible consequences of choroid plexus dysfunction in the context of neurological disorders. Finally, the implications of the choroid plexus in neurotoxicology and future research needs concerning the toxicology of the choroid plexus are discussed.

PHYSIOLOGICAL BASIS OF THE CHOROID PLEXUS AS A TARGET FOR XENOBIOTICS

As a blood-CSF barrier, the choroid plexus possesses several unique anatomical and physiological features that are entirely different from the rest of brain tissues. First, there appears a discrepancy between its physical size and the surface area. The size of the mature choroid plexus relative to brain varies widely among vertebrates, but is typically less than 5% of brain weight (Cserr et al., 1980). In normal human subjects, the choroid plexus weighs about 2 to 3 g. Given a small percentage of the choroid plexus in overall brain weight, one might conclude that the choroid plexus may contribute less than does the blood-brain barrier to the regulation of the homeostasis of the cerebral compartment. However, as far as the surface area is concerned, the blood-CSF barrier is by no means insignificant in

governing brain chemistry. In comparison to its small tissue mass, the choroid plexus has a very large surface area. Besides the primary microvilli, the entire choroid plexus is so pleated that it creates the secondary multiform macrovilli, which greatly increase the total surface area of the tissue. As estimated from the choroid plexus of 1-month-old rats, which is typically 2–3 mg of the wet weight, the total apical surface area of the choroidal epithelium approximates 75 cm², about one half that of the blood-brain barrier (155 cm²) (Keep and Jones, 1990). Thus, the broad surface area of the blood-CSF barrier ensures an efficient exchange of materials between the CSF and blood compartments. From the toxicological point of view, the extended surface area increases the chances of the tissue being exposed to xenobiotics from either side of the barrier.

Second, one of the important factors in determining CNS homeostasis pertains to the rate of entrance of the materials into the cerebral compartment. To many less lipid-soluble solutes, their disposition in brain relies largely on the equilibrium among a number of elements, which are coordinated by both blood-brain and blood-CSF barriers, such as the blood flow at local area, interstitial fluid (ISF) exchange rate, CSF production and circulation, and composition of both ISF and CSF. In fact, the blood flow to the choroid plexus of experimental animals (sheep and rats) ranges between 4 to 6 ml/min/g, which is about 3–5 times faster than those estimated to many other brain regions (0.9–1.8 ml/min/g) (Davson and Segal, 1996; Maktabi et al., 1990; Page et al., 1980; Schalk et al., 1989). The fast blood flow to the choroid plexus is expected with respect to its physiological role in secretion of CSF and in delivery of substances to the cerebral compartment. The rapid blood flow at the choroid plexus, on the other hand, warrants an efficient influx of chemicals, some of which are potentially toxic, into the plexus tissue and possibly into the CSF.

Third, there is a structural discrepancy in tight junctions between the blood-brain barrier and blood-CSF barrier. The tight junctions between the epithelial cells in the blood-CSF barrier seem less effective, or somewhat “leakier,” than those between endothelial cells of the blood-brain barrier (Davson and Segal, 1996; Johanson, 1995). Taking electrical resistance across the barrier as an example, a well-established epithelial barrier model in Trans-well culture device usually possesses a trans-epithelial resistance of 100–200 ohms · cm² (Zheng et al., 1998a, 1999a). In comparison, the trans-endothelial resistance of the endothelial cells co-cultured with astrocytes in the similar device can achieve a trans-endothelial resistance as high as 500–800 ohms · cm² (Abbruscato and Davis, 1999; Rutten et al., 1987). How this in vitro difference in trans-barrier resistance eventually resembles that in vivo is unclear. Conceivably, however, this leaky structure may provide an easy path for xenobiotics to enter the cerebral compartment.

Finally, the unique anatomical location of the choroid plexus within the cerebral ventricles may be associated with certain metal-induced neurotoxicities. For example, the hippocampus, an area that coordinates memory and learning, has been considered the primary target for Pb-induced learning defects (Campbell et al., 1982; Slomianka et al., 1989). Anatomically, the hippocampus extends from cortex and folds inward to become a part of the floor in the inferior horn of the lateral ventricles (Martin, 1996). Interestingly, the hippocampal formation is immediately adjacent to the lateral choroid plexus where the Pb

accumulates to an extraordinary amount (Friedheim et al., 1983; Zheng et al., 1991, 1996, 1998a).

CLASSIFICATION OF TOXICANTS ACTING ON THE CHOROID PLEXUS

The toxicants, including metals that act on the choroid plexus, are classified as general choroid plexus toxicants, selective choroid plexus toxicants, and sequestered choroid plexus toxicants according to the mechanisms of their actions.

General choroid plexus toxicants usually accumulate in the choroid plexus and cause the substantial damage to the choroid plexus structure. The deposition of toxic metals in the choroid plexus and the subsequent morphological changes in the structure may not only permit the metals themselves to diffuse into the brain, but also facilitate the entrance of other neuroactive toxicants to the brain. However, our knowledge on this aspect is still incomplete. Metals in this category include mercury, cadmium, and arsenic.

Selective choroid plexus toxicants usually do not directly alter choroid plexus' permeability, nor do they produce massive pathophysiological alterations seen in clinics. However, the metals en route to the brain may selectively act on certain critical regulatory functions of the choroid plexus, giving rise to profound neurotoxic consequences. This group of toxic metals include lead, manganese, copper, and tellurium.

Sequestered choroid plexus toxicants, which are also called barrier stored toxicants, may deposit in or be sequestered by the choroid plexus. Nevertheless, the sequestration of these metals in the tissue has not been associated with any known pathological or pathophysiological consequences to the blood-CSF barrier. Metals in this category include iron, silver, zinc, and gold.

GENERAL CHOROID PLEXUS TOXICANTS

Mercury (Hg)

Mercury is an important metal pollutant in the environment. The release of organic mercurial compounds from the heavy industry into the environment has led to human epidemic tragedies in Japan and Iraq (Clarkson, 1987). Mercury exists in the environment in inorganic mercurials and organic aryl- and alkyl-mercurials. In the body, inorganic mercurial salts poorly penetrate the blood-brain barrier and thus produce less neurotoxicity, whereas more neurotoxic mercury vapor can diffuse through lungs to blood and then into the brain. Organic mercurials, particularly alkyl-mercurials such as methylmercury (MeHg), have a high lipid solubility and therefore bear the highest toxicological significance.

Organic Mercury—Following exposure, MeHg accumulates in the choroid plexus. A patient suffering from Minamata disease in 1956 died about 26 years later. Autopsy data showed a high total mercury amount in the brain. Deposition of mercury was clearly exhibited histochemically in microglial cells and Bergmann's glial cells, neurons of specific brain areas, and in the epithelial cells of the choroid plexus (Takeuchi et al., 1989). Studies in MeHg-treated animals also demonstrate a significant accumulation of mercury in the choroid plexus of the third, fourth, and lateral ventricles (Moller-Madsen, 1990).

Subcellularly, mercury is present within the lysosomes of the epithelial cells (Moller-Madsen, 1991).

Using fluorescent Evans blue-protein complex as a permeability indicator, Steinwall and Olsson (1969) demonstrated that treatment with mercury produced a high permeability to the complex, resulting in heavy fluorescence in choroidal stroma and epithelia. The result indicated an impaired integrity of choroidal epithelial cells.

Whether and how MeHg is transported at the choroid plexus is unknown. Because of its high lipophilicity, MeHg by itself is capable of diffusing through the cell membrane without mandating a specific carrier system. Nonetheless, an active transport mechanism at the blood-brain barrier appears to drive MeHg into the brain. MeHg presumably conjugates with endogenous cysteine to form a complex, which has a structure similar to methionine. This complex can be transported by a carrier-mediated transporter for methionine in brain capillaries. The proposed mechanism was supported by the observations: (1) that injection of L-cysteine in vivo accelerated MeHg uptake into brain; (2) that the uptake of MeHg at the blood-brain barrier was saturable following injection of MeHg-cysteine complex; and (3) that concomitant administration of methionine inhibited MeHg-cysteine uptake (Aschner and Clarkson, 1988, 1989; Hirayama, 1980). Further, it was thought that plasma MeHg-glutathione complex may serve as a source of MeHg-cysteine (Kerper et al., 1992). Whether the similar transport mechanism operates in the choroid plexus remains unexplored.

Inorganic Mercury—Inorganic mercurial salts are generally less neurotoxic than organic mercurial compounds. Yet, some inorganic mercurial products such as mercuric chloride (HgCl_2) can act as a general or direct choroid plexus toxicant. Inorganic mercury compounds are highly concentrated in the choroid plexus. In one clinical case, a 63-year-old woman took inorganic mercury-containing laxative for 25 years for the treatment of chronic constipation. Postmortem pathological examination showed that many small punctuate granules, representing the deposits of mercury, accumulated in the choroid plexus. Further analysis of the tissue samples from different brain regions revealed that the choroid plexus contained mercury about 5 and 45 times higher than those seen in brain cortex and in the CSF, respectively (Davis et al., 1974). In animal studies, the choroid plexus accumulates mercury following administration of inorganic mercury and ruptures after a prolonged exposure (Berlin and Ullberg, 1963a; Brun et al., 1976; Moller-Madsen, 1990; Placidi et al., 1983; Steinwall and Olsson, 1969; Suda et al., 1989; Zheng et al., 1991). Using photo-emulsion histochemical techniques, Suda et al. (1989) identified condensed mercury granules in choroidal epithelial cells from rats. It is unclear, however, if these mercury-induced granules are similar to the cellular inclusion bodies as identified by other investigators.

Cadmium (Cd)

Cadmium is mainly used for electroplating (about 29% of year production) in modern industry. Kidney and bone are the primary target organs for cadmium toxicity. In severe poisoning, the Itai-Itai disease identified in the Japanese is believed to be due to cadmium

interference of skeletal calcium and phosphate balance. Few neurological symptoms, however, have been related to cadmium intoxication in clinics.

As a general choroid plexus toxicant, cadmium directly destroys the plexus ultrastructure. In both chronic (22 weeks) and acute (1–24 days) exposure models, the levels of cadmium in the choroid plexus found were high, while cadmium in the CSF fell below the detection limit (Arvidson, 1986; Arvidson and Tjalve, 1986; Valois and Webster, 1987, 1989a; Zheng et al., 1991). A postmortem human study revealed that the cadmium concentration in the choroid plexus was about 2–3 times higher than that found in the brain cortex (Manton et al., 1984). Cadmium-produced deterioration of the plexus structure can be characterized by the loss of microvilli, a rupture of the apical surface, and an increased number of blebs (Arvidson and Tjalve, 1986; Berlin and Ullberg, 1963a,b; Valois and Webster, 1987, 1989a,b). Cellular debris present in the ventricular lumen may result from the breaking of the apical membrane. Subcellularly, the epithelial cells display an abnormally high number of cytoplasmic vacuoles and lysosomes with condensed or irregular nuclei (Valois and Webster, 1987).

Upon exposure, cadmium induces and thereupon binds to metallothionein (MT) in various organs (Kagi and Kojima, 1987; Zheng et al., 1990). The choroid plexus also expresses MT proteins. Nishimura et al. (1992) observed a strong MT immunostaining in ependymal cells and choroid plexus epithelium in younger rats (1–3 weeks old) poisoned with cadmium. Thus, the sequestration of cadmium by MT may partly contribute to the high accumulation of cadmium in the choroid plexus.

Cadmium-induced injury in the cerebral microvessels is thought to be associated with oxidative stress. Following in vivo cadmium exposure, there was an early increase followed by a later decrease in microvessel enzymes involved in cellular redox reactions, such as superoxide dismutase, glutathione peroxidase, and catalase. Thus, a depletion of microvessel antioxidant defense systems and a resultant increase in lipid peroxidation may provoke microvessel damage (Christensen and Fujimoto, 1984; Shukla et al., 1996). It is possible, although not observed as yet, that the similar cytotoxic mechanism may occur in the choroid plexus of cadmium-intoxicated animals.

Arsenic (As)

Arsenic is a metal of wide occurrence. The intoxication can result from polluted soil, water, food, pesticides, herbicides, as well as occasional medicinal uses. Arsenic is a direct vascular toxicant. Following acute exposure in humans, petechial hemorrhage occurred in several brain areas. The resultant cerebral edema may explain the clinical symptoms such as headache, lethargy, delirium, coma, and intracerebral hemorrhage (Beckett et al., 1986).

From animal studies, the choroid plexus retains arsenic upon exposure to melaminylthioarsenite and sodium arsenate (Friedheim et al., 1983; Zheng et al., 1991). When rabbits received an intravenous injection of 2 mg As (as sodium arsenate)/kg, the arsenic concentration was much greater in the lateral choroid plexus than in the CSF (40-fold higher) and brain cortex (13-fold higher) (Zheng et al., 1991). Studies in dogs gave rise to similar outcomes. Arsenic concentration in the choroid plexus built up to high multiples

of that in the blood and CSF. The choroid plexus to blood ratio of arsenic ranged from 18 to 14,102. One study dog with extremely high choroid plexus arsenic had an extensive intracerebral hemorrhage (Friedheim et al., 1983). It seems likely that both intracerebral blood vessels with their special endothelium and the choroid plexus tend to store arsenic and rupture when overloaded.

Arsenic is a known mitochondrial phosphorylation uncoupler. As(III) molecules have a strong affinity to sulfhydryl groups of tissue proteins. As(V), on the other hand, possesses the molecular configuration enabling it to mimic the oxyanion phosphate. Both arsenic species inhibit mitochondrial energy production. How the mitochondrial toxicity initiated by arsenic affects the choroid plexus function is unclear, and deemed to be an interested subject for future research.

SELECTIVE CHOROID PLEXUS TOXICANTS

Lead (Pb)

Lead-induced learning defects in children represent a major global tragedy associated with environmental pollution. In the United States, it has been estimated that 1.7 million children have elevated blood lead (Brody et al., 1995). There appears to be no threshold with regard to the association between blood lead concentration and intelligence (Finkelstein et al., 1998). Various groups of investigators have hypothesized mechanisms by which Pb may alter biochemical, pharmacological, and molecular processes in the CNS. Those include (but are not limited to): Pb interactions with cellular proteins (Goering, 1993); inhibition of voltage-sensitive calcium channels (Oortgiesen et al., 1993); perturbation of synaptic transmission of a number of neurotransmitters (Cory-Slechta et al., 1993; Oortgiesen et al., 1993); alteration of the development and growth of neurons and astroglia in culture systems (Legare et al., 1993), and direct interaction with essential metal ions in the brain (Simons, 1993). It is not at all clear, however, which (if any) of these mechanisms is responsible for the developmental deficits attributable to low level Pb exposure.

Lead Exposure and Sequestration by the Choroid Plexus—Lead accumulates in the choroid plexus to a great extent. Friedheim et al. (1983) found that lead in human choroid plexus increased significantly with age, while lead in the brain did not. Manton et al. (1984) further reported a 100-fold increase of lead in human choroid plexus compared with that in the brain cortex. A significant aspect of these findings is that an age-related accumulation of lead in a particular tissue inside human brain was first emphasized, a phenomenon possibly associated with environmental exposure.

Animal studies also indicate that accumulation of lead in the choroid plexus is both dose-dependent and time-related (O'Tuama et al., 1976; Zheng et al., 1991). The concentrations of lead in the choroid plexus increased proportionally with the increase in dose, whereas lead concentrations in the brain cortex and CSF did not change. Following acute administration of lead acetate (50 mg/kg, ip), the lead concentration in the lateral choroid plexus was 57 times greater than in the brain cortex. This level did not reach a plateau even at 24 hours (Zheng et al., 1991). In a chronic lead exposure study, a dose regimen (50 and 250 µg Pb/ml in drinking water for 90 days) produced blood lead levels of 18 and 49 µg/dl,

respectively, in each group, which mimicked, to a more or less extent, the environmental exposure level in children. Rats under this long-term, low-level exposure scheme showed a significant accumulation of lead in the choroid plexus, which was about 7-fold compared to the control choroid plexus at day 30. Prolonged exposure for 60 and 90 days did not further increase Pb deposition in the choroid plexus, suggesting a possible saturation with Pb by 30 days (Zheng et al., 1996).

Effect of Lead on Transthyretin in CSF—The choroid plexus manufactures and secretes proteins for the extracellular compartment of the CNS. Of the proteins in the CSF, transthyretin (TTR or prealbumin) is exclusively produced and secreted by the choroid plexus in the CNS. TTR is a 55,000-Dalton protein consisting of four identical subunits in a tetrahedral symmetry (Herbert et al., 1986; Ingenbleek and Young, 1994). Plasma TTR (~180 µg/ml) originates from the liver. On a tissue weight basis, the choroid plexus contains 10 times more TTR mRNA than liver, and synthesizes TTR at a rate of 13 times faster than the liver (Dickson et al., 1985; Schreiber et al., 1990). TTR in CSF, however, is not derived from the liver, nor is its production by the choroid plexus dependent upon the hepatic activity (Aldred et al., 1995; Dickson et al., 1986; Weisner and Roething, 1983).

TTR is the major protein of CSF (~15 µg TTR/ml of CSF), making up 25% of total CSF protein (Aldred et al., 1995). Its importance for CNS development is likely evidenced by the fact that it is present in very high concentration during pre-natal and early post-natal life. In children, TTR transports thyroid hormones (thyroxine, T₄ and triiodothyronine, T₃) and retinol in the body. Thyroid hormones, upon entering the circulation, are reversibly bound to a set of proteins including thyroxine-binding globulin, TTR, and albumin. These binding proteins exert a “buffering” action that allows thyroid hormones to be released if thyroidal secretion decreases, or to be bound if their secretion increases. In human, TTR is the major thyroid hormone binding protein in the CNS conveying about 60–80% of CSF thyroxine (Herbert et al., 1986; Larsen and DeLallo, 1989). The binding of thyroxine to CSF TTR allows a fine control of the levels of thyroid hormone in the cerebral compartment. Evidence has shown that the choroid plexus transports thyroid hormones from blood to CSF via TTR synthesis in the choroidal epithelia (Chanoine et al., 1992; Dratman et al., 1991; Southwell et al., 1993); small portion of thyroxine may also enter the brain across the blood-brain barrier (Blay et al., 1993). It should be noted that the thyroid hormones must be transported and do not passively diffuse across brain barriers (Ingenbleek and Young, 1994; Schreiber et al., 1990).

Chronic lead exposure selectively reduces TTR levels in the CSF (Zheng et al., 1996). When the weanling rats were exposed to Pb in drinking water for a prolonged period (1 to 3 months), there was a 36 – 42% decrease of CSF concentrations of TTR in lead-treated animals, an effect that was highly significantly associated with the dose of lead, exposure time, and the dose-by-time interaction. The alteration of CSF TTR by lead can be characterized by (1) a rapid decline of CSF TTR, and (2) a direct inverse association between the % of reduction of CSF TTR and lead concentration in the choroid plexus. The decline of CSF TTR occurred early at the first time point, i.e., 30 days. Exposure to lead for 60 and 90 days did not further depress CSF TTR.

A decreased TTR in CSF could be due either to a diminished inflow from the source of the choroid plexus or an accelerated clearance of TTR in CSF, or both. Since the choroid plexus is the only source of cerebral TTR, a high accumulation of lead in this tissue may damage the capacity of the choroid plexus in producing TTR. To explore this possibility, the pulse-chase study offers technical benefits. It allows labeling the newly synthesized TTR molecules with [^{35}S]methionine in the cultured cells and then chase these [^{35}S]-labeled TTR molecules in cells as well as in culture medium within selected time frame. Upon lead exposure, there was a significant inhibition of total production of [^{35}S]TTR by 34–37% ($P < 0.01$). Moreover, lead exposure greatly suppressed the secretion of newly synthesized [^{35}S]TTR from epithelial cells to the culture media by 7.5 fold as compared to the controls (Zheng et al., 1999a).

As a carrier molecule, a decline in TTR production and secretion to the CSF would ultimately affect the transport of the molecules TTR carries. Thus, an in vitro two-chamber transport model, which allows the culture media in both chambers to be separated by a barrier of confluent monolayer of epithelial cells grown on a permeable membrane, was developed in this laboratory. [^{125}I]T₄ can be added to one side of the barrier. By monitoring [^{125}I]T₄ in both sides (or chambers) of barrier, examination of the transport kinetics of thyroxine across the choroidal epithelial barrier become possible. When [^{125}I]T₄ was added to the outer chamber (contacting the basement of epithelial cells), the radioactivity migrated from the outer to inner chamber (contacting apical surface of epithelial cells) to a higher concentration in the inner chamber, indicating an active transport of T₄ from basal to apical interface of the barrier (Southwell et al., 1993; Zheng et al., 1998a). On the same model, lead exposure reduced the initial release rate constant (k_r) of [^{125}I]T₄ from the cell monolayer to the culture media in outer chamber, and impeded the transepithelial transport of [^{125}I]T₄ from the basal to apical side of epithelial cells by 27%. These in vitro data indicate a selective effect of Pb on TTR-T₄ transport by the choroid plexus (Zheng et al., 1999a).

A recent human study conducted in this laboratory further substantiates that the thyroid hormone (T₄) in the CSF may be altered both as a function of CSF-lead and CSF-TTR (Cheung et al., 1999). Seventy-nine CSF samples were collected from randomly selected patients who were requested for clinical diagnosis of CSF chemistry. Among this human population, CSF concentrations of TTR ranged between 0.77–8.15 $\mu\text{g}/\text{mg}$ of CSF proteins; T₄ between 0.10 – 6.7 ng/mg ; and Pb between 0.05–3.84 $\mu\text{g}/\text{dl}$. One of the distinct outcomes was a significant association between CSF TTR and CSF T₄ ($r = 0.34$, $P < 0.005$), which strengthens the role of TTR in T₄ transport in the cerebral compartment. The decline in CSF TTR was inversely associated with the rise in CSF lead ($r = -0.31$, $P < 0.01$). An inverse correlation between CSF T₄ and CSF lead was also observed ($r = -0.28$, $P < 0.05$).

Thyroid hormones have striking effects on the CNS, particularly during the developmental period. Deficiency of thyroid hormones during this period produces multiple morphological, biochemical, and electrophysiological alterations of neurons and neuroglia (Dussault and Ruel, 1987). In children, deprivation of thyroid hormones causes irreversible mental retardation (Glorieux et al., 1983; Smith et al., 1957). Recently, Thompson (1996) has isolated and identified the genes that are expressed in response to thyroid hormones in developing rat brain. Notably, the TTR gene in the choroid plexus is expressed early in the

fetal development, a phenomenon consistent with the importance of the thyroid hormones in embryonic brain development (Cavallaro et al., 1993; Thomas et al., 1989). The results from this laboratory are consistent with the hypothesis that normal synthesis and secretion of TTR by the choroid plexus epithelia are needed to maintain normal T₄ transport at the blood-CSF barrier. A distorted TTR production/secretion by lead in the choroid plexus appears likely to impair the transport of thyroid hormones from the blood to the cerebral compartment. If this in vitro observation could be extended to the in vivo situation, then an impaired transport of thyroid hormones might putatively account for the known loss of cognitive abilities observed in Pb-poisoned children (Fig. 1). This notion, however, will require further experimental proof before it can be accepted.

Besides the epithelia in the choroid plexus, lead also accumulates in retinal pigment epithelia, which serves as a defensive barrier to the retina in the same way as the choroid plexus does to the brain. There was a weak correlation between lead concentration in retina and TTR in vitreous. The role of TTR in human eyes, by serving to deliver the useful molecules, e.g., thyroxine and retinoids and to maintain metabolic activity of the thinning peripheral retina, and the consequence of environmental exposure to toxic metals on eye TTR, have been discussed in a recent article from this laboratory (Eichenbaum and Zheng, 2000).

Effect of Lead on Signal Transduction in Choroid Plexus—Lead also intervenes in cellular phosphorylation processes by acting on the protein kinase system, particularly protein kinase C (PKC). PKC represents a family of Ca²⁺- and phospholipid-dependent protein kinases that catalyze the transfer of the γ -phosphate of ATP to phosphoacceptor serine or threonine residues of protein and peptide substrates. Activation of PKC is closely associated with the loss of epithelial barrier function (Gainer, 1985; Ojakian, 1981), increase in transendothelial permeability (Lynch et al., 1991), and inhibition of astroglia-induced endothelial differentiation (Lattera et al., 1992).

By Western blot analysis using specific antibodies against PKC isozymes (α , β , ζ , and ϵ), this laboratory has shown that rat choroid plexus expresses all four subtypes of PKC. Of the subtypes of PKC, the expression of ζ subtype in the choroid plexus is higher than that of α subtype. When tissue PKC is activated by phosphatidyl-L-serine and phorbol 12-myristate 13-acetate and compared among cerebral cortex, hippocampus, and choroid plexus, the hippocampus has the highest PKC activities, and the choroid plexus has the lowest. In cultured choroidal epithelial cells, incubation with lead in culture medium increases the membrane-bound PKC activities by 5.2-fold compared to the control, while the cytosolic PKC activities are decreased in parallel, the pattern of typical PKC activation (Zhao et al., 1998). It is unclear, however, whether the effect of lead on choroid plexus phosphorylation processes contributes to impaired regulation of TTR in the choroid plexus.

Manganese (Mn)

Manganese exists abundantly in a number of physical and chemical forms in the earth's crust, water, and atmosphere's particulate matter. Manganese compounds including its mixture with iron are widely used in the manufacture of steel, in the production of batteries,

in dietary supplements, and as an ingredient in some ceramics, pesticides, and fertilizers. A relationship between manganese intoxication and Parkinsonism has long been recognized (Barbeau et al., 1976; Mena et al., 1970; Tepper, 1961).

Manganese in Choroid Plexus and CSF Upon Exposure—There is no direct evidence toward manganese-associated damages in cerebral microvascular structure, nor can it be found about structural injury resulting from manganese toxicity in choroidal epithelial cells. However, manganese indeed accumulates in the choroid plexus (Ingersoll et al., 1995; Michotte et al., 1977; Valois and Webster, 1989b; Zheng et al., 1998b). The influx constant of manganese to the choroid plexus was about 150 and 1,000 times greater than that of cerebral cortex and CSF, respectively, when the plasma manganese was maintained at a constant level (Murphy et al., 1991).

The homeostasis of manganese in the CSF appears to be directly influenced by plasma manganese concentration (Zheng et al., 1998b). Some investigators have shown that the valence status of manganese ion determines the transport properties of manganese at the brain barrier systems. Mn(III), for example, enters the brain via a transferrin receptor-mediated mechanism, while Mn(II) appears to be readily taken up into the CNS, most likely as the free ion or nonspecific protein-bound species (Aschner et al., 1999; Rabin et al., 1993). By comparing the rates of metal transfer (K_{in}) into brain among 13 metal species, Smith et al. (1997) suggest that Mn(II) is the most permeable species among the metals studied. More recently, studies using transferrin knock-out mice indicate that deficiency in circulating transferrin has no apparent effect on tissue distribution of Mn(II) (Dickinson et al., 1996; Malecki et al., 1998). Thus, a non-transferrin-mediated mechanism appears to explain manganese movement across the brain barriers. The data from this laboratory show a parallel increase of manganese in CSF and plasma when Mn(II) was administered (Zheng et al., 1998b). Thus, Mn(II) species seem to be poorly restricted by the brain barrier systems.

Biochemical Basis of Manganese-Iron Interaction—Chemically and biochemically, manganese shares numerous similarities with iron in that (1) both are transition elements adjacent to each other in the Periodic Table; (2) both carry similar valent charges (2+ and 3+) in physiological conditions; (3) both have similar ionic radius; (4) both strongly bind transferrin (Tf); and (5) intracellularly, both preferentially accumulate in mitochondria. Because of these similarities, it is not surprising that manganese can interact directly with iron at the cellular and subcellular levels, particularly on certain enzymes that require iron as a cofactor in their active catalytic center, such as aconitase, NADH-ubiquinone reductase (Complex I), and succinate dehydrogenase (SDH).

Manganese Exposure and Cerebral Iron Regulation—Whereas manganese poisoning does not directly produce histopathological impairment to the blood-CSF barrier, manganese may selectively alter the barrier's ability in regulating cerebral iron homeostasis. When groups of rats received intraperitoneal injections of $MnCl_2$ at the dose of 6 mg Mn/kg/day for 30 days, manganese exposure resulted in a 32% decrease in plasma iron ($P < 0.01$) and no changes in plasma total iron binding capacity (TIBC). Surprisingly, the iron concentration in the CSF of the same rats did not decline as it did in plasma. Instead, it markedly increased by 3-fold. The ratio of CSF to plasma iron ($Fe_{CSF/plasma}$) was increased

from 0.16 in control rats to 0.72 in manganese-treated rats, reflecting an influx of iron from the systemic circulation to the cerebral compartment upon manganese exposure (Zheng et al., 1999b).

Under normal physiological conditions, the brain stringently regulates iron balance by three well-coordinated systems: (1) the influx of iron into brain, which is regulated by transferrin receptor (TfR)-mediated transport at brain barriers; (2) the storage of iron in which the cellular sequestration is largely dependent upon availability of ferritin; and (3) the efflux of iron whose rate is controlled by bulk CSF flow to the blood circulation (Connor and Benkovic, 1992; Jefferies et al., 1984). At the brain barriers that separate the systemic circulation from the cerebral compartment, the iron-transferrin complex binds to surface TfR followed by endocytosis into cerebral capillary endothelia (and possibly into choroidal epithelia) where the molecules subsequently dissociate. Apotransferrin is then recycled to the blood compartment, whereas the released iron crosses the abluminal membrane of the barriers into the cerebral compartment by binding to brain transferrin derived discretely from oligodendrocytes and choroidal epithelia. The cerebral transferrin-bound iron thus becomes available for neurons expressing transferrin receptors (Connor and Benkovic, 1992; Moos, 1996).

The post-translational modulation of TfR and ferritin synthesis is regulated by a [4Fe-4S] containing protein known as cytoplasmic aconitase (ACO1) or IRP-I (Fig. 2). When the cellular iron level is insufficient, cytoplasmic aconitase loses the fourth labile iron and assumes a [3Fe-4S] configuration. In that state, the enzyme forfeits its enzymatic activity and binds with high affinity to mRNAs that contain an iron responsive element (IRE) stem-loop structure, i.e., to the mRNAs of the major proteins in iron metabolism including ferritin, TfR, δ -aminolevulinic acid synthase, mitochondrial aconitase (ACO2), and succinate dehydrogenase (Beinert and Kennedy, 1993; Klausner et al., 1993). The net result of these interactions is a down-regulation of intracellular iron utilization and up-regulation of extracellular iron uptake.

Effect of Manganese on Iron Regulatory Process—Studies from this laboratory have shown that manganese can alter aconitase enzymatic activity, presumably, by competing with iron for the fourth, highly labile iron binding site of the [4Fe-4S] cube in enzyme's active center (Fig. 3) (Zheng et al., 1998b). Recent results further confirm that Mn(III) was evidently more effective than Mn(II) in enzyme inhibition. The ED₁₀ (10% inhibition of aconitase activity) was 124 and 1,164 μ M for Mn(III) and Mn(II), respectively (Tsao et al., 2000). Such an action, while suppressing the enzyme's catalytic function, may increase its binding affinity to the mRNAs encoding major proteins in iron metabolism such as ferritin and TfR.

This postulation was partly corroborated by our recent in vitro experiment (Zheng et al., 1999b). When the cultured choroidal epithelial cells derived from rat choroid plexus were incubated with manganese at 100 μ M overnight, an increased amount of TfR mRNA was found by Northern blot. Quantitative analysis of the optical abundance of the bands corresponding to TfR mRNA revealed that manganese treatment enhanced the expression of TfR mRNA inside choroidal epithelial cells by 50%. The question remains as to whether the

TfR proteins are produced in epithelial apical surface or in basal lamina. Nonetheless, the net result of manganese action on TfR is an up-regulation of cellular iron uptake. Thus, it appears likely that chronic manganese exposure might stimulate the expression of TfR in the choroid plexus, and possibly at brain capillary endothelia (as well as in other peripheral organs). The overexpression of TfR at brain barriers and the ensuing facilitated iron transport from blood to the cerebral compartment may explain a compartmental shift of iron from the blood to the CSF.

Consequence of Manganese-Iron Interaction—Abnormal metabolism of iron in systemic and cerebral compartments is reportedly associated with the etiology of a number of neurodegenerative diseases including idiopathic Parkinson's disease. A cellular iron overload in the basal ganglion region particularly in the substantia nigra may catalyze the generation of reactive oxygen species and enhance lipid peroxidation. Such an iron-mediated oxidative stress may ultimately lead to the degeneration of nigrostriatal dopamine neurons in patients (Jenner et al., 1992; Loeffler et al., 1995). Should this prove true, it is reasonable to speculate that manganese-induced Parkinsonism may be partly due to an impairment by manganese of iron homeostasis by selectively expediting unidirectional influx of iron from the systemic circulation to cerebral compartment.

Copper (Cu)

Copper is also an essential metal ion and participates in biochemical reactions as a cofactor in a number of enzymes including tyrosinase, cytochrome oxidase, and superoxide dismutase. Excessive accumulation of copper in brain, liver, and other major organs causes the pathological damage, clinically known as Wilson's disease. A low serum ceruloplasmin, due to a genetically inherited disorder, and a resultant high free copper are believed to be the causes of the disease. It is still unclear how copper gains access to the CNS and if the blood-brain barrier and blood-CSF barrier regulate copper homeostasis in the cerebral compartment at all. Nevertheless, copper deposits in the choroid plexus. A recent study on sheep demonstrates that chronic copper poisoning markedly induces metallothionein within astrocytes, cerebral endothelia, and choroid plexus, suggesting that these cell structures may stabilize copper and possibly modulate CNS copper homeostasis (Dincer et al., 1999). Nishihara et al. (1998) have recently cloned several mouse homologues of copper trafficking genes. By in situ hybridization, they found that these genes (mCTR1, mATX1, and mATP7a) were highly expressed in the choroid plexus. Presumably, the choroid plexus may use the trafficking pathway in regulating bi-directional (uptake or efflux) transport of copper to and from the cerebral compartment.

Copper itself is not a barrier destroyer; but the presence of copper may alter the transport of iron. This was seen in rats fed with copper-containing diet, where the influx of iron into brain was significantly decreased compared to that of rats fed with the control diet (Crowe and Morgan, 1996).

Tellurium (Te)

Tellurium has been found to accumulate in the choroid plexus following chronic administration. The mechanism for this accumulation may be due to intra-cellular binding

rather than active transport (Agnew, 1972; Agnew et al., 1974; Yuen et al., 1975). There is no distinct histopathological alteration seen in cerebral capillaries and in the choroid plexus. However, tellurium inhibits iodide transport in the choroid plexus (Agnew et al., 1974).

SEQUESTERED CHOROID PLEXUS TOXICANTS

Iron (Fe)

Iron is an essential metal for a variety of physiological processes. Excess cerebral iron, both intracellularly and extracellularly, has been linked to a number of neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's diseases, etc. (Connor and Benkovic, 1992; Jenner et al., 1992). Among the processes involved in brain regulation of iron, the entrance of iron to the cerebral compartment represents a critical step in monitoring cerebral iron homeostasis. It has been generally accepted that transferrin-bound iron gains access to brain via TfR on brain capillary endothelial cells (Jefferies et al., 1984; Kissel et al., 1998). A recent study, using GFAP-IL6 transgenic mice with a constitutive blood-brain barrier defect, reveals a 40% increase of total iron concentration in the brain. This is followed by a consequential lipid peroxidation in the neocortex and the cerebellum of symptomatic animals (Castelnau et al., 1998).

Iron molecules are selectively taken up by the choroid plexus following intravenous injection to rats (Morris et al., 1992). By an improved histochemical staining method, a substantial staining of iron can be seen in neuroglial cells and in the choroid plexus epithelial cells (Moos and Mollgard, 1993). In human subjects with a calcified choroid plexus, iron concentration in the choroid plexus was about 5 times greater than the values obtained from other brain tissues (Michotte et al., 1977).

The choroid plexus along with the oligodendrocytes are the only two cell types in the CNS capable of manufacturing transferrin. Thus, a possible role of the choroid plexus in participating in brain iron regulation has been suggested (Crowe and Morgan, 1992; Morris et al., 1992; Zheng et al., 1999b). Although some studies using antibody against TfR failed to reveal the receptors in choroid plexus capillaries (Broadwell et al., 1996; Kissel et al., 1998), others have elucidated their presence (Lu et al., 1995; Moos, 1996). The results from this laboratory confirm an abundant expression of TfR mRNA in cultured choroidal epithelial cells (Zheng et al., 1999b). It appears likely that the choroid plexus may co-regulate cerebral iron homeostasis. The degree to which the choroid plexus contributes to the overall brain iron regulation, as compared to the role of the blood-brain barrier, has not yet been established.

Silver (Ag)

Even though silver-induced neurotoxicity is rare, silver ions can readily penetrate the blood-brain barrier and blood-placental barrier (Danscher, 1981; Stoltenberg et al., 1994). Silver accumulates in the choroid plexus to a significant concentration. For example, exposure of pregnant rats to silver led to a substantial silver deposition in the choroid plexus in the offspring (Rungby and Danscher, 1983). Similar observations have been made in humans. One clinical case showed that a 72-year-old patient who had taken nose drops containing a

silver preparation for 2–5 years, developed argyria, symptoms representing a diffuse deposition of Ag in tissues after prolonged silver exposure. The necropsy data found that the choroid plexus displayed a distinct deposition of silver in the epithelial basal lamina. In contrast, the brain parenchymal tissues were generally free of the metal (Goebel and Muller, 1973).

Gold (Au) and Zinc (Zn)

Both gold and zinc are sequestered by the choroid plexus. The results of an experiment in which radioactive ^{198}Au was introduced into the lateral ventricle of experimental dogs, showed that the choroid plexus had a level of radioactivity about 550 times higher than that found in the brain cortex. Thus, the choroidal epithelium appears to take up gold from the CSF (Rish and Meacham, 1967).

Zinc also accumulates in the choroid plexus as determined by autoradiography (Franklin et al., 1992). The permeability of choroid plexus to zinc was about 12 times higher than that of the cerebral capillaries, although the overall contribution of the choroid plexus to zinc influx into the brain has been considered insignificant in comparison with that of the blood-brain barrier in the same study.

IMPLICATIONS OF CHOROID PLEXUS IN NEUROTOXICOLOGY

Given the fact that the choroid plexus upholds the chemical stability of the cerebral compartment, the toxicological relevance of choroid plexus in metal-induced encephalopathies should never be undermined. In fact, some neurological disorders, although not metal-related, have indeed been correlated to the clinical impairment of the blood-CSF barrier. For example, schizophrenia and certain forms of idiopathic mental retardation might be associated with dysfunction of the choroid plexus (Rudin, 1979, 1980, 1981). Other CNS disorders possibly associated with the choroid plexus dysfunction include Reye's syndrome (Levine, 1987), endogenous depression (Jorgensen, 1988), and African sleeping sickness (Ormerod and Venkatesan, 1970). More recently, a significant pathogenic relationship between the choroid plexus and brain cortex of Alzheimer's patients has been postulated (Miklossy et al., 1998, 1999; Sasaki et al., 1997). These findings suggest a greater role of the choroid plexus in the pathogenesis of certain CNS diseases than one previously envisioned.

Choroid Plexus in Early Brain Development

The choroid plexus develops early in embryogenesis primarily from spongioblasts. In humans, the choroid plexus first appears in the roof of the fourth ventricle at the ninth week of ontogeny; it subsequently appears in the lateral and third ventricles at approximately 9–10 weeks. During the 10th week, the choroid plexus becomes granulated and begins to perform its secretory functions, although the entire structure is not matured or fully developed until the sixth gestational month (Milhorat, 1976).

The early development of the choroid plexus may cope with the rapid growth of cerebral cortex during CNS development and maturation. The choroid plexus actively transports critical nutrients and hormones for the growth of neuronal and neuroglial cells. Besides TTR

as discussed earlier, the choroid plexus produces and secretes retinol-binding protein (RBP) to the CSF (Aldred et al., 1995; MacDonald et al., 1990; Zetterstrom et al., 1994). Both TTR and RBP transport retinoids in the body. TTR and RBP interact with retinol to form a trimolecular complex in a close equimolar stoichiometry over a wide concentration range. This interaction indicates that the bulk (90–95%) of retinol in human blood circulates by this complex (Blaner and Olson, 1994; Ingenbleek and Young, 1994). Strong evidence also suggests that retinol is transported by RBP across the blood-brain barrier and blood-CSF barrier to the CNS (Aldred et al., 1995; MacDonald et al., 1990; Ruberte et al., 1993; Zetterstrom et al., 1994), and that local TTR cooperates in the transport of retinol (Martone et al., 1988). The role of retinoids in brain development has recently received great attention. Far beyond its classically defined functions in vision, retinoids mediate neuronal cell differentiation (Maden et al., 1990), regulate the activity of type III thyroxine deiodinase in astroglial cells (Esfandiari et al., 1994), induce gene expression relevant to the development of specific neuronal structures or pathways (Maden et al., 1990; Ruberte et al., 1993), and selectively modulate hippocampal plasticity (Zetterstrom et al., 1994). More interestingly, some studies indicate that maternal vitamin A restriction causes an altered brain development in terms of tissue weight, DNA and RNA levels, and protein expression (Sharma and Misra, 1990).

Since the choroid plexus manufactures and secretes both TTR and RBP to CSF and is the possible site of retinol transport, it is compelling to explore whether or not accumulation of lead as well as other toxicants in the choroid plexus influences the production and transportation of RBP and retinol.

Choroid Plexus in Neurodegenerative Diseases

Alzheimer's disease is one of the most common neurodegenerative disorders of old age, affecting approximately 4 million Americans. The neuropathological hallmarks of Alzheimer's are two kinds of microscopic lesions, neuritic (or senile) plaques and neurofibrillary tangles, both of which are found in the autopsy brains. The major constituents of neuritic plaque and tangles are beta-amyloid protein and tau protein, respectively. Beta-amyloid is derived from a larger protein called the amyloid precursor protein (APP), which normally embeds in the neuronal membrane. Aggregation of highly insoluble toxic beta-amyloid is now believed to be one of the critical steps in the etiology of Alzheimer's disease. Recent data indicate that the choroid plexus may be a source of CNS APP (Kalara et al., 1996; Miklossy et al., 1998; Sasaki et al., 1997; Wen et al., 1999). On 292 human brain autopsies, there was a highly significant correlation between the occurrence of Alzheimer type of changes in the cortex and those in the choroid plexus (Miklossy et al., 1998). Miklossy and his associates (1999) have further observed that APP and Tauprotein can be produced by cells outside the CNS and thus suggested that these proteins may be derived from or transported by the choroid plexus.

The environmental factors associated with the risk of Alzheimer's disease have not yet been identified. Aluminum was suggested to be a pathogenic metal (Rifat et al., 1990); however, a larger epidemiological study failed to confirm such a cause-effect relationship (Anonymous, 1994). Instead, the same study indicates a risk associated with occupational

exposure to glues, pesticides, and fertilizers. By far, the possibility of toxic metal accumulation in the choroid plexus and the consequent effect on APP and Tau protein trafficking at this blood-CSF barrier have never been examined.

As discussed earlier, manganese appears to alter iron transport and cerebral regulation by the choroid plexus. Accumulation of excess iron in basal ganglia, a region being responsible for movement coordination, has been identified in Parkinson's patients (Connor and Benkovic, 1992), although the reason for iron deposition in this particular area remains unknown. Thus, a thorough understanding of manganese-iron interaction at the blood-brain and blood-CSF barriers will better define the role of manganese in cerebral iron metabolism, and will offer clues to the etiology of Parkinson's disease and its associated abnormal iron regulation.

How Does the Choroid Plexus Defend Against Metal Toxicities?

As a barrier, the choroidal epithelia are often the first and the most frequent ones to encounter metal insults from blood. How, then, do the barrier cells survive the toxic attack? Two mechanisms may aid resistance of choroid plexus to blood-borne toxicities. First, the choroid plexus contains abundant metal binding ligands that effectively sequester metal ions. Glutathione (GSH), a cysteine-containing tripeptide, has been suggested to protect against cadmium toxicity (Singhal et al., 1987; Zheng et al., 1990). The choroid plexus manufactures, secretes, and regulates GSH in the CSF (Anderson et al., 1989). Cysteine is a precursor of GSH and metallothionein. The concentration of total cysteine (cysteine + cystine) in the choroid plexus is 3-fold greater than that in the brain cortex. Moreover, the concentration of cystine in the choroid plexus is 4-fold higher than in the brain cortex (Zheng et al., 1991). Metallothioneins, which bind essential metals such as zinc and copper, and toxic metals such as cadmium, mercury, and lead, have also been identified in the choroid plexus.

Second, the choroid plexus owns an active defense system. The activities of superoxide dismutase and catalase are significantly higher in the choroid plexus than in cerebrum and cerebellum (Tayarani et al., 1989). A number of GSH-related detoxifying enzymes, such as GSH-S-transferase, gamma-glutamyltranspeptidase, GSH peroxidase and reductase, are present in the choroid plexus (Carder et al., 1990; Prusiner and Prusiner, 1978; Senjo et al., 1986; Tayarani et al., 1989). Conceivably, the presence of these protective enzymes effectively defends the barrier against free radical-initiated oxidative stress.

Taken together, the choroid plexus likely forms the first line of defense against neurotoxicants. It must be kept in mind, however, that none of the cellular defense mechanisms would operate in an unlimited capacity. The pathophysiological changes can occur either as the threshold above which the protective capacity of the choroid plexus is exceeded or saturated, or as a direct result of barrier dysfunction. Thus, the need arises for a more comprehensive understanding of the detoxification mechanisms, such as antioxidant systems, induction of protective macromolecules (heat shock proteins, etc.), formation of specific metal inclusion bodies or binding proteins, and biotransformation reactions (methylation, conjugation, etc.) that operate in the choroid plexus.

Disruption on Transport Function

The choroid plexus regulates bi-directional transport and provides a highly selective pathway for materials to communicate between the cerebral and blood compartments. The choroid plexus synthesizes proteins and releases them into the CSF. TTR, RBP, transferrin, and ceruloplasmin (Aldred et al., 1987), for example, are synthesized in the choroidal epithelial cells. The inhibitory effect of lead on TTR production and secretion by the choroid plexus is one of the examples whereby toxic metals may induce neurotoxic outcome by their effect on the blood-CSF barrier. The choroid plexus also transports amino acids. In an in vitro system where transport is considered to be primarily via the apical epithelium, lead significantly inhibits the uptake of L-tyrosine by the choroid plexus (Kim and O'Tuama, 1978). As tyrosine is a known precursor of several neurotransmitters, the authors raised an interesting hypothesis that lead poisoning may be accompanied by abnormal amino acid concentrations in the brain extracellular compartment. Besides these examples, there are many channels, transporters, carriers, and receptors in the choroid plexus. Our knowledge on how toxic metals interact with transport functions and to what degree these interactions contribute to neurological disorders remains incomplete.

CONCLUSIONS

Compelling evidence demonstrates a definite capacity of the choroid plexus in sequestering toxic heavy metal and metalloid ions. As the integrity of blood-brain and blood-CSF barriers, both structurally and functionally, is quintessential to brain chemical stability, the role of the choroid plexus in metal-induced neurotoxicities should never be undermined. Metals accumulated in the choroid plexus can directly damage the general plexus structure, or selectively impair the critical regulatory mechanisms, or impassively deposit in this highly vascularized tissue. Our current knowledge on the toxicological aspect of choroid plexus research is still incomplete. Thus, future research should be directed to explore the role of choroid plexus in early CNS development as affected by metal sequestration in this tissue, to investigate how metal accumulation in the choroid plexus alters its function in regulation of key elements involved in the etiology of neurodegenerative diseases, and to better understand the blood-CSF barrier as a defense mechanism in CNS functioning. These studies will be not only of scientific interest but also critical to our understanding of xenobiotic-induced neurotoxicities.

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Fig. 1.

Proposed mechanism of lead-induced neurotoxicity. Deficiency of thyroid hormones during early brain development period produces multiple morphological, biochemical, and electrophysiological alterations of neurons and neuroglia, leading to an irreversible mental retardation. Could Pb cause the deficit in children's cognitive abilities by depressing brain TTR and thus impairing the function of thyroid hormones in early brain development?

A. mRNA encoding Tf receptor

□ IRP-I, ACO1 ↑ IRE in mRNA

B. mRNA encoding ferritin, SDH, ACO2 **Fig. 2.**

Cellular regulation of intracellular iron homeostasis by IRP-I. **A:** mRNA encoding Tf receptor with five clustered iron responsive element (IRE) at 3' untranslated region. **B:** mRNA encoding ferritin with one clustered IRE at 5' cap site. In Fe-replete cells, IRP-I secures Fe in the form of a [4Fe-4S] cluster. While this form of IRP-I binds mRNA poorly, it can enzymatically catalyze the conversion of bound citrate to isocitrate. When cellular Fe levels are insufficient, however, IRP-I assumes a [3Fe-4S] configuration, loses its cluster and enzymatic activity, and is transformed into an mRNA binding-protein. At this state, the enzyme binds with high affinity to IRE-containing mRNAs, stimulates the expression of those mRNAs whose IRE's are 3' (e.g., TfR), and inhibits translation of those whose IRE's are 5' (e.g., ferritin, SDH, mitochondrial aconitase). The net result of this RNA:protein interaction is an increase in cellular Fe uptake. Thus, cytosolic aconitase or IRP-I is critical to cellular Fe regulation.

Fig. 3.

Possible competition between Mn and Fe for the binding site in aconitase. Since the coordination chemistry of Mn closely resembles that of Fe, Mn may insert itself into the fourth Fe site of aconitase and, therefore, while suppressing the enzyme's catalytic function, it increases the protein's ability to bind to mRNA, which favors the expression of transferrin receptor (TfR) and restrains the translation of ferritin.