REVIEW

Neurotoxicology of the Brain Barrier System: New Implications

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ABSTRACT

The concept of a barrier system in the brain has existed for nearly a century. The barrier that separates the blood from the cerebral interstitial fluid is defined as the blood-brain barrier, while the one that discontinues the circulation between the blood and cerebrospinal fluid is named the blood-cerebrospinal fluid barrier. Evidence in the past decades suggests that brain barriers are subject to toxic insults from neurotoxic chemicals circulating in blood. The aging process and some disease states render barriers more vulnerable to insults arising inside and outside the barriers. The implication of brain barriers in certain neurodegenerative diseases is compelling, although the contribution of chemical-induced barrier dysfunction in the etiology of any of these disorders remains poorly understood. This review examines what is currently understood about brain barrier systems in central nervous system disorders by focusing on chemical-induced neurotoxicities including those associated with nitrobenzenes, N-methyl-D-aspartate, cyclosporin A, pyridostigmine bromide, aluminum, lead, manganese, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and 3-nitropropionic acid. Contemporary research questions arising from this growing understanding show enormous promises for brain researchers, toxicologists, and clinicians.

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INTRODUCTION

The concept of a barrier between the blood and the brain was originally conceived in 1906 based on the observation that a large-molecular-weight dye by intravenous injection stained nearly all of the tissues and organs throughout the body except the brain; whereas a direct delivery of the same molecule to the cerebrospinal fluid (CSF) in the brain ventricles dyed exclusively brain parenchyma (1). Clearly, there must exist an anatomical entity that impedes the free movement of the dye between the blood and brain. Only with advances in electron microscopic technology nearly half a century later, did it become possible to visualize the tight junctions between cerebral capillary endothelia, thereby revealing the structural basis of the blood-brain barrier (BBB). The tight junctions between the choroidal epithelia in the choroid plexus, which are the foundation of the blood-CSF barrier (BCB), were identified somewhat later (1–3).

Major progress has since been made toward elucidation of essential roles of BBB and BCB in overall brain functions. For example, investigations have revealed that the brain barriers actively, not merely passively, nourish the immature brain during early development. Not only do brain barriers serve as the sites for transport of proteins, polypeptides, and hormones, but they also produce some of these macromolecules, lending themselves to a crucial role in a neuroendocrine regulation in the central nervous system (CNS). Moreover, brain barriers appear to contribute to the etiology of CNS diseases and so have become a new focus of investigation. Despite these imperatives to understand brain barriers, the potential toxicological significance of brain barriers has not been sufficiently addressed.

This article aims to review brain barrier systems in CNS disorders, particularly focusing on chemical-induced neurotoxicities and certain neurodegenerative diseases. For general reviews of structure and function of BBB and BCB, readers are referred to other publications (1,2,4).

Brain Barrier Systems in Chemical-Induced Neurotoxicities

Normal neurological function relies on the delicate chemical balance among neurons and their associated synaptic connections. Because such a balance is safeguarded in part by brain barriers, the damaging effect of a chemical to the barriers' structure or function can contribute substantially to chemical-induced neurotoxicity. The interactions between chemicals and brain barriers ex-

emplifying their role in neurotoxicities are summarized in Table 1 and discussed as follows.

Neurotoxicity Due to Injured Barrier Structures: Nitrobenzenes, *N*-Methyl-*D*-aspartate (NMDA), Cylosporin A, Tacrolimus, and Pyridostigmine Bromide

Many neurotoxicants can directly damage the barriers' structure and increase its permeability, allowing the toxicants to come into contact with brain parenchyma. 1,3,5-Trinitrobenzene (TNB, a soil and water contaminant at certain military installations) can produce encephalopathy characterized by symmetric vacuolation and microcavitation in selected brain regions. Prior to the tissue lesion, however, TNB breaks down the BBB and allows large serum proteins to diffuse through it. Thus, the selective regional vulnerability of brain capillaries to TNB has been suggested as a critical event in TNB-mediated neurotoxicity (5). Other nitrobenzene derivatives such as *m*-dinitrobenzene share similar toxic mechanisms by provoking oxidative cytotoxicity of the BBB, followed by neuronal and neuroglial injury (6). More examples include excitotoxin, NMDA, and immunosuppressants (cyclosporin A and tacrolimus). Although structurally different, all of these chemicals can induce apoptosis in brain capillary endothelial cells prior to their initiation of neuronal lesions (7,8).

Exposure to one chemical may compromise the integrity of the BBB, leading to an excess brain accumulation of other chemicals which normally do not reach the brain and thereby augmenting their neurotoxicity. In a study investigating the Gulf War Syndrome among Persian Gulf War veterans who had received pyridostigmine bromide to protect against organophosphorus nerve gas, Abou-Donia et al. (9) suggest that pyridostigmine bromide–type chemicals may damage the BBB and render it more permeable to other xenobiotics in circulation.

Neurotoxicities Due to Altered Barrier Functions: Aluminum and Lead

Certain chemicals do not directly disrupt the morphology of barriers, but rather alter their regulatory functions, bringing about neurotoxicity due to disordered cerebral homeostasis. Exposure to aluminum in laboratory animals results in the development of neurofibrillary tangles and degeneration of cerebral neurons. Although part of this effect represents direct toxicity of aluminum on neuronal pathways, aluminum ions also possess a high affinity for cerebral endothelia (10). Following exposure, aluminum-facilitated passage of endogenous, behavior-

Table 1

Mechanisms of Brain Barriers in Chemical-Induced Neurotoxicity

Type	Mechanism	Examples	Reference
I	Disrupt barrier structure, followed by increased influx and increased toxicity	Trinitrobenzene	(5)
		Dinitrobenzene	(6)
		NMDA	(7)
		Cyclosporin A	(8)
		Pyridostigmine bromide	(9)
		BCNU	(27)
II	Alter barrier functions, but do not directly damage barrier structure	Aluminum	(10,11)
		Lead	(19,20)
		Manganese	(46)
		Inorganic mercury	(28)
III	Biotransformation of xenobiotics at brain barriers as part of brain defense mechanism	MPTP	(24,25)
		BCB	(26)

ally active peptides across the BBB was noted (11). It is highly probable that aluminum acts as a direct barrier toxin. This may be a concern for patients undergoing chronic hemodialysis treatment and manifesting dialysis dementia (11,12).

Another example of direct barrier toxicity is seen in the inhibitory effect of lead on the production of transthyretin (TTR) by the BCB. Unlike plasma TTR, which is derived primarily from the liver, brain TTR is exclusively produced, secreted, and regulated by the choroid plexus (13–15). The importance of TTR in CNS development is evidenced by the fact that it is present in very high concentration during prenatal and early postnatal life (16). In the brain, TTR transports thyroid hormones and solublizes β-amyloid peptides. Our recent studies based on 82 human CSF samples reveal a significant correlation between TTR and T4 concentrations in the CSF, and therefore support the assertion that TTR from the choroid plexus participates in the regulation of thyroid hormone in the cerebral compartment (17).

Earlier animal studies in this laboratory have established that chronic exposure to lead results in an excess accumulation of this metal in the choroid plexus, which apparently correlates with a reduced TTR level in the CSF (18,19). By tracking newly-synthesized TTR molecules labeled with [^{35}S] methionine, we found that lead exposure reduced the synthesis and suppressed the secretion of [^{35}S] TTR from the choroidal epithelia into the CSF. In the end, this impeded the transepithelial transport of [^{125}I] T$_4$ from the blood to the CSF (20). It has long been known that thyroid hormones are critical to brain development and maturation. Deprivation of thyroid hor-

mone in children has repeatedly been linked to irreversible mental retardation (21–23). It appears plausible that lead exposure in children may affect the homeostasis of thyroid hormones in the cerebral compartment and possibly in the circulation as well. Such an alteration might account for the known loss of cognitive abilities observed in lead-poisoned children.

Biotransformation at the Brain Barriers: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

Similar to other cell types, both cerebral endothelia and choroidal epithelia contain a variety of drug metabolizing enzymes. Many are inducible and capable of converting xenobiotics to more water-soluble species, thereby preventing them from entering the brain. The term ''biochemical barrier'' appears useful in describing this property of brain barriers in protecting against xenobiotics as distinct from their structural role. MPTP has been extensively investigated as a prototypical compound in Parkinson's disease research. Systemic administration of MPTP causes Parkinsonism in humans and subhuman primates; but rats and other laboratory animals are much less susceptible to MPTP toxicity (24,25). Metabolism of MPTP by monoamine oxidase (MAO) generates a more water-soluble metabolite MPP+. To examine the species difference, Kalaria et al. (24) compared MAO activity in brain microvessels between various species and found that the rate of detoxifying MPTP metabolism by cerebral microvessels in rats was about 30-fold greater than that by humans. In other words, the marked species differ-

ences in susceptibility to systemic MPTP may be partly due to differences in metabolic ability of brain barriers to alter MPTP structure. Conceivably, if the blood level of MPTP reaches a threshold point above which the capacity of ''on-barrier'' metabolism is exceeded or saturated, there might be a ''spillover'' of lipophilic MPTP into the cerebral compartment, followed by the neuronal cytotoxicity distinct to MPTP exposure. It is not entirely clear, though, whether the BBB in the whole brain bears the same ''threshold'' level, or whether only the barrier at the basal ganglion has a low threshold, which consequently makes this region particularly vulnerable to MPTP. A similar biochemical barrier also exists in the BCB (26).

Alternatively, ''on-barrier'' metabolism may locally activate xenobiotics intracerebrally rather than deactivate them. The bioactivation of xenobiotics at BBB and BCB interfaces, however, is poorly understood at present.

Brain Barrier Systems in Neurodegenerative Diseases

Role of Altered Brain Barriers in Alzheimer's Disease

The neuropathological hallmark of Alzheimer's disease (AD) is the aggregation of β-amyloid (Aβ), a small peptide of about 42 amino acids, in senile plaques and to lesser degree in neurofibrillary tangles. Aβ peptides occur as an enzymatically cleaved product of the amyloid precursor protein (APP). In conjunction with several factors induced by advanced age, the soluble Aβ aggregates to form fibrillar Aβ and eventually precipitates in the extracellular space.

Brain barriers contribute to the etiology of AD possibly in three aspects: (i) the aging of cerebral vascular structure in the overall aging process of the brain, (ii) as the site of transport of extracerebral Aβ into the brain, and (iii) the ability to prevent Aβ aggregation by producing TTR. Regarding the first aspect, aging, the cerebral vasculature is prone to pathological changes during the aging process similar to peripheral vessels (29,30). Amyloid deposits have been observed on the walls of cerebral vessels as well as in the choroidal epithelia, and are sometimes associated with Alzheimer-type lesions (31–33). In addition, the cerebral microvessels in AD patients exhibit an aberrant protein kinase C (PKC-zeta) (34), increased permeability, and dysfunctional BCB (33,35). Although none of these have been noticeably associated with chemical exposure, it is essential to recognize that aging occurring at the barriers is not an independent process medi-

ated solely by genetics, but is affected by environmental factors as well. Thus, there is a potential role of chemicals in influencing the pathology of cerebral aging. For example, lead exposure has been shown to cause abnormal PKC activity in both the BBB (36) and the BCB (37).

Amyloid precursor protein exists widely in extracerebral tissues (38–40). The soluble monomeric form of Aβ peptide has been identified in blood and CSF. Recent evidence suggests that transport of extracerebral Aβ to the brain may be modulated by brain barriers. Moreover, the BCB appears to play a cleansing role, removing Aβ from the CSF (41). These findings suggest a role of brain barriers in modulating cerebral Aβ in normal and AD brains. It is possible to hypothesize that any alteration in the capacity of brain barriers in handling Aβ, presumably as a cumulative consequence of life-time chemical exposure, could ultimately affect the local concentrations of Aβ, thereupon promoting the aggregation of Aβ in specific brain areas. This conjecture, however, will require substantial scrutiny both clinically and experimentally.

As mentioned above, the choroid plexus produces TTR within the CNS. The presence of TTR in senile plaques and in neurofibrillary tangles of AD patients has been recognized for several decades (42). TTR conjugates with Aβ, and in so doing solublizes Aβ and prevents formation of characteristic amyloid fibrils in AD (43,44). Clinically, Riisoen (45) reported that TTR quantified in CSF was inversely correlated with the degree of dementia in 24 AD patients. Serot et al. (46) observed that the mean values of CSF TTR in AD patients were significantly lower than those of age-matched control subjects. The same group of investigators also observed an apparent ultrastructural anomaly in AD choroid plexus (47). Notably, data from both human and animal studies in this laboratory indicate that CSF levels of TTR decline as age advances. Moreover, the concentrations of TTR in the CSF appears to be inversely associated with lead, an environmental toxicant, in the CSF as well as in the choroid plexus (17,19).

Altered Brain Barriers in Parkinsonism: Manganese

A direct involvement of brain barriers in the etiology of idiopathic Parkinson's disease (IPD) is unclear. In certain chemical-induced forms of Parkinsonism, however, the contribution of dysfunctional brain barriers has been suggested. One example has been discussed in MPTP neurotoxicity above. Another example is manganese-induced Parkinsonism. Manganese can interfere with iron

metabolism at both systemic and subcellular levels. Chronic exposure to manganese in rats resulted in a decrease in plasma iron, but a marked increase in CSF concentrations of iron (48).

The brain regulates iron balance through three, well-coordinated systems: the influx of iron by transferrin receptor (TfR)–mediated transport at brain barriers, the storage of iron dependent on availability of ferritin in neurons and neuroglia, and the efflux of iron by bulk CSF flow to the blood circulation (49–51). The expression of TfR is posttranslationally regulated by a 4Fe-4S containing protein known as cytoplasmic aconitase (ACO1) or iron regulatory protein I (IRP-I). We have shown that manganese can alter aconitase enzymatic activity, presumably by competing with iron for the fourth, highly labile binding site of the 4Fe-4S cube in the enzyme's active center (52). Such action, while suppressing the enzyme's catalytic function, may increase its binding affinity to the mRNA encoding major proteins in iron metabolism such as TfR and increase its expression. Chronic manganese exposure may up-regulate TfR in the BCB and possibly in the BBB as well. The overexpression of TfR at brain barriers thus facilitates iron transport from the blood to the cerebral compartment.

Cellular iron overload in the basal ganglion, particularly in the substantia nigra, catalyzes the generation of reactive oxygen species. Such iron-mediated oxidative stress has been linked to the degeneration of nigrostriatal dopamine neurons in IPD patients (53,54). The fact that manganese and iron are immediate neighbors in the Periodic Table lends itself compellingly to the theory that manganese–iron interaction may contribute, at least in part, to manganese-induced Parkinsonism.

Brain Barrier Systems in Other Neurological Disorders

About 15 to 20% of AIDS patients eventually develop neurological manifestations of the disease including dementia, myelopathy, and peripheral neuropathy. In infected children, cognitive and motor deficits are apparent (55,56). AIDS virus, on the way to the CNS, can reside in the barrier cells and increase the permeability of the barriers to HIV-1 or other unwanted materials (57–60). At present, it remains unclear whether AIDS dementia is due to the direct action of HIV-1 on neuronal structure, or due to the action of virus on brain barriers, which subsequently permits blood-borne toxicants to access brain parenchyma, or both.

The impairment of the BCB has been associated with a number of particular clinical encephalopathies. For example, schizophrenia and certain forms of idiopathic mental retardation may result from dysfunction of the choroid plexus (61). According to Rudin's hypothesis, damage by immune complexes allows easy access of exogenous psychopeptides into the CSF and surrounding limbic brain tissue. This, in turn, induces abnormal behavior. Other CNS disorders, possibly associated with the BCB dysfunction, include Reye's Syndrome (62), endogenous depression (63,64), and African Sleeping Sickness (65).

New Perspectives in Brain Barrier Research

Concept of Blood-Brain Regional Barriers

Chemical-induced brain damage is highly selective to some brain areas but not others. The current literature offers no satisfactory explanation for these observations: Why, for example, are neurons in the basal ganglion, particularly in the substantia nigra, selectively affected by MPTP? Why is lead accumulated in the hippocampus but manganese in the striatum? Similarity among chemicals in their structure and physical properties should explain this disparity, at least to some degree. Accordingly, manganese may mimic iron's distribution and MPP+ mimic dopamine. Still, why aren't other dopamine-like xenobiotics neurotoxic to substantia nigra? Why doesn't cobalt, another iron neighbor, cause Parkinsonism? Conceivably the clinical outcomes of exposure may be determined by two factors in combination: selective chemical disposition in the CNS and unique, intrinsic sensitivity of various brain tissues to chemicals.

In this context, brain barriers may play key deterministic roles in chemical-induced neurotoxicities through their physical location within brain structures, their vulnerability to chemicals, their local structural variations, and the varying activity of "biochemical-barrier." Lead accumulates in and targets both choroid plexus and hippocampus (51,66–68) and this phenomenon may relate to the intimate anatomic contact of both tissues. Perhaps closer anatomical location renders the hippocampus more susceptible to lead diffused from the choroid plexus. It is possible to hypothesize that endothelial barriers are not uniform throughout the entire brain but have structural or functional variance such that a toxin can access a particular region and spare others. Perhaps there are blood-brain regional barriers such as a "blood-striatum" barrier or "blood-hippocampus" barrier. These observations

and hypotheses will be subjected to research and debate in years to come.

Age and Brain Barriers

The BBB in the early stage of brain development is immature and highly ''leaky.'' While its formation is nearly complete in most brain areas at birth, the barrier remains functionally immature and permeable, and in some species, it remains in such a state long after birth (69,70). An age-dependent maturation also occurs in the BCB (1).

Chemical exposure at a young age may have profound neurotoxic consequences. During early development, the leaky structure of brain barriers accommodates the high demand of blood-borne nutrients by the growing brain. However, this relative openness of the barriers in early life renders the brain highly susceptible to insults from exposure to toxic substances, typified by lead neurotoxicity in young children. Additionally, because brain barriers actively participate in brain development by supplying useful macromolecules such as TTR, impairment to this function further compromises normal development. Research in this area is inadequate.

In aging, cerebral vasculature undergoes substantial changes including increased permeability and weakened detoxification, efflux, and repair functions. These changes, among others, can greatly reduce the barriers' defensive mechanisms and turn themselves into a target for toxicants.

Astrocytic-Endothelial Interactions

Cumulative evidence has substantiated a central role of astrocytes in induction, growth, and maintenance of the BBB and the transport of substances across it. When co-cultured with endothelial cells in vitro, astrocytes are capable of extending their endfeet to come into contact with endothelia and endow them with BBB properties (71). Interruption of this intimate astrocytic-endothelial cooperation induces negative consequences to the BBB. A mitochondrial poison, 3-nitropropionic acid, selectively damages striatal astrocytes within a few hours of *in vivo* exposure. This is followed by extensive BBB breakdown and striatal lesions (72). Certainly, the interaction between astrocytes and cerebral endothelia can become a target for neurotoxicants.

CONCLUSIONS

A growing body of evidence suggests that the brain barriers are subject to toxic insults from chemicals in blood circulation. Aging processes and disease status make the barriers more vulnerable to chemical toxicity. A number of neurotoxicants target brain barriers giving them a foothold for their subsequent neurotoxicity that otherwise would not occur. The implication of brain barriers in certain neurodegenerative diseases is compelling, although the evidence for any particular disorder being mainly due to chemical-induced barrier dysfunction remains sporadic. There are, on the other hand, enormous promises for brain researchers, toxicologists, and clinicians in this area: the effect of aging on barrier functions as influenced by chemical exposure is largely unknown; and the susceptibility of regional brain barrier to xenobiotics has not been explored. With rapid advancement in clinical and laboratory technologies, a century-old subject of blood-brain barriers will become a new frontier in neurotoxicological research.

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