

Transthyretin, Thyroxine, and Retinol-Binding Protein in Human Cerebrospinal Fluid: Effect of Lead Exposure

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Transthyretin (TTR), synthesized by the choroid plexus, is proposed to have a role in transport of thyroid hormones in the brain. Our previous studies in animals suggest that sequestration of lead (Pb) in the choroid plexus may lead to a marked decrease in TTR levels in the cerebrospinal fluid (CSF). The objectives of this study were to establish in humans whether TTR and thyroxine (T₄) are correlated in the CSF, and whether CSF levels of Pb are associated with those of TTR, T₄, and/or retinol-binding protein (RBP). Eighty-two paired CSF and blood/serum samples were collected from patients undergoing clinical diagnosis of CSF chemistry. Results showed that the mean value of CSF concentrations for TTR was 3.33 ± 1.60 $\mu\text{g}/\text{mg}$ of CSF proteins (mean \pm SD, $n = 82$), for total T₄ (TT₄) was 1.56 ± 1.68 ng/mg ($n = 82$), for RBP was 0.34 ± 0.19 $\mu\text{g}/\text{mg}$ ($n = 82$), and for Pb was 0.53 ± 0.69 $\mu\text{g}/\text{dl}$ ($n = 61$ for those above the detection limit). Linear regression analyses revealed that CSF TTR levels were positively associated with those of CSF TT₄ ($r = 0.33$, $p < 0.005$). CSF TTR concentrations, however, were inversely associated with CSF Pb concentrations ($r = -0.29$, $p < 0.05$). There was an inverse, albeit weak, correlation between CSF TT₄ and CSF Pb concentrations ($r = -0.22$, $p = 0.09$). The concentrations of TTR, TT₄, and Pb in the CSF did not vary as the function of their levels in blood or serum, but RBP concentrations in the CSF did correlate to those of serum ($r = 0.39$, $p < 0.0005$). Unlike TTR, CSF RBP concentrations were not influenced by Pb. These human data are consistent with our earlier observations in animals, which suggest that TTR is required for thyroxine transport in the CSF and that Pb exposure is likely associated with diminished TTR levels in the CSF.

Key Words: transthyretin; thyroxine; retinol-binding protein; lead; cerebrospinal fluid; choroid plexus.

The choroid plexus constitutes the blood-cerebrospinal fluid (CSF) barrier. The structural and functional integrity of this barrier is crucial to the homeostasis of the internal milieu of the central nervous system (CNS; Johanson, 1995; Zheng, 2001). The choroid plexus regulates brain chemistry and function by

two essential mechanisms, i.e., by selectively limiting the access of blood-borne substances to the cerebral compartment and by serving as a unique source of essential materials to the CNS (Davson and Segal, 1996; Nilsson *et al.*, 1992). The impairment of this barrier has been suggested to be associated with certain clinical encephalopathies (Levine, 1987; Ormerod and Venkatesan, 1970; Rudin, 1981; Sullivan *et al.*, 1999).

Research in both humans and animals has established that the choroid plexus accumulates lead (Pb) to an extraordinary degree following Pb exposure. Thus, the choroid plexus appears to function as a "sink" for Pb and other toxic metals (Friedheim *et al.*, 1983; Manton *et al.*, 1984; Zheng *et al.*, 1991, 1996). Accumulation of Pb in the choroid plexus in a low-dose, long-term Pb exposure model in rats results in a significant reduction in the concentration of transthyretin (TTR, previously named prealbumin) in the cerebrospinal fluid (Zheng *et al.*, 1996). Our *in vitro* studies of newly synthesized TTR molecules further reveal that Pb treatment inhibits the production and secretion of TTR by the choroidal epithelial cells in culture, leading to a diminished thyroxine transport at the blood-CSF barrier (Zheng *et al.*, 1998, 1999).

TTR is a 55,000-Dalton protein consisting of four identical subunits in a tetrahedral symmetry (Ingenbleek and Young, 1994). Whereas plasma TTR originates primarily from the liver, brain TTR is exclusively produced, secreted, and regulated by the choroid plexus (Aldred *et al.*, 1995; Herbert *et al.*, 1986; Wade *et al.*, 1988). On a tissue weight basis, the choroid plexus contains 11 times more TTR mRNA than liver, and synthesizes TTR at a rate 13 times faster than the liver (Schreiber *et al.*, 1990). CSF TTR makes up 25% of total CSF protein (Aldred *et al.*, 1995). 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 (Larsen and DeLallo, 1989). One of the possible functions of TTR in the brain is to transport thyroid hormones mainly in the form of thyroxine (3,5,3',5'-tetraiodothyronine, T₄). In humans, TTR conveys about 60–80% of CSF thyroxine (Hagen and Elliott, 1973; Herbert *et al.*, 1986; Larsen and DeLallo, 1989). Evidence has also suggested that thyroxine may enter

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the brain across the blood–brain barrier and/or blood–CSF barrier (Blay *et al.*, 1993; Chanoine *et al.*, 1992; Dratman *et al.*, 1991; Schreiber *et al.*, 1990), and the choroid plexus might facilitate the transport of thyroid hormones from blood to CSF via TTR synthesis in the choroidal epithelia (Schreiber *et al.*, 1990; Southwell *et al.*, 1993).

The thyroid hormones have striking effects on the CNS, particularly during the developmental period (Dussault and Ruel, 1987; Legrand, 1984). Thompson (1996) has identified several thyroid hormone-responsive genes in rat brain and further suggests a unique effect of thyroid hormone in initiating a finely tuned program of gene expression for neural development. Deficiency of thyroid hormones during early brain development produces multiple morphological alterations, including disturbance in the establishment of the normal wiring pattern in the brain that results in permanent impairment of neuronal connectivity (Legrand, 1984; Ruiz-Marcos *et al.*, 1979). Deficiency of thyroid hormones during development also leads to biochemical and electrophysiological alterations, such as delayed electrical activity in the peripheral auditory system (Hebert *et al.*, 1985) and diminished gene expression of myelin protein (Farsetti *et al.*, 1991). In children, the end point of the deprivation of thyroid hormones is irreversible mental retardation (Glorieux *et al.*, 1983; Legrand, 1984; Smith *et al.*, 1957).

In addition to the manufacture of TTR, 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). RBP, in coordination with TTR, interacts with retinol to form a trimolecular complex in a close equimolar stoichiometry over wide concentration ranges. About 90–95% retinol in human blood circulates by this complex (Vogel *et al.*, 1999). Evidence indicates that retinol is transported by RBP across the blood–brain barrier and blood–CSF barrier to the CNS (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). Many studies have established that retinoids are essential to brain development (Esfandiari *et al.*, 1994; Maden *et al.*, 1990; Ruberte *et al.*, 1993; Sharma and Misra, 1990; Zetterstrom *et al.*, 1994).

Given that accumulation of Pb in the choroid plexus diminishes CSF TTR concentrations in animals, we postulated that environmental Pb exposure in humans may alter CSF TTR levels. As the choroid plexus also provides RBP to the CSF, we further postulated that accumulation of Pb in this tissue may interfere with RBP concentrations in CSF. Thus, the purpose of this study was to investigate *a*) whether TTR, total T₄ (TT₄), and/or RBP in human CSF were altered in a manner that correlated to CSF Pb concentrations; *b*) whether the status of TTR in the CSF correlated to that of TT₄; and *c*) whether the variations of TTR, TT₄, RBP, and/or Pb in the CSF were associated with serum concentrations of these parameters or with age.

MATERIALS AND METHODS

Subjects and sample collection. Paired CSF and blood samples were collected when and where available from the patients who were admitted to the Hangzhou First Hospital in Hangzhou, China. Most of the patients lived in the metropolitan area of Hangzhou City, the capital of Zhejiang Province, which had a population of 5.8 million in 1996. The patients were requested for evaluation of CSF chemistry for the following reasons: motor accidents (*n* = 15), brain tumors (*n* = 18), cerebrovascular diseases (*n* = 6), meningitis (*n* = 5), spinal cord injury or diseases (*n* = 5), peripheral nervous diseases (*n* = 5), neurodegenerative diseases (*n* = 3), or clinical diagnosis for other disorders (*n* = 25) such as viral infection and high cranial pressure. Patients with clinical or pathological evidence of renal failure and hepatodysfunction were excluded from this study. There were 32 women and 50 men, with ages ranging from 10 to 82 years. These and other demographic data are presented in Table 1.

CSF samples were collected, free of blood contamination, either during a surgical process or by lumbar puncture for clinical diagnosis. Portions of the leftover CSF samples were used for this study. Blood samples from the same patients were collected and prepared for serum. All collected samples were placed in polystyrene tubes and stored at –20°C until analysis. The study protocol received official approvals by the Office of Clinical Investigation at the Hangzhou First Hospital and the Internal Review Board at Columbia-Presbyterian Hospital.

Atomic absorption spectrophotometry analysis of Pb. All samples were thawed at room temperature. Pb concentrations in the CSF and whole blood were determined by graphite furnace atomic absorption spectrometry. CSF samples were thoroughly mixed and then diluted with 1% Ultrex HNO₃ containing 0.2% ammonium phosphate in a 1:5 ratio. If necessary, the samples were further diluted after the initial analysis. Standards for all determinations were prepared freshly daily from a Pb nitrate AA working stock solution in 0.5% Ultrex HNO₃ (Zheng *et al.*, 1996, 1999). For blood Pb (BPb) concentrations, the procedure of Fernandez and Hillgoss (1982) was used. Reference materials for BPb (#SRM-2670) from the New York State Department of Health were used as internal quality control standards. A Perkin-Elmer Model 3030 Zeeman atomic absorption spectrophotometer, equipped with an HGA-600 graphite furnace, was used for quantification. The detection limit of CSF-Pb and BPb was 0.1 ng/ml and 1 ng/ml, respectively.

Radioimmunoassay (RIA) of TTR and RBP. RIA procedures for determining serum and CSF concentrations of TTR and RBP are well established and previously described in detail (Blaner, 1990). The RIA for TTR employs purified human plasma TTR (both as standard and for use as [¹²⁵I]-TTR) and a monospecific rabbit antihuman polyclonal TTR antibody. A standard displacement curve was established by plotting the percentage of maximal binding of [¹²⁵I]-TTR with known amounts of homogeneously purified human plasma TTR for a standard dilution of anti-TTR. The purified human TTR was iodinated by the lactoperoxidase procedure as described by Blaner (1990). TTR concentrations in CSF and serum were quantitated using this procedure. The procedures for iodination and RIA of RBP were essentially the same as the above described for TTR, except that a rabbit antihuman polyclonal RBP antibody was used. The methods used for both TTR and RBP radioimmunoassay have proven to be sensitive, specific, and reproducible (Blaner, 1990). As little as 2–5 μl CSF was usually sufficient for the assay of CSF TTR or RBP by this method. Within- and between-assay coefficients of correlation for these RIAs were 4.8 and 6.2%, respectively.

Radioimmunoassay of total thyroxine (TT₄). The method of Black *et al.* (1975) was used for determination of TT₄ in serum and CSF. In brief, the T₄ standards were prepared in hormone-free serum, which was obtained by treatment of serum with activated charcoal followed by centrifugation and then filtration. Human serum samples were incubated with known amount of T₄ standard and mouse antihuman monoclonal T₄ antibody (1:3000) in an RIA buffer consisting of 0.025% 8-anilino-1-naphthalene sulphonic acid and 50 mM barbital. The antigen/antibody complex was precipitated by addition of 1 ml of 30% polyethylene glycol and 100 μl of 10 mg/ml gamma globulin. After

TABLE 1
Summary of TTR, TT₄, RBP, and Pb in Human Blood and CSF

	Male	Female	Total		
	Mean ± SD	Mean ± SD	Mean ± SD	Median	Range
Age	48.4 ± 20.0 (49)	50.7 ± 16.9 (33)	49.6 ± 18.7 (82)	45.0	10–82
BPb (μg/dl)	15.4 ± 8.07 (50)	14.2 ± 8.76 (32)	14.9 ± 8.31 (82)	12.6	2.5–40.3
Serum					
TTR (μg/ml)	250 ± 68.3 (50)	235 ± 76.3 (32)	244 ± 71.4 (82)	234.8	94.2–490
TTR (μg/mg)	3.87 ± 2.40 (50)	3.39 ± 1.96 (32)	3.69 ± 2.24 (82)	3.16	1.16–12.1
TT ₄ (ng/ml)	126 ± 59.9 (50)	120 ± 56.4 (32)	123.8 ± 58.2 (82)	112.6	10.5–276
TT ₄ (ng/mg)	1.92 ± 1.44 (50)	1.64 ± 0.91 (32)	1.81 ± 1.26 (82)	1.53	0.33–8.27
RBP (μg/ml)	32.9 ± 10.4 (50)	30.2 ± 10.4 (32)	31.9 ± 10.4 (82)	31.3	13.9–58.4
RBP (μg/mg)	0.51 ± 0.33 (50)	0.44 ± 0.25 (32)	0.48 ± 0.3 (82)	0.38	0.14–1.69
CSF					
Pb (μg/dl)	0.57 ± 0.73 (37)	0.46 ± 0.64 (24)	0.53 ± 0.69 (61)	0.26	0.01–3.84
TTR (μg/ml)	0.95 ± 0.26 (49)	1.03 ± 0.23 (33)	0.98 ± 0.25 (82)	0.93	0.52–1.90
TTR (μg/mg)	3.18 ± 1.36 (49)	3.57 ± 1.89 (33)	3.33 ± 1.60 (82)	3.21	0.73–8.15
TT ₄ (ng/ml)	0.44 ± 0.47 (49)	0.54 ± 0.52 (33)	0.48 ± 0.49 (82)	0.24	0.03–1.92
TT ₄ (ng/mg)	1.49 ± 1.75 (49)	1.66 ± 1.57 (33)	1.56 ± 1.68 (82)	0.78	0.03–6.66
RBP (μg/ml)	0.14 ± 0.20 (49)	0.11 ± 0.08 (33)	0.13 ± 0.16 (82)	0.11	0.01–1.41
RBP (μg/mg)	0.35 ± 0.20 (49)	0.32 ± 0.17 (33)	0.34 ± 0.19 (82)	0.36	0.03–1.07

Note. Numbers in parentheses represent sample size. The sources of variation could be due to the wide range of ages, the CSF sampling sites, and/or disease status. No significant difference ($p > 0.4$) was identified in any of these parameters between males and females.

centrifugation, the radioactivity in pellet was determined and converted to concentrations from a standard displacement curve.

TT₄ in CSF was assayed using the same procedure, except that the standard curve was established in the RIA buffer containing 1% bovine serum albumin. The method was repeatable and reliable. The intraday precision at 50 pg/tube was 4% ($n = 12$) and interday precision 5% ($n = 8$). The detection limit of TT₄ in serum and CSF was 15 pg/tube, which corresponded to 0.4 ng/ml serum and 0.075 ng/ml CSF.

Determination of protein contents. Total protein concentrations in CSF and serum were determined using a Bio-Rad Protein Assay Kit (Bio-Rad Lab, Richmond, CA) with bovine serum albumin as the standard.

Statistical analyses. Hospital records and other clinical reports were reviewed and abstracted for demographic data, clinical diagnoses, and neuropathological diagnoses. Data are expressed as the mean ± SD unless otherwise stated. Associations between Pb, TTR, and TT₄ in the CSF and serum were analyzed by a linear regression or by multiple linear regressions when the multiple factors were considered, following the data transformation to logarithm. This transformation is valid with regard to the symmetric distribution and the linearity of variables following the logarithm transformation. The differences between two means were analyzed by a standard, parametric ANOVA.

Materials. Chemicals were obtained from the following sources: bovine lactoperoxidase, barbital, 8-anilino-1-naphthalene sulphonic acid, polyethylene glycol, and thyroxine (3,5,3',5'-tetraiodothyronine, T₄, M.W. = 777) in free acid from Sigma Chemical Co., St. Louis, MO; ¹²⁵Iodide (specific activity: 17 mCi/μg), ¹²⁵I-labeled T₄ (specific activity: 1.2 mCi/μg) from Du Pont, Boston, MA; PD-10 Sephadex G-25 column from Pharmacia Biotech; and AAS standards of Pb from Alfa Products, Danvers, MA. Human TTR (M.W. = 55,000) and RBP (M.W. = 21,000) and rabbit antihuman TTR or RBP antiserum were prepared according to the procedures described earlier (Blaner, 1990). All reagents were of analytical grade, HPLC grade, or the highest available pharmaceutical grade.

RESULTS

TTR and TT₄ in Human CSF and Serum

The average CSF concentration of TTR among this Chinese population ($n = 82$) was 0.98 μg/ml, which was about 250-fold lower than serum TTR (244 μg/ml). If, however, both values were normalized by protein contents in CSF and serum, the TTR concentrations of the CSF (average 3.3 μg/mg protein) were approximately the same as those seen in serum (average 3.7 μg/mg; Table 1). Similarly, while TT₄ in serum (124 ng/ml) was about 258-fold higher than TT₄ in CSF (0.5 ng/ml), both were similar when adjusted with protein contents (Table 1). There was no direct correlation between serum TTR and CSF TTR (correlation coefficient $r = -0.015$, $p = 0.892$), nor was a correlation between serum TT₄ and CSF TT₄ observed ($r = 0.17$, $p = 0.123$). Thus, the variation in either TTR or TT₄ concentration in the CSF appears to be independent of that parameter in serum.

CSF TT₄ concentrations were highly significantly associated with CSF TTR concentrations, having an r of 0.33 ($p = 0.003$; Fig. 1). The age of the population played an insignificant role in this relationship, as verified by multiple linear regression analyses. CSF TTR tended to decline with an increase in age, but this relationship did not achieve statistical significance ($r = -0.21$, $p = 0.06$; Fig. 2). Similarly, there was no significant age-related change in the concentration of CSF TT₄ ($r = 0.129$, $p = 0.254$), serum TTR ($r = -0.008$, $p = 0.940$), or serum TT₄ ($r = 0.085$, $p = 0.449$). No gender-based differences were

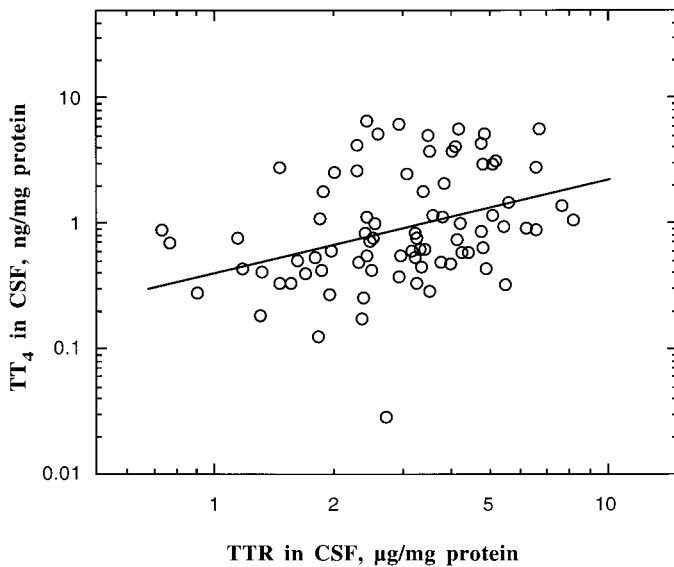


FIG. 1. TTR concentrations in human CSF are significantly associated with TT₄ concentrations in CSF. Both TTR and TT₄ were determined by radioimmunoassay. Data were analyzed by a simple linear regression ($r = 0.33$, $p < 0.005$, $n = 82$).

observed for TTR and TT₄ concentrations in either the CSF or the serum (Table 1).

As expected, TT₄ concentrations in serum were significantly associated with TTR concentrations in serum ($r = 0.32$, $p = 0.003$).

Pb in Human Blood and CSF

Blood Pb (BPb) varied widely among this population, ranging from 2.5 μg/dl to 40.3 μg/dl, with an average of about 15

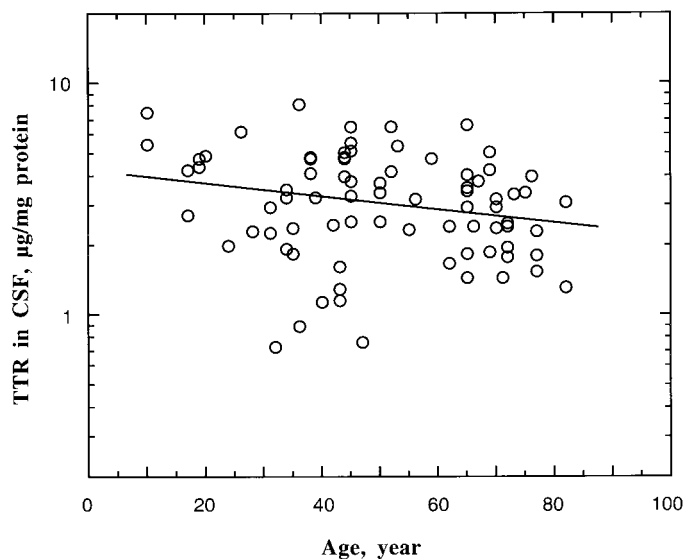


FIG. 2. TTR concentrations in human CSF are weakly and inversely associated with age. Data were analyzed by a simple linear regression ($r = -0.21$, $p = 0.06$, $n = 82$).

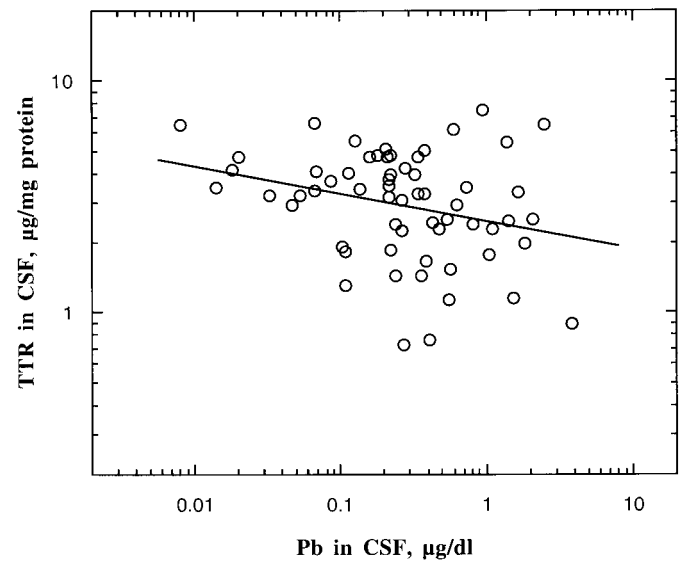


FIG. 3. TTR concentrations in human CSF correlates inversely with CSF Pb concentrations. Data were analyzed by a multiple linear regression where the age effect was excluded ($r = -0.29$, $p < 0.05$, $n = 61$).

μg/dl (Table 1). BPb concentrations were not statistically associated with age ($r = -0.140$, $p = 0.212$). The mean value of BPb observed in this study is lower than those observed among Pb-exposed Chinese workers (25–33 μg/dl; Ho *et al.*, 1998), but higher than those reported in general population in Beijing (10 μg/dl; Tang *et al.*, 1990) and Shanghai (9.2 μg/dl; Shen *et al.*, 1997).

The range of CSF Pb (0.05 to 3.8 μg/dl) was at least 10 times less than that observed for BPb. The average concentration of CSF Pb was 0.53 μg/dl. CSF Pb concentrations did not change with increasing age ($r = -0.068$, $p = 0.603$). Regression analysis revealed that CSF Pb concentrations did not change as the function of BPb ($r = 0.102$, $p = 0.439$).

Although CSF Pb concentrations in males was on average 23% higher than those of females, this apparent difference did not achieve statistical significance ($p > 0.4$). There was no significant gender difference in BPb concentrations.

Effect of CSF Pb and BPb on TTR and TT₄ in the CSF

The presence of Pb in the CSF was inversely associated with CSF TTR concentrations ($r = -0.30$, $p = 0.023$; Fig. 3). Multiple regression analyses excluded the possible contribution of an age factor in this relationship. Serum TTR concentrations, however, did not change as a function of BPb ($r = -0.11$, $p = 0.31$).

TT₄ levels in the CSF tended to decline with an increase in CSF Pb concentrations ($r = -0.22$; Fig. 4). However, this apparent inverse correlation between CSF TT₄ and CSF Pb concentrations was not statistically significant ($p = 0.090$).

Pb levels in the blood were not related to the CSF concentration of TTR ($r = 0.028$, $p = 0.801$), nor were they associ-

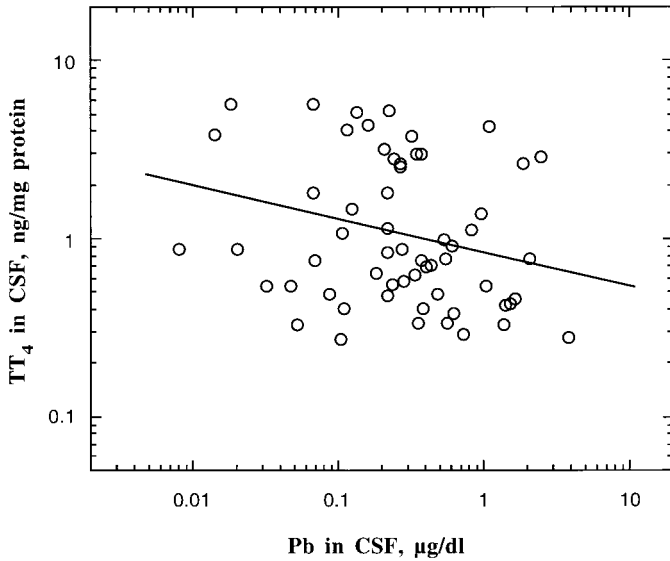


FIG. 4. TT₄ concentrations in human CSF tend to be inversely associated with Pb concentrations in CSF. Data were analyzed by a simple linear regression ($r = -0.22, p < 0.09, n = 61$).

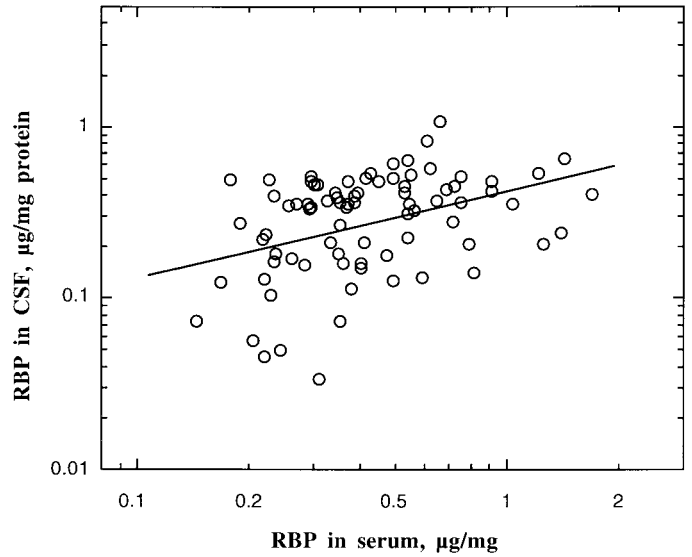


FIG. 6. RBP concentrations in human CSF are positively associated with serum RBP concentrations. Data were analyzed by a simple linear regression ($r = 0.39, p < 0.0005, n = 81$).

ated with CSF concentration of TT₄ ($r = 0.043, p = 0.704$). Change in CSF TTR concentrations appeared to be solely related to CSF Pb levels.

RBP in Human CSF and Serum and the Effect of Pb

Among this Chinese population, CSF RBP concentrations ranged from 0.03 to 1.1 µg/mg CSF proteins, a variation much less than that observed for CSF TTR. Per milligram of protein, RBP concentrations in both serum and CSF were about 10 times less than corresponding TTR concentrations (Fig. 5). In

contrast to CSF TTR, RBP concentrations in the CSF were highly significantly associated with RBP in serum ($r = 0.39, p = 0.0004$; Fig. 6). The levels of RBP in serum appeared to affect directly its level in the CSF. In addition, RBP concentrations in CSF showed a significant increase as a function of age ($r = 0.24, p = 0.03$; Fig. 7), while RBP concentrations in serum did not change with age ($r = 0.10, p = 0.36$).

Pb concentrations in the CSF were not significantly associated with CSF concentrations of RBP ($r = -0.145, p = 0.265$).

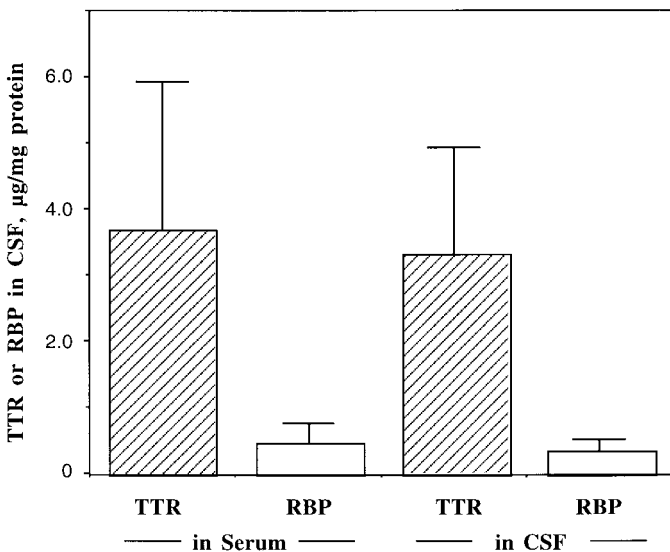


FIG. 5. Concentrations of RBP in serum and CSF are much lower than TTR concentrations in serum and CSF. Data represent mean + SD ($n = 82$).

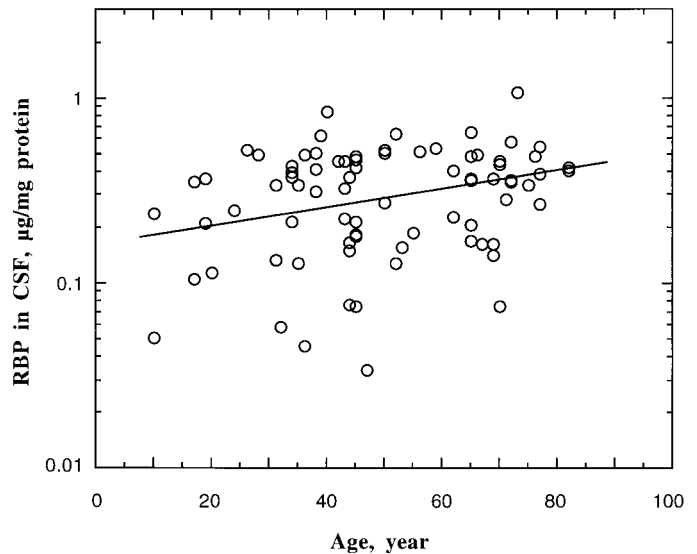


FIG. 7. RBP concentrations in human CSF increases as a function of age. Data were analyzed by a simple linear regression ($r = 0.27, p < 0.05, n = 81$).

Effect of BPb on TTR, TT₄, and RBP in Serum

By linear regression analyses, there was no significant correlation between BPb and serum concentrations of TTR ($r = -0.114$, $p = 0.307$), TT₄ ($r = -0.160$, $p = 0.152$), or RBP ($r = -0.096$, $p = 0.393$).

DISCUSSION

Results from these paired human CSF-serum samples indicate that the variation of TTR concentrations in CSF is not at all correlated with serum TTR concentrations. Schreiber and his colleagues have suggested that a faster rate of TTR production, due to greater TTR mRNA expression in the choroid plexus than in the liver, may afford the choroid plexus with the capacity sufficient to provide most, if not all, of the TTR found in the CSF. These investigators further demonstrated that CSF-derived TTR is secreted unidirectionally and exclusively from the choroid plexus into the CSF (Schreiber *et al.*, 1990). Other investigators have shown that except for choroidal epithelial cells essentially all other brain cell types, including neurons, astrocytes, oligodendrocytes, or cerebral endothelial cells, lack the capacity to synthesize TTR (Blay *et al.*, 1993; Herbert *et al.*, 1986). Accordingly, our finding of a lack of relationship between CSF TTR and serum TTR supports the view that human CSF TTR is primarily derived from the choroid plexus.

Our data further demonstrate that CSF TT₄ concentrations do not vary as a function of serum TT₄ concentrations. Given the lipid solubility of thyroxine, one might speculate that thyroxine would be able to cross the blood-brain barrier through a simple diffusion mechanism responsive to mass balance. However, data from human and animal studies indicate that the transport of thyroxine between serum and the CSF is highly restricted (Ingenbleek and Young, 1994; Kirkegaard and Faber, 1991; Schreiber *et al.*, 1990). Alteration of thyroxine binding to TTR results in altered patterns of thyroxine distribution in the choroid plexus and other regions of the brain (Chanoine *et al.*, 1992). *In vivo* exposure to certain toxic fungicides (hexachlorobenzene and pentachlorophenol) can also cause a diminished uptake of ¹²⁵I-T₄ into CSF and into specific brain structures such as the occipital cortex, thalamus, and hippocampus. This phenomenon has been taken to suggest a fine control of thyroxine availability to the CSF by the brain barriers (Kirkegaard and Faber, 1991; van Raaij *et al.*, 1994). Based on our results from this human study, it appears that brain TT₄ economy is probably not governed by serum thyroxine concentrations, but rather by the mechanisms that control its entrance, movement, and metabolism in the CNS.

The results of our human study reveal a significant correlation between thyroxine and TTR in the CSF. The question as to whether TTR plays an essential role in transport of thyroxine to the brain remains to be a subject of controversy. Some investigators have suggested that thyroid hormones are taken up in the choroid plexus from the blood via fenestrated capillaries

and the loose stroma of choroid plexus into the choroidal epithelial cells, where TTR is synthesized. Thyroxine then binds with a high affinity to TTR, which is subsequently secreted into the CSF (Chanoine *et al.*, 1992; Schreiber *et al.*, 1990; Southwell *et al.*, 1993). Other investigators have argued that the CNS acquires thyroid hormone mainly through the blood-brain barrier (Blay *et al.*, 1993; Dratman *et al.*, 1991). Our observation of the positive relationship between TTR and TT₄ in human CSF does not necessarily prove a possible function of TTR in transport of thyroxine across the blood-brain barrier and/or blood-CSF barrier. However, it may indicate that TTR is required to convey thyroxine within human cerebral compartment, and thus is essential in maintaining the homeostasis of thyroxine in human CSF.

Although long postulated, it is still startling that there is a significant inverse association between Pb and TTR concentrations in the CSF among this population. As the CSF and blood samples in this study were obtained primarily from patients with diverse diseases, possible interference in our results due to disease status cannot be ruled out. Nevertheless, the results of this human study appear to be consistent with our previous observations from animal experiments. Our earlier studies have shown that low-dose, long-term exposure of weanling rats to Pb in drinking water reduces TTR levels in the CSF (Zheng *et al.*, 1996). Using a pulse-chase technique to follow the newly synthesized TTR molecules labeled with [³⁵S]methionine in rat choroidal epithelial cells, we have also shown that Pb treatment inhibits the synthesis of total [³⁵S]TTR by these cells and greatly suppresses the rate of polarized secretion of [³⁵S]TTR from the cultured cells into the extracellular space (Zheng *et al.*, 1999). Taken together, these data and other published data suggest that the accumulation of Pb in the choroid plexus following Pb exposure alters one of the important functions of the choroid plexus in neuroendocrine regulation, i.e., suppression of TTR production and its secretion into the CNS. As TTR carries most of thyroxine in the CSF, it seems possible that the toxic action of Pb on TTR might ultimately alter thyroid hormone status in the CSF. This may, in turn, impair brain development and functional maturation and account in part for the toxic effects of Pb on brain development and function.

In contrast to TTR in the CSF, no statistically significant association between Pb exposure and the levels of RBP in human CSF was observed. The choroid plexus produces and secretes RBP into the CSF (Aldred *et al.*, 1995; MacDonald *et al.*, 1990; Zetterstrom *et al.*, 1994). RBP, probably along with TTR, assists in the transport of retinol across the blood-brain barrier and blood-CSF barrier to the CNS, although at present mechanistic details of this process remain unclear (MacDonald *et al.*, 1990; Martone *et al.*, 1988; Ruberte *et al.*, 1993). One of the interesting observations from this study in humans was that CSF RBP concentrations were closely related to serum concentrations of RBP. In other words, RBP in the cerebral compartment, unlike TTR, may be importantly derived from the

blood source. The brain barriers, while capable of synthesizing RBP, e.g., in the choroid plexus to the CNS, may be more permeable to the smaller RBP molecules than to TTR. How this would affect retinol homeostasis within the cerebral compartment is a subject worthy further investigation.

In summary, for a total of 82 randomly selected, paired human CSF/serum samples, we observed a significant correlation between TTR and TT₄ concentrations in the CSF and a significant inverse association between CSF Pb and TTR concentrations. These results suggest that CSF TTR may be importantly involved in thyroxine transport in the cerebral compartment and that Pb exposure may alter this function of TTR in humans.

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