Measurement of the Estradiol Concentration in Cerebrospinal Fluid from Infants and Its Correlation with Serum Estradiol and Exosomal MicroRNA-126-5p

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Estradiol has an important role in the brain, such as in neuronal development and protection, but estradiol levels in the human brain have not been well investigated. In this study, we measured the estradiol concentration in the cerebrospinal fluid (CSF) of infants to reveal the relationships between the estradiol concentrations in the serum and the CSF and further determined exosomal microRNAs in serum. Estradiol in the CSF was strongly correlated with serum estradiol and moderately correlated with miR-126-5p in the serum exosomes. This report is the first to determine the estradiol concentration in CSF from infants and showed that the levels of miR-126-5p as well as serum estradiol can be candidates to predict brain estrogen status.

Key words estradiol; cerebrospinal fluid; microRNA; infant

INTRODUCTION

Estradiol is a sex steroid hormone synthesized in and secreted from peripheral endocrine glands that has an important role in reproduction. Recently, the brain was shown to possess an inherent endocrine system and synthesize steroid hormones. Estradiol was reported to induce dendritic growth and synaptogenesis.1) Estradiol also modulates long-term depression and spinogenesis in hippocampal principal neurons.2) We previously showed the protective effects of estradiol on several neuronal damages using hippocampal slices isolated from rats in the developmental stage.3) Therefore, estradiol is a multifunctional molecule, especially in the developing brain.

Given the important roles of estradiol in the brain, it is important to determine or extrapolate the brain estradiol levels. Caruso et al. determined the estrogen levels in the plasma and cerebrospinal fluid (CSF) in adult rats and reported a positive relationship of the estradiol levels in the plasma and in the CSF of female rats.4) However, in humans, few reports have measured CSF estrogen levels except for one study on adult females by Kawwass et al.5) According to their report, estradiol levels in the CSF were approximately 20% of those in the serum. Brain estrogen can be derived from peripheral steroidogenic organs via the bloodstream and from de novo synthesis at specific brain regions from steroid precursors or cholesterol. Thus, peripheral steroid hormones might affect the levels of estradiol in the CSF. In addition, growing evidence has shown that the levels of several microRNAs are correlated with estradiol contents.6) In this study, we measured the estradiol levels in the CSF of infants and then assessed the correlation with not only serum estradiol levels but also microRNAs in serum exosomes to predict brain estradiol levels from peripheral measurements.

MATERIALS AND METHODS

Subjects and Sample Collection This study was approved by the review boards of the University of Occupational and Environmental Health (H30-083), the Kitakyushu General Hospital (H30-8-2) and Hiroshima University (E-1402). Children from 1 to 4 years of age with febrile seizures were recruited at the Hospital of the University of Occupational and Environmental Health and the Kitakyushu General Hospital between November 2018 and September 2019. The final number of subjects for analysis was 8 children, 2 boys and 6 girls. Blood and CSF samples were collected within several hours after arrival at the hospitals. CSF samples were obtained by lumbar puncture and serum was separated from blood by centrifugation. Isolated serum and CSF were frozen and stored before use.

Quantification of 17β-Estradiol in the Serum and the CSF The estradiol contents in the serum and CSF were measured by Aska Pharma Medical Co., Ltd., (Kawasaki, Japan) using liquid chromatography-tandem mass spectrometry (LC-MS/MS).7)

Exosomal RNA Extraction and Real-Time PCR RNA in serum exosomes was isolated from 500 µL of human serum by using an exoRNeasy Serum/Plasma Midi Kit according to the manufacturer’s protocol (Qiagen, Hilden, Germany). A synthetic cel-miR-39 microRNA mimic was added to each sample to serve as a spike-in control for monitoring microRNA purification and amplification. RNA concentration...
was determined by a Nanodrop 1000 system (Thermo Scientific, Waltham, MA, U.S.A.), and cDNA was synthesized using a miScript II RT Kit (Qiagen). MicroRNAs were measured by miScript CYBR Green PCR Kit with miScript Primer Assays for human miR-27b-3p (MS00031668, Qiagen), miR-126-5p (MS00006636, Qiagen), miR-148a-3p (MS00003556, Qiagen) and cel-miR-39 (MS00019789, Qiagen). Cp values for each microRNA were divided by those for cel-miR-39 to calculate the relative microRNA levels, which were represented as AU. 8)

**Statistical Analysis**  Correlation analysis was performed by using the Spearman rank correlation coefficient.

**RESULTS AND DISCUSSION**

The average estradiol concentration in the serum was $857 \pm 303$ fg/mL and that in the CSF was $11.0 \pm 2.6$ fg/mL, indicating that the estradiol concentration in the serum is approximately 80 times higher than that in the CSF in infants. A significant positive correlation was found between the estradiol levels in the serum and the CSF (Fig. 1, Spearman rank correlation coefficient was $0.83, p = 0.0093$). Estradiol is mainly synthesized in ovarian granulosa cells and is released into the bloodstream in adult females, while several tissues, such as the adrenal cortex, ovary, testis and brain, can contribute to estradiol concentration in the blood in infants. Peripheral estradiol was reported to be transported to the brain through the blood–brain barrier (BBB) in rats because of its hydrophobicity. 9) In contrast, estradiol is actively synthesized in the brain, especially the hippocampus. Rat hippocampal estradiol levels were shown to be 6 times higher than those in the plasma. 10) In this study, although the CSF concentration of estradiol was much lower than that in the serum, the concentration of estradiol in the CSF was highly correlated with that of the serum. Thus, a portion of estradiol in the blood is considered to be transported constantly to the brain through mechanisms such as permeabilization of the BBB, although certain regulation of estradiol transport can be presented. Estradiol concentrations in CSF of women at the average age of 25.6 years were 76 pg/mL, 5) which are much higher than CSF estradiol levels in infant measured in this study. Blood estradiol concentrations in adult females are higher than those in infants. Therefore, these knowledge also support that a part of estradiol in the blood is transferred to the brain.

We used serum and CSF residues from infants with febrile seizures after several clinical laboratory tests in this study. We and other investigators reported that seizures can cause temporary BBB leakage. 11,12) This change could affect estradiol transport between the peripheral blood and the central nervous system (CNS), although it is difficult to recruit healthy voluntary infants for the collection of CSFs.

Estimation of brain estradiol has become important because increasing evidence shows that brain estradiol is involved in normal neuronal development as well as neuronal protection by harmful stimuli such as stroke or convulsion. 7,13,14) A previous report showed that miR-27b-3p, miR-126-5p and miR-148a-3p in serum exosomes are involved in the systemic estradiol concentration using samples from premenopausal women and monozygotic postmenopausal twins after estrogenic hormone replacement therapy. 6) Therefore, we measured the levels of miR-27b-3p, miR-126-5p and miR-148a-3p in the exosomes from infant serum and then compared the levels with the estradiol concentrations in the serum and the CSF presented in Fig. 1.

miR-27b-3p and miR-148a-3p were not detected in any of the samples by real-time PCR, suggesting that these microRNAs rarely exist in exosomes from the serum of infants with febrile seizure. The levels of miR-126-5p were significantly correlated with the estradiol levels in the serum and showed the tendency of positive correlation to the estra-
diol levels in the CSF (Figs. 2A, B, Spearman rank correlation coefficients were 0.76 and 0.65, and p values were 0.028 and 0.0082, respectively). miR-126 is expressed in vascular endothelial cells\(^5\) and estradiol was reported to upregulate miR-126-3p expression via increased expression of a transcription factor, Ets-1 in vascular endothelial cells.\(^6\) Therefore, these findings might explain the positive correlation between serum estradiol levels and miR-126-5p contents.

**CONCLUSION**

We determined for the first time the estradiol concentration in CSF from infants. The estradiol concentration in the CSF was strongly correlated with serum estradiol concentration and moderately correlated with miR-126-5p in the serum exosomes. These novel findings could be useful to predict brain estrogen status from peripheral measurements.

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**Conflict of Interest** The authors declare no conflict of interest.

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