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Various levels of plasma brain-derived neurotrophic factor in patients with tinnitus

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ABSTRACT

Objective: Thus far, no objective measure has been developed to evaluate tinnitus severity. There is a close relationship between tinnitus and depression, in which brain-derived neurotrophic factor (BDNF) has a pathophysiological role. To determine whether BDNF levels could be used to evaluate tinnitus severity, we evaluated plasma BDNF levels in patients with tinnitus.
Methods: Plasma BDNF levels were measured in 43 tinnitus patients and 30 healthy control patients. The severities of tinnitus, depression, and anxiety were measured using the tinnitus handicap inventory (THI) and the hospital anxiety and depression scale (HADS), respectively. Patients with tinnitus were divided into 2 groups depending on their THI scores: mildly handicapped (<36) and severely handicapped (>38). We also divided our subjects into 2 groups depending on the HADS score, which represents patient mood, including depression and anxiety.
Results: Plasma BDNF levels were significantly higher in the mildly handicapped group than in the severely handicapped and control groups ($P < 0.01$). Patients with HADS scores of $\leq$14 had significantly lower THI scores ($P < 0.05$) and higher BDNF levels ($P < 0.01$).
Conclusions: Our findings show for the first time that plasma BDNF levels vary with the severity of tinnitus, suggesting that plasma BDNF level is a useful tool for objective evaluation of tinnitus.

1. Introduction

Tinnitus is the perception of sounds in the absence of external noise. Subjective tinnitus is defined as the perception of phantom sounds. Tinnitus can affect the entire life of an individual, preventing intellectual work and generally impairing quality of life. In some cases, tinnitus can cause suicidal behavior [2]. Severe tinnitus is often accompanied by affective disorders such as depression. Psychiatric comorbidity occurs especially in individuals with severe tinnitus and adds considerably to patient suffering [2]. Major depressive disorder and anxiety disorder occur most frequently in individuals with chronic disabling tinnitus, with a prevalence of 60% or more [11,34]. Several studies have shown that tinnitus severity and tinnitus-related distress are correlated with depression [32].

In general, subjective tinnitus has no physical signs, and there are no objective clinical diagnostic tests to evaluate its severity. Currently, only patient descriptions can serve as a basis for clinical evaluation. It is therefore very important to develop objective tools for evaluation of tinnitus. The tinnitus handicap inventory (THI) is a very useful test to evaluate the handicap caused by tinnitus [21]. In a consensus meeting (additional) use of the THI was recommended for clinical studies in order to facilitate comparison of results from different studies [17].

Brain-derived neurotrophic factor (BDNF) is a member of the "neurotrophin" protein family of growth factors, which are related to the prototypical "nerve growth factor" NGF [3]. Neurotrophic factors are found in the brain and the periphery. BDNF acts on certain neurons of the central nervous system (CNS) and peripheral nervous system, supporting the growth, differentiation, and survival of neurons and synapses [3]. BDNF plays a central role in synaptic plasticity and neurogenesis in general. It was reported that brain BDNF levels correlate with serum BDNF concentrations [8];

Abbreviations: BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; HADS, hospital anxiety and depression scale; HRP, horseradish peroxidase; NGF, nerve growth factor; NT-3, neurotrophin-3; NT-4, neurotrophin-4; THI, tinnitus handicap inventory; TMB, tetramethylbenzidine; BDI, Beck Depression Inventory.

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therefore, blood levels of BDNF may serve as an indirect measure of brain BDNF levels. According to Lommatzsch et al. [19], changes in plasma BDNF levels reflect BDNF concentration changes in the brain. It was recently reported that increased plasma BDNF concentrations observed during physical exercise in humans were due to the enhanced release of BDNF from the brain. These results indicate that serum BDNF is a non-specific trait marker of depression [25], whereas plasma BDNF is a state marker [8].

Strong evidence suggests that serum BDNF levels are abnormally low in patients suffering from major depressive disorder [29]. In addition, close relationships between BDNF gene expression levels and tinnitus have been reported [12,28,31]. Because tinnitus and depression tend to coexist, we reasoned that plasma BDNF levels may serve as tools for the evaluation of tinnitus.

BDNF levels may serve as markers for activity changes in the auditory system. Increased expression of BDNF and gamma-aminobutyric acid (GABA) in the inferior colliculus [24] as well as a reduction in local field potentials in the auditory cortex [30] were reported to be associated with tinnitus. BDNF-induced changes in glutamatergic signaling suggest that BDNF exerts modulatory effects on spontaneous neuronal firing rates in the auditory cortex [13].

In the present study, we examined plasma levels of BDNF in patients with tinnitus and in healthy controls. We tested for any correlations between plasma BDNF levels and clinical characteristics, including tinnitus severity. The objective of the study was to investigate the plasma levels of BDNF in patients with tinnitus.

2. Materials and methods

To investigate whether alterations in neurotrophin levels can be detected in subjects with tinnitus, we determined the peripheral levels (plasma) of BDNF in patients with tinnitus ($N = 43$; 14 male and 29 female) and healthy controls ($N = 30$; 15 male and 15 female). Our subjects included tinnitus patients without other inner ear disorders like sudden deafness or otitis interna. We carefully excluded subjects with tinnitus due to acute inner ear disorder by asking patients about recent history of hearing deterioration. The average age was 57.1 ± 15.2 years (average $\pm$ SD) and 50.7 ± 10.1 years, respectively. The severity of tinnitus was evaluated by the THI score. The proposed severity according to THI score was as follows: no handicap (0–16), mild handicap (18–36), moderate handicap (38–56), severe handicap (58–76) and catastrophic handicap (78–100) [22]. Patients with THI scores of less than 36 were classified as having mild tinnitus. Patients were classified as having severe tinnitus if the THI score was more than 38. Subjects were first-visit patients complaining of tinnitus at the Hino Municipal hospital. None of the patients had psychiatric disorders at the time of the first visit, and none reported taking drugs for the treatment of psychiatric disorders, including antidepressants and anxiolytics. Conventional audiological evaluation was conducted by pure tone audiometry. Severity of tinnitus was evaluated by the THI score. Mood, including anxiety and depression, was evaluated using the hospital anxiety and depression scale (HADS) [33]. We divided our subjects into 2 groups depending on the HADS score. In our previous study, we determined that a total score of 15 or more is indicative of mood disorder [10]. We classified patients with total scores of 14 or below as HADS ≤ 14 and those with scores of 15 or above as HADS ≥ 15. In addition we collected data on hearing threshold, site of tinnitus (left ear, right ear, bilateral, or intracranial), and duration of tinnitus from the initial onset. The hearing threshold was calculated as the average of 4 consecutive frequencies of 500, 1000, 2000, and 4000 Hz.

2.1. Plasma BDNF measurements

Blood samples from all subjects were drawn between 0900 and 1000 h. Approximately 4 mL of blood was collected in a vacuum tube with lithium heparin and immediately centrifuged at 3800 rpm for 10 min. Plasma was stored at $-70\,^{\circ}C$ prior to use. Human BDNF was detected by sandwich ELISA according to the manufacturer's instructions (CYT306; Millipore Co., Billerica, MA, USA). All assays were performed in F-bottom 96-well plates (Nunc, Wiesbaden, Germany). Tertiary antibodies were conjugated to horseradish peroxidase (HRP). Color was developed with tetramethylbenzidine (TMB) and measured at 450/570 nm. BDNF content was quantified against a standard curve calibrated with known amounts of BDNF. The detection limit was <4 pg/mL. All samples were tested twice, and mean values were calculated. Cross-reactivity to related neurotrophins (NGF, NT-3, and NT-4) was less than 3%. Intra-assay and inter-assay coefficients of variation were 3.7% and 8.5%, respectively. Concentration was expressed as pg/mL. The relationship between the THI score and plasma BDNF concentration was investigated.

We tested for correlations between plasma BDNF levels, tinnitus handicap, depression, and anxiety. All experiments were carried out in accordance with the guidelines of the ethics committee of the Hino Municipal Hospital and the Declaration of Helsinki.

2.2. Statistical analysis

All data were analyzed using Microcal Origin R version 6.0 software (Microcal Software Inc., Northampton, MA, USA) and GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA). Statistical analyses were performed using *t*-tests, repeated measures ANOVA, and chi-square tests. If the *P* value was less than 0.05, the results were considered statistically significant.

3. Results

Thirteen patients reported tinnitus in the right ear and 12, in the left ear. Eleven patients reported bilateral tinnitus, and 7 reported intracranial tinnitus. The average hearing threshold was 22.9 ± 20.3 dB for the right ear and 20.9 ± 14.8 dB for the left. THI ranged from 2 to 90 (average: 38.2 ± 23.4). The duration of tinnitus ranged from 2 days to 312 months (average duration: 25.5 ± 59.6 months). Initially, patients with tinnitus were divided into 2 groups depending on the duration of tinnitus. Tinnitus with duration of less than 2 months was defined as acute tinnitus. Tinnitus with duration of more than 3 months was defined as chronic tinnitus [20]. Twenty patients had acute tinnitus, and 22 patients had chronic tinnitus. When we compared BDNF levels, HADS scores, and THI scores between these groups, there were no significant differences. Therefore, we combined these 2 groups and treated them as 1 group for comparison purposes. The total HADS scores of tinnitus patients (14.5 ± 7.5) were significantly higher than those of controls (7.8 ± 5.4; $P < 0.0001$). Plasma BDNF levels ranged from 48.6 to 4045.4 pg/mL (average, 768.7 ± 961.4 pg/mL) in tinnitus patients and from 44.8 to 1289.9 pg/mL (average, 338.5 ± 287.7 pg/mL) in the controls (Table 1 and Fig. 1). The site of tinnitus, hearing threshold, and the duration of tinnitus did not correlate with the THI scores, HADS scores, or BDNF levels.

There were 25 patients with mild tinnitus and 18 with severe tinnitus. Fig. 1 shows that mild tinnitus patients showed significantly higher plasma levels of BDNF than severe tinnitus patients (1321.9 ± 1266.1 vs. 385.1 ± 524.9 pg/mL; $P < 0.01$) and controls ($P < 0.01$; Fig. 1). Plasma BDNF levels were negatively correlated with HADS scores ($R = -0.35$, $P < 0.05$), while THI and HADS scores were positively correlated ($R = 0.55$ $P < 0.0001$). After adjusting for