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ORIGINAL ARTICLE

Evaluation of Fluoxetine Induced Brain-derived Neurotrophic Factor on Facial Nerve Healing (Experimental Study)

Mustafa Gunes, Hakan Cincik, Salim Dogru, Evren Erkul, Mehmet Guney Senol, Fatih Ozdag, Atila Gungor

Department of Otorhinolaryngology and Department of Neurology (*), GATA Haydarpasa Training Hospital, Istanbul, Turkey (MG, HC, SD, EE, MGS, FO, AG)

Objective: Our aim is to evaluate the effect of fluoxetine treatment on serum Brain Derived Neurotrophic Factor levels as well as facial nerve healing by an experimental facial nerve crush injury in rat model.

Materials and Methods: 38 rats were allocated and rats were divided into five groups (Group A, B, A1, B1, C). Temporary facial nerve crush injury was performed in Group A, B, A1 and B1. Groups A1 and B1 were not treated with any drug. Fluoxetin, at a dose of 1 mg/ kg/ day, was injected intraperitoneally for 7 days in Group A, and for 28 days in Group B. Group C did not have any injury or treatment. Electromyography evaluation was performed at 7th day in Group A and A1, and at 28th day in Group B and B1. Brain Derived Neurotrophic Factor levels were measured from serum samples.

Results: There were no statistically significant increase of Brain Derived Neurotrophic Factor levels after fluoxetine treatment in Group A and B. Fluoxetine treatment did not affect the latency period on 7th day. On 28th day, it shortened the latency, though not significantly. Amplitude values revealed significant improvement between 7th day (Group A1 and A) and 28th day (Group B1 and B).

Conclusion: No significant effect of fluoxetine has been shown on serum Brain Derived Neurotrophic Factor levels or on facial nerve recovery following facial nerve injury. Further studies should be performed to ascertain the effective dose of Fluoxetine and proper application method.

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Introduction

Peripheral facial palsy (PFP) is the most frequent cranial neuropathy and may originate from various kinds of damage to the seventh nerve including its motor nucleus. In the majority of cases, investigations fail to establish a definite etiology. Idiopathic PFP is commonly referred as Bell's palsy (BP). The second most common cause is trauma [1].

Neurotrophic factors are one of the factors contributing to healing process of a nerve following an injury [21]. Brain Derived Neurotrophic Factor (BDNF) is an effective neurotrophic factor of which serum levels are found to be elevated after a nerve injury. It is a member of the neurotrophins family such as Nerve Growth Factor (NGF) and Neurotrophine 3-4-5-6 (NT 3-4-5-6) [2,3]. In many experimental studies, external application of BDNF has been used to increase axonal regeneration [2,3]. Serum BDNF levels of patients with major depression have been found to be lower than those of healthy persons. Many studies have showed that antidepressant treatment induces BDNF levels [4-6].

Since the BDNF is an expensive agent which cannot be easily obtained, we tried an antidepressant agent,

Corresponding address:
Evren Erkul
GATA Haydarpasa Egitim Hastanesi, KBB Klinigi
34668 Kadikoy / ISTANBUL / TURKEY
Phone: 90 216 542 20 20-4353 , Fax: 90 216 348 78 80
E-mail: evrenerkul@yahoo.com

Fluoxetin. We planned to administer this drug to rats with nerve injury in order to see its effects on BDNF levels and on healing of facial nerve injury in this experimental study.

Materials and Methods

Thirty-eight 38 male Wistard rats (12-24 week) weighing 250–300 gram were selected for the study. The study was approved by Animal Studies Ethics Committee at Haydarpasa Training Hospital, Istanbul, Türkiye (05.11.2008/116) and GATA Haydarpasa Training Hospital Hospital Epidemiology Committee, Istanbul, Türkiye The rats were divided into five groups. Two experimental groups (Group A and B) and two control groups (Group A1 and B1) had 8 rats. Negative control group (Group C) had 6 rats. Right facial nerve was dissected in all rats. Temporary facial nerve crush injury was performed in all groups except Group C. International standards regarding the animal care and handling have been followed during the experiments.

Standard Nerve Injury

Rats were anesthetized for 30–45 minutes by intramuscular injection of Ketamine (Ketalar®, Pfizer, İstanbul, Türkiye) in 150mg/kg dose. Depth of anesthesia was checked with corneal reflex and limb withdrawal response. Right side of their face was shaved and cleaned with antiseptic solutions. Then the facial nerve was exposed by transecting the skin from post auricular area to the angle of the mouth. Parotid gland was removed and facial nerve branches were followed proximally through the facial nerve trunk. The nerve trunk was pressed using a vascular clamp for 40 minutes without disrupting the nerve integrity (Figure 1). 4/0 silk was used for skin suturation. No prophylactic antibiotics were used. After awakening, total right facial paralysis were observed in all rats of study groups.

Groups:

Group A: After the standard nerve injury, Fluoxetin (Prozac®, Lilly İlaç Tic. Ltd. Şti., Istanbul, Türkiye), at a dose of 1 mg/kg/day, was injected intraperitoneally for 7 days. EMG's were performed on 7th day and serum samples were taken to assess BDNF levels.

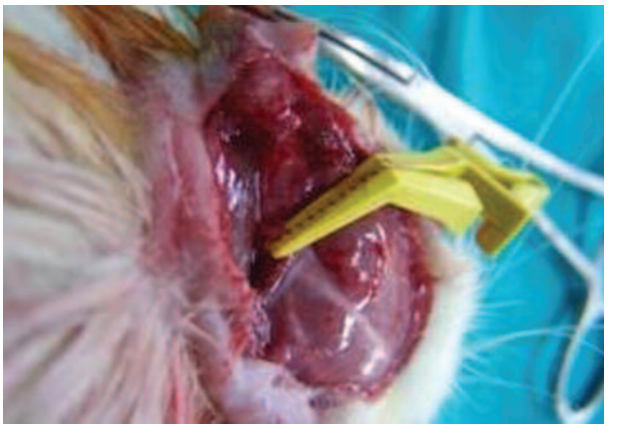

Figure 1. The nerve trunk being pressed with a vasaular clamp for 40 min.

Control Group A1: Facial nerve crush injury was performed without any medical treatment. EMG's were performed on 7th day and serum samples were taken to assess BDNF levels.

Group B: After the standard nerve injury, Fluoxetin, at a dose of 1 mg/kg/day, was injected intraperitoneally for 28 days. EMG's were performed on 28th days and serum samples were taken to assess BDNF levels.

Control Group B1: Facial nerve crush injury was performed without any medical treatment. After 28 days, EMG's were performed on 28th day and serum samples were taken to assess BDNF levels.

Group C: This group was planned as the negative control group. Facial nerve dissection was performed without any injury or treatment. EMG's were performed and serum samples were taken to assess BDNF levels.

Preparation and administration of the drug:

Fluoxetine 20 mg capsule was dissolved in warm sterile saline immediately before use. The animals received Fluoxetine at a dose of 1 mg/kg intraperitoneally at noon with a total volume of 3 ml over 30 seconds. Saline was used as placebo in rats in the control group (Group A1, B1 and C). All the procedures were performed blind by a single investigator and with randomization of treatments.

Electromyography (EMG): Rats were anesthetized and operated again for electrophysiological analysis of the nerve. Facial nerve (FN) trunk and branches was

identifed by dissection through the previous incision scar. Main trunk of the facial nerve was dissected from surrounding tissues, slightly suspended and prepared for stimulation. The transmission effect of surrounding tissues was eliminated by suspension of the trunk by a specially designed stimulating electrode. This electrode was made by our hospital's Biomedical Services. During CMAP (compound muscle action potential) recordings, the recording electrode (Medtronics; 0.45 mm diameter, 20 mm long, concentric fine needle electrodes, Catalog; 9013-L-0512, MN, USA) was placed in orbicularis oris muscle whereas the ground electrode was placed within sternocleidomastoid muscle (Medtronics; 0.7 mm diameter, 35 mm long, catalog; 9013-L-0611, MN, USA). Facial nerve EMG's were performed by the same neurologist with the same EMG device (Medelec, Syngery, London, UK)

Motor unit action potentials were obtained from orbicularis oris after stepwise increasing of stimulus intensity starting from 0.1 mA square waves electrical pulses lasting for 0.1 msec. Latency and amplitude of the evoked potentials were measured. Latency was defined as the elapsed time from the beginning of the stimulation to the appearance of first deflection from the isoelectrical line. In latency measurement, distance between stimulus and record point was kept constant at 30 mm. Amplitude was measured from negative to positive peaks of the compound muscle potential.

Assessment of serum BDNF level: In order to determine BDNF changes in serum, thoracotomy was performed following the EMG procedure and blood was taken from heart directly by syringes. The blood centrifugated at 3000 rpm for 10 minutes and serum was obtained. The serum BDNF levels were determined by using " Rat Brain Derived Neutrophic Factor (BDNF) ELISA kit (USCN Life Science Inc., Wuhan, China, catalog no: E0011 Ra) as immunological mediator.

Analytical sensivity and measuring limits of Rat BDNF ELISA kit:

Sensitivity: 0.031 ng / ml, Measuring range: 0.156-10 ng / ml, Cross reactions: No cross reactions were mentioned.

Statistical study: SPSS (Statistical Package for Social Sciences) for Windows 15.0 statistical software package was used for converting raw data into data and analyzing. While analysis of data, the arithmetic average (AA), standard deviation (SD), confidence intervals, minimum and maximum were used for descriptive statistics. Appropriateness of research data and normal distribution were checked by Kolmogorov-Smirnov test and graphical representation. Because of data does not fit the normal distribution, data obtained from the results of the study was compared by Mann-Whitney U test. Results were evaluated in 95% confidence interval, significance $p < 0.05$ level.

Results

One rat in Group A1 and one rat Group B1 died after surgery. Study was completed with seven rats in Group A1 and B1.

Serum BDNF levels Results: Due to hemolysis in serum, two rats of Group C were excluded from study. This analysis was performed in four rats Group C. Regression analysis was performed between absorbance and concentration. As a result, the correct equation $y=6.258\ x^{[2,5188]}$, and coefficient of determination (R^2) was 0.9924.

The highest value of serum BDNF concentrations was 3.814 ng/ml in Group C whereas the lowest value was 0.164 ng/ml in Group B.

Serum BDNF concentration average in Group C was statistically significantly higher than that of other groups ($p_{C-A1}= 0.02$; $p_{C-A}= 0.03$; $p_{C-B1}= 0.02$; $p_{C-B} = 0.01$). Serum BDNF concentrations (ng/ml) is shown in Table 1.

Serum BDNF concentration average in Group A was higher than that of Group A1, control group, however this elevation was not statistically significant ($p_{A1-A}= 0.77$). Serum BDNF concentration average in Group B was higher than that of Group B1, control group. This elevation was not statistically significant, either ($p_{B1-B}=0.48$).

Table 1. BDNF serum concentrations (ng/ml)

	N	Mean ± SD	95%Confidence Interval		Min.	Max.
			Lowest Level	Highest Level		
Group A	8	0.76 ± 0.60	0.25	1.26	0.19	1.89
Group B	8	0.72 ± 0.33	0.44	0.99	0.16	1.19
Group A1	7	0.72 ± 0.40	0.35	1.08	0.29	1.17
Group B1	7	0.69 ± 0.47	0.26	1.13	0.29	1.68
Group C	4	2.30 ± 1.20	0.40	4.21	1.10	3.81

EMG Findings: For the electrophysiological evaluation of groups, latency (ms) and amplitude (mV) values were measured. There were no response from two rats in Group A. Thirty-four rats were studied, totally (Group A; 6 rats, Group B; 8 rats, Group A1; 7 rats, Group B1; 7 rats, Group C; 6 rats).

Latency: Latencies were measured to be 4.15 ms in Group B1 as highest value and 1.05 ms in Group C as the lowest value. The statistical distribution of latency values are shown in Tables 2-3. The average latency measurements in Group C was statistically significantly shorter than the other groups (p_{C-A1}= 0.02; p_{C-A}= 0.01; p_{C-B1}= 0.01; p_{C-B} = 0.004). The mean average latency in Group A was longer than Group A1, but this was not statistically significant (p_{A1-A}= 0.28). The mean average in Group B was shorter than Group B1, this was not statistically significant, either (p_{B1-B} = 0.10).

Table 2. Latency values (ms)

	N	Mean ± D	95%Confidence Interval		Min.	Max.
			Lowest Level	Highest Level		
Group A	6	2.17 ± 0.75	1.38	2.95	1.35	3.45
Group B	8	1.66 ±0.16	1.52	1.79	1.45	1.90
Group A1	7	1.84 ±0.46	1.41	2.27	1.20	2.55
Group B1	7	2.12 ±0.93	1.26	2.98	1.30	4.15
Group C	6	1.17 ±0.36	0.79	1.54	1.05	1.5

Table 3. EMG latency values of facial nerves (msn)

	Group A	Group B	Group A1	Group B1	Group E
1. Rat	3.45	1.45	1.65	2.05	1.50
2. Rat	2.60	1.90	1.60	1.70	1.30
3. Rat	2.00	1.65	1.70	1.80	1.25
4. Rat	1.90	1.75	2.35	2.00	1.40
5. Rat	1.70	1.75	1.85	4.15	1.05
6. Rat	1.35	1.75	1.20	1.85	1.50
7. Rat		1.55	2.55	1.30	
8. Rat		1.45			
Mean ± SD	**2.17±0.75**	**1.66±0.16**	**1.84±0.46**	**2.12±0.93**	**1.17±0.36**

Table 4. Amplitude values (mV)

	N	Mean ± SD	95%Confidence Interval		Min.	Max.
			Lowest Level	Highest Level		
Group A	6	0.68 ± 0.72	-0.07	1.44	0.10	2.00
Group B	8	2.34 ± 1.61	1.00	3.68	1.00	5.20
Group A1	7	0.57 ± 0.39	0.21	0.94	0.20	1.30
Group B1	7	1.29 ± 0.93	0.43	2.14	0.60	2.80
Group C	6	4.12 ± 1.88	2.14	6.09	2.8	7.8

Values of amplitude: Amplitudes were measured to be 7.80 mV in Group C as the highest value and 0.10 mV in Group A as the lowest value. The statistical distribution of amplitude values are shown in Table 4.

The average value of the amplitude in Group C was significantly higher than that of Group A, A1 and B1 ($_{C-A}$= 0.003; p_{C-A1}= 0.002; p_{C-B1}= 0.003). Group C average was higher than Group B average, but this was not statistically significant (p_{C-B} = 0.09).

The average value of the amplitude of Group A was greater than that of Group A1, whereas the average of Group B was greater than that of Group B1 (p_{A1-A}= 0.89, p_{B1-B}= 0.07), though both differences were not statistically significant. Group B average was statistically significantly greater than that of the average of Groups A1 and A (p_{A1-B}= 0.003 p_{A-B}= 0.02). Amplitude values revealed significant improvement between 7th day (Group A1 and A) and 28th day (Group B1 and B).

Discussion

Many drugs and treatment methods like gangliosids, hormones, electromagnetic field studies [7], hyperbaric oxygen therapy [8], and corticosteroids [9] have been used to accelerate nerve healing after facial nerve injury. Human body derived neurotrophic factors may also contribute to the healing of nerve [9]. The most abundant neurotrophin in the brain is BDNF [10]. BDNF, a small dimeric protein, is synthesized mainly within neurons and responsible for the growth of neurons. The most BDNF concentrated regions are hippocampus and cerebral cortex in the brain [11]. BDNF levels increase following a nerve injury and it mainly affects motor neurons [2,3]. Local or systemic application of neurotrophic factors have been shown to decrease neuronal death rate and promotes axonal growth [2,3]. However, since the possible toxic-carcinogenic-teratogenic effects, proper dosage and application methods of the BDNF have not been defined, it is still not used routinely. When neuron cultures derived from mouse cortex was applied with BDNF, it was observed that BDNF promoted the development of dendrite and synapses [12]. In another study, a BDNF gene was implanted in a group of cells within mouse striatum. Then, a neurotoxin was applied to all cells. There were no serotonin or dopamine loss in BDNF implanted cells compared to the nonimplanted cells. It has been shown that BDNF, when applied externally, increases axonal regeneration, as well [13].

A large part of serum BDNF is stored within platelets. Platelets cannot produce BDNF; however, they gain it from outside sources and release it by a specific stimulus [14]. So, platelets are considered as BDNF transport system within human body [15]. There is close relationship between serum and central nervous system concentrations of BDNF. Serum BDNF originates from central nervous system by passing the blood-brain barrier and BDNF releases from platelets, so this helps to evaluate and guess the quantity of BDNF in central nervous system [14, 16, 17].

Brain BDNF levels decrease in neurodegenerative diseases such as Alzheimer's and Parkinson's disease [18, 19]. It has been shown that there is a close relationship between serum and brain concentrations of BDNF in patients with depression [20]. BDNF levels have been

shown to be lower in patients with major depressive disorder compared to the control subjects. It is also observed that there is an inverse correlation between serum BDNF levels and depression severity [21].

After extended application of various antidepressant medicines, BDNF levels and its receptor; tyrosine kinase B (TrkB) found to be increased in rat hippocampus [22]. Various antidepressants which were applied to various limbic areas, particularly into the hippocampus, have been shown to increase BDNF expression, as well [23-25]. Patients with depression who were not treated with any antidepressant have significantly lower serum BDNF levels than those who were treated with antidepressants [21]. These findings support the suggestion that antidepressant treatment increases BDNF expression.

Selective serotonin reuptake inhibitor (SSRI) which has been widely used to treat depression increases serotoninergic activity in synaptic gap [26]. Fluoxetine, a prototype of the SSRI group, is a specific and strong neuronal reuptake inhibitor [27]. Farahani et al. [6] shortened the wound healing period in rats by accelerating wound healing with Fluoxetine. In the present study, we aimed to show the effect of Fluoxetine treatment on serum BDNF levels and on facial nerve healing on 7th and 28th days by means of electrophysiological measurements.

It was reported that following a nerve injury in rats, serum BDNF levels increase within a week and then, get back to normal in 6 months [2]. However, serum BDNF levels came down to 1/3 of normal rat's BDNF levels in our study. We suggest that this may be due to anxiety or depression in rats.

Following nerve injury, BDNF levels in fluoxetine injected groups for 7 and 28 days were higher than those of noninjected groups. However, this difference was not statistically significant. Serum BDNF levels have not been shown to rise significantly with Fluoxetin application in this study. This may be due to ineffective dose of fluoxetine and/or the application method. Oral form of Fluoxetine was diluted with saline and then injected intraperitoneally rather than applying intraoral in this study.

Farahani et al.[6] reported that fluoxetine significantly reduced mean healing period in rats by accelerating wound healing with the same dose of intraperitoneal injection. Although stress decreased the linear healing rate by 48%, fluoxetine treatment increased it by 68% and 31%, both in stressed and nonstressed rats, respectively. Nerve regeneration was not evaluated by EMG at Farahani's study.

In the present study, fluoxetine treatment did not affect the latency period on 7th day. On 28th day, it shortened the latency, though not significantly. After nerve injury, there were no statistically significant difference between fluoxetin treated rats and control group on 28th days. However, there were a statistically significant difference between other groups and control group. In addition, the amplitudes were significantly increased at fluoxetine group after 28 days when compared with seventh day and also there was no statistically significant difference between control group and fluoxetine treated group with 28 days. We believe that the positive effect of fluoxetine arises after 28 days.

In conclusion, fluoxetine did not increase serum BDNF levels significantly with the given dose. However, there was positive effect on healing when used for 28 days. This is the first study of evaluating fluoxetine on facial nerve healing after injury and further studies are needed to ascertain proper application method and dose.

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No conflict of interest.

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