The Effect of Estrogen, Progesterone, and Its Combination on The Expression of Brain-Derived Neurotrophic Factor Medula Spinalis in Regeneration Process of Peripheral Nerve

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Abstract

Background: The presence of modulation factors in the regeneration process of peripheral nerve cells influenced by estrogen and progesterone. Peripheral nerve injury is a fairly common case of trauma. Brain-derived neurotrophic factor (BDNF) is the most active group of neurotrophins in stimulating neurogenesis. **Objectives:** To proves the effect of estrogen and progesterone on the expression of BDNF on the spinal cord in peripheral nerve regeneration process. **Methods:** Laboratory experimental study with the completely randomized design. Total transitory nerve ischiadicus was performed on four groups of rats. Each group received hormone therapy according to the group. Hormone therapy is administered every 3 days for 28 days, and on the 29th day is termination, spinal cord sampling, and followed by BDNF expression examination by Immunohistochemistry method. **Results:** In the control group, BDNF expression of spinal cord neuron cells was 93.0±14.0. In the treatment group, BDNF expression was obtained after estrogen therapy was 77.25±19.19, progesterone was 84.5±20.61, and in a combination of estrogen and progesterone was 77.75±16.54. After statistical tests, no significant differences were found between the treatment groups ($p = 0.316$). **Conclusion:** The administration of estrogen, progesterone, or a combination of both did not significantly increase BDNF expression when compared to the control group.

Keywords: *Peripheral nerves, Ischiadicus nerve, Estrogen, Progesterone, Neurotrophin, Brain-derived Neurotrophic Factor*

Introduction

Peripheral nerve injury is a fairly common case. The incidence rate of peripheral nerve injury in general is covering 2% of all cases of trauma[1]. A study in 2011 of 16,753 trauma patients, 219 (1.3%) of whom suffered peripheral nerve injury; 182 patients (83.1%) were men, where the most cause of peripheral nerve injury was laceration from sharps (61%), followed by second by traffic accident (22%)[2]. Peripheral nerve damage also happened in infection cases, such as leprosy[3]. In the United States, 11,000 cases of paralysis occur each year. This case costs up to seven billion US dollars each year. Until 1995, more than 50,000 surgical procedures were performed to repair peripheral nerve injuries[4]. Injury to peripheral nerves can cause severe functional disturbances that will greatly disrupt the daily and professional activities[5].

The treatment of peripheral nerve injuries is a difficult clinical problem. Because nerve cells are unique cells compared to other cells, their inability to proliferate and only regenerate their axons. Epineurial neurorrhaphy has been widely practiced as a procedure to repair peripheral nerve injury, with the ultimate goal of improving functional conditions such as the time

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before the injury is still unsatisfactory[6]. Unlike injury to the central nervous system, if the axons in the peripheral nerves are injured, regenerative responses may produce a good functional outcome. However, the results are not very satisfactory in cases of proximal nerve injury, due to slower regeneration and the occurrence of changes caused by chronic denervation. Strategies to increase axon growth can provide benefits in peripheral nerve regeneration[7].

Neurotrophin is a growth factor group consisting of Nerve Growth Factor (NGF), Brain-derived neurotrophic factor (BDNF), Neurotrophin-3 (NT-3), NT-4/5, and NT-6 [8]. Neurotrophin serves to protect nerve cells from degeneration and trigger nerve regeneration of the injury and increase the differentiation of nerve cell stem[9]. Based on another study, it was found that functional improvement was associated with increased levels of neurotrophin in muscle, serum, and nerves[10]. BDNF is a protein found in the central and peripheral nervous system. Part of the group Neurotrophin is most active in stimulating neurogenesis, and the most widely distributed in the nerve system[11,12].

Previous research found that prednisolon administration could improve nerve function impairment[11]. In the study, it was found that endogenous BDNF is absolutely necessary for the regeneration and remyelination process after peripheral nerve injury. BDNF is strongly expressed in peripheral nerves injured and also in denatured muscles. Provision of exogenous BDNF therapy actually lowered the expression of BDNF on peripheral nerves and resulted in inhibition of regeneration and re-myelination process after significant peripheral nerve injury[12]. In some other studies found the existence of modulation factor in the regeneration process of peripheral nerve cells, which is influenced by estrogen and progesterone hormones. In the study, it was found that administration of estradiol preparations in female rats post-treatment of crush injury on the ischiadicus nerve modulated the process of nerve cell regeneration[13].

Progesterone has functions other than the reproductive organs, also functions on other target organs such as the central nervous system and the peripheral nervous system. In the study found progesterone and its derivatives can cause the remyelination of axons by oligodendrocyte[14]. The effect of progesterone in the nervous system involves various signaling mechanisms. The identification of classic intracellular progesterone receptors as a therapeutic target for myelin repair demonstrates new health benefits for synthetic progestins, designed specifically for contraceptive use and replacement hormone replacement therapy. There is a great advantage in the use of natural progesterone in myelin improvement strategies because progesterone is converted into biologically active metabolites in neural networks and interacts with some target proteins. The formation of endogenous progesterone is currently explored as an alternative strategy of neuroprotection, axonal regeneration, and myelin repair. Progesterone modulates the regeneration process of peripheral nerve cells post crush injury. In the treatment group, rats' motor results returned after 21 days post-treatment, while the control group took longer time[15]. Therefore, in this study we measured the effect of estrogen and progesterone on the expression of BDNF as the peripheral nerve regeneration process in the spinal cord.

Methods

Laboratory experimental research with the completely randomized design of a factorial pattern were used in this study. The research was conducted in sample animal at Laboratory Department of Phytochemistry, Universitas Airlangga. The data were taken at Pathology Anatomy Faculty of Medicine, Universitas Airlangga - Dr.Soetomo Teaching Hospital during September 2014 to February 2015. The subjects were divided into four groups: control group, treatment group 1, treatment group 2, and treatment group 3. The experimental unit was a 2-3-month-old Wistar male rats that weigh 200-300 grams and in a good health. Animal health can be observed from the movement that quite agile, not lethargic, clean skin and no injuries, bright eyes and not wistful. Total sample was 12 rats.

The study procedure consisted of acclimation, peripheral nerve lesion model, estrogen preparation test, progesterone, and estrogen-progesterone combination and examination of BDNF spinal cord expression. In the acclimation of weighted, included in a complete randomized treatment group, acclimatized for seven days were fed and drank ad libitum; Furthermore, in peripheral nerve model models using a total transect

model, starting with a skin incision as high as the major trochanter, then the incision is extended in the distal direction, followed by a muscle-splitting incision. After nerve immobilization, the transaction model (neurotmesis) is performed using scissors, at the lowest possible level, just above the terminal nerve branching. The next step, Proximal and distal from the neural pieces was then reconnected end to end with simple interrupted suture stitches of two stitches on the epineural layer. After that, the skin was closed and stitched, in the control group, the rats was operated on the same part but not administered the drug preparation.

The examination of estrogen preparations, progesterone, and a combination of estrogen and progesterone. The administration of the drug was administered over a period of 28 days with a pause between the administration of the drug preparation for three days. The experimental group 1 (P1) was injected with an estrogen preparation, the treatment group 2 (P2) was injected with progesterone preparation, and treatment group 3 (P3) was injected with estrogen and progesterone preparations parallel to the leg then pushed through the abdominal wall into the peritoneal cavity. While the control group injected 0.9% NaCl and treated for 28 days; subsequent examination of BDNF expression of the spinal cord on the 29th day was taken by a sample of spinal cord tissue. Rats were given anesthesia using eter before L4 - L6 intumesensia lumbosakral tissues were taken. These area consists of ischiadicus neuron. These tissues were send to the Pathology Anatomy Faculty of Medicine, Universitas Airlangga - Dr.Soetomo Teaching Hospital for further examination of the BDNF expression of spinal cord using IHC. This study has been approved ethically by the Medical Research Ethics Committee of Faculty of Medicine, Universitas Airlangga Surabaya (177/EC/KEPK/FKUA/2017).

The collected data is analyzed by using the Shapiro-Wilk test. When using normal data distribution, the data were analyzed by parametric test, i.e., the factorial variance analysis (ANOVA) to determine the potency of estrogen, progesterone, and the combination of both, by comparing each estrogen, progesterone and combination group with the control group. The results are said to be significant if the obtained price $p < 0.05$, however, if obtained abnormal data distribution, then the data will be analyzed by non-parametric test Kruskal-Wallis.

Results

The total number of samples included in this study were sixteen rats which then divided into four groups. So for each group both treatment and control consists of four rats. After treatment and painting, an evaluation is performed on all preparations and the whole preparation is well-grounded, neuron cells can be well differentiated, whether expressed either BDNF or non-BDNF.

In this study, a spinal medallion preparation was performed using IHC technique to assess BDNF expression in neurons of the ischial nerve cells found in the spinal cord. Assessment of BDNF expression was done on the gray matter of the spinal cord, using a microscope with 400 times magnification. BDNF expression was examined in three fields of view, calculated in the percentage of the number of neuron cells that are colored by the total number of neuron cells in each field of view. The results of BDNF IHC dye preparation can be seen in Figures 1.

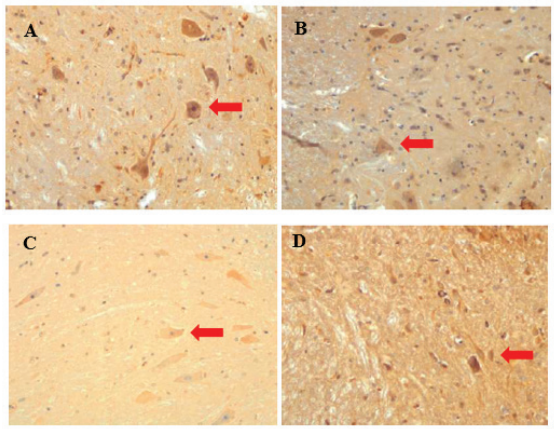

Figure 1. The results of colored IHC BDNF in rat's spinal cord: (A) control treatment; (B) estrogen therapy; (C) progesteron therapy; (D) estrogen and progesteron combination therapy

After the painting and interpretation of BDNF expression results in spinal cord rats try. In the control group, BDNF expression of spinal cord neuron cells was 93.0±14.0. In the treatment group, BDNF expression was obtained after estrogen therapy was 77.25±19.19, progesterone was 84.5±20.61, and in the combination of estrogen and progesterone was 77.75±16.54. The first data analysis was performed to test the normality of data distribution by using Shapiro-Wilk test, control group

obtained p = 0.001. Thus it can be concluded that the data has abnormal distribution, and continued with the non-parametric test.

Comparison of BDNF expressions between groups

Table 1 showed the results of the BDNF expression assessment of each group. To compare inter-group BDNF expression, Kruskal-Wallis non-parametric test was used to determine the effect of estrogen, progesterone, or estrogen and progesterone combination compared with the control group on BDNF expression on neuron cells in the spinal cord. From the result of Kruskal-Wallis test obtained p-value equal to 0.316, meaning there is no significant difference between treatment group. Thus it can be concluded from the test, that statistically no effect of giving estrogen, progesterone, or combination of estrogen and progesterone to the expression of BDNF cells of spinal cord neurons in this study.

Table 1. Comparison of BDNF expressions between groups

Groups	n	BDNF Expression (%)					P
		Mean	SD	Medium	Min	Max	
Control	4	93.0	14.0	100	72	100	0.316
Estrogen	4	77.25	19.19	81	54	93	
Progesterone	4	84.50	20.61	93	54	98	
Combination	4	77.75	16.54	75	60	100	

BDNF: Brain-derived neurotrophic factor

Discussion

Based on data obtained from the results of this study, it was obtained that giving estrogen, progesterone, or combination therapy did not statistically increase the expression of BDNF cells of spinal cord neurons. In the control group, BDNF expression of spinal cord neuron cells was quite high (93.0±14.0%), while in the treatment group, BDNF expression was obtained after estrogen therapy, progesterone therapy, and combination therapy showed lower number. From these data, it can be seen that on estrogen therapy, progesterone, or combination of both the relatively lower BDNF expression, although not statistically significant.

Previous research on the effect of estrogen and progesterone therapy on mRNA and BDNF protein levels in rat brain, in which we found the significant increase in mRNA and BDNF protein levels in some areas of the rat brain after estrogen and progesterone hormone therapy[15]. Low level of plasma estrogen also increases Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL) and Osteoprogeterin (OPG)[16]. In this study, BDNF was evaluated by quantitative method by measuring BDNF mRNA by Reverse Transcription Polymerase Chain Reaction (RT-PCR) method, and BDNF protein content by Enzyme-linked Immunosorbent Assay (ELISA) method, while in this study, BDNF was evaluated using anatomical pathology examination with IHC painting, which was more subjective.

There was a significant increase in mDNA and BDNF levels after estrogen and progesterone hormone therapy in the hippocampus and piriformis cortex areas, whereas, for the frontal and olfactory cortical area, the study itself found no significant effect[17]. It can be concluded that the effect of estrogen and progesterone hormone therapy on elevated levels of mRNA and BDNF protein was specific depending on which area being examined. This may also apply to peripheral nerves, although in other studies estrogen and progesterone therapy has been shown to increase levels of mRNA and BDNF proteins in certain areas of the brain, but not necessarily when applied to the spinal cord.

Expression of BDNF, NT3, and NT4 on nerves and muscles, after being treated with various ischiadic nerve injuries (neuropraxia, axonotmesis, and neurotmesis). From the research, it was found that in the treatment of neurotmesis BDNF mRNA levels of iskiadicus nerve increased significantly more than 10-fold on days 7 to 28 when compared with the control group that was not given treatment[18]. From these findings, it can be concluded that BDNF levels will increase in peripheral nerve injury conditions, in this case, neurotmesis even without therapy. This may explain the findings of this study in the control group where only given peripheral nerve treatment (neurotmesis) without hormonal therapy, on day 29 after treatment was obtained results of high BDNF expression of 93.0 ±14.0 %, in which the results were not statistically significant when compared with the group with estrogen, progesterone, or combination therapy. As with other studies also using quantitative methods with RT-PCR with more measurable results when compared with IHC method used in this study[17,18].

Conclusion

The administration of estrogen, progesterone, or combination of both did not increase BDNF expression. The effect of estrogen and progesterone hormone therapy on elevated levels of mRNA and BDNF protein was specific depending on which area being examined. The BDNF levels will increase in peripheral nerve injury conditions such as neurotmesis.

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