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Pre and Post-ischemic Low Level Laser Irradiation as a Protective and Curative Procedure in Brain Stroke in Rats

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ABSTRACT

Introduction: These days, stroke is one of the leading causes of disability in develop countries which could be responsible for death in any age. Although the importance of this health issue, there is no definite treatment for it. The phenomenon of biostimulation induced by low level laser (LLL) has different usages in medical situations such as prevention from cell death and elimination of pain. In this study we used low level laser therapy in pre-ischemic and post-ischemic condition in stroke model of rats.

Materials and Methods: The brain stroke was induced in rats by middle cerebral artery occlusion according to Koizumi method. The LLLT-treated groups received a single diode laser exposure with 830 mW power output , 810 nm continuous-wave mode with the use of energy density of about 36 J/cm2 (35.891 j/cm2) at a power density of 149.549~ 150 mW/cm2 at the brain tissue level. Two groups received LLLT in different condition, one group before stroke and the other one after stroke.

Result: Both groups (Pre-ischemic and Post-ischemic) low level laser therapy have significant neurological improvement, less apoptosis (due to Caspase 3 activity assay) and brain lesion volume.

Conclusion: Low level laser therapy could be used as a prophylaxis and treatment before and after brain stroke. It might have protective and curative effects on stroke. Also the neurological outcomes could be better by low level laser therapy.

KEYWORDS Literature; English; Period.

Introduction

Despite ongoing development in treatments, stroke is still a leading root cause that ends up serious long term disability(Go AS *et al*., 2014)responsible for even more deaths each year than those resulting from tuberculosis, AIDS, and malaria combined. It is possible for stroke to occur at any age. However, as age increases the risk of stroke enhances correspondingly. It is reported by Hall *et al.* that 34% of individuals who were hospitalized due to stroke in 2009 were younger than the age of 65(Hall *et al*., 2012).Treatment of acute ischemic stroke has been minimal at best. Treatments that have been shown to be effective, have been minimally impactful on this critical public health issue(Gonzalez, 2006).

One field which is appealing consideration is the application of low level laser (LLL) to treat brain disorders and diseases (Lampl, 2007; Naeser and Hamblin, 2011). The phenomenon of photo-biostimulation induced by LLL (light) therapy (LLLT), has been applicable in numerous medical situations. In such circumstances, prevention from cell and tissue death, stimulation of therapeutic process and afterward cure of injuries, and possible elimination of pain, as well as swelling and inflammation is required(Chung *et al*., 2012).It has been proven that a variety of photo-biostimulation effects will take place when near-infrared (NIR) light with specific wavelengths (i.e. 808 nm), penetrates deeply into the scalp, skull, and brain tissues due to its intrinsic potential(Zhang *et al*., 2000).The NIRlighthas an insignificant proportion of incident power density to the cortical exterior surface in human beings(Wan *et al*., 1981).This kind of light triggers the biological effects which are not due to thermal effects of the laser beam (Anders *et al*., 1993; Castro-e-Silva *et al*., 2003; Mochizuki-Oda *et al*., 2002).It has been suggested that human brain cells are optimally developed to respond to light therapy because of having cortical neurons which are rich in mitochondria; the primary photoreceptors for red and NIR light(Naeser and Hamblin, 2011).

Recently, several publications have been found to publish many articles on photo-biostimulation effects of infrared (IR) laser therapy both in vitro and in vivo(Byrnes et al., 2005; Desmet et al., 2006; Ilic et al., 2006; Lapchak and Araujo, 2007; Oron et al., 2001).IRlaser therapy, through in vivo studies, has also been shown to be useful for the care of acute myocardial infarctions, acute ischemic strokes, injured peripheral nerves, and spinal cord injuries.(DeTaboada *et al*., 2006; Leung *et al*., 2002; Nissan *et al*., 1986).

Despite wide-ranging investigation for stroke treatment method during the past several years, limited number of neuroprotectants have been appropriately translated into medical-related administration from basic medical sciences (Xing *et al*., 2008).The risk of a repeat stroke at the first month following a stroke is higher than at any other time(Mohan *et al*., 2011). The risk of stroke patients suffering from another stroke is evident in the fact that approximately 25% of individuals who suffer from a stroke have had a previous stroke history (Go *et al*., 2014).Regardlessof the economic and social effectsof stroke related to mortality; it has a huge economic impact and produces significant family burden imposed by the large majority of stroke patients who survivebutwithare physical and mental disability(Association, 2005; McCulloch and Dewar, 2001).

Even though the efficiency of LLLT in brain disease has been proved in recent years, most improvements will be achieved by minimizing the amount of individual suffering from brain disease.Nonetheless; it is possible to lessen brain stroke pa-

tients by prevention in vulnerable individuals. For this reason, the aim of this study was to assess the efficacy of low level laser therapy in prevention and diminishing the complications of brain stroke in rats.

Materials and Methods

Animals and Experiment Design

The entire animal procedures were authorized by the Ethical Committee of Animal Care and Use Protocol, Shiraz University of Medical Sciences. The study was performed on Adult male Sprague-Dawley rats weighting 250-300 grams (Laboratory Animal Center, Shiraz University of Medical Sciences).The rats were kept in pathogen-free environmental conditions in an air-conditioned, temperature-controlled room. They were maintained on a 12-hr on/12-hr off light cycle with open accessibility to a constant supply of water and food .The rats were randomly divided to forth group (n=10) described as below:

Control Group: This received no intervention and treatment

Sham group: This underwent MCAO operation

Pre – Ischemic LLLT: This received LLLT 1 day before MCAO

Post – ischemic LLLT: This received LLLT 1 day after MCAO

Laser Treatment

Mice were anesthetized with … and . . following a moderate sedation with ether and positioned on a clean bench completely coated with black opaque cover . The LLLT-treated groups received a single diode laser exposure with 830 mW power output , 810 nm continuous-wave mode (DioDent Micro 810 , HOYA ConBio , Fremont , CA) with the use of energy density of about 36 J/cm2 (35.891 j/cm2) at a power density of 149.549- 150 mW/cm2 at the brain tissue level (Wu *et al*., 2010).

The laser was installed centrally on the top of the exposed (shaved skin) skull by placing the tip of the laser head (beam size = 1.5×3.7 cm2) over the rats' head at the distance of 35.5 cm. Alaser power meter and an IR viewer was utilized to evaluate the laser irradiance and the laser beam diameter respectively at the brain tissue level after dispersion through the skull in order to determine transmission via the skull. The duration of laser irradiation was taking four minutes (240 seconds) recorded by a digital hand-held stopwatch. After LLLT treatment the mice were permitted to recover from anesthesia in their cages. Moreover, no pathologic changes upto eight weeks were observed in rat brains.

Animal model for middle cerebral artery occlusion (MCAO)

Inducing middle cerebral artery occlusion was performed according to Koizumi method(Koizumi J *et al.*, 1986). Briefly, the rats were anesthetized with halothane (5% for induction and 2% for maintenance) and then, a vertical incision was performed in the middle of the neck, the right common carotid, internal carotid and external carotid arteries were exposed and isolated from vagus nerve. Two loose sutures were prepared on common carotid artery below bifurcation and external carotid was clamped, the silicone-coated nylon suture 4.0 was passed through a little incision in common carotid artery and after 30 min the nylon suture was removed and the sutures were tightened up so that the blood could flow via external carotid artery by removing the clamp.

Histology

After 28 days, the rats were killed with deep anesthesia and perfused with normal saline and after that Paraformaldehyde 4%. The specimens were prepared for cryosections (at a thickness of 10µm) and then the sections were mounted on silicon pre-coated slides(EZ Longa *et al*., 1989a). The specimens were stained with Hematoxin& Eosin. The data was analyzed with one-way ANOVA SPSS 16.00

Neurological Function Assessment

Neurological performances were examined every two days for all rats during 28 days of experiment. The neurological examination was scored on six-score scale. The scores are following as below:

Score of 0: No neurological deficit

Score of 1: Failure to extend left forepaw completely. It shows mild focal neurological deficit

Score of 2: Circling to the left. It means a moderate focal neurological deficit

Score of 3: Falling to the left. It indicates a sever focal neurological deficit

Score of 4: Not walking spontaneously and decreasing level of consciousness.

Score of 5: Death due to brain ischemia(EZ Longa *et al*., 1989b).

Apoptosis evaluation with measurement of Caspase 3 activity

Activation of Interleukin-1ß-converting enzyme (ICE) family proteases/caspases initiates apoptosis in mammalian cells. This assay is based on spectrophtorometric assessment of chromophore p-nitroaniline (p-NA) after cleavage from labeled substrate DEVD-p-NA. The p-NA light emission could be measured by using spectrophotometer at 400-405 nm. For this assay Caspase 3 assay kit was bought from Abcam Company. (Abcam, cat. no ab39401)(Haghighi F *et al*., 2014).

Statistical analysis

All data were analyzed with One-Way ANOVA test by SPSS 16.00. The P vale was 0.05.

Results

Histological evaluation

The coronal sections were selected with 1 mm interval and from the first one was 2 mm posterior to frontal pole. Damaged area was distinguished by some ischemic signs including eosinophilic cytoplasm and pyknotic nuclei. The infracted volume in sham operated group was 187.238 ± 10.50 m, in Pre-Ischemic LLLT was 122.50± 7.25 m and in the post ischemic LLLT it was 113.26± 8.77 m (figure 1). There were significant differences between the sham group and two other groups.

Neurological Examination

The rats in each group were examined every 2 days for 28 days after MCAO according the score scale which previously described. The average of neurological scores for sham group was 3.97± 0.49, for Pre-ischemic LLLT was 2.55 ± 0.48 and Post-Ischemic LLLT was 2.84 ± 0.46. There were significant differences between sham group and the other two groups, however; the neurological scores between pre-ischemic LLLT and post –ischemic LLLT have no significant differences (figure 2).

Apoptosis Assessment

For apoptosis assessment, Caspase 3 activity was evaluated by spectrophotometer at 405nm. The higher absorbance value indicated the higher Caspase 3activity and as a result the higher incidence of apoptosis. The average of absorbance for sham group was 1.19±0.11, for Pre-Ischemic LLLT was 0.77 ± 0.11 and for Post-Ischemic LLLT was 0.65±0.12. There were significant differences between the groups which received LLLT and sham operated group. ($P< 0.05$; figure 3).

Discussion

The present study was designed to assess the protective effects of LLL irradiation before stroke in order to study the reduction of stroke complications. This was accomplished through neurological examination as well as assessment of Histological features and apoptosis assay outcomes. The

overall direction of results, demonstrated that pre-ischemic LLL irradiation provides a set of neurological rehabilitation which are approximately the same as what can be obtained from post-ischemic LLL irradiation. The overall result of laser treatment is postulated to induce improved energy metabolism, increased cellular respiration and, enhanced cell viability(Lapchak, 2010b) via stimulation mitochondrial adenosine triphosphate (ATP) formation by mitochondrial chromophore cytochrome c oxidase(COX)(Drochioiu, 2010; Karu, 2010; Karu, 2000). COX plays a central role in the eukaryotic cellular bioenergetics by providing protons across the interior membrane after energy absorption inside mitochondria (Desmet et al., 2006; Eells et al., 2003; Karu, 2000). This terminal enzyme complex in the cellular respiratory chain contains two copper centers, CuA and CuB. The CuA center is the primary chromophore which includes a wide absorption peak about 830 nm in its oxidized form (Lampl *et al*., 2007; Lapchak, 2010a). In addition to the enhancement in mitochondrial ATP production, photo-biostimulation might additionally stimulate secondary cell-signaling pathways and cytoprotective gene transcription such as antioxidant enzymes, heat shock proteins, anti-apoptotic proteins(Lapchak and De Taboada, 2010; Naeser and Hamblin, 2011)and modulation of oxidative stress and nitric oxide production (Chen *et al*., 2011). Improvement is witnessed in energy metabolism and cell viability enhancements as well as prevention of apoptosis. This is done by maintenance of homeostatic functions of cells and prevention of the increase of ischemia-induced death as a result of the normalization of ATP concentrations in cells within the "penumbra" (Lampl *et al*., 2007; Lapchak, 2010a) . The second mechanism operating in LLLT which enhances recovery of function (Oron *et al*., 2006) is increased neurogenesis and production of endogenous neurotrophic factors and stimulation of neuro-recovery mechanism(Leung *et al*., 2002). Neurogenesis is the generation of neuronal precursors and creation of newest neural cells (Galvan and Jin, 2007). The sub-granular layer (SGL) of the dentate gyrus in the hippocampus and the subventricular zone (SVZ) of the lateral ventricles are two main sites for adult neurogenesis(Eriksson *et al*., 1998). Physiological factors including growth factors and environmental enrichment can be a source of neurogenesis stimulation. Additionally, pathological processes such as neurodegeneration and ischemia could also contribute in neurogenesis stimulation(Jin *et al*., 2006).

In line with these, we took the advantages of histological evaluation in order to assess the stroke lesion area in different groups. As it has been expected, lesion areas were reduced in both laser-irradiated groups compare to the sham group. This is obviously attributed to the reduction of Caspase 3 activity rising from photo-biostimulation effects of LLLT. This is in complete agreement with the findings of Oron, et al. (Oron *et al*., 2006) and DeTaboada, *et al*. (DeTaboada et al., 2006) who have demonstrated the encouraging consequence of infrared laser therapies on the two different animal models of ischemic stroke including rabbit small clot embolic stroke model [RSCEM] and permanent middle cerebral artery occlusion in rat.(Zhang *et al*., 1997). This also confirmed the previous findings published by Zhang RL, *et al*. which was one of the earliest studies using LLLT (808 nm) to treat acute stroke in rat models. Their results were obtained following only one treatment at 24 hour post-stroke in a way that there seemed to be no distinction in the stroke lesion area between laser-irradiated and control rats. Furthermore, considerable improved outcomes of LLLT were developed when it has been applied 18 hours post-stroke to human acute stroke patients, over the entire surface of the head(Lampl *et al*., 2007; Stemer *et al*., 2010; Zivin *et al*., 2009).

Regarding the data obtained from neurological examination, it is recognized that both LLL treated groups, show a statistically significant (P value less than 0.05) improvement compared to the sham group. These values correlate favorably with the Lapchak, et al. which found significant behavioral improvement, by studying the effect of a 10-minute LLLT using high CW energy (25 mw/cm^2, 15 J/ cm^2) at midline of the rabbit embolic stroke brain (Lapchak *et al*., 2004). Although it was not curative enough in severe stroke patients(Zivin *et al*., 2009), significant improvements ($p< 0.04$) were observed in the moderate and moderate-severe stroke patients only who received the real laser protocol(Zivin *et al*., 2009).

The latter finding was particularly important when it came to neurological outcomes. Interestingly, not only the rats in pre-ischemic group did not show any statistical significant (P value < 0.05) higher levels of Caspase 3 activity compared to those in post-ischemic group, but also it appears that they were approximately as well-recovered as the other group. prevention looks more efficient than the treatment in high-risk patients. As the Stroke Council of the

American Heart Association stated, despite there has been many potential treatment of acute stroke available, prevention is still a more effective method for reduction of stroke probability(Wolf PA et al., 1999) in high-risk patients. Although there are certainly rooms to optimize physical parameters as well as choosing appropriate patients in applying the pre-ischemic LLLT as a preventive method, it seems that it has a number of advantages. Firstly, since the LLLT mechanism has been completely distinguished(Hamblin MR et al., 2006), its tolerability and safe approach, has made it a reliable procedure potential for an efficient treatment of neurological disordersParticularly(Lampl Y et al., 2007; Leung et al., 2002; Naeser and Hamblin, 2011; Nissan et al., 1986), considering elders or severe liver disease patients who are poor in managements of drug interactions. Besides, high-risk patients can benefit from non-aggressive profile of LLLT, low expenses, and availability of the modality.

Conclusion

Low level laser therapy could be used as a prophylaxis and treatment before and after brain stroke. It might have protective and curative effects on stroke. Also the neurological outcomes could be better by low level laser therapy.

Conflict of interest

The authors report no conflict of interests.

Figure 1.Histology evaluation.

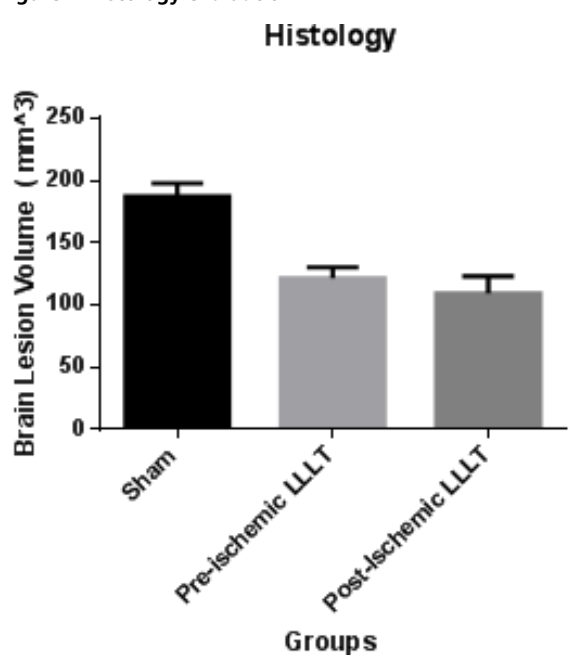


Figure2. Neurological function assessment

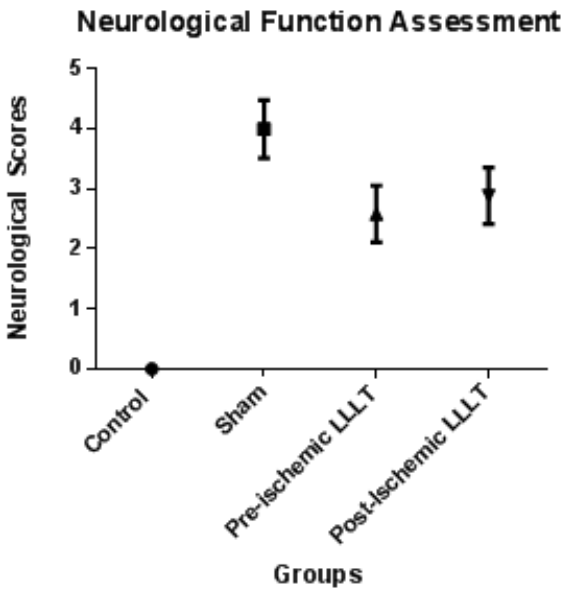
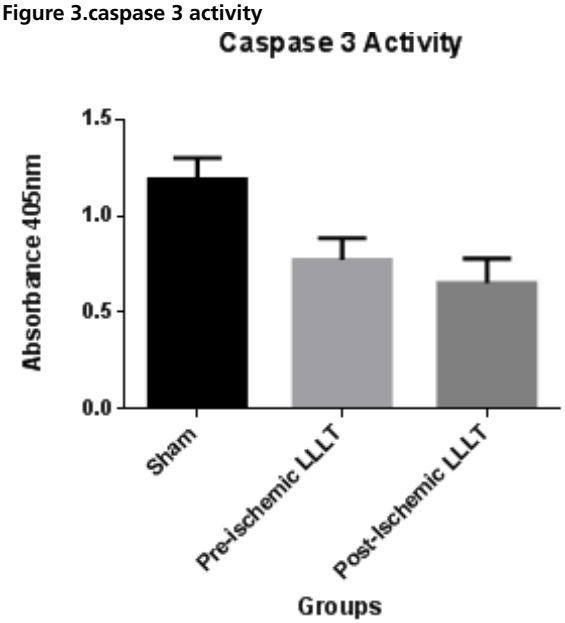


Figure 3.caspase 3 activity



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