Int J Clin Exp Physiol. 2024;11(1):32-37.
https://www.ijcep.org

Original Article

Optimal Parameters of Magnetic Field Action and their Influence on Some Index Improvement in Traumatic Brain Injury Rat Model

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ABSTRACT

Background and Aim: Traumatic Brain Injury (TBI) remains a significant medical challenge with limited effective treatments. Recent studies suggest that magnetic field therapy can enhance neural recovery. However, the optimal parameters for magnetic field application in TBI models are not well established. The aim of this study was to find out the optimal parameters for applying magnetic field to the recovery of TBI rats by using Taguchi method and to determine the effect of magnetic field on survival rate and some functional index of the TBI rats. **Methods:** Fourteen albino Wistar male rats weighed 220-250 g were used in the experiment. An experimental model of TBI rats was constructed by Feeney's method. Optimal parameters of magnetic field was identified by Taguchi orthogonal array. Magnetic field was applied to the experimental group TBI rats for 5 days. Modified Neurological Severity Score (mNSS), Brain Water Content (BWC), electrical stimulation threshold and survival rate were measured. **Results:** The optimal magnetic field parameters, determined by using one-factor experiment and Taguchi method were magnetic induction at 20 mT, frequency at 20 Hz, duration for 25 min and daily application frequency of 2 times per day. When applied to a TBI rat model, magnetic field of the optimal parameters decreased brain water content and increased survival rate. In addition, modified Neurological Severity Score (mNSS), electrical stimulation threshold were decreased. **Conclusion:** Optimal parameters of alternating magnetic field for functional recovery of TBI rats improved neuro dysfunction and increased survival rate in TBI rats.

Keywords: Magnetic field, Modified neurological severity score, Survival rate, Taguchi method, Traumatic brain injury model.

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Received: 28-12-2023;
Revised: 02-02-2024;
Accepted: 26-02-2024.

INTRODUCTION

Traumatic brain injury due to head trauma is increasing in the world every year and the mortality because of those is also high with malignant tumor, heart disease and stroke. The cause of traumatic brain injury includes falls (35.2%), traffic accidents (17.3%), sports injury (16.5%) and other accidents (31%).[1]

Currently, it is common to use decompressive dehydration to reduce intracranial pressure as a primary measure in cerebral contusion worldwide, which is important to prevent cerebral herniation and secondary brain damage. However, decompressive dehydration is effective in preventing cerebral herniation by lowering intracranial pressure and preventing secondary brain damage, but the effect of restoring nerve function and eliminating sequelae is not clear.

Thus, in recent years, various therapies have been studied worldwide to prevent brain incarceration and secondary brain damage in cerebral contusions, as well as to restore neurological function and treat sequelae. Treatment for cerebral contusion include pharmacological therapy, surgical treatment, hyperbaric oxygen therapy, hypothermia and psychotherapy. Despite advances in medical science, effective treatments for TBI are still limited, underscoring the need for novel therapeutic approaches. One promising area of research involves the use of Magnetic Field (MF) therapy to enhance neuroplasticity and functional recovery following TBI.[2-4] Magnetic fields have already been widely applied in clinical practice due to their potent effects on analgesia, anti-edema, anti-inflammatory and microcirculatory restitution effects.[5-8] MF therapy has been explored for various neurological conditions, including stroke, neurodegenerative diseases and spinal cord injuries, showing potential in promoting neural repair and functional recovery.[9,10] However, the application of

DOI: 10.5530/ijcep.2024.11.1.5

MF therapy in TBI is relatively nascent and optimal parameters such as field strength, frequency and duration of exposure remain to be systematically evaluated. Recent studies suggest that MF therapy can modulate neuro-inflammatory responses, reduce oxidative stress and enhance neurogenesis, which are critical factors in the recovery process post-TBI. For instance, a study by Martínez-Sánchez *et al.*[11] demonstrated that low-frequency pulsed electromagnetic fields significantly improved cognitive function and reduced lesion volume in a rat model of TBI. Similarly, Wang *et al.*[12] reported that MF exposure facilitated synaptic plasticity and improved motor function in TBI-affected rodents. Despite these promising findings, the translation of MF therapy from preclinical studies to clinical practice requires a thorough understanding of the optimal parameters that maximize therapeutic benefits while minimizing potential risks.

Therefore, this study was undertaken to evaluate the optimal parameters of magnetic field application for the recovery of TBI rats by using Taguchi method[13-15] and to determine the effect of magnetic field on the survival rate and some functional indices of the TBI rat.

MATERIALS AND METHODS

Animals

14 adult male Wistar rats weighing 220-250 g were included in the study. All the rats were allowed for free access to food and water before surgery under optimal conditions (12 hr light/12 hr dark with humidity 60±5%, 22±3°C). All animals were taken care as recommended in the Guide for the Care and Use of Laboratory Animals issued by the D.P.R.K Association of Laboratory Animal Care.

Grouping

Control group (n=7): Adult male Wistar rats with TBI but without magnetic field application.

Study group (n=7): Adult male Wistar rats with TBI and underwent optimal magnetic field application for 5 days.

Experimental Model of TBI

Animal model of TBI using rats was constructed by Feeney's method.[16-18] The rats were weighed and anesthetized by intraperitoneal injection of 5% ketamine 0.05 g/kg and fixed on the operating table with the abdomen facing downward. After removal of the parietal hairs, the skin was incised 2 cm along the mid-sagittal line, the subcutaneous fascia was removed and the membrane was removed to expose the skull. A hole of 5 mm diameter was drilled in the center of the left posterior cortical motor area. In the model group, the impact head of the beater was placed over the exposed dura. After the stroke, erection of rats' hairs, limb cramp and urinary incontinence were observed. The drilled site was filled with wax and the skin was sutured.

Parameters Assessed

Modified neurological severity score (mNSS)

In the TBI model, motor function, sensory function, balance function, degree of reflex loss and limb strength were assessed using the modified Neurological Severity Score (mNSS).[19,20] The higher the mNSS, the greater the neuro dysfunction and the normal is zero.

Brain water content (BWC)

According to the wet-dry method, Brain Water Content (BWC) was determined.[21,22] The rat's head was cut off to remove the brain. The brain was cut along the midline and weighed using an electronic balance to the left hemisphere. After incubation in a drying oven at 100°C until no further change in weight was observed, the dry weight was measured and the brain water content was calculated according to the following formula:

$$\text{Brain water content (BWC)} = \frac{(\text{wet weight} - \text{dry weight})}{\text{wet weight}} \times 100\%$$

Electrical stimulation threshold

By using the current stimulator (ELECTROSTIMULATOR ST-3, Hungary), electrical stimulation threshold of rats were measured. The electrodes were placed on the forelimbs and hind limbs of rats and the electrical stimulation threshold was measured as the threshold for muscle contraction when pulsed electrical stimulation (duration 1 ms, stimulation frequency 2 Hz) gave.

Application of Alternating Magnetic Field

Rats in the experimental groups were exposed to alternating magnetic fields generated in the cylindrical applicator of an Ambit 2000 apparatus for magneto therapy (Ambit, Poland). Rats in control group were subjected to sham exposure. During sham exposure, the applicator was remained unconnected; therefore, no magnetic field was generated.

Statistical Analysis of Data

All data were expressed as mean±SD. Inter-group differences were compared through two-tailed independent sample *t* test using SPSS 10.0 statistical software. Differences were considered statistically significant at a probability level equal to or less than 0.05.

RESULTS

Table 1 showed the changes of mNSS in rats exposed to different intensity of magnetic field after traumatic brain injury. The mNSS of the TBI rats decreased significantly compared to the control group when the magnetic field intensity was 10 mT, 20 mT and 30 mT. Table 2 showed the changes of mNSS in rats exposed to different frequencies of magnetic field after traumatic brain injury. The results of the experiments showed that the mNSS was significantly reduced in the rats which received magnetic field at

frequencies of 20, 30 and 40 Hz compared to the control group. Table 3 showed changes of mNSS in rats exposed to the different duration of magnetic field after traumatic brain injury. The application of the magnetic field for 15 to 25 mins had a positive effect.

In Table 4, the control elements and their respective levels were given. Magnetic induction (mT), frequency (Hz), duration (min) and number of times (times/day) were control alternatives that were taken into account for the optimization process. Table 5 showed the L9 orthogonal array that was taken into account for the analysis along with a three-level design, four factors and a total of 9 experimental runs. Table 6 presents the effect of magnetic field parameters on grey relational grade. The parameter level that leads to optimal values of the process performance characteristics is the level of maximum grey relational grade. From the response table for the grey relational grade, the optimal magnetic field parameters were: magnetic induction at level 2 (20 mT), frequency at level 2 (20Hz), duration at level 3 (25 min) and number of actions at level 2 (2 times per day).

Table 7 showed changes of BWC in TBI rats exposed to optimal parameters of the magnetic field. BWC in TBI rats exposed to the magnetic field was 82.35±0.43%, which was significantly lower than control model group after 5 days. Table 8 showed changes of mNSS in TBI rats exposed to the magnetic field. mNSS was significantly reduced in the TBI rats which received magnetic field compared to the control group rats that did not receive magnetic field. The mNSS in the experimental group decreased significantly 3 day after magnetic field action compared to the control group and continued to decrease significantly until 14 days.

Table 9 showed the change in the electrical stimulation threshold to assess the recovery of neuronal function of traumatic brain injury by magnetic field. When magnetic field of 20 mT and 20

Table 1: Changes of mNSS in rats exposed to the different intensity magnetic field after traumatic brain injury.

Groups	Magnetic induction	mNSS	DR (%)
Control (n=7)		10.13±0.51	
Experimental (n=7, respectively)	5	7.69±0.27*	24.09
	10	6.39±0.26*#	36.92
	15	5.67±0.36*#	44.03
	20	6.58±0.23*#	35.04

Values were expressed as mean±SD. $p<0.05$ was considered as statistically significant.* $p<0.05$ versus control group; #$p<0.05$ versus 5mT group. mNSS: Modified Neurological Severity Score; DR: Decrement rate.

Table 2: Changes of mNSS in rats exposed to the different frequency magnetic field after traumatic brain injury.

Groups	Frequency (Hz)	mNSS	DR (%)
Control (n=7)		10.13±0.51	
Experimental (n=7, respectively)	10	6.41±0.26*#	36.72
	20	5.56±0.33*#	45.11
	30	6.13±0.32*#	39.49
	40	7.23±0.23*	28.62

Values were expressed as mean±SD. $p<0.05$ was considered as statistically significant.*$p<0.05$ versus control group; #$p<0.05$ versus 40 Hz experimental group. **mNSS:** Modified Neurological Severity Score; **DR:** Decrement rate.

Table 3: Changes of mNSS in rats exposed to the different duration magnetic field after traumatic brain injury.

Groups	Duration (min)	mNSS	DR (%)
Control		10.13±0.51	
Experimental (n=7, respectively)	10	7.68±0.24*	24.19
	15	6.42±0.27*#	36.62
	20	5.64±0.34*#	44.32
	25	6.09±0.24*#	39.88
	30	7.35±0.30*	27.44

Values were expressed as mean±SD. $p<0.05$ was considered as statistically significant. *$p<0.05$ versus control group; #$p<0.05$ versus 10 min experimental group. mNSS: Modified Neurological Severity Score; DR: Decrement rate.

Table 4: Magnetic field parameters and their levels.

Sl. No.	Factors	Symbols	Units	Levels		
				1	2	3
1	Magnetic Induction	A	mT	10	20	30
2	Frequency	B	Hz	10	20	30
3	Duration	C	min	15	20	25
4	Numbers of action	D	times/day	1	2	3

mT: Milli Tesla; Hz: Hertz.

Table 5: Experimental layout using L_9 (3^4) orthogonal array and SN ratios.

No	Factors				Experimental values (K)			SN ratio η (dB)
	A	B	C	D	y_1	y_2	y_3	
1	1	1	1	1	6.62	6.58	6.45	-16.3254
2	1	2	2	2	5.45	5.43	5.67	-14.8352
3	1	3	3	3	5.44	5.67	5.48	-14.8559
4	2	1	2	3	6	5.98	5.89	-15.5003
5	2	2	3	1	5.35	5.52	5.27	-14.6173
6	2	3	1	2	5.55	5.78	5.51	-14.9864
7	3	1	3	2	6.33	6.24	6.18	-15.918
8	3	2	1	3	6.22	5.98	6.01	-15.6651
9	3	3	2	1	6.38	6.45	6.41	-16.1418

SN ratio: Signal-to-noise ratio.

Table 6: Response table from grey relational grade.

Levels	Factors			
	A	B	C	D
1	-46.0165	-47.7437	-46.9768	-47.0844
2	-45.104	-45.1176	-46.4773	-45.7396
3	-47.7249	-45.9841	-45.3912	-46.0214

Table 7: Changes of BWC in TBI rats exposed to the magnetic field.

Groups	BWC (%)
Control (n=7)	79.57±0.43
Experimental (n=7)	82.35±0.43*

Values were expressed as mean±SD. $p<0.05$ was considered as statistically significant. *$p<0.05$ versus control. BWC: Brain water content; TBI: Traumatic brain injury.

Hz were applied to the TBI rats for 5 days at 25 min, the electrical stimulation threshold of the rats were decreased significantly in both the forelimb and hind limb (Table 9). Table 10 showed the survival rate in TBI rats exposed to magnetic field for 5 days compared to control group. The effect of magnetic field on the survival rate of the TBI rats was found to be 91.8% with a significant increase in survival rate compared to the control group that did not receive magnetic field (Table 10).

DISCUSSION

To achieve the therapeutic effect of magnetic field application, the parameters should be chosen and used appropriately. The therapeutic effect of magnetic field is different when the intensity, frequency and duration of magnetic field application are different.[7]

To study the effect of alternating magnetic field on the TBI rat model, we first conducted experiments to determine the optimal intensity, frequency, duration and the numbers of application of a day. Therefore, we conducted one-factor experiment to determine the optimal intensity, frequency and duration of the magnetic field based on modified Neurological Severity Score (mNSS) in the TBI rats. mNSS is an index to evaluate neuro dysfunction due to traumatic brain injury, which is an objective and sensitive indicator of brain dysfunction.[19] The mNSS is a composite of the motor(muscle status and abnormal movement), sensory (visual,

Table 8: Change of mNSS in TBI rats exposed to the magnetic field.

Groups	Before injury	Day after injury			
		1	3	7	14
Control (n=7)	0.00±0.00	16.12±0.29	11.59±0.45	9.15±0.35	7.45±0.36
Experimental (n=7)	0.00±0.00	15.72±0.31	9.87±0.26*	6.77±0.36*	3.52±0.28*

p<0.05 was considered as statistically significant. *p<0.05 versus control group. mNSS: Modified neurological severity score; TBI: Traumatic brain injury.

tactile and proprioceptive sensibilities), beam balance, reflex absence and abnormal movement tests.[20] In the test, each score point is awarded for the inability to perform the test or for the lack of a tested reflex; thus, the higher the score, the severer the injury is (normal score 0, maximal deficit score 18). The results showed that the mNSS of the TBI rats decreased significantly compared to the control group when the magnetic field intensity was 10 mT, 20 mT and 30 mT respectively (Table 1). From biophysical point of view, the biological mechanism of action of the magnetic field is related to the effect of the electromotive force by the bio-currents formed in the nervous system and vascular system of the organism and thus there is a need to focus on the different types of bio-current rhythms and their resonance phenomena occurring in tissues and organs of the organism. Therefore, we conducted experiments to rationally determine the frequency, which is a major parameter of alternating magnetic fields that can alleviate traumatic brain injury. The results of the experiments showed that the mNSS in the 20, 30 and 40 Hz groups were significantly reduced compared to the control group (Table 2).

Too short magnetic field exposure time may lead to no therapeutic effect and too long time may lead to adverse effects. The duration of the magnetic field, which may have a positive effect on brain injury due to traumatic injury, was 15-25 min as shown in Table 3. The results of the above experiments showed that appropriate magnetic field intensity for traumatic brain injury was 10-30 mT,

Table 9: Change of electrical stimulation threshold (v) in TBI rats exposed to the magnetic field.

Groups	Forelimb	Hindlimb
Control (n=7)	9.30±0.35	16.95±0.79
Experimental (n=7)	6.41±0.41*	8.78±0.37*

p<0.05 was considered as statistically significant. *p<0.05 versus control group.TBI: Traumatic brain injury.

Table 10: Changes of 5-day survival rate in TBI rats exposed to the magnetic field.

Groups	Total number	Survivors	Mortality	Survival rate (%)
Control (n=7)	82	62	20	75.6
Experimental (n=7)	73	67	6	91.8*

p<0.05 was considered as statistically significant. *p<0.05 versus control group. TBI: Traumatic brain injury.

frequency was 10-30 Hz, the duration was 15-25 min and the numbers of daily application was 1-3 times.

We found the optimal parameters of magnetic field application by using Taguchi method, based on a range of magnetic field parameters representing therapeutic effects through one-factor experiment (Tables 4-6). As a result, the optimal parameters of the magnetic field were: magnetic induction 20 mT, magnetic field frequency 20 Hz, duration 25 min and numbers of applications 2 per day.

And then, we observed changes in some functional indices of rats when the magnetic field of the optimal parameters was applied to the TBI rats. Changes of BWC after 5 days in TBI rats exposed to the magnetic field was 82.35±0.43%, which was significantly lower than control model group (Table 7). Free radicals from traumatic brain injury cause brain edema by enhancing vascular permeability as well as disrupting vascular endothelial cell and ion channels.[23] Thus, the results suggest that the magnetic field may reduce brain water content by inhibiting endothelial damage or promoting repair by secreting various cytokines, including Vascular Endothelial Cell Growth Factor (VEGF) at the site of brain injury. When the rats were exposed to a magnetic field, mNSS was also significantly reduced compared to the groups without magnetic field. As shown in Table 8, the mNSS in the

experimental group was started to decrease significantly, 3 days after magnetic field application compared to the control group and continued to decrease significantly until 14 days.

We measured the change in the electrical stimulation threshold to assess the recovery of neuronal function of traumatic brain injury by magnetic field. When magnetic fields of 20 mT and 20 Hz were applied to the TBI rats for 5 days at 25 min, the electrical stimulation threshold of the model animals decreased significantly in both the forelimb and hind limb (Table 9). This was consistent with previous reports that alternating magnetic fields, together with cortical neuronal repair, enhance nervous system excitability and lower axonal excitation threshold, thereby creating new motor conduction pathways by excluding axons that were originally inactive into activated axons.[24] The effect of magnetic field on the survival rate of the TBI rats was found to be 91.8% with a significant increase in survival rate compared to the non-magnetic field group (Table 10). The results of the present study suggested that the magnetic field of optimal parameters improved the motor disturbance of TBI animal model.

CONCLUSION

The optimal parameters of alternating magnetic field for functional recovery of TBI rats were magnetic induction at 20 mT, frequency of 20 Hz, duration for 25 min and 2 times per day. The magnetic field of optimal parameters improved neuro dysfunction and increased the survival rate in TBI rats.

ACKNOWLEDGEMENT

We are grateful to Dr. Han Song Chol from National Academy of Sciences, DPR Korea, for his technical assistance in making animal model.

CONFLICT OF INTEREST

The authors declare that there is no conflict of Interest.

ABBREVIATIONS

MF: Magnetic field; **TBI:** Traumatic Brain Injury; **mNSS:** Modified Neurological Severity Score; **BWC:** Brain Water Content; **DR:** Decrement Rate; **SN ratio:** Signal-to-Noise Ratio.

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Cite this article: Won J, Gwang KH. Optimal Parameters of Magnetic Field Action and their Influence on Some Index Improvement in Traumatic Brain Injury Rat Model. Int J Clin Exp Physiol. 2024;11(1):32-7.