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Original Article

Study on the Optimum Parameters of Alternating Magnetic Field Treatments against Cerebral Ischemia/Reperfusion Injury in Rats

Won Jong*, Myong Hui Kim

ABSTRACT

Background and Aim: Cerebral ischemia/reperfusion injury is a major cause of acute brain injury. The pathogenetic mechanisms underlying ischemia/ reperfusion injury involve apoptosis, inflammation and oxidative stress. The aim of this study is to clarify the optimum parameters of low-frequency alternating magnetic field treatment for the functional recovery after MCAO reperfusion injury in rats by using Taguchi method. **Methods:** Brain samples from ischemic hemisphere were removed and weighed to measure the brain water content as brain water content is considered as index to estimate the ischemic-reperfusion injury. **Results:** Neuroprotective effects of low-frequency alternating magnetic fields after the middle cerebral artery occlusion reperfusion injury in rats are most likely to be exerted on parameters with intensity of 15mT, frequency of 20Hz and duration of 15 min for one time. **Conclusion:** Contribution ratio of frequency among parameters of alternating magnetic field is the highest for neuroprotective effects.

Key words: Alternating magnetic field, Middle cerebral artery occlusion, Taguchi method, Cerebral ischemia, Neuroprotective effect.

INTRODUCTION

More than 5 million people are suffering with stroke, the disease with the second high mortality rate and a leading cause of long-term disability and a further 5 million become permanently disabled among 15 million stroke patients each year worldwide.[1-3] The quick restoration of blood supply to the ischemic brain tissue is the best treatment. By the way, most of the cells in the ischemic core after focal cerebral ischemia and reperfusion undergo necrosis. And cell death in the ischemic penumbra is a process that leads to apoptosis.[4] Here there is a lot of evidence to prove that apoptosis contributes significantly to cell death both in the ischemic core and in the surrounding penumbra region subsequent to cerebral ischemia/reperfusion (I/R) as well as necrosis.[3,5,6] Clinical researches on pathogenic area of human stroke show that pathogenic area of human stroke is usually middle cerebral artery and their branches. So middle cerebral artery occlusion (MCAO) is a widely accepted model for cerebral ischemia.[7] Recently researchers are trying to apply the magnetic field treatment which is noninvasive and doesn't have side-effect for the improvement of cerebral function.[8] It is well known that the major effects of magnetic field treatment are analgesia, anti-edema, anti-inflammation and improvement of microcirculation system.[9-11] Especially the alternating magnetic field gives rise to an antioxidant effect by depressing the effect of free radical and inhibit the apoptosis process of cerebral nerve cell that is a main cause of cerebral damage in an ischemic reperfusion physiopathology.[12]

The aim of this study is to clarify the optimum parameters of low-frequency alternating magnetic field treatment for the functional recovery after MCAO reperfusion injury in rats by using Taguchi method.[13-15]

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing 210–260 g were included in the study. All the rats were allowed for free access to food and water before surgery under optimal conditions (12/12 h light/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.

Model of Middle Cerebral Artery Occlusion (MCAO)

Temporary cerebral ischemia was induced by middle cerebral artery occlusion in rats.[3,16,17] Rats were anesthetized with chloral hydrate (350 mg/kg, intraperitoneally), the left common carotid artery was exposed and isolated. And the middle cerebral

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artery was occluded by inserting a nylon filament (diameter 0.24–0.28 mm) into the internal carotid artery. The nylon filament was slowly removed for reperfusion after 2 h of ischemia. The body temperature of the rats was maintained at 36.5°C to 37.5°C with a thermostatically controlled infrared lamp throughout the procedure. Rats with no neurological deficits after 24 h of reperfusion were excluded from the study.

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 and, therefore, generated no magnetic field.

Measurement of Brain Water Content

According to the wet-dry method, cerebral edema was determined by measuring the brain water content (BWC) and this is done 24 hr after MCAO.[3,18] Brain of the rat was immediately removed after being killed with chloral hydrate anesthesia. Brain samples from the ischemic hemisphere were rapidly weighed on an electronic balance to obtain wet weight. And then brain samples were dried in an oven at 100°C for 24 h to obtain the dry weight. The brain water content was calculated as follows:

BWC = [(wet weight-dry weight)/wet weight]×100%

To calculate the decrement rate (DR) of BWC following formula was used.

$$DR = \frac{cBWC\text{-}rBWC}{cBWC} \times 100$$

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

Experiments of One Factor

Intensity of magnetic field, frequency and duration for one time were considered as important factors for functional recovery effects of magnetic field to MCAO reperfusion injury in rats and experiments of one factor were carried out. To study the change of BWC on the different intensity, rats in experimental groups were exposed to low-frequency sinusoidal magnetic fields at a frequency of 10Hz and induction in the range from 5 to 30 mT intensity for 5 min each time with the interval of 6 hr. The control rats were subjected to sham exposure. As shown in the Table 1, when the magnetic fields of different intensity were individually exposed to the rats after MCAO reperfusion injury, the BWCs of rats in AM_{10mT}, AM_{20mT} groups were significantly decreased (P<0.05) compared with control.

When the sinusoidal magnetic fields of different frequencies (magnetic fields of 10, 20, 30, 40, 50 Hz, 10mT intensity, 5min for each time with the interval of 6 hr) were respectively exposed to rats after MCAO reperfusion, the BWCs of rats in $AM_{10\ HZ,}$ $AM_{20\ HZ}$ and $AM_{30\ HZ}$ groups were significantly decreased (P<0.05) by comparison with control (Table 2). And when the sinusoidal magnetic fields of different duration for one time (1, 5, 10, 15, 20 min, 10mT intensity, frequency of 10 $_{HZ}$, interval of 6 hr) were respectively exposed to rats after MCAO reperfusion, the BWCs of rats in $AM_{5\ min,}$ $AM_{10\ min}$ and $AM_{15\ min}$ groups were significantly decreased (P<0.05) by comparison with control (Table 3).

Study on the Optimal Parameters by Using Taguchi Method

Through the results in the experiment of one factor, the regulatory factors and their levels of magnetic fields for MCAO model rats were decided as given in Table 4. On the basis of Table 4, $L_9(3^4)$ orthogonal array was constructed (Table 5) and signal-to-noise ratio (SN ratio) was calculated.

We calculated η by using formula as given below.

$$\text{SN ratio } (\eta) = -10 \times \lg\left(\frac{1}{n}\sum_{i=1}^{n} y_i^2\right)$$

The sub-table was constructed by calculating the sum of SN ratio on the level of each factor. As shown in Table 6, the optimal parameters are $A_2B_2C_3$. That is, the intensity of sinusoidal magnetic field was 15 mT, frequency was 20 Hz and duration for one time is 15min.

According to reliability assessment by checking in the optimal parameters of magnetic field, BWC was 81.13±0.15 (%), SN ratio is -38.1837. As a result the optimal parameters have reliability of 95%.

DISCUSSION

Clinical researches of pathogenic area of stroke in human show that pathologic area of stroke is usually middle cerebral artery and their branch. Therefore, MCAO is a widely accepted model of cerebral ischemia. The standard treatment for acute ischemic stroke is reperfusion of the occluded vessels as soon as possible.[19] But reperfusion may

Table 1: Changes of BWC in rats exposed to the different intensity magnetic field after the middle cerebral artery occlusion reperfusion.

Groups	BWC (%)	DR (%)
Control (*n*=7)	83.08±0.17	0.00
AM_{5mT} (*n*=7)	82.75±0.56	0.40
AM_{10mT} (*n*=7)	81.87±0.37*	1.45
AM_{20mT} (*n*=7)	82.39±0.23*	0.82
AM_{30mT} (*n*=7)	82.61±0.18	0.57

Values were expressed as mean±SD. *P<0.05 was considered as statistically significant. BWC: Brain water content; DR: Decrement rate

Table 2: Changes of BWC in rats exposed to the different frequency magnetic field after the middle cerebral artery occlusion reperfusion.

Groups	BWC (%)	DR (%)
Control (*n*=7)	83.08±0.17	0.00
$AM_{10\ HZ}$ (*n*=7)	81.87±0.37*	1.45
$AM_{20\ HZ}$ (*n*=7)	81.11±0.27**	2.37
$AM_{30\ HZ}$ (*n*=7)	82.22±0.31*	1.03
$AM_{40\ HZ}$ (*n*=7)	82.82±0.27	0.31
$AM_{50\ HZ}$ (*n*=7)	82.34±0.36	0.89

Values were expressed as mean±SD. P<0.05 was considered as statistically significant. * P<0.05 versus control group; ** P<0.01 versus control group; BWC: Brain water content; DR: Decrement rate.

Table 3: Changes of BWC in rats exposed to the magnetic field for different duration after the middle cerebral artery occlusion reperfusion.

Groups	BWC (%)	DR (%)
Control (n=7)	83.08±0.17	0.00
AM_{1min} (n=7)	82.91±0.35	0.20
AM_{5min} (n=7)	81.87±0.37*	1.46
AM_{10min} (n=7)	81.68±0.55*	1.68
AM_{15min} (n=7)	81.04±0.28**	2.46
AM_{20min} (n=7)	82.62±0.51	0.56

Values were expressed as mean±SD. P<0.05 was considered as statistically significant. * P<0.05 versus control group; ** P<0.01 versus control group; BWC: Brain water content; DR: Decrement rate.

Table 4: Action factors and their levels of magnetic field parameters.

No	Factors	Mark	Unit	Levels		
				1	2	3
1	Intensity	A	mT	10	15	20
2	Frequency	B	Hz	10	20	30
3	Duration	C	min	5	10	15
4	Error	D				

still subject to further damage of the surrounding tissue by an active process that leads to apoptosis and neurodegenerative cascades.[20] This phenomenon is called cerebral I/R injury. Apoptosis and radical damage during I/R injury plays a major role in brain injury associated with stroke.[21,22] The aim of the present study was to clarify the optimum parameters of low-frequency alternating magnetic field treatment for functional recovery after MCAO reperfusion injury in rats. To expect a curative effect of magnetic field after the MCAO reperfusion injury in rats, the optimal parameters for therapeutic application of low- frequency magnetic field must be found. The functional recovery effects of a magnetic field is usually variable when intensity, frequency and duration for one time application are different.[10] Hence, Taguchi method was applied on research to clarify the optimum parameters of the low-frequency alternating magnetic field treatment for the recovery after MCAO reperfusion injury in rats.[13-15]

In the ischemic reperfusion injury of brain, the radicals rupture endothelial cells of blood vessel and ion pathway and increased permeability to cause edema in brain. It is usual that the condition of the patient is aggravated because of cerebral edema.[8,23] Therefor BWC is an important index that shows inflammatory and oxidant injury of cerebral cell objectively in stroke. As a result, we considered BWC as index to estimate the ischemic-reperfusion injury and had experiments on the basis of Taguchi method (quality engineering approach).[13-15]

First of all, intensity of magnetic field, frequency and duration for one time were considered important factors for functional recovery of the magnetic field to MCAO reperfusion injury in rats and experiments of one factor were carried out. According to our experiments, when the rats were exposed to 10mT and 20mT intensity of magnetic fields (frequency of 10 Hz, duration for one time of 5min) after MCAO reperfusion, the BWC in rats of AM_{10mT}, AM_{20mT} groups were significantly decreased (P<0.05) compared with the controls.

And then, when the rats after middle cerebral artery occlusion reperfusion were exposured to 10, 20 and 30Hz frequency magnetic field (intensity

Table 5: $L_9(3^4)$ orthogonal array, experimental values 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	82.61	81.91	82.5	-38.312
2	1	2	2	2	81.07	81.34	81.24	-38.193
3	1	3	3	3	81.35	81.05	80.67	-38.172
4	2	1	2	3	81.21	82.34	81.56	-38.245
5	2	2	3	1	80.72	80.87	81.12	-38.159
6	2	3	1	2	81.62	81.34	81.23	-38.212
7	3	1	3	2	82.21	81.78	81.68	-38.265
8	3	2	1	3	81.78	81.68	81.72	-38.247
9	3	3	2	1	82.14	81.86	81.89	-38.272

SN ratio: Signal-to-noise ratio

Table 6: Sub-table.

Levels	Factors			
	A	B	C	D
1	-114.677	-114.822	-114.772	-114.744
2	-114.616	-114.600	-114.710	-114.670
3	-114.784	-114.657	-114.596	-114.664

10mT, duration for one time 5min), the BWC in rats of $AM_{10\ HZ,}$ $AM_{20\ HZ}$ and $AM_{30\ HZ}$ groups were significantly decreased (P<0.05) compared with the controls. If the duration for one time of the alternating magnetic field is very short, the treatment effects of magnetic field couldn't be expected. And if it was too long, side effects might appear. Through the experiments we found out that the effective times of magnetic field was 5-15min. Through the results from the experiments of one factor we decided the regulatory factors and their levels of magnetic field for MCAO reperfusion injury as in Table 4. We located the factors and their levels in $L_9(3^4)$ orthogonal array as in Table 5. And we calculated η from experimental values.

We calculated η by using

$$\eta = -10 \times \lg\left(\frac{1}{n}\sum_{i=1}^{n} y_i^2\right).$$

As a result we clarified that the optimal parameters of magnetic fields for therapeutic application of low-frequency magnetic field to expect a curative effect of magnetic field after the middle cerebral artery occlusion (MCAO) reperfusion injury are A_2, B_2, C_3. Therefor the optimal intensity of magnetic field was 15mT, frequency is 20Hz and the duration for one time is 15min. And we could also see that contribution ratio of frequency was the highest by calculating variations and dispersions for factors.

This suggests that low–frequency alternating magnetic fields provide neuronal protection, at least partly by conferring anti-apoptotic mechanisms and anti-radicals effects. In conclusion, our results demonstrate that low–frequency alternating magnetic fields has neuroprotective effects on focal cerebral I/R injury and that these effects were mostly likely to be exerted on parameters with intensity 15 mT, frequency 20Hz and duration for one time 15min. However, the actual mechanisms by which the low–frequency alternating magnetic fields decrease BWC after the middle cerebral artery occlusion reperfusion injury require further investigation.

CONCLUSION

The low-frequency alternating magnetic field improves edema after the middle cerebral artery occlusion reperfusion injury in rats. Neuroprotective effects of low–frequency alternating magnetic fields after the middle cerebral artery occlusion reperfusion injury in rats are mostly likely to be exerted on parameters with intensity of 15 mT, frequency of 20 Hz and duration for one time of 15min. And contribution ratio of frequency among parameters of alternating magnetic field is the highest for neuroprotective effects.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of Interest.

ABBREVIATIONS

I/R: Ischemia/ Reperfusion; **MCAO:** Middle Cerebral Artery Occlusion; **BWC:** Brain Water Content; **DR:** Decrement Rate; **SN ratio:** Signal-to-Noise Ratio.

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