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Exercise attenuates oxidative stress in patients with stroke

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Abstract

In stroke patients, excessive oxidative stress impairs brain nerve cells and leads to arteriosclerosis. On the other hand, rehabilitative exercise is necessary for the functional improvement and maintenance after stroke, and exercises themselves increase reactive oxygen species production simultaneously. Therefore, it is essential to elucidate how exercises influence oxidative stress in stroke patients. We assessed the effects of exercises on 29 Japanese subacute-phase stroke patients (exercise group, 20; control group, 9), in terms of oxidative stress by examining changes in reactive oxygen metabolite (ROM) level (i.e., oxidative stress) and biological antioxidant potential (BAP) level (i.e., antioxidant capacity) in blood plasma. The exercise group performed two sets of 1-hour exercises 6 days/week for 56 days. The control group performed the same 1-hour exercises, but only on days 1 and 56. ROM and BAP levels in blood plasma in both groups were measured immediately before and after the exercises and at rest on days 1 and 56. ROM level significantly decreased and BAP level significantly increased at rest from days 1 to 56 in the exercise group. However, no significant change was observed in these levels in the control group.

Conclusion: Regular rehabilitative exercise can improve antioxidant capacity and attenuate oxidative stress even in stroke patients.

INTRODUCTION

Oxidative stress is a state of imbalance between reactive oxygen species (ROS) and antioxidant capacity, which defends against ROS, leading to oxidative tissue damage. Overproduced ROS in cytosolic compartments, subcellular organelles, and mitochondria exacerbate brain cell damage in the acute phase of stroke with their excessive consumption of antioxidants.[1,2] Moreover, in the chronic phase, inflammation is accompanied by mobilization and activation of leukocytes and by activation of platelets and endothelium, resulting in an excessive production of ROS and in oxidative endothelial and blood cell damage. These events lead to atherogenesis and initiation of thrombosis.

On the other hand, therapeutic exercises are generally considered to be necessary for functional improvement and functional maintenance after stroke.[3-7] However, there is a possibility that the exercises themselves would increase ROS production simultaneously. Therefore, it is essential to elucidate the relationship between these contradictory issues, namely, "therapeutic exercises are needed for functional improvement and functional maintenance" and "exercises themselves increase ROS production, which leads to atherogenesis and initiation of thrombosis activities."

The time course of ROS production after the onset of cerebral infarction in animal models was examined by measuring ROS production by various methods, such as microdialysis[8], electron spin trapping[9], electron spin spectroscopy[10], chemiluminescence spectroscopy[11], and fluorescence spectroscopy[12], and using cytochrome electrodes.[13] However, there have been no studies in which the time courses of the changes in the amount of ROS and antioxidant capacity in stroke patients who exercise regularly have been investigated. Therefore, in this study, we assessed the effects of exercises on Japanese stroke patients from the viewpoint of oxidative stress by examining changes in reactive oxygen metabolite (ROM) level (i.e., oxidative stress, the

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amount of total metabolites of ROS) and biological antioxidant potential (BAP) level (i.e., antioxidant capacity) in blood plasma.

In humans, it is impossible to directly measure the amount of ROS, because ROS are highly reactive and short-lived. Hence, the methods of indirectly estimating the amount of ROS using a substance that reacts with ROS are generally employed, and the amount of reactive oxygen metabolites such as 8-hydroxyl-2'-deoxyduanosine[14,15] and malondialdehyde[16,17] is measured. However, the measurement of only a single hyperoxidated lipid or nucleotide is of little clinical significance. As the methods of estimating antioxidant capacity, the amount of antioxidants such as superoxide dismutase (SOD)[18,19] , glutathione peroxidase (GPX)[18,19], catalase (CAT)[19], vitamin C[20], or vitamin E[21] is measured, but the measurement of only a single antioxidant is of little clinical significance. Recently, numerous studies have been carried out in which ROM level is used as a comprehensive index of oxidative stress and BAP level is used as a comprehensive index of antioxidant capacity.[22-24] Moreover, measurement of ROM level involves a small variation, is reproducible, and is convenient.[25] Therefore, in this study, ROM level and BAP level in blood plasma were measured. However, in further studies, it is necessary to measure the individual reactive oxygen metabolites and antioxidants.

METHODS

This study was approved by the local research ethics committee of Okuma Central Hospital. Following the explanations about the purpose of the study, protection of privacy, and the use of personal information, written informed consent was obtained from all the patients in accordance with the Declaration of Helsinki.

Twenty-nine Japanese patients who had had a stroke 2 weeks to 6 months previously were enrolled in this study. Exclusion criteria included inability to undergo rehabilitation due to heart failure (New York Heart Association Classification class 3 or higher), renal failure requiring maintenance dialysis, loss of consciousness (Glasgow Coma Scale score of 12 or lower), and cognitive impairment or severe spasticity. Of the 64 stroke patients admitted to the inpatient rehabilitation ward of Okuma Central Hospital between June 2010 and December 2010, 20 who met all inclusion criteria and gave written informed consent were allocated to the exercise group. Of a further 96 stroke patients admitted to the care facilities of Okuma Hospital between June 2010 and December 2010, 9 who met all inclusion criteria and gave written informed consent were allocated to the control group. It was considered that these 9 patients would not have functional improvement in spite of rehabilitation. They were orally administered an antihypertensive, antiplatelet drugs, and/or anticoagulants. None of the patients underwent surgery. No antioxidant supplements were used in either group during the study period. Demographic data of all the participants are shown in the table.

We designed a non-randomized clinical trial with two groups (exercise group, control group). The exercise group consisted of 20 patients who were able to perform regular exercises, consisting of 14 with cerebral infarction and 6 with cerebral hemorrhage. The control group consisted of 9 patients who were unable to perform regular exercises because of their environment (e.g., being admitted to care facilities): 6 with cerebral infarction and 3 with cerebral hemorrhage. At study entry, the two groups did not significantly differ with regard to age, but did significantly differ by Barthel index ($p = 0.438$ and $p < 0.05$, respectively; Table 1).

The exercise group performed two sets of 1-hour exercises daily 6 days/week for 56 days. The control group performed the same 1-hour exercises, but only on days 1 and 56. ROM and BAP levels in blood plasma were measured immediately before and after the 1-hour exercises and at rest at 7 am on days 1 and 56. In Japan, hospital admission for stroke-related rehabilitation is covered by the national health insurance for up to 180 days, and stroke patients are typically admitted for 60-90 days. Because stroke patients require long-term rehabilitation, it would be preferable to investigate the effects of long-term rehabilitation. However, given that coverage is limited and that therapeutic exercise for stroke patients is typically conducted on 6 days per week, we considered that a study period of 56 days (8 weeks) was suitable. In addition, all patients were able to remain hospitalized for these 56 days. To account for the possibility that oxidative stress is affected by exercise or circadian changes throughout the day, blood was drawn at 7 a.m., when subject movement is likely to be relatively low. All patients in both groups completed the study.

Table 1: Demographic data of participants. At study entry, patient groups did not significantly differ by age, but significantly differed by Barthel index ($p = 0.438$ and $p < 0.05$, respectively).

	Exercise group (n=20)	Control group (n=9)
Male, female	12, 8	5, 4
Age (mean ± SD)	74.0 ± 10.0 years	77.0 ± 6.8 years
Stroke type		
– Heamorrhagic	6	3
– Ischemic	14	6
Number of medical co-morbidities		
– None	8	5
– < 2	11	2
– ⩾ 2	1	2
Initial Barthel Index score (mean ± SD)	20.5 ± 4.4	0.7 ± 1.7

Exercises

The exercises consisted of 1) functional training, including flexibility exercises such as range of motion of muscles and joints and muscle strengthening exercises for the limbs, which consisted of up to about 10 repetitions for 20 minutes; and 2) movement training, consisting of the repeated sequence of rolling, sitting up, lying down, sitting, standing up, standing, and walking for 40 minutes. Functional training was performed first, followed by movement training. The exercise group performed two sets of exercise of 1- hour each 6 days/week for 56 days. The control group performed the same exercises on days 1 and 56 only. Exercise intensity was in the range from 11 to 13 on the Borg Scale. All exercises were supervised by a physical therapist or an occupational therapist.

Measurements

Venous blood samples were collected from both groups immediately before and after the 1-hour exercises and at rest at 7 am on days 1 and 56. ROM level was measured as an index of oxidative stress, i.e., the amount of total metabolites of ROS. BAP level was measured as an index of antioxidant capacity, using free-radical analytical system 4 (FRAS4, Wismerll Co., Ltd., Tokyo, Japan). ROM level is presented in arbitrary unit U.CARR.[26] ΔROM level was calculated as the ROM level immediately after the 1-hour exercises minus the ROM level immediately before the exercises. ΔBAP level was calculated as the BAP level immediately after the 1-hour exercises minus the BAP level immediately before the exercises.

Measurement of ROM level

Blood samples were collected from the peripheral veins. Whole-blood samples were centrifuged (6000 *g*, 5 min). Ten microliters of blood plasma was mixed with an acetic acid buffer solution of pH 4.8 in a pipette to maintain the hydrogen ion concentration. The samples were transferred into a cuvette containing a colorless chromogen (*N*, *N*-diethylpara-phenylenediamine) (Wismerll Co., Ltd., Japan), which is oxidized by free radicals and changes into a radical cation with a magenta color. The intensity of the magenta color reflects the concentration of hydroperoxides in a blood sample, which is proportional to ROM level. The intensity of the magenta color is measured using a photometer (505 nm, 5 min).

Measurement of BAP level

BAP level was also simultaneously measured using blood samples that were collected from the peripheral veins. Whole-blood samples were centrifuged (6000 *g*, 5 min). The salt of a trivalent iron, $FeCl_3$, was dissolved in a given colorless solution containing a chelation acid derivative.

(The solution turns red as a result of the action of trivalent iron (Fe^{3+}) ions, but is decolorized following the addition of blood plasma owing to the reduction of Fe^{3+} ions to bivalent iron (Fe^{2+}) ions). The color intensity of the solution was measured using a photometer (505 nm, 3 sec). Ten microliters of blood plasma was mixed with the solution. The BAP level in blood plasma can be evaluated by measuring the degree of decolorization using a photometer (505 nm, 5 min).

Statistical analyses

Descriptive data are presented as mean ± SD. In statistical significance testing, a normality test was performed on the data using the Shapiro-Wilk test. The data that showed normality were analyzed using the *t*-test, and those that showed no normality were analyzed using the Wilcoxon signed- rank test. Differences were considered significant when the *p*-value was < 0.05. Statistical analyses were performed using SPSS Statistics 19 for Windows (SPSS Japan Inc., Tokyo, Japan).

RESULTS

ROM and BAP levels immediately before and after the 1-hour exercises

Day 1

In both the exercise group and the control group, the ROM levels immediately after the 1-hour exercises increased significantly compared with those before the exercises ($p < 0.05$, $p < 0.05$, respectively; Figure 1 A, B). In both the exercise group and the control group, the BAP levels immediately after the 1-hour exercises increased significantly compared with those before the exercises ($p < 0.05$, $p < 0.05$, respectively; Figure 1 C, D). In addition, at study entry, there were no significant differences in the ROM or BAP levels immediately before and after the

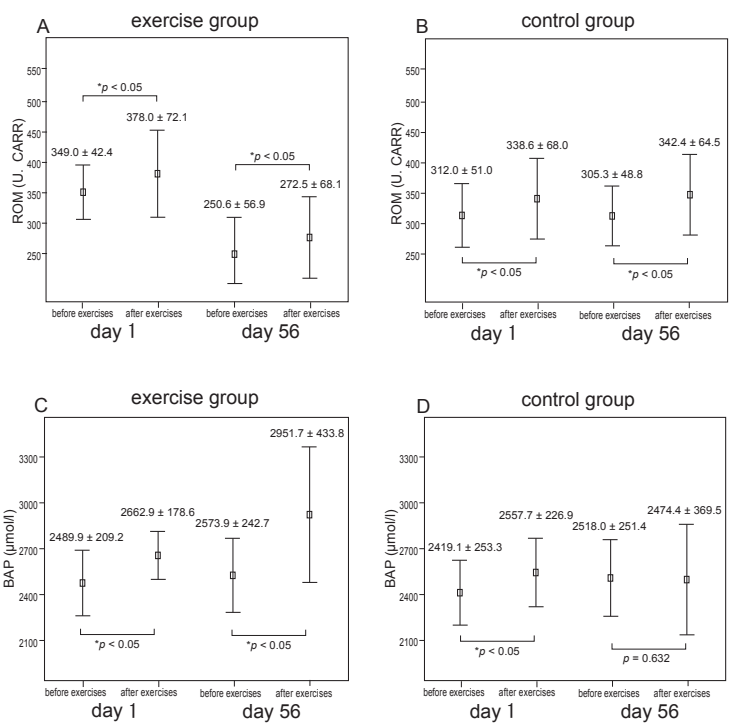

Figure 1. Mean (±SD) ROM and BAP levels immediately before and after the exercises on days 1 and 56, ROM level on days 1 and 56 in exercise group (A), ROM level on days 1 and 56 in control group (B), BAP level on days 1 and 56 in exercise group (C), BAP level on days 1 and 56 in control group (D). On days 1 and 56 in both groups, ROM levels immediately after the 1-hour exercises significantly increased compared with those before the exercises. On the other hand, on days 1 and 56 in the exercise group and day 1 in the control group, BAP levels immediately after the 1-hour exercises significantly increased compared with those before the exercises. However, on day 56 in the control group, there was no change in BAP level.
* p < 0.05.

1-hour exercises, between the exercise group and the control group ($p = 0.281$, $p = 0.621$).

Day 56

In both the exercise group and the control group, the ROM levels immediately after exercises increased significantly compared with those before exercises ($p < 0.05$, $p < 0.05$, respectively; Figure 1 A, B). In the exercise group, the BAP level immediately after the 1-hour exercises increased significantly compared with that before the exercises, whereas no significant change in the control group was observed ($p < 0.05$, $p = 0.632$, respectively; Figure 1 C, D).

On days 1 and 56, the transient exercises increased oxidative stress in stroke patients in both groups. On day 56 in the control group, the increase in antioxidant capacity induced by transient exercises was not obtained, because the control group did not exercise regularly. Thus, it is considered that regular exercises were essential to maintain the increase in antioxidant capacity induced by transient exercises.

ΔROM and ΔBAP levels

The ΔROM level in the exercise group decreased significantly from day 1 to day 56, whereas no significant change in the control group was observed ($p < 0.05$, $p = 0.226$, respectively; Figure 2 A, B). The ΔBAP level in the exercise group increased significantly from day 1 to day 56, whereas no significant change in the control group was observed ($p < 0.05$, $p = 0.343$, respectively; Figure 2 C, D). On day 1, at study entry, there was no significant difference in ΔROM or ΔBAP level between the exercise group and the control group ($p = 0.494$, $p = 0.888$).

ROM and BAP levels at rest

The ROM level at rest decreased significantly from day 1 to day 56 in the exercise group, whereas no significant change in the control group was observed ($p < 0.05$, $p = 0.480$, respectively; Figure 3A, B). The BAP level at rest increased significantly from day 1 to day 56 in the exercise group, whereas no significant change in the

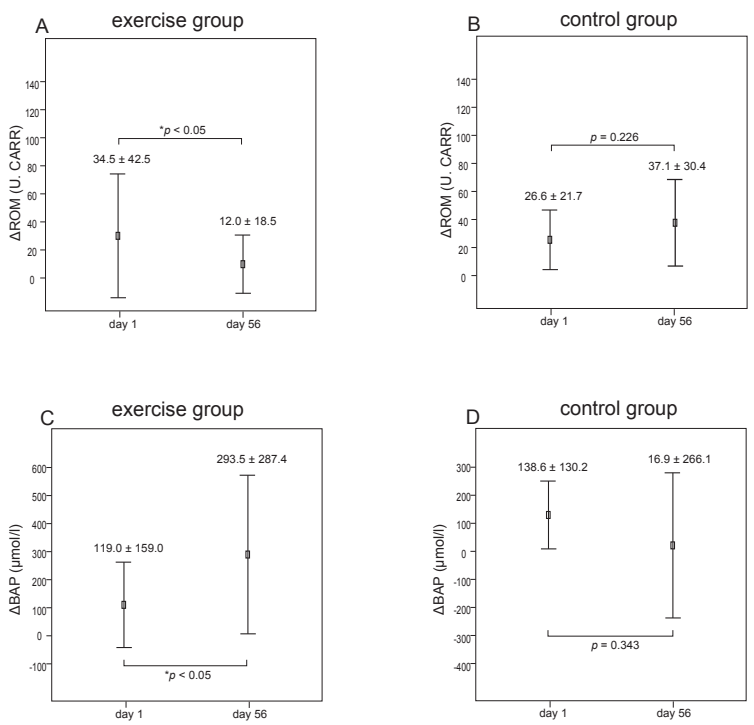

Figure 2. Mean (±SD) ΔROM and ΔBAP levels on days 1 and 56. ΔROM level in exercise group (A), ΔROM level in control group (B), ΔBAP level in exercise group (C), ΔBAP level in control group (D). ΔROM level decreased significantly, and ΔBAP level increased significantly from day 1 to day 56 in the exercise group. However, no significant change was observed in ΔROM level or ΔBAP level in the control group. * p < 0.05.

control group was observed ($p < 0.05$, $p = 0.981$, respectively; Figure 3C, D). On day 1, at study entry, there was no significant difference in the ROM or BAP level at rest between the exercise group and the control group ($p = 0.195$, $p = 0.944$).

We are also uncertain whether the decrease in ROM level at rest from day 1 to day 56 was caused by the decrease in ROS production and the increase in antioxidant capacity or just the increase in antioxidant capacity. However, as an effect of the 56-day exercises, at rest, antioxidant capacity increased and the increase in oxidative stress was minimized.

DISCUSSION

Rehabilitative exercise is considered to be very important to restore function in stroke patients. Actually, many meta-analyses of the effects of exercises on the functional recovery of the stroke patients showed the improvement of their function and ADL.[27-30] However, it has been unclear how oxidative stress or antioxidant capacity is affected by rehabilitative exercise in stroke patients. In fact, the brain is particularly susceptible to ROS damage for the following reasons: (1) It is only 2% of the total body weight, but consumes 20% of oxygen in the whole body. (2) It is abundant in materials that are easily oxidizable including unsaturated fatty acids and amino acids. Polyunsaturated fatty acids account for one-third of fatty acids in the brain, and many of which have a double bond particularly including arachidonic acid and docosahexaenoic acid. (3) It is not particularly rich in antioxidants (GPX, CAT). (4) It is an organ that contains all three types of nitric oxide synthase (NOSs), namely, neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS), and NO is easily produced. In addition, peroxynitrite (ONOO-), a reaction product of NO and peroxyl radical (O_2^-), and hydroperoxyl radical (·OH) like material produced thereof show high cytotoxicity. (5) Many parts of the brain are rich in iron. Moreover, oxidative stress impairs brain nerve cells directly leading to stroke exacerbation[31-34] and cerebrovascular endothelial cells[35,36], lipid[37], smooth muscle cells[38-42], blood cells[43], and coagulation fibrinogenolysis system[44, 45] indirectly leading to arteriosclerosis formation and stroke recurrence. Therefore, in stroke patients, it is very important to understand

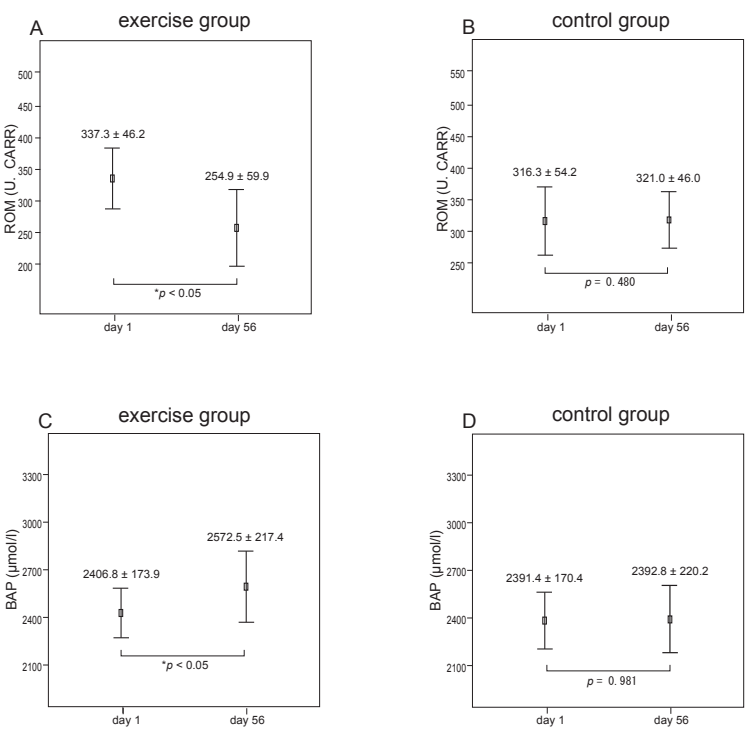

Figure 3. Mean (±SD) ROM and BAP levels at rest on days 1 and 56. ROM level in exercise group (A), ROM level in control group (B), BAP level in exercise group (C), BAP level in control group (D). At rest, ROM level decreased significantly, and BAP level increased significantly from day 1 to day 56 in the exercise group. However, no significant change was observed in ROM or BAP level in the control group. * $p < 0.05$.

the dynamics of oxidative stress and antioxidant capacity due to rehabilitative exercise.

In this study, oxidative stress was increased in both groups immediately after transient exercises on days 1 and day 56. To our knowledge, this is the first clinical study to investigate changes in oxidative stress after exercise in stroke patients. Generally, in healthy people, aerobic metabolic energy originating from oxidative phosphorylation is used during physical activity, and oxygen consumption during exercise reaches 10 to 20 times that at rest.[46] Because ROS such as superoxides (O_2^-) are generated in aerobic metabolism at a constant rate (2-5%), ROS production and oxidative stress increase in accordance with exercise intensity and duration.[47] The results of this study suggest that rehabilitative exercises increase oxidative stress in stroke patients. During rehabilitative exercise, excessive ROS production, which leads to atherogenesis and initiation of thrombosis activities, should be avoided. No study has so far researched in detail the production of ROS or antioxidant capacity under various exercise conditions such as various types, intensities, and durations of exercises even in healthy subjects. Therefore, in the future, we should clarify optimal exercise conditions for stroke patients.

The ΔROM level in the exercise group was significantly lower on day 56 than on day 1 compared to that in the control group. This appears to suggest that an increase in oxidative stress caused by rehabilitative exercises can be reduced if stroke patients regularly perform rehabilitative exercises for 56 days. To our knowledge, this is the first report of this effect in stroke patients. In a preliminary study, rats performing aerobic exercise for 60 minutes once a day for 8 weeks showed a reduced glutathione (GSH) level which was twice as high as that before the start of aerobic exercises and the decrease in ROS production induced by exercise.[48] In another preliminary study, elderly rats (21 months old) also performed aerobic exercise for 60 minutes once a day for 8 weeks. At the beginning of the study, 8-oxodeoxyguanosine levels in the nucleus and mitochondrial DNA in the liver just after aerobic exercise were approximately 2 and 1.5 times, respectively, as high as before aerobic exercises. At the end of the 8-week exercise period, in contrast, increases in 8-oxodeoxyguanosine levels in the nucleus and mitochondrial DNA in the liver after aerobic exercises were comparable between elderly rats and young rats.[49] In the present study, we are uncertain whether the decrease in ΔROM level from day 1 to day 56 was caused by the decrease in ROS production and increase in antioxidant capacity or just the increase in antioxidant capacity. However, as an effect of the 56-day exercises, immediately after a transient exercise, antioxidant capacity increased and the increase in oxidative stress was minimized. With regard to oxidative stress, we therefore consider that regular rehabilitative exercises will induce tolerance to exercise even in stroke patients.

From day 1 to day 56 in the exercise group, the ROM level at rest became significantly lower and the BAP level became significantly higher. We consider that a 56-day rehabilitative exercise program will produce a decrease in oxidative stress at rest and an increase in antioxidant capacity in stroke patients. To date, this effect has also not been investigated in stroke patients. But, regarding the effects of exercises on antioxidant capacity, many authors reported that SOD and GPX activities increase following regular aerobic exercises.[18, 19] In the present study, we are also uncertain whether the decrease in ROM level at rest from day 1 to day 56 was caused by the decrease in ROS production and the increase in antioxidant capacity or just the increase in antioxidant capacity. However, as an effect of the 56-day exercises, at rest, antioxidant capacity increased and the increase in oxidative stress was minimized. We recommend that stroke patients perform rehabilitative exercises to reduce oxidative stress, increase antioxidant capacity and prevent arteriosclerosis and embolism.

On day 1, the BAP level significantly increased immediately after transient exercise in both groups. On day 56, however, the BAP level significantly increased immediately after transient exercise only in the exercise group, and did not significantly increase in the control group. We consider that regular rehabilitative exercises enhanced ROS production and exacerbated oxidative stress, which in turn resulted in a strong and sustained increase in antioxidant capacity. Indeed, Li has demonstrated that increased ROS production during exercise can activate antioxidants for defense, such as mitochondrial manganese SOD mainly by upregulation of antioxidant gene expression.[50] Moreover there is nuclear factor E2 p45-related factor 2 (Nrf-2) that binds to keap1 in the cytoplasm. It deviates and migrates to the nucleus by oxidation of thiol due to the increase in ROS production. In the nucleus, Nrf-2 binds to the antioxidant responsive element (ARE) and is involved in the induction of the expression of antioxidant enzyme production factors such

as NADPH[51], oxidoreductase[52], and glutathione *S*-transferase.[53-54] Moreover, it was reported that intake of an antioxidant supplement leads to ROS breakdown, inhibition of the induction of peroxisome proliferator-activated receptor gamma (PPAR-γ) and PPAR-γ co-activator-1a (PGC-1a) expressions, and also inhibition of the induction of expression of antioxidant enzymes, such as SOD and GPX. It was also reported that an increased ROS production immediately after exercises is required for the subsequent increase in antioxidant capacity to increase.[55, 56] Thus, antioxidant supplements during exercise are useful for minimizing transient oxidative stress, but may hinder patients from acquiring greater antioxidant capacity.

Although no patients in our present study used antioxidant supplements, rehabilitative exercises for 56 days improved antioxidant capacity and resistance to oxidative stress not only during exercises but also at rest, and attenuated oxidative stress even in stroke patients. No increase in oxidative stress-related recurrence of stroke or aggravation of clinical symptoms was seen. We consider that rehabilitative exercise is useful for reducing oxidative stress, increasing antioxidant capacity and preventing arteriosclerosis and embolism in stroke patients.

In conclusion, regular rehabilitative exercise improves antioxidant capacity not only during exercise but also at rest, and attenuates oxidative stress even in stroke patients. However, there is a possibility that ROS production due to exercise increases to the level that can induce stroke exacerbation and recurrence. Therefore, in the future, it is necessary to set the optimal exercise conditions, namely, the type, intensity, and duration of exercises for stroke patients from the viewpoint of oxidative stress.

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DISCLOSURE

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