ORIGINAL ARTICLE

Wheelchair half-marathon race increases natural killer cell activity in persons with cervical spinal cord injury

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Study design: Non-randomized study.

Objective: We reported that individuals with cervical spinal cord injury (CSCI) showed no increase in natural killer cell activity (NKCA) in response to 20-min arm exercise. It could be argued that this lack of response was owing to the short duration and intensity of the exercise.

Setting: The 29th Oita International wheelchair marathon race.

Methods: The present study compared the effects of wheelchair half-marathon race on natural killer (NK) cell count, NKCA and other hematological and hormonal parameters in six subjects with CSCI and seven control subjects with spinal cord injury between T4 and L1 (SCI), before, immediately after and 2 h after recovery.

Results: NK cell counts increased at both time points after the race in SCI, but not in CSCI, compared with before the race. NKCA increased immediately in both groups of subjects after the race, and then returned to the pre-race level at 2 h after the race. Plasma cortisol did not change in both groups throughout the study. Plasma adrenaline increased sharply in SCI after the race, then returned to the pre-race level at 2 h after the race, whereas no change was observed in CSCI throughout the study.

Conclusion: The present study demonstrated that wheelchair half-marathon race increases NKCA despite the lack of increase in plasma adrenaline in CSCI, suggesting the activation of NKCA by mechanisms other than circulating adrenaline level.

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Keywords: immune reaction; wheelchair half-marathon race; plasma adrenaline; plasma cortisol; sympathetic nervous system

INTRODUCTION

Natural killer (NK) cells are the most responsive among the leukocytes to physiological and psychological stress.¹ It is widely known that moderate exercise increases NK cell activity (NKCA), whereas prolonged exercise initially increases and finally decreases NKCA to below pre-exercise levels in healthy humans.¹ Adrenaline infusion in healthy volunteers produces an instantaneous increase in NKCA accompanied by a selective increase in circulating NK cells.² Selective β_2 adrenoreceptor antagonists inhibit the catecholamine-dependent detachment of NK cells.³ Therefore, in response to physical exercise, the rise in plasma epinephrine contributes to the increase in NKCA. On the other hand, the mechanism of NKCA suppression after prolonged exercise remains unknown, but several studies have reported that NKCA is suppressed by cortisol⁴ and inhibited by prostaglandins⁵ after prolonged exercise.

In individuals with cervical spinal cord injury (CSCI), plasma adrenaline does not increase during exercise because the peripheral sympathetic nervous system terminates at the cervical spinal cord and is not under the control of the central nervous system.⁶ In a previous study, we reported that 20-min arm exercise at 60% maximal oxygen consumption in CSCI did not increase NKCA, and such lack of response was probably mainly because of a dysfunctional sympathetic nervous system.⁷

It is possible that both the number and activity of circulating NK cells in CSCI individuals fail to remain constant or even decrease during and after prolonged exercise, similar to the response to

prolonged exercise in able-bodied (AB) persons.^{8–10} Unfortunately, our CSCI subjects examined in our previous study could not perform prolonged arm-crank ergometer exercise (more than 20 min) at 60% of VO_2max in the laboratory setting.⁷ However, it is known that at least few CSCI individuals are able to complete wheelchair half-marathon race and that a few international wheelchair sporting competitions hold half-marathon race for CSCI athletes.

The purpose of the present experiment was to examine the exercise-induced NKCA response in CSCI athletes with dysfunctional sympathetic nervous system. In all, six athletes with CSCI and seven spinal cord injury (SCI) were studied during the wheelchair half-marathon race.

MATERIALS AND METHODS

Subjects and procedure

A total of seven persons with thoracic and lumbar SCI (spinal lesions between T4 and L1) and six persons with CSCI (spinal lesions between C6 and C8) participated in the present study. All subjects joined and completed the 21 097.5 m half-marathon division of the 29th Oita International wheelchair marathon race (held in October 25, 2009). Table 1 lists the anthropometric characteristics of the two groups. The selection criteria of persons with SCI and CSCI included that the half-marathon racers: (1) were males, to exclude the possible influence of menstrual cycle-related hormonal changes on the immune system, (2) had no current health problems and were not on medications known to affect the immune and endocrine response, (3) had an

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activity profile of recreational athletes with no formal exercise training history, and (4) were not splenectomized. The Research Ethics Committee of Wakayama Medical University approved the protocol of this study in advance. Informed written consent was obtained from all participants. The Oita International Wheelchair Marathon Organization confirmed the official race records of all participants (time and position) and the published official results were used in the present study as the subjects' race time.

Venous blood samples were collected from the antecubital vein using heparinized tubes and EDTA-2K-containing tubes on the following three occasions: (a) 'before', at between 0600 and 0800 hours on the race day, (b) 'after', immediately after the race within 10 min of each racer's finishing time (around noon on the race day), and (c) 'recovery', at 2 h after the racer finishing time (around 1400 hours on the race day).

NKCA assay

Peripheral blood mononuclear cells were isolated from heparinized peripheral blood using a lymphocyte separation medium. The harvested cells were washed with 10 mM phosphate-buffered saline, pH 7.2, and resuspended at a concentration of $8 \times 10^5 \text{ ml}^{-1}$ in RPMI 1640 medium containing 10% fetal bovine serum. NKCA was assayed using a T-cell leukemia cell line (MT2) (as target cells) labeled with sodium 51 chromate. The labeled MT2 cells ($1 \times 10^4 \text{ ml}^{-1}$) were cultured with the peripheral blood mononuclear cell ($8 \times 10^5 \text{ ml}^{-1}$) in microtiter plates for 4.5 h at 37 °C. After incubation, the culture supernatant was harvested and radioactivity was measured in an auto well gamma system. Spontaneous release was determined by incubating the same amount of cells in medium alone and maximum release was determined by treating the same amount of target cells in the presence of 10% octylphenol ethylene oxide condensate (Triton X-100).

Table 1 Anthropometric data of participating subjects with spinal cord injury (SCI) and cervical spinal cord injury (CSCI)

	SCI	CSCI	P value
<i>n</i>	7	6	
Age (years) (mean $\pm$ s.e.m.)	42.7 $\pm$ 3.2	34.5 $\pm$ 3.4	NS
Spinal lesion	T4–L1	C6–C8	
ASIA Impairment Scale	A	A	
Average race time (min) (mean $\pm$ s.e.m.)	60.6 $\pm$ 4.6	88.0 $\pm$ 4.9	

Abbreviation: NS, not significant.

The percentage of cell lysis was calculated from the formula:

$$\% \text{ lysis} = \frac{[(\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})] \times 100, \text{ and averaged using triplicate readings.}}$$

NK cell count

Three-color flow cytometry was used to determine the total number of NK cells (CD3–CD16+CD56+). Briefly, 50 μl of whole blood was incubated at room temperature for 20 min with 10 μl of antibody cocktail in a polypropylene tube. Antibodies were purchased from Becton-Dickinson (San Jose, CA, USA). Erythrocytes were then lysed with 1 ml FACSlyse and incubated for further 9 min. Finally, the tube was centrifuged at 2500 r.p.m. for 3 min before removal of the supernatant. The cells were resuspended in 250 μl 1% formalin until analysis. Samples were sent to a commercial pathology laboratory (Sullivan Nicolaides, Taringa, Queensland, Australia) for analysis by a FACScan flow cytometer (Becton-Dickinson). The flow cytometer was calibrated daily by using different microbeads (QC Windows and Quantum 1000 (Flow Cytometry Standards, San Juan, PR, USA) and CaliBRITE (Becton-Dickinson)), as per the standard methods for quantifying cellular antigens by flow cytometry. A total of 20 000 events were collected and the data recorded as percentage of positive cells. The total number of lymphocytes determined from a full blood count was multiplied by the percentage of CD3–CD16+CD56+ cells.

Other blood tests

Total blood cell counts, the percentage of neutrophils, total lymphocytes and monocytes, hemoglobin, hematocrit, plasma catecholamines and cortisol levels were measured using standard methods.

Statistical analysis

Data were expressed as mean $\pm$ s.e.m. and analyzed using a repeated measures analysis of variance. When the results of analysis of variance tests were significant ($P < 0.05$), we used Fisher's LSD test to determine differences between pre-exercise levels and each subsequent time period. Differences between SCI and CSCI were examined for statistical significance using the Student's unpaired *t*-test. A *P* value less than 0.05 denoted the presence of a significant difference between two groups.

RESULTS

Changes in blood parameters

Table 2 lists the blood cell counts of the CSCI and SCI groups at three different time points: 'before', 'after' and 'recovery'. Erythrocyte count,

Table 2 Changes in blood cell counts, hemoglobin and hematocrit, in wheelchair half marathon race

	SCI			CSCI		
	Before	After	Recovery	Before	After	Recovery
Leukocytes ($10^3 \mu\text{l}^{-1}$)	6.2 $\pm$ 0.5	11.7 $\pm$ 0.6*	13.3 $\pm$ 0.8	5.9 $\pm$ 0.4	9.9 $\pm$ 1.0*	9.3 $\pm$ 0.8*
Neutrophils ($10^3 \mu\text{l}^{-1}$)	3.4 $\pm$ 1.1	8.9 $\pm$ 2.1*	11.1 $\pm$ 1.9*	3.2 $\pm$ 1.3	7.6 $\pm$ 2.0*	7.5 $\pm$ 2.0*
Lymphocytes ($10^3 \mu\text{l}^{-1}$)	2.4 $\pm$ 0.7	2.1 $\pm$ 0.9	1.6 $\pm$ 0.9	2.2 $\pm$ 0.9	1.8 $\pm$ 0.7	1.4 $\pm$ 0.2
Monocytes ($10^3 \mu\text{l}^{-1}$)	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.2	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2	0.3 $\pm$ 0.1
Erythrocytes ($10^4 \mu\text{l}^{-1}$)	487.0 $\pm$ 31.0	512.0 $\pm$ 22.0	529.0 $\pm$ 14.0	463.0 $\pm$ 19.0	487.0 $\pm$ 20.0	468.0 $\pm$ 17.0
Platelet ($10^4 \mu\text{l}^{-1}$)	23.6 $\pm$ 2.6	27.7 $\pm$ 2.4*	21.5 $\pm$ 2.7	16.3 $\pm$ 2.4	16.9 $\pm$ 2.0	13.1 $\pm$ 2.6
Hemoglobin (g l ⁻¹)	1.54 $\pm$ 0.10	1.64 $\pm$ 0.07	1.69 $\pm$ 0.05	1.43 $\pm$ 0.07	1.49 $\pm$ 0.06	1.46 $\pm$ 0.06
Hematocrit (%)	42.8 $\pm$ 2.4	45.4 $\pm$ 1.7	46.6 $\pm$ 1.3	40.2 $\pm$ 1.7	42.3 $\pm$ 1.9	40.6 $\pm$ 1.4

Abbreviations: CSCI, cervical spinal cord injury; SCI, spinal cord injury.

Values are mean $\pm$ s.e.m. * $P < 0.05$, compared with before the race.

Before: the morning of the race day, after: immediately after completion of the race, recovery: 2 h after completion of the race.

hemoglobin level and hematocrit remained constant throughout the experiment in both groups. On the other hand, the leukocyte count increased ($P < 0.05$) immediately after the race and 2 h after the race in both groups. The race significantly increased the absolute numbers of peripheral neutrophils and lymphocytes in both groups.

Changes in NK cells count and activity

In SCI subjects, the NK cell count increased immediately after the race and returned to the pre-race level at 2 h after the race, whereas in CSCI subjects, the absolute number of NK cells did not change significantly throughout the study (Figure 1).

There was no significant difference in pre-race NKCA values between SCI and CSCI subjects (Figure 2). In SCI subjects, the NKCA increased immediately after the race and decreased 2 h after the race compared with the pre-race level, whereas in CSCI subjects,

NKCA increased immediately after the race, and then returned to the pre-race level at 2 h after the race (Figure 2). NKCA at 2 h after the race was significantly lower in SCI than CSCI.

Changes in hormonal profiles

The mean pre-race concentration of cortisol was similar in the two groups (Figure 3). Although the half marathon wheelchair race resulted in similar changes in plasma cortisol level in both groups, these changes were not statistically significant (Figure 3).

Plasma adrenaline concentration was significantly lower in CSCI than SCI before the race (Figure 4). The race significantly increased the concentration immediately after the race in SCI subjects, but not in CSCI (Figure 4). Plasma adrenaline returned to the pre-race level at 2 h after the race in SCI subjects.

Figure 1 Number of NK cells in wheelchair half-marathon racers before (before), immediately after (after) and 2 h after (recovery) the race. Data are mean $\pm$ s.e.m. * $P < 0.05$, compared with before the race.

Figure 3 Plasma cortisol levels in wheelchair half-marathon racers before (before), immediately after (after) and 2 h after (recovery) the race. Data are mean $\pm$ s.e.m.

Figure 2 Peripheral NKCA in wheelchair half-marathon racers before (before), immediately after (after) and 2 h after (recovery) the race. Data are mean $\pm$ s.e.m. * $P < 0.05$, compared with before the race. # $P < 0.05$, SCI vs CSCI.

Figure 4 Plasma adrenaline levels in wheelchair half-marathon racers before (before), immediately after (after) and 2 h after (recovery) the race. Data are mean $\pm$ s.e.m. * $P < 0.05$, compared with before the race. # $P < 0.05$, SCI vs CSCI.

DISCUSSION

This study is the first to examine changes in NKCA during the wheelchair half-marathon race in CSCI persons. The major findings of the present study were the following; (1) wheelchair half-marathon race increased NKCA in CSCI persons despite the stable concentration of plasma adrenaline and constant number of NK cells, (2) the race increased plasma adrenalin concentration, NK cell count and NKCA in SCI persons, and (3) plasma cortisol did not change throughout the study in both groups of subjects. The results suggest lack of activation of the sympathetic nervous system and cortisol system in CSCI persons during and after wheelchair half-marathon race, and that NKCA in CSCI seems to be activated by yet an unknown factor, but not adrenaline, during the exercise.

The direct mechanism of exercise-induced increase in NKCA is not clear even in AB persons, however; one possible mechanism is the plasma catecholamine concentrations.⁸ The increase in plasma adrenaline concentration accounts for at least part of the exercise-induced modulation of NKCA. NK cells are rapidly mobilized into the peripheral circulation most likely through various mechanisms including catecholamine-induced downregulation of adhesion molecules and shear stress due to a substantial increase in peripheral blood flow.¹¹ NK cells are produced mainly by the spleen and are recruited into the circulation by sympathetic outflow and catecholamine action.⁸ *In vitro* studies have demonstrated that NK cells attached to the vascular endothelium detach themselves after brief incubation with catecholamines (as short as 5 min), and that the response was catecholamine-dose-dependent.¹² A more recent report has demonstrated that exercise-induced catecholamines modulate the expression of adhesion molecules on NK cells, resulting in the mobilization of NK cells into the circulation.¹³ In the present study, persistent changes in adrenaline levels, NK cell numbers, and NKCA in SCI were observed during the race. These results suggest that increases in plasma adrenaline during the half-marathon race in SCI subjects resulted in the observed increases in NK cell count and NKCA. These findings are similar to those reported previously by Furusawa *et al*.¹⁴ On the other hand, in subjects with CSCI, circulating adrenaline concentrations remained constant during the wheelchair half-marathon race although NKCA increased immediately after the race. These subjects suffer from transection of the sympathetic nerves at the cervical level, connecting the peripheral and central nervous systems. Therefore, it seems that NKCA was increased by mechanism(s) other than the sympathoadrenal activity.

In the present study, peripheral NK cell count did not tend to increase after the race in CSCI subjects. Many studies have observed parallel changes in the number and activity of NK cells during submaximal exercise in AB persons.^{8–10} However, other studies reported changes in NKCA on a per NK cell basis after the exercise in AB individuals.¹⁵ Our findings also suggested that the post exercise increase in NKCA was due to not only the recruitment of NK cells into the peripheral circulation but also to the augmentation of NKCA on a single-cell level.

In a previous study, we reported that 20-min arm-crank ergometer exercise at 60% of $\text{VO}_{2\text{max}}$ increased NKCA in AB but did not change throughout the study in eight subjects with CSCI at C6–C7.⁷ The difference between CSCI and AB was the pattern of plasma adrenaline, that is, exercise-induced increase in plasma adrenaline in AB but not in CSCI.⁷ The CSCI athletes completed the wheelchair half-marathon race in about 80–110 min. The results of the present study suggest that such prolonged physical activity significantly increased NKCA even in CSCI. Thus, we consider the exercise protocol followed in our previous study was probably too short to increase NKCA in CSCI.

The intensity and duration of exercise influence the mobilization of NK cells in AB persons.^{8–10} Several investigators demonstrated that NKCA is enhanced during exercise, but that immunodepression follows severe exercise.⁹ It has been reported that in subjects with SCI, NKCA immediately after prolonged exercise such as wheelchair full marathon race decreased below the baseline (pre-exercise) level.¹⁶ The present study also demonstrated that in SCI group, NKCA increased immediately after the half-marathon race and decreased to below pre-exercise 2 h after the race. While in CSCI group of the present study, NKCA increased immediately after the race and returned to pre-exercise level 2 h after the race. These findings suggested that the wheelchair half marathon might be not excessively intense though of extremely long duration for CSCI participants. We consider that the race participants in only CSCI group maintained their optimal pace during the race. Cortisol is known to impair the immune response.^{17–19} However, cortisol levels remained constant throughout the study in the both groups of subjects. Evidence suggests that secretion of prostaglandin by monocytes as well as certain cytokines after exercise influences NKCA in AB individuals.⁵ Unfortunately, we did not examine the effects of the wheelchair half marathon on these factors in our subjects owing to the limited amount of collected blood.

The wheelchair half-marathon race was selected in the present study, because it represented more than 1 h exercise in subjects with CSCI. However, sympathetic innervation in CSCI terminates at the level of the cervical lesion. This deficit results in limitation of the peak heart rate in CSCI and often such subjects can neither complete intense exercise nor exercise longer than 20 min at 60% of $\text{VO}_{2\text{max}}$, as demonstrated in our previous study.⁷ Therefore, the subjects of the present study, that is, the wheelchair half marathon athletes with CSCI, who completed the race in about 80–110 min, are different to those who participated in our previous study.⁷

In summary, the present study demonstrated that the wheelchair half-marathon race resulted in increase of NKCA despite the lack of increase in plasma adrenaline concentration in CSCI athletes. The intensity of the wheelchair half-marathon race activates NKCA in athletes with CSCI between C6 and C8, through mechanisms other than circulating adrenaline concentrations. Further studies are necessary to determine those factors responsible for the increased NKCA during such exercise in CSCI.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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