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Original Article

Effects of Mental Arithmetic Stress on Blood Cell Counts and the Immune System

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Abstract

To determine whether acute mental stress alters the composition of peripheral blood cells or components of the immune system, we determined blood cell counts and leukocyte differential counts, and examined lymphocyte subsets, before, during, and after 10 min. of mental arithmetic in 20 healthy female students. During mental stress the absolute number of leukocytes, lymphocytes, CD8+ cells, and CD16+ cells increased significantly, and the rate of CD8+ cell increase was higher than CD4+ cell increase. Therefore the CD4+/CD8+ ratio decreased significantly during mental stress. Erythrocyte counts, hemoglobin concentrations and packed cell volumes remained unchanged during and after mental stress. These results demonstrate that acute mental stress is associated mainly with rapid peripheral blood lymphocyte changes, including the release of CD8+ and CD16+ cells into circulation. The absence of significant increases in erythrocyte counts, hemoglobin concentrations, and packed cell volumes during stress indicates that changes in lymphocyte composition cannot be explained by the hemoconcentration. Thus, acute mental stress has a detectable influence on peripheral blood cell counts and the immune system.

Key words: Mental stress, Lymphocyte subset, Blood cell count, Flow cytometry

Introduction

Previous investigations have demonstrated that psychological and physical stresses lead to changes in the immune systems of healthy subjects [1-6]. For example, bicycle exercise in healthy subjects leads to mobilization of leukocytes and lymphocytes, and to characteristic changes in lymphocyte subpopulations [6, 7]. During and immediately after exercise, a marked increase in CD8+ cells, which suppress cell-mediated immunity, is more likely to be observed than an increase in CD4+ cells [1, 5].

To investigate the effect of mental stress on blood cells and the immune system, numerous types of stress have been utilized such as acoustic stress in the form of environmental noise from a tape cassette [8], and examination stress in students [2, 3, 9]. Decreases in the CD4/CD8 ratio [8], enrichment of the circulating CD20+ cell population [8], and decreased T lymphocyte proliferation responses were observed during mental stress [3, 9].

We previously demonstrated that a combination of various lifestyle factors is significantly associated with increased NK cell activity. Lifestyle factors such as subjective mental stress, physical exercise, and sleeping hours had a relatively large effect on NK cell activity [10]. However, we could not clearly evaluate the effects of daily mental stress on lymphocytes and lymphocyte subsets in a previous study [10]. It has been demonstrated that blood cell counts return to pre-stress levels after mental stress [11]. To our knowledge, although another group reported that lymphocyte subsets change quickly in response to acute mental stress, they did not observe changes in lymphocyte subsets and blood cell counts after acute mental stress [1, 8, 12]. Furthermore, there have been relatively few studies on the effects of ordinary mental stress on the blood and immune system in Japan [13], especially considering that Japanese society has many stresses. Hematological parameters may be useful in stress management. Therefore, in this study, blood cell counts, leukocyte differential counts, and lymphocyte subpopulations before, during, and after mental stress, plus related plasma cortisol levels in healthy young subjects, were investigated, and the relationships between these biochemical measurements and mental stress were examined.

Materials and Methods

1. Subjects

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A total of 29 female medical technology students from the Osaka College of Public Health participated in this study. The experimental stress group consisted of 20 subjects whose median age was 21.3years. The other 9 subjects made up the control group whose median age was 21.2years. All subjects were healthy nonsmokers, were not on any medication, and did not drink alcohol regularly. The subjects were instructed to wake up between 7.00 and 8.00 in the morning and not to eat breakfast. None of subjects slept for less than 7h the night before the experiment.

We explained the content of our research, and informed consent was obtained from all subjects.

2. Design and Procedures

All subjects entered the laboratory where room temperature was kept from 18 to 25°C at 9.30a.m., and the experimental procedure was explained to them. The stress experiments were started at 9.45a.m. according to the procedures standardized by Jern [11]. One or two subjects were examined per day. Initially the subjects rested for 30min. in the laboratory; then they performed mental arithmetic for 10min. by serially subtracting 7 from 700, trying to keep pace with a metronome set on andante (i.e. 90 beats per min.), and then they rested for 10min. after the mental arithmetic. Measurements of blood pressure, heart rate, and collection of blood samples were performed before, during and after mental arithmetic. The control group was examined 30min. and 40min. later in the resting state.

Blood pressure and heart rate were measured with HEM-706Fuzzy automatic blood pressure monitor (Omron Corp., Tokyo, Japan). A few minutes before the end of each period, 5ml of blood was drawn from each subject. Three ml of the blood was treated with EDTA for determination of the blood cell count, leukocyte differential count, and characterization of the lymphocyte subpopulations, and the other 2ml of blood was used to obtain serum for the determination of the cortisol level. The EDTA-treated blood samples were analyzed within 3hour of blood collection. However, in one subject, CD20+ and serum cortisol after stress could not be measured, as her blood sample was too small.

Blood cell counts were determined with a Coulter model T-660 counter (Coulter Electronics Inc., Hialeah, FL, U.S.A.). For evaluation of the leukocyte differential count, blood smears were treated with Pappenheim's stain and were counted under a microscope.

To characterize lymphocyte subpopulations, 100 μl of EDTA-treated blood was added to 100 μl of phosphate-buffered saline (PBS) containing 0.1% sodium azide (NaN_3), and incubated for 60 min. at 4°C with 10 μl of fluorescein labeled monoclonal antibodies OKT3 (CD3), OKT4 (CD4), OKT8 (CD8), OKNK (CD16) and OKT20 (CD20) (Ortho Pharmaceutical, NJ, U.S.A.), or with PBS containing 0.1% NaN_3. After the first incubation, samples were treated with a lysing solution for 15 min. at 20°C, washed and resuspended in PBS containing 0.1% NaN_3. Samples were analyzed by laser flow cytometry (Spectrum III, Ortho).

Serum cortisol levels were determined using a radio-enzymatic assay kit (Incstar Corp., Stillwater, MN, U.S.A.), which utilizes a competitive reaction.

Two-way repeated measures (before stress, during stress, after stress) ANOVAs were used to determine the effects of the mental stress on peripheral blood cell counts, leukocyte differential counts, lymphocyte subsets, and serum cortisol levels. Further, Scheffé's multiple comparison tests were used to assess specific differences between repeated measures. The results from the control group were analyzed by a nonparametric test (Wilcoxon's signed rank test). Probability values of less than 0.05 (two-tailed) were regarded as significant. All values are presented as mean±standard error of the mean.

Results

Hemodynamic Changes

Changes in the heart rate, and systolic and diastolic blood pressures were taken in the experimental stress group before, during, and after mental stress, and they are shown in Table 1. The heart rate ($F(2,19)=15.21$, $p<0.001$) and systolic blood pressure ($F(2,19)=5.51$, $p<0.01$) increased significantly during mental stress. Although diastolic blood pressure changed significantly ($F(2,19)=5.03$, $p<0.05$) between repeated measures, there was no significant difference between "before stress" and "during stress." After mental stress, the hemodynamic values quickly returned to pre-stress values. Changes in the heart rate, and systolic and diastolic blood pressures in the control group were not significant (data not shown).

Blood cell count and leukocyte differential count

As shown in Table 2, the leukocyte count ($F(2,19)=6.77$, $p<0.01$) and lymphocyte count ($F(2,19)=4.39$, $p<0.05$) increased significantly during mental stress. After mental stress, the lym-

Table 1 Hemodynamic variables before, during, and after mental stress

	Before stress	During stress	After stress	*F*
Heart rate (bpm)	67.1±1.8	71.9±1.5†	65.2±1.5†	15.21***
Systolic blood pressure (mmHg)	92.4±2.1	96.3±2.0†	91.8±1.7†	5.51**
Diastolic blodd pressure (mmHg)	60.5±1.3	63.7±1.7†	60.0±1.5†	5.03*

Values are mean±standard error of the mean.
Significance levels (two-way ANOVA):*P<0.05, **P<0.01,***P<0.001
Significance levels (Scheffé's multiple comparison test):†P<0.05

Table 2 Leukocyte counts and differential counts before, during,and after mental stress

	Before stress	During stress	After stress	*F*
Leukocytes ($\times 10^9$/l)	5.13±0.27	5.35±0.26†	5.40±0.25†	6.77**
Lymphocytes ($\times 10^9$/l)	1.72±0.10	1.84±0.09†	1.78±0.08	4.39*
Neutrophils ($\times 10^9$/l)	2.91±0.25	3.00±0.27†	3.14±0.26†	7.34**
Eosinophils ($\times 10^9$/l)	0.22±0.07	0.26±0.09	0.23±0.07	[a]N.S.
Basophils ($\times 10^9$/l)	0.05±0.01	0.04±0.01	0.05±0.01	N.S.
Monocytes ($\times 10^9$/l)	0.23±0.02	0.22±0.02	0.22±0.02	N.S.

Values are mean±standard error of the mean.
Significance levels (two-way ANOVA):*P<0.05 , **P<0.01
Significance levels (Scheffé's multiple comparison test):†P<0.05
[a]N.S., not significant.

phocyte count almost returned to pre-stress values, but leukocyte and neutrophil counts (F (2,19)=7.34, p<0.01) continued to rise significantly even though mental stress had ceased. Eosinophil, basophil and monocyte counts were not significantly different during mental stress. There was little or no change in the erythrocyte count, hemoglobin concentration, or packed cell volume during mental stress (Table 3). Changes in the blood cell count and leukocyte differential count in the control group were not significant (data not shown).

Lymphocyte Subsets

There were significant decreases in the percentages of CD3+ cells (F (2,19)=6.01, p<0.01), CD4+ cells (F (2,19)=9.62, p<0.001), and the CD4+/CD8+ ratio (F (2,19)=7.67, p<0.01) during mental stress, while the percentage of CD16+ cells (F (2,19)=6.72, p<0.01) increased (Table 4). The change in the percentage of CD8+ cells approached but did not reach significance (F (2,19)=3.14, p=0.055). The absolute number of CD8+ cells (F (2,19)=5.94, p<0.01) and CD16+ cells (F (2,19)=9.46, p<0.001) increased during mental stress (Table 5). After mental stress, the lymphocyte subsets that changed during mental stress returned to pre-stress levels except for the percentage of CD4+ cells. The other lymphocyte subsets studied did not change significantly during mental stress. Changes in the lymphocyte subsets in the control group were not significant (data not shown).

Serum cortisol

The serum cortisol level in the experimental group slightly decreased from the pre-mental stress level (9.7±0.6 μg/dl) to 9.4±0.6 μg/dl during mental stress, and decreased further after mental stress to 8.9±0.9 μg/dl; however this change was not statistically significant. In the control group, the serum cortisol level decreased significantly from 12.1±1.1 μg/dl (p<0.01), 30 min. into the rest period to 10.4±1.0 μg/dl 40 min. into the rest period.

Table 3 Erythrocyte counts, hemoglobin concentrations, and packed cell volumes before, during, and after mental stress

	Before stress	During stress	After stress	*F*
Erythrocytes ($\times 10^{12}$/l)	4.15±0.05	4.16±0.06	4.13±0.06	[a]N.S.
Hemoglobin concentrations (g/dl)	12.2±0.3	12.2±0.3	12.1±0.3	N.S.
Packed cell volumes (%)	36.0±0.7	36.1±0.7	35.9±0.7	N.S.

Values are mean±standard error of the mean.
[a]N.S, not significant.

Table 4 Lymphocyte subsets before, during, and after mental stress

	Before stress	During stress	After stress	*F*
CD3 (%)	77.1±1.4	73.9±1.6	75.7±1.3	6.01**
CD20 (%)	15.0±0.8	15.3±0.9	15.4±0.9	[a]N.S.
CD3/CD20 (ratio)	5.28±0.49	5.00±0.44	5.05±0.43	N.S.
CD4 (%)	43.7±1.8	41.6±1.8	42.2±1.8	9.62***
CD8 (%)	30.6±1.3	31.6±1.2	30.8±1.2	3.14*
CD4/CD8 (ratio)	1.52±0.12	1.40±0.11	1.45±0.11	7.67**
CD16 (%)	10.3±0.7	12.8±1.1	11.3±0.8	6.72**

Values are mean±standard error of the mean.
Significance levels (two-way ANOVA):*P=0.055, **P<0.01, ***P<0.001
Significance levels (Scheffé's multiple comparison test):†P<0.05
[a]N.S., not significant.

Discussion

Mental stress causes substantial psycho-physiological arousal[11,12]. This response pattern is characterized by marked increases in heart rate and blood pressure, suggesting the involvement of combined adrenergic activation[1]. Our experiments showed that the heart rate increased an average of 5 beats/minute, and that systolic and diastolic blood pressures increased 4mmHg and 3mmHg, respectively, during mental stress. Ten minutes after finishing the mental arithmetic, these increased hemodynamic values returned to pre-stress levels. Thus, mental stress is characterized by transitory hemodynamic changes[11].

In this study, we found significant changes in immune cells during mental stress. The CD4/CD8 ratio decreased significantly and the number of CD20+ cells slightly increased. These changes may well be explained by the action of increased plasma catecholamine concentrations during mental stress. A previous report showed that increases in several lymphocyte subsets positively correlate with rises in plasma catecholamine concentrations [1]. Interestingly, β-adrenergic receptor measurements from human lymphocyte subsets showed that these receptors are predominantly localized to B cells, as compared to T cells, and that the binding activity of CD8+ cells is greater than that of CD4+ cells[14]. The decrease in the CD4/CD8 cell ratio observed in this study might help explain another group's observation that lymphocyte responses to mitogens are diminished during stress[2].

We previously reported that overall lifestyle is significantly associated with NK cell activity[10]. Our results showed that moderate stress slightly increased NK cell activity. Weiss et al.[15] found that NK cell activity in animals increases during moderate stress, but decreases during extreme stress. Our study showed that the number of CD16+ cells increased significantly during mental arithmetic, but quickly returned to the pre-stress level after the stress. Thus, mental arithmetic may be classified as a moderate stress, because the subset of NK cells bearing the CD16+ surface antigen is reported to be the most cytolytic[16-18]. Moreover, high numbers of NK cells might be related to NK cell activity, which has been observed in healthy subjects after administration of noradrenaline[19]. The changes in lymphocyte subsets

Table 5 Lymphocyte subsets before, during, and after mental stress

	Before stress	During stress	After stress	*F*
CD3 ($\times 10^9$/l)	1.32±0.07	1.36±0.07	1.35±0.06	[a]N.S.
CD20 ($\times 10^9$/l)	0.32±0.06	0.35±0.07	0.34±0.06	N.S.
CD4 ($\times 10^9$/l)	0.76±0.05	0.77±0.05	0.75±0.05	N.S.
CD8 ($\times 10^9$/l)	0.53±0.04	0.58±0.04	0.55±0.03	5.94*
CD16 ($\times 10^9$/l)	0.18±0.02	0.24±0.03	0.20±0.02	9.46**

Values are mean±standard error of the mean.
Significance levels (two-way ANOVA):*P<0.01, **P<0.001
Significance levels (Scheffé's multiple comparison test):†P<0.05
[a]N.S., not significant.

during stress demonstrate redistribution of the lymphocyte subpopulations in circulation versus the marginal lymphocyte pools [2], which suggests that the sympathetic nervous system has an immunoregulatory effect during mental stress [1].

Increased leukocyte counts during mental stress concur with results from previous studies [20, 21] using other stressors, such as physical stress. Physical stress is known to increase plasma cortisol [21] and catecholamine levels [20]. Landmann et al.[1] suggested that cortisol either regulates blood granulocytes independently of the adrenergic system or has a permissive role in the mobilization of granulocytes after adrenergic activation during physical stress. Administration of adrenaline induced diphasic leukocytosis. Initially, the number of lymphocytes increased, and the second phase was characterized by an increased neutrophil count [22]. Our results showed a similar tendency; the number of lymphocytes increased significantly during mental stress and almost returned to pre-stress levels after the stress, but the neutrophil counts increased during and after stress.

Conversely, administration of cortisol induced decreases in the number of lymphocytes, and suppressed the function of CD4+ cells [23]. Serum cortisol levels in the stress experimental group decreased slightly during and after mental stress as previously reported [1]. While the serum cortisol level in the control group decreased significantly. This tendency was also observed in the experimental group. As the serum cortisol level is generally highest in the early morning, this decrease might have been caused by circadian rhythm. Therefore, under the experimental conditions we used, the effects of the cortisol level were unlikely to influence the results we obtained. In addition, since the blood cell count, leukocyte differential count, and lymphocyte subsets in the control group did not change significantly, it is unlikely that the repeated venepuncture and the laboratory conditions affected these results. We speculate that rapid hematological changes are mainly induced by adrenergic activation.

The increased leukocyte, lymphocyte, neutrophil, and lymphocyte subset counts cannot be solely explained by the changed hemo-concentrations induced by mental stress, because there was little or no change in erythrocyte count, hemoglobin concentration, or packed cell volume during mental stress [11].

This study has demonstrated that rapid hematological changes occur in response to transitory acute mental stress. This response is characterized by a decrease in the CD4/CD8 ratio, and by increases in the number of CD16+ cells, leukocyte count, lymphocyte count, and neutrophil count. The mechanisms underlying these immune responses to mental stress are unclear. This study supports the theory that hematological components, including immune cells in humans, are affected by mental stress dependent on lifestyle.

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