

Influence of Self-Stimulation of the Parasympathetic Nervous System on Lymphocyte Function and DNA Repair Function in Family Caregivers

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Abstract

The purpose of this study was to determine the effect of self-stimulation of the parasympathetic nervous system on immune function and DNA repair function in family caregivers. Seven female family caregivers engaged in individually tailored stimulation of the parasympathetic nervous system in everyday life for 2 weeks. Although we observed effects on lymphocyte function and secretory immunoglobulin A concentration in saliva compared with the control period, there was no effect on DNA repair function.

Keywords: Family caregiver; Stress; Immune function; DNA repair function; Parasympathetic nervous system

Introduction

According to a report from the Japanese Ministry of Health, Labour and Welfare, with the aging of the population, the number of people needing long-term care, including those with dementia or intractable diseases, is increasing every year. Similarly, the number of people who use the public care insurance system is increasing (more than 5 million people in 2011). In particular, the use of home care services is rising. Thus, as the number of people who require home care has increased, the number of family caregivers has also likely increased. In addition, the family caregiver's burden may have become heavier because of the rise in the percentage of patients who require high-level medical care in association with advances in medicine, the shortening average length of hospital stays, and improvement in home environments. In addition, Tsuboi and Murakami [1] reported that family caregivers who performed home care for more than 3 years have a significantly higher feeling of caregiver burden compared to for less than 3 years. Thus, prolonged home care may lead an increase in caregiver stress.

In conditions of persistent stress, the cortisol concentration is maintained at a high level because of a decline in the negative feedback system [2]. Investigators have shown that family caregivers, people in high-stress jobs, and patients with depression, all of whom are under chronic stress, have high cortisol levels [3-5]. We focused on impaired immune function and impaired DNA repair function in a high-stress condition (high cortisol concentrations). These functions are difficult to be conscious of because they have no subjective symptoms, but they are important problems with the potential to cause infection or cancer.

Regarding immune function, several studies have reported impaired lymphocyte function due to chronic stress. For example, the lymphocytes of family caregivers who care for the elderly or patients with dementia show poorer cytokine production compared to those of non-caregivers [6,7]. These negative influences may be affected by a reduction in the CD4/CD8 ratio [8] or a shift in cytokine production from Th1-type to Th-2 type induced by stress or glucocorticoids [9,10]. In addition, stress decreases the concentration of secretory immunoglobulin A (sIgA) in saliva [11]. Salivary sIgA inhibits bacterial adherence [12], and salivary sIgA levels are negatively correlated with the frequency of respiratory infections [13]. Also, Yokoyama [14] reported that family caregivers of the elderly feel greater fatigue and have a higher frequency of upper respiratory infections compared to noncore givers. These reports suggest that the immune function in family caregivers is impaired by chronic stress, and that they have increased risk of infectious diseases.

Some reports have demonstrated a relationship between stress

and the risk of cancer. In an epidemiological report, Lillberg et al. [15] reported that stressful life events are associated with an increased risk of breast cancer. Also, depression is associated with an increased risk of cancer [16,17]. At the cellular level, stress hormones (epinephrine, norepinephrine, and cortisol) increase DNA damage [18]. In addition, lymphocytes from a stressed population were more sensitive to the induction of DNA damage and had a higher level of residual damage [19]. Moreover, cortisol and norepinephrine inhibit DNA repair [18,20]. Kiecort-Glaser et al. [21] reported that a high-distress group had poorer DNA repair in lymphocytes exposed to X-irradiation than that in a low-distress group. These reports suggest that stress may play a role in cancer due to increased DNA damage and decreased DNA repair.

Recent findings about the bidirectional interaction between the nervous and immune systems via neurotransmitters suggest that the level of immune suppression in chronic stress can be reduced by stimulating the parasympathetic nervous system. Autonomic nerves innervate immune organs [22,23], and investigators have demonstrated the presence of both muscarinic and nicotinic receptors on mononuclear leukocytes [24]. Numerous studies have revealed that stimulation of the parasympathetic nervous system is essential for maintaining lymphocyte function. For example, Antonica et al. [25] showed that proliferative responses to mitogen stimulation tend to decline in lymph node cells and in thymic cells isolated from vagotomized mice. Parasympathetic decentralization of lymph nodes also results in a significant reduction of interferon-gamma release compared to that found in innervated lymph nodes [26].

On the other hand, acetylcholine (Ach), a neurotransmitter in the central and peripheral nervous systems, significantly enhances the mitogen-induced proliferation of T cells and interleukin (IL)-2 production [27,28]. Moreover, treatment of lymphocytes with Ach or with a muscarinic Ach receptor agonist enhances mitogen-induced IL-2 production [29-31]. These reports suggest that stimulation of the parasympathetic nervous system in individuals experiencing chronic

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stress may improve lymphocyte function that has been suppressed by elevated serum cortisol levels. In addition, the effect of Ach is seen even with only 1 hour of treatment [27], and even muscarinic receptors in lymphocytes are decreased in chronic stress [32-34].

Despite numerous studies showing that the parasympathetic nervous system is stimulated by nursing care (passive stimulation by a nurse) including footbaths, massage, shampooing, and music [35-38], it is not clear whether continued stimulation or active stimulation (self-stimulation) of the parasympathetic nervous system is effective in improving immune function. Moreover, in these reports, the same stimulus method was employed for all individuals.

Regarding DNA repair function, De Rosa et al. [39] reported that incubating lymphocytes with nicotine, which is a nicotinic Ach receptor agonist, decreases the percentage of apoptotic lymphocytes that is increased following exposure to cortisol. Because apoptosis is caused by DNA that is damaged beyond repair, stimulation of the parasympathetic nervous system has the potential to effectively enhance DNA repair function. However, no studies about the relationship between stimulation of the parasympathetic nervous system and DNA repair have been reported.

Family caregiver health is beneficial for care-receivers well as caregivers, considering the continuation of home care and maintenance of its quality. In the present study, the researcher investigated whether immune function and DNA repair function in family caregivers were improved by tailored self-stimulation of the parasympathetic nervous system in everyday life for 2 weeks.

Method

Participants

The target population included family caregivers with 3 years or more of care experience, and for whom the symptoms of the care-receiver were stable. After securing approval by the Ethics Committee of the Graduate School of Nursing, Chiba University, participants were recruited through a home-visit nursing station and a hospital with a pediatric department in Chiba. The researcher approached the caregiver through the nurses and informed each person of the purpose of the study, the experimental schedule, and the right to terminate the experiment at any time at their home. Seven female family caregivers ranging in age from 53 to 80 years (mean \pm SD: 61.4 \pm 9.3 years; Table 1) participated in this study. They spent 12.8 \pm 8.5 hours per day in care giving-related activities, and they had been providing care for 14.7 \pm 9.1 years.

Intervention procedure

The participants were instructed to perform parasympathetic nervous system stimulation for more than 5 minutes, once a day or more, for 2 weeks (intervention period) at their home. Stretching exercise (shoulder or lumbar regions), rest with eye warming, taking a lukewarm bath, and rest with leg elevation were used as parasympathetic nervous system stimulation methods. At the beginning of the intervention period, the researcher proposed 2-4 methods to each participant based on an assessment of the type of care provided and the individual's physical condition. Illustrations prepared by the researcher were used to explain the stretching exercise and rest with leg elevation. A warm eye mask (Megurism, Kao Corporation, Tokyo, Japan) was used for rest with eye warming. Stimulation methods were selected following consultation with the participants and measurement of heart rate variability (HRV). HRV was displayed graphically at the time it was measured, and was explained to each participant by the researcher.

For confirmation of implementation time, self-measurement of blood pressure was performed every day during the intervention period before and after each stimulation using an automated sphygmomanometer (HEM-6051, Omron Healthcare, Kyoto, Japan). In addition, the researcher asked the participant to record which stimulation methods were used each day.

Ten milliliters of peripheral blood and 60 μ l of saliva were obtained both before and after the intervention period at their home to measure lymphocyte function (mitogen-induced IL-2 production), DNA repair function, blood cell count, leukocyte classification, serum cortisol concentration, and sIgA concentration. All samples were drawn between 10:00 a.m. and 12:00 noon to control for circadian variations.

Study design

The study used an interpersonal comparison design of the intervention. All sample data before and after the intervention period was compared with the data from before and after the control period (ordinary two weeks). The order of the intervention period and the control period was randomly assigned, and thus, four participants started the control period and the other three started the intervention period (Figure 1).

Measures

HRV: Parasympathetic nervous system stimulation was confirmed through HRV for each caregiver before the intervention period. The high frequency (HF) components of HRV are related to parasympathetic activity, whereas the low frequency (LF) components are associated with both parasympathetic and sympathetic activities [40]. The LF/HF ratio of HRV has been proposed as an index of sympatho-vagal balance [41,42]. LF and HF were integrated at 0.05-0.15Hz and 0.15-0.40Hz of the power spectra, respectively. Heart rate was recorded using a training computer (RS800CS, Polar Electro Oy, Kempele, Finland). Spectral analysis of HRV was performed every minute with the maximum entropy method using time series analysis software (MemCalcVersion 2.0, Suwa Trust, Tokyo, Japan).

Blood data: Kotobiken Medical Laboratories (Tsukuba, Japan) performed the measurement of serum cortisol concentrations and the number of white blood cells, lymphocytes, and neutrophils for assessment of the stress condition or to determine the influence on lymphocyte function.

Mitogen-Induced IL-2 production: To evaluate the effect on lymphocyte function, mitogen-induced IL-2 production was measured. Phytohemagglutinin-L (PHA, Sigma-Aldrich Inc., St. Louis, MO) was used for mitogenic stimulation. Blood was diluted 1:1 with phosphate buffered saline (PBS), and peripheral lymphocytes were isolated with density gradient centrifugation using Pancoll Human (PAN Biotech GmbH, Aidenbach, Germany). After three washes with PBS, cells were suspended in RPMI 1640 (Sigma-Aldrich) supplemented with 10% fetal calf serum and adjusted to 1×10^6 cells/ml. One hundred

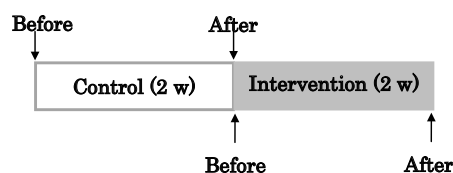


Figure 1: Experimental schedule.

microliters of each cell suspension was transferred to a 96-well plate, and equal volumes of medium containing PHA (10 µg/ml) were then added. After 72-hr incubation at 37°C in 5% CO₂, the culture supernatants were collected and stored at -80°C. IL-2 concentrations in the supernatants were determined using the Human IL-2 enzyme-linked immunosorbent assay kit (Bender MedSystems GmbH, Vienna, Austria) according to the manufacturer's specifications. IL-2 concentration was calculated from the standard curve obtained from the optical density of the calibrators.

IgA in Saliva: Saliva samples were collected by passive drool, refrigerated immediately, and stored at -80°C (according to the manufacturer's specifications). sIgA concentrations in the saliva were determined using the salivary secretory IgA indirect enzyme immunoassay kit (Salimetrics, PA, USA). Concentration analysis was performed according to the manufacturer's specifications.

DNA repair: DNA repair function was measured by the recovery of apurinic-apyrimidinic (AP) site counts following irradiation. Isolated peripheral lymphocytes were exposed to X-irradiation of a dose of 2Gy per minute (total 4Gy) in microcentrifuge tubes at a concentration of 1.5 × 10⁶ cells/ml in cold medium. Each cell suspension was then transferred to a 24-well plate and incubated at 37°C with 5% CO₂ for 2 hours. Immediately following irradiation (baseline) and 2 hours after incubation, genomic DNA was extracted from the lymphocytes using the Get pure DNA Kit (Dojindo Molecular Technologies Inc., Rockville, MD). The APsite count (the number of AP sites per 1 × 10⁵ base pairs) was performed using the DNA damage quantification kit (Dojindo) according to the manufacturer's specifications. The DNA repair rate was calculated as the number of remaining APsites (the number of APsites 2 hours after incubation subtracted from baseline) divided by the number of APsites at baseline.

Interviews

After the intervention period, the participants were interviewed

to obtain their impression, promotional factor, and interference factor regarding continual parasympathetic nervous system stimulation for 2 weeks.

Statistical analysis

First, to eliminate the order effect, all data were analyzed with two-way repeated-measures analysis of variance (ANOVA), incorporating a 2 × 2 design (order of the two periods × before and after the period) using Visual Stat for Windows version 4.5 (Stat Soft Inc., Tokyo, Japan) before the analysis of effect of intervention. ANOVA of all data showed no significant main effect or interaction. These results confirmed that the order of the two periods did not affect the data.

Then, the data were analyzed with two-way repeated-measures ANOVA, incorporating a 2 × 2 design (intervention and control period × before and after the period). Tukey's honestly significant difference post-hoc comparisons were conducted as a multiple comparison test to assess the significance of main effects or interactions. In all statistical analyses, *p* values <0.05 were considered statistically significant.

Results

The stimulation methods are shown in Table 1. In parasympathetic nervous system stimulation confirmed through HRV for each participant before the intervention period, HRV in each person showed higher parasympathetic activity and lower sympathetic activity during the stimulation compared with before and after stimulation.

The caregivers performed parasympathetic nervous system stimulation for 29.9 ± 10.9 minutes per day (Table 1). All average systolic blood pressure values decreased after stimulation compared to before stimulation.

Table 2 shows the mean values for the sample data before and after both periods (intervention and control). The mean value of serum

No.	Age (years)	Duration of care (years)	Patient	Utilized relaxation method	Total time / day (min.)	Variation in systolic blood pressure (mmHg)
1	80	7	Parkinson syndrome	Stretching, hot eye mask	22 ± 6	-12.7 ± 15.4
2	60	12	Cervical spine injury	Stretching, hot eye mask, lying position	54 ± 15	-9.9 ± 14.5
3	61	6	Dementia	Stretching, hot eye mask	26 ± 3	-5.7 ± 7.8
4	66	6	Dementia	Hot eye mask, lying position	27 ± 10	-8.1 ± 10.2
5	53	22	Severely retarded children	Stretching, hot eye mask, tub bath	28 ± 9	-4.1 ± 7.3
6	56	23	Severely retarded children	Stretching, hot eye mask, tub bath	29 ± 10	-5.6 ± 9.9
7	54	27	Severely retarded children	Stretching, hot eye mask, tub bath, lying position	23 ± 8	-2.6 ± 7.8

Note: Variation in systolic blood pressure refers to a comparison of the value after stimulation to the value before stimulation.

Table 1: Characteristics of the participants, method of stimulation and time, and blood pressure.

	Intervention		Control	
	before	after	before	after
Cortisol (µg/dl)	7.8 ± 1.0	8.7 ± 1.9	8.6 ± 1.4	9.3 ± 2.7
White blood cells (cells/µl)	5457.1 ± 1271.3	5628.6 ± 1397.3	5642.9 ± 1595.7	5528.6 ± 1241.9
Lymphocytes (cells/µl)	2094.4 ± 822.8	2033.3 ± 652.7	2088.9 ± 656.1	2198.9 ± 788.9
Neutrophils (cells/µl)	2880.0 ± 475.5	3146.6 ± 906.3	3124.7 ± 1046.2	2832.4 ± 805.1
IL-2 concentration (pg/ml)	3395.9 ± 1960.2	5288.5 ± 2515.1*	4706.1 ± 2516.0	3518.3 ± 2105.7
sIgA (µg/ml)	215.7 ± 104.3	246.4 ± 81.4*	258.6 ± 98.5	216.4 ± 101.9
DNA repair (%)	33.5 ± 21.5	31.9 ± 18.2	34.0 ± 21.1	32.7 ± 21.6

* *p* < 0.05 compared to before the intervention

Table 2: Mean value of sample data before and after both periods.

cortisol concentration over the entire period was $8.7 \pm 2.0 \mu\text{g/dl}$. No significant main effect or interaction was shown for the changes (rates of increase) in serum cortisol concentration. Similarly, no significant main effect or interaction was shown for the changes in the number of white blood cells, lymphocytes, neutrophils, and DNA repair rate.

Figure 2 shows the changes in mitogen-induced IL-2 production before and after both periods. After the intervention period, the IL-2 concentration was significantly higher than in the control period. Figure 3 shows the changes in sIgA concentration before and after both periods. After the intervention period, the sIgA concentration was significantly higher than during the control period.

Table 3 shows the participants' impressions regarding continual parasympathetic nervous system stimulation after the intervention period. They perceived regional and systemic effects during the 2 weeks. In addition, they understood the variability in their blood pressure and how to relax their body. The graphical explanation of each person's own HRV data was the promotional factor regarding continuation for four participants. The factors that interfered with continuation of the stimulation included self-measurement of blood pressure, recording the stimulation method used every day during the intervention period, and interruption by phone or child.

Discussion

The focus of the present study was to clarify the effect of nursing intervention (individually tailored self-stimulation of the parasympathetic nervous system) for 2 weeks on immune function and DNA repair function in family caregivers. The validity of the method of stimulating the parasympathetic nervous system was confirmed with the HRV data and the blood pressure data.

The mean value of serum cortisol concentration that reflects stress

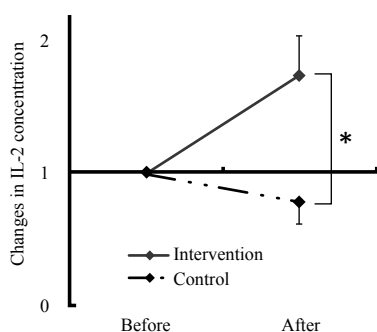


Figure 2: Changes in IL-2 concentration. * $p < 0.05$.

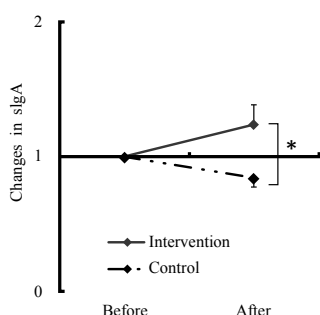


Figure 3: Changes in sIgA concentration. * $p < 0.05$.

over the entire period ($8.7 \pm 2.0 \mu\text{g/dl}$) was higher than the value in healthy controls in previous reports ($5.4 \mu\text{g/dl}$, Weber et al. [43], $7.5 \mu\text{g/dl}$, O, Brien et al. [20]). Thus, our participants were in a stressed condition.

Although no significant difference was observed in serum cortisol concentrations (indicating an equivalent stress condition as during the control period), the changes in lymphocyte function (mitogen-induced IL-2 production) after the intervention period were significantly higher than those after the control period (Figure 2). Similarly, the sIgA concentration improved after the intervention period, although the difference was slight (Figure 3). According to the report of Osaka Prefectural Institute of Public Health [6], the mean value of IL-2 production stimulated with same method was $2098.1 \pm 144.0 \text{ pg/ml}$. However, making a simple comparison is difficult because of the difference in age (61 years or more age) and the absence of cortisol data. The mean value of sIgA measured with same method (using Salimetrics) showed $379.4 \mu\text{g/ml}$ (in the manual: $n=21$, adults). The mean value in emergency department nurses was $148.5 \mu\text{g/ml}$ [11]. Thus, the sIgA levels for the caregivers in the present study were not low. However, the findings suggest that stimulating the parasympathetic nervous system in everyday life is effective in improving lymphocyte function and sIgA concentration. Preganglionic parasympathectomy has been reported to decrease salivary sIgA secretion rates from the rat submandibular gland [44], which is consistent with our results.

The DNA repair function was observed as recovery from X-irradiation after 2 hours. No significant difference was noted between the control and the intervention periods. This may reflect individual variability at the level of gene repair function or sensitivity differences associated with the number of Ach receptors and cortisol receptors per cell, which depends on the species and cell type [45-47]. In addition, this function is difficult to compare because various methods have been used to measure DNA repair or damage, and these data were not reported under the same conditions as the present study.

According to the subjective evaluation of continuing the parasympathetic nervous system stimulation routine for 2 weeks, the caregivers perceived regional and systemic effects (Table 3). These sensations of comfort may have been the main reasons that all caregivers agreed to cooperate in the present study and continued the stimulation. Deciding on the stimulation methods by consulting with the participants and performing them in a number of ways are also possible reasons for continuing. In addition, the graphical explanation of each person's HRV data was the promotional factor. They were convinced that the method they chose was reasonable for parasympathetic nervous system stimulation by watching their own graph before the intervention period. Visualization of automatic nervous system function with the recognition of the physical sense may have promoted continuing because they had never been aware of such changes in automatic nervous system function before. The interference factors were particular to the study and not due to the parasympathetic nervous system stimulation per se.

These findings suggest that stimulating the parasympathetic nervous system in everyday life is effective for improving lymphocyte function and sIgA concentration, but ineffective for improving DNA

Regional effect	Reduced eye fatigue or backache
Systemic effect	Relaxed body, calmed down, better quality sleep
Other	Understood her tension by measuring blood pressure, understood a personalized method of relaxation, became conscious of relaxation time, became aware of the state of her own tension

Table 3: Subjective effect after intervention period.

repair function. In addition, effects were shown even with a variety of different methods of parasympathetic nervous system stimulation. Although most previous studies used a single method, with the method used in the present study, family caregivers had the benefit of adjusting what they did and when they did it, in accordance with their lifestyle or physical condition. In addition, it may be more effective for a nurse to recommend methods based on assessment of personal needs (type of care performed or physical condition).

Side benefits of intervention were also observed. The participants understood their own tension due to measurement of their blood pressure, became conscious of relaxation time, and became aware of the state of their own tension (Table 3). These types of awareness may impact each person's future healthcare management. The findings of the present study may be applicable to providing guidance for mitigating the influence of stress. They may also be effective in providing guidance regarding concrete methods, during health counseling of those who are already under stress or as general information in primary preventive care in health education.

The limitations of the present study are the small sample size and observance of only females. Further investigation is therefore required to include larger trials with both males and females. Another limitation is the length of the experimental period. Self-stimulation in the present study was performed for 2 weeks because of participant burden. In addition, the researcher only measured mitogen-induced IL-2 production in lymphocytes and sIgA in saliva as indicators of immune function. Further investigation is required to clarify the influence of continuing stimulation for a much longer period and whether this method will be effective for preventing infectious disease. Studying the frequency of upper respiratory infections [14] may be useful for examining the prevention of infectious disease.

In conclusion, individually tailored self-stimulation of the parasympathetic nervous system for 2 weeks is effective for improving lymphocyte function and sIgA concentration in family caregivers, but has no effect on DNA repair function.

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