Healthy forest parks make healthy people: Forest environments enhance human immune function

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Abstract

Humans have enjoyed forest environments for a long time because of the quiet atmosphere, beautiful scenery, mild climate, and fresh, clean air.

In the present study, we found that visiting forest parks enhanced human natural killer cell (NK) activity, increased anti-cancer proteins, and reduced stress. This effect lasted for more than 30 days. Phytoncides released from trees and the decreased production of stress hormones may partially contribute to the increased NK activity. Because NK cells can kill tumor cells by releasing anti-cancer proteins, and visiting forest parks increases NK activity and the amount of anti-cancer proteins, therefore the above findings suggest that visiting forest parks may have a preventive effect on cancer generation and progression.

Introduction

Forest environments have been enjoyed by humans for a long time because of the quiet atmosphere, beautiful scenery, mild climate, and clean fresh air found in forests. A forest bathing trip is a short, leisurely trip to visit a forest park, called “Shinrinyoku” in Japanese, which is similar to natural aromatherapy. A forest bathing trip involves visiting a forest park for relaxation and recreation while breathing in volatile substances, called phytoncides (wood essential oils), which are volatile organic compounds derived from trees, such as α-pinene and limonene (Li et al. 2006, 2007a, 2008ab, 2009, 2010, Li 2010). Incorporating forest bathing trips into a good lifestyle was first proposed in 1982 by the Forest Agency of Japan (Li 2010). It has now become a well-recognized relaxation and/or stress management activity in Japan (Li et al. 2007a, 2008ab, 2010, Li 2010). Forest bathing trips significantly increased the score for vigor and decreased the scores for anxiety, depression, anger, confusion, and fatigue as investigated by the Profile of Mood States (POMS) test (Li et al. 2007a, 2008b, 2010, Li 2010). Customary forest bathing may help to decrease the risk of psychosocial stress-related diseases (5). Moreover, forest bathing trips can decrease blood glucose, reduce blood pressure (Ohtsuka et al., 1998), reduce the concentration of cortisol in saliva, reduce prefrontal cerebral activity, and stabilize autonomic nervous activity in humans (Park et al., 2010). Because forests occupy 67% of the land in Japan, forest bathing is easily accessible (Li 2010, Li et al. 2008c). Moreover, forest bathing is possible in similar environments throughout the world. It is well known that the immune system including natural killer (NK) cells plays an important role on the defense against bacteria, viruses and tumors (Wu and Lanier, 2003). It is also well known that stress inhibits immune function (Li et al. 2005a). Empirically, forest environments may reduce stress. Therefore, we speculate that forest environments may have beneficial effects on immune function by reducing stress. However, there have been no reports to date

investigating the effect of forest environments on human immune function except for the studies conducted by authors.

Effect of forest environments on human immune function

Based on the background mentioned above, since 2005 a series of investigations to study the effect of forest bathing on human immune function have been conducted by measuring human NK activity, the numbers/proportions of NK and T cells, and perforin-, granzymes A and B (GrA/B)-, and granulysin (GRN)-expressing lymphocytes in human peripheral blood lymphocytes (PBLs) during visits to forest parks with both male and female subjects in Japan (Li et al. 2007a, 2008ab, 2010, Li 2010).

In the first study (Li et al. 2007a), 12 healthy male subjects, aged 37-55 years (43.1 ± 6.1), were selected from three large companies in Tokyo, Japan. The information of the subjects gathered from a self-administered questionnaire, including age and lifestyle habits, has been reported previously (Li et al. 2007b). None of the subjects had any signs or symptoms of infectious diseases, used drugs that might affect immunological analysis, or were taking any medication at the time of the study. The subjects participated in a three-day/two-night trip to forest areas at Iiyama in Nagano prefecture located in northwest Japan in early September, 2005. On day 1, the subjects walked about 2.5 km. This level of exertion was selected because it closely resembles the average amount of physical activity in one work day. This walk was conducted in the forest park during the afternoon. Participants were allowed to rest anywhere and anytime they chose. On day 2, they walked about 2.5 km over two hours both in the morning and afternoon, respectively, in two different forest parks; and on day 3, the subjects finished the trip and returned to Tokyo after blood was drawn and a questionnaire survey was completed. The forests included Japanese cedar (Cryptomeria), Japanese beech, and Japanese

oak. Blood was sampled on the second and third days. White blood cell (WBC) counts, NK activity, numbers of NK and T cells, and numbers of GRN, perforin, and GrA/B-expressing lymphocytes were measured in the blood samples. The same measurements were made before the trips on a normal working day as a control. Blood was sampled at 8:00 am on all occasions. To control for the effect of alcohol on NK activity, the subjects did not consume alcohol for 2 days before blood was drawn. The subjects did not take hot spring baths or eat any special foods such as herbs and alcoholic drinks during the trip, as these can have an effect on immune function. Phytoncide concentrations in forest air samples were also measured.

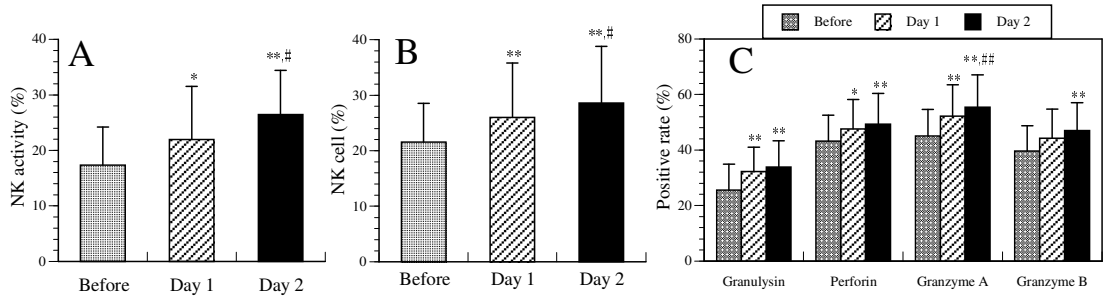

Fig. 1. Effect of a forest bathing trip on NK activity (A), the number of NK cells (B), and the number of GRN, perforin, GrA/B-expressing cells in PBL (C). Data are presented as the mean+SD (n=12). *: $p<0.05$, **: $p<0.01$, significantly different from before the trip, #: $p<0.05$ significantly different from day 1 by paired t-test. Cited from Li et al. Int J Immunopathol Pharmacol. 2007;20:3-8.

The forest bathing trip significantly increased human NK activity and the numbers of NK cells (Fig. 1AB). It has been reported that NK cells kill tumor or virus-infected cells by the release of perforin, granzymes, and GRN via the granule exocytosis pathway (Li et al. 2002, 2004, 2005b, 2008d, Okada et al. 2003). In order to explore the mechanism of enhancement of NK activity induced by forest bathing, the effect of forest bathing on the intracellular levels of perforin, GRN, and GrA/B in PBL was investigated, and it was found that the forest bathing trip also significantly increased the numbers of intracellular perforin-, GRN-, and GrA/B-expressing lymphocytes (Fig. 1C). Taken together, these findings indicate that a forest bathing trip can increase NK activity, and that this effect might be at least partially mediated

by increasing the number of NK cells and by the induction of intracellular perforin, GRN, and GrA/B (Li et al. 2007a).

How long does the increased NK activity last after a forest park visiting?

Two questions remain to be resolved: (i) will a trip to places without forest (a city tourist visit) also increase NK activity? (ii) How long does the increased NK activity last after a forest bathing trip or a city tourist visit? Thus, two investigations were conducted to address these two questions (Li et al. 2008a). Twelve healthy male subjects, aged 35-56 years (mean 45.1±6.7), were selected from four large companies in Tokyo, Japan. Information on the subjects was gathered from a self-administered questionnaire, including age and lifestyle habits as described previously (Li et al. 2007b). None of the subjects had any signs or symptoms of infectious disease, used drugs that might affect immunological analysis, or were taking any medication at the time of the study. The subjects experienced a three-day/two-night trip to three different *Chamaecyparis obtuse* (Japanese cypress, Hinoki in Japanese) forest parks around Agematsu town in Nagano prefecture located in northwest Japan in early September, 2006. The schedule of the forest bathing trip was similar to that described previously (Li et al. 2007a). Briefly, on day 1, the subjects walked about 2.5 km; on day 2, they walked about 2.5 km over two hours both in the morning and afternoon, respectively, in two different forest parks; and on day 3, the subjects finished the trip and returned to Tokyo after blood was drawn and a questionnaire survey was completed. On the city tourist visit, eleven out of twelve of the same subjects experienced a three-day/two-night trip to Nagoya city in mid-May, 2006 (Li et al. 2008a). On the first day, the subjects walked for two hours in the afternoon along a tourist route through a historic district in Nagoya, and then stayed at a hotel also in Nagoya. On the second day, the subjects walked for 2 hours around the Nagoya

Baseball Dome in the morning and 2 hours around/in Nagoya Airport in nearby Nagoya city in the afternoon. There are some areas with trees in Nagoya city, but there are almost no trees in the areas visited. The class of hotel was the same and the lifestyle of the subjects during the stays in the hotels was the same for the city and the forest trips. The walking courses in both trips were 2.5 km, which was the same as the previous study (Li et al. 2007a). Blood was sampled at 8:00 am on the second and third days, on days 7 and 30 after the forest bathing trip, and three days prior to the trips as a control. WBC counts, NK activity, proportions of NK and T cells, and GRN-, perforin-, and GrA/B-expressing cells in PBL were measured. Adrenaline concentration in urine was also measured. To control for the effect of alcohol on NK activity, the subjects did not consume alcohol for 2 days before blood was drawn including before and during the trips, and after the trip on days 7 and 30. The purpose of setting the control experiment in a city in this study was to determine whether taking a trip (city tourist visit) can also affect NK activity.

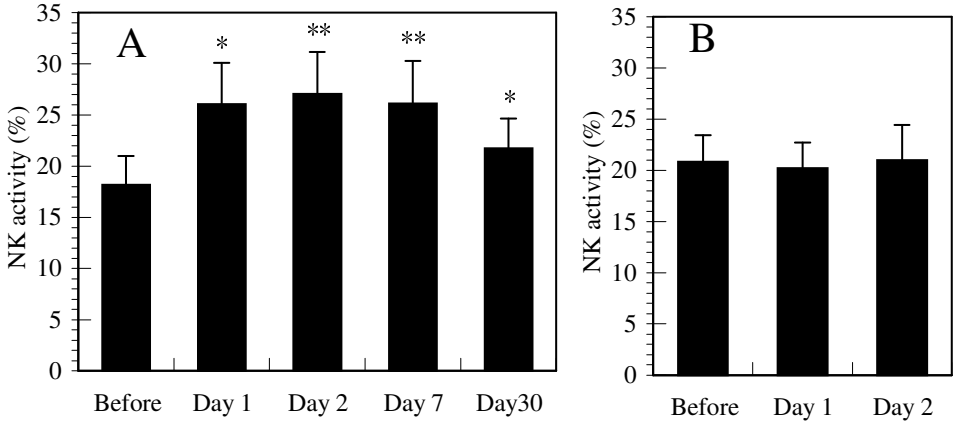

Fig. 2. Effect of a forest bathing trip (A) and a city tourist visit (B) on NK activity. Data are presented as the mean+SE (n=12 for A, n=11 for B). *: $p<0.05$, **: $p<0.01$, significantly different from before the trip by paired t-test. Cited from Li et al. Int J Immunopathol Pharmacol. 2008;21:117-128.

The forest bathing trip significantly increased human NK activity, the numbers of NK cells (Fig. 2), and the percentages of GRN-, perforin-, and GrA/B-expressing cells in PBL, which

confirmed the previous findings (Li et al. 2007a). The increased NK activity, number of $CD16^+$ NK cells, and percentages of GRN-, perforin-, and GrA/B-expressing cells lasted more than 7 days and even for 30 days in the cases of NK activity, the number of NK cells, and GRN- and GrB-expressing cells. In contrast, the city tourist visit did not increase human NK activity, numbers of NK cells, or the expression of the selected intracellular perforin, GRN, and GrA/B . Phytoncides, such as alpha-pinene and beta-pinene, were determined by gas chromatography-mass spectrometry (GC-MS). We detected alpha-pinene and beta-pinene in forest air, but almost not in city air. These findings indicate that a forest bathing trip increased NK activity, the number of NK cells, and the levels of intracellular perforin, GRN, and GrA/B, and that these effects lasted for at least 7 days after the trip (Li et al. 2008a). The important finding is that visiting a forest, rather than a city, increases NK activity and the intracellular levels of perforin, GRN, and GrA/B. Phytoncides released from the trees may partially contribute to the increased NK activity (Li et al. 2006, 2009).

A day trip to a forest park also increased human NK activity

Recently, we found that a day trip to a forest park also increased human NK activity and the expression of anti-cancer proteins in male subjects (Li et al. 2010). In this study, twelve healthy male subjects, aged 35-53 years, were selected after giving informed consent. The subjects experienced a day trip to a forest park in the suburbs of Tokyo. They walked for two hours in the morning and afternoon, respectively, in the forest park on Sunday. Blood and urine were sampled the following morning and again 7 days after the trip. The NK activity, numbers of NK and T cells, and GRN, perforin, and GrA/B-expressing lymphocytes, the concentration of cortisol in blood samples, and the concentration of adrenaline in urine were measured. Similar measurements were made before the trip on a weekend day as the control.

Phytoncide concentrations in the forest were measured. The day trip to the forest park significantly increased NK activity and the numbers of NK, perforin, GRN, and GrA/B-expressing cells while significantly decreasing the concentrations of cortisol in the blood and adrenaline in urine. The increased NK activity lasted for 7 days after the trip. Phytoncides, such as isoprene, alpha-pinene and beta-pinene, were detected in the forest air. These findings indicate that the day trip to the forest park also increased the NK activity, number of NK cells, and levels of intracellular anti-cancer proteins, and that this effect lasted for at least 7 days after the trip. Phytoncides released from trees and decreased stress hormone levels may partially contribute to the increased NK activity.

Effect of forest environments on NK activity in female subjects

Although it has been demonstrated that forest bathing trips enhance human NK activity in male subjects, it still remained to be resolved whether or not forest bathing trips also increase NK activity in female subjects. It has been reported that menstrual cycle significantly affects NK activity (Souza et al. 2001), therefore, the influence of menstrual cycle on NK activity should be controlled for in experiments with female subjects.

In this study (Li et al. 2008b), thirteen healthy nurses, aged 25-43 years (mean 28.8±4.6), professional career 4-18 years (mean 6.7±3.8), were selected with informed consent. None of the subjects had any signs or symptoms of infectious disease, used drugs that might affect immunological analysis, or were taking any medication at the time of the study. The subjects experienced a three-day/two-night trip to forest fields around Shinano town in Nagano prefecture located in northwest Japan in early September of 2007. The schedule of the forest bathing trip and blood sampling was similar to that described previously (Li et al. 2007a, 2008a). WBC counts, NK activity, numbers of NK and T cells, and GRN, perforin, and

GrA/B-expressing lymphocytes in the blood samples, the concentrations of estradiol and progesterone in serum, and the concentrations of adrenaline and noradrenaline in urine were measured. The same control measurements were made before the trip on a normal working day. Blood was sampled at 8:00 am on all days. The concentrations of phytoncides in the forests were also measured.

The forest bathing trip significantly increased NK activity and the positive rates of NK (Fig. 3), perforin-, GRN-, and GrA/B-expressing cells. The increased NK activity and the positive rates of NK, perforin, GRN, and GrA/B-expressing cells lasted for more than 7 days after the trip (Li et al. 2008b), which confirmed the previous findings in male subjects (Li et al. 2008a). Phytoncides, such as alpha-pinene and beta-pinene were detected in forest air. These findings indicate that a forest bathing trip also increased NK activity, the number of NK cells, and the levels of intracellular anti-cancer proteins in female subjects, and that this effect lasted for at least 7 days after the trip. Phytoncides released from trees may partially contribute to the increased NK activity (Li et al. 2006, 2010).

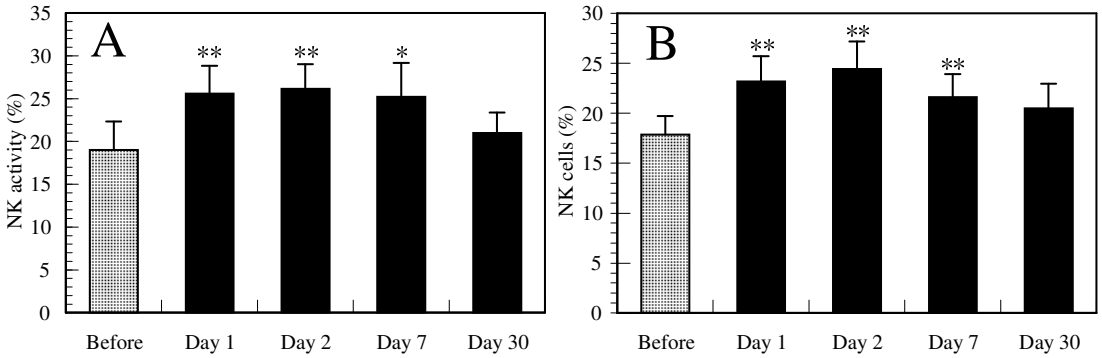

Fig. 3. Effect of a forest bathing trip on NK activity (A) and the percentage of NK cells (B) in female subjects. Data are presented as the mean+SE (n=13). *: $p<0.05$, **: $p<0.01$, significantly different from before the trip by paired t-test. Cited from Li et al. J Biol Regul Homeost Agents. 2008;22:45-55.

It has been reported that the menstrual cycle and the levels of estradiol and progesterone in serum may affect human NK activity in female subjects (Souza et al. 2001, Roszkowski et al.

1997, Szekeres-Bartho et al. 2005). To control for the influence of menstrual cycle on NK activity, a questionnaire was administered to obtain information on the menstrual cycle of the subjects. The ratios of subjects who were in the follicular phase during the experiment were 5/13, 6/13, 6/13, 7/13, and 6/13 on the day before the trip, days 1 and 2 during the trip, and days 7 and 30 after the trip, respectively, indicating that there was no significant difference in the proportion of the menstrual cycles of the subjects between the different days. This suggests that the menstrual cycle had a similar influence on the average of NK activity on the different days. Therefore, statistical analyses to compare the mean values of NK activity on the different days by paired t-test should be appropriate. In addition, the concentrations of estradiol and progesterone in the serum of the subjects were also measured to confirm the influence of estradiol and progesterone on NK activity. In this study, there was no significant difference in the concentration of estradiol in the serum in the days before, during, and after the forest bathing trip, indicating that estradiol had a similar effect on NK activity on different days in the subjects in this case. Although the levels of progesterone on days 1 and 2 were higher than that before, the difference in the concentration of progesterone in the serum between days 1 or 2 and before the trip was not significant, suggesting that progesterone had a similar effect on NK activity on different days in the subjects in this case (Li et al. 2008b).

Many factors, including circadian variation (Angeli 1992), physical exercise (Li et al. 2007b), and alcohol consumption (Li et al. 2007b) can affect human NK activity. In order to control for the effect of circadian rhythm on NK activity, blood was sampled at 8:00 am on all days (Li et al. 2007a, 2008ab, 2009, 2010, Li 2010). To control for the effect of physical exercise on NK activity, the walking steps during the trips were limited to the average normal workday distances as monitored by a pedometer. The levels of physical activity among all trips were also matched. To control for the effect of alcohol on NK activity, the subjects did not consume

alcohol for 2 days before blood was drawn during the study period for both trips including before the trips and after the trips on days 7 and 30. The sleeping hours during the trips were slightly longer than that on average working days; however, the difference was not significant in either type of trip. Li et al. (2007b) found that there was no difference in the numbers of NK cells, nor the levels of perforin-, GRN-, or GrA/B-expressing cells in PBL among subjects who slept for 5, 6, or 7 hours. In addition, although the sleeping hours during the city tourist visit were slightly longer than on average working days, the NK activities during the trip were almost the same as for working days, indicating that the longer sleeping hours did not affect NK activity in the city tourist visit (Li et al. 2008a). Taken together, although the sleeping hours during the trips were slightly longer than those on average working days, this difference did not affect NK activity or cell numbers in the city tourist trip.

Effect of forest environments on stress hormones

Measurement of free adrenaline and noradrenaline in urine provides a reliable measure of the circulating concentration of adrenaline and noradrenaline in the bloodstream and thus is a measure of sympathoadrenal medulla activity (Moleman et al. 1992). The concentrations of adrenaline and noradrenaline in urine have been used to evaluate work related stress in nurses (Brown et al. 2006) and lorry drivers (van der Beek et al. 1995), in which the subjects showed decreases in adrenaline and/or noradrenaline in urine with the lower stress. It was found that forest bathing trips significantly decreased adrenaline and noradrenaline concentrations in urine in both male (Li et al. 2008a) and female (Li et al. 2008b) subjects, while a city tourist visit had no effect (Li et al. 2008a), suggesting that the subjects were under conditions of lower stress during the forest bathing trips (Li et al. 2008ab). It has been reported that adrenaline and noradrenaline inhibit human NK activity (Garland et al. 2003, Yokota et al.

2004). Li et al. (2005a) found previously that physical and/or psychological stress decreased NK activity, NK receptor levels, and mRNA transcription levels of granzymes and perforin by increasing plasma corticosterone level in mice. The increase in NK activity during forest bathing trips may be related to an attenuated stress hormone response (adrenaline) associated with the forest bathing trip, whereas increased sympathetic activity may have an immunosuppressive effect through the release of adrenaline. Other studies have reported that forest bathing trips reduce the concentration of cortisol in saliva, reduce prefrontal cerebral activity, reduce blood pressure, and stabilize autonomic nervous activity in humans (Park et al. 2010). In addition, forest bathing trips significantly increased scores for vigor and decreased the scores for anxiety, depression, and anger in the POMS test, suggesting that the subjects were physiologically relaxed during the forest trips (Li et al. 2007a, 2008b, 2009).

Effect of phytoncides on human NK function

Why did the forest environment increase human NK activity? What kind of factors in the forest environment activated human NK cells? We speculate that aromatic volatile substances (phytoncides) derived from trees such as α-pinene and limonene play an important role. We detected several phytoncides, such as isoprene, α-pinene, β-pinene, and *d*-limonene, in the forest parks during the trip. To investigate the effect of phytoncides on human NK function, NK-92MI cells, a human NK cell line, were incubated in the presence of phytoncides such as α-pinene, 1,8-cineole, *d*-limonene, and essential oils extracted from trees including Japanese cedar and *Chamaecyparis obtusa* (*Hinoki* in Japanese), then NK activity and the intracellular levels of perforin, GrA, and GRN were measured (Li et al. 2006). Phytoncides significantly increased the cytolytic activity of NK-92MI cells in a dose-dependent manner and significantly increased the intracellular levels of perforin, GrA, and GRN in NK-92MI cells.

Phytoncides also partially, but significantly, restored the decreased human NK activity and the decreased perforin, GrA, and GRN levels in NK-92MI cells induced by dichlovos, an organophosphorus pesticide. Pretreatment with phytoncides partially prevented dichlovos-induced inhibition of NK activity. Taken together, these data indicate that phytoncides significantly enhanced human NK activity and this effect is at least partially mediated by the induction of intracellular perforin, GrA, and GRN (Li et al. 2006). Moreover, we also found that *in vivo* exposure to phytoncides from *Chamaecyparis obtusa* stem oil for 3 nights significantly increased human NK activity and the percentages of NK cells (Fig. 4), perforin, GRN, and GrA/B-expressing cells, and significantly decreased the percentage of T cells and concentrations of adrenaline and noradrenaline in urine (Li et al. 2009). These findings suggest that phytoncides contribute to the enhanced NK activity during the forest trip.

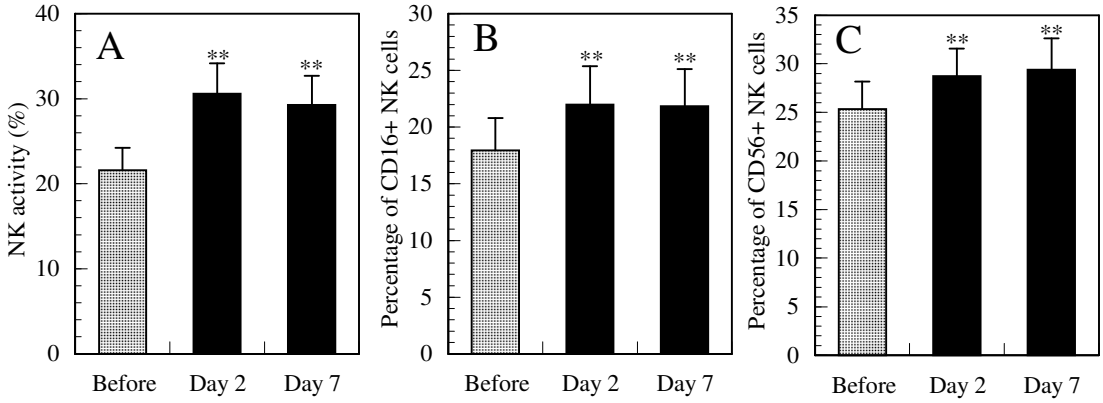

Fig. 4. Effect of a day trip to a forest park on human NK activity (A), the percentages of $CD16^+$ (B) and $CD56^+$ (C) NK cells. Data are presented as the mean+SE (n=12). **: $p<0.01$, significantly different from before the trip by the paired t-test. Cited from Li et al. Int J Immunopathol Pharmacol, 22; 951-9.

In addition, we found that people living in areas with lower forest coverage had significantly higher standardized mortality ratios for cancers compared with the people living in areas with higher forest coverage in Japan, suggesting that forest environments may partially contribute to decreased mortality ratios for some cancers (Li et al. 2008c).

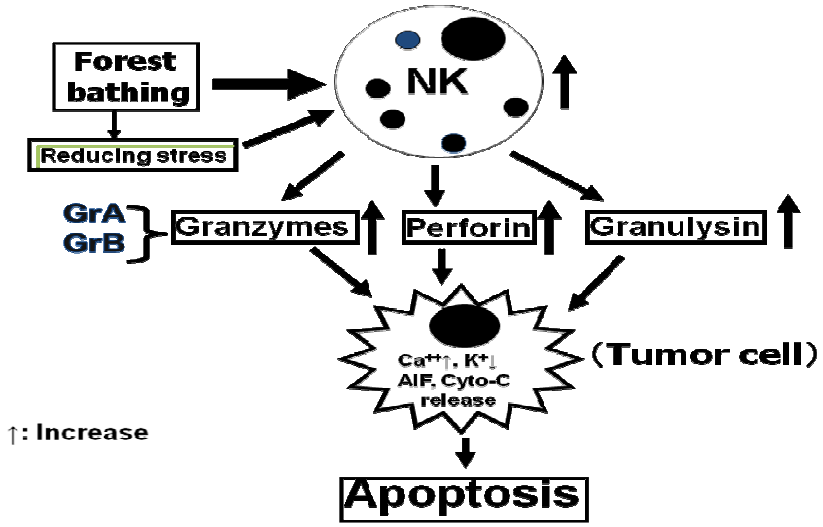

Fig. 5. Mechanism of forest bathing-induced induction in NK activity. Cited from Li. Environmental health and preventive medicine, 15, 9-17, 2010.

Conclusions

These findings indicate that forest bathing trips increase NK activity, which was mediated by increases in the number of NK cells and the levels of intracellular anti-cancer proteins (Li, 2010) (Fig. 4). Phytoncides released from trees as well as decreased production of stress hormones may also partially contribute to the increased NK activity. However, this conclusion would need to be tested with a larger sample size and stratified against the other variables that could influence the cellular responses.

Because NK cells can kill tumor cells by releasing anti-cancer proteins, such as perforin, GRN, and GrA/B, and forest bathing trips increase NK activity and the intracellular level of anti-cancer proteins, the above findings suggest that forest bathing trips may have a preventive effect on cancer generation and development. In this manner, we believe that our findings suggest that time spent in natural, green forest parks can promote better health.

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References

Angeli, A. (1992). Circadian rhythms of human NK cell activity. *Chronobiologia*, 19, 195-8.

Brown, D.E., James, G.D., & Mills, P.S. (2006). Occupational differences in job strain and physiological stress: female nurses and school teachers in Hawaii. *Psychosomatic Medicine*, 68, 524-30.

Garland, M., Doherty, D., Golden-Mason, L., Fitzpatrick, P., Walsh, N., & O'Farrelly, C. (2003). Stress-related hormonal suppression of natural killer activity does not show menstrual cycle variations: implications for timing of surgery for breast cancer. *Anticancer Research*, 23, 2531-5.

Li, Q., Nagahara, N., Takahashi, H., Takeda, K., Okumura, K., & Minami, M. (2002). Organophosphorus pesticides markedly inhibit the activities of natural killer, cytotoxic T lymphocyte and lymphokine-activated killer: a proposed inhibiting mechanism via granzyme inhibition. *Toxicology*, 172, 181-90.

Li, Q., Nakadai, A., Takeda, K., & Kawada, T. (2004). Dimethyl 2,2-dichlorovinyl phosphate (DDVP) markedly inhibits activities of natural killer cells, cytotoxic T lymphocytes and lymphokine-activated killer cells *via* the Fas-ligand/Fas pathway in perforin-knockout (PKO) mice. *Toxicology*, 204, 41-50.

Li, Q., Liang, Z., Nakadai, A., & Kawada, T. (2005a). Effect of electric foot shock and psychological stress on activities of murine splenic natural killer and lymphokine-activated killer cells, cytotoxic T lymphocytes, natural killer receptors and mRNA transcripts for granzymes and perforin. *Stress*, 8:107-16.

Li, Q., Nakadai, A., Ishizaki, M., Morimoto, K., Ueda, A., Krensky, A.M., & Kawada, T. (2005b). Dimethyl 2, 2-dichlorovinyl phosphate (DDVP) markedly decreases the expression of perforin, granzyme A and granulysin in human NK-92CI cell line. *Toxicology*, 213, 107-16.

Li, Q., Nakadai, A., Matsushima, H., Miyazaki, Y., Krensky, A.M., Kawada, T., & Morimoto, K. (2006). Phytoncides (wood essential oils) induce human natural killer cell activity. *Immunopharmacology and Immunotoxicology*, 28, 319-33.

Li, Q., Morimoto, K., Nakadai, A., Inagaki, H., Katsumata, M., Shimizu, T., Hirata, Y., Hirata, K., Suzuki, H., Miyazaki, Y., Kagawa, T., Koyama, Y., Ohira, T., Takayama, N., Krensky, A.M., & Kawada, T. (2007a). Forest bathing enhances human natural killer activity and expression of anti-cancer proteins. *International journal of immunopathology and pharmacology*, 20, 3-8.

Li, Q., Morimoto, K., Nakadai, A., Qu, T., Matsushima, H., Katsumata, M., Shimizu, T., Inagaki, H., Hirata, Y., Hirata, K., Kawada, T., Lu, Y., Nakayama, K., & Krensky, A.M. (2007b). Healthy lifestyles are associated with higher levels of perforin, granulysin and granzymes A/B-expressing cells in peripheral blood lymphocytes. *Preventive Medicine*, 44, 117-23.

Li, Q., Morimoto, K., Kobayashi, M., Inagaki, H., Katsumata, M., Hirata, Y., Hirata, K., Suzuki, H., Li, Y.J., Wakayama, Y., Kawada, T., Park, B.J., Ohira, T., Matsui, N., Kagawa, T., Miyazaki, Y., & Krensky, A.M. (2008a). Visiting a forest, but not a city, increases human natural killer activity and expression of anti-cancer proteins. *International journal of immunopathology and pharmacology,* 21, 117-128.

Li, Q., Morimoto, K., Kobayashi, M., Inagaki, H., Katsumata, M., Hirata, Y., Hirata, K., Shimizu, T., Li, Y.J., Wakayama, Y., Kawada, T., Ohira, T., Takayama, N., Kagawa, T., & Miyazaki Y. (2008b). A forest bathing trip increases human natural killer activity and

expression of anti-cancer proteins in female subjects. *Journal of biological regulators and homeostatic agents*, 22, 45-55.

Li, Q., Kobayashi, M., Kawada, T. (2008c). Relationships between percentage of forest coverage and standardized mortality ratios (SMR) of cancers in all prefectures in Japan. *The Open Public Health Journal*, 1, 1-7.

Li, Q., Kobayashi, M., & Kawada, T. (2008d). DDVP markedly decreases the expression of granzyme B and granzyme 3/K in human NK cells. *Toxicology*, 243, 294-302.

Li, Q. (2010). Effect of forest bathing trips on human immune function. *Environmental health and preventive medicine*, 15, 9-17.

Li, Q., Kobayashi, M., Inagaki, H., Hirata, Y., Hirata, K., Li, Y.J., Shimizu, T., Suzuki, H., Wakayama, Y., Katsumata, M., Kawada, T., Ohira, T., Matsui, N., & Kagawa, T. (2010). A day trip to a forest park increases human natural killer activity and the expression of anti-cancer proteins in male subjects. *Journal of biological regulators and homeostatic agents*, (submitted).

Li, Q., Kobayashi, M., Wakayama, Y., Inagaki, H., Katsumata, M., Hirata, Y., Hirata, K., Shimizu, T., Kawada, T., Ohira, T., Park, B.J., Kagawa, T., & Miyazaki, Y. (2009). Effect of phytoncide from trees on human natural killer function. *International journal of immunopathology and pharmacology,* 22; 951-9.

Moleman, P., Tulen, J.H., Blankestijn, P.J., Man in 't Veld, A.J., & Boomsma, F. (1992). Urinary excretion of catecholamines and their metabolites in relation to circulating catecholamines. Six-hour infusion of epinephrine and norepinephrine in healthy volunteers. *Archives of general psychiatry*, 49, 568-72.

Morita E., Fukuda, S., Nagano, J., Hamajima, N., Yamamoto, H., Iwai, Y., Nakashima, T., Ohira, H., Shirakawa, T. (2007). Psychological effects of forest environments on healthy adults: Shinrin-yoku (forest-air bathing, walking) as a possible method of stress reduction. *Public Health,* 121, 54.

Ohtsuka, Y., Yabunaka, N., Takayama, S. (1998). Shinrin-yoku (forest-air bathing and walking) effectively decreases blood glucose levels in diabetic patients. *International Journal of Biometeorology,* 41, 125-127.

Okada, S., Li, Q., Whitin, J.C., Clayberger, C., & Krensky, A.M. (2003). Intracellular Mediators of Granulysin-induced Cell Death. *Journal of Immunology*, 171, 2556-62.

Park, B.J., Tsunetsugu, Y., Kasetani, T., Kagawa, T., & Miyazaki, Y. (2010). The physiological effects of Shinrin-yoku (taking in the forest atmosphere or forest bathing): evidence from field experiments in 24 forests across Japan. *Environmental health and preventive medicine*, 15, 18-26.

Roszkowski, P.I., Hyc, A., Stopińska-Głuszak, U., & Malejczyk, J. (1997). Natural killer cell activity and sex hormone levels in mastopathy. *Gynecological endocrinology*, 11, 399-404.

Souza, S.S., Castro, F.A., Mendonça, H.C., Palma, P.V., Morais, F.R., Ferriani, R.A., & Voltarelli, J.C. (2001). Influence of menstrual cycle on NK activity. *Journal of reproductive immunology*, 50, 151-9.

Szekeres-Bartho, J., Polgar, B., Kozma, N., Miko, E., Par, G., Szereday, L., Barakonyi, A., Palkovics, T., Papp, O., & Varga, P. (2005). Progesterone-dependent immunomodulation. *Chemical immunology and allergy*, 89, 118-25.

van der Beek, A.J., Meijman, T.F., Frings-Dresen, M.H., Kuiper, J.I., & Kuiper, S. (1995). Lorry drivers' work stress evaluated by catecholamines excreted in urine. *Occupational and Environmental Medicine*, 52, 464-9.

Wu, J., Lanier, L.L. (2003). Natural killer cells and cancer. *Advances in cancer research,* 90, 127-56.

Yokota, T., Uehara, K., & Nomoto, Y. (2004). Addition of noradrenaline to intrathecal morphine augments the postoperative suppression of natural killer cell activity. *Journal of anesthesia,* 18, 190-5.