Life Sciences 85 (2009) 408–411

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Relaxant effect of microtitan via regulation of autonomic nerve activity in mice

Wataru Aoi [a,b,*], Yoshikazu Takanami [c], Yukari Kawai [c], Takeshi Otsuki [d], Toshiyuki Kawake [e], Yuji Naito [b], Toshikazu Yoshikawa [b,c]

[a] Laboratory of Health Science, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Shimogamo Hangi-cho 1-5, Sakyo-ku, Kyoto 606-8522, Japan
[b] Department Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kajii-cho 465, Kamigyo-ku, Kyoto 602-8566, Japan
[c] Department of Preventive Medicine for Health Science, Kyoto Prefectural University of Medicine, Kyoto, Japan
[d] Faculty of Health and Welfare Human Services, Saint Catherine University, Matsuyama, Ehime 799-2496, Japan
[e] Phiten Co., Ltd., Tearaimizu-Cho 678, Nakagyo-ku, Kyoto 604-8152, Japan

ARTICLE INFO

Article history:
Received 21 January 2009
Accepted 17 July 2009

Keywords:
Spontaneous activity
Sympathetic nerves
Parasympathetic nerves
Noradrenalin
Heart rate variability

ABSTRACT

Aims: It has been shown that microtitan may possibly affect the nervous system. In the present study, we examined the effect of microtitan on spontaneous activity during the sleeping period and on autonomic nervous activity in mice.
Main methods: Institute of Cancer Research (ICR) mice were divided into placebo and microtitan groups that were housed in chambers with rubber sheets impregnated with microtitan or placebo sheets. In both groups, spontaneous active movement, metabolic parameters, and heart rate variability (HRV) were measured.
Key findings: Spontaneous activity during the light period was decreased for mice housed with microtitan sheets compared with placebo sheets. The urinary noradrenalin level was also reduced by microtitan. Heart rate variability was assessed by using a telemetry system and autonomic nervous activity was estimated. Power spectral analysis of R–R interval data revealed that the high frequency band, which shows parasympathetic activity, was significantly increased by microtitan, while the low frequency to high frequency power spectral ratio was decreased in the mice housed with microtitan sheets compared to the mice housed with placebo sheets.
Significance: Microtitan promoted rest during the sleeping period by regulating autonomic nervous activity, which indicates that microtitan has a relaxant effect.

Introduction

Titanium is a metal that is one of the transition elements and it is utilized as a component of various materials, including metal alloys and paints. In the medical field, it is used for the fabrication of implantable devices because it is highly biocompatible. In addition, there are some beneficial effects of titanium, including anti-inflammatory and anti-oxidant activity, as well as promotion of nerve cell growth (Carballo-Vila et al. 2008; Overgaard et al. 1998; Sahlin et al. 2006; Suzuki et al. 2003). Further, it has been suggested that some processed titanium can affect the nervous system in mammals, as compound action potential and signal transduction speed are reduced in sciatic nerve (Cehreli et al. 2003; Onur et al. 2004). Recently, a previous study (Korte 2008) demonstrated that microtitan (titanium particles that are only microns in diameter) treatment reduced the resting membrane potential and the action potential firing rate of pyramidal neurons in mouse hippocampal slices even if there is space between the metal and the neurons. Such effects of microtitan on pyramidal neurons could relieve a sensation of pain. These observations led us to a hypothesis that the processed titanium may suppress neuronal excitability and may also regulate the membrane potential of other neurons such as those in autonomic nerves.

The autonomic nervous system is one of the major factors controlling the cardiovascular system (Julius and Johnson 1985; Kawashima 2005; Schultz et al. 2007), the functions of internal organs (Kiba 2002; Tougas 1999), and immunity (Bellinger et al. 2008; Nance and Sanders 2007). Thus, sustained disturbance of the balance between sympathetic and parasympathetic activity leads to various disorders. The autonomic nervous system is also closely associated with emotions, excitement, and stress, and its activity reflects the mental state. Mental stress leads to the elevation of sympathetic nerve activity (SNA) and causes excitation (Ziegler et al. 1993). If microtitan could suppress SNA, it might regulate abnormal responses such as cardiovascular abnormalities caused by psychological stress. That is, if microtitan induces the predominance of parasympathetic activity (PSNA) over sympathetic activity, it may exert a relaxant effect.

To investigate this hypothesis, we studied the effect of microtitan on the autonomic nervous system and the emotional state in mice.

* Corresponding author. Laboratory of Health Science, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Shimogamo Hangi-cho 1-5, Sakyo-ku, Kyoto 606-8522, Japan. Tel.: +81 75 703 5417; fax: +81 75 703 5417.
E-mail address: waoi@koto.kpu-m.ac.jp (W. Aoi).

0024-3205/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2009.07.007

When their housing is changed, mice become excited and move actively around their cages, and their sleeping time is markedly decreased. If housing mice in a cage containing microtitan could decrease sympathetic activity, it could reduce their excitation and contribute to prolongation of the sleeping time. Therefore, we examined the effect of titanium in a murine psychological stress model.

Materials and methods

Preparation of the microtitan rubber sheet

Microtitan was produced by breaking titanium to a mean diameter of 26.7 μm (Phiten Co., Ltd. Kyoto, Japan). A microtitan rubber sheet was prepared that contained microtitan at a concentration of 0.94% w/w and a rubber sheet free of microtitan was also prepared as a placebo. The sheets were attached to the walls of the cages both inside and outside.

Animals and experimental design

The present study complied with the principles and guidelines of the Japanese Council on Animal Care and it was also approved by the Committee for Animal Research of Kyoto Prefectural University of Medicine (permission No. M19-42). Institute of Cancer Research (ICR) mice (10 weeks old) were obtained from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan) and were acclimatized for 6 weeks in an air-conditioned (22 ± 2 °C) room with a 12-h light/dark cycle (lights on from 07:30 to 19:30 h). We used male mice only because spontaneous activity is affected by the menstrual cycle in females. The mice were divided into two groups, which were a control group and a microtitan group. In both groups, spontaneous active movement, metabolic parameters, and heart rate variability (HRV) were measured. Mice in the microtitan group were measured in cages with rubber sheets containing microtitan, while mice in the control group were measured in cages with the placebo rubber sheets. In addition, to examine the effect of microtitan, mice were exposed to a microtitan sheet wrapped in aluminum. At 07:30, mice were transferred into the cages individually, and were allowed to move about freely and had access to normal chow and drinking water. All measurements were started from 19:30.

Determination of spontaneous activity and metabolic rate

The spontaneous activity of each mouse was examined as described previously (Shibakura et al. 2006) with multiple lenses (Kyohritsu Electronic Industry Co.,Ltd., Osaka, Japan) that detect the infrared radiation emitted by animals for 12 h from 19:30. Active movement was assessed as a single count when the animal moved from one region of the measurement area, which was optically divided by the multiple lenses, to a neighboring region. The mice were sedentary and not subjected to any exercise before measurements were made. Total counts were calculated by summing all counts over 12 h period.

At the same time, indirect calorimetry analysis was performed for assessment of resting metabolic activity. Details of the indirect calorimetry measurement for mice have been described previously (Aoi et al. 2008). Briefly, both oxygen consumption and carbon dioxide production were measured every 3 min for 12 h with a metabolic analyzer for small animals (MK-5000R, Muromachi Kikai, Tokyo, Japan). Then the respiratory exchange ratio (RER) was calculated from the level of oxygen consumption (VO_2) and carbon dioxide production (VCO_2).

HRV analysis

To evaluate autonomic activity, we performed power spectral analysis of heart rate variability (Lanfranchi and Somers 2002; Stein and Kleiger 1999). Electrocardiograms were obtained by using a telemetry system for mice (Daiya Medical Co., Ltd., Tokyo Japan). A small transmitter was implanted subcutaneously in the back of each mouse under anesthesia, and electrodes were tunnelled through under to the upper chest and abdomen at 5 days before recording. The heart rate (HR) was recorded from the ECG in mice that are awake from 19:30 to 20:30. ECG data were analyzed using A/D conversion, and the power spectrum of the R–R interval was calculated by the maximum entropy method with MemCalc software (Suwa Trust Co. Ltd., Sapporo, Japan). The frequency bands adopted for analysis in mice were a low frequency band (LF) of 0–0.75 Hz and a high frequency band (HF) of 0.75–2.4 Hz.

Urinary noradrenalin level

Mice were housed in metabolic cages with the rubber sheets (microtitan or placebo) and urine was collected for 24 h. Then the urinary content of noradrenalin was measured.

Statistics

All data are reported as the mean ± SE. Differences between groups were evaluated by one-way ANOVA or Student's *t*-test. If ANOVA indicated a significance difference, Fisher's PLSD test was used to determine the significance of differences between mean values. In all analyses, $p<0.05$ was considered to indicate statistical significance.

Results

Spontaneous activity and indirect calorimetry

Spontaneous activity was measured and indirect calorimetry analysis was performed for 12 h during the light period. Spontaneous activity over 12 h was significantly decreased in the microtitan group compared with the control group ($p=0.031$) (Fig. 1). However, the decrease was not found when the titan sheet was wrapped in aluminum. The mean oxygen consumption did not differ between the two groups and RER was also not altered by microtitan (Table 1).

HRV analysis

HRV analysis, which was measured from heart beats R–R intervals, was performed during initial 1 h after movement to avoid the effect of

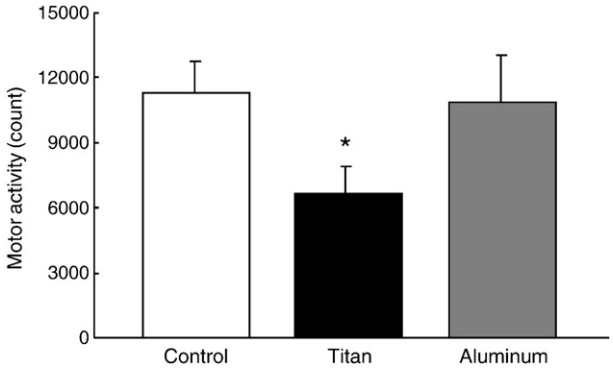

Fig. 1. Spontaneously active movement was assessed in light period. Motor count was measured with multiple lenses that detect the infrared radiation emitted by animals. Integrated value for 12 h was calculated for each mouse. Values are expressed as the mean ± SE for 8 mice. *Statistically significant differences from the control at the level of $p<0.05$.

Table 1
Respiratory metabolic performance.

	Control	Titan
Oxygen consumption (ml/kg BW/min)	36.4 ± 0.8	36.4 ± 0.6
Respiratory exchange ratio	0.88 ± 0.04	0.91 ± 0.03

Indirect calorimetry was examined in lighting period. Respiratory exchange ratio was measured by oxygen consumption and carbon dioxide exhaustion. Mean value for 12 h was calculated for each mouse. Values are expressed as the mean ± SE for 8 mice.

sleep on autonomic activity. The mean hourly values HR, LF, HF, and ratio of power (LF/HF) were calculated for each mouse after implantation of the transmitter and electrodes. HR was lower in the microtitan group more than placebo group (Table 2). Although LF power was not changed by microtitan, HF power was significantly increased in the mice exposed to microtitan ($p = 0.001$) (Table 2). In mice housed with the microtitan sheets, the LF/HF ratio was also decreased compared with that in mice housed with the placebo sheets ($p = 0.017$) (Table 2).

Urinary noradrenalin

To evaluate SNA, the noradrenalin level was measured in urine collected for 24 h. Urinary noradrenalin was markedly lower in the microtitan group compared with the placebo group ($p = 0.035$) (Fig. 2). However, the decrease was not found when the titan sheet was wrapped in aluminum.

Discussion

We examined the relaxant effect of microtitan in a mouse model of psychological stress. Mice sleep during the daylight hours, so spontaneous activity is always decreased in this period. Therefore, the further reduction of spontaneous movement noted in the microtitan group during the present study should be due to prolongation of the sleeping time in the sleeping period and might indicate a relaxant effect of microtitan. We also evaluated the effect of microtitan on autonomic activity by power spectral analysis of the HRV, which has long been used as a noninvasive marker of autonomic activity (Ohnuki et al. 2001; Stein and Kleiger 1999). In the present study, 0–0.75 Hz was defined as the LF power spectrum and 0.75–2.4 Hz as the HF power spectrum according to previous studies of small animals (Marusato et al. 1998; Waki et al. 2003). Autonomic activity has been evaluated from R–R variability at these frequencies under various stressful conditions, such as tilt, mental stress, and physical activity (Lanfranchi and Somers 2002; Terkelsen et al. 2005). The LF/HF ratio, which reflects SNA, was reduced in mice housed with microtitan sheets compared to its level in mice housed with placebo sheets, along with elevation of HF power that reflects PSNA. In addition, the urinary noradrenalin level was lower in the microtitan group than the control group. Noradrenalin is mainly secreted by sympathetic nerves, so a decrease of urinary noradrenalin supported

Table 2
Heart rate variability.

	Control	Titan
Heart rate (beats/min)	652 ± 7	634 ± 6
LF (ms^2)	1.74 ± 0.14	1.65 ± 0.15
HF (ms^2)	2.83 ± 0.16	3.77 ± 0.18*
LF/HF	0.60 ± 0.05	0.45 ± 0.03*

Autonomic nerve activity was determined by power spectral analysis. Heart rate was recorded from ECG obtained by telemetry system from 19:30 to 20:30 in mice that are awake. The frequency bands were adapted for analysis in mice: a low frequency band (LF) of 0–0.75 Hz and a high frequency band (HF) of 0.75–2.4 Hz. Mean value for 1 h was calculated for each mouse. Values are expressed as the mean ± SE for 7–8 mice. *Statistically significant differences from the control at the level of $p < 0.05$.

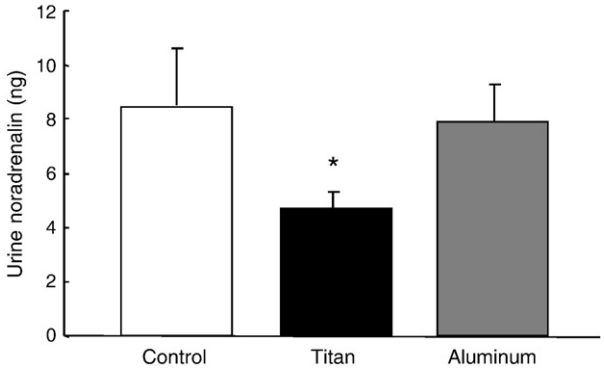

Fig. 2. Noradrenalin content in urine collected for 24 h was measured. Values are expressed as the mean ± SE for 8 mice. *Statistically significant differences from the control at the level of $p < 0.05$.

the suppression of sympathetic activity by microtitan. These observations suggest that microtitan could suppress the sympathetic activation generated in mice by a change of environment, thus promoting relaxation and prolongation of the sleeping time.

The mechanism of action of microtitan remains unclear, but an influence on the neuronal depolarization threshold may be one of the main factors. Korte (2008) reported that tape containing titanium could influence synaptic plasticity and that it significantly decreased the firing rate of single pyramidal neurons in comparison with placebo tape. Thus, the regulation of membrane potential by microtitan may be also caused in SNA, as was found for pyramidal neurons in the previous study. On the other hand, the HF power (indicating parasympathetic activity) was higher in mice housed with microtitan than in mice housed with placebo. The sympathetic and parasympathetic systems can directly interact with each other (Benthem et al. 2001; Berthoud and Neuhuber 2000; Smith et al. 2002; Vanhoutte and Levy 1980), so the apparent increase of PSNA may have been partly caused by inhibition of SNA. Although further studies will be needed to determine the mechanism by which microtitan influences the autonomic nervous system in detail, it is considered a possibility that modification effect on membrane potential of sympathetic nerve would alter the autonomic balance and promote the predominance of PSNA over SNA in a state of emotional stress.

We found that the decrease of SNA and noradrenalin did not occur when mice were exposed to a microtitan sheet wrapped in aluminum. A previous study (Korte 2008) also showed that an effect of tape containing microtitan on pyramidal neurons was blocked by placing a lead plate of 0.5 cm in diameter between the neurons and the titanium source. Thus, the influence of microtitan on neurons seems to be mediated via some factor that can cross an open space, but does not penetrate metal, such as an electromagnetic wave, or that directly affects body through the skin in contact with the microtitan sheet. In that study, titanium exerted the effect even when the microtitan tape was not in contact with neurons, indicating that microtitan can affect neurons even if there is space between the metal and its target. In the present study, we only examined a 0.94% concentration of microtitan in the rubber sheet, which is the highest concentration that could be added to the sheet without disturbing its properties. It should be also considered studying the dose-response effect of microtitan in further study because the effect of microtitan on neurons may depend on its concentration as shown in the previous study (Korte 2008).

In conclusion, microtitan decreased spontaneous activity during the daylight period in mice exposed to mental stress. SNA was also decreased in the mice exposed microtitan according to power spectral analysis of HRV, along with a reduction of the urinary noradrenalin level. These observations suggest that microtitan promoted a longer sleeping period by regulating autonomic nervous activity and indicate that microtitan has a relaxant effect.

References

Aoi W, Naito Y, Takanami Y, Ishii T, Kawai Y, Akagiri S, Kato Y, Osawa T, Yoshikawa T. Astaxanthin improves muscle lipid metabolism in exercise via inhibitory effect of oxidative CPT I modification. Biochemical and Biophysical Research Communications 366 (4), 892–897, 2008.

Bellinger DL, Millar BA, Perez S, Carter J, Wood C, ThyagaRajan S, Molinaro C, Lubahn C, Lorton D. Sympathetic modulation of immunity: relevance to disease. Cellular Immunology 252 (1–2), 27–56, 2008.

Benthem L, Mundinger TO, Taborsky Jr GJ. Parasympathetic inhibition of sympathetic neural activity to the pancreas. American Journal of Physiology Endocrinology and Metabolism 280 (2), E378–E381, 2001.

Berthoud HR, Neuhuber WL. Functional and chemical anatomy of the afferent vagal system. Autonomic Neuroscience 85 (1–3), 1–17, 2000.

Cehreli MC, Onur MA, Sahin S. Effects of hydroxyapatite-coated and commercially pure titanium oral implant surfaces on compound nerve action potentials. Clinical Oral Implants Research 14 (3), 269–272, 2003.

Carballo-Vila M, Moreno-Burriel B, Chinarro E, Jurado JR, Casañ-Pastor N, Collazos-Castro JE. Titanium oxide as substrate for neural cell growth. Journal of Biomedical Materials Research Part A, 2008.

Julius S, Johnson EH. Stress, autonomic hyperactivity and essential hypertension: an enigma. Journal of Hypertension 3 (4), S11–S17, 1985.

Kawashima T. The autonomic nervous system of the human heart with special reference to its origin, course, and peripheral distribution. Anatomy and Embryology 209 (6), 425–438, 2005.

Kiba T. The role of the autonomic nervous system in liver regeneration and apoptosis—recent developments. Digestion 66 (2), 79–88, 2002.

Korte M. Influence of Aquatitan Tape on nerve cells of the central nervous system. Journal of Clinical Biochemistry and Nutrition 43, 1–4, 2008.

Lanfranchi PA, Somers VK. Arterial baroreflex function and cardiovascular variability: interactions and implications. American Journal of Physiology Regulatory, Integrative and Comparative Physiology 283 (4), R815–R826, 2002.

Marusato Y, Hirakawa H, Harada Y, Nakamura T, Hayashida Y. Effects of systemic hypoxia on R–R interval and blood pressure variabilities in conscious rats. American Journal of Physiology (3), H797–H804, 1998.

Nance DM, Sanders VM. Autonomic innervation and regulation of the immune system (1987–2007). Brain, Behavior, and Immunity 21 (6), 736–745, 2007.

Ohnuki K, Moritani T, Ishihara K, Fushiki T. Capsaicin increases modulation of sympathetic nerve activity in rats: measurement using power spectral analysis of heart rate fluctuations. Bioscience, Biotechnology, and Biochemistry 65 (3), 638–643, 2001.

Onur MA, Taş Z, Gürpinar A, Sahin S, Cehreli MC. Morphologic, functional and behavioral effects of titanium dioxide exposure on nerves. Clinical Oral Implants Research 15 (5), 513–519, 2004.

Overgaard L, Danielsen N, Bjursten LM. Anti-inflammatory properties of titanium in the joint environment. An experimental study in rats. The Journal of Bone and Joint Surgery British 80 (5), 888–893, 1998.

Sahlin H, Contreras R, Gaskill DF, Bjursten LM, Frangos JA. Anti-inflammatory properties of micropatterned titanium coatings. Journal of Biomedical Materials Research Part A 77 (1), 43–49, 2006.

Schultz HD, Li YL, Ding Y. Arterial chemoreceptors and sympathetic nerve activity: implications for hypertension and heart failure. Hypertension 50 (1), 6–13, 2007.

Shibakura T, Iwai Y, Mizunoya W, Matsumura S, Nishizawa Y, Inoue K, Fushiki T. The physiological and behavioral effects of subchronic intracisternal administration of TGFb in rats: comparison with the effects of CRF. Biomedical Research 27 (6), 297–305, 2006.

Smith PG, Warn JD, Steinle JJ, Krizsan-Agbas D, Hasan W. Modulation of parasympathetic neuron phenotype and function by sympathetic innervations. Autonomic Neuroscience 96 (1), 33–42, 2002.

Stein PK, Kleiger RE. Insights from the study of heart rate variability. Annual Review of Medicine 50, 249–261, 1999.

Suzuki R, Muyco J, McKittrick J, Frangos JA. Reactive oxygen species inhibited by titanium oxide coatings. Journal of Biomedical Materials Research Part A 66 (2), 396–402, 2003.

Terkelsen AJ, Mølgaard H, Hansen J, Andersen OK, Jensen TS. Acute pain increases heart rate: differential mechanisms during rest and mental stress. Autonomic Neuroscience 121 (1–2), 101–109, 2005.

Tougas G. The autonomic nervous system in functional bowel disorders. Canadian Journal of Gastroenterology 13, 15A–17A, 1999.

Vanhoutte PM, Levy MN. Prejunctional cholinergic modulation of adrenergic neurotransmission in the cardiovascular system. American Journal of Physiology Heart and Circulatory Physiology 238 (3), H275–H281, 1980.

Waki H, Kasparov S, Wong LF, Murphy D, Shimizu T, Paton J. Chronic inhibition of endothelial nitric oxide synthase activity in nucleus tractus solitarii enhances baroreceptor reflex in conscious rats. Journal of Physiology 546 (1), 233–242, 2003.

Ziegler MG, Ruiz-Ramon P, Shapiro MH. Abnormal stress responses in patients with diseases affecting the sympathetic nervous system. Psychosomatic Medicine 55 (4), 339–346, 1993.