Japanese Journal of Veterinary Research 64(4): 265–271, 2016

JJVR

NOTE

Does the routine handling affect the phenotype of disease model mice?

Maiko Ono[1,#], Hayato Sasaki[2,4], Kenichi Nagasaki[5], Daisuke Torigoe[2,6], Osamu Ichii[3], Nobuya Sasaki[2,4] and Takashi Agui[2,*]

[1] Animal facility, [2] Laboratory of Laboratory Animal Science and Medicine, Department of Disease Control, and [3] Laboratory of Anatomy, Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818, Japan

[4] Laboratory of Laboratory Animal Science and Medicine, Faculty of Veterinary Medicine, Kitasato University, Towada 034–8628, Japan

[5] Section of Biological Safety Research, Chitose Laboratory, Japan Food Research Laboratories, Chitose 006–0052, Japan

[6] Division of Microbiology and Genetics, Institute of Resource Development and Analysis, Kumamoto University, Kumamoto 860–0811, Japan

Received for publication, May 31, 2016; accepted, August 24, 2016

Abstract

The three different mouse handling methods, picking up by tails, tunnels, and open hands were performed using the ICGN glomerulonephritis mouse and the severity of symptoms was evaluated. The handling groups exhibited a tendency of more severe symptoms than the non-handling control group. Female mice handled by their tails showed significantly more severe symptoms than the control group. In addition, we subjected the normal laboratory mice, C57BL/6 and BALB/c mice to tail and tunnel handling to assess the stress conditions. The plasma corticosterone level in the tail-handled mice was higher than that in control mice. These results indicate that handling causes stress and may affect the phenotype of disease model mice.

Key Words: handling, mouse, stress

Routine handling for laboratory animals is a conventional procedure in animal facilities and is performed when changing cages, measuring body weight, and restraining animals for experimental procedures. The principal handling method for mice is picking up by the tail. However, in the

*Corresponding author: Takashi Agui, Laboratory of Laboratory Animal Science and Medicine, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818, Japan
Phone: +81–11–706–5106. E-mail: agui@vetmed.hokudai.ac.jp

[#] Present address: Institute for Animal Experimentation, Graduate School of Medicine, Hokkaido University, Sapporo 060–0818, Japan

doi: 10.14943/jjvr.64.4.265

recent study, it was demonstrated that this manipulation induced aversion and anxiety in the mouse, and affected the phenotype in the ethology[10].

Laboratory animals play an important role in the biomedical research to develop prevention and treatments of human diseases as disease models. However, little is known about the effects of ordinary daily handling on the phenotype of the disease model, and there may be errors in the phenotype due to different handling methods by experimenters and technical staff.

The objective of this study was to identify influences of long-term handling on the phenotype of the disease model mouse, in particular, chronic disease models. We also aimed to improve the well-being of laboratory mice and reproducibility of experimental results.

Chronic kidney disease (CKD) is an independent risk factor for cardiovascular disease events[14] and all cases of deaths[13] in the general Japanese population, suggesting that CKD represents a major public health problem. The ICGN mouse is an ICR-derived glomerulonephritis model mouse for the human idiopathic nephrotic syndrome. The ICGN mouse is a superior model showing most of the characteristics of human CKD such as glomerulosclerosis, renal tubule stroma fibrosis, renal anemia, albuminuria, and edema[12,15].

We investigated the influence of handling on the phenotype of the disease model using the ICGN mouse as a model of chronic disease by evaluating the severity of the disease symptoms based on the different handling methods (Experiment 1). Furthermore, we assessed whether handling causes any stress for animals using normal laboratory mice, C57BL/6 (B6) and BALB/c (BALB) mice (Experiment 2).

Male and female ICGN mice were bred at our animal facility. Twenty female and male mice were divided into 4 groups (n = 5 each), three different handling groups and control. Three-week-old male B6 (n = 5) and BALB (n = 5) mice were obtained from CLEA Japan Inc. (Tokyo, Japan) to assess the stress level by two different handling methods. Two to three mice were housed in an individually ventilated cage (W 196 mm × D 306 mm × H 166 mm, Allentown Inc., Allentown, NJ, U.S.A) with bedding (TEK-FRESH, Harlan, Indianapolis, IN, U.S.A). Environmental enrichment was provided in the form of transparent polycarbonate tunnels with red color, 10 cm in length and 5 cm inside diameter (Bio-Serv, Flemington, NJ, U.S.A). All experimental procedures were approved by the President of Hokkaido University after review by the Institutional Animal Care and Use Committee of Hokkaido University. Animals were maintained in specific pathogen-free conditions with standard pellet feed (Lab MR standard, Nosan, Yokohama, Japan) and water *ad libitum*. The housing room was maintained at 22 ± 4°C on a 12 h light-dark cycle, but humidity was not controlled. In the laboratory animal care and handling, the investigators adhered to the Regulations for the Care and Use of Laboratory Animals, Hokkaido University.

Experiment 1 (Exp. 1)

Four-week-old ICGN mice were handled for 5 days a week by three different handling methods. Mice were picked up by their tails and lifted up (tail method), lifted up after voluntarily entering into the plastic tunnel (tunnel method), or hand-scooped and lifted up by both hands moving freely over the palm (hand method). Control mice were undisturbed (control). In each handling, mice were lifted up for 20 sec. All cages were changed once a week. During cage-changing, each group of mice was handled in the same way. The control mice were moved by holding their tails for a short period during cage-changing.

After handling for 4 weeks, blood was collected from 8-week-old mice under isoflurane anesthesia and mice were euthanized. Measurements of hematocrit value (HCT), concentration of plasma creatinine (Cre), and blood urea nitrogen (BUN)

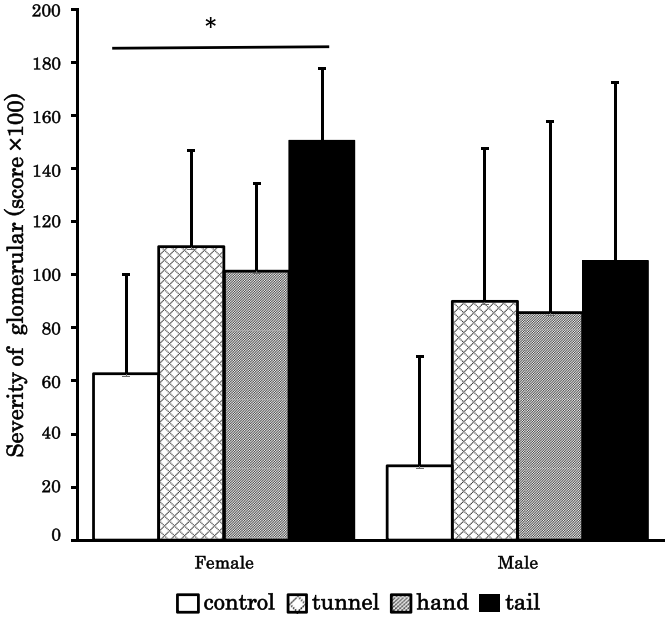

Fig. 1. Effects of handling, control, tail-, hand-, and tunnel-handling, on glomerular damage score in ICGN mice (Exp. 1). Each value represents the mean ± S.D. *, Significant difference in multiple comparisons following the Kruskal-Wallis test (Steel-Dwass method, $P < 0.05$)

were taken. Kidneys were fixed with 10% formalin and subjected to the histopathological examination. Paraffin-embedded sections of 4 μm thickness were stained with Periodic acid-Schiff (PAS). To assess the severity of glomerular damage, 30 glomeruli per kidney were examined and scored according to the following criteria: grade 0, no abnormality or nearly normal; grade 1, a little expansion of the mesangial matrix, mild cell proliferation, and/or mild membranous hypertrophy; grade 2, segmental expansion of the mesangial matrix, cell proliferation, membranous hypertrophy, and/or glomerular hypertrophy; grade 3, global expansion of the mesangial matrix, cell proliferation, membranous hypertrophy, and/or glomerular hypertrophy and hyalinosis; grade 4, disappearance of capillaries and capsular lumina, and/or periglomerular infiltration of inflammatory cells and fibrosis[11,18].

Experiment 2 (Exp. 2)

We also performed tail and tunnel handling on normal laboratory mice, B6 and BALB mice. After acclimatization for 1 week, we started handling when the mice were 4 weeks old. Mice were handled for 4 weeks, and at 8 weeks old, blood was collected by tail vain puncture and plasma corticosterone (CORT) level was examined using an Assay Max CORT ELISA Kit (Assaypro, St. Charles, MO, U.S.A.). Blood was collected at 20 min after handling between 11 : 00 and 12 : 00. Blood sampling took within 2 min.

In the histopathological examination in Exp. 1, the scores of the glomerulus lesions of tail-handled mice were significantly higher than that of female control mice (Fig. 1. $P < 0.05$). The scores of female tunnel and hand groups were approximately 1.5-fold greater than those of the control group. The scores of the male tail, tunnel, and hand groups were also approximately 3-fold greater than those of the control group, although the difference was not statistically significant. In both male and female mice, handled mice appeared to show a more severe lesion grade level than the control mice regardless of the handling method. In the blood examination (Fig. 2), there were no significant differences between the control and the handling groups. In females, each

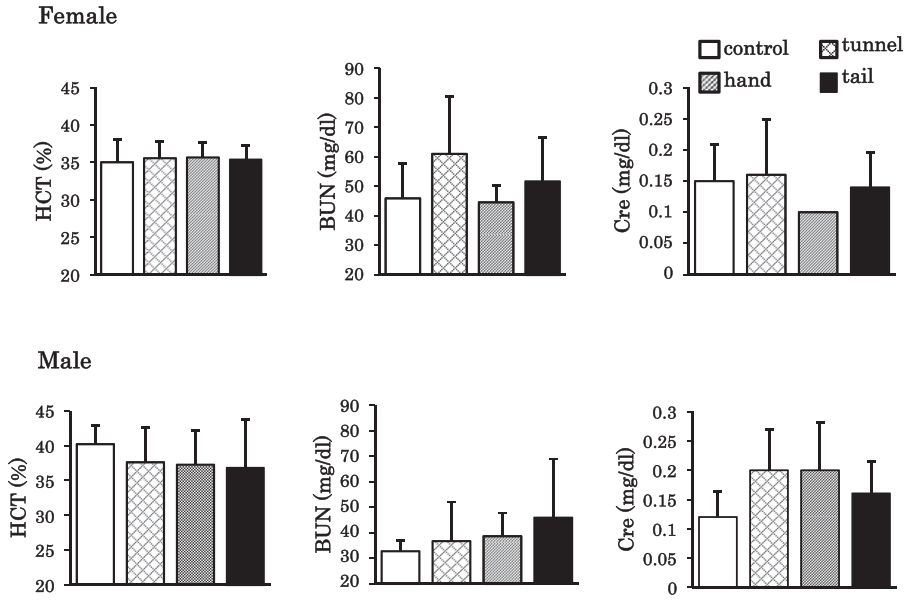

Fig. 2. The results of the blood examination in ICGN mice of each handling group.

group showed low HCT values compared with those of males. The BUN concentration was increased in both sexes of each handling group, which is considered as a result of renal failure. No significant relationship was seen between the blood parameters and the severity of the glomerular legions. This is consistent with the previous reports that glomerulosclerotic lesions in renal histopathology were observed earlier than the exhibition of blood parameter changes[12,15].

In Exp. 2, mice handled by their tails showed a significantly higher plasma corticosterone level in comparison with that of control in B6 mice. In BALB mice, the plasma corticosterone level of mice handled by tunnels was higher than that of mice handled by the tail method and the control (Fig. 3. $P < 0.05$).

Data were expressed as the mean $\pm$ standard deviation and statistical analysis was performed using the nonparametric Kruskal-Wallis test ($P < 0.05$). The correlation between two parameters was analyzed using the Steel-Dwass test ($P < 0.05$).

The results suggest that all handling methods have an influence on the phenotype of the chronic renal disease model mouse (Exp. 1). Both tail and tunnel handling methods induced stress responses in the two inbred mouse strains (Exp. 2). In addition, tail-handling caused higher stress than tunnel-handling in B6 mice, whereas tunnel-handling caused higher stress than tail-handling in BALB mice (Exp. 2). Considering the results of Exp. 2, either tail-handled or tunnel-handled ICGN mice may get stressed. The glomerulus lesions of the ICGN mouse may worsen due to stress caused by handling. Stress is a condition whereby environmental burdens exceed the adaptive capacity of individuals to a point where psychological and physiological responses may place them at risk for disease. Long-term exposure to distressing environmental conditions may be associated with tissue damage and disease. A previous study has presented evidence suggesting that stress such as hypertension is directly associated with CKD risk factors[5].

As for the remarkable differences seen in females, females are generally considered to be emotionally high-responsive under stressful conditions and have higher corticosterone levels and activity than males[1,9].

It was demonstrated that picking up mice by the tail induced aversion and high anxiety in a test using an elevated plus maze[10]. A significantly

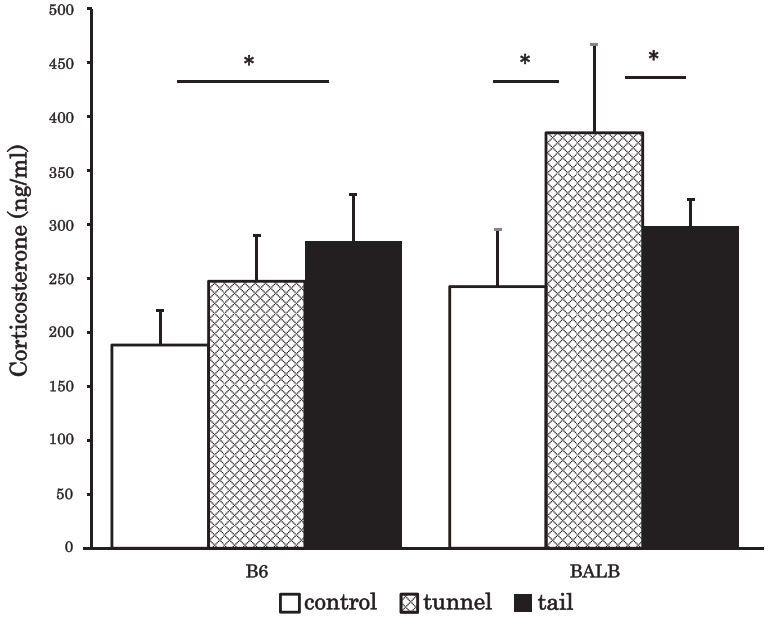

Fig. 3. Effects of three different handling methods on corticosterone plasma levels in B6 and BALB mice (Exp. 2). Each value represents the mean ± S.D. ＊, Significant differences in multiple comparisons following the Kruskal-Wallis test (Steel-Dwass method, $P < 0.05$)

higher corticosterone level seen in the tail group in B6 mice in Exp. 2 was consistent with the previous report. On the other hand, the corticosterone level in the tunnel group was higher than that in the tail group in BALB mice. This result was contrary to our expectation. We predicted that transferring mice by tunnel would result in lower stress, as previous studies indicated[8]. The period of human contact correlates with stress in BALB mice. The BALB mice needed much more time than B6 mice to enter the plastic tunnel voluntarily. BALB mice needed more than 5 min to enter the tunnel voluntarily, whereas B6 mice needed only 10 to 15 sec to enter the tunnel voluntarily. Handling duration may be an important factor in inducing stress. BALB mice showed a higher corticosterone level than did B6 in control and both tail and tunnel groups (Fig. 3). This result is consistent with other reports indicating that BALB mice display more pronounced circulating corticosterone elevations than the B6 strain in stress responses[3,9].

In the tunnel handling of ICGN mice in Exp. 1, ICGN mice did not show any differences in the time to enter the tunnel compared with B6 mice. Therefore, the time required for each handling was constant in Exp. 1.

It was recommended that mice should be handled with a tunnel located in their home cage as a simple practical method to minimize handling stress. Mice handled by a home cage or external tunnel showed less anxiety in an elevated plus maze than those picked up by the tail[8]. However, previous and our current studies have shown that animal handling *per se* induces an increase in circulating corticosterone levels[6,16]. This suggests that the habituation for 4 weeks is not sufficient to reduce animals' corticosterone activation. Moreover, it is suggested that handling may have an influence on the phenotype of disease model mice. Recently, Ghosal *et al.* have reported that handling mice with a cup and massage produces good results with respect to the behavioral and metabolic parameters compared with tail-handling, such as reducing anxiety behavior, elevating glucose tolerance in mice fed a high calorie diet, reducing blood glucose in the fasting conditions, and reducing blood corticosterone level[7].

Previous and present findings indicate that

many parameters, such as handling method, period of handling, genetic, and sex differences may participate in the emotional stress reactivity of mice[2,4,17]. We therefore suggest more careful consideration of the different manipulations of laboratory mice. Proper handling must conduce to the welfare of laboratory animals as well as reliable experimental results.

We thank Dr. Yuko Okamatsu, Associate Professor, Laboratory of Biochemistry, Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, for giving generous support on CORT measurement and Ms. Marui for her help collecting blood samples. This work was supported by JSPS KAKENHI (Grants-in-Aid for Scientific Research No. 25925011), the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1) Aoki, M., Shimozuru, M., Kikusui, T., Takeuchi, Y. and Mori, Y. 2010. Sex differences in behavioral and corticosterone responses to mild stressors in ICR mice are altered by ovariectomy in peripubertal period. *Zoolog. Sci.*, **27**: 783–789.
2) Balcombe, J. P., Barnard, N. D. and Sandusky, C. 2004. Laboratory routines cause animal stress. *Contemp. Top. Lab. Anim. Sci.*, **43**: 42–51.
3) Benedetti, M., Merino, R., Kusuda, R., Ravanelli, M. I., Cadetti, F., dos Santos, P., Zanon, S. and Lucas, G. 2012. Plasma corticosterone levels in mouse models of pain. *Eur. J. Pain*, **16**: 803–815.
4) Boleij, H., Salomons, A. R., van Sprundel, M., Arndt, S. S. and Ohl, F. 2012. Not all mice are equal: Welfare implications of behavioural habituation profiles in four 129 mouse substrains. *PLoS One*, **7**: e42544.
5) Bruce, M. A., Beech, B. M., Sims, M., Brown, T. N., Wyatt, S. B., Taylor, H. A., Williams, D. R. and Crook, E. 2009. Social environmental stressors, psychological factors, and kidney disease. *J. Investig. Med.*, **57**: 583–589.
6) Gariépy, J. L., Rodriguiz, R. M. and Jones, B. C. 2002. Handling, genetic and housing effects on the mouse stress system, dopamine function, and behavior. *Pharmacol. Biochem. Behav.*, **73**: 7–17.
7) Ghosal, S., Nunley, A., Mahbod, P., Lewis, A. G., Smith, E. P., Tong, J., D'Alessio, D. A. and Herman, J. P. 2015. Mouse handling limits the impact of stress on metabolic endpoints. *Physiol. Behav.*, **150**: 31–37.
8) Gouveia, K. and Hurst, J. L. 2013. Reducing mouse anxiety during handling: effect of experience with handling tunnels. *PLoS One*, **8**: e66401.
9) Harizi, H., Homo-Delarche, F., Amrani, A., Coulaud, J. and Mormède, P. 2007. Marked genetic differences in the regulation of blood glucose under immune and restraint stress in mice reveals a wide range of corticosensitivity. *J. Neuroimmunol.*, **189**: 59–68.
10) Hurst, J. L. and West, R. S. 2010. Taming anxiety in laboratory mice. *Nat. Methods*, **7**: 825–826.
11) Ichii, O., Konno, A., Sasaki, N., Endoh, D., Hashimoto, Y. and Kon, Y. 2008. Autoimmune glomerulonephritis induced in congenic mouse strain carrying telomeric region of chromosome 1 derived from MRL/MpJ. *Histol. Histopathol.*, **23**: 411–422.
12) Mizuno, S., Yue, B. F., Okamoto, M., Horikawa, Y. and Kurosawa, T. 1997. Diffuse glomerulosclerosis without tubular injury does not directly manifest renal dysfunction in nephrotic mice (ICGN strain). *Exp. Nephrol.*, **5**: 498–507.
13) Nakayama, M., Metoki, H., Terawaki, H., Ohkubo, T., Kikuya, M., Sato, T., Nakayama, K., Asayama, K., Inoue, R., Hashimoto, J., Totsune, K., Hoshi, H., Ito, S. and Imai, Y. 2007. Kidney dysfunction as a risk factor for first symptomatic stroke events in a general Japanese population--the Ohasama study. *Nephrol. Dial. Transplant.*, **22**: 1910–1915.
14) Ninomiya, T., Kiyohara, Y., Kubo, M., Tanizaki, Y., Doi, Y., Okubo, K., Wakugawa, Y., Hata, J., Oishi, Y., Shikata, K., Yonemoto, K., Hirakata, H. and Iida, M. 2005. Chronic kidney disease and cardiovascular disease in a general Japanese population: the Hisayama Study. *Kidney Int.*, **68**: 228–236.
15) Ogura, A., Asano, T., Matsuda, J., Takano, K., Nakagawa, M. and Fukui, M. 1989. Characteristics of mutant mice (ICGN) with spontaneous renal lesions: a new model for human nephrotic syndrome. *Lab. Anim.*, **23**: 169–174.
16) Pacák, K., Palkovits, M., Kvetnanský, R., Yadid, G., Kopin, I. J. and Goldstein, D. S.

1995. Effects of various stressors on in vivo norepinephrine release in the hypothalamic paraventricular nucleus and on the pituitary-adrenocortical axis. *Ann. N. Y. Acad. Sci.*, **771**: 115–130.
17) Salomons, A. R., Arndt, S. S. and Ohl, F. 2012. Impact of anxiety profiles on cognitive performance in BALB/c and 129P2 mice. *Cogn. Affect. Behav. Neurosci.*, **12**: 794–803.
18) Sasaki, H., Sasaki, N., Nishino, T., Nagasaki, K.-i., Kitamura, H., Torigoe, D. and Agui, T. 2014. Quantitative trait loci for resistance to the congenital nephropathy in tensin 2-deficient mice. *PLoS One*, **9**: e99602.