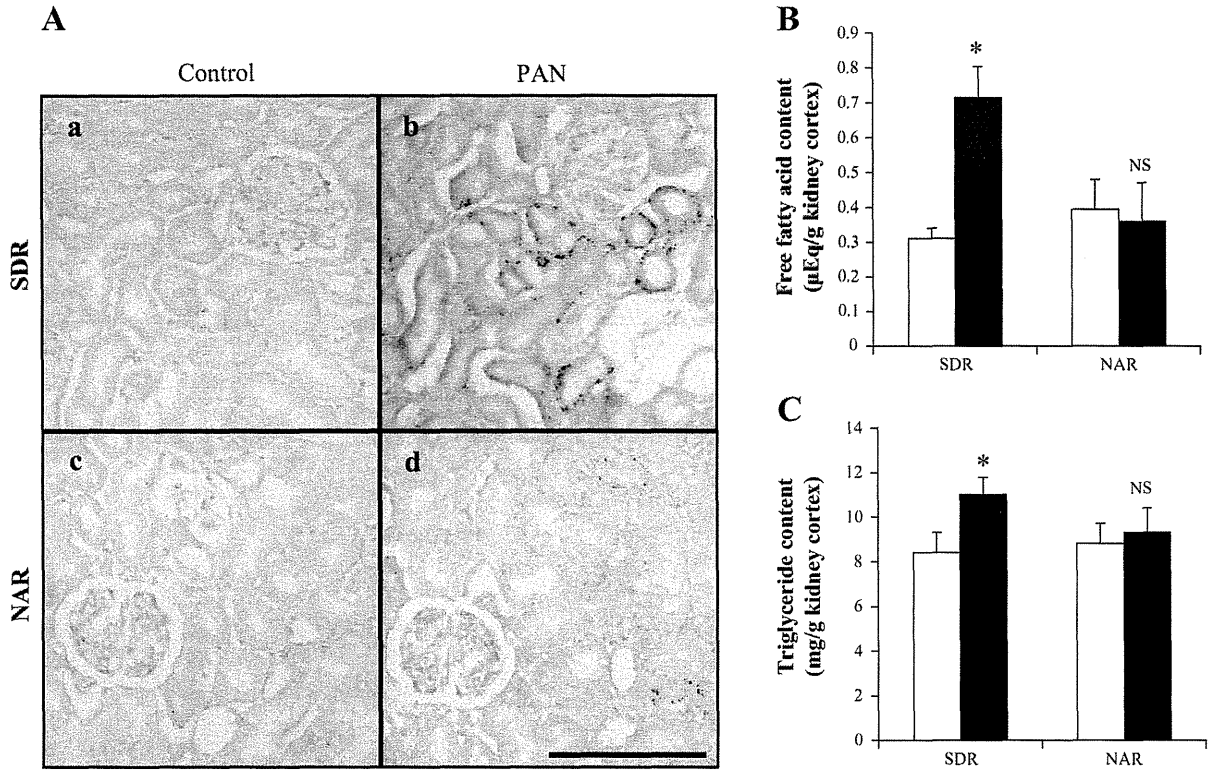

Fig. 4. Effects of PAN treatment on lipid accumulation in the kidney cortex of SDRs and NARs on the 14th day after PAN treatment. *A*: representative photomicrographs of the oil red O-stained kidney cortex in the control SDR group (*a*), PAN-SDR group (*b*), control NAR group (*c*), and PAN-NAR group (*d*) (magnification: ×200). Bar = 100 μm. Noteworthy amounts of lipoid deposits were identified in proximal tubular epithelial cells (PTECs) of the PAN-SDR group (*b*), and little lipid staining was identified in PTECs of the PAN-NAR group (*d*). *B* and *C*: free fatty acid content (*B*) and triglyceride content (*C*) in the kidney cortex were compared between the control group (open bars) and PAN-treated group (closed bars) of either SDRs or NARs. Data are presented as means ± SE; $n = 6$ in rats/group. *$P < 0.05$ compared with the corresponding control group.

DISCUSSION

The present study determined the tubular damage and disorder of FA metabolism in the nephrotic kidney. PAN treatment induced proteinuria and dyslipidemia in both SDRs and NARs through damage of glomerular podocytes, but the PTEC damage, apoptosis, and lipid accumulation concomitant with albuminuria were found only in SDRs, suggesting that albuminuria causes the disorder of FA metabolism in PTECs. To clarify mechanisms for the disorder of FA metabolism, the present study examined expressions of enzymes for FA synthesis, transport, and oxidation and disclosed a specific downregulation of MCAD and CYP4A expressions in the nephrotic kidney. Therefore, the lipid accumulation might be explained in part by the downregulation of MCAD and CYP4A.

MCAD is essential for complete FA β-oxidation, and MCAD-null mice develop severe hepatosteatosis and cardio-

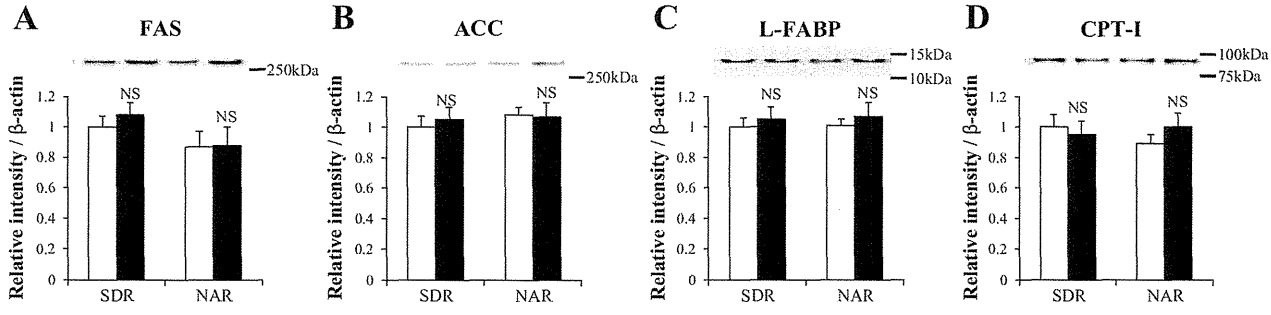

Fig. 5. Effects of PAN treatment on expressions of enzymes for fatty acid catabolism in the kidney cortex of SDRs and NARs on the 14th day after PAN treatment. Protein levels of fatty acid synthase (FAS; *A*), acetyl-CoA carboxylase (ACC; *B*), liver-type fatty acid-binding protein (L-FABP; *C*), and carnitine palmitoyltransferase (CPT)-I (*D*) in the kidney cortex were compared between the control group (open bars) and PAN-treated group (closed bars) of either SDRs or NARs. Representative Western blots are shown at the *top*, and each lane from the *left* to the *right* was loaded with a protein sample prepared from the control SDR group, PAN-SDR group, control NAR group, and PAN-NAR group. Intensities of the bands for each protein were normalized to those for β-actin (*bottom*), and the intensity of the band in the control SDR group was assigned a value of 1. Data are presented as means ± SE; $n = 6$ rats/group.

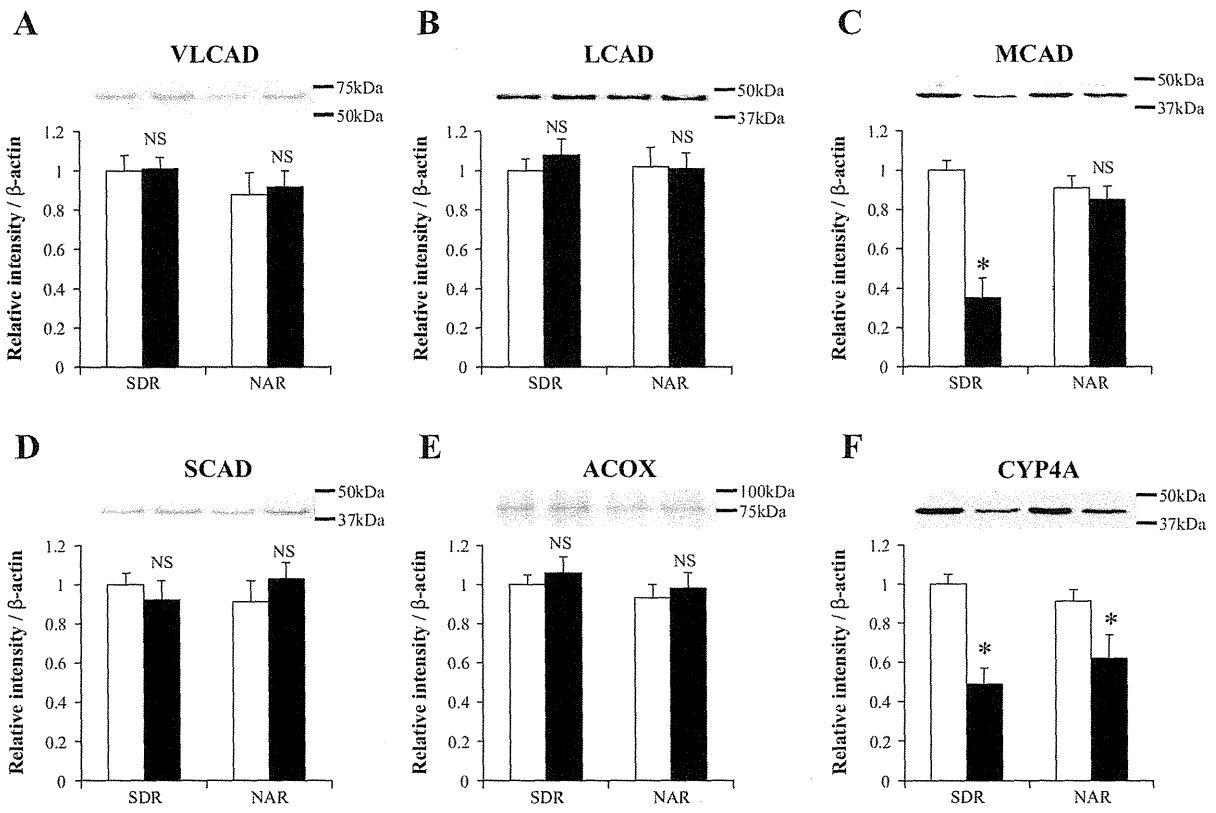

Fig. 6. Effects of PAN treatment on expressions of enzymes for fatty acid metabolism in the kidney cortex of SDRs and NARs on the 14th day after PAN treatment. Protein levels of very long-chain acyl-CoA dehydrogenase (VLCAD; *A*), long-chain acyl-CoA dehydrogenase (LCAD; *B*), medium-chain acyl-CoA dehydrogenase (MCAD; *C*), short-chain acyl-CoA dehydrogenase (SCAD; *D*), acyl-CoA oxidase (ACOX; *E*), and cytochrome *P*-450 (CYP)4A (*F*) in the kidney cortex were compared between the control group (open bars) and PAN-treated group (closed bars) of either SDRs or NARs. Representative Western blots are shown on the *top*, and each lane from the *left* to the *right* was loaded with a protein sample prepared from the control SDR group, PAN-SDR group, control NAR group, and PAN-NAR group. Intensities of the bands for each protein were normalized to those for β-actin (*bottom*), and the intensity of the band in the control SDR group was assigned a value of 1. Data are presented as means ± SE; $n = 6$ rats/group. *$P < 0.01$ compared with the corresponding control group.

myopathy (40). MCAD expression reduced in the mouse kidney with cisplatin-induced acute renal failure and ischemia-reperfusion injury (20, 21, 26). In contrast to the present study, albumin-overload proteinuria reduced VLCAD expression and LCAD activity in the kidneys of PPAR-α-null and wild-type mice (12). The constitutive expressions and activities of VLCAD and LCAD were significantly lower in the kidneys of PPAR-α-null mice (1, 12), but expression and activity of MCAD were not different between PPAR-α-null and wild-type mice (1, 21), suggesting that VLCAD and LCAD might serve as rate-limiting enzymes for FA β-oxidation in PPAR-α-null mice. The PAN-induced nephrosis caused hypoalbuminemia with increased plasma cholesterol and triglyceride, and the albumin overload caused hyperalbuminemia with increased plasma free FAs (38). The discrepancy of expressions of FA β-oxidation enzymes in the two nephrotic models can be explained by differences in dyslipidemia and plasma albumin levels.

CYP4A is expressed in PTECs, thick ascending limbs of the loop of Henle's loop (TALs), glomeruli, and renal microvessels (10) and catalyzes the ω-hydroxylation of medium-chain and long-chain FAs. CYP4A metabolizes arachidonic acid to 20-HETE (31). 20-HETE constricts renal microvessels and inhibits Na^+ reabsorption in PTECs and TALs. It regulates kidney functions and activates a number of intracellular signal transduction pathways involved in cell growth and survival (23, 31). Reduction of 20-HETE may contribute to increasing Na^+ reabsorption and O_2 consumption after tubular hypoxia and damage in the nephrotic kidney. In agreement with this speculation, a CYP4A inhibitor exacerbated ischemia-reperfusion kidney injury, and 20-HETE analogs attenuated the injury (29).

The present study further examined the expressions of transcriptional regulators for MCAD and CYP4A and disclosed that PGC-1α and ERRα but not PPAR-α expressions were downregulated in the nephrotic kidney. The PPAR-α ligand clofibrate induced MCAD and CYP4A expressions in the rat kidney (9, 25). PPAR-α expression was reduced in the remnant kidneys of 5/6 nephrectomized rats (4, 15) and in the kidneys of mice with cisplatin-induced acute renal failure (20). The PPAR-α ligand and transgene prevented acute tubular necrosis in mice with cisplatin-induced acute renal failure and ischemia-reperfusion injury (20, 21, 27). Acute FA toxicity induced by albumin-overload proteinuria was exaggerated in the kidneys of PPAR-α-null mice (12).

PGC-1α has been recently pointed as an important regulator of FA metabolism. PGC-1α upregulates FA oxidation and expressions of mitochondrial β-oxidation enzymes such as

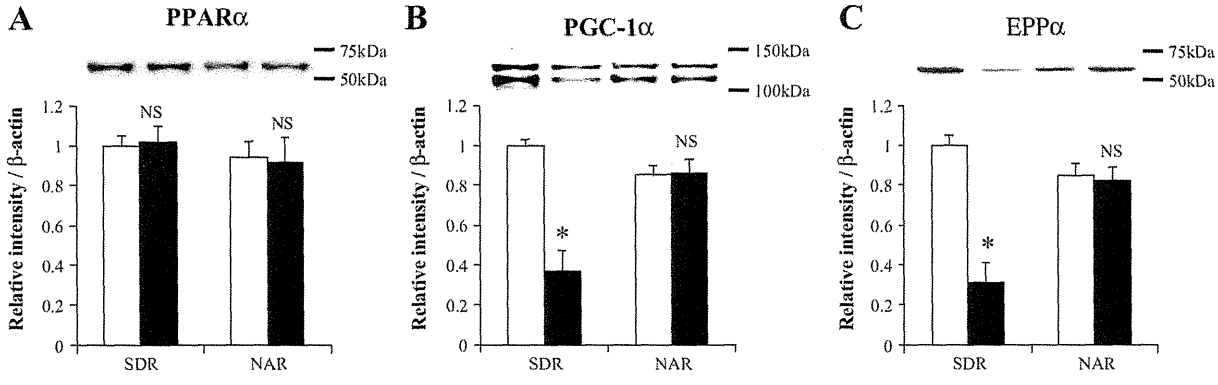

Fig. 7. Effects of PAN treatment on the expressions of peroxisome proliferator-activated receptor (PPAR)-α, PPAR-γ coactivator (PGC)-1α, and estrogen-related receptor (ERR)α in the kidney cortex of SDRs and NARs on the 14th day after PAN treatment. Protein levels of PPAR-α (*A*), the lower band of PGC-1α (*B*), and ERRα (*C*) in the kidney cortex were compared between the control group (open bars) and the PAN-treated group (closed bars) of either SDRs or NARs. Representative Western blots on the *top*, and each lane from the *left* to the *right* was loaded with a protein sample prepared from the control SDR group, PAN-SDR group, control NAR group, and PAN-NAR group. Intensities of the bands for each protein were normalized to those for β-actin (*bottom*), and the intensity of the band in the control SDR group was assigned a value of 1. Data are presented as means ± SE; $n = 6$ rats/group. *$P < 0.01$ compared with the corresponding control group.

MCAD, LCAD, and CPT-I, particularly when it coactivates PPAR-α (43). PGC-1α also activates mitochondrial biogenesis and energy production (19) and can induce MCAD expression in an ERRα-dependent manner (34, 36). PGC-1α is abundantly expressed in tissues with high activity of FA oxidation, such as in the heart, brown fat, liver, and kidney. In the kidney, PGC-1 expression is localized in PTECs and TALs (26). PGC-1 mRNA was diminished in PTECs of mice with cisplatin-induced acute renal failure (26). PGC-1α, ERRα, and MCAD mRNA were reduced in the kidneys of mice treated with lipopolysaccharide (17). Furthermore, PGC-1α expression was proportionally suppressed with the degree of renal impairment, and the reduced PGC-1α expression involved O_2 consumption in response to TNF-α (41). PGC-1α reduces ROS accumulation and apoptosis with upregulation of the mitochondrial antioxidant defense system in vascular endothelial cells under basal and oxidative stress conditions (42). Additionally, suppression of PGC-1α would be a critical event that influences

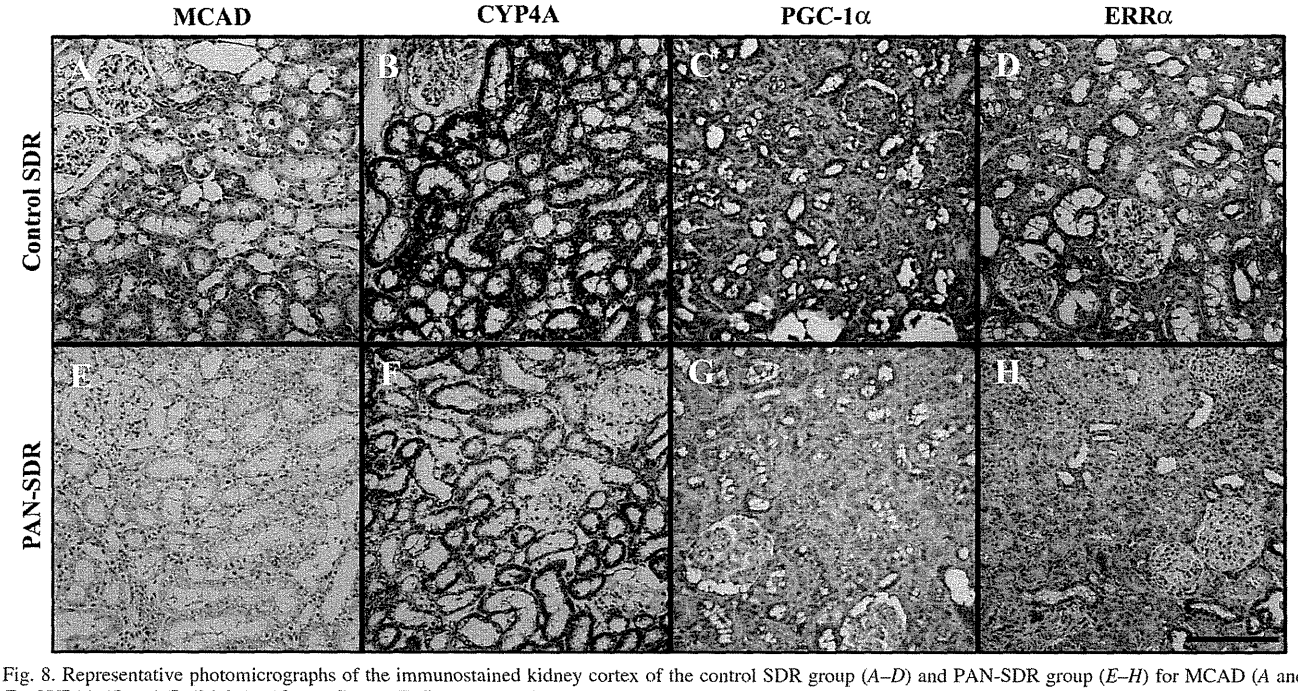

Fig. 8. Representative photomicrographs of the immunostained kidney cortex of the control SDR group (*A–D*) and PAN-SDR group (*E–H*) for MCAD (*A* and *E*), CYP4A (*B* and *F*), PGC-1α (*C* and *G*), and ERRα (*D* and *H*) on the 14th day after PAN treatment (magnification: ×200). Bar = 100 μm. The expression of MCAD protein was localized in PTECs of the control SDR group (*A*) and was decreased in the PAN-SDR group (*E*). The expression of CYP4A protein was localized in PTECs of the control SDR group (*B*) and was decreased in the PAN-SDR group (*F*). The expression of PGC-1α protein was localized in PTECs of the control SDR group (*C*) and was decreased in the PAN-SDR group (*G*). The expression of ERRα protein was localized in PTECs of the control SDR group (*D*) and was decreased in the PAN-SDR group (*H*).

the duration of functional impairment (41). Indeed, in our study, the reduced PGC-1α expression improved on the 28th day after PAN treatment when the urinary protein excretion normalized (data not shown).

Proteinuria is considered to play an essential role in the progression of tubulointerstitial damage and end-stage renal disease (28, 30). PPAR-α has been considered a therapeutic target for FA toxicity associated with proteinuria (12), as in tubular injury after ischemia-reperfusion or toxic insults (20, 21, 26, 27). The present study revealed unchanged PPAR-α expression in the nephrotic kidney and suggested a novel mechanism of proteinuria-induced tubular damage with the disorder of FA oxidation through the downregulation of PGC-1α and ERRα. PGC-1α and ERRα may play key roles in the control of FA toxicity in animals with constitutive PPAR-α expression and in humans with lower PPAR-α activity.

Although MCAD, CYP4A, PGC-1α, and ERRα were downregulated in the nephrotic kidney, functional roles of these molecules in kidney damage remain unclear. To the best of our knowledge, there is no report of the effective activator of PGC-1α in the kidney, whereas a β_2-adrenergic receptor agonist induced PGC-1α-b and PGC-1α-c expressions in skeletal muscle (22). Our preliminary study showed that the PPAR-α ligand clofibrate prevented kidney damage in the PAN-SDR group with upregulation of MCAD and CYP4A expressions in the kidneys (data not shown). These results suggest that activation of PPAR-α functions and maintenance of FA β- and ω-oxidations could protect the kidney from albuminuria-induced damage. To clarify the roles of PGC-1α in kidney damage, future studies using genetically modified animals for PGC-1α (3, 22) will be required.

In conclusion, the present study demonstrated that albuminuria had the specific effect of inducing tubular damage, apoptosis, and lipid accumulation with downregulation of MCAD, CYP4A, PGC-1α, and ERRα in PTECs of PAN-induced nephrotic rats. The disorder of FA metabolism in PTECs may contribute the development of albuminuria-induced tubulointerstitial damage.

GRANTS

This work was supported in part by Ministry of Education, Culture, Sports, Science, and Technology Grants 20590694 and 20300184 and by a grant from the Miyagi Prefecture Kidney Association.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Y.M., O.I., and K.T. conception and design of research; Y.M., R.R., D.I., P.C., Y.N., and K.J. performed experiments; Y.M. analyzed data; Y.M. and K.J. interpreted results of experiments; Y.M. prepared figures; Y.M. drafted manuscript; Y.M. and O.I. edited and revised manuscript; Y.M., O.I., and M.K. approved final version of manuscript.

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CLINICAL INVESTIGATIONS

Fall Prevention Using Olfactory Stimulation with Lavender Odor in Elderly Nursing Home Residents: A Randomized Controlled Trial

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OBJECTIVES: To investigate the effects of lavender olfactory stimulation intervention on fall incidence in elderly nursing home residents.

DESIGN: Randomized placebo-controlled trial.

SETTING: Three randomly selected nursing homes in northern Japan.

PARTICIPANTS: One hundred and forty-five nursing home residents aged 65 and older.

INTERVENTION: Participants were randomly assigned to the lavender (n = 73) or placebo group (n = 72) for a 360-day study period. The lavender group received continuous olfactory stimulation from a lavender patch. The placebo group received an unscented patch.

MEASUREMENT: The primary outcome measure was resident falls. Other measurements taken at baseline and 12 months included functional ability (assessed using the Barthel Index), cognitive function (Mini-Mental State Examination (MMSE)), and behavioral and psychological problems associated with dementia (Cohen-Mansfield Agitation Inventory (CMAI)).

RESULTS: There were fewer fallers in the lavender group (n = 26) than in the placebo group (n = 36) (hazard ratio (HR)=0.57, 95% confidence interval (CI) = 0.34–0.95) and a lower incidence rate in the lavender group (1.04 per person-year) than in the placebo group (1.40 per person-year) (incidence rate ratio = 0.51, 95% CI = 0.30–0.88). The lavender group also had a significant decrease in CMAI score ($P = .04$) from baseline to follow-up in a per protocol analysis.

CONCLUSION: Lavender olfactory stimulation may reduce falls and agitation in elderly nursing home residents; further research is necessary to confirm these findings. J Am Geriatr Soc 60:1005–1011, 2012.

Key words: fall prevention; lavender; nursing home residents

Falls are recognized as a major problem in community-dwelling elderly adults and even more so in frail elderly adults residing in institutions.[1,2] Approximately half of nursing home residents fall annually, two to three times that of community residents.[3] Falls are associated with morbidity and mortality in nursing home residents and linked to poorer overall functioning. A high risk of falling can considerably compromise the ability to perform activities of daily living (ADLs) and participate in social activities.[4] Reducing or minimizing the risk of falling can positively affect residents' quality of life.

Important underlying risk factors for falls include lower extremity weakness, gait and balance instability, poor vision, cognitive and functional impairment, and sedating and psychotropic medications.[1] Cognitive impairment is a strong risk factor for falls in nursing homes that may increase the risk of falls in multiple ways through the behavioral and psychological symptoms of dementia (BPSD), as well as gait and balance disturbances.[5–7]

A systematic review revealed that effective measures to prevent falls in nursing homes are seriously lacking.[7] Some challenges to incorporating fall prevention into practice include intervention feasibility, staff time constraints, competing demands, and inadequate reimbursement.[8,9]

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Other barriers include a perceived lack of skills by healthcare professionals in managing complex, multifactorial health conditions and a lack of coordination in the nursing home setting. To overcome these difficulties, a new innovative, easy-to-execute intervention is warranted.

It is hypothesized that lavender (*Lavandula angustifolia*), used in aromatherapy as a relaxant, has multiple ameliorating effects on fall-related risk factors in elderly adults. A previous study showed that olfactory stimulation using lavender oil improved balance in elderly people.[10] In addition, another recent study reported that gait performance as measured using the Timed Up and Go test and 10-m walking speed significantly improved after exposure to lavender olfactory stimulation.[11] Although these studies demonstrated a transient effect of lavender olfactory stimulation, long-term exposure to continuous lavender olfactory stimulation has not been investigated. It is conceivable that, if individuals were exposed continuously to lavender olfactory stimulation, the stabilizing effects of lavender odor on gait performance might prevent falls in frail elderly people.

Lavender odor has soothing properties affecting anxiety and agitation underlying BPSD.[12,13] BPSD, such as physically nonaggressive behaviors (including pacing and wandering) and aggressive behaviors (leading to increases in prescription neuroleptic medications), may lead to and increase in fall risk. Because of the difficulty in treating individuals with BPSD, prescription tranquilizers and other psychotropic medications are common,[14,15] but such medications have shown modest efficacy but can have adverse effects such as confusion, gait disturbance, and falls. Therefore, increasingly more attention is being paid to nonpharmacological interventions specific to agitation. A recent review identified aromatherapy with lavender as a potential treatment for BPSD in nursing home residents.[16]

Olfactory stimulation with lavender may prevent falls in nursing home residents by ameliorating behavioral and psychological problems and consequently reducing the need to prescribe psychotropic medications, thereby ameliorating gait and balance disorders. The aim of this study was to test the effects of continuous lavender olfactory stimulation on the incidence and risk of falls in elderly nursing home residents. To this end, a randomized placebo-controlled trial was conducted using a paper patch with or without lavender attached daily by care staff to the inside of the clothes near the neck of nursing homes residents.

METHODS

Study Design, Participants, and Setting

The trial was conducted in three nursing homes randomly selected from 24 nursing homes in Aomori city, northern Japan. Inclusion criteria for eligible subjects were aged 65 and older and the ability to transfer independently regardless of assistive devices used. Recruitment occurred between September 10, 2009, and January 27, 2010. Of the 155 residents meeting the eligibility criteria, 10 were excluded; three did not provide informed consent, three moved before the trial began, and four had pica disorder. Residents with pica disorder, the unusual desire to eat "unnatural" things for food, were excluded because of the risk that they would eat the patch.

In each nursing home, the eligible residents were randomized to the lavender group or placebo group at a 1:1 ratio. An independent statistician performed resident allocations using computer-generated randomization of numbers at each nursing home. Treatment allocation status was delivered to the head nurse at each nursing home, and patches were prepared accordingly. Participants and study staff were blinded to the treatment groups and outcome measurements. One hundred and forty-five residents were randomized: 73 to the lavender group and 72 to the placebo group.

The ethics board of Tohoku University Graduate School of Medicine approved the study protocol, and the study design took into account the principles set out in the Helsinki Declaration (Seoul, 2008). The protocol was registered to UMIN Clinical Trials Registry identifier (UMIN000004222).

Intervention

Lavender olfactory stimulation was provided using a commercially available white patch (1 cm × 2 cm, Aromaseal Lavender; Hakujuji Co., Tokyo, Japan). This patch, attached to the inside of the resident's clothes near the neck, was originally developed to make busy and stressful people relax by providing continuous olfactory exposure to lavender for 24 hours. The odor is so faint that only the person wearing the patch can sense it. The price of one patch is 25 cents U.S. The placebo patch was an Aromaseal that had not been processed and was unscented. Nursing home staff, blinded to which Aromaseal was the placebo, affixed the lavender or placebo patch to the resident's clothing and replaced the patch daily. The head nurse prepared the appropriate patches and distributed them to the nursing home staff accordingly. Residents wore the patch for the whole day. At the time the patch was changed, the nursing home staff confirmed the existence of the prior day's patch; if the patch was missing, it was reported. The intervention finished 360 days after the start unless a resident dropped out. The final participants finished follow-up on January 14, 2011.

Measurements

The primary outcome measure was resident falls. For this study, a fall was defined in accordance with the World Health Organization's definition: "an event which results in a person coming to rest inadvertently on the ground or floor or other lower level."[17,18] The nursing home staff, blinded to group allocation, were trained to identify falls according to this definition and recorded falls daily using fall calendar sheets. The head nurse supervised the recording of falls regularly, and the calendar sheets were audited monthly to ensure agreement with incident reports. Individual nursing notes were also cross-checked for duplication and missed falls.

Trained research assistants, blinded to group allocation and information from previous evaluations, collected demographic and behavioral measures at baseline and 12-month follow-up. Behavioral measurements included the Cohen-Mansfield Agitation Inventory (CMAI) to quantify

BPSD,[19] the Barthel Index to assess level of functional ability, the MMSE to assess cognitive function, and the Vitality Index to assess activity of daily living (ADL)-related vitality.[20] The resident and caregiver assessed fall history in the previous year, and the staff was consulted and nursing notes and resident charts reviewed. To predict the probability of falling, visual, transfer, and mobility status were assessed using the St. Thomas's Risk Assessment Tool in Falling Elderly Inpatients (STRATIFY).[21] Medication status was assessed from medical chart reviews.

Statistics

Initial comparisons of outcome measures between groups were performed using chi-square tests or Mann–Whitney tests, as appropriate. Kaplan–Meier plots were used to compare time to first fall between groups.

Analyses for main outcomes, including time to first fall and number of falls per person-year, were based on an intention-to-treat analysis. Kaplan–Meier analyses and log-rank statistics were used to compare the proportion of fallers to non-fallers over time between groups. For consideration of covariance in time to first fall analysis between groups, a multivariate Cox proportional hazards regression was performed. A comparison of the number of falls per person-year between groups was performed using a multivariate Poisson regression model regarding the observation time as the offset variable. To confirm robustness in the Poisson regression model, the standard errors of each coefficient were adjusted by multiplying the unadjusted standard errors by the square root of the multiplicative overdispersion factor.

In multivariate analyses, age category (65–74 vs ≥ 75), sex, history of fall in a previous year (presence vs absence), cognitive function (MMSE score < 24 vs ≥ 24), agitation status (CMAI 22 = not agitated vs ≥ 23 = shows signs of agitation), transfer status (STRATIFY transfer and mobility score 0, 1, 2, 5, 6, or 3, 4), visual status (STRATIFY 1 or 2), number of medications (<5 vs ≥ 5), and use of tranquilizers (yes vs no) were regarded as possible covariates for the Model 1 multivariate analysis. In the Model 2 analysis, variables that achieved a significance level of $P < .2$ in the univariate analysis were subsequently included in a multivariate analysis using the stepwise forward Cox regression procedure and the Poisson regression procedure, respectively. To elucidate the mechanisms underlying the effects of lavender olfactory stimulation, an analysis for secondary outcomes, such as changes in CMAI, Barthel Index, MMSE, and Vitality Index were performed using a per protocol analysis. Normality of the data was assessed using the Shapiro-Wilk test. Comparisons between groups were performed using the Mann–Whitney test. Comparisons within groups at different time points were performed using the Wilcoxon signed-rank test or the paired Student *t*-test.

The analysis of outcomes for fallers and falls (Table 2) was done on the intention-to-treat analysis set, whereas the comparison of treatment groups at baseline and follow-up (Table 3) used the per protocol analysis set. All *P*-values were two-sided to detect a significance level of $P < .05$. Analyses were performed using SAS software version 9.2 (SAS Institute. Inc., Cary, NC).

Sample Size

To calculate the required sample size, the number of falls per person-year was focused on, based on data from similar nursing homes in Japan.[22] When the sample size in each group is 69, with a total number of events required (E) of 55, an exponential maximum likelihood test of equality of survival curves with a .05, two-sided significance level will have an 80% power to detect the difference between a placebo exponential parameter (l_1) of 0.8500 and an active exponential parameter (l_2) of 0.4000 (constant hazard ratio (HR) = 2.125); this assumes an accrual period of 0.10, a maximum follow-up time of 1.00, and no dropouts.[23]

RESULTS

A flowchart of enrollment, randomization, and follow-up is shown in Figure 1. No significant differences were observed between the lavender and placebo groups in the proportion who withdrew or in their reasons for withdrawal. No participants refused the lavender-scented patch, and there were no adverse effects reported due to exposure to the lavender. The baseline and demographic characteristics of residents allocated to each group are summarized in Table 1. The groups did not differ significantly according to age or risk factors for falls. No participants had missing values on primary outcome measures before death or discharge from nursing homes.

There were 62 falls reported during the follow-up period (Table 2); only two resulted in injury, a subdural hemorrhage in the lavender group and a femoral neck fracture in the placebo group. The percentages of participants who

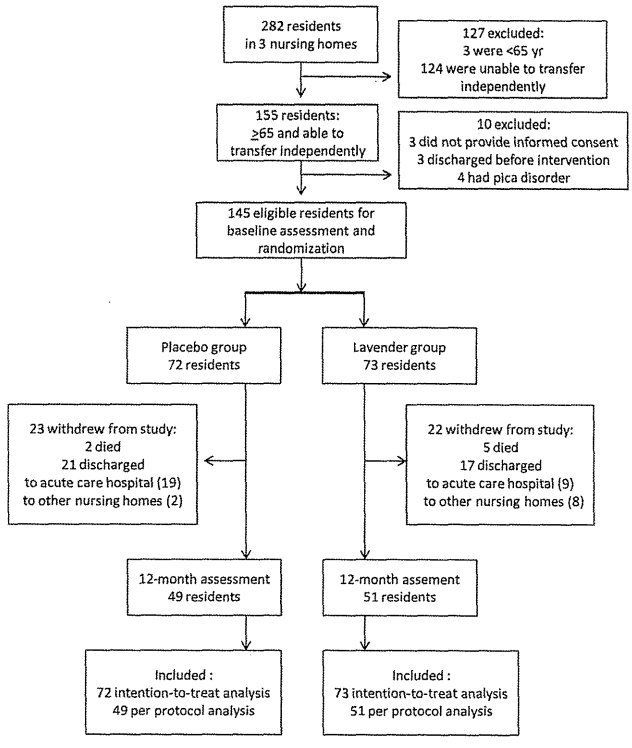

Figure 1. Flowchart for enrollment, randomization, and follow-up of study participants.

Table 1. Baseline Characteristics of Participants (n = 145)

Characteristic	Placebo, n = 72	Lavender, n = 73	*P*-Value
Age, mean ± SD	84.1 ± 7.7	84.2 ± 7.8	.93[a]
≥75 years, n (%)	62 (86.1)	64 (87.7)	.81[b]
Female, n (%)	13 (18.1)	14 (19.2)	>.99[b]
Comorbidity, n (%)			
History of stroke	23 (31.9)	24 (32.9)	>.99[b]
Diabetes mellitus	12 (16.7)	18 (24.7)	.31[b]
Osteoarthritis	1 (1.4)	1 (1.4)	>.99[c]
Parkinson's disease	1 (1.4)	1 (1.4)	>.99[c]
Visual impairment	6 (8.3)	10 (13.7)	.42[b]
Barthel Index, mean ± SD	49.6 ± 19.2	50.3 ± 18.5	.82[a]
Mini-Mental State Examination score, mean ± SD	14.6 ± 8.1	15.3 ± 8.4	.51[a]
<24, n (%)	59 (81.9)	60 (82.2)	>.99[b]
Cohen-Mansfield Agitation Inventory score, mean ± SD	24.6 ± 6.9	24.2 ± 5.2	.61[a]
≥23, n (%)	18 (25.0)	23 (31.5)	.46[b]
Vitality Index, mean ± SD	8.1 ± 1.9	8.1 ± 2.0	.73[a]
History of falls, n (%)	30 (41.7)	31 (42.5)	>.99[b]
History of recurrent falls, n (%)	11 (15.3)	10 (13.7)	.49[b]
Transfer risk, n (%)	34 (47.2)	41 (56.1)	.32[b]
Mobility status[d], n (%)			
Walk without aids	52 (72.2)	53 (72.6)	.88[b]
Walk with aids	16 (22.2)	17 (23.3)	
Use a wheelchair	4 (5.6)	3 (4.1)	
Number of medications, mean ± SD	4.9 (2.7)	5.0 (2.3)	.85[a]
≥5, n (%)	37 (51.4)	37 (9.6)	>.99[b]
Prescription medications, n (%)			
Tranquilizer	15 (20.8)	10 (13.7)	.28[b]
Antidepressant	1 (1.4)	2 (2.7)	>.99[c]
Yokukansan	6 (8.3)	5 (6.8)	.77[b]
Diuretics	11 (15.3)	15 (20.5)	.52[b]
Antihypertensive	43 (59.7)	45 (61.6)	.87[b]
Antidiabetic drugs	7 (9.7)	11 (15.1)	.45[b]

SD, standard deviation.
[a] Mann–Whitney *U*-test.
[b] Chi-square test.
[c] Fisher exact test.
[d] Moving to the bathroom.

fell at least once during the 12-month study period were 35.6% (lavender group) and 50% (placebo). There were no significant differences observed when examining Kaplan–Meier plots of time to first fall between treatment groups ($P = .11$) or in relation to tranquilizer use ($P = .16$).

The crude results of the Cox proportional hazards analysis on the intention-to-treat analysis set were not significant (Table 2), although after adjustment for covariates between the lavender and placebo groups, the differences for first fall were significant for Models 1 ($P = .04$) and 2 ($P = .03$). The HR of the intervention to placebo group was 0.59 (95% confidence interval (CI) = 0.35–0.99) after adjustment for age, sex, fall history, MMSE, CMAI, transfer and visual status, and tranquilizer use (Model 1). The HR decreased to 0.57 (95% CI = 0.34–0.95) after adjustment for MMSE, fall history, and transfer (Model 2).

Table 2. Outcomes for Fallers and Falls

Outcome	Placebo, n = 72	Lavender, n = 73	*P*-Value
Intervention days, mean ± standard deviation	313.8 ± 76.3	287.5 ± 114.5	.78[a]
Faller, yes/no	36/36	26/47	.08[b]
Recurrent faller, yes/no	23/49	14/59	.08[b]
Total number of falls, n	88	46	
Fall rate per person-year	1.40	1.04	
Hazard ratio for fallers (95% CI)			
Crude	1	0.67 (0.40–1.10)	.11[c]
Adjusted (Model 1)	1	0.59 (0.35–0.99)	.04[c]
Adjusted (Model 2)	1	0.57 (0.34–0.95)	.03[c]
Incidence rate ratio for fallers (95% CI)			
Crude	1	0.57 (0.32–0.99)	.04[d]
Adjusted (Model 1)	1	0.54 (0.31–0.95)	.03[d]
Adjusted (Model 2)	1	0.51 (0.30–0.88)	.02[d]

CI, confidence interval.
Model 1 adjusted for age, sex, fall history, Mini-Mental State Examination (MMSE) score, Cohen-Mansfield Agitation Inventory, transfer status, visual status, tranquilizer. Model 2 adjusted for MMSE score, fall history, transfer (selected using stepwise variable selection).
[a] Mann–Whitney *U*-test.
[b] Chi-square test.
[c] Cox proportional hazard regression.
[d] Poisson regression model.

The number of falls per person during the follow-up period ranged from zero to five in the lavender group and zero to seven in the placebo group. As shown in Table 2, the incidence rate for the lavender group was significantly lower than for the placebo group even before adjustment for possible covariates ($P = .04$). The incidence rate ratio (IRR) in crude analysis was 0.57 (95% CI = 0.32–0.99). After adjustment for age, sex, fall history, MMSE, CMAI, transfer and visual status, and tranquilizer use (Model 1), the IRR decreased to 0.54 (95% CI = 0.31–0.95). After adjustment for MMSE, fall history, and transfer status (Model 2), the IRR further decreased to 0.51 (95% CI = 0.30–0.88).

Table 3 shows the results of per protocol analyses for changes in functional ability (Barthel Index), cognitive function (MMSE), volition (Vitality Index), and agitation (CMAI) after 12 months of treatment. No differences were observed between groups at baseline or 12 months for any of the indexes analyzed. The lavender and placebo groups showed a significant decrease in cognitive functioning at 12-month follow-up. When comparing CMAI scores at 12-month follow-up, the lavender group showed a significant decrease in agitated status ($P = .04$) from baseline, but the placebo group did not. The Barthel and Vitality indexes did not change significantly from follow-up in either group. The average number of medications at 12-month follow-up was 4.73 ± 2.17 in the lavender group and 4.57 ± 2.17 in the placebo group.

During the study period, one resident from each group was newly prescribed tranquilizers. At 12-month follow-up, six residents in the lavender group and 10 in the placebo group were prescribed tranquilizers. No significant difference was observed in the number of residents

Table 3. Comparison of Groups at Baseline Versus Follow-Up in Per Protocol Analyses

	Placebo (n = 49)			Lavender (n = 51)		
	Baseline	Follow-Up		Baseline	Follow-Up	
Test	Mean ± SD		*P*-Value	Mean ± SD		*P*-Value
Barthel Index	50.0 ± 1.91	47.5 ± 21.0	.09[a]	49.6 ± 18.3	49.5 ± 18.5	.94[b]
Mini-Mental State Examination score	14.6 ± 21.0	11.9 ± 8.4	<.001[a]	15.3 ± 9.2	13.4 ± 9.1	<.001[a]
Cohen-Mansfield Agitation Inventory score	24.5 ± 6.7	24.0 ± 3.7	.82[a]	24.3 ± 5.4	22.9 ± 2.3	.04[a]
Vitality Index	8.2 ± 1.7	8.1 ± 2.3	.76[a]	8.2 ± 2.0	8.1 ± 2.2	.90[a]

SD, standard deviation.
No difference was observed between groups at baseline and after 12-month interventions for each index according to the Mann–Whitney *U*-test.
P-value was comparison between baseline and post intervention according to [a]Wilcoxon rank test or [b]paired Student t-test.

prescribed tranquilizers between the groups at baseline ($P = .78$) or the end of the trial ($P = .71$). One resident from the lavender group and one from the placebo group took vitamin D (1 μg) daily; neither of them fell during the study period.

DISCUSSION

This study highlights the beneficial effects of lavender odor on fall prevention in elderly nursing home residents. This multifacility randomized placebo-controlled study showed that daily use of a lavender patch was associated with a lower incidence rate of falls. Although not significant, the number of residents who fell during the observation period ($P = .08$) and those who fell two or more times during the 12-month study ($P = .11$) was less in the lavender group. After adjustment for possible confounding factors, the proportion of residents who were nonfallers over time was significantly lower in the lavender group.

The mechanism by which lavender prevents falls is speculative. Lavender oil is used extensively in aromatherapy and is described as therapeutic for insomnia, headaches, migraines, anxiety, nervousness, and melancholy.[24] Lavender has been used as a sleep aid and can be a useful nonpharmacological alternative to traditionally prescribed medications for insomnia, which are strong risk factors for falls in elderly adults.[25] Because lavender is thought to have soothing properties, it is logical to assume it may also affect the anxiety and agitation that underlie BPSD. The lavender group showed a significant decrease in agitated status, whereas the placebo group did not, suggesting the involvement of a soothing effect of lavender odor. There was not significant less tranquilizer use in the lavender group than in the placebo group, so tranquilizer use was not viewed as a potential confounding factor in the present study. The frequency of tranquilizer use was lower in the current study than in other studies in nursing homes, probably because of Yokukansan use, a traditional Asian medicine commonly prescribed to treat BPSD.[26,27] Although there was no difference in Yokukansan use between the lavender and placebo groups, further study is warranted to elucidate the relationship between Yokukansan, tranquilizers, and lavender olfactory stimulation.

Another possible explanation for why lavender prevents falls might be attributed to its stabilizing effects on balance. In previous work, the application of olfactory stimulation by an essential oil such as lavender and black pepper during quiet standing was associated with less postural sway in frail elderly adults.[10] Multiple sensory and motor mechanisms ranging from peripheral to cortical sensory-motor integration regulate the control of posture and motion.[28] In addition to vestibular afferents, visual and proprioceptive inputs contribute to postural stability. Although several multisensory vestibular cortical areas, which process signals provided from multiple thalamic nuclei, were identified using imaging studies, the core vestibular cortical region is thought to be located in the insular cortex.[29] Odor is one of the strongest stimuli over a wide area of the cerebral cortex including the insular cortex.[30] Olfactory stimulation may stabilize balance by activation of the insular cortex. Unfortunately, a limitation of the present study is the lack of balance data. Further studies are needed to clarify the contribution of the balance-stabilizing effects of lavender on fall prevention.

Only two residents were prescribed vitamin D (1 in each group). Vitamin D supplementation is an easy pharmacotherapy to prevent falls in nursing home residents.[1,7] The current evidence recommends that vitamin D be prescribed in a dosage of 1,000 IU for nursing home residents. Vitamin D may be effective in reducing falls and increasing muscle strength in persons with severe vitamin D deficits,[1] but current evidence of risk reduction of falls with vitamin D supplementation is inconsistent.[7]

Several current guidelines recommend multifactorial risk assessment of falls and interventions customized to an individual's risk factor profile as a primary treatment strategy in community-dwelling elderly people.[1,31] Several randomized controlled trials have investigated the effectiveness of this strategy in nursing home residents,[32–39] and only some of the trials showed efficacy in reducing falls.[36–39] It is unclear whether differences in effectiveness may be attributed to a variation in the type of intervention or selection bias. The sample population recruited into trials may not be representative of the general elderly population (e.g., lack of studies that include participants with multiple comorbidities or cognitive decline). It is important to develop a suitable program for multifactorial intervention in each facility setting.

The present study has several limitations. First, it was conducted with nursing home residents, so results cannot be generalized to community-dwelling elderly people. Second, although the study showed that lavender olfactory

stimulation prevents falls in elderly nursing home residents, it was not powered to detect a clinically relevant reduction in injurious falls because the incidence of such events was low. Third, as is the nature of odor application, nursing home residents and staff may not have been completely blinded, which may have resulted in reporting bias. Finally, the olfactory functioning of the participants was not tested. Difficulty in identifying odor has been reported not only in individuals with Alzheimer's and Parkinson's diseases,[40] but also in elderly persons without cognitive impairment.[41] Therefore, it was possible there were residents who could not sense the lavender odor.

A meta-analysis showed that a multifactorial intervention including exercise training for balance stability reduced the risk and rate of falls in community-dwelling elderly adults.[25] Moreover, gradual withdrawal of some types of drugs for improving sleep, reducing anxiety, and treating depression have been shown to reduce the rate of falls.[25] Lavender olfactory stimulation acts on balance and psychological status, suggesting that it may have the ability to reduce falls in nursing home residents and community dwelling-elderly adults.

CONCLUSION

Daily olfactory stimulation with lavender may prevent falls in elderly nursing home residents. Further studies with large sample sizes comprising multiple ethnic groups are warranted to confirm these findings.

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Effect of cigarette smoking on cough reflex induced by TRPV1 and TRPA1 stimulations

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Summary
Background: Recent studies have shown that neurogenic inflammation induced by cigarette smoke is inhibited by TRPA1 antagonist, but not by TRPV1 antagonist. Since cough reflex sensitivity is known to be modified by smoking status, we investigated the effects of cigarette smoking on TRPA1- and TRPV1-induced cough and urge-to-cough in healthy males.
Methods: Twenty-six healthy never-smokers and 30 healthy current smokers were recruited via public postings. Cough reflex thresholds and urge-to-cough were evaluated by inhalation of capsaicin, a TRPV1 agonist, and cinnamaldehyde, a TRPA1 agonist. The cough reflex thresholds were defined as the lowest concentrations of capsaicin and cinnamaldehyde that elicited two or more coughs (C_2) and five or more coughs (C_5), respectively. The urge-to-cough was evaluated using the modified Borg scale.
Results: In capsaicin-induced cough, the cough reflex thresholds, as expressed by C_2 and C_5, in current smokers were significantly higher than those in never-smokers ($p < 0.01$ and $p < 0.001$, respectively). The urge-to-cough log–log slopes in current smokers were significantly lower than those of never-smokers ($p < 0.001$). There were no significant differences in the thresholds of the urge-to-cough between never-smokers and current smokers. In cinnamaldehyde-induced cough, there were no significant differences in cough reflex thresholds in C_2 and C_5 between never-smokers and current smokers, nor were there any significant differences in urge-to-cough log–log slope between never-smokers and current smokers. There were no significant differences in the thresholds of the urge-to-cough between never-smokers and current smokers.
Conclusion: The study suggests that smoking has a differential effect on cough responses between TRPV1 and TRPA1 stimulations.

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Introduction

Cigarette smoke is one of the most common inhaled irritants of the respiratory tract. In never-smokers, inhaling only a small puff of cigarette smoke can evoke airway irritation and vigorous cough responses.[1] In addition, cigarette smoking is well known to be a risk factor for chronic cough and chronic obstructive pulmonary disease, which are associated with the symptom of cough.[2] However, several studies have shown that chronic smokers without a history or symptoms of airway disease have a significantly diminished cough reflex sensitivity to capsaicin (they cough less in response to inhaled capsaicin) compared to that of healthy never-smokers.[3–7]

Cough results from the stimulation of sensory receptors within the respiratory tract, the afferent impulses of which activate the brainstem and higher cortical centers for cough. Both TRPV1 (transient receptor potential cation channel, subfamily V, member 1) and TRPA1 (transient receptor potential cation channel, subfamily A, member 1) channels have been implicated in the afferent sensory loop of the cough reflex.[8,9] TRPA1, like TRPV1, is expressed by trigeminal and nodose/jugular ganglia neurons.[10] Both channels are most often found in the same neuron.[11] Although this means that activation of TRPA1 will likely exert effects similar to those observed following the activation of TRPV1,[10] recent studies have shown that neurogenic inflammation induced by cigarette smoking is inhibited by HC-030031, a TRPA1 antagonist, but not by capsazepine, a TRPV1 antagonist.[12]

Although there are various chemical components among the approximately 5000 constituents of cigarette smoke, nicotine, reactive oxygen species, and, unsaturated aldehydes included in cigarette smoke have been found to activate the TRPA1 channel, but not the TRPV1 channel.[12–14] Therefore, these components may lead to up-regulation of peripheral afferent activity for cough induced by TRPA1 stimulation but not by TRPV1 stimulation. However, there has been a report that nicotine acts on central neural mechanisms as an anxiolytic,[15] suggesting that it may cause the down-regulation of the cortical facilitatory pathway for cough.

The cough reflex thresholds and perception of urge-to-cough in response to TRPV1 or citric acid stimulations are known to be modified by smoking status.[16,17] However, the effect of cigarette smoking on cough reflex sensitivity to TRPA1 stimulation is unknown. Since TRPA1 plays a role in tobacco smoking-induced neurogenic airway inflammation, we investigated the effects of cigarette smoking on cough reflex thresholds in response to TRPV1 and TRPA1 stimulations. Simultaneously, we demonstrated the effects of smoking on perception of urge-to-cough induced by TRPV1 and TRPA1 stimulations.

Methods

Subjects

Twenty-six healthy male never-smokers and 30 healthy male current smokers were recruited to evaluate cough related responses to inhaled capsaicin, a TRPV1 agonist, and cinnamaldehyde, a TRPA1 agonist. All were recruited via public postings in and around the Tohoku University School of Medicine campus. Never-smoker males and current smoker males were entered into a randomized, crossover study of inhalational cough challenge of two tussive agents. The study was approved by the Institutional Review Board of the Tohoku University School of Medicine. Subjects were without a history of pulmonary and airway diseases, recent (within 4 weeks) suggestive symptoms, respiratory tract infection, and seasonal allergies. Subjects did not take any regular medication.

Cough reflex thresholds to capsaicin or cinnamaldehyde and urge-to-cough

Cough reflex thresholds in response to capsaicin and cinnamaldehyde were measured on different days using a modification of the methods of capsaicin-induced cough reported by Fujimura et al.,[18] and the cinnamaldehyde-induced cough reported by Birrell et al.[9] The current smokers smoked more than one cigarette within 2 h of each cough challenge. Capsaicin (30.5 mg) (Sigma Aldrich, Seatle, USA) was dissolved in Tween 80 (1 ml) and ethanol (1 ml) and then dissolved in physiological saline (8 ml) to make a stock solution of 0.01 M, which was stored at −20 °C. This solution was diluted with physiological saline to make testing solutions starting at a concentration of 0.49 μm and increasing it by doubling the concentration up to 1000 μm. On the other hand, based on the method reported by Birrell et al., cinnamaldehyde (Sigma Aldrich, Seatle, USA) was dissolved in 50% ethanol to make a stock solution of 800 mM.[9] This stock solution was diluted with 50% ethanol to make solutions ranging from 60 to 800 mM.[9]

Each subject inhaled the control solution followed by progressively increasing concentrations of capsaicin or cinnamaldehyde solutions. Subjects inhaled solutions for 15 s every 60 s, by tidal mouth-breathing, while wearing a nose-clip of an ultrasonic nebulizer (MU-32; Sharp Co Ltd; Osaka, Japan).[19,20] The nebulizer generated particles with a mean mass median diameter of 5.4 μm at an output of 2.2 mL/min. By tidal breathing, one-half of the particles were expected to be deposited in the lungs.[21] The cough reflex threshold and suprathreshold were estimated by the lowest concentrations of capsaicin or cinnamaldehyde that elicited two or more coughs (C_2) and the lowest concentrations of capsaicin or cinnamaldehyde that elicited five or more coughs (C_5). In a preliminary experiment, we assessed data reproducibility from two consecutive TRPA1-induced cough challenges, spaced 1 week apart. We obtained strong correlations in C_2 and C_5 between the initial application and the second application in eight healthy subjects ($r = 0.85$, $p < 0.01$; $r = 0.83$, $p < 0.01$, respectively).

Immediately after the completion of each nebulizer application, the subject made an estimate of the urge-to-cough on the modified Borg scale. The scale ranged from "no need to cough" (rated 0) and "maximum urge-to-cough" (rated 10). The urge-to-cough scale was placed in front of the subjects and the subject pointed at the scale number, which was recorded by the experimenter. To assess the intensity of the urge-to-cough, subjects were recommended to ignore other sensations such as dyspnea,

burning, irritation, choking, and smoke in their throat. Subjects were told that their sensation of an urge-to-cough could increase, decrease, or stay the same during the capsaicin or cinnamaldehyde challenges, and that their use of the modified Borg scale should reflect this.

In each subject, the estimated urge-to-cough scores were plotted against the corresponding capsaicin or cinnamaldehyde concentrations using a log–log transformation. Since it is known that there is a linear relationship between estimated urge-to-cough scores and tussive agent concentration on a log–log scale,[22,23] the slope and intersection were determined by linear regression analysis on a log–log scale.[16,24,25] The thresholds of the urge-to-cough in each subject was estimated as an intersection with the *x*-axis (citric acid concentration axis), indicating the dose of the urge-to-cough score = 1.[16,24,25] We also determined the initial concentrations of capsaicin or cinnamaldehyde that induced urge-to-cough sensations without provoking associated motor cough events as a threshold of urge-to-cough, termed C_U.[26]

Statistical analysis

Data are expressed as mean ± SD. The cough reflex threshold was log transformed. The Mann–Whitney *U* test was used to compare never-smokers and current smokers variables. A $p < 0.05$ was considered significant.

Results

All 56 subjects completed the experiments without any difficulty or side effects. The characteristics of subjects are summarized in Table 1. There were no statistically significant differences in age, height, weight, and spirometric measurements between never-smokers and current smokers.

Figure 1 shows comparisons of cough reflex thresholds in response to capsaicin between never-smokers and current smokers. As shown in Figure 1a, the cough reflex threshold to capsaicin, as expressed by log C_2, in current smokers (1.12 ± 0.49 μM) was significantly greater than that in never-smokers (0.75 ± 0.41 μM, $p < 0.01$). Similarly, Figure 1b shows that current smokers (0.95 ± 0.36 μM) had a significant enhancement of log C_5 compared to never-smokers (1.42 ± 0.36 μM, $p < 0.001$).

The log–log slope between capsaicin concentration and the Borg scores of the urge-to-cough were estimated for each subject. As shown in Figure 2a, the urge-to-cough log–log slope in current smokers (0.54 ± 0.27 point μM) was significantly lower than that in never-smokers (0.89 ± 0.31 point μM, $p < 0.001$). The urge-to-cough thresholds in response to capsaicin were estimated as an intersection with the *x*-axis of the linear regression equation of the log–log relationships between capsaicin concentration and the Borg scores of the urge-to-cough.[16,24,25] As shown in Figure 2b, there were no significant differences in log urge-to-cough threshold induced by capsaicin between never-smokers (−0.20 ± 0.49 μM) and current smokers (−0.50 ± 1.36 μM). We also defined the initial concentration of capsaicin that induced a urge-to-cough sensation without provoking an associate motor cough event, termed C_U.[26] As shown in Figure 2c, capsaicin-induced log C_U in current smokers (0.21 ± 0.34 μM) did not differ from that of never-smokers (0.25 ± 0.31 μM). These data indicate that smoking causes no significant changes in bronchopulmonary sensors involved in the urge-to-cough induction induced by capsaicin and that there is a larger contribution of central gain mechanisms than that of peripheral ones.

Figure 3 shows comparisons of cough reflex thresholds to cinnamaldehyde between never-smokers and current smokers. As shown in Figure 3a, cinnamaldehyde-induced log C_2 was similar in never-smokers (2.61 ± 0.30 mM) and current smokers (2.68 ± 0.20 mM). Figure 3b shows that cinnamaldehyde-induced log C_5 was also similar in never-smokers (2.70 ± 0.25 mM) and current smokers (2.76 ± 0.16 mM).

In the same way as capsaicin, the log–log slope between cinnamaldehyde concentration and the Borg scores of the urge-to-cough was estimated for each subject. Figure 4a shows that the urge-to-cough log–log slope induced by cinnamaldehyde in current smokers (0.98 ± 0.32 point mM) did not differ from that of never-smokers (1.04 ± 0.51 point mM). In addition to this phenomenon, there were no significant differences in log urge-to-cough threshold and C_U between never-smokers (1.86 ± 0.79 mM and 2.24 ± 0.29 mM, respectively) and current smokers (2.08 ± 0.27 mM; and 2.24 ± 0.28 mM, respectively).

Table 1 Comparison of characteristics between never-smokers and current smokers.

Variable	Never-smokers	Current smokers	*P* value
Number	26	30	–
Age (years)	26.0 ± 7.6	26.6 ± 8.5	n.s.
Height (cm)	171.6 ± 5.2	173.3 ± 6.7	n.s.
Weight (kg)	66.0 ± 8.2	67.4 ± 12.0	n.s.
Brickman index	0	121.3 ± 117.1	–
FEV_1 (L)	4.10 ± 0.53	4.20 ± 0.70	n.s.
FVC (L)	4.68 ± 0.71	4.81 ± 0.75	n.s.
FEV_1/FVC (%)	87.4 ± 5.82	87.4 ± 6.79	n.s.

Data are mean ± SD. *P* value by the Mann–Whitney *U* test. FEV_1, forced expired volume in 1 s; FVC, forced vital capacity.

Discussion

In this study, we found that the increased cough reflex thresholds induced by capsaicin in current smokers were accompanied by decreased urge-to-cough log–log slope, whereas the thresholds for urge-to-cough did not differ between never-smokers and current smokers. In cinnamaldehyde-induced cough, we showed that cough reflex thresholds were not significantly different between never-smokers and current smokers. Simultaneously, urge-to-cough log–log slope, log urge-to-cough threshold and C_U were similar in never-smokers and current smokers.

There have been several studies concerning the effect of smoking on the cough reflex[3–7,16] which applied capsaicin and citric acid cough challenge methods. In this study,

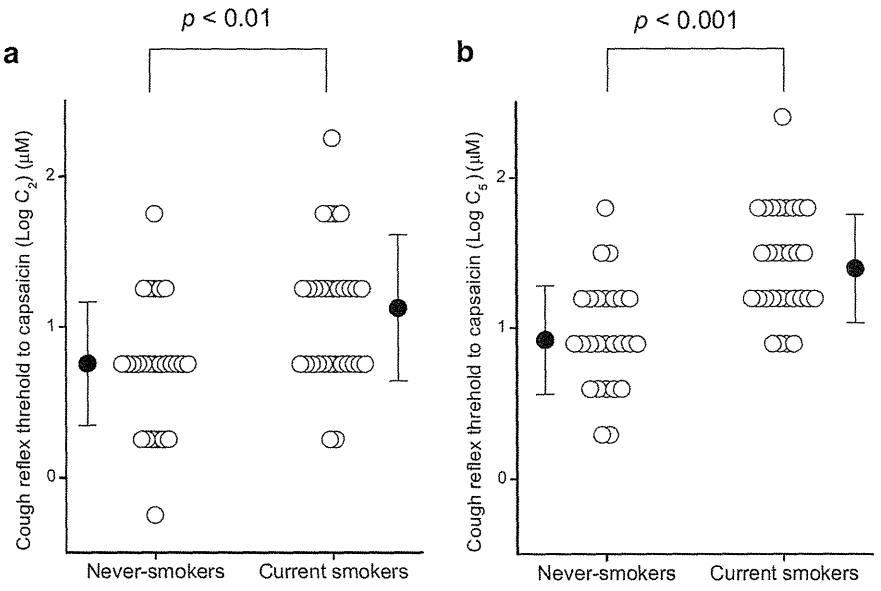

Figure 1 Comparisons of cough reflex thresholds to capsaicin between never-smokers and current smokers. (a) Cough reflex threshold expressed as the log transformation of the lowest concentration of capsaicin that elicited two or more coughs (C_2). (b) Cough reflex threshold expressed as the log transformation of the lowest concentration of capsaicin that elicited five or more coughs (C_5). Closed circles and error bars indicate the mean value and the standard deviation in each group, respectively.

based on the capsaicin-induced cough challenge method reported by Fujimura,[17] we demonstrated that our observations are consistent with past studies which investigated the effect of smoking on cough reflex sensitivity to capsaicin.[3–7]

In contrast, the effect of smoking on cinnamaldehyde-induced cough has not been investigated. There is only one study, that by Birrell et al.,[9] concerning cinnamaldehyde-evoked coughing in humans. According to ERS guidelines on the assessment of cough,[27] they employed the dosimeter method in assessing cough response to cinnamaldehyde. We, however, used the tidal breathing method. Since there has been a report that the capsaicin cough threshold in the tidal breathing method is a strongly correlated with that in

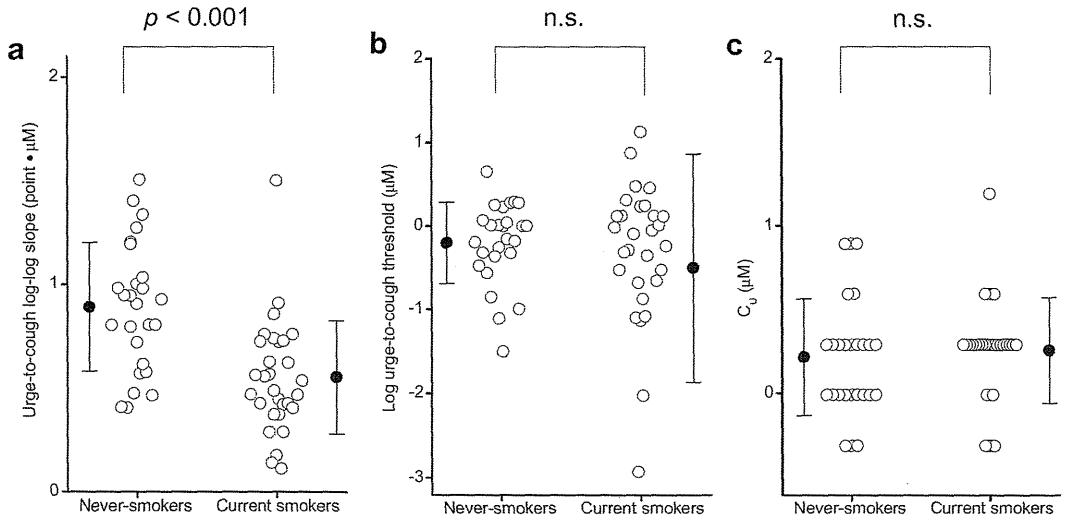

Figure 2 Comparisons of urge-to-cough induced by capsaicin between never-smokers and current smokers. (a) The urge-to-cough log–log slope by linear regression between capsaicin concentration and log Borg scores. (b) The urge-to-cough threshold estimated by log capsaicin concentration at the log Borg score of urge-to-cough = 0. (c) The C_U induced by capsaicin indicates the concentration of capsaicin provoking perception of urge-to-cough without associated motor cough. Open circles indicate the value of each subject. Closed circles and error bars indicate the mean value and the standard deviation in each group, respectively. n.s. denotes not significant.

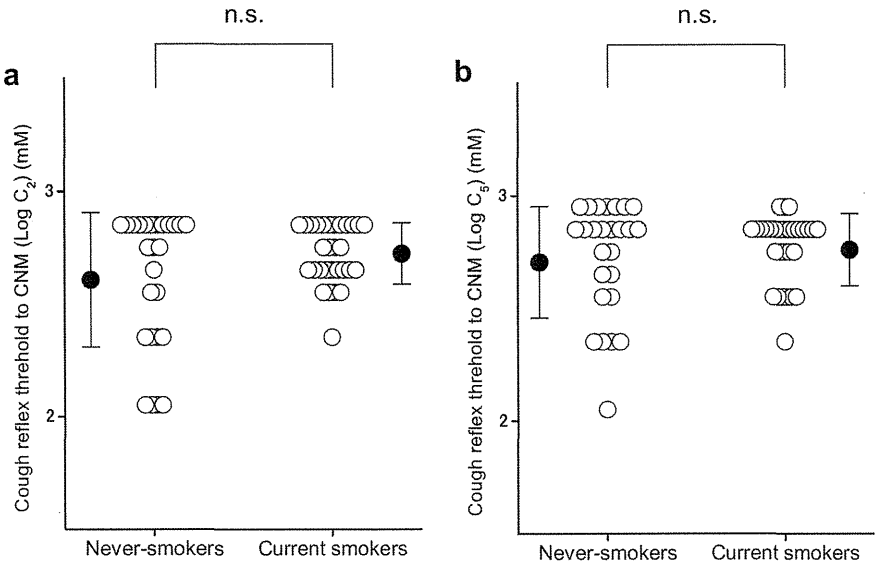

Figure 3 Comparisons of cough reflex thresholds to cinnamaldehyde (CNM) between never-smokers and current smokers. (a) Cough reflex threshold expressed as the log transformation of the lowest concentration of cinnamaldehyde that elicited two or more coughs (C_2). (b) Cough reflex threshold expressed as the log transformation of the lowest concentration of cinnamaldehyde that elicited five or more coughs (C_5). Closed circles and error bars indicate the mean value and the standard deviation in each group, respectively.

the dosimeter method,[28] the cinnamaldehyde-induced cough response may also have a strong correlation between two methods. Although we recruited only male subjects in our protocol, Birrell et al. recruited both males and females.[9] Therefore, since the cough reflex is known to be modified by the gender factor,[24] the difference in cinnamaldehyde-induced cough between our study and the experiment of Birrell et al. probably resulted from the gender factor.

In experimental animals (e.g., guinea pigs), chronic exposure of the airways to cigarette smoke induced cough hypersensitivity to various tussive inhalation challenges. In

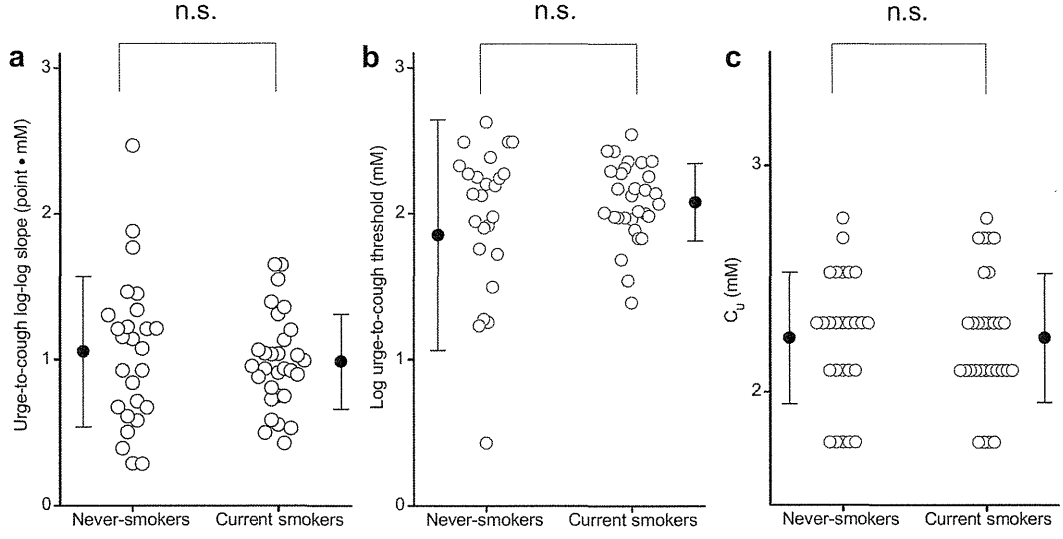

Figure 4 Comparisons of urge-to-cough induced by cinnamaldehyde between never-smokers and current smokers. (a) The urge-to-cough log–log slope by linear regression between cinnamaldehyde concentration and log Borg scores. (b) The urge-to-cough threshold estimated by log cinnamaldehyde concentration at the log Borg score of urge-to-cough = 0. (c) The C_U induced by cinnamaldehyde indicates the concentration of cinnamaldehyde provoking perception of urge-to-cough without associated motor cough. Open circles indicate the value of each subject. Closed circles and error bars indicate the mean value and the standard deviation in each group, respectively. n.s. denotes not significant.

spite of this, there have been conflicting results regarding the changes in cough reflex sensitivity to capsaicin among human chronic smokers.[3–7,16] It is widely reported that capsaicin- and citric acid-induced cough reflex sensitivities in current smokers were significantly lower than those of never-smokers. In the present study, we demonstrated that capsaicin-induced cough reflexes in current smokers were significantly lower than those of never-smokers. However, the underlying mechanisms for the down-regulation of cough reflex sensitivity to capsaicin in current smokers are not fully understood.

Although cough is usually referred to as a reflex controlled by the brainstem, coughing can also be controlled via the higher cortical center and be related to cortical modulations.[29] Therefore, depression of the cough reflex could be due to both the cortical facilitatory pathway for cough and the medullary reflex pathway. Since the urge-to-cough is a brain component of the cough motivation-to-action system,[30] depressed urge-to-cough in capsaicin-evoked cough suggests impairment of motivation and the reward pathway for cough, which is located in the supra-medulla.

Unpleasant respiratory sensations such as the urge-to-cough can be the result of sensory activation of subcortical and cortical neural pathways. Some of these pathways are shared across respiratory modalities while activation of some neural areas are modality specific.[31] Brain imaging studies have shown that ascending cough sensory information recruits neuronal activity in a variety of higher order brain regions, including primary sensory, insula, cingulate, premotor, motor, and orbitofrontal cortices.[32,33] These regions likely contribute to the perceptual awareness of airway irritation by TRPV1 and TRPA1 stimulations, and the resulting cognitive, emotional, and behavioral consequences that arise (unpleasantness, anxiety, spatial awareness, and the motivational drive to respond). In addition, since it has been reported that nicotine acts on central neural mechanisms as an anxiolytic,[15] primary sensory, insula, cingulate, premotor, motor, and orbitofrontal cortices could be suppressed by nicotine. It must still be clarified whether the central processing of afferent neural input from TRPA1 on airway nerve ending is consistent with that from TRPV1.

In the present study, we demonstrated that cinnamaldehyde-evoked cough motor responses and urge-to-cough were similar in never-smokers and current smokers (Figure 3a–c). These phenomena have not been reported in other studies of induced cough and urge-to-cough in smokers.[16,17] Reflex cough is initiated by sensory stimulation from peripheral afferents.[34–36] These afferents are known to project to the cerebral cortex, affective and cognitive centers.[31,33] Thus, since nicotine can play a role in the central neural mechanism for cough, it seems to facilitate the down-regulation factor of the cough reflex in response to cinnamaldehyde due to the depression of the cerebral cortex, and the affective and cognitive centers.

However, although there are various chemical components among the approximately 5000 constituents of cigarette smoke, recent studies have found that nicotine, reactive oxygen species, and α,β-unsaturated aldehydes included in cigarette smoke directly activate TRPA1 but not TRPV1 channels.[11–13] In addition to these findings, Talavera et al. have reported that TRPV1 is inhibited by nicotine.[12] Taken together, it is indicated that TRPA1 is more likely to cause up-regulation of peripheral afferent activity for cough by smoking.

Stimulation of these afferents is also the first step in eliciting the cough sensation that precedes the motor action of cough. Thus, since the cough reflex in current smokers can be modified by down-regulation of the central neural facilitatory pathway for cough, the dissociation between the enhanced peripheral afferent activity and the depression of central neural facilitatory pathway is related to be results of the cinnamaldehyde-induced cough responses that are similar in never-smokers and current smokers.

It has not been fully elucidated whether there is cooperation between TRPV1 and TRPA1 channels. The dependence of TRPA1 on Ca^{2+} may result in the desensitization of TRPA1 channels by decreased intracellular Ca^{2+} in the locale of desensitized TRPV1 channels.[38] However, since the selective TRPV1 antagonist could not inhibit the cough response induced by the TRPA1 selective agonist in animals, whether this sort of cooperation exists in generating a cough reflex has yet to be clarified.[8]

Finally, in the present study, our data show that the $\log C_5$ value for cinnamaldehyde in all subjects is a relatively high concentration compared with that of capsaicin. Several evidences have been presented indicating that expression of TRPA1 restricts a small sub-population of neurons involved in cough.[39,40] As shown by existing results, including experimental studies (naïve animals),[8,9] TRPA1 activation initiates cough that is relatively modest compared to the cough initiated by the TRPV1 activation (increased concentration TRPA1 agonist vs. TRPV1 agonist). Taken together, this is likely due to the lower efficacy of TRPA1 stimulation to induce sustained activation of the cough-triggering airway afferent nerves.

The present study shows that smoking has differential effects on cough responses to TRPV1 and TRPA1 stimulations. Smoking inhibits TRPV1-induced but not TRPA1-induced cough, suggesting that the effect of smoking status on cough reflex sensitivity is dependent on tussigen. Cigarette smoking is a risk factor for diverse respiratory diseases.[37] Our study suggests that we should pay attention to the smoking status and the receptor responsible for challenged tussigen in order to assess the cough reflex sensitivities in respiratory disease.

Conflict of interest statement

All the authors declare that they have no competing interests that might be perceived to influence the results and discussion reported in the present manuscript.

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Urge-to-cough and dyspnea conceal perception of pain in healthy adults

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ABSTRACT

Although dyspnea has been shown to attenuate pain, whether urge-to-cough, a respiratory sensation preceding cough, exerts a similar inhibitory effect on pain has not been clarified. We examined the effects of both urge-to-cough and dyspnea on pain induced by thermal noxious stimuli. Urge-to-cough was induced by citric acid challenge and dyspnea was induced by external inspiratory resistive loads. During inductions of two respiratory sensations, perception of pain was assessed by thermal pain threshold (TPTh) and tolerance (TPTo). TPTh and TPTo were significantly increased accompanied by increases in perception of both urge-to-cough and dyspnea. Fractional change in TPTh during dyspnea was significantly correlated with that during urge-to-cough. Fractional change in TPTo during dyspnea was significantly correlated with that during urge-to-cough. The study suggests that both two distinct respiratory sensations, i.e., urge-to-cough and dyspnea may harbor perception of pain. Further studies investigating interactions among these sensations in clinical settings are warranted.

1. Introduction

Subjective symptom usually plays a critical role in the diagnosis, management and prevention of disease states. Pain, dyspnea and cough, as three debilitating troubling symptoms, frequently coexist in many clinical situations (Gracely et al., 2007; Hastings et al., 2009). If cough and dyspnea, as common symptoms, are prominent, symptom of pain may be overlooked and result in a delay of diagnosis and treatment (Hastings et al., 2009; Oster and Bindman, 2003; Goldberg et al., 2010). Since a number of analogies exist among pain, dyspnea and cough (Gracely et al., 2007), it is of importance in clinical settings to know whether perceptual interactions exist among these three symptoms. However, our knowledge about interactions regarding their perception is seriously sparse.

Although dyspnea are unpleasant respiratory sensation, cough is a motor action typically preceded by a respiratory sensation such as an awareness of an irritating stimulus and is perceived as a need to cough, termed the urge-to-cough (Davenport et al., 2002). Urge-to-cough is a component of the brain motivation system that mediates the cognitive responses of cough stimuli (Davenport, 2008). Cough reflex sensitivity is severely diminished during general anesthesia and sleep (Canning et al., 2004; Nishino et al., 1990). In patients with aspiration pneumonia, both the cough reflex and the cognition of cough are significantly impaired (Yamanda et al., 2008). These studies suggest that the initiation of a reflex cough response is facilitated by the cognition of the urge-to-cough.

Both the urge-to-cough and dyspnea are uncomfortable respiratory sensations. The perceptions of urge-to-cough and dyspnea may share common pathways and somatosensory areas (Gracely et al., 2007). It has been shown that dyspnea has an inhibitory influence on the pain sensation (Morélot-Panzini et al., 2007; Nishino et al., 2008). Whether urge-to-cough, also exerts a similar inhibitory effect on pain has never been tested. Moreover, recent brain imaging studies have shown that cortical regions activated by both the perceptions of pain and urge-to-cough are similar to each other, suggesting that there may be some neurophysiological links between them (Mazzone et al., 2007; Farrell et al., 2005).

In the present study, we therefore investigated pain perception in response to the cognition of urge-to-cough using citric acid as a tussive stimulus. Furthermore, in order to compare with another respiratory sensation, we also investigated the response of thermal pain perception to dyspnea during external inspiratory resistive loads.

2. Method

2.1. Subjects

Forty-eight healthy never-smokers (29 males and 19 females) were originally recruited through public postings in and around

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