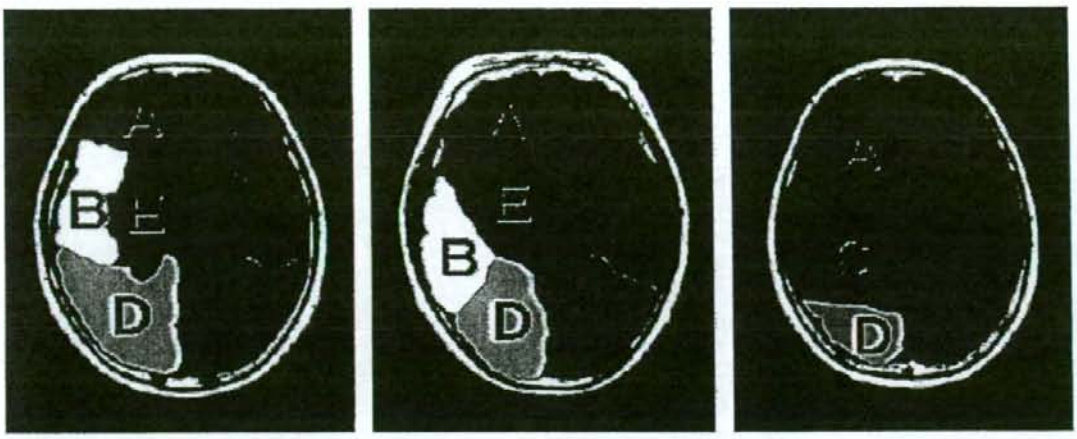

Figure 3 Evaluation of deep white matter hyperintensities (DWMH). DWMH were evaluated in the (A) frontal, (B) temporal, (C) parietal and (D) occipital lobes, and (E) in the basal ganglia in both hemispheres. Each lesion was rated as three grades according to diameter by the method of de Groot *et al.*: (1) 1–3 mm; (2) 3–10 mm; and (3) >10 mm.[4] The sum of all grades in five regions in both hemispheres was defined as the DWMH score.

Periventricular hyperintensity score or DWMH score was compared between subjects who did or did not exhibit each symptom of geriatric syndrome and analyzed by Student's *t*-test. When the difference was considered to be significant ($P < 0.05$), the difference was further assessed by means of multivariate logistic regression analysis with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease.

Ethical considerations

This study was approved by the ethical committees of the institutes involved in this project. We explained this study clearly, and obtained written consent from all participants and their guardians (mainly family members). All the data were stored and analyzed carefully to preserve the subjects' anonymity and protect their privacy.

Results

Clinical data

The clinical characteristics of the study subjects are shown in Table 1. The mean age of subjects was 74.5 ± 7.8 years (mean ± SD), and subjects aged 65 or older comprised 88.1%. The mean body mass index was 21.8 ± 3.3 kg/m^2 and none of the subjects were obese. Of the subjects, 10.1% had experienced stroke or other cerebrovascular disease and 22.7% were smokers. Hypertension, diabetes and hyperlipidemia were present in 50.7%, 27.3% and 50.0% of the subjects, respectively.

White matter lesions

Periventricular hyperintensities and DWMH were observed in 77.7% and 96.7% of the total subjects, respectively. The mean score of PVH and DWMH was 5.5 ± 4.8 and 35.5 ± 39.8, respectively (Table 1). Pearson's correlation analysis showed a strong positive correlation between PVH score and DWHM score ($r = 0.56$, $P < 0.0001$). In relation to aging, a positive correlation was found between PVH score and age ($r = 0.34$, $P < 0.0001$), and between DWMH score and age ($r = 0.28$, $P < 0.0001$).

Cognitive and psychological assessment

The mean score of MMSE, GDS-15 and vitality index was 23.1 ± 5.3, 5.0 ± 3.5 and 9.4 ± 1.2 points, respectively, indicating that the subjects showed cognitive decline, depression and decreased vitality, all to a mild extent. Given that a score of 23 or below on MMSE is regarded as the presence of cognitive impairment,[19] 47.5% of the subjects fell into this category. The causes of cognitive impairment were Alzheimer disease (AD; 53.3%), vascular dementia (VaD; 16.4%), combined dementia of AD and VaD (9.0%) and other types of dementia (21.3%). Pearson's correlation analysis revealed a negative correlation between PVH score and MMSE, PVH score and vitality index, DWMH score and MMSE, and DWMH score and vitality index,

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Table 1 Clinical characteristics of study subjects

	Prevalence ($n = 286$)	Mean ± standard deviation
Clinical characteristics		
Age (years)		74.5 ± 7.8
Women (%)	74.0	
Height (m)		1.55 ± 0.08
Bodyweight (kg)		52.4 ± 10.6
Body mass index (kg/m^2)		21.8 ± 3.3
Systolic blood pressure (mmHg)		135.3 ± 20.2
Diastolic blood pressure (mmHg)		76.3 ± 11.8
Prevalence of complications		
Hypertension (%)	50.7	
Diabetes (%)	27.3	
Hyperlipidemia (%)	50.0	
Past history of cerebrovascular disease (%)	10.1	
Smoking (%)	22.7	
Cognitive and psychological assessment		
Mini-Mental State Examination (0–30 points)		23.1 ± 5.3
Geriatric depression scale (0–15 points)		5.0 ± 3.5
Vitality index (0–10 points)		9.4 ± 1.2
White matter lesions		
Periventricular hyperintensities (points)	5.5 ± 4.8	
Deep white matter hyperintensities (points)	35.5 ± 39.8	

Table 2 Relationship between white matter lesions and global cognition (MMSE), depressive state (GDS-15) and vitality (vitality index)

	Linear regression	
	PVH score	DWMH score
MMSE	−0.380**	−0.272**
GDS-15	0.022	−0.066
Vitality index	−0.432**	−0.184*

Univariate linear regression analysis: *$P < 0.01$, **$P < 0.0001$. DVMH, deep white matter hyperintensity; GDS-15, 15-item Geriatric Depression Scale; MMSE, Mini-Mental State Examination; PVH, periventricular hyperintensity.

respectively (Table 2). It was also found that calculation (serial subtraction of 7 from 100) was negatively correlated with PVH score ($r = -0.156$, $P = 0.04$, data not shown), and verbal fluency (naming as many vegetables as possible) was negatively correlated with PVH score ($r = -0.216$, $P < 0.01$, data not shown). On the other hand, no significant correlation was found between PVH score and GDS-15, or between DWMH score and GDS-15. Multiple logistic analysis revealed that PVH score and DWMH score remained significant determinants of cognitive impairment (MMSE, ≤23) and low vitality (vitality index, ≤9) after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 3).

One hundred and ninety subjects reported symptoms of geriatric syndrome. The frequency is shown in Table 4. Frequent symptoms (>20%) were tripping (32.1%), constipation (26.3%), gait disturbance (23.2%) and pollakiuria (22.1%). Student's *t*-test showed that PVH score was significantly greater in subjects who exhibited the following symptoms of geriatric syndrome: hallucinations, delusions, gait disturbance, tripping, falls, pollakiuria, urinary incontinence, weight loss, apathy, swallowing difficulty, tremor and muscle stiffness. Multiple logistic analysis revealed that PVH score remained a significant determinant of hallucinations, tripping, pollakiuria, urinary incontinence, weight loss, apathy and swallowing difficulty after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 5). By the same method, DWMH score was

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Table 3 Periventricular hyperintensity and deep white matter hyperintensity scores as determinants of cognitive impairment and low vitality

	PVH score			DWMH score		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Cognitive impairment	1.185	1.084–1.295	<0.001	1.010	1.001–1.021	<0.05
Low vitality	1.260	1.133–1.401	<0.0001	1.025	1.012–1.039	<0.001

Cognitive impairment and low vitality were defined as MMSE ≤23 and vitality index ≤9, respectively. Multiple logistic analysis was performed after adjustment for age, sex, hypertension, diabetes, hyperlipidemia, and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; DVMH, deep white matter hyperintensity; OR, odds ratio; PVH, periventricular hyperintensity.

significantly greater in subjects who exhibited the following symptoms of geriatric syndrome: hallucinations, delusions, gait disturbance, tripping, falls, pollakiuria, urinary incontinence and constipation. Multiple logistic analysis revealed that DWMH score remained a significant determinant of hallucinations, delusions, tripping, urinary incontinence and constipation after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 6).

Discussion

Elderly persons are affected by multiple chronic diseases. Once they are affected by serious illness, full recovery cannot be expected with medical treatment, because elderly patients are often trapped in a vicious circle of illness and poor quality of life (QOL). This is the reason why care and welfare contribute to the total well-being of the elderly. Physicians need to pay great attention to improving QOL as well as treating illness. Thus, it is important to comprehend the whole picture of their life by means of comprehensive geriatric assessment, which evaluates multiple aspects of an elderly person's life, such as activities of daily living, cognition, mood, vitality, communication and social environment.

The present study confirmed a negative correlation between the severity of WML and MMSE score. Multivariate analysis showed that the presence of WML was a significant risk factor for cognitive impairment, even after adjustment for confounding factors of age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease. The mechanism and the size and location of WML that impair cognitive function are not yet clear. However, from previous studies, it seems convincing that a reduction of blood flow in the frontal lobe plays an important role in cognitive impairment in elderly people who exhibit WML.[20,21] Clinical manifestations of WML include attention deficit and a decline in information-processing ability.[4,13,22] Junque *et al.* reported the reappearance of primitive reflexes, one of the symptoms of frontal lobe dysfunction, in patients with WML.[11] In this study, patients with PVH showed attention deficit (incapability of calculation) and verbal inarticulacy (naming less vegetables), implying the impairment of frontal lobe function. WML, as reported previously,[6,23] were negatively correlated with vitality. Multiple logistic regression analysis, using potential risk factors including advanced age as confounding variables, found that the presence of WML was an independent risk factor for low vitality. Additionally, a relation between PVH score and apathy, a significant symptom of geriatric syndrome, was also found. From previous studies showing the importance of frontal lobe function in vitality,[24–26] we assume that blood flow reduction in the frontal lobe may account for the apathy and low vitality in patients with WML. More precisely, WML disrupting the frontal-subcortical circuit may result in dysfunction in the anterior cingulate and dorsolateral prefrontal circuits, thereby leading to apathy and decreased vitality.[5,6,20] Increase in PVH score or DWMH score was not apparently correlated with depression, probably because depression is associated with many factors such as aging, female sex, hyperlipidemia and medication.[27–29] The subjects in this study were mostly elderly (88.1%) and female (74.0%). We assume that these confounding conditions made it difficult to prove a true relation between WML and depression. From analysis of the association of WML with geriatric syndrome, it appears that WML have a relation to psychiatric symptoms (hallucinations and delusions), gait abnormalities (gait disturbance, tripping and falls), urinary symptoms (pollakiuria and urinary incontinence) and possibly with parkinsonism (swallowing difficulty, tremor and muscle stiffness). It was reported that WML were related to gait abnormalities,[5–7] presumably caused by disruption of the frontal-subcortical circuit.[30] Some other studies suggested that parkinsonism is also a contributing factor to gait disturbance in patients with WML.[6,31] Interestingly, we found that both gait abnormalities and symptoms of parkinsonism were associated with WML.

The present study confirmed an association between WML and voiding dysfunction (pollakiuria and incontinence). It was reported that urinary dysfunction was derived from damage to the frontal-subcortical

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Table 4 Comparison of periventricular hyperintensity and deep white matter hyperintensity scores between subjects who did or did not exhibit each symptom of geriatric syndrome

Geriatric syndrome	Prevalence (%)	PVH score Symptom Present	Absent	*P*-value	DWMH score Symptom Present	Absent	*P*-value
Hallucination	6.8	**8.5 ± 5.9**	**4.4 ± 4.7**	**<0.01**	**59.8 ± 43.9**	**28.6 ± 35.4**	**<0.01**
Delusion	9.5	**7.6 ± 5.2**	**4.4 ± 4.8**	**0.01**	**56.1 ± 37.6**	**28.2 ± 35.9**	**<0.01**
Insomnia	18.9	4.2 ± 3.6	4.7 ± 4.9	0.56	31.4 ± 36.0	31.3 ± 37.6	0.98
Vertigo	18.9	6.1 ± 6.5	4.4 ± 4.4	0.06	33.4 ± 38.1	30.7 ± 37.0	0.70
Paralysis	2.1	8.5 ± 4.8	4.6 ± 4.9	0.12	59.5 ± 47.2	30.1 ± 36.3	0.11
Numbness	16.6	5.1 ± 4.6	4.6 ± 4.8	0.62	34.6 ± 40.0	29.9 ± 36,0	0.52
Gait disturbance	23.2	**6.7 ± 5.1**	**4.2 ± 4.7**	**<0.01**	**43.3 ± 41.7**	**27.5 ± 34.9**	**0.01**
Tripping	32.1	**6.4 ± 4.5**	**3.9 ± 4.9**	**<0.01**	**42.1 ± 43.7**	**25.9 ± 32.4**	**<0.01**
Falls	17.9	**6.6 ± 4.9**	**4.3 ± 4.8**	**0.01**	**45.8 ± 43.1**	**28.0 ± 35.0**	**0.01**
Pollakiuria	22.1	**8.0 ± 5.8**	**3.8 ± 4.2**	**<0.01**	**41.5 ± 41.0**	**41.5 ± 41.0**	**0.04**
Urinary incontinence	13.8	**7.5 ± 5.1**	**4.3 ± 4.8**	**<0.01**	**52.4 ± 44.9**	**52.4 ± 44.9**	**<0.01**
Constipation	26.3	5.8 ± 4.3	4.4 ± 5.1	0.08	**44.5 ± 45.1**	**44.5 ± 45.1**	**<0.01**
Decreased appetite	14.7	6.1 ± 4.4	4.5 ± 5.0	0.12	42.1 ± 42.6	42.1 ± 42.6	0.11
Weight loss	14.2	**6.9 ± 4.1**	**4.4 ± 5.0**	**0.01**	40.7 ± 41.3	40.7 ± 41.3	0.15
Apathy	7.6	**7.4 ± 3.6**	**4.4 ± 5.0**	**0.03**	30.7 ± 28.1	30.7 ± 28.1	0.97
Speech impairment	2.7	5.6 ± 5.2	4.5 ± 4.7	0.62	35.3 ± 48.0	35.3 ± 48.0	0.80
Swallowing difficulty	14.7	**12.2 ± 4.4**	**4.5 ± 4.8**	**<0.01**	44.6 ± 34.6	44.6 ± 34.6	0.40
Tremor	5.3	**9.1 ± 6.5**	**4.4 ± 4.7**	**<0.01**	45.0 ± 38.1	45.0 ± 38.1	0.24
Muscle stiffness	3.2	**9.2 ± 4.8**	**4.5 ± 4.9**	**0.02**	48.7 ± 43.4	48.7 ± 43.4	0.23

PVH and DWMH score are shown as mean ± SD. Boldface values are statistically significant ($P < 0.05$ by Student's *t*-test). DVMH, deep white matter hyperintensity; PVH, periventricular hyperintensity.

Table 5 Periventricular hyperintensity score as determinant of geriatric syndrome

	OR	*P*-value	95% CI
Hallucination	1.12	0.043	1.004–1.248
Tripping	1.11	0.005	1.032–1.194
Pollakiuria	1.17	0.001	1.067–1.278
Urinary incontinence	1.11	0.022	1.015–1.207
Weight loss	1.14	0.007	1.036–1.246
Apathy	1.14	0.027	1.015–1.276
Swallowing difficulty	1.35	0.019	1.050–1.741

Multiple logistic analysis was performed to analyze each symptom of geriatric syndrome, with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; OR, odds ratio.

Table 6 Deep white matter hyperintensity score as determinant of geriatric syndrome

	OR	*P*-value	95% CI
Hallucination	1.017	0.020	1.003–1.032
Delusion	1.016	0.024	1.002–1.030
Tripping	1.011	0.020	1.002–1.020
Urinary incontinence	1.016	0.008	1.004–1.028
Constipation	1.011	0.025	1.001–1.021

Multiple logistic analysis was performed to analyze each symptom of geriatric syndrome, with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; OR, odds ratio.

circuit.[5,20] In relation to the symptoms of parkinsonism (swallowing difficulty, tremor and muscle stiffness), this association was previously explained by dysfunction of the frontal-subcortical circuit.[6,31] The importance of this lesion was also suggested by a study showing that swallowing difficulty occurs with dysfunction of internuncial neurons that link the brainstem to the cerebral cortex.[32]

Considering the cause of manifestation of geriatric syndrome in patients with WML, it appears that damage to associative pathways in the frontal and subcortical regions due to ischemic hypoperfusion is an important mechanism.[5,20,21] It is necessary to localize the responsible connecting pathway for each symptom by a sophisticated approach in the future.

In conclusion, we showed that WML were associated with cognitive impairment, low vitality and geriatric syndrome of psychological disorders, gait disturbance,

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urinary problems and parkinsonism. Evaluating WML in relation to geriatric syndrome and building a preventive measure against WML is an important future task for maintaining the independence of elderly people.

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References

1 Breteler MM, van Swieten JC, Bots ML *et al.* Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. *Neurology* 1994; **44**: 1246–1252.
2 Hachinski VC, Potter P, Merskey H. Leuko-araiosis. *Arch Neurol* 1987; **44**: 21–23.
3 Hunt AL, Orrison WW, Yeo RA *et al.* Clinical significance of MRI white matter lesions in the elderly. *Neurology* 1989; **39**: 1470–1474.
4 de Groot JC, de Leeuw FE, Oudkerk M *et al.* Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Ann Neurol* 2000; **47**: 145–151.
5 Kuo HK, Lipsitz LA. Cerebral white matter changes and geriatric syndromes: is there a link? *J Gerontol A Biol Sci Med Sci* 2004; **59**: 818–826.
6 Starkstein SE, Sabe L, Vazquez S *et al.* Neuropsychological, psychiatric, and cerebral perfusion correlates of leuko-araiosis in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 1997; **63**: 66–73.
7 Baloh RW, Ying SH, Jacobson KM. A longitudinal study of gait and balance dysfunction in normal older people. *Arch Neurol* 2003; **60**: 835–839.
8 Sakakibara R, Hattori T, Uchiyama T, Yamanishi T. Urinary function in elderly people with and without leukoaraiosis: relation to cognitive and gait function. *J Neurol Neurosurg Psychiatry* 1999; **67**: 658–660.
9 Tarvonen-Schroder S, Roytta M, Raiha I, Kurki T, Rajala T, Sourander L. Clinical features of leuko-araiosis. *J Neurol Neurosurg Psychiatry* 1996; **60**: 431–436.
10 Pantoni L, Garcia JH. The significance of cerebral white matter abnormalities 100 years after Binswanger's report. *Stroke* 1995; **26**: 1293–1301.
11 Junque C, Pujol J, Vendrell P *et al.* Leuko-araiosis on magnetic resonance imaging and speed of mental processing. *Arch Neurol* 1990; **47**: 151–156.
12 Fazekas F. Magnetic resonance signal abnormalities in asymptomatic individuals: their incidence and functional correlates. *Eur Neurol* 1989; 29: 164–168.
13 Ylikoski R, Ylikoski A, Erkinjuntti T, Sulkava R, Raininko R, Tilvis R. White matter changes in healthy elderly persons correlate with attention and speed of mental processing. *Arch Neurol* 1993; **50**: 818–824.
14 Fu JH, Lu CZ, Hong Z, Dong Q, Luo Y, Wong KS. Extent of white matter lesions is related to acute subcortical infarcts and predicts further stroke risk in patients with first ever ischaemic stroke. *J Neurol Neurosurg Psychiatry* 2005; **76**: 793–796.
15 Taylor WD, MacFall JR, Provenzale JM *et al.* Serial MR imaging of volumes of hyperintense white matter lesions in elderly patients: correlation with vascular risk factors. *Am J Roentgenol* 2003; **181**: 571–576.
16 Folstein MF, Folstein SE, McHugh PR. "Mini-Mental State". A practical method for grading the cognitive state of patients for the clinician. *J Psychiat Res* 1975; **12**: 189–198.
17 Sheikh JI, Yesavage JA. Geriatric Depression Scale (GDS): recent evidence and development of a short version. *Clin Gerontol* 1986; **56**: 165–173.
18 Toba K, Nakai R, Akishita M *et al.* Vitality index as a useful tool to assess elderly with dementia. *Geriatr Gerontol Int* 2002; **2**: 23–29.
19 Cullen B, Fahy S, Cunningham CJ *et al.* Screening for dementia in an Irish community sample using MMSE: a comparison of norm-adjusted versus fixed cut-points. *Int J Geriatr Psychiatry* 2005; **20**: 371–376.
20 Pugh KG, Lipsitz LA. The microvascular frontal-subcortical syndrome of aging. *Neurobiol Aging* 2002; **23**: 421–431.
21 Yao H, Sadoshima S, Kuwabara Y, Ichiya Y, Fujishima M. Cerebral blood flow and oxygen metabolism in patients with vascular dementia of the Binswanger type. *Stroke* 1990; **21**: 1694–1699.
22 Burton EJ, Kenny RA, O'Brien J *et al.* White matter hyperintensities are associated with impairment of memory, attention, and global cognitive performance in older stroke patients. *Stroke* 2004; **35**: 1270–1275.
23 Thomas P, Hazif-Thomas C, Saccardy F, Vandermarq P. Loss of motivation and frontal dysfunction. Role of the white matter change. *Encephale* 2004; **30**: 52–59.
24 Okada K, Kobayashi S, Yamagata S, Takahashi K, Yamaguchi S. Poststroke apathy and regional cerebral blood flow. *Stroke* 1997; **28**: 2437–2441.
25 Craig AH, Cummings JL, Fairbanks L *et al.* Cerebral blood flow correlates of apathy in Alzheimer disease. *Arch Neurol* 1996; **53**: 1116–1120.
26 Benoit M, Koulibaly PM, Migneco O, Darcourt J, Pringuey DJ, Robert PH. Brain perfusion in Alzheimer's disease with and without apathy: a SPECT study with statistical parametric mapping analysis. *Psychiatry Res* 2002; **15**: 103–111.
27 Stordal E, Mykletun A, Dahl AA. The association between age and depression in the general population: a multivariate examination. *Acta Psychiatr Scand* 2003; **107**: 132–141.
28 Terao T, Iwata N, Kanazawa K *et al.* Low serum cholesterol levels and depressive state in human dock visitors. *Acta Psychiatr Scand* 2000; **101**: 231–234.
29 Noble RE. Depression in women. *Metabolism* 2005; **54**: 49–52.
30 Hennerici MG, Oster M, Cohen S, Schwartz A, Motsch L, Daffertshofer M. Are gait disturbances and white matter degeneration early indicators of vascular dementia? *Dementia* 1994; **5**: 197–202.
31 Piccini P, Pavese N, Canapicchi R *et al.* White matter hyperintensities in Parkinson's disease. Clinical correlations. *Arch Neurol* 1995; **52**: 191–194.
32 Daniels SK, Foundas AL. Lesion localization in acute stroke patients with risk of aspiration. *J Neuroimaging* 1999; **9**: 91–98.

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β_2-Adrenergic receptor regulates Toll-like receptor-4-induced nuclear factor-κB activation through β-arrestin 2

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Summary

Toll-like receptors (TLRs) play an important role in innate immunity while, β_2-adrenergic receptors (β_2AR) provide the key linkages for the sympathetic nervous system to regulate the immune system. However, their role in macrophages remains uncertain. Here, we demonstrate the cross-talk between β_2AR and TLR signalling pathways. Expression of β_2AR was down-regulated by TLR4 ligand lipopolysaccharide (LPS) stimulation. To investigate the physiological consequence of this down-regulation RAW264 cells, a macrophage cell line, were transfected with a β_2AR expression vector (RAWar). Both LPS-stimulated inducible nitric oxide synthase (NOS II) expression and NO production were markedly suppressed in the RAWar cells. The activation of nuclear factor-κB (NF-κB) and degradation of the inhibitor of NF-κB (IκBα) in response to LPS were markedly decreased in these cells. The level of β-arrestin 2, which regulates β_2AR signalling, was also reduced in RAW264 cells after stimulation with LPS, but not in RAWar cells. Overexpression of β-arrestin 2 (RAWarr2) also inhibited NO production and NOS II expression. Furthermore, we demonstrated that β-arrestin 2 interacted with cytosolic IκBα and that the level of IκBα coimmunoprecipitated by anti-β-arrestin 2 antibodies was decreased in the RAW264 cells but not in RAWar or RAWarr2 cells. These findings suggest that LPS-stimulated signals suppress β_2AR expression, leading to down-regulation of β-arrestin 2 expression, which stabilizes cytosolic IκBα and inhibits the NF-κB activation essential for NOS II expression, probably to ensure rapid and sufficient production of NO in response to microbial attack.

Keywords: β_2-adrenergic receptor; monocytes/macrophages; nitric oxide; nuclear factor-κB; toll-like receptor

Introduction

The ability of the innate immune system to recognize and respond to microbial components has been chiefly attributed to a family of type I transmembrane receptors termed Toll-like receptors (TLRs) that are expressed abundantly on antigen-presenting cells such as macrophages and dendritic cells and can discriminate among the distinct molecular patterns associated with microbial components.[1,2] The TLR-initiated activation of nuclear factor-κB (NF-κB) is essential for the regulation of inducible nitric oxide synthase (NOS II) and several proinflammatory cytokines, which are produced in response to invading pathogens. The NO produced by NOS II has a number of important biological functions, including roles in host defence against intracellular pathogens and tumour-cell killing. Although this basic definition is still accepted, over the past decade NO has been shown to play a much more diverse role not only in the immune system but also in other organ systems, including both beneficial and detrimental effects.[3,4] For example, the systemic inflammatory response syndrome, which includes

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severe septic shock and multiple organ system failure, remains a leading cause of death in critically ill patients. Therefore, it is necessary to clarify the molecular mechanisms of TLR-initiated signalling that lead to NO production in response to microbial components.

Nuclear factor-κB is found predominantly in the cytoplasm complexed with members of the inhibitor of NF-κB (IκB) family. The release of NF-κB from IκB proteins is an essential step in the generation of transcriptionally competent NF-κB. The consensus is that IκB proteins mask the nuclear localization signals of NF-κB proteins, thereby regulating NF-κB activity, primarily by limiting their nuclear translocation. Recent studies, however, have indicated that IκBα is detected in both the nucleus and cytoplasm and that although the NF-κB complexes shuttle between the nucleus and cytoplasm under all conditions, they are unable to bind DNA because of their association with proteins of the IκB family.[5–7] Nuclear IκBα is not sensitive to signal-induced degradation. Therefore, following stimulation, NF-κB activities are dependent on the level of cytoplasmic NF-κB/IκBα complexes.

Recently, we demonstrated that the level of β_2-adrenergic receptor (β_2AR) expression influences TLR4 signalling.[8] β_2AR is a member of a family of G protein-coupled receptors (GPCRs) and is the key link involved in immune system regulation via the sympathetic nervous system.[9,10] Primary and secondary lymphoid organs, such as the thymus, spleen and lymph nodes, receive extensive sympathetic/noradrenergic innervation, and lymphocytes, macrophages and many other immune cells bear functional β_2AR. Therefore, β_2AR stimulation regulates pro-inflammatory cytokine production, lymphocyte traffic and proliferation, and antibody secretion through cyclic adenosine monophosphate (cAMP) generation and protein kinase A (PKA) activation.[10,11] However, the role of β_2AR in the TLR signalling pathway in macrophages remains vague. On the other hand, arrestins are cytosolic proteins that play a critical role in the regulation of GPCR signalling.[12,13] Recent studies have shown that they also interact with their partner molecules in a variety of signalling pathways, including NF-κB signalling.[14–16] In the present study, we investigated the physiological consequence of the down-regulation of β_2AR expression in macrophages and analysed the cross-talk between the signalling of β_2AR and TLRs.

Materials and methods

Cell culture

The murine macrophage cell line RAW264 (RCB0535) was purchased from RIKEN Cell Bank (Ibaraki, Japan) and cultured as described in our previous study.[17] The cells were stimulated with 1 μg/ml lipopolysaccharide (LPS) from *Escherichia coli* 055 (Sigma-Aldrich, St Louis, MO). Cell viability was assessed using the trypan blue dye exclusion test and cell size was measured by flow cytometric analysis of forward light scatter characteristics using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA).

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared as described elsewhere.[18] The NF-κB oligonucleotide probe (5′-AGT TGA GGG GAC TTT CCC AGG-3′) was purchased from Promega (Madison, WI) and labelled with biotin at its 3′ end. The nuclear protein (2 μg) and excess amounts of labelled oligonucleotide probes were incubated in 20 μl EMSA buffer [20 mM HEPES, pH 7·6, 10 mM $(NH_4)_2SO_4$, 1 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid (EDTA), 0·2% Tween, 30 mM KCl, 1 μg poly (dI-dC), 1 μg poly L-lysine] at room temperature for 15 min, electrophoresed in 7% polyacrylamide gels, transferred onto the Biodyne Plus Membane (Pall BioSupport Division, Port Washington, NY), and cross-linked in ultraviolet light. To detect signals, the blots were incubated with streptavidin–horseradish peroxidase conjugate in a blocking reagent for 15 min and with a chemiluminescent reagent for 5 min. The blots were then exposed to Kodak X Omat AR film (GE Healthcare Bio-Science, Piscataway, NJ).

Western blotting analysis

Cell membrane proteins were prepared using the Plasma Membrane Protein Extraction Kit (Bio Vision, Mountain View, CA). Cytoplasmic protein extracts were prepared as described previously (30). The protein concentration was determined using the Bradford reagent (BioRad, Hercules, CA), and equal amounts of membrane proteins or cytoplasmic proteins were loaded. The samples were separated by 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred on to polyvinylidene difluoride membranes (Applied Biosystems, Foster City, CA). The membranes were blocked with 10% non-fat dried milk in Tris-buffered saline and incubated with goat polyclonal antibodies against β_2AR, goat polyclonal antibodies against β-arrestin 2, or rabbit polyclonal antibodies against IκBα and NOS II (Santa Cruz Biotechnology, Santa Cruz, CA); this was followed by incubation with appropriate secondary antibodies (horseradish peroxidase-conjugated rabbit anti-goat or goat anti-rabbit immunoglobulin G; Dako, Kyoto, Japan). To ensure equal protein loading, the membranes were incubated with rabbit anti-actin or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotechnology) for the detection of cytoplasmic or cell surface GAPDH[19] after stripping. Immunoreactivity was visualized using an enhanced chemiluminescence reagent (ECL; GE Healthcare Bio-Science).

Immunoprecipitation

The cells were lysed with lysis buffer (20 mM Tris–HCl, pH 7·6, 150 mM NaCl, 2 mM EDTA, 0·5% Nonidet P-40 and protease inhibitors). The samples were clarified by centrifugation at 21 000 *g* at 4° for 30 min. The protein concentration was determined using the Bradford reagent (Bio-Rad). β-Arrestin 2 was immunoprecipitated with anti-β-arrestin 2 monoclonal antibodies (Santa Cruz Biotechnology) from equal samples, followed by treatment with 10 μl protein G–Sepharose beads (GE Healthcare Bio-Science). After extensive washing, the complexes were analysed by SDS–PAGE and Western blotting by using rabbit polyclonal antibodies against IκBα.

Determination of nitrite concentration

Nitrite in the cell culture supernatants was measured using the assay system of Ding *et al.*[20] The nitrite concentration was calculated by comparison with sodium nitrite, which was used as a standard. In some experiments, 200 μM pyrrolidine dithiocarbamate (PDTC, Sigma) was added to the cultures.

Determination of intracellular cAMP concentration

Cells were cultured with or without LPS for 6 hr and were stimulated with Salbutamol (1×10^{-6} M) for the final 30 min. Cell supernatants were then removed and cells were lysed. Intracellular cAMP was determined with a commercially available enzyme immunoassay (GE Healthcare Bio-Science).

Real-time polymerase chain reaction (PCR)

Total cellular RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany), and aliquots of 2 μg were reverse-transcribed with ReverScript I (Wako Pure Chemical Industries, Osaka, Japan) and an oligo-dT(15-mer) (Roche Diagnostics, Indianapolis, IN) at 42° for 50 min. The complementary DNAs (cDNAs) were amplified by PCR under the following conditions using the oligonucleotide primers and cycles listed in Table 1: 94° for 30 seconds, 55° for 30 seconds, and 72° for 30 seconds for NOS II and 18S ribosomal RNA (rRNA), and 94° for 30 seconds, 60° for 30 seconds, and 72° for 30 seconds for total and transfected β_2AR and β-arrestin 2. The quantity of the cDNA template included in these reactions and the number of amplification cycles were optimized to ensure that the reactions were stopped during the linear phase of product amplification, thus permitting semiquantitative comparisons of messenger RNA (mRNA) abundance between different RNA preparations.

β_2AR and β-arrestin 2 plasmid constructs and stable transfection

Full-length murine β_2AR (*$\beta_2$ar*) and β-arrestin 2 (*βarrestin2*) cDNAs were obtained by PCR using the primers 5′-GCTGAATGAAGCTTCCAGGA-3′ (sense) and 5′-GCCTGTATTACAGTGGCGAG-3′ (antisense) for β_2AR and 5′-GGCGGGCGGAGGGCGGCGAG-3′ (sense) and 5′-CGTCCTAGCAGAACTGGTCA-3′ (antisense) for β-arrestin 2. The amplified β_2AR and β-arrestin 2 fragments were subcloned into the pGEM-T Easy vector (Promega) and then into *Not*I-digested pcDNA4 (Invitrogen, Carlsbad, CA). The amplified PCR products were sequenced using an automatic DNA sequencer (Applied Biosystems). The plasmid DNA used for transfection was prepared using the EndoFree Plasmid Kit (Qiagen). RAW264 cells were transfected with the pcDNA4 vector, pcDNA4-$\beta_2$ar, or pcDNA4-βarrestin2 using LipofectAMINE Reagent (Invitrogen). Selection was initiated in a medium containing 500 μg/ml Zeocine (Invitrogen).

Luciferase assays

The full-length murine NOS II promoter fragment was cloned into the pGL3-enhancer luciferase reporter gene vector (Promega) (pGL3-NOS II) as described previously.[21] RAW264 cells were transfected using the LipofectAMINE Reagent with constructs containing the luciferase reporter gene, and luciferase activity was determined using the Dual Luciferase Assay System Kit (Promega) as described elsewhere.[21] Activity was normalized relative to an internal cotransfected constitutive control (*Renilla* luciferase expression vector, pRL-TK; Promega). In some

Table 1. Oligonucleotide sequences used for polymerase chain reaction

	Forward	Reverse	Cycle
β_2AR	GGAGCAGGATGGGCGGACGG	GCCTTCCATGCCTGGGGGAT	34
Transfected β_2AR	GGAGCAGGATGGGCGGACGG	TGGTGATGGTGATGATGACC	34
β-arrestin 2	GCAGCCAGGACCAGAGGACA	CCACGCTTCTCTCGGTTGTC	35
NOS II	CTTCCGAAGTTTCTGGCAGCAGCG	GAGCCTCGTGGCTTTGGGCTCCTC	26
18S	GAGAAACGGCTACCACATCC	CCCAAGATCCAACTACGAGC	26

β_2AR, β_2-adrenergic receptor; NOS II, nitric oxide synthase II.

experiments, RAW264 cells were transiently cotransfected with the NF-κB-responsive promoter reporter–luciferase construct pNF-κB-Luc (Clontech, Palo Alto, CA) or pGL3-NOS II and pcDNA4-$\beta_2$ar or IκBα dominant-negative vector pCMV-IκBαM (Clontech).

Statistical analysis

Student's *t*-test for unpaired samples was used to compare two means. For more than two groups, statistical significance of the data was assessed by analysis of variance. Where significant differences were found, individual comparisons were made between groups using the *t*-statistic and adjusting the critical value according to the Bonferroni method. Differences were considered significant at $P < 0{\cdot}05$. Data in the text and figures are expressed as means ± SEM.

Results

Preventing the down-regulation of β_2AR inhibits LPS-stimulated NOS II expression

Levels of both β_2AR protein and β_2AR mRNA were markedly decreased in RAW264 cells following LPS stimulation (Fig. 1a). To investigate the role of β_2AR down-regulation in response to LPS, a stable β_2AR transfectant (RAWar) and a vector control (RAWvec) were established. Although the levels of both β_2AR protein and mRNA expression were notably decreased in RAWvec cells following LPS stimulation, the down-regulation of β_2AR expression was prevented in the RAWar cells (Fig. 1b). The transfected β_2AR protein did not have a tag sequence capable of modifying β_2AR function so the protein levels of only transfected β_2AR could not be analysed. The mRNA levels of transfected β_2AR were low in unstimulated RAWar cells but markedly increased in the cells following LPS stimulation (Fig. 1c). In our previous study, we showed that the levels of both protein and mRNA of transfected cDNA cloned into the pcDNA4 vector were low in unstimulated RAW264 cells but were markedly increased in the cells following LPS stimulation.[17] Therefore, it appears that total β_2AR expression in unstimulated RAWar cells was not much higher than in RAWvec cells and that the decrease in intrinsic β_2AR expression in the LPS-stimulated RAWar cells was masked by the increased expression of transfected β_2AR as the result of the LPS stimulation. Although, the intracellular cAMP concentration in RAWar cells stimulated with salbutamol was similar to that in RAWvec cells, LPS

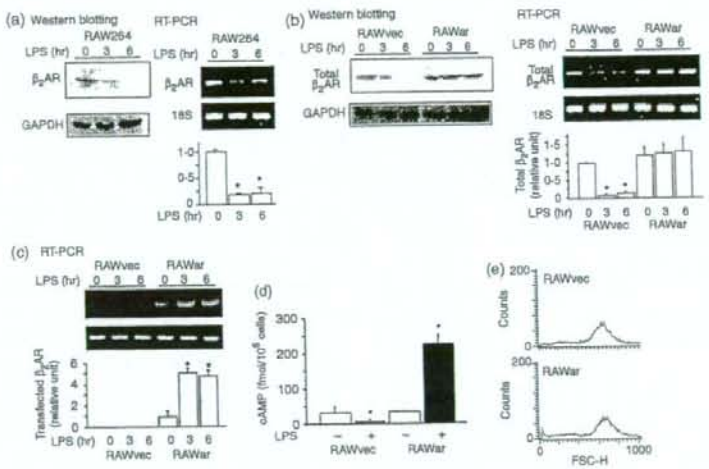

Figure 1. Lipopolysaccharide (LPS) stimulation down-regulates β_2-adrenergic receptor (β_2AR) expression. (a) RAW264 cells were stimulated with LPS. The protein levels of β_2AR and GAPDH (loading control) in the plasma membrane were analysed by Western blotting (left panel). The β_2AR messenger RNA (mRNA) and 18S ribosomal RNA (rRNA; loading control) were analysed by reverse transcription–polymerase chain reaction (RT-PCR; right upper panel). Bar graphs show the relative intensity of the PCR bands from three separate experiments (mean ± SEM) (right lower panel). *$P < 0{\cdot}01$ versus 0 hr. (b) RAW264 cells were transfected with the $\beta_2$ar construct or vector alone. The protein levels of β_2AR and GAPDH (left panel) and mRNA expressions of β_2AR and 18S rRNA (right upper panel) were analysed as in (a). Bar graphs show the relative intensities of the PCR bands from three separate experiments (mean ± SEM) (right lower panel). *$P < 0{\cdot}01$ versus 0 hr. (c) mRNA expressions of β_2AR and 18S rRNA (upper panel) were analysed as in (a). Bar graphs show the relative intensities of the PCR bands from three separate experiments (mean ± SEM) (lower panel). *$P < 0{\cdot}01$ versus 0 hr. (d) Cells were cultured with or without LPS for 6 hr and were stimulated with salbutamol (1×10^{-6} M) for the final 30 min. Then, intracellular cyclic AMP concentrations were analysed. *$P < 0{\cdot}05$ versus without LPS. (e) Cell size was measured by flow cytometric analysis of forward light scatter characteristics (FSC).

stimulation decreased the accumulation of intracellular cAMP in RAWvec cells but increased it in RAWar cells (Fig. 1d), suggesting that the transfected β_2AR was functionally active. Similar histograms of the distribution of forward light scatter characteristics were observed in RAWvec and RAWar cells, suggesting that the β_2AR transfection did not alter the cell size (Fig. 1e). In addition, cell viabilities were more than 98% in both cells.

The effects of forced β_2AR expression on NO production were examined. The nitrite concentration in the culture supernatants of the LPS-stimulated RAWar cells was considerably lower than in the culture supernatants of the RAWvec cells (Fig. 2a). After stimulation with LPS for 6 hr, a distinct 130 000 molecular weight NOS II protein band was observed in the RAWvec cells but not in the RAWar cells (Fig. 2b). Although a protein band corresponding to NOS II was observed in the RAWar cells after stimulation with LPS for 24 hr, the expression level was apparently lower than in the RAWvec cells. Similar results were obtained on reverse transcription PCR analysis of NOS II mRNA expression (Fig. 2b).

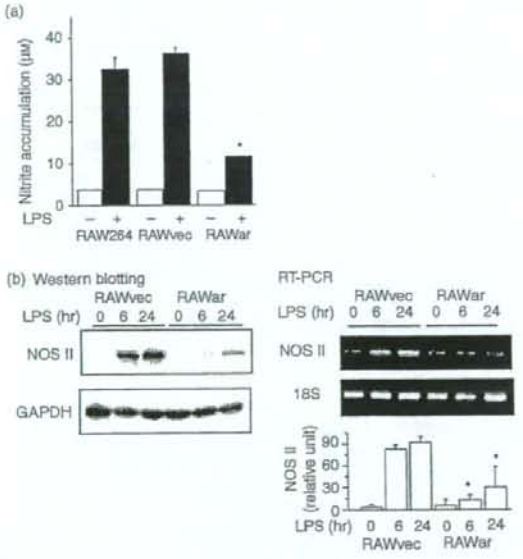

Figure 2. Forced β_2-adenergic receptor (β_2AR) expression suppresses nitric oxide (NO) production and nitric oxide synthase II (NOS II) expression. (a) Cells were stimulated with lipopolysaccharide (LPS) for 24 hr, and nitrite accumulation in the supernatants was measured using the Griess reagent. The results are expressed as means ± SEM from three-well cultures. *$P < 0{\cdot}001$ versus LPS-stimulated RAW264 or RAWvec cells. (b) The protein levels of NOS II and GAPDH (left panel) and messenger RNA expressions of NOS II and 18S ribosomal RNA were analysed as in A (right upper panel). Bar graphs show the relative intensity of the polymerase chain reaction bands from four separate experiments (mean ± SEM) (right lower panel). *$P < 0{\cdot}01$ versus corresponding RAWvec cells. Data shown are representative of three or four separate experiments.

Preventing the down-regulation of β_2AR inhibits LPS-stimulated NF-κB activation.

Next, the effects of forced β_2AR expression on NF-κB activation in response to LPS were analysed. As illustrated in Fig. 3(a), marked NF-κB activation was observed in the RAWvec cells stimulated with LPS for 3 and 6 hr but not in the RAWar cells. The level of cytoplasmic IκBα was decreased in the RAWvec cells after LPS stimulation for 6 hr but this level was not decreased in the RAWar cells (Fig. 3b). To further confirm the role of β_2AR in LPS-stimulated NF-κB activation, the effects of forced β_2AR expression on NF-κB-dependent gene transcription were analysed. NF-κB-mediated-luciferase reporter activity (Fig. 3c) and NOS II promoter activity (Fig. 3d) after stimulation with LPS were inhibited in cells that were cotransfected with the pcDNA4-$\beta_2$ar construct (AR) as well as in cells cotransfected with pCMV-IκBαM (DN-κB). These findings suggested that β_2AR functions as a negative regulator of NF-κB activation by inhibiting IκBα degradation in LPS-stimulated macrophages. Previously, it has been shown that PDTC blocks NF-κB activation by inhibiting IκBα degradation and subsequently the translocation of NF-κB subunits to the nucleus.[22] To elucidate the effects of NF-κB activation on the expression of the responsive gene, *Nos2*, PDTC was added to the RAW264 cell cultures at several time-points after the addition of LPS, and accumulation of NO in the supernatants was analysed after LPS stimulation for 24 hr. As illustrated in Fig. 3(e), when PDTC was added to cultures at 0–9 hr after the addition of LPS, the NO concentrations in these cultures were markedly lower than those in cultures stimulated with LPS for 24 hr without PDTC (right column), indicating that continuous NF-κB activation is essential for adequate NOS II induction.

β_2AR regulates NF-κB activation through β-arrestins

As β-arrestin 2 has been reported to interact with IκBα,[15,16] we examined whether β-arrestin 2 participates in the β_2AR-mediated regulation of IκBα degradation and NF-κB activation in response to LPS. The expression of β-arrestin 2 was also down-regulated in the LPS-stimulated RAW264 cells (Fig. 4, left panels). Forced β_2AR expression abolished the down-regulation of β-arrestin 2 expression (middle panels), suggesting that β-arrestin 2 expression was regulated by β_2AR. Deletion of β_2AR by small interfering RNA (siRNA) decreased β-arrestin 2 expression (data not shown), supporting the theory that β-arrestin 2 expression is regulated by β_2AR. To investigate the role of β-arrestin 2 down-regulation in response to LPS, a stable β-arrestin 2 transfectant (RAWarr2) was

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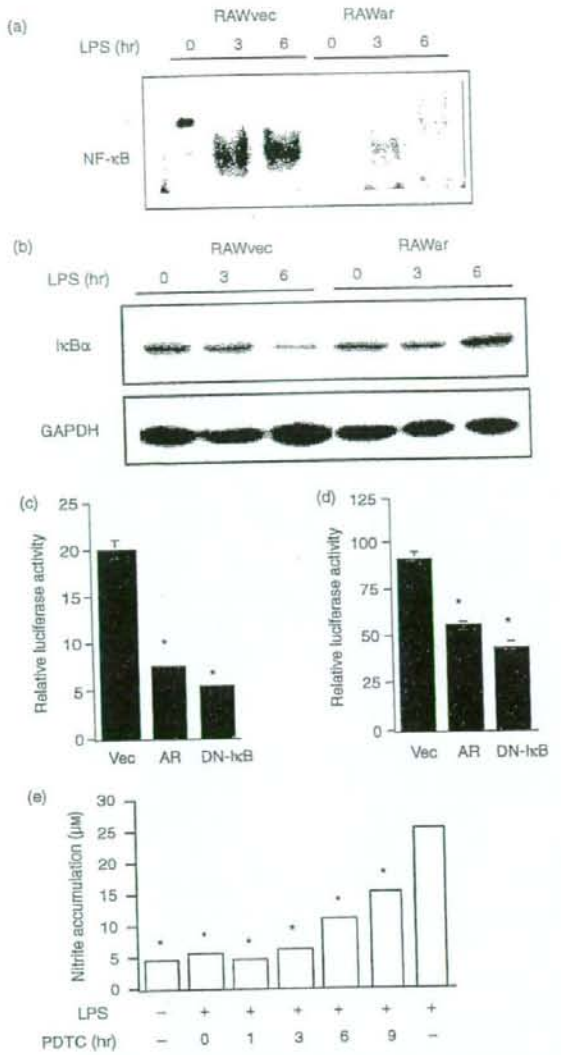

established (Fig. 4, right panels). Since transfection with the vector did not influence NO production (Fig. 1c), cells transfected with β-arrestin 2 were compared with RAW264 cells. As shown in the RAWar cells (Fig. 2), NO production (Fig. 5a) and NOS II protein and mRNA expressions (Fig. 5b) were definitely decreased in the RAWarr2 cells.

Anti-β-arrestin 2 antibodies coimmunoprecipitated IκBα in RAW264 cells before, but not after, LPS stimulation for 6 hr (Fig. 6). On the other hand, the amount of IκBα coprecipitated by anti-β-arrestin 2 antibodies was not reduced but rather was increased in the RAWar and RAWarr2 cells after LPS stimulation, indicating that the LPS-stimulated down-regulation of β_2AR and β-arrestin 2 is essential for IκBα degradation.

Figure 3. Forced β_2-adrenergic receptor (β_2AR) expression suppresses nuclear factor-κB (NF-κB) activation. (a) The vector control cells and β_2AR transfectant were stimulated with lipopolysaccharide (LPS), and NF-κB activation was analysed by electrophoretic mobility shift assay. (b) The vector control cells and β_2AR transfectant were stimulated with LPS, and cytoplasmic inhibitor of NF-κB (IκBα) and GAPDH (loading control) were analysed by Western blotting. (c, d) RAW264 cells were cotransfected with the pNF-κB-Luc vector (c) or the NOS II promoter–luciferase construct (d) and vector (Vec), pcDNA4-β_2AR (AR) or pCMV-IκBαM (DN-IκB). The cells were cultured with LPS for 24 hr, and luciferase activities were determined. The results are expressed as means ± SEM from six-well cultures. *$P < 0.001$ versus cells cotransfected with Vec. (e) Pyrrolidine dithiocarbamate (PDTC) was added to the cultures at the indicated time-points after addition of LPS. Nitrite accumulation in the supernatants at 24 hr of culture was measured using the Griess reagent. The results are expressed as means ± SEM from three-well cultures. The error bars are too small to be distinguishable in the figure (numeric data from the left bar: 3·75 ± 0·18, 5·07 ± 0·22, 4·22 ± 0·07, 5·69 ± 0·12, 10·38 ± 0·06, 15·00 ± 0·05, and 25·20 ± 0·28). *$P < 0.001$ versus LPS-stimulated cells without PDTC. Data shown are representative of two or three separate experiments.

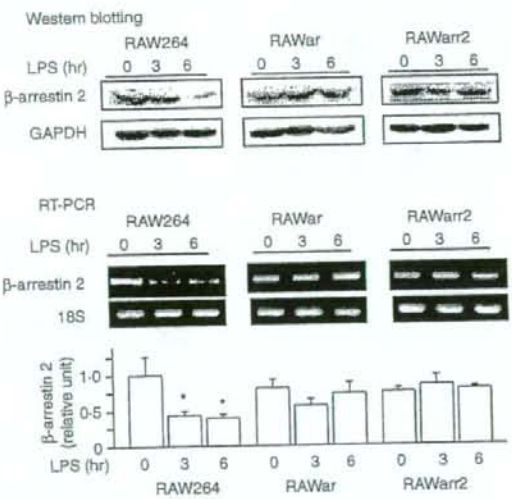

Figure 4. Lipopolysaccharide (LPS) stimulation down-regulates β-arrestin 2 expression. RAW264, RAWar, and RAWarr2 cells were stimulated with LPS, and the protein levels of β-arrestin 2 and GAPDH (upper panel) and messenger RNA expressions of β-arrestin 2 and 18S ribosomal RNA (middle panel) were analysed as in Fig. 1(a). Bar graphs show the relative intensity of the band from three separate experiments (mean ± SEM) (lower panel). *$P < 0.01$ versus 0 hr.

Discussion

In this study, we investigated the role played by β_2AR in the antimicrobial responses of macrophages. First, we demonstrated that β_2AR expression is decreased by LPS

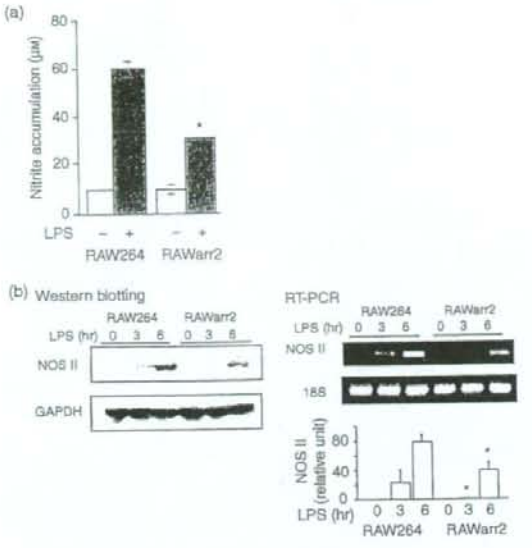

Figure 5. Forced β-arrestin 2 expression suppresses nitric oxide (NO) production and nitric oxide synthase II (NOS II) expression. (a) Cells were stimulated with lipopolysaccharide (LPS) for 24 hr, and nitrite accumulation in the supernatants was measured using the Griess reagent. The results are expressed as means ± SEM from three-well cultures. *$P < 0.001$ versus LPS-stimulated RAW264 cells. (b) The protein levels of NOS II and GAPDH (left panel) and messenger RNA expressions of NOS II and 18S ribosomal RNA (light upper panel) were analysed as in Fig. 1(a). Bar graphs show the relative intensity of the polymerase chain reaction bands from three separate experiments (mean ± SEM) (right lower panel). *$P < 0.01$ versus corresponding RAW264 cells. Data shown are representative of three to four separate experiments.

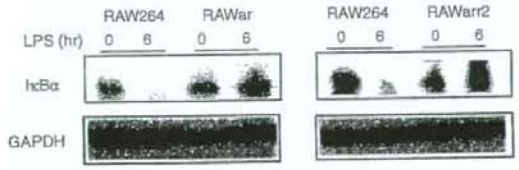

Figure 6. β-arrestin 2 interacts with cytosolic inhibitor of NF-κB (IκBα). Before and after stimulation with lipopolysaccharide (LPS) for 6 hr, cells were lysed and immunoprecipitated with anti-β-arrestin 2 antibodies. Western blotting analysis was performed using anti-IκBα antibodies (upper panel). The protein levels of GAPDH in equal amounts of lysates were used for control (lower panel).

stimulation. To investigate the role of β_2AR down-regulation in response to LPS directly, we established a macrophage cell line, RAWar. Prevention of the down-regulation of β_2AR expression in RAWar cells resulted in reduced NO production, suggesting that the LPS-associated down-regulation of β_2AR expression plays an important role in NO production in macrophages.

Decreases in NOS II mRNA expression were observed in the RAWar cells, indicating that NOS II expression was transcriptionally down-regulated by forced β_2AR expression. Prevention of the down-regulation of β_2AR expression in the RAWar cells resulted in a marked decrease in NF-κB activation and inhibited cytosolic IκBα degradation, indicating that the forced β_2AR expression inhibited LPS-induced NF-κB activation by IκBα stabilization.

On the other hand, β-arrestins, which are universally expressed members of the arrestin family, are the major regulators of GPCR signalling and bind to activated GPCRs, causing receptor desensitization and internalization.[14] Recently, β-arrestins have been shown to play functional roles in the regulation of a variety of signalling pathways and in the mediation of cross-talk between signalling pathways. Moreover, there is accumulating evidence that β-arrestin 2, which is expressed abundantly in the spleen, is functionally involved in some important immune responses.[23–26] We have demonstrated that β-arrestin 2 is down-regulated in LPS-stimulated RAW264 cells. Down-regulation of β-arrestin 2 was abolished in RAWar cells, suggesting that β-arrestin 2 expression is regulated by β_2AR. These findings suggest that β_2AR participates in signal transduction pathways from TLR4 by regulating the level of β-arrestin 2 expression. Meanwhile, the amount of IκBα coimmunoprecipitated by anti-β-arrestin 2 antibodies was decreased in the RAW264 cells after their stimulation with LPS but not in the RAWar or RAWarr2 cells, suggesting that β_2AR inhibited LPS-induced NF-κB activation by stabilizing IκBα through β-arrestin 2. The release of NF-κB following the degradation of IκBα proteins is an essential step in the generation of transcriptionally competent NF-κB. In addition, NF-κB activity following stimulation is dependent on the level of cytoplasmic NF-κB/IκBα complexes free from stabilizing factors. Therefore, the following appear likely: (1) LPS-stimulated signals suppress β_2AR expression, (2) the reduction of β_2AR results in the down-regulation of β-arrestin 2 expression, (3) β-arrestin 2 stabilizes cytoplasmic IκBα and inhibits NF-κB activation (so reduction in the level of β-arrestin 2 accelerates IκBα degradation and NF-κB activation in LPS-stimulated cells) and (4) nuclear translocation of NF-κB enhances NOS II expression.

The cross-talk between β_2AR and the TLR signalling pathways is schematically summarized in Fig. 7.

Catecholamines increase cAMP via β_2AR activation, and PKA activation inhibits NF-κB-induced transcription by phosphorylating cAMP responsive element binding protein (CREB), which competes with p65 for the limited amounts of CREB-binding protein (CBP) (Fig. 7a(ii)).[27] However, β_2AR agonists did not suppress NO production (unpublished observation). In the present study, we demonstrated that LPS stimulation suppressed the cAMP accumulation in RAWvec cells stimulated with a β_2AR

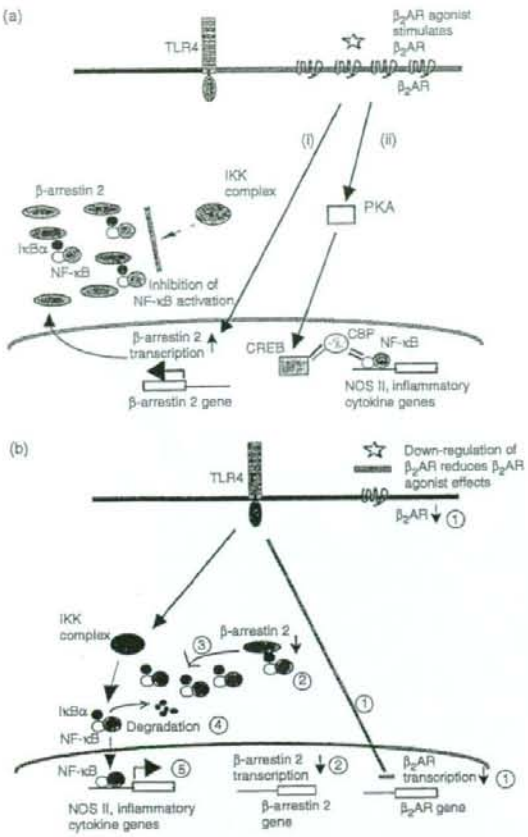

Figure 7. Cross-talk between β_2-adrenergic receptor (β_2AR) and Toll-like receptor (TLR) signalling pathways. (a) β_2AR agonists suppress nuclear factor-κB (NF-κB) activation by increasing cytoplasmic β-arrestin 2, which stabilizes the NF-κB/inhibitor of NF-κB (IκBα) complexes in cytoplasm (i) or by activating cAMP response element binding protein (CREB), which then produces competition between CREB-binding protein (CBP) and NF-κB in the nucleus (ii). (b) TLR4-dependent signals lead to the following steps both in the presence or absence of β_2AR agonists: ① TLR4-dependent down-regulation of β_2AR expression, ② down-regulation of β-arrestin 2, ③ release of NF-κB/IκBα complexes in the cytoplasm, ④ degradation of IκBα, and ⑤ translocation of NF-κB to the nucleus and transcription of its target genes.

agonist. In addition, we showed that prevention of the down-regulation of β_2AR inhibits the degradation of IκBα through β-arrestin 2, which stabilizes IκBα in the steady state (Fig. 7a(ii)). Therefore, the down-regulation of expression of both β_2AR and β-arrestin 2 by the TLR4-dependent pathway might provide a mechanism for 'escaping' anti-proinflammatory signals, such as the β_2AR–cAMP–PKA pathway[27] or the β_2AR–β-arrestin 2–IκBα pathway. As the levels of β_2AR ligands vary under different conditions, understanding the cross-talk between TLRs and β_2AR pathways may have both physiological and pathophysiological importance. Taken together, the observations of the present study regarding the regulation of TLR4 signalling through β_2AR appear to provide another therapeutic target for the regulation of inflammatory disease conditions.

Acknowledgements

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References

1 Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol* 2003; **21**:335–76.
2 Janeway CA Jr, Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002; **20**:197–216.
3 Hierholzer C, Harbrecht B, Menezes JM *et al.* Essential role of induced nitric oxide in the initiation of the inflammatory response after hemorrhagic shock. *J Exp Med* 1998; **187**:917–28.
4 Bogdan C. Nitric oxide and the immune response. *Nat Immunol* 2001; **2**:907–16.
5 Lain de Lera T, Folgueira L, Martin AG *et al.* Expression of IkappaBalpha in the nucleus of human peripheral blood T lymphocytes. *Oncogene* 1999; **18**:1581–8.
6 Tergaonkar V, Correa RG, Ikawa M, Verma IM. Distinct roles of IkappaB proteins in regulating constitutive NF-kappaB activity. *Nat Cell Biol* 2005; **7**:921–3.
7 Rodriguez MS, Thompson J, Hay RT, Dargemont C. Nuclear retention of IkappaBalpha protects it from signal-induced degradation and inhibits nuclear factor kappaB transcriptional activation. *J Biol Chem* 1999; **274**:9108–15.
8 Itoh CE, Kizaki T, Hitomi Y *et al.* Down-regulation of beta2-adrenergic receptor expression by exercise training increases IL-12 production by macrophages following LPS stimulation. *Biochem Biophys Res Commun* 2004; **322**:979–84.
9 Downing JE, Miyan JA. Neural immunoregulation: emerging roles for nerves in immune homeostasis and disease. *Immunol Today* 2000; **21**:281–9.
10 Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve – an integrative interface between two supersystems: the brain and the immune system. *Pharmacol Rev* 2000; **52**:595–638.
11 Kohm AP, Sanders VM. Norepinephrine and beta 2-adrenergic receptor stimulation regulate $CD4^+$ T and B lymphocyte function *in vitro* and *in vivo*. *Pharmacol Rev* 2001; **53**:487–525.
12 Ferguson SS, Downey WE 3rd, Colapietro AM, Barak LS, Menard L, Caron MG. Role of beta-arrestin in mediating agonist-promoted G protein-coupled receptor internalization. *Science* 1996; **271**:363–6.

13 Ogasawara J, Sanpei M, Rahman N, Sakurai T, Kizaki T, Hitomi Y, Ohno H, Izawa T. β-adrenergic receptor trafficking by exercise in rat adipocytes: roles of G-protein-coupled receptor kinase-2, β-arrestin-2, and the ubiquitin–proteasome pathway. *FASEB J* 2006; **20**:350–2.

14 Luttrell LM, Lefkowitz RJ. The role of β-arrestins in the termination and transduction of G-protein-coupled receptor signals. *J Cell Sci* 2002; **115**:455–65.

15 Witherow DS, Garrison TR, Miller WE, Lefkowitz RJ. β-arrestin inhibits NF-κB activity by means of its interaction with the NF-κB inhibitor IκBα. *Proc Natl Acad Sci U S A* 2004; **101**:8603–7.

16 Gao H, Sun Y, Wu Y, Luan B, Wang Y, Qu B, Pei G. Identification of β-arrestin2 as a G protein-coupled receptor-stimulated regulator of NF-κB pathways. *Mol Cell* 2004; **14**: 303–17.

17 Kizaki T, Suzuki K, Hitomi Y *et al.* Uncoupling protein 2 plays an important role in nitric oxide production of lipopolysaccharide-stimulated macrophages. *Proc Natl Acad Sci U S A* 2002; **99**:9392–7.

18 Kizaki T, Ookawara T, Iwabuchi K *et al.* Age-associated increase of basal corticosterone levels decreases $ED2^{high}$, $NF\text{-}\kappa B^{high}$ activated macrophages. *J Leukoc Biol* 2000; **68**:21–30.

19 Raje CI, Kumar S, Harle A, Nanda JS, Raje M. The macrophage cell surface glyceraldehyde-3-phosphate dehydrogenase is a novel transferrin receptor. *J Biol Chem* 2007; **282**:3252–61.

20 Ding AH, Nathan CF, Stuehr DJ. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. comparison of activating cytokines and evidence for independent production. *J Immunol* 1988; **141**:2407–12.

21 Kizaki T, Suzuki K, Hitomi Y *et al.* Negative regulation of LPS-stimulated expression of inducible nitric oxide synthase by AP-1 in macrophage cell line J774A.1. *Biochem Biophys Res Commun* 2001; **289**:1031–8.

22 Staal FJ, Roederer M, Herzenberg LA, Herzenberg LA. Intracellular thiols regulate activation of nuclear factor κB and transcription of human immunodeficiency virus. *Proc Natl Acad Sci U S A* 1990; **87**:9943–7.

23 Sun Y, Cheng Z, Ma L, Pei G. β-arrestin2 is critically involved in CXCR4-mediated chemotaxis, and this is mediated by its enhancement of p38 MAPK activation. *J Biol Chem* 2002; **277**:49212–9.

24 Barlic J, Andrews JD, Kelvin AA *et al.* Regulation of tyrosine kinase activation and granule release through β-arrestin by CXCRL *Nat Immunol* 2000; **1**:227–33.

25 Fong AM, Premont RT, Richardson RM, Yu YR, Lefkowitz RJ, Patel DD. Defective lymphocyte chemotaxis in β-arrestin2- and GRK6-deficient mice. *Proc Natl Acad Sci U S A* 2002; **99**:7478–83.

26 Walker JK, Fong AM, Lawson BL, Savov JD, Patel DD, Schwartz DA, Lefkowitz RJ. β-arrestin-2 regulates the development of allergic asthma. *J Clin Invest* 2003; **112**:566–74.

27 Parry GC, Mackman N. Role of cyclic AMP response element-binding protein in cyclic AMP inhibition of NF-κB-mediated transcription. *J Immunol* 1997; **159**:5450–6.

骨粗鬆症治療

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特集：転倒と骨粗鬆症

転倒リスク評価とリスクを高める薬剤

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転倒は，心身などの内的要因と居住環境や履物などによる外的要因に起因する複雑な老年症候群の1つである．多様な危険因子を個人評価する場合には，時間と人手がかかる．これを解決するため，簡便な「転倒スコア」および「転倒スコア短縮版」を開発した．後者は高齢者健診の1次スクリーニングに，前者はリスク別のケアプラン策定に有用である．数種類以上の薬剤処方は，転倒のリスクを高める．なかでも精神神経用薬は，おおむね50％以上転倒危険率を増す．転倒にかぎっていえば，短期間作動型の睡眠薬でも，非定形抗精神病薬，新しい抗うつ薬のいずれも転倒の危険を除外できていない．精神神経用薬の減量や中止は，転倒防止の第一級のエビデンスである．

Key words 転倒予測技術，簡便性と実用性，多剤服用，精神神経用薬，薬物中止介入

はじめに

転倒・骨折は高齢者における寝たきり要因の第3位に位置づけられ，骨粗鬆症性骨折のなかで最も重い骨折である大腿骨頸部骨折は，その90％以上が転倒によって生ずるとされている[1]．転倒の際に骨折を生じなくとも，数度の転倒を経験すると，意欲や日常生活動作能力（ADL）を低下させる[2]．地域住民におけるADL依存の危険因子として，転倒は約2倍のリスクであり[2]，転倒予防は寝たきり予防にきわめて重要である．従来の転倒リスク評価研究の問題点を克服し，そこから得られた「多剤服用が転倒リスク」である結果を踏まえ，最近の薬剤と転倒のレビューを紹介する．

転倒リスク，これまでの研究

従来，転倒危険因子は，特定のフィールドでの横断的，あるいは縦断的解析によってなされてきたが，抽出された危険因子は，身体的脆弱性，歩行機能の低下など共通の危険因子がある一方，めまいや認知症などは成績が一致していない[2]．転倒は，内的要因である身体的側面と外的要因である環境要因による複合的症候群と捉えられるが，後者は地域や文化的，生活習慣的側面により大きく異なる可能性もある．

従来の転倒危険因子は，病歴，現症，血液検査，生活能力などの簡便な検査，専門調査員による測定検査，特殊な機器を用いた検査などが統一性なく調査され，一般健康診断に適応できるかどうかの観点に著しく欠けてい

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表❶ 測定方法の難易度で分けた，転倒の危険因子

特殊機器，医師の問診などが必要な専門検査
・歩行運動系（関節症，ミオパチーなど）
歩行速度遅延
バランス低下
下肢筋力低下
・心血管系障害（不整脈，起立性低血圧など）
・神経系障害（認知症，パーキンソンニズムなど）
・薬剤（鎮静薬，睡眠薬など）
問診表などで可能な簡易な方法
・老研式活動能力指標低下
（手段的ADL，知的能動性，社会的役割の13項目で構成）
・過去の転倒歴
・環境要因　照明不良，障害物，段差，不適切な履物など

（鈴木隆雄，2002[1]より改変引用）

た（表❶）[1]．本研究では，国内外の文献的レビューをもとに，転倒ハイリスク者の早期発見の評価方法作成ワーキンググループの研究班によって簡易な「転倒リスク評価表」（表❷）[3]を作成し，その妥当性，有効性を検証した．

方法

2003年度厚生労働科学研究費効果的医療技術の確立推進臨床研究事業，転倒骨折班の合同討議，国内外のレビュー[1]から，筋力低下，バランス欠如，歩行障害，視力障害，移動障害，認知機能障害，ADL障害，起立性低血圧，加齢，転倒の既往，慢性疾患，薬剤，段差が必須項目としてあげられた（表❶）[1]．これらの項目を具体的に質問表のみで被験者が内容を理解し，かつ因子のもつ意味が変容しないよう議論を重ね，問診表を完成させた（表❷）．くり返し再現性，季節変動などの基本的検討はすでになされ，良好な結果を得ている[3]．

調査対象

全国7地域（浦臼町，仙台市，塩尻市，多摩地区，香北町，相良村）の住民2,439名（男性932名，女性1,507名：76.3±7.4歳）に対し，問診表の意味を説明し調査の同意を得た後，自記式にて回答，自記不可能な場合は調査員が聞き取り調査をおこなった．

解析

1）過去の転倒歴を従属変数として，多変量解析をおこなった．

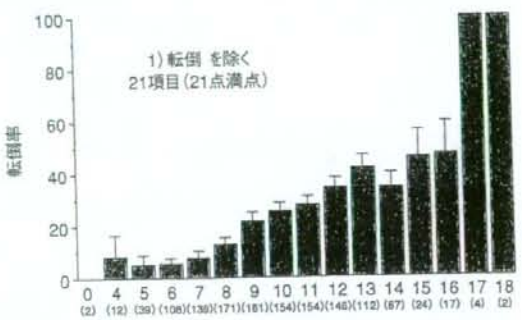
2）観察期間中の転倒歴を従属変数として，過去の転倒歴を含む，調査票の項目を独立とし重回帰分析をおこなった．

年齢，性は強制注入した．p値が0.05未満を統計学的に有意とした．なお有意な傾向として，$p<0.1$の項目も記載した．

表❷ 質問項目と陽性頻度

1）転倒：解答数2,439名で708例（4.7±1.0回/年）	28.8%
2）つまずくことがある	56.5%
3）手摺につかまらず，階段ののぼり降りができない	50.6%
4）歩く速度が遅くなってきた	65.2%
5）横断歩道を青のうちに渡りきれない	17.0%
6）1キロメートルくらいつづけてあるけない	35.8%
7）片足で5秒くらい立てない	38.6%
8）杖をつかっている	28.3%
9）タオルを固く絞れない	16.8%
10）めまい，ふらつきがある	32.4%
11）背中が丸くなってきた	44.9%
12）膝が痛む	47.3%
13）目が見えにくい	53.1%
14）耳が聞こえにくい	42.5%
15）物忘れが気になる	63.7%
16）転ばないかと不安になる	45.8%
17）毎日薬を5種類以上飲んでいる	31.2%
18）家のなかで歩くとき暗く感ずる	11.4%
19）廊下，居間，玄関に障害物	20.8%
20）家のなかに段差がある	69.1%
21）階段を使わなくてはならない	27.7%
22）生活上家の近くの急な坂道を歩く	33.3%

（鳥羽研二ら，2005[3]より引用）



図❶ 転倒評価表スコアと転倒率（過去1年）
（鳥羽研二ら，2005[3]より引用）

結果

各項目の陽性頻度：過去1年の転倒歴は708名（男性229名，女性479名，平均年齢77.5±7.4歳），転倒率は28.8%，観察期間中は25%であり，骨折は1.8%にみられた．質問項目と陽性頻度を表❷に，転倒評価表スコアと転倒率を図❶に示す．スコアが大きくなるほど転倒率が高くなることが示された．

ほかの検査方法との比較

本スコアの実用性に関し，松林らは，この問診表を用い，北海道浦臼町で転倒調査を施行した．転倒の従属変数として，転倒危険を察知するカットオフポイントは，転倒スコア10点以上で，感度，特異度とも70％以上の結果であった．また，従来のUp&Goテストや，歩行速度，Functional Reachより，転倒予測の感度，特異度にすぐれている結果を得ている[4]．

より多数の症例で前向き研究による観察期間中の転倒を従属変数とする多変量解析によって，独立した有意な項目は，5項目に絞られた．オッズ比を加味した，簡易式「転倒チェック」シートを示す（表❸）[5]．簡易式「転倒チェック」シートの転倒予測の感度，特異度は70％以上である．

表❸ 簡易式の「転倒チェック」シート（該当項目に✓をつける）

□ 過去１年間に転んだことがある	5点
□ 背中が丸くなってきた	2点
□ 歩く速度が遅くなってきたと思う	2点
□ つえを使っている	2点
□ 毎日５種類以上の薬を飲んでいる	2点
合計	点

※６点以上は「要注意」

（Okochi J *et al*，2006[5]より引用）

薬剤と転倒

精神神経用薬

地域住民における薬剤，とくに精神神経用薬の転倒リスクに関する研究は多数なされている．最近の母集団が大きい研究では，イタリアの2,854名の在宅ケアプログラムを受給している高齢者において，いずれかの精神神経用薬を服用している者の転倒危険率（オッズ比）は1.47（95％CI：1.24～1.74）でほぼ5割り増しであった．非定形精神病薬でもオッズ比は1.45倍と有意であった[6]．

従来，短期間作動型のベンゾジアゼピン系睡眠薬は，長期間作動型にくらべ安全と考えられてきた．しかし転倒にかぎっては，この研究では，長期間作動型1.45倍に対し，短期間作動型1.32倍で有意差はなかった．アメリカボルチモアの女性8,127名参加の多施設コホート研究[7]でも，長期間作動型が1.56倍，短期間作動型は1.42倍で有意差を認めなかった．高齢者の肝臓での代謝に起因するか，腎排泄遅延が影響するかなど機序の不明な点は多いが，従来考えられてきたように「短期間作動型睡眠導入薬なら安全」の神話は壊れたといってよい．

抗うつ薬に関しては，2つの研究では異った結果を得ている．イタリアにおいてはオッズ比0.92だったのに対し，ボルチモアでは1.54倍と有意であった．さらにこの研究では，SSRIは3.45倍の転倒率を示し，高齢者に副作用が多いと批判されている3環系抗うつ薬の1.28倍より有意に高頻度で転倒をおこした．従来，抗うつ薬は口渇，排尿障害などが副作用として重視されてきたが，今後は転倒を含めた冷静な比較が求められよう．

抗痙攣薬に関しては，従来より高齢者の生命予後にエビデンスがないことが知られてきた．今回ボルチモアの研究では，抗痙攣薬の転倒危険率は2.56倍と高く，より適応を慎重に選ぶ必要がある．この研究で麻薬は転倒危険率に影響がなかったことも重要である（オッズ比＝0.99）．

循環器用薬と鎮痛薬

Tinetti ら[8]は1999年に，60歳以上の症例に関して循環器用薬や鎮痛薬と転倒に関連があった29の研究のメタアナリシスを発表している．精神神経用薬にくらべ，危険度が増す率は低いが，高齢者には好んで処方されるため参考までに表❹に記した．

この研究では，75歳以上と未満で，結果に差がなかった．また，転倒率の多い集団と少ない集団でも薬剤の作用に差がなく，ジゴキシン，Ia型抗不整脈薬，利尿薬は転倒の危険を増すことに注意を喚起した．いずれの疾患集団でも3または4薬剤以上の処方で転倒が増すことが示されており，単一薬剤より，重層的な危険を増す「多剤服用は転倒の危険がある」ということがメタアナリシスでも示されている．われわれの結果と一致するものであり，高齢者の処方にあたっては，転倒リスクを考慮し，患者・家族に説明したうえで処方しなくてはならない．

表❹ 各種循環器用薬，鎮痛薬の転倒危険率

薬剤	オッズ比	95%CI	有意*
利尿薬	1.08	1.02～1.16	*
サイアザイド	1.06	0.97～1.16	
ループ	0.90	0.73～1.12	
βブロッカー	0.93	0.77～1.14	
中枢性交感神経抑制薬	1.16	0.87～1.55	
ACE阻害薬	1.20	0.92～1.58	
Ca 拮抗薬	0.94	0.77～1.14	
亜硝酸薬	1.13	0.78～1.36	
抗不整脈薬(Ia)	1.59	1.02～2.48	*
ジゴキシン	1.22	1.05～1.42	*
鎮痛薬			
麻薬	0.97	0.78～1.20	
非麻薬	1.09	0.88～1.34	
NSAIDs	1.16	0.97～1.38	
アスピリン	1.12	0.80～1.57	

(Tinetti ME *et al*, 1999[8]より引用)

表❺ 向精神薬の使用と転倒リスク
(N=1,845； n=204 Fallers)

Medication	Multivariable-Adjusted Analysis (ハザード比95%CI)	P-value
オランザピン(yes/no)	1.74(1.04～2.90)	p=0.04
リスペリドン(yes/no)	1.32(0.57～3.06)	p=0.52
定型抗精神病薬(yes/no)	1.35(0.87～2.09)	p=0.19
抗うつ薬(yes/no)	1.45(1.09～1.93)	p=0.01
抗不安薬(yes/no)	1.19(0.94～1.50)	p=0.15

(Hien le TT *et al*, 2005[9]より引用)

認知症薬と転倒

認知症の周辺症状に対する「非定形抗精神病薬」に関しては，死亡率が1.7倍になることから，FDA(米国食品医薬品局)では長期処方を原則禁止している．Hienら[9]は，これらに先立ち，非定形抗精神病薬と転倒リスクを大規模研究でまとめている(表❺)．

薬剤中止の介入と転倒予防

精神神経用薬の中止は転倒を70%減少させることが知られている[10]．米国の「転倒ガイドライン」でも，4種類以上の投薬を受けている患者の投薬数を減らすことは，地域においても，長期介護施設においても第一級のエビデンスと位置づけられている[11]．

おわりに

転倒予防事業で，今後の転倒危険者を抽出する検査を考える場合，従来のように，環境因子の問診表と下肢筋力検査(歩行速度，かた足立ち時間)などに時間を費やすより，過去の転倒回数を十分聴取し，身体的側面(骨粗鬆症，認知症，膝関節症)の情報を得るため，「転倒スコア」を活用することが簡易で，有用であると示唆された．

転倒予防には，まず多剤処方の見直しが最も安価な治療法であり，とくに，睡眠薬の安易な処方は慎むべきであり，代替手段を考えてからという処方態度を心がけたい．

本研究は，厚生労働科学研究長寿科学総合研究事業の助成によっておこなわれた．

文 献

1) 鈴木隆雄：転倒の疫学．日老医誌 **40**：85-94, 2002
2) 鳥羽研二，松林公蔵，西永正典：効果的医療技術の確立推進臨床研究．2003年度班研究報告書，2004
3) 鳥羽研二，大河内二郎，高橋泰：転倒リスク予測のための「転倒スコア」の開発と妥当性の検証．日老医誌 **42**：346-362, 2005
4) 松林公蔵：地域在住高齢者における転倒の実態に関する研究．厚生労働科学研究費補助金(長寿科学総合研究事業)分担研究報告書，2007
5) Okochi J, Toba K, Takahashi T *et al* : Simple screening test for risk of fall in the elderly. *Geriatr Gerontol Int* **6** : 223-227, 2006
6) Landi F, Onder G, Cesari M *et al* : Psychotropic medications and risk for falls among community-dwelling frail older people : an observational study. *J Gerontol A Biol Sci Med Sci* **60** : 622-626, 2005
7) Ensrud KE, Blackwell TL, Mangione CM *et al* : Central nervous system-active medications and risk for falls in older women. *J Am Geriatr Soc* **50** : 1744-1745, 2002
8) Tinetti ME, Leipzig RM, Cumming RG : Drugs and falls in older people : a systematic review and meta-analysis : II. Cardiac and analgesic drugs. *J Am Geriatr Soc* **47** : 40-50, 1999
9) Hien le TT, Cumming RG, Cameron ID *et al* : Atypical Antipsychotic Medications and Risk of Falls in Residents of Aged Care Facilities. *J Am Geriatr Soc* **53** : 1290-1295, 2005

10) Rubenstein LZ : Falls. Ambulatory Geriatric Care, ed. by Yoshikawa TT, 1993
11) Guideline for the Prevention of Falls in Older Persons. American Geriatrics Society, British Geriatrics Society, and American Academy of Orthopaedic Surgeons Panel on Falls Prevention. *J Am Geriatr Soc* **49** : 664-672, 2001

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