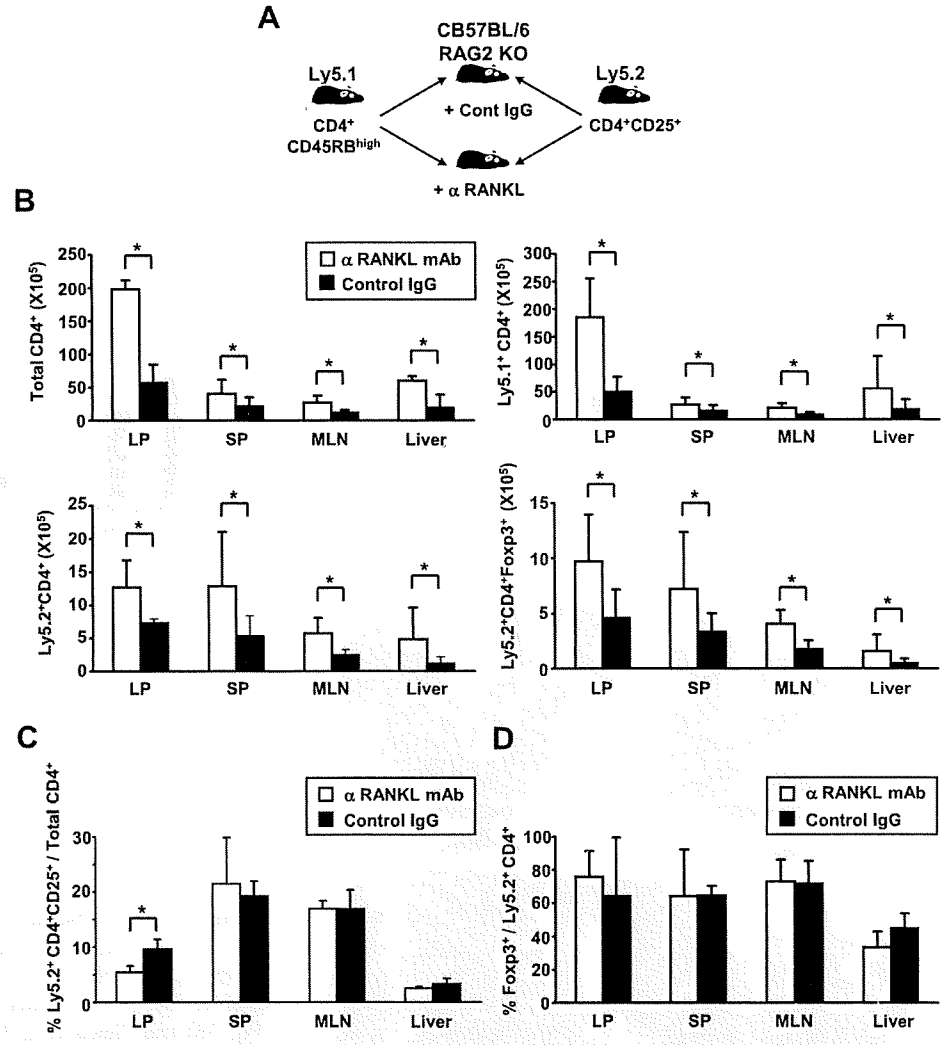

FIGURE 5. Blockade of the RANK/RANKL pathway induces the dysregulated cell balance between effector $CD4^+$ T cell and T_R cells in the inflamed mucosa of colitic mice. *A*, C57BL/6 RAG-$2^{-/-}$ mice were injected i.p. with $Ly5.1^+CD4^+CD45RB^{high}$ (3×10^5/mouse) alone or $Ly5.1^+CD4^+CD45RB^{high}$ (3×10^5) + $Ly5.2^+CD4^+CD25^+$ T cells (1×10^5) and treated with control IgG or anti-RANKL mAb by i.p. injection at a dose of 250 μg three times per week over 6 wk starting at the time of transfer. *B*, Absolute number of total $CD4^+$ T cells ($Ly5.1^+$ + $Ly5.2^+$ cells), $Ly5.1^+CD4^+$ T cells, $Ly5.2^+CD4^+$ T cells, $Ly5.2^+CD4^+Foxp3^+$ T_R cells in LP, SP, MLN, and liver of control IgG- or anti-RANKL mAb-treated mice. Data are indicated as the mean ± SEM of 12 mice per group for LP, SP, and MLN and six mice per group for liver. *, $p < 0.01$. *C*, Ratio of $CD4^+CD25^-$ T_R ($Ly5.2^+$) cells per total $CD4^+$ T cells ($Ly5.1^+$ + $Ly5.2^+$) at 6 wk after transfer was analyzed by flow cytometry. Data are indicated as the mean ± SEM of six mice per group. *, $p < 0.01$. *D*, Ratio of $Foxp3^+$ cells to total $Ly5.2^+CD4^+$ T cells at 6 wk after transfer was analyzed by flow cytometry. Data are indicated as the mean ± SEM of six mice per group. *, $p < 0.01$.

lamina propria lymphocyte from the four groups of mice. As shown in Fig. 4*C*, IFN-γ and IL-17 production by LP $CD4^+$ T cells was markedly suppressed by cotransfer of $CD4^+CD25^+$ T_R cells with $CD4^+CD45RB^{high}$ T cells, but this suppression was partially but significantly abrogated by the treatment with anti-RANKL mAb to the level of LP $CD4^+$ T cells from the mice transferred with $CD4^+CD45RB^{high}$ T cells alone and treated with anti-RANKL mAb or control IgG.

Balance between effector $CD4^+$ T cells and $CD4^+CD25^+$ T_R cells in the inflamed mucosa was dysregulated by treatment with anti-RANKL mAb

To further assess the balance in cell numbers between effector $CD4^+$ T cells and $CD4^+CD25^+$ T_R cells in recipient SCID mice, we transferred $Ly5.1^+$ $CD4^+CD45RB^{high}$ cells and $Ly5.2^+$ $CD4^+CD25^+$ T_R cells into C57BL/6 RAG-$2^{-/-}$ mice to distinguish between effector $CD4^+$ T cells and T_R cells (Fig. 5*A*). We first confirmed that C57BL/6 RAG-$2^{-/-}$ mice transferred with $CD4^+CD45RB^{high}$ T cells and $CD4^+CD25^+$ T_R cells did develop colitis with the marked expansion of $CD4^+$ T cells when treated with anti-RANKL mAb, but not with control IgG (data not shown). As expected, the absolute number of total $CD4^+$ T cells ($Ly5.1^+$ plus $Ly5.2^+$ cells) and $Ly5.1^+$ effector $CD4^+$ T cells in LP of anti-RANKL mAb-treated mice was significantly increased due to the presence of colitis as compared with that in control IgG-treated mice with no colitis (Fig. 5*B*). Also, the absolute number of $Ly5.2^+$ T cells and $Ly5.2^+CD4^+Foxp3^+$ T_R cells in LP of colitic anti-RANKL mAb-treated mice was significantly higher than that in non-colitic control IgG-treated mice (Fig. 5*B*), suggesting that both effector $CD4^+$ T cells and T_R cells extensively proliferated in LP of anti-RANKL mAb-treated colitic mice. Interestingly, however, the ratio of $Ly5.2^+CD4^+CD25^+$ T_R cells to total $CD4^+$ T cells in the recipient mice treated with anti-RANKL mAb was markedly decreased in the intestine as compared with that in the recipient mice treated with control IgG, although there were no significant differences in the spleen, MLN, or liver between the two groups (Fig. 5*C*). These results suggested that the blockade of the RANK/RANKL signaling pathway affected the expansion and/or migration of $CD4^+CD25^+$ T_R cells, resulting in dysregulated cell balance between effectors and T_R cells in the inflamed mucosa.

Blockade of the RANK/RANKL pathway does not affect migration of $CD4^+CD25^+$ T_R cells to the inflamed mucosa, but suppresses their expansion in the inflamed mucosa

To assess why blockade of the RANK/RANKL pathway abolished T_R function in mice transferred with $CD4^+CD45RB^{high}$ T cells and $CD4^+CD25^+$ T_R cells, we conducted several in vitro and in vivo experiments. First, to assess the possibility that blockade of the RANK/RANKL pathway skews the expression of gut-homing receptors on T_R cells, splenic $CD4^+$ T cells were cultured for 72 h in the presence or absence of anti-RANKL mAb with a stimulating

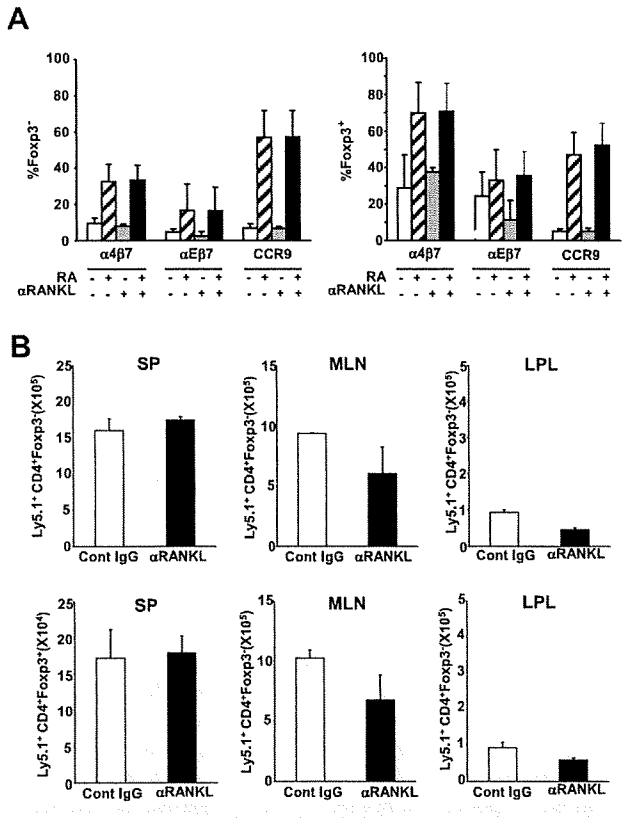

FIGURE 6. Blockade of the RANK/RANKL pathway does not affect the migration of $CD4^+CD25^+$ T_R cells to the inflamed mucosa of colitic mice. *A*, $CD4^+$ T cells (2×10^5) purified from normal BALB/c mice were cultured with purified SP $CD11c^+$ DC (2×10^3) from colitic mice in addition to soluble 1 μg/ml anti-CD3 mAb, 10 ng/ml human rTGF-β, all-*trans* RA, and 5 ng/ml rIL-2 with or without 1 μg/ml anti-RANKL mAb. On day 4, cells were stained with PerCP-conjugated anti-CD4 mAb and PE-conjugated anti-integrin $\alpha_4\beta_7$, PE-conjugated anti-integrin $\alpha_E\beta_7$, or PE-conjugated anti-CCR9 mAb, followed by intracellular staining by allophycocyanin-conjugated Foxp3 mAb. Data are indicated as the mean ± SEM of six samples per group. *, $p < 0.01$. *B*, Anti-RANKL mAb treatment does not affect the migration of $CD4^+Foxp3^+$ and $CD4^+Foxp3^-$ T cells in vivo. To assess the effect of anti-RANKL mAb on the trafficking of T_R cells to inflamed mucosa of colitic mice, RAG-$2^{-/-}$ mice were transferred with $CD4^+CD45RB^{high}$ T cells and, 4 wk after transfer, they were treated with 250 μg of anti-RANKL mAb or control IgG two times in 1 day (or on 2 consecutive days?). They were then transferred with splenic $Ly5.1^+CD4^+$ T cells from normal mice and, 24 h after the second transfer, the cell number of $Ly5.1^+CD4^+Foxp3^+$ (*lower*) or $Foxp3^-$ (*upper*) T cells recovered from LP, SP, and MLN was evaluated by flow cytometry.

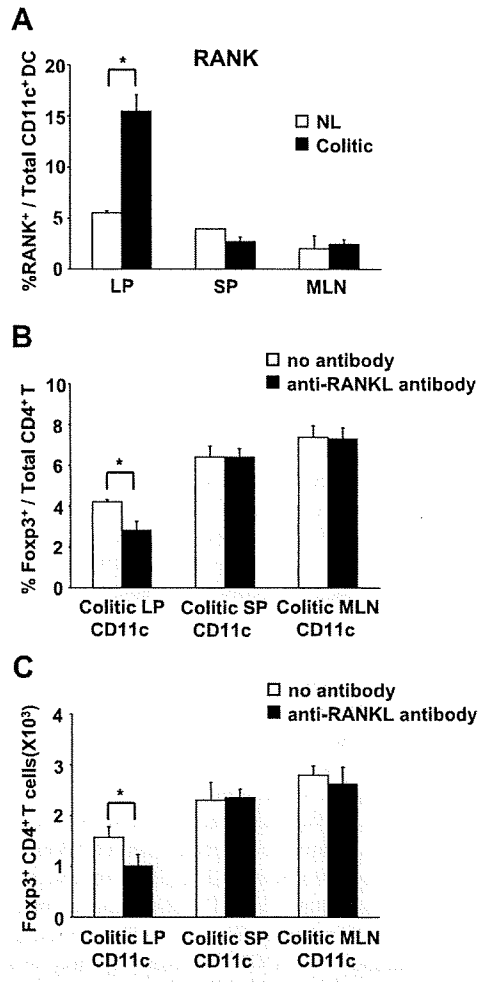

FIGURE 7. Blockade of the RANK/RANKL pathway suppresses the expansion of $CD4^+CD25^+$ T_R cells in the inflamed mucosa. *A*, Expression of RANK on LP, SP, or MLN DC cells obtained from colitic (□) or normal (■) mice. *B* and *C*, Splenic $CD4^+$ T cells from normal mice were cultured with colitic LP, MLN, or SP $CD11c^+$ DC in the presence of anti-CD3 mAb with control IgG (□) or anti-RANKL mAb (■) for 72 h, and the ratio of $CD4^+Foxp3^+$ T_R cells per total $CD4^+$ T cells (*B*) and the number of $CD4^+Foxp3^+$ T_R cells (*C*) recovered from culture with colitic LP, MLN, or SP $CD11c^+$ DC in the presence or absence of anti-RANKL mAb were evaluated by flow cytometry. Data are indicated as the mean ± SEM of seven samples per group. *, $p < 0.05$.

mixture including anti-CD3mAb, soluble IL-2, TGF-β, and RA, which is known to induce the gut-homing receptors (integrin $\alpha_4\beta_7$, $\alpha_E\beta_7$, and CCR9) (23). As expected, the mixture strongly induced integrin $\alpha_4\beta_7$, $\alpha_E\beta_7$, and CCR9, but the addition of anti-RANKL mAb did not affect the expression of these molecules (Fig. 6*A*).

To further assess the possibility that blockade of the RANK/RANKL pathway affected the T_R cell migration to the inflamed mucosa, we performed an additional in vivo adoptive transfer experiment, since it has been reported that RANK is expressed on endothelial cells (24). To this end, RAG-$2^{-/-}$ mice were transferred with $Ly5.2^+CD4^+CD45RB^{high}$ T cells and 4 wk after the transfer they were treated with control IgG or anti-RANKL mAb twice in 1 day. On the following day, they were transferred with splenic $CD4^+$ T cells obtained from normal C57BL/6-Ly5.1 mice (Fig. 6*B*). One day after the second transfer, the number of $Ly5.1^+CD4^+Foxp3^+$ or $Ly5.1^+CD4^+Foxp3^-$ T cells in SP, MLN, and LP was found not to be modified by anti-RANKL-treatment at all (Fig. 6*B*).

To finally assess the possibility that colitic LP DC cells modulate the expansion of T_R cells in the inflamed mucosa in a RANK/RANKL-dependent manner, we evaluated the expression of RANK on $CD11c^+$ DC cells obtained from the spleen, MLN, and LP of colitic and normal mice. As shown in Fig. 7*A*, the expression of RANK on colitic LP DC was significantly increased as compared with that on normal LP DC. In contrast, the expression of RANK in SP and MLN was similar in normal and colitic mice. Given the up-regulated expression of RANK on colitic LP DC cells, we next assessed the possibility that the RANK/RANKL pathway is involved in the expansion of T_R cells. To this end, splenic $CD4^+$ T cells obtained from normal mice were cultured with colitic LP, MLN, or SP $CD11c^+$ DC in the presence of anti-CD3 mAb with or without anti-RANKL mAb for 72 h. The ratio of $CD4^+Foxp3^+$ T_R cells per the total of $CD4^+$ T cells (Fig. 7*B*) and the number of $CD4^+Foxp3^+$ T_R cells (Fig. 7*C*) recovered from culture with colitic LP, but not MLN

or SP, $CD11c^+$ DC in the presence of anti-RANKL mAb was significantly decreased as compared with that in the presence of control IgG, suggesting that the RANK/RANKL pathway is critically involved in the expansion of LP T_R cells through the direct interaction with colitic RANK-expressing LP DC and T_R cells.

Discussion

In the present study, we demonstrated that 1) $CD4^+CD25^+$ T cells including $CD4^+CD25^{high}$ T_R cells and activated $CD4^+CD25^{low}$ effector cells rather than $CD4^+CD25^-$ T cells preferentially express the RANKL molecule and 2) blockade of the RANK/RANKL signaling pathway suppresses the expansion of $CD4^+CD25^+$ T_R cells and subsequently abolishes the T_R cell-mediated suppression of colitis due to dysregulation of the cell balance between effector $CD4^+$ T cells and T_R cells in the inflamed intestine. Interestingly, although activated effector $CD4^+$ T cells and inducible $CD4^+CD25^+$ T_R cells also express RANKL molecules in SCID mice transferred with $CD4^+CD45RB^{high}$ T cells alone, the administration of this mAb did not affect the course of colitis. Collectively, these findings indicate that the RANK-RANKL signaling pathway is critically involved in intestinal mucosal tolerance by controlling the expansion and function of $CD4^+CD25^+$ T_R cells in the inflamed mucosa.

Although many previous reports have established the role of the RANK/RANKL signaling pathway in osteoclastogenesis and bone loss in various chronic T cell-mediated inflammatory diseases including IBDs (20, 25–29), the role of this pathway in the local inflammation in various models remains unknown. For example, Kong et al. (26) initially reported that the blockade of this pathway by using soluble recombinant osteoprotegerin (OPG) protein at the onset of disease prevented bone and cartilage destruction, but interestingly not inflammation in a T cell-dependent model of rat adjuvant arthritis. In contrast, Ashcroft et al. (30) demonstrated that the administration of RANK-Fc protein not only reverses the bone loss in $IL\text{-}2^{-/-}$ mice, which is a spontaneous model of osteoporosis and colitis, but also reduces the development of colitis by blocking the interaction between RANK-expressing DC and RANKL-expressing activated $CD4^+$ T cells in the inflamed mucosa of the colon. In our colitis model induced by adoptive transfer of $CD4^+CD45RB^{high}$ T cells into SCID mice, however, administration of neutralizing anti-RANKL mAb did not prevent the development of colitis, although inducible $CD4^+CD25^{high}$ T_R cells and previously activated $CD4^+CD25^{low}$ T cells expressed RANKL. Consistent with this finding, Byrne et al. (31) previously demonstrated that administration of human osteoprotegerin-Fc increased bone density in this model, but had no effects on the intestinal inflammation). Several explanations have been advanced for the discrepancy, including differences in the species, the type of animal model, the type of blocking agents, and dosing regimens used. Furthermore, it has recently been demonstrated that in vivo administration of neutralizing anti-cytokine mAbs, such as anti-IL-2 mAb, enhances the corresponding cytokine activity due to the formation of cytokine/anti-cytokine mAb complexes, which are more stable and stimulatory (32). Although we previously demonstrated that our anti-RANKL mAb used in vivo successfully worked as a blocking mAb in a model of collagen-induced arthritis (21), further studies will be needed to address this issue.

Although we could not detect a suppressive effect of neutralizing anti-RANKL mAb on the development of colitis in SCID mice transferred with $CD4^+CD45RB^{high}$ T cells alone, we found that this treatment induced colitis in mice transferred with $CD4^+CD45RB^{high}$ T cells and $CD4^+CD25^+$ T_R cells at a ratio of 3:1, while mice transferred with $CD4^+CD45RB^{high}$ T cells and $CD4^+CD25^+$ T_R cells at the same ratio and given control IgG did not develop colitis. This strongly suggested that the target cells for anti-RANKL mAb are $CD4^+CD25^+$ T_R cells rather than $CD4^+CD45RB^{high}$ T cells or the differentiated effector $CD4^+$ T cells. Consistent with this notion, we found that RANKL was expressed on $CD4^+CD25^{high}$ T_R cells, but not on $CD4^+CD25^-$ cells (Fig. 1). In an in vitro coculture assay to further evaluate the role of RANKL on $CD4^+CD25^+$ T_R cells in modulating the T_R activity of $CD4^+CD25^{high}$ cells in vitro, however, the addition of anti-RANKL mAb or anti-RANK mAb produced no detectable reduction of T_R activity, suggesting that the direct interaction between RANKL-expressing $CD4^+CD25^+$ T_R cells or activated $CD4^+$ T cells and RANK-expressing APCs including DC is not essentially important for abolishing T_R activity at least in vitro. Since in vitro assays do not always reflect the T_R function in vivo, we next performed another adoptive transfer experiment using $Ly5.1^+CD4^+CD45RB^{high}$ T cells and $Ly5.2^+CD4^+CD25^+$ T_R cells to evaluate the possibility that blockade of the RANK/RANKL signaling pathway affects the recruitment and expansion of specific populations in our model. In this experiment, we found that the ratio of the Ly5.2-derived $CD4^+CD25^+$ T_R cell population per total $CD4^+$ T cells in anti-RANKL mAb-treated mice was significantly decreased in the LP, but not in the MLN, spleen, or liver, as compared with the ratio in control-IgG-treated mice. This finding suggests three points. First, it is possible that the migration of $CD4^+CD25^+$ T_R cells to the inflamed mucosa is regulated by the interaction between RANKL-expressing T_R cells and possibly RANK-expressing endothelial cells. Consistent with this hypothesis, it has been reported that RANK is expressed on murine and human endothelial cells (24). However, this is unlikely because our short-term in vivo adoptive transfer experiment (Fig. 7) demonstrated that treatment of anti-RANKL mAb did not affect the recovered cell number of $CD4^+CD25^+$ T_R cells in the inflamed mucosa. Second, it is possible that the in vivo expansion of RANKL-expressing $CD4^+CD25^+$ T_R cells or colitogenic $CD4^+$ effector/memory T cells is modulated by RANK-expressing DC in the inflamed mucosa. Consistent with this hypothesis, we found that 1) the expression of RANK on colitic LP DC is significantly increased as compared with that on normal LP DC and 2) the ratio of $CD4^+Foxp3^+$ T_R cells to total $CD4^+$ T cells after stimulation with colitic LP, but not MLN or splenic, $CD11c^+$ DC was significantly suppressed by the addition of anti-RANKL mAb. Thus, it is possible that the interaction of RANKL-expressing $CD4^+CD25^+$ T_R cells and RANK-expressing activated DC in the inflamed mucosa plays an important role in the maintenance of T_R cells. Third, not only the first suppression of T cell priming in draining lymph nodes by $CD4^+CD25^+$ T_R cells, but also the second line of suppression in the inflamed mucosa by these cells is critically involved in the intestinal homeostasis to suppress the development of colitis. Consistent with our finding, Green et al. (33) previously reported that the blockade of the RANK/RANKL pathway resulted in a decreased frequency of $CD4^+CD25^+$ T_R cells in the draining lymph nodes and pancreas in the NOD mouse (33). However, it remains unknown why the ratio of $CD4^+CD25^+$ T_R cells in MLNs was unchanged in our adoptive transfer model. The exact mechanism of the function of $CD4^+CD25^+$ T_R cells in the inflamed mucosa warrants further investigation.

Finally, the suppressive site of colitis should be discussed. Denning et al. (34) previously demonstrated that integrin β_7-deficient ($\beta7^{-/-}$) $CD4^+CD25^+$ T_R cells that preferentially migrate to MLNs, but are impaired in their ability to migrate to the intestine because of the lack of the gut-homing integrin $\alpha_4\beta_7$ and $\alpha_E\beta_7$ molecules, are capable of preventing intestinal inflammation, suggesting that T_R accumulation in the intestine is dispensable for the protection of this colitis model. In their protection protocol, indeed, it is possible

that $\beta_7^{+/+}$CD4$^+$CD25$^+$ T_R cells are not needed to suppress the development of colitis, because $\beta_7^{-/-}$ CD4$^+$CD25$^+$ T_R cells directly migrate to MLNs and can inhibit naive CD4$^+$CD45RB^{high} T cell activation and proliferation within Ag-draining MLNs, resulting in suppression of the development of the gut-seeking activated effector CD4$^+$ T cells instructed to express the gut-homing receptors such as integrins $\alpha_4\beta_7$ and $\alpha_E\beta_7$. However, it remains unknown whether mucosal CD4$^+$CD25$^+$ T_R cells are necessary for the suppression of mucosal pathogenic effector CD4$^+$ T cells ex vivo, especially in an ongoing colitis system in which it can be assessed whether LP CD4$^+$CD25$^+$ T_R cells as effector T_R cells can suppress the surrounding LP effector CD4$^+$ T cells ex vivo.

In this regard, we have previously demonstrated that human CD4$^+$CD25^{bright} and mouse CD4$^+$CD25$^+$ T cells reside in the intestinal LP, express CTLA-4, GITR, and Foxp3 and possess T_R activity in vitro (11, 12). We also found that the clinical score in SCID mice transferred with CD4$^+$CD45RB^{high} T cells and intestinal LP CD4$^+$CD25$^+$ T cells at a ratio of 3:1 was significantly decreased as compared with that in SCID mice transferred with CD4$^+$CD45RB^{high} T cells alone (12), indicating that the murine intestinal LP CD4$^+$CD25$^+$ T cells maintain intestinal homeostasis to suppress the development of colitis. Having evidence that the murine intestinal LP CD4$^+$CD25$^+$ T cells suppressed the development of colitis induced by the adoptive transfer of CD4$^+$CD45RB^{high} T cells, we further asked whether MLNs are fully essential for the suppression of colitis by splenic CD4$^+$CD25$^+$ T cells. As a second approach to this issue, we also found that the cotransfer of splenic CD4$^+$CD25$^+$ T_R cells prevented the development of colitis in the lymph node-null LT$\alpha^{-/-}$ × RAG-2$^{-/-}$ mice transferred with CD4$^+$CD45RB^{high} T cells, indicating that splenic CD4$^+$CD25$^+$ T cells can suppress the development of colitis in the absence of MLNs (12). Moreover, we demonstrated that CD4$^+$CD25$^+$ T_R cells actually migrated and resided in the colon in LT$\alpha^{-/-}$ × RAG-2$^{-/-}$ mice cotransferred with Ly5.2-derived CD4$^+$CD45RB^{high} T cells and Ly5.1-derived splenic CD4$^+$CD25$^+$ T cells, suggesting that the LP might be a regulatory site between colitogenic effector/memory cells and T_R cells to suppress intestinal inflammation, probably as a second line of suppression (yy). Along with the present findings that the RANK/RANKL interaction is critically involved in the function of CD4$^+$CD25$^+$ T_R cells in the intestine, our research suggests that therapeutic approaches enhancing the migration of CD4$^+$CD25$^+$ T_R cells, such as the specific induction of RANKL on CD4$^+$CD25$^+$ T_R cells, may be feasible in the treatment of IBD.

Disclosures

The authors have no financial conflict of interest.

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Cardiac parasympathetic dysfunction concurrent with cardiac sympathetic denervation in Parkinson's disease

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ABSTRACT

We aimed to characterize the relationship between cardiac sympathetic and parasympathetic dysfunction employing cardiac ^{123}I-meta-iodobenzylguanidine (MIBG) uptake and other autonomic function parameters in Parkinson's disease (PD). 79 PD patients were studied. We performed ^{123}I-MIBG myocardial scintigraphy to assess the extent of cardiac sympathetic denervation. Electrocardiogram readings at rest and postural change in blood pressure were also examined. Coefficient variation of RR intervals (CVR-R) was used as an index for cardiac parasympathetic activity. Cardiac ^{123}I-MIBG uptake did not vary significantly among the Hoehn–Yahr (H–Y) stages. There was a significant correlation between cardiac ^{123}I-MIBG uptake and CVR-R (early, $r=0.457$, $p<0.001$; late, $r=0.442$, $p<0.001$). While the correlation was present among the patients who had had the disease less than two years (early, $r=0.558$, $p<0.001$; late, $r=0.530$, $p<0.001$), the patients with the disease duration longer than two years did not have such a significant correlation. Age, disease duration, corrected QT interval, or postural blood pressure change did not correlate with cardiac ^{123}I-MIBG uptake. Orthostatic hypotension was observed in 13 out of 72 subjects, and reduced CVR-R was a major determinant for the development of orthostatic hypotension. We conclude that cardiac parasympathetic dysfunction occurs concurrent with sympathetic denervation as revealed by ^{123}I-MIBG myocardial scintigraphy in PD and contributes to the development of orthostatic hypotension.

1. Introduction

Recent evidence shows that Parkinson's disease (PD) is a neurodegenerative disease that manifests a constellation of neurological symptoms beyond classic Parkinsonian features, such as resting tremor and rigidity, and forms a continuum with dementia with Lewy bodies (DLB) characterized by limbic and neocortical degeneration responsible for cognitive impairment [1]. Braak et al. [2] clarified that pathological processes in PD begin in the anterior olfactory nucleus and medulla, the latter of which harbors the dorsal motor nucleus of the vagal nerve, one of major autonomic centers. Accordingly, hyposmia and autonomic dysfunction, particularly constipation, are now appreciated as early clinical manifestations relevant to PD. Recognition of these symptoms will become more important with attempts to institute preventive therapy against this disabling disease.

The peripheral autonomic system is also affected in PD. Pathological studies have demonstrated the presence of Lewy bodies in myenteric and submucosal plexuses [3–5]. As for the cardiac autonomic system, many nuclear radiological studies using ^{123}I-meta-iodobenzylguanidine (MIBG) or 6-[^{18}F]fluorodopamine have reported cardiac sympathetic denervation in PD patients, and the degeneration of postganglionic sympathetic fibers was confirmed by postmortem pathological examinations [6,7]. However, the absence of obvious cardiovascular symptoms, like orthostatic hypotension, in many of cases displaying reduced cardiac ^{123}I-MIBG uptake is an enigma [8,9]. This implies a need for a comprehensive understanding of cardiovascular autonomic status including cardiac parasympathetic and peripheral vasomotor activity and cardiac sympathetic function. Despite the presence of several hemodynamic studies showing the involvement of cardiac parasympathetic system in PD [9–13], its incidence and temporal profile relative to cardiac sympathetic denervation remain elusive.

Here, we demonstrate that cardiac parasympathetic dysfunction occurs with sympathetic denervation in PD by examining heart rate variability at rest and cardiac ^{123}I-MIBG uptake. Concurrent development of parasympathetic and sympathetic dysfunction is obvious in the early stages of PD.

2. Methods

2.1. Subjects

79 PD patients (39 men and 40 women) who visited our outpatient clinic were studied. All patients fulfilled United Kingdom Parkinson's

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Table 1
Comparisons of demographic and autonomic function parameters other than H/M ratio of ^{123}I-meta-iodobenzylguanidine (MIBG) among the Hoehn–Yahr stages

Hoehn–Yahr stage	I	II	III	IV	V	p
N	20	29	23	5	2	
Age, y	71.0±5.2	72.5±6.9	76.4±5.5	75.2±5.5	74.0±4.2	NS
Gender (M:F)	8:12	15:14	10:13	3:2	0:2	NS
Disease duration, y	1.4±1.1	3.5±4.0	4.2±4.6	6.0±6.1	10.5±6.4	NS
						p=0.017[a]
Age of onset, y	69.4±5.4	69.0±8.7	72.3±8.0	69.2±10.7	63.5±10.6	NS
WR (%)	32.7±7.1	33.0±6.3	33.2±6.1	29.9±3.5	26.2±5.0	NS
CVR-R (%)	2.50±1.26	2.11±0.89	2.26±1.23	1.40±0.40	0.81±0.13	NS
QTc (ms)	412±15	408±16	411±15	425±21	433±1	NS
ΔSBPp (mm Hg)	−4.2±15.3	−6.5±17.6	−4.9±16.5	−11.0±15.7	−5.5±16.2	NS

Data are mean±SD. NS=not significant.
[a] *Post-hoc* comparison I vs. V.

Disease Society Brain Bank Clinical Diagnosis Criteria [14]. Other Parkinsonian disorders such as vascular parkinsonism, multiple system atrophy, and progressive supranuclear palsy were excluded from clinical features and MRI findings. None of the subjects exhibited marked dementia or visual hallucination. Those who had ischemic heart disease, diabetes mellitus, or were undergoing treatment with selegiline hydrochloride (L-deprenyl) or tricyclic antidepressants were not included in consideration of the potential effects of these factors on cardiac ^{123}I-MIBG uptake. We employed the Hoehn–Yahr (H–Y) stage to assess the disease severity. 44 of the 79 subjects were on medication for PD at the time of investigation. We evaluated motor symptoms during the "off" period when we examined medicated patients. The mean age±SD was 73.4±6.2 years, and the mean disease duration±SD, 3.5±4.1 years.

Informed consent was obtained from every patient prior to enrollment. This study was approved by the institutional review board for clinical research of the National Hospital Organization Tokyo Medical Center.

2.2. ^{123}I-MIBG myocardial scintigraphy

The patients were asked not to have breakfast on the day of examination. Each subject was relaxed in the supine position for 20 min and was intravenously injected with 111 MBq of ^{123}I-MIBG (Daiichi Radioisotope Laboratories Co., Tokyo, Japan) around 10:00 AM. A thoracic planar image was acquired in a static fashion for 5 min using a dual-headed rotating scintillation camera (HITACHI gamma-view-i RPC-DC) equipped with low-energy, high-resolution parallel-hole collimators after 15 min (early phase) and 3 h (late phase) following the administration of ^{123}I-MIBG. The acquisition matrix was 256×256. Energy discrimination was centered on 159 keV with a 10% window. In each case, an oval region of interest (ROI) was set on the left ventricular part of the heart with a rectangular reference ROI placed on the upper mediastinum of the anterior ^{123}I-MIBG planar image. Average counts per pixel were made in these ROIs. The heart-to-mediastinum (H/M) ratios for the early and late images were calculated to evaluate the integrity of cardiac sympathetic nerve fiber densities. The washout ratio (WR) was defined as 100×(Ec−Lc)/Ec % (Ec: the early cardiac count density, Lc: the decay-corrected late cardiac count density).

2.3. Electrocardiogram analysis and postural change in systolic blood pressure

After a bed rest for 5 min, an electrocardiogram (EKG) recording in the supine position with normal breathing was carried out for 5 min using ECG-1550 (Nihon Kohden). For analysis of coefficient variation of RR intervals (CVR-R), successive 200 RR intervals were sampled during the recording period. CVR-R was automatically calculated as a percentage of the standard deviation of the RR intervals divided by their mean. CVR-R measured at rest under normal breathing is an established index for parasympathetic activity [15,16]. QTc was computed according to Bazett's formula; $QTc=QT/(RR)^{1/2}$. Blood pressure was measured in the supine position. Subsequently, blood pressure in the upright position was recorded after 60-second-long orthostasis. Postural change in systolic blood pressure (ΔSBPp) was also calculated.

2.4. Statistical analysis

The data were analyzed using the SPSS software, version 15.0 Family (SPSS Inc., Chicago, IL). Inter-group differences were evaluated using one-way analysis of variance (ANOVA) combined with Tukey's *post-hoc* test or unpaired *t*-test. χ^2 calculations were used for frequency data. Correlations for the H/M ratio of MIBG uptake, CVR-R, QTc, heart rate (HR), and postural change in systolic blood pressure were assessed using Pearson's correlation coefficient. Multiple regression analyses were performed on the correlation of H/M ratio of MIBG uptake with other parameters. p value <0.01 was considered statistically significant.

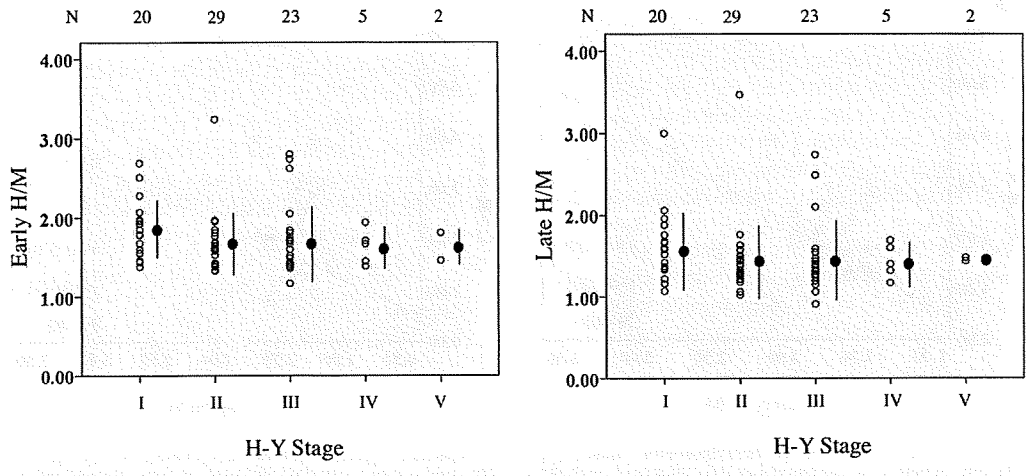

Fig. 1. Comparisons of H/M ratio of ^{123}I-meta-iodobenzylguanidine (MIBG) uptake among the Hoehn–Yahr (H–Y) stages. The dot and bar data represent mean±SD. The data were analyzed using one-way analysis of variance and Tukey's *post-hoc* test.

3. Results

3.1. Comparisons of cardiac ^{123}I-MIBG uptake and autonomic function-related parameters among the H–Y stages

Of the 79 PD patients, we rated 20 patients as H–Y stage I, 29 as H–Y stage II, 23 as stage III, 5 as H–Y stage IV, and 2 as H–Y stage V, on the basis of their clinical manifestations. Using EKGs, we detected atrial fibrillation (Af) in two subjects, premature atrial contractions (PACs) in one, premature ventricular contractions (PVCs) in two, and both Af and PVC in one patient. Such cases were excluded from the analysis of CVR-R and QTc. Additionally, 5 subjects had right bundle branch block (RBBB), and they were eliminated from the QTc analysis. Blood pressure data were available in 72 cases. Orthostatic hypotension (ΔSBPp ≤ −20 mm Hg) was observed in 13 cases (three in H–Y stage I, three in H–Y stage II, four in H–Y stage III, two in H–Y stage IV, and one in H–Y stage V). As shown in Table 1, among the H–Y stages, we found no statistically significant differences in age, disease duration, or sex ratios. There was a trend for a disease stage-dependent reduction in the H/M ratio in the early image (early H/M), and late image (late H/M) of ^{123}I-MIBG uptake (Fig. 1), but statistical significance was not reached because of considerable variations. We did not find any statistically significant difference in WR, CVR-R, QTc, or ΔSBPp (Table 1). These findings suggest that cardiovascular autonomic dysfunction in PD is likely to develop independent of motor impairment, although our data contain only two patients with H–Y stage V.

3.2. Correlations of cardiac ^{123}I-MIBG uptake with autonomic function parameters

In a univariate analysis, there was a significant correlation between H/M ratio and CVR-R (early, $r=0.457$, $p<0.001$; late, $r=0.442$, $p<0.001$; Fig. 2A). We confirmed that there was no significant correlation between CVR-R and age in our subjects ($r=0.086$, $p=0.469$), which excluded the possibility that aging was a significant confounding factor in our study. Subsequently, we performed a sub-analysis using the data obtained only from the PD patients with a duration of illness less than two years. Interestingly, significant correlations between H/M ratio and CVR-R were demonstrated in these patients (early, $r=0.558$, $p<0.001$; late, $r=0.530$, $p<0.001$; Fig. 2B), while such correlations were not detected in subjects having a disease duration beyond two years (data not shown).

On the other hand, WR did not correlate with CVR-R ($r=-0.108$, $p=0.365$). QTc did not correlate significantly with the H/M ratio (early, $r=0.001$, $p=0.995$; late, $r=0.065$, $p=0.597$).

We also looked at the correlation between HR and either of H/M ratio or CVR-R. There was no correlation between HR and H/M ratio (HR vs. early H/M, $r=0.063$, $p=0.583$; HR vs. late H/M, $r=0.058$, $p=0.613$). Meanwhile, there was a weak correlation between HR and CVR-R ($r=-0.227$, $p=0.053$), although the correlation did not reach statistical significance (Fig. 3).

Obvious orthostatic hypotension (ΔSBPp ≤ −20 mm Hg) was observed in 13 out of 72 cases in which blood pressure data were available. One reason for such a low frequency might be that we did

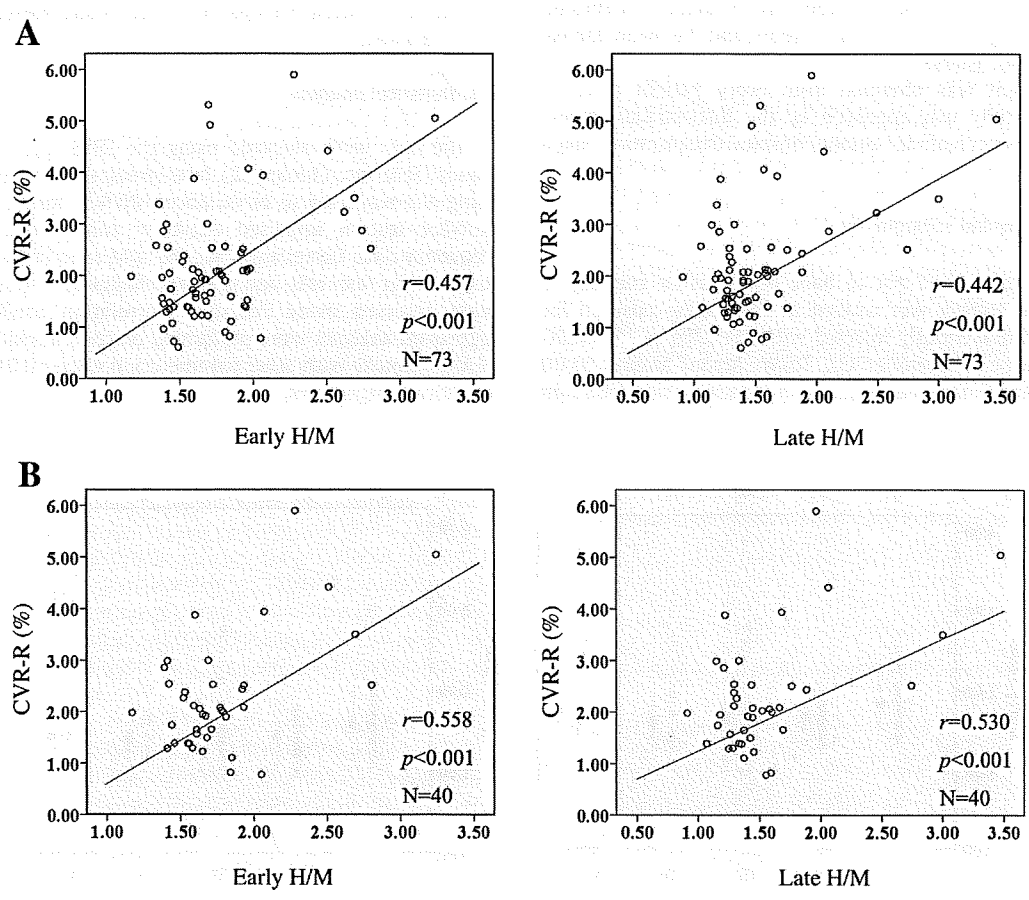

Fig. 2. Correlations between H/M ratio of ^{123}I-meta-iodobenzylguanidine (MIBG) uptake and coefficient variation of RR intervals (CVR-R). (A) Significant correlations were found between H/M ratio of ^{123}I-MIBG at both early and late stages and CVR-R (early, $r=0.457$, $p<0.001$; late, $r=0.442$, $p<0.001$). (B) Using the data obtained only from the subjects with a duration of illness less than two years, the correlations H/M ratio of ^{123}I-MIBG at both early and late stages and CVR-R were significant (early, $r=0.558$, $p<0.001$; late, $r=0.530$, $p<0.001$).

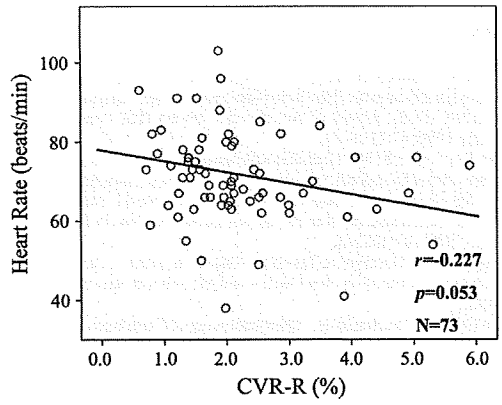

Fig. 3. Correlations between heart rate (HR) and coefficient variation of RR intervals (CVR-R). There was a weak correlation between HR and CVR-R, although the correlation did not reach statistical significance ($r=-0.227$, $p=0.053$).

not adopt a head-up tilt technique [17]. However, our method where the subjects rose on their own appeared more relevant to their natural setting. We compared the demographic and autonomic function parameters between subjects with and without orthostatic hypotension. As shown in Table 2, among those factors, the difference in CVR-R was most prominent. We also performed multiple regression analysis concerning the correlation of cardiac ^{123}I-MIBG uptake and other parameters. Consistent with the univariate analysis, CVR-R exhibited a statistically significant correlation with both early and late H/M ratio (early, $p=0.004$; late, $p=0.003$). Nevertheless, age, disease duration, ΔSBPp, and QTc failed to show any significant correlation (Table 3).

4. Discussion

The present study demonstrates a significant positive correlation between the H/M ratio in the ^{123}I-MIBG myocardial scintigraphy and the CVR-R in patients with PD. Furthermore, our sub-analysis indicates that such a correlation can be attributed to the data obtained from the patients with a disease duration of less than two years. Our findings suggest that cardiac parasympathetic dysfunction occurs in parallel with cardiac sympathetic denervation in early PD patients. Plus, as the severity of cardiac autonomic parameter alterations was not proportional to the degree of motor impairment, the development of cardiac autonomic dysfunction is likely to be independent of the dopaminergic neurodegeneration responsible for motor symptoms. Our data also suggest that cardiac parasympathetic dysfunction plays a major role in the emergence of orthostatic hypotension in PD.

Recently, the importance of non-motor symptoms associated with PD, like hyposmia and autonomic dysfunction, has been emphasized.

Table 2
Comparisons of demographic data, H/M ratio of cardiac ^{123}I-meta-iodobenzylguanidine (MIBG) uptake, and other autonomic function parameters between patients with and without orthostatic hypotension (OH)

	Without OH (n=59)	With OH (n=13)	p
Age, y	72.9±6.4	74.0±5.0	0.480
Gender (M:F)	26:33	7:6	0.525
Disease duration, y	3.3±3.7	5.0±6.3	0.356
Age of onset, y	69.4±7.7	68.9±8.9	0.850
Early H/M ratio	1.77±0.39	1.64±0.17	0.062
Late H/M ratio	1.51±0.44	1.41±0.21	0.197
WR (%)	33.0±6.7	30.7±4.8	0.137
CVR-R (%)	2.30±1.20	1.65±0.50	0.004*
QTc (ms)	410±15	419±18	0.124

*$p<0.01$.
Data are mean±SD.

Table 3
Multiple regression analysis of correlation of cardiac ^{123}I-meta-iodobenzylguanidine (MIBG) uptake with demographic data and other autonomic function parameters

	Parameter estimate	SD	p
a. Early H/M			
Age	0.010	0.007	0.175
Disease duration	−0.012	0.010	0.271
CVR-R	0.118	0.040	0.004*
QTc	−0.002	0.003	0.494
ΔSBPp	0.004	0.003	0.113
b. Late H/M			
Age	0.010	0.008	0.250
Disease duration	−0.013	0.012	0.281
CVR-R	0.139	0.045	0.003*
QTc	0.001	0.003	0.871
ΔSBPp	0.007	0.003	0.036

*$p<0.01$.

These symptoms can be clinical manifestations attributable to early pathological processes in PD [2,18]. As for cardiac autonomic abnormalities, sympathetic denervation has been demonstrated by numerous studies employing either ^{123}I-MIBG myocardial scintigraphy or 6-[^{18}F] fluorodopamine cardiac PET [8,9,11–13,19,20]. Nevertheless, little information is available as to how the involvement of cardiac parasympathetic system develops in PD. Goldstein et al. [12] demonstrated that PD patients with orthostatic hypotension had a parasympathetic dysfunction, as evidenced by a reduced reflexive cardiovagal gain during the Valsalva maneuver. Moreover, Kallio et al. [21] demonstrated an abnormal cardiac parasympathetic activity in their 50 untreated PD patients by examining the high frequency component of RR intervals (RR-HF). Their results are in agreement with our finding that cardiac parasympathetic activity begins to decline with cardiac sympathetic denervation even in early PD patients. A recent report studying 44 untreated PD patients shows that RR-HF decreases with increasing disease severity, thus suggesting that cardiac parasympathetic dysfunction was a late event in PD [9]. There was no correlation between the RR-HF and the severity of cardiac sympathetic denervation despite ^{123}I-MIBG myocardial scintigraphy in their study. In another study, a weak correlation was found between heart rate variability and the latency of sympathetic skin response, the latter of which is a marker for sympathetic sudomotor activity [22]. Although it is accepted that there is cardiac parasympathetic dysfunction in PD, there seems to be a controversy as to the temporal profile of its development in relation to sympathetic abnormalities.

The heart rate variability at rest under normal breathing has been established as a reliable index of cardiac parasympathetic activity [15,16]. We measured the heart rate variability of 200 consecutive heartbeats, which took only a few minutes. This approach has several strengths. First, it does not require any strenuous act, such as the Valsalva maneuver, which can be unfeasible for PD patients with overt hypokinesia. A brief EKG recording in a supine position is suitable for PD patients, who often have motor impairment. Second, the heart rate variability at rest is almost free of the baroreflex function exerted by the glossopharyngeal afferent fibers from the carotid sinus and aortic arch, while that obtained during the Valsalva maneuver is susceptible to modulation by the vagal baroreflex. Our results raise the possibility that the abnormality lies in the vagal efferent system. Although some authors have detected abnormal changes in the heart rate variability indicative of parasympathetic dysfunctions during the Valsalva maneuver in PD, it was difficult to determine the site of abnormality in its complicated reflex arc [9,12]. Furthermore, destruction of nigrostriatal dopaminergic system *per se* has been demonstrated to affect baroreflex sensitivity in rats [23]. Despite the difference in species, this result suggests caution in interpreting the results from the Valsalva maneuver in PD. Third, our short-term EKG recording is less susceptible to the effect of arrhythmias. A recent study using a

45-minute-long EKG recording has disclosed a higher incidence of extrasystoles in 40 PD patients as compared to 80 normal subjects (55% vs 16.25%) [24]. Thus, a shorter recording should avoid the effect of arrhythmias, which render the accurate measurement of heart rate variability impossible. Lastly, as the CVR-R can be calculated very quickly, and does not entail any complicated analysis, it can provide immediate information on cardiac parasympathetic function. For these reasons, our method for measuring the CVR-R is an excellent means to assess cardiac parasympathetic activity of PD patients.

The parasympathetic abnormalities underlying the decreased heart rate variability in PD are unknown. From a physiological viewpoint, it can reflect a loss of function or a hypertonic state of the cardiac parasympathetic system. However, it is impossible to determine which of these is responsible from our findings alone. It was shown that the decrement of RR intervals in response to a decrease in blood pressure during the early second phase of the Valsalva maneuver was compromised, whereas the reflex bradycardia in the fourth phase was preserved in their de novo PD patients [9]. This favors the hypertonic state hypothesis, because the finding can be well explained by the inability of parasympathetic tone to diminish with declining blood pressure.

Concerning the underlying pathological changes, Benarroch et al. [25,26] showed that the ventrolateral nucleus ambiguus, a major locus of cardiac preganglionic vagal neurons [27,28], is spared in PD. Meanwhile, the dorsal motor nucleus of the vagal nerve provides a minor preganglionic parasympathetic projection to the cardiac ganglia, and cell loss and aberrant α-synuclein depositions identified as Lewy bodies and Lewy neurites in the brainstem nucleus are prominent in PD [2,26]. Consistent with the MIBG myocardial scintigraphy findings, the cardiac postganglionic sympathetic fiber degeneration has been verified by several pathological studies [6,7].

In contrast, information about the involvement of cardiac postganglionic vagal neurons in PD remains scanty [29,30]. Iwanaga et al. [29] noted the presence of Lewy bodies and Lewy neurites in the cardiac plexus. Intriguingly, those inclusions resided in both tyrosine hydroxylase-positive and -negative nerve processes, which suggested that PD affects parasympathetic fibers as well as sympathetic nerves in the heart. As incidental Lewy body disease cases were included in their study, there is the likelihood that the involvement of cardiac postganglionic parasympathetic fibers occurs even in the early stage of PD.

The paucity of cardiovascular symptoms, such as orthostatic hypotension, in PD patients with cardiac sympathetic denervation identifiable with ^{123}I-MIBG myocardial scintigraphy seems paradoxical, and therefore obscures the significance of the cardiac sympathetic denervation. Haensch et al. [31] also reported that reduced ^{123}I-MIBG myocardial uptake occurred irrespective of the presence of orthostatic hypotension. Our study suggests that the advancement of cardiac parasympathetic dysfunction in addition to sympathetic denervation may be a requirement for the development of orthostatic hypotension. Additionally, our data imply that impaired cardiac parasympathetic activity contributes more to determine HR than cardiac sympathetic denervation, although the impact of CVR-R change on HR is relatively small (Fig. 3). This is likely to explain why most of the PD patients with a low CVR-R value did not exhibit tachycardia at rest. Moreover, the possibility remains that concurrent cardiac sympathetic denervation may offset the effect of parasympathetic dysfunction.

In summary, this study provides important evidence for cardiac parasympathetic dysfunction in parallel to cardiac sympathetic denervation in early PD. Thus, our findings expand the spectrum of non-motor aspects of PD and highlight the character of PD as a multisystemic disorder even in its early stage.

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パーキンソン病
—基礎・臨床研究のアップデート—

V. 治　　療

薬物療法

主な治療薬の種類と特徴

ドパミン放出促進薬—概要，薬理作用，用法・用量，有用性，副作用とその対策—

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ドパミン放出促進薬—概要，薬理作用，用法・用量，有用性，副作用とその対策—

Drug related to an increase of dopamine release: amantadine hydrochloride

高橋一司

Key words：amantadine hydrochloride, Parkinson's disease, therapy, dopamine release, NMDA antagonist, dyskinesia

はじめに

本稿では，塩酸アマンタジン(amantadine hydrochloride: Am)に関して記載する．Amは当初1日200mgの投与によりparkinsonismに効果を示すことが判明し，その後1日300mg以上の投与によってパーキンソン病(PD)進行期のL-dopa induced dyskinesia(LID)に抑制効果を示すことが知られるようになった．その機序として，前者にはドパミン(dopamine: DA)放出促進作用・取り込み抑制作用が関連し，後者にはNMDA(N-methyl-D-aspartate)受容体拮抗作用が関連すると考えられている．薬理学的にも多様な機序をもち，PD治療においては維持量の違いによってdual effectsを示す不思議な薬剤である．本稿ではその作用機序ごとに分けて記載を試みた．

1. ドパミン放出促進薬としての塩酸アマンタジン

a. 概　　要

Amは，1959年米国のDu Pont社において，A型インフルエンザウイルスに対する抗ウイルス剤として開発され，1968年にSchwabらによりPDへの有効性が発見された．PD患者(58歳，女性)がインフルエンザ予防のため，本剤を内服(1日200mg，分2)，PD症状の改善を自覚した．6週間の内服後に終了，それに伴い症状が内服以前の状態へ戻ってしまったことを訴えた．Schwabら[1]は，この興味ある報告を重視し，163人の患者で本剤の効果を評価し，6カ月間のAm 1日200mg(2回に分服)により，66％の患者でPD症状の改善が認められた．投与開始後，筋強剛，無動に効果を示し，4-8週後に改善効果はやや低下するが，その効果は消失することなく継続した．また内服中止に伴い症状は増悪し，再投与にて改善効果が得られた．

これに続き，1970年代にplaceboを対照にした二重盲検の検討が施行され，Amの抗PD作用が確認された．そして我が国では，抗PD剤として1975年に発売された(**図1**)．

b. 薬理作用

抗PD薬としてのAmの作用部位・機序はまだ十分に解明されていない．

(1) カテコールアミン放出促進作用：ラットの虹彩および線条体を摘出し，それぞれに^{3}H-NA(ノルアドレナリン)，^{3}H-DA(ドパミン)を添加しプレインキュベート後，Amを含む緩衝液(10^{-5}M)で表面灌流を行うと，虹彩からの^{3}H-NA，線条体からの^{3}H-DAの放出増加がみら

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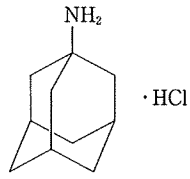

図1　アマンタジンの化学構造式

分子式および分子量：$C_{10}H_{17}N \cdot HCl$(187.71)．商品名のSymmetrel®は，化学構造式が左右対象(symmetry)であることに由来する．

れた[2]．最近Araiらは，6-hydroxydopamine(6-OHDA)のPDモデルラットにおいて，L-dopa(50 mg/kg)/benserazide(10 mg/kg)投与による細胞外DA濃度を測定し(*in vivo* microdialysisによる)，Am(30 mg/kg)の前投与により，線条体の細胞外DA濃度が250％に増加したことを報告した[3]．更にその作用機序が，aromatic l-amino acid decarboxylase(AADC)活性の変化によるものではないことも示した．

(2) ドパミン取り込み抑制作用：ラット脳から線条体・尾状核・被殻を摘出し，遠心分離後，顆粒分画溶液にAmと^{3}H-DAを添加したところ，Am(3.8×10^{-6} M)にてDA取り込みに50％の抑制効果がみられた[4]．

(3) ドパミン合成促進作用：Am 40 mg/kgをラットに皮下注射し，2時間後に線条体を摘出し^{3}H-チロシンを加えてインキュベートすると，溶媒と線条体切片の^{3}H-DAの量は有意に増加し，DA合成の活性化作用を指摘した報告があるが[5]，一方，前述のAraiらの報告のようにAADC活性の関与は否定的な結果がある．

c. 薬物動態

健常男性5人にAm(50 mg，100 mg)を早朝空腹時に1回経口投与し，血漿中濃度を測定した結果，約2-3時間で最高血漿中濃度(約130および260 ng/mL)に達し，その後半減期約10-12時間で血中から消失した[6](**図2**)．消化管からの吸収は良好で，また排泄部位は主に腎臓である．したがって腎機能低下例では血漿中濃度が上昇し[7]，蓄積により副作用が発現する可能性がある．投与後約24時間で投与量の約60％，48時間までに約70％が未変化体で尿中に排泄された[6]．ヒトの尿中ではN-アセチル体が5-15％に認められた．透析による除去率は低く，4時間の透析で投与量の5％以下しか除去されなかった[8]．なお分布に関しては，血液-脳関門通過性は良好，胎児と乳汁中への移行性もみられる．

血漿中Am濃度に関しては，Nishikawaらによる最新のデータが注目される[9]．2週間以上Amを内服している78人のPD患者(平均年齢：66.6歳)で，朝食後の内服(平均用量：135.1 mg/日)から3時間後の血漿中濃度は812.5 ng/mL(91-4,400 ng/mL)で，94％の症例では2,000 ng/mL以下であった．濃度は腎機能低下(creatinine clearance: Ccrにて評価)に伴って上昇していた．3症例でmyoclonus，幻覚，せん妄などの副作用がみられ，濃度がいずれも3,000 ng/mL以上に上昇していた．血清creatinine値は1例では1.1 mg/dLと正常範囲，他の1例も1.4 mg/dLと軽度高値にとどまっていたことに注意を払う必要がある(いずれの例でもCcrは27 mL/minと著明に低下)．更にCcr>60 mL/minの14例(100 mg/日内服)の検討では，血漿中濃度に191-800 ng/mL(平均475.3 ng/mL)と約4倍の差がみられた点も銘記すべきであろう．また血漿中濃度3,000 ng/mLが中枢神経系の副作用発現の閾値と考えられると指摘されている．なお，副作用は可逆的で，3例ともAm内服中止後，3-5日で副作用が消失した．腎機能低下がみられる例，ことに高齢者でのAm内服の際は，副作用の発現に特に注意する必要がある．また，低体重の高齢者では体重あたりの投与量が多くなりやすいと考えられる．

d. 用法・用量

Amの剤形は50 mg錠，100 mg錠，細粒(10％：1 g中に100 mg含有)がある．通常，成人には初期量1日100 mgを1-2回に分割経口投与し，1週間後に維持量として1日200 mgを2回に分割経口投与する．なお，症状，年齢に応じて適宜増減可能だが，1日300 mgを3回分割経口投与までとなっている．

e. 有用性

Amの臨床成績は，我が国で2002年に策定さ

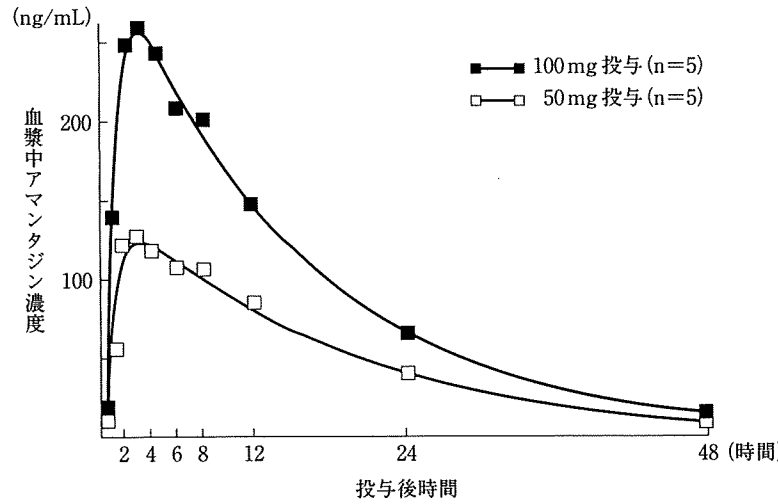

図2　アマンタジン(50 mg, 100 mg)を1回投与後の血漿中濃度の推移(文献[6]より引用)

表1　アマンタジンに関する'パーキンソン病治療ガイドライン'委員会(2002年)の結論
(文献[10]より作成)

A. 有用性	
1) パーキンソン病症例に対する対症効果	症状改善に有効である.
	症状別有効症例率では, 振戦, 筋固縮, 無動のいずれにも有効である.
	多くの症例に有効であるが, 症状改善率は高くないと結論される.
	無効例もある.
	効果が一過性である(長期にわたれば消失)ことを明らかに支持する報告はない.
2) 進行期パーキンソン病症例に対する対症効果	dyskinesiaに対して, 恐らく有効である.
	症状変動に対する効果は, エビデンスが不十分である.
3) パーキンソン病進行抑制効果	神経保護作用の判定は, 十分なエビデンスがなく, 結論を述べるには時期尚早である.
B. 安全性	安全に投与できる薬剤である.
C. 臨床への応用	パーキンソン病治療に有用である.
	初期, 軽症例には, 投与の意味がある.
	進行例では, L-dopaの併用が必要となる.
	dyskinesiaへの効果が注目されている.

れたPD治療ガイドラインおいて詳細に検討がなされている[10](**表1**). Level Iaに該当するmeta-analysisによる検討はない. PubMedから選択された論文は, 1966-74年：8文献(Level I), 3文献(Ib), 1975-2000年：6文献(I)で, これに我が国で実施された治験の報告が2件加えられ, 19文献に基づいて検討がなされている. 1970年代の検討のほとんどは, 1日200 mg投与を2-4週間継続したもので, 無動, 筋強剛の改善効果が共通して示されている. 我が国での他剤との比較では, 塩酸トリヘキシフェニジルとの有意差はなく, ブロモクリプチン

が優れているとの結果である．L-dopaとの併用効果を示した検討も4つあるが，併用による上乗せ効果の持続は不明である．また長期投与の効果持続に関しては，Factorらの検討がある[11]．様々なLevelの報告のまとめとなっているが，効果が一過性であるとの確定した知見はないとの結論である．

なお，本剤の承認された効能または効果[12]として，特にパーキンソン症候群においては，筋強剛，振戦，無動，歩行障害などの主要四症状のいずれにも有効である．効果発現が早い(通常，数日から1週間以内に治療効果がみられる)．PD症状に伴う，うつ気分を改善するとあるが，PD治療ガイドラインでは，うつ症状に関する研究は極めて不十分とされた[10]．

f. 副作用とその対策

パーキンソン症候群での副作用調査(承認時までおよび新開発医薬品の副作用頻度のまとめの集計，2006年12月改訂)において，2,278例中23.4％に副作用が認められ，その発現頻度は，消化器系12.8％，精神神経系16.2％，皮膚1.0％，全身症状3.1％，泌尿器系0.3％，心・血管系1.0％，筋骨格系0.2％，呼吸器系0.1％，感覚器系0.5％，その他1.4％であった．

(1) 精神神経系：幻覚は本剤を高用量投与時に発現しやすく(200-300mg/日)，特に高齢者や腎機能障害者には低用量からの漸増が薦められる．本剤によるせん妄の発現機序はまだ明確にはなっていない．いずれの症状も投与開始1-2週間後から発現し，中止1週間程度で軽快する症例が多い．

(2) その他：下肢浮腫の発現機序としては，総水分量やNaなどの電解質に変化がみられないことから，血管壁の透過性が変化し，体液が末梢へ移行することによるとの説がある．また，網状皮斑(livedo reticularis)は，真皮と皮下脂肪組織の境界部にある小血管において，静脈側の緊張低下と動脈側の緊張亢進により生じる淡紅色の網の目状の紅斑である．本剤による網状皮斑は浮腫と同時に発現する場合が多く，本剤減量や利尿剤の投与により消失するとされる．

【重大な副作用と初期症状】

(1) 悪性症候群(syndrome malin)：急激な減量や中止，また投与継続中に出現．

(2) 視力低下を伴うびまん性表在性角膜炎，角膜上皮浮腫様症状

(3) 心不全

項目(2)のびまん性表在性角膜炎とは，非常に微細な角膜上皮の脱落が多発する状態を指し，通常，結膜異物，コンタクトレンズの長期間装用などにより発症する．本剤では，点状表層角膜炎の病態が報告されているが，頻度も発生機序も不明である．角膜病変そのものは原因除去により早く回復するが，薬剤投与の継続により重篤な転帰(視力障害など)をとる可能性もあり，速やかな中止と眼科医による診断・処置が重要である．

g. 禁　　忌

(1) 透析を必要とするような重篤な腎障害のある患者

(2) 妊婦または妊娠している可能性のある婦人および授乳婦

(3) Amに対し過敏症の既往歴のある患者

h. 過量投与

過量投与の際の徴候・症状：神経筋障害(反射亢進，運動不穏，けいれん，ジストニー姿勢など)と急性精神病徴候(錯乱，見当識障害，幻視など)が，急性中毒の顕著な特徴である．そのほか洞性頻脈，嘔吐，尿閉などがみられることがある．

処置：特異的な解毒薬は知られていない．必要に応じて対症的な処置を行う．

i. そ の 他

PDにおけるうつ状態の発生頻度は，一般的に40％前後とされている．症状の特徴は軽度の抑うつ傾向，意思や思考の抑制で，apathy(無感情)，anhedonia(失快楽感)，anxiety(不安)がみられることが多く，自責感や自殺企図などは多くない．しかし，Am服用中のPD患者において，自殺企図が国内外を通じ報告されており，長期投与の際には患者の精神症状に注意する．

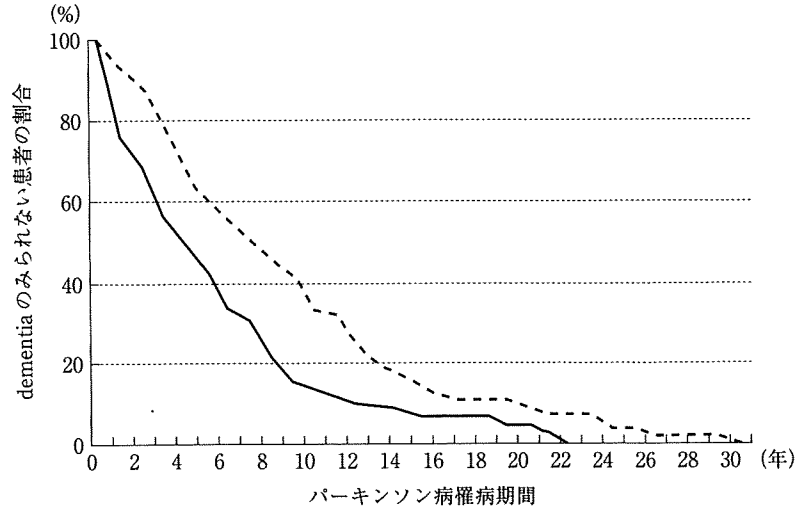

図3　Dementia 発症をエンドポイントとした Kaplan–Meier 曲線
（文献[18]より引用）
破線：アマンタジン内服群，実線：アマンタジン非内服群．

2. NMDA 受容体拮抗薬としての塩酸アマンタジン

大脳皮質から線条体への入力神経で最も多いのはグルタミン酸作動性線維である．近年，dyskinesia 抑制効果に関する Am の作用機序として，線条体における NMDA 受容体の拮抗作用が推定されている[13]．PD 治療ガイドラインでは，LID に関する臨床成績として 3 文献が検討され[10]，LID の軽減は 17–60 % とされる．Verhagen Metman ら[14]は，LID と wearing off がみられる PD 患者 14 例（平均罹病期間：13 年，L–dopa 平均投与期間：12 年，平均投与量：1,074 mg）において，300–400 mg の Am を 3 週間投与し，dyskinesia score と off 時間の改善を認めた．投与量は多いが，副作用の記載はない．1 日 300 mg/日以上の Am が LID に対して有効とされる基礎となった検討である（注：我が国での承認用量は 1 日 300 mg/日まで）．有効例での血漿中濃度は，平均 1,126 ng/mL（>6 μM）まで上昇していたとされ，効果発現には十分な血漿中濃度が必要と考えられるが，前述したとおり腎機能低下がみられる症例では，やはり慎重な内服量の設定が望まれる．

また米国神経学会（AAN）の定めた PD 治療に関する practice parameter（evidence–based review）では，Am は dyskinesia の減少に効果を示す可能性がある（Level C）とされている[15]．根拠となる文献（Class II）として Snow らの検討があげられている[16]．LID がみられる PD 患者 24 例（平均罹病期間：10.6 年，L–dopa 平均投与量：834 mg）において，200 mg の Am を 3 週間投与し，total dyskinesia score の改善と dyskinesia 出現時間の短縮を認めた．

興奮性アミノ酸の神経毒性に対して，Am の NMDA 受容体拮抗作用による神経保護効果も類推されている．Uitti らは，Am 内服 PD 患者（250 人）と非内服 PD 患者（586 人）を比較し（Kaplan–Meier 曲線を用いた生存率の検討），Am 内服患者の寿命が有意に長いと報告している[17]．もちろん，この結果を直接神経保護効果と結びつけることはできないが，今後の検討が期待される領域である．また dementia 発症に関する効果の検討もある．Inzelberg らは，PD 患者 593 例（平均罹病期間：9.2 年，Am 内服患者は 263 人，44 %）において，dementia の発症をエンドポイントとし，Kaplan–Meier 曲線を用いて検討した[18]．全体の 20 % にあたる 116 人

にてdementiaが発症し，発症までの期間はAm非内服群では5.9年に比し，Am内服群では9.1年と有意に延長していたことが示された(**図3**)．ラット大脳皮質におけるcholine acetyltransferase活性はNMDA刺激により低下することが知られるが，NMDA受容体拮抗作用をもつ本剤やmemantineの投与により，その活性低下の程度が減少し，acetylcholineの利用率が保たれる機序の可能性も指摘されている[19]．

おわりに

AmはPD患者における偶然の投与の結果，抗PD作用が発見・確認されるに至った．注意深い診察と鋭い洞察が，臨床医学の発展にいかに寄与できるかが，如実に示された，臨床医として銘記すべきエピソードである．著者らは臨床医として患者から多くを学び，それを医学的に研究・発展させ，有効な治療法として確立し，患者へ還元していく責務がある．薬効の臨床的な確認後に様々な薬理作用が検討されているが，現時点でそのいずれも十分とはいえない．NMDA受容体の拮抗薬としてLIDの抑制効果もPDモデル動物で確認されているが，Am単独投与では運動量はむしろ低下するとの報告があり，その作用機序にはグルタミン酸受容体のsubtypeの関与も考えられ[20]，今後も検討が必要であろう．

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神経変性疾患の診断と治療　脳の時代21世紀の克服にむけて　（診断編）

パーキンソン病

高橋　一司*
たかはし　かずし

- パーキンソン病は，中脳黒質―線条体ドパミン作動性神経細胞の変性・脱落による錐体外路症状を示し，病理学的にはLewy小体の出現を特徴とする神経変性疾患である．
- 安静時振戦（Tremor at rest），筋強剛（Rigidity），無動（Akinesia）・運動緩慢（Bradykinesia），姿勢反射障害（Postural instability）が，4大症状（TRAP）である．
- これまで付随的な症候とみられてきた非運動症状（精神神経症状，自律神経症状，感覚症状）を包括的にとらえ，全身性のneuropsychiatric disorderとして，疾患概念が変貌している．
- 運動症状発現以前（premotor）に，すでに一部の非運動症状が出現している．
- "PDがどこから発症するのか"という問いは，Braakらの病理進展仮説とともに現在大きく注目されている．

Key Words　パーキンソン病，診断，非運動症状，ドパミン神経細胞，Lewy pathology

はじめに

パーキンソン病（PD）は，中脳黒質―線条体ドパミン作動性神経細胞の変性・脱落による錐体外路症状を示し，病理学的にはLewy小体の出現を特徴とする神経変性疾患とされてきた．脳内での神経伝達物質としてのドパミンの発見からわずかに50年，21世紀を迎えた今，PDの疾患概念は大きな変革を遂げつつある．ひとつは，これまで付随的な症候とみられてきた非運動症状（精神神経症状，自律神経症状，感覚症状）を包括的にとらえ，全身性のneuropsychiatric disorderとしての疾患概念の変貌である．もう一点は，"PDの病理変化であるLewy pathologyはどこからはじまるのか？"という問いに対する答え，まさしく病態生理の本質に迫った研究成果の蓄積である．このふたつは密接に関連している．運動症状発現以前（premotor）に，すでに一部の非運動症状が出現していることは，臨床的に多くの裏付けがある．今後，PDの診断そのものが，古典的な運動症状の臨床評価でなされている現状から，劇的な変貌を遂げる可能性がある．診断精度を向上させるbiomarkerの発見，画像診断や発症に関連する因子（環境因子や遺伝的因子）の研究の進歩には瞠目すべきものがある．本稿では，古典的な4大症状に加え，Braakらの病理進展仮説をふまえ，非運動症状のうち特に発症前診断への道を開く可能性のある症状や検査を中心に概説する．

□ 運動症状と診断基準

安静時振戦（Tremor at rest），筋強剛（Rigidity），無動（Akinesia）・運動緩慢（Bradykinesia），姿勢反射障害（Postural instability）が4大症状（TRAP）[1]とされる．本邦のPD治療ガイドライン（2003年，マスターエディション）[2]では，診断の要点として，

① 前記の4大症状のうち少なくとも二つが存在すること
② L-ドーパまたはドパミンアゴニストにて明らかな症状の改善を認めること
③ 頭部CTまたはMRIの所見に原則として明らかな異常を認めないこと
④ 感染，薬物や中毒などによるパーキンソン症候群を除外できることである

としている．PDの診断の基本は，詳細な病歴聴取と神経学的診察である．各国で種々の診断基準が提唱されているが（United Kingdom Parkinson's Disease Society Brain Bankの診断基準[3]，

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表1　パーキンソン病診断基準（厚生省特定疾患・神経変性疾患調査研究班 1996 年）

(1) 自覚症状
　A：安静時のふるえ（四肢または顎にめだつ）
　B：動作がのろく拙劣
　C：歩行がのろく拙劣
(2) 神経所見
　A：毎秒 4～6 回の安静時振戦
　B：無動・寡動（a：仮面様顔貌，b：低く単調な話し方，c：動作の緩徐・拙劣，d：姿勢変換の拙劣）
　C：歯車現象を伴う筋固縮
　D：姿勢・歩行障害；前傾姿勢（a：歩行時に手の振りが欠如，b：突進現象，c：小刻み歩行，d：立ち直り反射障害）
(3) 臨床検査所見
　A：一般検査に特異的な異常はない
　B：脳画像（CT，MRI）に明らかな異常はない
(4) 鑑別診断
　A：脳血管障害のもの
　B：薬物性のもの
　C：その他の脳変性疾患

診断の判定（次の 1～5 のすべてを満たすものをパーキンソン病と診断する）
1．経過は進行性である
2．自覚症状で，上記のいずれか一つ以上がみられる
3．神経所見で，上記のいずれか一つ以上がみられる
4．抗パーキンソン病薬による治療で，自覚症状・神経所見に明らかな改善がみられる
5．鑑別診断で上記のいずれでもない

参考事項（診断上次の事項が参考になる）
1．パーキンソン病では神経症状に左右差を認めることが多い
2．深部反射の著しい亢進，バビンスキー徴候陽性，初期から高度の痴呆，急激な発症はパーキンソン病らしくない所見である
3．脳画像所見で，著明な脳室拡大，著明な大脳萎縮，著明な脳幹萎縮，広範な白質病変などはパーキンソン病に否定的な所見である

（文献[5]より引用）

Calne らの診断基準[4]など），ここでは厚生省（現厚生労働省）特定疾患・神経変性疾患調査研究班が作成した診断基準（1996 年）を示す（**表 1**）[5]．重症度分類は，通常 Hoehn & Yahr の分類が用いられる．PD は緩徐進行性であるが，その進行速度は linear ではなく，病初期により速く，高齢発症にて速く，また振戦優位型の subtype より，姿勢反射障害・歩行障害優位型（postural instability gait difficulty：PIGD）の subtype にて速いことが指摘されている[6,7]．詳細な臨床評価法としては，Unified Parkinson's Disease Rating Scale（UPDRS）が国際的に使用され，日本語版の妥当性も確認されている[8]．2008 年 6 月の Movement Disorder Society（MDS）の年次集会では，MDS-UPDRS として改訂版が発表され，その日本語版も近い将来に完成される予定である．

1．安静時振戦（Tremor at rest）

PD の初発症状としてもっとも気付かれやすく，もっとも多い．発症時に 69％の患者でみられ，全経過では 75％の患者にみられたとの報告がある[3]．上肢の遠位にみられ，安静時の振戦（4～6 Hz），指で丸薬をこねるような運動（pill-rolling tremor）が特徴的であるが，下肢，口唇，下顎にも出現する．動作時には消失・軽減し，睡眠時には消失する．姿勢時にも同様の周波数の振戦がみられることがあり，上肢を水平に挙上し保持してから数秒から数十秒の delay をもって出現することもあり（re-emergent tremor）[9]，診察時には注意が必要である．患者の ADL には，むしろ姿勢時の振戦が影響する．

2．無動（Akinesia）・運動緩慢（Bradykinesia）

PD でもっとも特徴的な症状である．歩行や起

立を含め，あらゆる動作が緩慢となり，日常生活の障害となる．動作の回数，スピードの低下とともに動作の振幅も減少し，進行すると無動となる．巧緻運動障害，小字症（micrographia），仮面様顔貌（hypomimia, masked face），瞬目の減少，構語障害（単調な小声，monotonic & hypophonic dysarthria），唾液の嚥下頻度の低下とともにみられる流涎（drooling），歩行時の腕振り（arm swing）の減少・消失などもこの症状に含まれる．

3．筋強剛（Rigidity）

患者の関節を他動的に屈曲・伸展させる時に感じる筋トーヌスの亢進で，PD の場合は発症側が優位であることも重要な点である．筋強剛には，ガクガクと歯車が噛み合うような抵抗がある歯車現象（cogwheel phenomenon）を伴うことが多く，PD で特徴的とされる．その所見は，遠位（手首や足首）から近位（頸部や体幹）まで認められる．筋強剛には，しばしばしびれや疼痛の訴えが伴う．特に病初期に他疾患と誤って診断される例も稀ではなく，十分注意を払う必要がある．

4．姿勢反射障害（Postural instability）

典型的には前傾前屈姿勢となり，肘と膝は軽度屈曲姿勢となる．検者が患者の後方にまわり，肩を軽く引くと（pull test），立ち直り反射の消失により後方突進現象（retropulsion）が見られる．前方や側方でも見られることがある．また，歩行はすり足となり，歩幅が小さくなる．症状が進行すると最初の一歩が出ず（すくみ現象；freezing phenomenon），歩き出すと止まれなくなる．進行期には体軸症状として，首下がり（dropped head）や腰曲がり（camptocormia）が出現することもある．症例によっては，ドパミンアゴニストの投与が誘因となることも知られ，臨床的には注意を払う必要がある．

□ 補助検査としての画像所見

1．^{123}I-MIBG（metaiodobenzylguanidine）心筋シンチグラフィー

本邦では本検査が，PD を含んだ Lewy 小体病の臨床的補助診断法となり得る可能性から広く活用されている[10,11]．Lewy 小体病では，高率に心臓の MIBG 集積が低下し，他の parkinsonism との鑑別に有用とされ，近年臨床現場において汎用されている（**図 1**）．ただし，病初期では低下がみられない症例もみられ，検査結果は臨床所見とあわせ，慎重に評価する必要がある．発症初期に集積がみられても，経時的に検査を繰り返すことが大切である．臨床的に心血管系での自律神経障害を示す徴候のない患者群でも subclinical に心筋の交感神経支配の障害が存在する点が認識され，本検査が広く注目されるに至った．

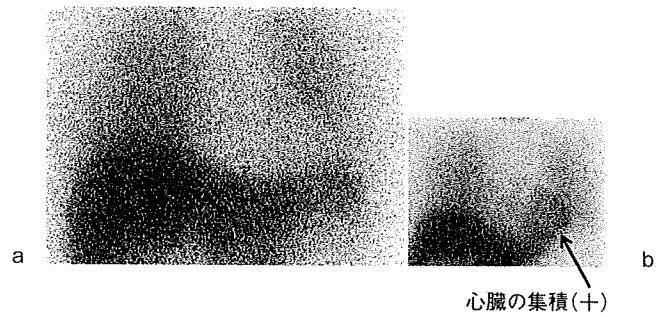

図 1
a：PD 患者における MIBG 心筋シンチグラフィー画像（後期像）
b：同画像（後期像）の正常所見

2．経頭蓋超音波検査

PD 患者の黒質は高輝度に描出され，これには鉄の沈着の可能性が指摘されている．PD に特徴的な変化とされ，発症前診断にも有用とされるが，本検査の短所として，閉経後の高齢女性（特に邦人）では側頭骨の状態から超音波が脳実質に到達せず，黒質を観察することが困難な場合が多いことがあげられる．

3．頭部 MRI

拡散強調画像を含め PD 以外の parkinsonism で特異度が高い所見が知られ，鑑別診断に有用である．通常の 1.5 Tesla の MRI では PD に特異的な所見は知られていない．

4．脳血流シンチグラフィー（SPECT）

PD 以外の parkinsonism の鑑別に有用である．

5．その他

SPECT や PET を用いた，線条体におけるドパミン系の神経伝達機能に関する画像検査が，海外では臨床診断に活用されている．PD では，ドパミン神経の節前機能が著明に低下しているが，