

Relationship Between 3-O-methyldopa and the Clinical Effects of Entacapone in Advanced Parkinson's Disease

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ABSTRACT

The aim of this study is to clarify the relationship between serum 3-O-methyldopa (3-OMD) and the clinical effects of entacapone. The 3-OMD and maximum serum concentration (C_{max}) of levodopa were measured in 21 Parkinson's Disease patients who took 100 mg levodopa / dopa decarboxylase inhibitor. After the administration of entacapone, the 3-OMD concentration and percentage of "on" time during waking hours (% of "on" time) were studied for 8 weeks. The 3-OMD concentration was reduced by 34%, and the increase in % of "on" time was 28% at the 8th week compared with baseline. We defined the COMT-index as [baseline 3-OMD concentration] / [levodopa C_{max} when 100 mg levodopa was administered alone]. The COMT-index was significantly correlated with the increase in % of "on" time at the 8th week. In conclusion, the measurement of baseline 3-OMD and levodopa pharmacokinetics is useful for predicting the clinical effects of entacapone.

Key words: Parkinson's disease, 3-OMD, Entacapone, Levodopa AUC

Parkinson's disease (PD) is caused by the degeneration of nigrostriatal neurons, resulting in a deficiency of dopamine in the central nervous system (CNS). As dopamine does not readily cross the blood-brain barrier, the dopamine prodrug levodopa and dopamine agonists are mainly used to treat PD. Despite the development of dopamine agonists, levodopa is still the most effective treatment for PD¹⁵⁾. However, long-term levodopa treatment causes motor complications such as wearing-off and dyskinesia⁴⁾. These motor complications occur with an annual incidence of about 10% among PD patients¹⁾. Furthermore, the wearing-off phenomenon impairs the quality of life of Parkinson's disease patients⁶⁾. Therefore, it is important that it is managed.

Levodopa is metabolized to dopamine by dopa decarboxylase and to 3-O-methyldopa (3-OMD) by catechol-O-methyltransferase (COMT) in the periphery. When levodopa is administered together with dopa decarboxylase inhibitor (DCI), levodopa availability in the CNS is increased³⁾, and the COMT pathway of levodopa metabolism becomes predominant in the periphery⁴⁾. While the half-life of levodopa is approximately 1 hr, the half-life of 3-OMD is about 15 hr, which leads to the

accumulation of 3-OMD in the plasma and brain under chronic levodopa treatment¹⁰⁾. Like levodopa, 3-OMD is transported across the blood-brain barrier by the large neutral amino acid (LNAA) transporter and consequently competes with levodopa for uptake into the brain¹⁴⁾. Moreover, a recent study indicated that 3-OMD damages neuronal cells¹¹⁾. Thus, it is assumed that the serum concentration of 3-OMD plays an important role in levodopa-treated PD patients. Entacapone, a peripheral COMT inhibitor, prolongs the retention time of levodopa in the plasma¹⁴⁾ and is used in PD patients with the wearing-off phenomenon. However, the determinants of serum 3-OMD concentration and the relationship between motor symptoms and serum 3-OMD concentration are not fully understood. Since entacapone was approved for use in Japan in April 2007, there is little data about its clinical effects and the relationship between its clinical effects and serum 3-OMD concentration in Japanese patients. Furthermore, there is no study about predictive factors for its clinical effect. The aims of this study are: first, to clarify the factors that determine the serum 3-OMD concentration; second, to investigate the change in serum 3-OMD concentration that occurs in Japanese PD patients administered

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entacapone; and third, to clarify the relationship between serum 3-OMD concentration and the clinical effects of entacapone.

METHODS

Patients

The study subjects were PD patients who visited our hospital from August 2007 to September 2008. The study was conducted according to the Declaration of Helsinki. The patients were given oral and written information about the study and gave their written consent.

All patients fulfilled the clinical diagnostic criteria of the UK Brain Bank for PD⁸⁾ and exhibited signs of wearing-off motor fluctuations. All patients were taking levodopa and had shown a positive response to levodopa treatment. Patients who had previously received entacapone or were suffering from dementia were excluded.

The clinical data collected included age, sex, disease duration, duration of therapy, duration of wearing-off, antiparkinsonian drug doses, and levodopa equivalent daily dose (LEDD). LEDD was calculated according to a previous report⁹⁾.

Study Design

1. First study (pharmacokinetics of the first administration of entacapone)

In order to assess the pharmacokinetics of levodopa, the patients took a tablet orally containing 100 mg levodopa and DCI (10 mg carbidopa or 25 mg benserazide) in the morning following an overnight fast. Blood specimens were then collected through an intravenous catheter at 0, 15, 30, 45, 60, 90, 120, and 180 min after the levodopa/DCI administration. The serum levodopa and 3-OMD concentrations were measured. The area under the concentration-time curve (AUC) of levodopa was calculated by the trapezoid method up to 180 min, and the maximum serum concentration (C_{max}) of levodopa was calculated using the one compartment model.

The pharmacokinetic evaluation was performed the next day using the same method, but 100 mg of entacapone was now added to the regimen.

2. Second study (clinical effects and pharmacokinetics during long term entacapone therapy)

After the first study, the same patients were administered one 100 mg entacapone tablet orally with each dose of their levodopa/DCI preparation for 8 weeks. The patients completed an "on/off" self-rating diary on a daily basis at the baseline and during the 2nd, 4th, 6th, and 8th weeks. For each 30-min period between 05:00 and 24:00, the patients rated their motor physical condition by choosing: "on" (good mobility), "off" (worse to bad mobility), or asleep. The mean "on" time duration of at least 3 days was calculated from the self-rating diary. When the "on" time duration was not

prolonged by more than 30 min at the 4th week, 200 mg entacapone was administered with each dose of their levodopa/DCI preparation. The doses of other antiparkinsonian drugs were not changed throughout the study. The levodopa dose was reduced when a patient's symptoms necessitated it. Unified Parkinson's Disease Rating Scale (UPDRS) motor scores were assessed, and serum 3-OMD concentrations were measured at the baseline and in the 2nd, 4th, 6th, and 8th weeks.

Serum analysis

The serum levodopa and 3-OMD concentrations were determined by the high-performance liquid chromatography-electrochemical detection method²⁾ with minor modifications. In brief, 100 μ l aliquot serum samples were processed by adding 100 μ l of 1 M perchloric acid and 20 μ l of 50 μ M dihydroxybenzylamine hydrobromide as an internal standard. Then, the precipitated proteins were removed by centrifugation at 13,000 rpm for 5 min. The resultant supernatants were applied to the chromatographic system.

The chromatographic system consisted of a LC-10AT pump, a CTO-10A column oven, and a DGU-14A degasser (Shimadzu, Kyoto, Japan). The system was connected to an ECD-100 electrochemical detector (Eicom, Kyoto, Japan). The voltage was set at 750 mV vs Ag/AgCl, and chromatographic separation was performed using an EICOMPAK SC-5ODS column (Eicom, Kyoto, Japan) with a PC-03 guard column (Eicom, Kyoto, Japan) in a column oven. The mobile phase (per liter) contained 150 ml of methanol, 850 ml of citrate-acetate buffer, 400 mg of sodium octane sulfate, and 20 mg of EDTA-2Na, pumped at a fixed flow rate of 0.5 ml/min. The citrate-acetate buffer consisted of 0.017 M of sodium acetate and 0.083 M of citric acid monohydrate, and the pH of the buffer was adjusted to 2.8 with perchloric acid. Levodopa, 3-OMD, and dihydroxybenzylamine were eluted at 5.2, 11.8, and 7.2 min, respectively.

Statistical Methods

Statistical analysis was performed using a nonparametric method (Wilcoxon signed-rank test, Spearman's rank correlation and Friedman test), and statistical significance was set at $p < 0.05$. Calculations were performed using the JMP 5.0.1J software (SAS Institute Inc., Cary, N.C., USA) and SPSS 16.0J for Windows (SPSS Inc, Chicago).

RESULTS

Patients

Twenty-one advanced PD patients, comprising 9 men and 12 women, were recruited. The characteristics of the patients are shown in Table. At the baseline, 16 patients took levodopa/carbidopa and

5 patients took levodopa/benserazide preparation. The concomitant antiparkinsonian medications included anticholinergics (n = 6), selegiline (n = 13), dopamine agonists (n = 19), amantadine (n = 6), and droxidopa (n = 3). The median "on" time duration of these patients was 7.7 hr and the median percentage of "on" time during waking hours (% of "on" time) was 48%.

Table: Patient baseline characteristics (n=21)

Age (yrs)	68	(52 - 80)
Sex (n)		
Men	9	
Women	12	
Duration of PD (yrs)	12	(5 - 19)
Duration of wearing-off (yrs)	2	(1 - 10)
Duration of antiparkinsonian medication (yrs)	11	(3 - 18)
"on" time duration (hrs)	7.7	(2.2 - 10.3)
% of "on" time during waking hours (%)	48	(12 - 61)
Hoehn and Yahr stage at "on" phase	3	(1 - 5)
UPDRS motor score at "on" phase	24.5	(9 - 42)
Daily dose of levodopa/DCI (mg)	400	(250 - 625)
Dosing frequency of levodopa/DCI	4	(3 - 8)
Total LEDD (mg)	840	(467.5 - 1346)

All data are shown as median (minimum - maximum).

UPDRS : unified Parkinson's disease rating scale

DCI : dopa decarboxylase inhibitor

LEDD : levodopa equivalent daily dosage

First study

The baseline serum 3-OMD concentration was positively correlated with duration of therapy ($p = 0.045$), levodopa AUC ($p = 0.001$) and levodopa Cmax ($p = 0.002$) in the absence of entacapone (Fig. 1). However, the baseline serum 3-OMD concentration was not correlated with age, sex, duration of wearing-off, the daily levodopa dosage, LEDD, Hoehn & Yahr stage, UPDRS motor score, the "on" time duration, nor % of "on" time at the baseline.

The AUC of levodopa was significantly increased by 22% (median) after the first entacapone administration compared with the control value for levodopa/DCI alone ($p < 0.001$).

Second study

After the initiation of entacapone administration, 3 patients withdrew from the study due to study protocol deviation (2 patients did not complete the "on/off" self-rating diary, and another patient stopped taking entacapone of her own volition because of dyskinesia). The remaining 18 patients completed the study. The daily levodopa dose was not changed at any point during the study period. In 1 patient (5.6%), the dose of entacapone was increased to 200 mg because the "on" time duration was not prolonged by more than 0.5 hr at the 4th week.

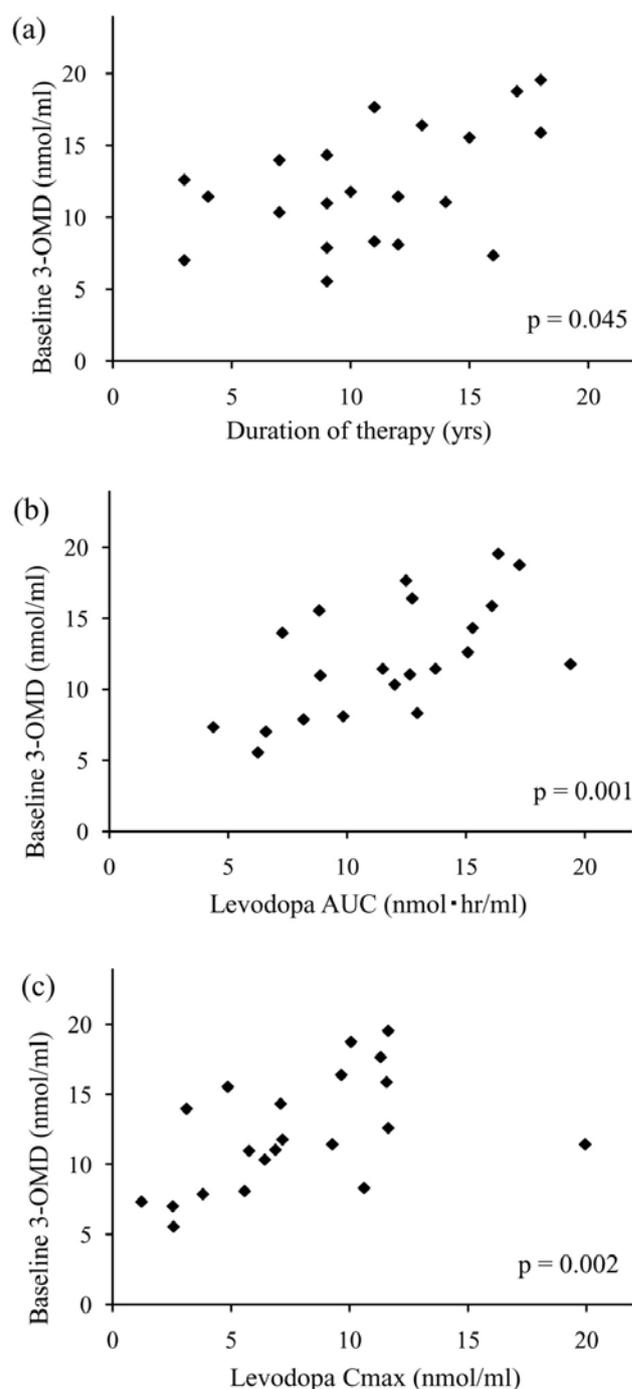


Fig. 1. The baseline serum 3-OMD concentration was positively correlated with (a) the duration of therapy ($p = 0.045$), (b) levodopa AUC ($p = 0.001$), and (c) levodopa Cmax ($p = 0.002$). (Spearman's rank correlation)

The median serum 3-OMD concentration, the UPDRS motor score and median % of "on" time was significantly decreased with entacapone (Friedman test, $p < 0.001$, respectively). The median serum 3-OMD concentration was significantly decreased from the 2nd week to the 8th week and was decreased by 3.5 nmol/ml (34%) at the 8th week (Wilcoxon's signed rank test, $p < 0.001$) (Fig. 2a). The UPDRS motor score was also

significantly decreased by 7 points (median) at the 8th week (Wilcoxon's signed rank test, $p < 0.001$) (Fig. 2b) compared with the baseline. The median "on" time duration was significantly improved by 4.4 hr at the 8th week (Wilcoxon's signed rank test, $p < 0.001$) and the median % of "on" time was

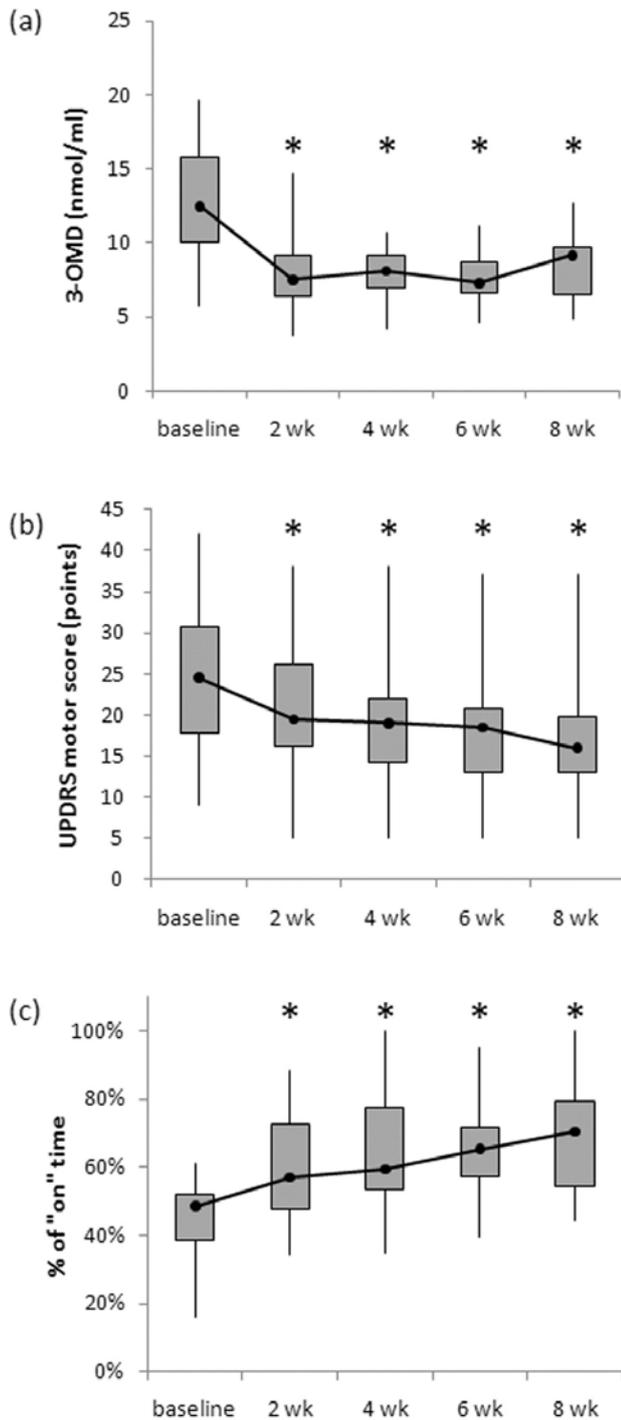


Fig. 2. Changes in (a) serum 3-OMD, (b) UPDRS motor score, and (c) % of "on" time from the baseline to the 8th week after entacapone therapy (n=18).

* Wilcoxon's signed rank test, $p < 0.001$, compared with the baseline.

also significantly improved by 28% at the 8th week (Wilcoxon's signed rank test, $p < 0.001$) (Fig. 2c) compared with the baseline.

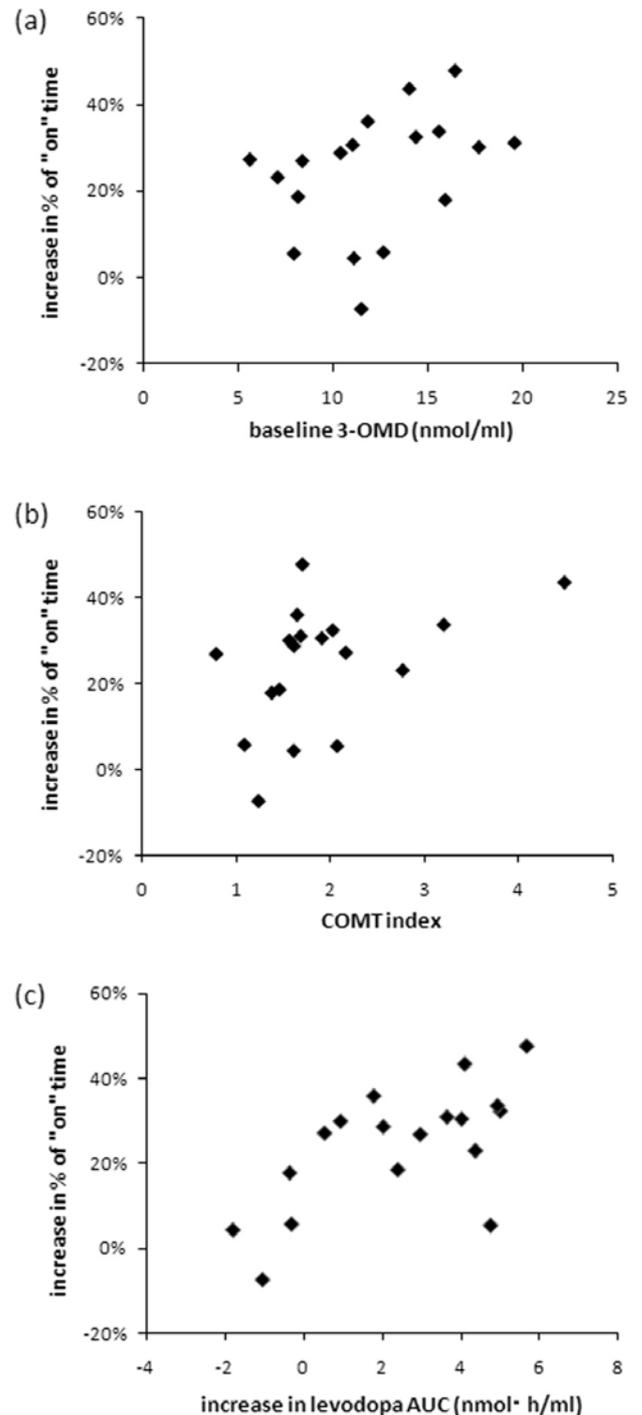


Fig. 3. (a) Association between the increase in % of "on" time and serum 3-OMD concentration. ($p=0.053$)

(b) Association between the increase in % of "on" time and COMT index. ($p=0.027$)

(c) Association between the increase in % of "on" time and the change in levodopa AUC. ($p=0.006$)

Evaluation at the 8th week (n=18).

(Spearman's rank correlation)

The baseline serum 3-OMD concentration tended to be correlated with the increase in % of “on” time at the 8th week ($p = 0.053$) (Fig. 3a). Neither the rate of decline in the serum 3-OMD concentration nor the reduction in the serum 3-OMD concentration was correlated with the increase in % of “on” time. None of age, sex, disease duration, duration of therapy, duration of wearing-off, LEDD, Hoehn & Yahr stage, or UPDRS motor score was correlated with the increase in % of “on” time.

Since levodopa is metabolized to 3-OMD by COMT, the concentration of 3-OMD is determined by COMT activity and the amount of levodopa absorbed. The ratio of the serum 3-OMD concentration to the levodopa concentration might reflect COMT activity more correctly than serum 3-OMD concentration. Thus, we defined the COMT-index as [baseline 3-OMD concentration] / [levodopa C_{max} when 100 mg levodopa was administered alone]. The COMT-index was significantly correlated with the increase in % of “on” time at the 8th week ($p = 0.027$) (Fig. 3b).

The increase in levodopa AUC after the first entacapone administration was positively correlated with the increase in % of “on” time at the 8th week ($p = 0.006$) (Fig. 3c).

DISCUSSION

We prospectively studied the association between the 3-OMD, a levodopa metabolite, concentration and the effects of entacapone in Japanese advanced PD patients.

We clarified that the baseline serum 3-OMD concentration was associated with the duration of therapy. Our study suggested that long-term levodopa therapy increases COMT activity. Serum 3-OMD concentration was associated with the AUC and C_{max} of levodopa, but not with the daily levodopa dosage. The rationale for this was that most patients (15 of 21 patients) took a daily dosage of 300 - 400 mg levodopa, but each of them would have had a different levodopa absorption rate. Because the AUC and C_{max} of levodopa reflect the amount of levodopa absorption, serum 3-OMD, a levodopa metabolite, concentration was associated with the AUC and C_{max} of levodopa.

Our study showed that the 3-OMD concentration was decreased by about 30% and the UPDRS motor score at the “on” phase was also decreased by 7 points by treatment with entacapone in Japanese patients. As entacapone generally does not increase the C_{max} of levodopa after a single coadministration of levodopa¹⁶⁾, motor function is not improved by a single administration of entacapone. However, repeated administration of entacapone may increase the overall levodopa concentration and improve the motor function of patients. Moreover, a recent

study¹¹⁾ showed that 3-OMD impaired the locomotor activity of rats and that 3-OMD can damage neuronal cells. In another study¹²⁾, when treatment with controlled-release carbidopa and levodopa was compared with treatment with a combination of carbidopa, levodopa and entacapone, the mean pharmacokinetic values of levodopa were similar, but the “off” time duration was shorter for the entacapone group than the controlled-release levodopa group. As it is assumed that the 3-OMD concentration was decreased in the entacapone group, the difference between the clinical effects observed in the 2 groups might have been due to differences in the 3-OMD concentration.

Therefore, we assumed that PD patients benefit from entacapone for the following reasons: first, entacapone increases the overall serum levodopa concentration; second, the amount of levodopa crossing the blood-brain barrier might be increased due to reduced competition with 3-OMD for the LNAA transporter; and finally, neuronal cell damage caused by 3-OMD also might be reduced.

In a previous study of Japanese Parkinson's disease patients with wearing-off motor fluctuations, the mean “on” time duration improvement was 1.4 hr for the entacapone group¹³⁾. The reason why patients obtained a longer “on” time duration in our study may be attributable to differences in the categories in the self-rating diary. In a previous study¹³⁾, “on” was defined as good to excellent mobility and “off” as bad mobility. On the other hand, our diary defined “on” as good mobility and “off” as worse to bad mobility (partial to complete “off”). Partial “off” is a condition in between “off” and “on”⁷⁾; thus, partial “off” might be easily converted to the “on” state by entacapone. In addition, open-label study also contributed to the longer “on” time. We defined the COMT-index as an index of COMT activity. The COMT-index was significantly correlated with the increase in the % of “on” time, in other words, patients with high COMT activity would show increased clinical effects of entacapone. We considered that patients with low COMT activity would show poor clinical effects of entacapone because there might be other factors for the wearing-off phenomenon in those patients. Therefore, the COMT-index could predict the clinical effect of entacapone before the administration of entacapone.

The increase in levodopa AUC after the first entacapone administration was also correlated with the increase in % of “on” time at the 8th week. We considered that patients with a good pharmacological response on first entacapone administration would have a good clinical response and that the increase in levodopa AUC could also predict the clinical effect of entacapone.

However, a two-day blood study is necessary to calculate the increase in levodopa AUC after the first entacapone administration. On the other hand, the COMT-index can be calculated with only a one-day blood study and might be more clinically useful than the increase in levodopa AUC.

The limitations of this study were as follows. The sample size was small, and we conducted an open-label study. We excluded patients with dementia because the "on" time parameter was designed so that it could be recorded by the patients themselves. Therefore, selection bias could not be avoided and might have interfered with the collection of accurate information.

In conclusion, our study showed that the measurement of serum 3-OMD concentration and levodopa pharmacokinetics and the calculation of COMT index are useful for predicting the clinical effects of entacapone. A further large study will be needed to confirm our conclusion.

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