Effects of 3-O-Methyldopa, L-3,4-Dihydroxyphenylalanine Metabolite, on Locomotor Activity and Dopamine Turnover in Rats

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It has been well known that 3-O-methyldopa (3-OMD) is a metabolite of L-3,4-dihydroxyphenylalanine (L-DOPA) formed by catechol O-methyltransferase (COMT), and 3-OMD blood level often reaches higher than physiological level in Parkinson’s disease (PD) patients receiving long term L-DOPA therapy. However, the physiological role of 3-OMD has not been well understood. Therefore, in order to clarify the effects of 3-OMD on physiological function, we examined the behavioral alteration in rats based on locomotor activity, and measured dopamine (DA) and its metabolites levels in rats at the same time after 3-OMD subchronic administration. The study results showed that repeated administrations of 3-OMD increased its blood and the striatum tissue levels in those rats, and decreased locomotor activity in a dose dependent manner. Although 3-OMD subchronic administration showed no significant change in DA level in the striatum, DA metabolite levels, such as 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), and homovanillic acid (HVA) were significantly decreased. After 3-OMD washout period (7d), locomotor activity and DA turnover in those rats returned to normal levels. Furthermore, locomotor activity and DA turnover decreased by 3-OMD administration were recovered to normal level by acute L-DOPA administration. These results suggested that 3-OMD affect to locomotor activity via DA neuron system. In conclusion, 3-OMD itself may have a disadvantage in PD patients receiving L-DOPA therapy.

Key words 3-O-methyldopa; L-3,4-dihydroxyphenylalanine; locomotor activity; dopamine turnover; rat

Parkinson’s disease (PD) is characterized by a progressive loss of dopaminergic neurons in the substantia nigra region of the basal ganglia, which results in movement-related symptoms. Current treatment for PD includes dopamine replacement therapy in the form of L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor to dopamine (DA) in its synthesis pathway that has been the gold standard of care for decades. More recently, DA receptor agonists, such as pramipexole, pergolide, and ropinirole, have become more commonly prescribed for the treatment of PD. Approximately 50% of PD patients, when treated with L-DOPA for more than 5 years, experience motor fluctuations such as the “wearing-off” phenomenon or the “no-on” phenomenon.1) L-DOPA is commonly administered with aromatic amino acid decarboxylase (AADC) inhibitor, such as carbidopa or benserazide. 3-O-methyldopa (3-OMD) is a major metabolite of L-DOPA. 3-OMD is formed by catechol O-methyltransferase (COMT) in many organs including blood, peripheral tissues and brain.2) 3-OMD easily accumulates in several tissues (liver, kidney, brain, blood) because 3-OMD has much longer half-life (approximately 15h) than L-DOPA.2,3) It is reported that L-DOPA upregulates the expression and activity of COMT.4) Therefore, blood level of 3-OMD is continually high and 3-OMD accumulates in PD patients receiving long term L-DOPA therapy.5) COMT inhibitor, such as entacapone, increases the area under the L-DOPA plasma concentration–time curve6) and thus prolongs its clinical effect.7) 3-OMD inhibits distribution of L-DOPA into the brain, since 3-OMD competes with L-DOPA at the blood–brain barrier transporter system.8) In addition, 3-OMD might inhibit DA release because DA efflux from striatal tissue slices was significantly reduced after L-DOPA superfusion with 3-OMD.9) Some studies reported that 3-OMD blood level in PD patients with motor complications, such as dyskinesia and wearing-off, is higher than that of patients without motor fluctuations.10–12) The physiological role of 3-OMD in L-DOPA therapy is poorly understood10) and needs further investigation.

A previous study has reported that single intracerebroventricular (icv) injection and subacute administration (5d, icv) of 3-OMD impaired locomotor activity.13) Single injection (icv) of 3-OMD decreased DA turnover (3,4-dihydroxyphenylacetic acid (DOPAC)/DA) in the striatum.14) The purpose of this study is to clarify the effects of 3-OMD on physiological function in chronic L-DOPA treatment focused on the locomotor activity and DA turnover in the rats. The present study shows that the changes in behavior, DA and its metabolites brain tissue levels in the rats after repeated 3-OMD peripherally administration. In addition, we measured the locomotor activity and DA turnover, to find out the effect of L-DOPA on the rats administrated 3-OMD.

MATERIALS AND METHODS

Chemicals 3-O-MD, DA, DOPAC, 3-methoxytyramine (3-MT) and homovanillic acid (HVA) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). L-DOPA, carbidopa, methanol, citric acid monohydrate, sodium acetate, ethylene-diaminetetraacetic acid (EDTA), perchloric acid (PCA) and other materials were obtained from Wako Pure Chemical Ind., Co. (Osaka, Japan).

Animals Male Wistar rats weighing 250–350g were purchased from Japan SLC. Animals were kept in the animal care facility until the beginning of experiments and had access to...
3-OMD was administrated intraperitoneally for 7d and 24h after the last injection, rats were placed in the open field for the measurement of locomotor activity and rearing. They were recorded for 1h. The values represent the means±S.E.M. (*p<0.05, **p<0.01 vs. control group.

Fig. 1. Effect of 3-OMD (3, 10, 30 mg/kg) on Locomotor Activity (A) and Number of Rearing (B)

3-OMD, after the measurement of locomotor activity and number of rearing, blood was drawn from the tail vein and animals were sacrificed by decapitation and the striatum was dissected. Preparation of tissue and blood for HPLC analysis was performed as previously described.15,16 The HPLC system (LC-10 AT, Shimadzu, Kyoto, Japan) was connected with an electrochemical detector (ECD-100, EICOM, Kyoto, Japan) using a C-18 reverse-phase column (5µm, 150×3.0 mm, EICOM). The mobile phase consisted of citrate–acetate buffer, 4% octanesulfonic acid and 0.05% disodium EDTA, in 15% methanol in water, pH 2.8 and pumped at a rate of 0.5 mL/min. Detector potential is at 750mV.

**RESULTS**

In experiment 1, at first, the effects of 3-OMD on locomotor activity and number of rearing were investigated. The repeated intraperitoneal injection of 3-OMD decreased locomotor activity and number of rearing (Figs. 1A, B). Dose of 3-OMD at 3, 10 and 30 mg/kg decreased locomotor activity by 32.4, 29.0 and 33.2% and in number of rearing by 21.3, 24.4 and 38.4%, respectively. Secondly, the effects of 3-OMD on 3-OMD blood concentration and levels of 3-OMD, DA and its metabolite in the striatum were investigated. The intraperitoneal injection of 3-OMD increased dose-dependently its levels in the striatum and blood. Only little 3-OMD was detected in control rats blood and the striatum. 3-OMD blood concentrations at 3, 10 and 30 mg/kg were 2.6±0.35, 4.9±0.44 and 11±1.4 nmol/mL, respectively (Fig. 2A). 3-OMD tissue levels in the striatum at 3, 10 and 30 mg/kg were 0.44±0.089, 0.85±0.17 and 3.0±0.31 ng/mg wet weight, respectively (Fig. 2B). At least, in 3-OMD dose examined in this study, blood and the striatum levels increased in parallel.

When the rats were decapitated after repeated injection of 3, 10 or 30 mg/kg 3-OMD, there was no significant difference in DA striatal level between 3-OMD treated rats and controls (Fig. 3A). However, the levels of DOPAC, 3-MT and HVA were significantly decreased by all doses of 3-OMD in the rat striatum (Figs. 3B–D). The ratio (DOPAC+HVA)/DA is considered a marker of DA turnover. Concentrations of 3-OMD at 3, 10 and 30 mg/kg decreased DA turnover by 30.5, 18.9 and

**Behavioral Experimental Schedule and Pharmacological Treatments**

**Experiment 1.** Effect of 3-OMD: 3-OMD was dissolved in saline. 3-OMD (3, 10, 30 mg/kg) or saline were administrated intraperitoneally for 7d. Seven days administration would be enough to achieve a steady-state 3-OMD blood level, because 3-OMD half-life was 15h. Twenty-four hours after the last injection, the rats were placed in the open field for measurement of locomotor activity and number of rearing. After measurement, blood was drawn from the tail vein. The rats were decapitated and the striatum were dissected.

**Experiment 2.** Effect of Washout of 3-OMD: 3-OMD (3, 10, 30 mg/kg) or saline were administrated intraperitoneally for 7d. Following a washout period of 7d, the rats were placed in the open field for measurement of locomotor activity and number of rearing. After measurement, blood was drawn from the tail vein. The rats were decapitated and the striatum were dissected.

**Experiment 3.** Effect of L-DOPA on the Behavior of Rats Treated by 3-OMD: 3-OMD (30 mg/kg) or saline were administrated intraperitoneally for 7d. After that, L-DOPA/carbidopa (10 : 1) was administrated orally 5 min before measuring locomotor activity and number of rearing. The rats fasted at least 12h were administrated L-DOPA. L-DOPA (3, 10 mg/kg) was suspended in saline. After the measurement of locomotor activity, the rats were immediately decapitated and the striatum were dissected.

**Measurement of Locomotor Activity and Rearing**

Measurement of locomotor activity was performed as previously described.14 The rats were placed at the center of a cubic chamber (48 cm×48 cm×48 cm). The animal’s horizontal movements (locomotor activity) and number of rearing (standing upright on the hind legs) measured by automatic actography (SCANET MV-10; Melquest, Toyama, Japan) were estimated. All animals were habituated to the testing room for 30 min. Locomotor activity and number of rearing were determined for 1h.

**Sample Preparation and HPLC Assay**

For the measurements of DA, its metabolites (DOPAC, 3-MT, HVA) and food and water ad libitum, under a 12h light/dark cycle. Temperature and humidity were maintained at 23±2°C and 50±10%. This study was carried out in accordance with Guideline for the Committee of Animal Experimentation, Hiroshima University and the Committee of Research Facilities for Laboratory Animal Experimentation, Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University.

For the determination of DA, its metabolites (DOPAC, 3-MT, HVA), the striata were dissected. Preparation of tissue and blood for HPLC analysis was performed by Student’s t-test or the one-way analysis of variance with the Dunnet test for post hoc analysis. A difference of p<0.05 was considered statistically significant.
In experiment 2, the effect of 3-OMD after washout period (7 d) was investigated. Locomotor activity and number of rearing which were decreased by subchronic treatment of 3-OMD were returned to the control level, after washout period (Table 1). 3-OMD concentrations in the striatum and blood were not significantly different compared to physiological levels (date not shown). The levels of DA metabolites in 3-OMD treated rats were reversed to control levels. Moreover, no significant change in striatal DA turnover was observed after washout period (Table 1).

In experiment 3, the effect of L-DOPA on the behavior and DA turnover of the rats treated by 3-OMD was investigated. Repeated injection of 3-OMD (30 mg/kg) significantly decreased locomotor activity. Acute L-DOPA administration (10 mg/kg per os (p.o.)) to control rats did not affect locomotor activity. Locomotor activity decreased by 3-OMD treatment 40.2%, respectively (Fig. 4).

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Repeated injection of 3-OMD (30 mg/kg) significantly decreased DA turnover. Acute l-DOPA administration to control rats did not affect DA turnover. DA turnover decreased by 3-OMD treatment significantly reversed by acute l-DOPA treatment (10 mg/kg p.o.) (Fig. 6).

**DISCUSSION**

The present study demonstrated that the effects of 3-OMD, l-DOPA metabolite, on locomotor activity and DA turnover in the rats, in order to clarify the physiological roles of 3-OMD. This study showed that the 7 d 3-OMD peritoneal administration significantly decreased locomotor activity and DA turnover in rat. The decrease of locomotor activity and DA turnover induced by 3-OMD was reversed by acute administration of l-DOPA and after 3-OMD washout period.

In the PD patients receiving l-DOPA therapy, blood 3-OMD level reach to 5–15 nmol/mL,17 and 3-OMD might easily accumulate in several tissues including brain. When 30 mg/kg of 3-OMD was administered to the rats in the present study, 3-OMD blood level was found to be almost the same level as previously reported.17 Therefore, it was suggested that 3-OMD might penetrate into the brain in the rats as well as the PD patients.

A previous study has reported that single icv injection and subacute administration (5 d, icv) of 3-OMD impaired locomotor activity.13 In contrast, this study demonstrated the effect of the peripheral administration of 3-OMD on locomotor activity. It reflected clinical situation which l-DOPA was converted to 3-OMD by peripheral COMT in PD patient receiving l-DOPA therapy. After washout period, locomotor activity and number of rearing returned to control level. Moreover, 3-OMD levels of blood and the striatum returned to physiological levels after 7 d washout. Therefore, the present study suggested that 3-OMD which penetrated into brain decreased locomotor activity. 3-OMD is competitive substrate at the site of transporter against l-DOPA. Consequently, it was suggested that 3-OMD produced motor fluctuation in PD patients receiving l-DOPA therapy.

3-OMD decreased the levels of DA metabolite (DOPAC, 3-MT, HVA) and DA turnover, however, 3-OMD did not alter the tissue level of DA. 3-MT, a metabolite of DA by COMT, is considered to be a marker of DA release.18,19 DA efflux from striatal tissue slices was induced by l-DOPA superfusion was inhibited in the presence of 3-OMD.20 In addition, Neurotoxic effect of 3-OMD might have little association with reduction of DA metabolites, since millimolar order 3-OMD induced the neurotoxic effect.13 In these points of view, it was suggested that 3-OMD might inhibit DA release in the rat striatum, however, the mechanism of the DA release inhibition has been still unclear.

Moreover, the value of (DOPAC+HVA)/DA is used as one

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**Table 1. Effect of 3-OMD on the Locomotor Activity, Number of Rearing and DA Turnover after Washout of 3-OMD**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>3-OMD (mg/kg)</th>
<th>3-OMD (mg/kg)</th>
<th>30</th>
</tr>
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<tbody>
<tr>
<td>Locomotor activity (count)</td>
<td>3355±790</td>
<td>3680±841</td>
<td>3107±623</td>
<td>2974±637</td>
</tr>
<tr>
<td>Number of rearing</td>
<td>228±35</td>
<td>286±29</td>
<td>235±28</td>
<td>238±12</td>
</tr>
<tr>
<td>DA turnover (% of Control)</td>
<td>100±4.9</td>
<td>109±13</td>
<td>96±1.45</td>
<td>100±4.4</td>
</tr>
</tbody>
</table>

3-OMD was administrated intraperitoneally for 7 d. Following a washout period of 7 d, rats were placed in the open field for the measurement of locomotor activity and rearing. It was recorded for 1 h. Furthermore, rats were decapitated and the striatal regions were dissected. After sample preparations for HPLC, the levels of DA and its metabolites were measured in HPLC with an electrochemical detector. DA turnover represents (DOPAC+HVA)/DA. The values represent the means±S.E.M. (n=12–16). *p<0.05 vs. L-DOPA non-treated group without 3-OMD. **p<0.05 vs. L-DOPA non-treated group with 3-OMD.

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**Fig. 5. Effect of l-DOPA on the Locomotor Activity with or without 3-OMD Treatment**

3-OMD (30 mg/kg) or vehicle were injected by intraperitoneal administration for 7 d, and rats were placed in the open field for the measurement of locomotor activity. It was recorded for 1 h, 5 min post-administration of l-DOPA (3 or 10 mg/kg) or saline. The values represent the means±S.E.M. (n=12–16). *p<0.05 vs. L-DOPA non-treated group without 3-OMD. **p<0.05 vs. L-DOPA non-treated group with 3-OMD.

**Fig. 6. Effect of l-DOPA on the DA Turnover with or without 3-OMD Treatment**

3-OMD (30 mg/kg) or vehicle were injected by intraperitoneal administration for 7 d. After measurement of locomotor activity, rats were immediately decapitated and the striatal regions were dissected. After sample preparations for HPLC, the levels of DA and its metabolites were measured in HPLC with an electrochemical detector. DA turnover represents (DOPAC+HVA)/DA. The values represent the means±S.E.M. (n=3–4). *p<0.05 vs. l-DOPA non-treated group without 3-OMD. **p<0.05 vs. l-DOPA non-treated group with 3-OMD.
of indicator of DA turnover and as an index of the changes in DA utilization.\textsuperscript{20} Since 3-OMD failed to interact with AADC,\textsuperscript{21} 3-OMD had little impact on conversion into DA. Therefore, those evidences suggested that the reduction of DA utilization induced by inhibition of DA release might associate with locomotor impairment.

This study also investigated the effects of l-DOPA on locomotor activity and DA turnover decreased by 3-OMD treatment. Single l-DOPA treatment did not affect locomotor activity and number of rearing. Uretsky \textit{et al.} also demonstrated that the locomotor activity of intact rats was not affected by l-DOPA (50 mg/kg, intraperitoneally (i.p.)).\textsuperscript{22} After subchronic 3-OMD treatment, acute co-administration of l-DOPA returned locomotor activity and DA turnover to control level. These results suggested that l-DOPA might counteract the action of 3-OMD since l-DOPA promote DA availability decreased by 3-OMD treatment.

Since l-DOPA has short elimination half-life (approximately 1–2 h), l-DOPA level in blood is low in PD patients at the time of awakening even when patients take l-DOPA before bedtime. In contrast, 3-OMD level in blood is considered to be high (5–15 nmol/mL) and in steady state in PD patient receiving l-DOPA therapy. To decrease 3-OMD level in blood is considered to set up the motor fluctuation. The present results suggested that the motor fluctuation might be partially associated with existence of 3-OMD.

In conclusion, the present study showed that 3-OMD decreased locomotor activity and DA turnover in rats. In addition, washout of 3-OMD and acute l-DOPA administration with subchronic 3-OMD treatment returned locomotor activity and DA turnover to the control level. Therefore, it was suggested that 3-OMD itself may have a disadvantage in PD patients receiving l-DOPA therapy. To decrease 3-OMD level to the utmost might become a new treatment strategy in l-DOPA therapy. This study examined the effect of 3-OMD on locomotor activity and DA turnover and the relationship between 3-OMD and behavior in rat. Further study is required to investigate the mechanisms of how 3-OMD actually works.

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