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S-Methyl-L-Cysteine Levels in Plasma and Striatum Following its Intraperitoneal Administration and The Effects on Striatal D-serine Levels in Rats: An *In Vivo* Microdialysis Study

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Research Article

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ABSTRACT

Several recent studies have attempted to treat schizophrenia by increasing brain levels of D-serine (D-Ser), a co-agonist of *N*-methyl-D-aspartate receptors. Here, we intraperitoneally (i.p.) administered *S*-methyl-L-cysteine (SMLC), an inhibitor of alanine-serine-cysteine transporter 1 (Asc-1), to male Sprague-Dawley rats and investigated changes in plasma levels. Extracellular D-Ser levels and SMLC levels in the striatum were investigated using an *in vivo* microdialysis technique. Changes in endogenous L-serine (L-Ser), glycine (Gly), dopamine (DA), homovanillic acid (HVA), and 5-hydroxyindole acetic acid (HIAA) levels were also assessed. The maximum concentrations of SMLC in plasma and microdialysis samples were achieved within 60 and 90 min, respectively. SMLC was able to penetrate the blood-brain barrier and reach the striatum in a short time. It caused a dose-dependent increase in endogenous D-Ser levels, which then remained constant in the striatum, suggesting that peripheral administration of SMLC effectively increased endogenous D-Ser levels. SMLC also significantly increased L-Ser levels by inhibiting Asc-1, with limited effects on Gly, DA, HVA, and HIAA levels. These results suggest that the i.p. SMLC increased striatal D-Ser levels without affecting the release of other neurotransmitters.

INTRODUCTION

Schizophrenia is a severe mental disorder with a lifetime prevalence of 1%; it is characterized by positive symptoms (hallucinations and delusions), negative symptoms (withdrawal, dullness, and lack of motivation), and cognitive deficits (decreased attention, working memory, and executive function) [1]. It was recently reported that the levels of some endogenous compounds (e.g., polyunsaturated fatty acids and amino acids) were altered in peripheral samples (e.g., serum) of patients with schizophrenia [2-4]. Recent studies reported significantly decreased concentrations of D-serine (D-Ser), an endogenous co-agonist of the *N*-methyl-D-aspartate receptor (NMDAR) [5]. Notably, D-Ser levels and the ratio of D-Ser to total Ser in the plasma or serum [3,6,7] and cerebrospinal fluid [8] of patients with schizophrenia were significantly lower compared to a control group of healthy individuals. Therefore, decreased D-Ser levels in the central nervous system (CNS) may contribute to schizophrenia onset. Moreover, co-administration of D-Ser with conventional or atypical antipsychotics significantly ameliorated the negative and cognitive symptoms in patients with schizophrenia [9,10]. These results suggest that D-Ser administration may be an effective method of treating schizophrenia symptoms; however, the amount that can be administered clinically is strictly limited due to its nephrotoxic effects [11,12]. It is therefore essential to establish a low-dose administration method to maintain therapeutic effects of D-Ser in the CNS.

Recent research on schizophrenia treatments has focused on using chemical compounds to modulate levels of amino acid

transporters in the cellular membrane that are responsible for the incorporation of D-Ser [13-16]. The sodium (Na^+)-independent alanine-serine-cysteine transporter 1 (Asc-1) [17,18] is a small neutral amino acid transporter with high affinity for Ser, alanine (Ala), cysteine (Cys), threonine (Thr), glycine (Gly), and other small neutral D- and L-amino acids [13,19]; however, large acidic or polar amino acids (e.g., glutamate [Glu], glutamine [Gln], an aspartate [Asp]) are less efficiently transported by Asc-1 [13]. Na^+-dependent alanine-serine-cysteine-threonine transporter 2 (ASCT2), has high affinity for Ala, Cys, Thr, Gln, and Asp, and comparatively lower affinity for D-Ser [20]. Ishiwata [14] recently reported that an infusion of S-methyl-L-cysteine (SMLC), which is an inhibitor of Asc-1, increased endogenous D-Ser concentrations in the extracellular fluid of the rat medial frontal cortex. Similarly, Maucler [15] reported that SMLC infusion reduced the uptake of endogenous D-Ser in neurons and/or astrocytes. Furthermore, *in vitro* experiments have assessed the effect of Compound 1 (BMS-466442), which was synthesized by Bristol-Myers Squibb as an inhibitor of Asc-1 for D-Ser uptake [13].

In previous *in vivo* studies [14,15], 100–1000 μM SMLC was locally administered into the rat brain by direct infusion through a micro syringe pump. Therefore, it remains unknown whether peripheral or systemic administration of an Asc-1 inhibitor would lead to an SMLC-induced increase in D-Ser. In the present study, we employed SMLC as the Asc-1 inhibitor and investigated its distribution profiles in rat plasma after intraperitoneal (i.p.) administration. We evaluated the effects of peripheral SMLC administration on endogenous D-Ser concentrations in the rat striatum. We also performed *in vivo* microdialysis to examine the rat striatal levels of D-Ser and other neurotransmitters including dopamine (DA) and Gly, after the administration of SMLC. Changes in the striatal levels of L-serine (L-Ser), which has a high affinity for Asc-1, were simultaneously investigated.

MATERIALS AND METHODS

Intra-peritoneal SMLC Administration

All animal experiments used in this study were approved by the Animal Care Committee of Toho University (14-53-165). Male Sprague-Dawley rats (7 weeks old) were purchased from Charles River Japan (Kanagawa, Japan) and housed for at least 1 week in a temperature- and humidity-controlled room prior to experimentation.

Blood (~0.2 mL) was drawn from the left jugular vein just before the administration of SMLC (Sigma Co. Ltd., St Louis, MO, USA) dissolved in phosphate-buffered saline (PBS) (Dulbecco's PBS (-) "*Nissui*," Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) (50 or 100 mg/kg, i.p.). Blood samples were subsequently collected at 15, 30, 60, 120, 180, and 240 min after SMLC administration. The blood samples were centrifuged at 3,000 *g* for 15 min at 4°C to obtain the plasma supernatant, which was transferred to another tube and stored at -80°C until analysis.

Animal Surgery and Microdialysis

Rats were subjected to surgical implantation of the microdialysis guide cannula (Bio analytical Systems [BAS*i*], West Lafayette, IN, USA) in the striatum (anteroposterior, +1.0 and lateral, -2.0 from bregma; ventral, +3.6 from the dura) according to previously published methods [21,22].

The rats were allowed to recover from the surgery for 2 days before beginning the experiments. A brain probe (4 mm, BAS*i*) was inserted into the implanted guide cannula. Ringer's solution adjusted to pH 7.4 and supplemented with 2.0 mM phosphate buffer was perfused at the rate of 1.0 μL/min with a micro infusion pump (Bee Syringe Pump, BAS*i*). The rats were stabilized for 2.5 h after probe insertion before perfusion was initiated. The microdialysis samples (~30 μL each) were collected every 30 min under ice cooling and immediately stored at -80°C until analysis. The first three samples collected over 1.5 h were used as baseline samples.

SMLC Administration

After collecting the third microdialysis sample fraction, rats received i.p. injections of SMLC dissolved in PBS (50 or 100 mg/kg) at time 0. In the group that did not receive SMLC, PBS (1.0 mL/kg) was i.p. administered at time 0. PBS was administered 0.5 h after i.p. SMLC or PBS administration. Sample collection was continued until 3.5 h after SMLC administration.

Determination of Compounds

To determine the changes in plasma and extracellular SMLC concentrations, 10 μL plasma or 5 μL microdialysis sample was subjected to fluorescence derivatization with 4-fluoro-7-nitro-2, 1, 3-benzoxadiazole (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) [23] according to previously published methods [21,24]. The concentration of SMLC in the plasma or microdialysis sample was calculated based on the calibration curve constructed for the individual case. The intra- and inter-day precisions for plasma and microdialysis samples were satisfactory **(Table 1).**

Table 1. Intra- and inter-day precision of SMLC in (a) plasma and (b) microdialysis samples.

(a) Plasma sample

Concentration (μM)	Intra-day RSD (%)	Inter-day RSD (%)
50	1.35	0.40
250	1.09	1.53

500	0.94	2.02
750	1.00	1.39
1000	0.79	3.14
Mean RSD (%)	1.03	1.70

(b) Microdialysis sample

Concentration (μM)	Intra-day RSD (%)	Inter-day RSD (%)
1	0.65	8.12
5	4.33	7.01
12.5	1.73	5.86
25	3.65	2.25
50	1.09	9.92
Mean RSD (%)	2.29	6.63

The concentrations of the other compounds including D-Ser, L-Ser, Gly, DA, homovanillic acid (HVA), and 5-hydroxyindole acetic acid (HIAA), were determined using high-performance liquid chromatography (HPLC), as published previously [21,24,25] .

In Vitro Recovery Study for Microdialysis Sampling

For the *in vitro* recovery study, Ringer's solution was perfused at the rate of 1.0 μL/min into the microdialysis probe, which was inserted into the test solution containing D-Ser (100 μM), L-Ser (100 μM), Gly (25 μM), DA (7.5 μM), HVA (7.5 μM), HIAA (1.25 μM), and SMLC (50 μM) in PBS. The three microdialysis samples were collected every 30 min. The same sampling procedure was repeated once more using a different lot of probes. Each compound in the microdialysis samples was analyzed, and we calculated the peak area ratio against an internal standard. The recovery rates were calculated using the following formula:Recovery rate (%) = (peak area ratio of the analyte in the microdialysis sample)/(peak area ratio of the analyte in the test solution). The recoveries of these compounds are listed in **Table 2** and were in the range of 16.4–29.1% (*n* = 6).

Table 2. *In vitro* recovery rates (*n* = 6).

	Mean ± SD (%)
D-Ser	28.22 ± 2.15
L-Ser	24.72 ± 1.48
Gly	28.54 ± 2.52
DA	26.43 ± 2.33
HVA	16.59 ± 1.78
5-HIAA	16.42 ± 1.67
SMLC	29.07 ± 2.54

Statistical Analysis

Changes in the concentrations of extracellular D-Ser, L-Ser, Gly, DA, HVA, and HIAA are expressed as percentages of baseline. The average concentration of the first three samples was defined as 100%, and the variances of the concentrations are shown as percentages (mean ± the standard error of the mean). At each time point, a repeated measures two-way analysis of variance followed by Tukey's test was performed to compare the differences between the groups. A *p* value below 0.05 was considered significant.

RESULTS

SMLC Concentrations in Rat Plasma and Microdialysis Samples

Figure 1a shows the time-course profiles of SMLC concentrations in the plasma samples collected following i.p. SMLC administration (50 or 100 mg/kg). HPLC with fluorescence detection was performed to investigate plasma levels of SMLC after its peripheral administration [21, 24, 25]. SMLC concentrations rapidly increased at 15 min after administration and then gradually decreased for both doses (50 or 100 mg/kg, **Figure 1a**).

Figure 1b shows the time-course profiles of SMLC concentrations in the microdialysis samples collected from the rat striatum. No SMLC peak was observed in the first three microdialysis samples acquired before SMLC administration **(Figure 2).** This suggests that endogenous SMLC was not present in the rat striatum prior to its administration. However, the concentrations gradually increased after i.p. SMLC administration, reaching their maximums within 1.5 to 2.0 h for both doses (50 or 100 mg/kg) of SMLC to rats **(Figure 1b).**

Effects of SMLC on D-Ser, L-Ser, and Gly

Figures 1c and 1d show the effects of SMLC administration on the concentrations of endogenous D-Ser and L-Ser (expressed

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as a percent of baseline in the rat striatum). Significant increases in endogenous D-Ser levels were observed depending on the i.p. dose of SMLC **(Figure 1c).** Both D-Ser and L-Ser, which can be used as a potential indicator of SMLC-induced Asc-1 inhibition [14], were more abundant in the high-dose SMLC group (100 mg/kg) compared to the low-dose group (50 mg/kg) **(Figures 1c and 1d). Figure 3a** shows the effects of SMLC on Gly levels in the rat striatum. Minimal changes in Gly levels were observed following SMLC administration (50 or 100 mg/kg).

Effects of SMLC on DA, HVA, and HIAA

Regarding the levels of the other neurotransmitters investigated here, DA, HVA, and HIAA concentrations were not drastically changed following i.p. SMLC administration **(Figures 3b-3d).** These findings imply that DA, HVA, and HIAA levels might not be influenced by peripheral SMLC administration.

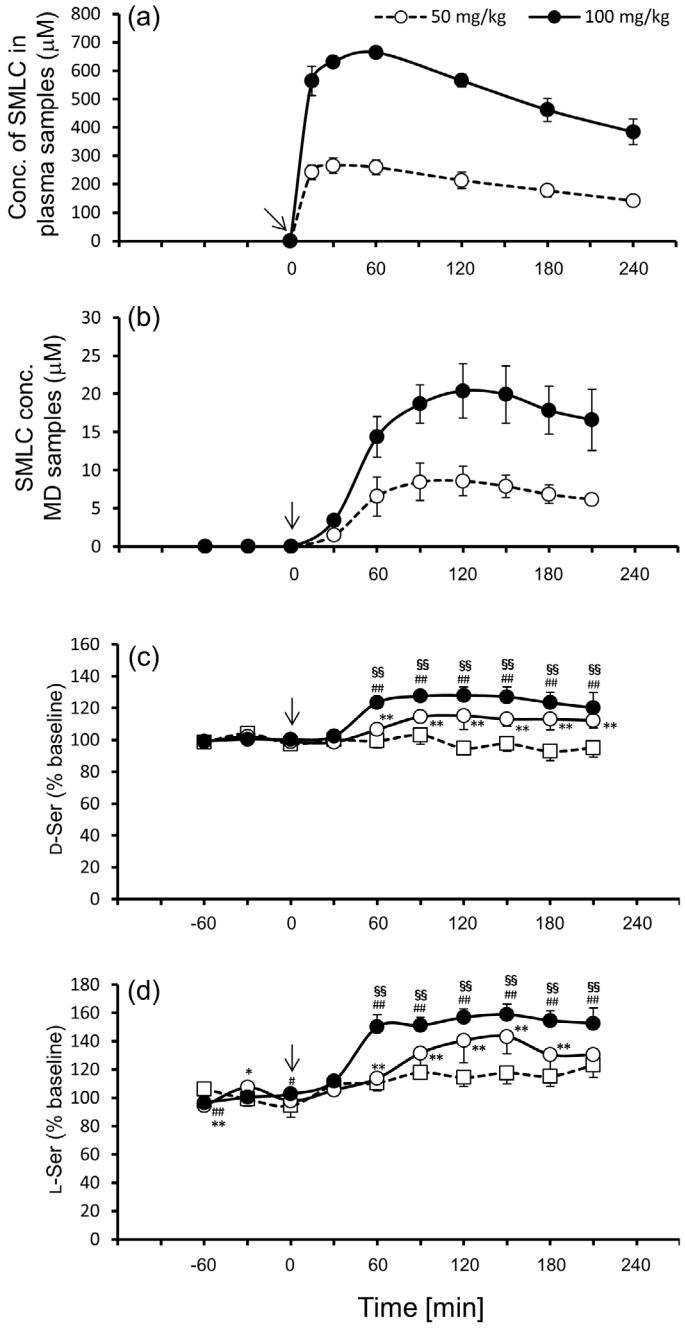

Figure 1. Time-course concentration profiles of SMLC in the plasma samples (a, *n* = 4 and 4) and microdialysis samples (b, *n* = 5 or 6) after intraperitoneal SMLC administration (50 or 100 mg/kg). Each point represents the mean ± the standard error of the mean. The time point of SMLC administration is indicated by the arrow. Effects of SMLC on the concentration of D-Ser (c), L-Ser (d) in striatal microdialysis samples. The open squares with broken lines represent PBS administration (control group, *n* = 6). The open circles represent intraperitoneal SMLC administration (50 mg/kg, *n* = 5). The closed circles represent intraperitoneal administration of SMLC (100 mg/kg, *n* = 6). **p* < 0.05, ***p* < 0.01 (for 50 mg/kg), #*p* < 0.05, ##*p* < 0.01 (for 100 mg/kg) vs. the control group, respectively; §*p* < 0.05, §§*p* < 0.01 vs. the SMLC group (50 mg/kg).

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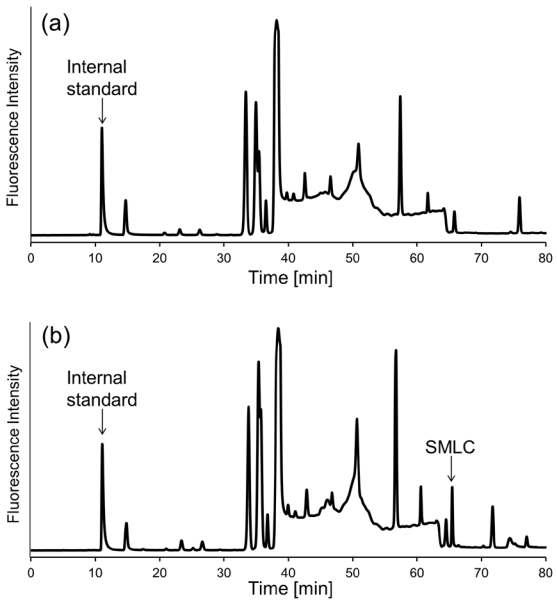

Figure 2. Representative chromatograms of SMLC from the microdialysis samples obtained at (a) baseline (-1.5 - -1.0 h) and (b) 1.5 h after (1.5–2.0 h) SMLC administration (100 mg/kg). (Internal standard: D, L-cysteine acid).

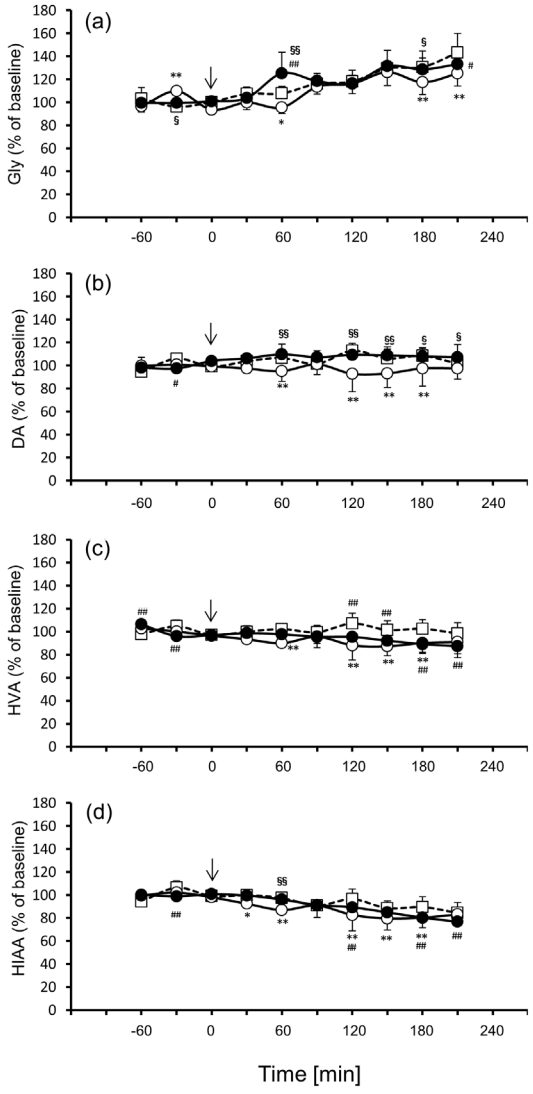

Figure 3. Effects of SMLC on the striatal microdialysis sample concentrations of Gly (a), DA (b), HVA (c), and HIAA (d). The open squares with broken lines represent PBS administration (control group, $n = 6$). The open circles represent intraperitoneal SMLC administration (50 mg/kg, $n = 5$). The closed circles represent high-dose SMLC administration (100 mg/kg, $n = 6$). $^{*}p < 0.05$, $^{**}p < 0.01$ (for 50 mg/kg), $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ (for 100 mg/kg) vs. the control group, respectively; $^{\S}p < 0.05$, $^{\S\S}p < 0.01$ vs. the SMLC group (50 mg/kg).

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DISCUSSION

The aim of the present study was to investigate changes in striatal concentrations of D-Ser and the neurotransmitters Gly and DA using *in vivo* microdialysis following the i.p. administration of SMLC. Although SMLC has previously been infused into the rat brain [13-15], our results revealed the following: 1) i.p. administered SMLC was detected in rat plasma and microdialysis samples from the striatum and 2) i.p. SMLC increased endogenous D-Ser levels in a dose-dependent manner.

Peripheral administration of SMLC, an Asc-1 inhibitor, might be transported into the CNS via ASCT2, because the chemical structure of SMLC is similar to that of L-Cys, which can reportedly penetrate the blood-brain barrier via ASCT2 [26]. Here, i.p. SMLC increased the striatal levels of both D-Ser and L-Ser in a dose-dependent manner. The increase in L-Ser was larger than that for D-Ser, but the tendency was similar to that reported previously when SMLC was infused into the rat brain [14]. Accordingly, these results suggest that it might be possible to use peripherally administered SMLC as an inhibitor of Asc-1 in the rat striatum.

In terms of pharmacological action of SMLC, time-course profiles of SMLC levels between plasma and microdialysis sample revealed a large difference at 0–30 min after administration. The increases in both D-Ser and L-Ser levels in microdialysis samples were closely synchronized with striatal SMLC levels. Furthermore, SMLC remained at nearly maximal concentrations until 210 min after i.p. administration, even though SMLC in rat plasma gradually decreased since 60 min after injection. These results suggest that the pharmacological action of SMLC (Asc-1 transporter inhibition), persisted for a relatively long time in the rat striatum.

On the other hand, it was previously reported that D-Ser release from Asc-1-transfected HEK293 cells was induced by D-Ile, which is a transportable competitive substrate of Asc-1 [27]. As for the mechanisms involved in the release of D-Ser by D-Ile, it has been proposed that Asc-1 is an antiporter and that Asc-1 hetero-exchange between D-Ser and D-Ile is activated by D-Ile to release D-Ser [27,28].

Sason [29] reported that authentic non-transportable inhibitors of Asc-1 (Lu AE00527) decreased D-Ser release in rat neocortical slices, thus inhibiting long-term potentiation and NMDAR activity. Collectively, these reports [27-29] do not rule out the possibility that i.p. administered SMLC might induce a hetero-exchange of Asc-1-like D-Ile to increase D-Ser release. Therefore, the precise mechanism of SMLC on Asc-1 to release D-Ser should be elucidated with future *in vivo* studies.

Minimal changes in DA levels were observed in the present study. Increasing extracellular DA levels is not a preferred method of treating schizophrenia because enhanced DA neurotransmission may exacerbate the positive symptoms of schizophrenia [1]. Therefore, the minimal changes in DA and its metabolite HVA observed following i.p. SMLC administration are preferable characteristics. In addition, the combined administration of SMLC with D-Ser might also affect serotonin neurotransmission, because levels of the serotonin metabolite HIAA were decreased. Serotonin receptor blockade in the CNS is one of the pharmacological actions of second-generation antipsychotic drugs [30]; therefore, changes in serotonin should be investigated in the future.

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