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Original Article

Association between Plasma Metabolic Profiles of the Antidepressant Escitalopram and Clinical Response in Subjects with Depression

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Liquid chromatography/electrospray ionization-mass spectrometry revealed plasma metabolic profiles for the antidepressant drug escitalopram (ECTP) and associated clinical responses in subjects with major depressive disorder (MDD). Metabolic profiles contribute to variations in responses to drug treatment of depression. To assess clinical responses and treatment outcomes, we quantified the levels of metabolites, including those of the parent drug, in plasma samples collected at different time points (days 0, 7, 14, and 42) during treatment of seven patients with MDD. Results showed that mean plasma levels of key metabolites and ECTP at day 7 were significantly associated with the clinical response at 42 days after treatment. Statistical analyses, including principal component analysis, of key metabolites and ECTP samples at different time points clearly distinguished the clinical responders from non-responder subjects. Although further validation with a larger cohort is necessary, our results indicate that early improvement and plasma levels of key metabolites and ECTP are predictive of therapeutic treatment outcomes and thus can be used to guide the use of ECTP.

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INTRODUCTION

Major depressive disorder (MDD) is a common and serious psychiatric mood disorder that has many negative social and economic impact effects, including functional impairment, disability, or lost work productivity; increased use of healthcare services; and higher than average suicide rates.[1)] The global point MDD prevalence adjusting for methodological differences was estimated to be 4.7% (4.4–5.0%) and the pooled annual incidence was 3.0% (2.4–3.8%).[2)] The aim of the pharmacological treatments for the relief of the symptoms rather than a pathological cure and each effectiveness of treatment regimes can vary significantly for individual MDD patients, and moreover, physicians most commonly prescribe antidepressant drugs based on their past experiences rather than a single consistent set of standards.[3)] Till now, there has been no practical laboratory-based test available in the labs to support the efforts of physicians for the selection of the

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most effective antidepressant drug. However, blood samples can be drawn at minimal risk and less cost to the patient through a simple venous blood stick and work on either plasma or serum to see whether peripheral metabolic perturbations have been implicated in MDD. Nowadays, identification of characteristic blood plasma metabolic patterns associated with the pathophysiology of MDD has become an important research goal because of the patterns that could be remarkably used to conclude a detectable molecular phenotype, which is an indicative of responsiveness to certain medications.[4)]

Escitalopram (ECTP) is a potent and highly selective serotonin reuptake inhibitor (SSRI), and one of the most commonly prescribed drugs for moderate to severe MDD patients.[3,5)] A mass spectrometry-based metabolomics approach to establishing sensitive high-throughput molecular screening has already achieved promising results in the diagnosis of a variety of neuropsychiatric disorders, including stroke, bipolar disorder, and autism.[6–10)] Some clinical studies on symptom-based diagnosis of depression in subjects with MDD are available,[11)] although few of those studies include ECTP blood serum concentration and response prediction.[12,13)] A previous study demonstrated that early improvement and ECTP blood serum concentration could be used to predict the effectiveness of antidepressant drug response in MDD patients.[13)] Another study demonstrated that the decrease in Hamilton Depression Rating Scale (HAMD) scores is greater in patients with high ECTP serum concentrations than in patients with low blood serum drug levels.[12)] Assessing psychopathology and quantifying metabolites and ECTP levels in blood in the early phase can help guide antidepressant drug treatment. However, no studies on metabolic profiles and quantitation of metabolites in MDD patients' blood samples are available. Sufficiently high metabolite levels of an antidepressant must reach target structures in the brain for the drug to take effect. Brain metabolite levels are assumed to be well correlated with blood levels.[14)] Therefore, blood levels of major metabolites and ECTP are valid surrogate markers of brain levels. The information from quantified metabolites can be used for dose optimization in psychopharmacotherapy through therapeutic drug monitoring.[15)]

Until now, no study has assessed the feasibility of mass spectrometry (MS)-based metabolomics to gauge clinical responses in MDD. We recently reported metabolic profiles of ECTP in plasma samples of posttreated MDD subjects.[16)] In continuation of our previous work on MDD, we carried out the present study to examine whether plasma metabolic profiles in MDD subjects could be used to predict and understand patient responses to treatment. The MS-based plasma metabolomics method that we report here can distinguish between clinical responder and non-responder MDD subjects.

MATERIALS AND METHODS

Study design

This study was designed to find early predictors of treatment responses to the antidepressant drug ECTP. During a 42-day treatment course, blood samples at different time points were obtained to determine if any metabolite marker candidates can predict later responses to ECTP. This study was performed at three sites in Korea from December 2012 through October 2015. According to the study protocol, ECTP monotherapy was initiated at a starting dose of 5 or 10 mg per day, with subsequent dosage adjustments based on clinical assessments. Concurrent medications such as benzodiazepines (lorazepam and alprazolam) were allowed for the treatment of agitation, anxiety, or insomnia. Subjects treated with other antidepressants, antipsychotics, or mood stabilizers were not included. All study protocols were in accordance with hospital principles and approved by an ethics committee. The main objectives of this study were establishment of plasma metabolic profiles and quantitation of metabolites to predict clinical responses in MDD subjects.

Subjects

Participants were interviewed according to the structured Mini-International Neuropsychiatric Interview.[17)] A diagnosis of MDD was based on the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition.[18)] Subjects with additional significant medical or neurological conditions present within one year prior to screening, including mental retardation, organic brain damage, or history of a substance use disorder, were excluded from this study. Seven patients (female=3 and male=4) completed the 42-day trial and were assessed for this study. All subjects gave written informed consent. Subjects ranged in age from 22 to 66 years. Clinical interviews and ratings were performed by trained psychiatrists.

Assessments

Post-fasting blood samples were collected at different time points (days 0, 7, 14, and 42) after ECTP treatment had been initiated. Each sample was analyzed for ECTP metabolites. Sampling time, drug dosage, and patient medication compliance were documented according to established protocols. Fasting blood samples were collected in plain tubes and serum was separated within an hour of collection. Plasma aliquots were stored at −78°C until analysis. At each time point, depression severity was assessed according to the 17-item HAMD questionnaire, which is commonly used to consistently assess depressive symptoms.[19)]

Analysis of plasma metabolites of ECTP

To complete the plasma metabolic profiles and quantitation of plasma levels of ECTP and its metabolites, blood samples were collected in the morning of days 0, 7, 14, and 42 at least 12 hours after the most recent daily drug intake. All blood samples collected at various time points were centrifuged to separate plasma from whole blood and were then processed for the extraction of ECTP and its metabolites as described in our previous study.[16)] After extraction, samples were injected into a liquid chromatography–mass spectrometry (LC–MS) system. Plasma levels of ECTP and its metabolites were quantified in different time-point samples using the liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS)/mass spectrometry multiple reaction monitoring (MS MRM) method. Treatment response prediction and clinical improvements were completed by considering plasma levels of metabolites and ECTP.

LC/ESI-MS analysis and conditions

Quantitative LC/ESI-MS metabolite analysis was performed using a triple quadrupole mass spectrometer (Triple Quad LC/MS 6490 series; Agilent Technologies, Santa Clara, CA, USA) equipped with an ESI source. Data were acquired using MassHunter Workstation software. Chromatographic

Table 1. Optimized MRM transitions for key metabolites and ECTP in different time-point plasma samples of MDD subjects.

Metabolite/drug	Q1	Q3	MRM transition	CE (eV)	Rt (min)
ECTP	*m/z* 325	*m/z* 307	*m/z* 325>*m/z* 307	34	15.7
DECTP	*m/z* 311	*m/z* 293	*m/z* 311>*m/z* 293	34	15.1
DDECTP	*m/z* 297	*m/z* 279	*m/z* 297>*m/z* 279	34	14.8
ECTPO	*m/z* 341	*m/z* 305	*m/z* 341>*m/z* 305	30	16.2
ECTPPA	*m/z* 310	*m/z* 266	*m/z* 310>*m/z* 266	32	9.5
ECTPO-GlcN	*m/z* 517	*m/z* 341	*m/z* 517>*m/z* 341	30	6.1
ECTPPA-GlcA	*m/z* 486	*m/z* 310	*m/z* 486>*m/z* 310	30	4.4
ECTPPA-OH	*m/z* 326	*m/z* 282	*m/z* 326>*m/z* 282	32	9.1

CE, collision energy; DDECTP, *N*, *N*-didesmethylated escitalopram; DECTP, *N*-desmethylated escitalopram; ECTP, escitalopram; ECTPO, escitalopram *N*-oxide; ECTPO-GlcN, ECTPO-glucuronide; ECTPPA, escitalopram propionic acid; ECTPPA-GlcA, ECTPPA-acylglucuronide; ECTPPA-OH, hydroxylated-ECTPPA; MDD, major depressive disorder; MRM, multiple reaction monitoring; Rt, retention time.

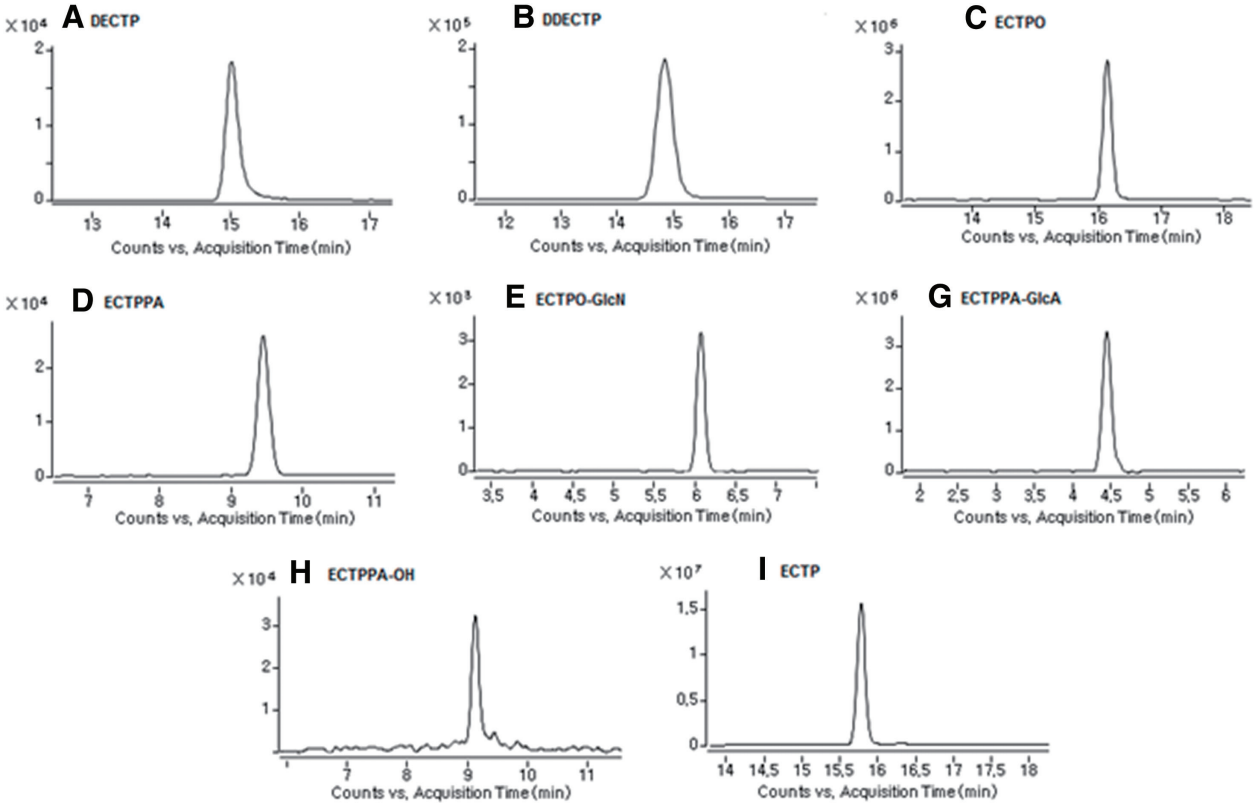

Fig. 1. LC/ESI-MS/MS MRM chromatograms of (a) DECTP, (b) DDECTP, (c) ECTPO, (d) ECTPPA, (e) ECTPO-GlcN, (f) ECTPPA-GlcA, (g) ECTPPA-OH, (h) ECTPPA-OH, and (i) ECTP. DDECTP, *N*, *N*-didesmethylated escitalopram; DECTP, *N*-desmethylated escitalopram; ECTPO, escitalopram *N*-oxide; ECTPO-GlcN, ECTPO-glucuronide; ECTPPA, escitalopram propionic acid; ECTPPA-GlcA, ECTPPA-acylglucuronide; ECTPPA-OH, hydroxylated-ECTPPA; LC/ESI-MS, liquid chromatography/electrospray ionization-mass spectrometry; MS MRM, mass spectrometry multiple reaction monitoring.

separation of ECTP and its metabolites was performed using an Agilent ZORBAX SB C-18 column (2.1×50 mm, 1.8 µm) with a mobile phase consisting of 0.1% formic acid in water (solvent A) and methanol (solvent B) in gradient elution mode. The gradient program was optimized as follows: T_{min}/% solution of B: $_0$/30, $_5$/30, $_{12}$/75, $_{16}$/90, and $_{20}$/10. The mobile phase flow rate was 0.4 mL/min, the column temperature was 30°C, and the injection volume was 5 µL. Typical operating source conditions for MS scans in the ESI mode were optimized as follows: the fragmentor voltage was set at 175 V, the capillary at 3000–3500 V, the sheath gas temp at 350°C, and the sheath gas flow at 11 L/min. Gaseous nitrogen was used for drying (250°C; 13 L/min) and nebulizing (45 psi), and ultra-pure nitrogen was used as the collision gas. All metabolites were analyzed using LC/ESI-MS to obtain retention times and to identify corresponding unique protonated/deprotonated ions. For collision-induced dissociation experiments, the precursor ion of interest was selected using a quadrupole (Q1) analyzer and the product ions were analyzed by another quadrupole (Q3). Product ion scans were carried out for each metabolite precursor ion using different collision energies (10, 15, 20, 25, 30, and 35 eV) to identify product ions. For the generated product ions, the collision energy for each MRM transition was optimized using a series of collision energies between 0 and 35 eV. Further, MRM transitions, dwell time, and collision energy for each metabolite were optimized in both positive- and negative-ion modes. Optimized MRM transitions and representative LC-ESI-MS/MS MRM chromatograms for key metabolites and ECTP are provided in Table 1 and Fig. 1, respectively.

Table 2. ECTP doses in different time-point plasma samples of MDD subjects.

Subjects		Age	Sex	ECTP dose			
				Day 0	Day 7	Day 14	Day 42
Responders	Subject 1	66	Female	5 mg	15 mg	15 mg	15 mg
	Subject 2	26	Male	5 mg	10 mg	10 mg	10 mg
	Subject 3	50	Male	5 mg	20 mg	20 mg	20 mg
	Subject 4	22	Male	5 mg	20 mg	20 mg	20 mg
Non-Responders	Subject 5	36	Female	5 mg	10 mg	15 mg	15 mg
	Subject 6	22	Female	10 mg	10 mg	10 mg	10 mg
	Subject 7	27	Male	10 mg	15 mg	20 mg	20 mg

ECTP, escitalopram; MDD, major depressive disorder.

Table 3. HAMD scores and % improvement on HAMD (from day 0) in different time-point plasma samples of MDD subjects.

Subjects		HAMD scores				HAMD improvement % from day 0		
		Day 0	Day 7	Day 14	Day 42	Day 7	Day 14	Day 42
Responders	Subject 1	16	7	3	2	56.3%	81.3%	87.5%
	Subject 2	10	5	5	3	50.0%	50.0%	70.0%
	Subject 3	26	21	12	5	19.2%	53.3%	80.8%
	Subject 4	24	15	10	7	37.5%	57.9%	62.5%
Non-Responders	Subject 5	15	7	10	8	53.3%	30.8%	46.7%
	Subject 6	19	3	8	14	84.2%	37.5%	26.3%
	Subject 7	20	11	11	11	45.0%	45.0%	45.0%

HAMD, Hamilton Depression Rating Scale; MDD, major depressive disorder.

RESULTS AND DISCUSSION

In this study, we included seven MDD subjects (female=3 and male=4; ages ranged from 22 to 66 years), treated with the antidepressant drug ECTP who completed the 42-day trial (Table 2). The psychopathology of participating MDD subjects was assessed on the HAMD as shown in Table 3. All subjects met the criteria for a major depressive episode and their mean HAMD score was 18.6 (range: 10 to 26) at day 0. In MDD subjects, doses were adjusted between 10 and 20 mg according to clinical decisions. Concurrent medications, such as benzodiazepines (lorazepam and alprazolam), were allowed for the treatment of agitation, anxiety, or insomnia. Subjects treated with other antidepressants, antipsychotics, or mood stabilizers were excluded from this study.

We performed LC/ESI-MS/MS plasma metabolic profiles of different time-point (days 0, 7, 14, and 42) samples collected from posttreated MDD subjects who were medicated with ECTP. After LC-ESI-MS identification of metabolites, their structures were determined based on MS/MS and accurate mass measurement data combined with deuterated experiments as described in our previous study.[16] The biotransformation of ECTP is catalyzed by human hepatic cytochrome P450 (CYP) enzymes and glucuronosyltransferase enzymes (Fig. 2).[20–24] Phase I metabolites are formed by the initiation of CYP enzymes, whereas Phase II metabolites such as glucuronides are formed due to glucuronosyltransferase enzymes.

To investigate the association between plasma metabolic profiles and clinical response in MDD subjects, we quantified the levels of 19 metabolites formed in different time-point samples using LC/ESI-MS/MS MRM. MDD subjects treated with ECTP have different plasma concentrations of ECTP and its metabolites,[25] which may contribute to individual clinical outcomes. We summarized the data of quantified metabolites by heat map analysis, which was performed using the Web-based MetaboAnalyst 3.0 data analysis software (Fig. 3). As shown in Fig. 3, amounts of metabolites varied in different time-point samples. Some metabolites were observed in higher amounts, some in adequate amounts, and some in negligible amounts. Initially, we took all 19 quantified metabolites into account to study the clinical response of MDD subjects. However, quantified amounts of most of the metabolites did not significantly affect or correlate with clinical response. For example, metabolites such as the carbonylated metabolites of hydroxylated-ECTP (ECTP-(OH)-CO), di-hydroxylated-ECTP (ECTP-$(OH)_2$-CO), and escitalopram propionic acid (ECTPPA) (ECTPPA-CO); the *N*,*N*-di-glucuronide metabolite of N, N-didesmethylated escitalopram (DDECTP) (DDECTP-GlcNN); the glycine-conjugated metabolite of ECTPPA (ECTPPA+Gly); and the glycine-conjugated metabolite of ECTPPA-OH (ECTPPA-OH+Gly) (indicated with a black color in heat map data; Fig. 3) were observed at negligible amounts in different time-point samples from all subjects. We therefore mainly considered seven major metabolites (key metabolites) such as N-desmethylated escitalopram (DECTP), DDECTP, Escitalopram N-oxide (ECTPO), ECTPPA, ECTPO-glucuronide (ECTPO-GlcN), ECTPPA-acylglucuronide (ECTPPA-GlcA), and hydroxylated-ECTPPA (ECTPPA-OH) (top portion of heat map; Fig. 3) to study clinical response.

Based on our data, mean plasma levels of key metabolites and ECTP on day 7 and more than 50% clinical improvement on the HAMD on day 14 correlate significantly with the clinical response after final treatment (on day 42) (Figs. 4 and 5). From these data, we categorized participated MDD subjects into two groups: responders and non-responders. Subjects showing good clinical response had greater mean plasma levels of key metabolites and ECTP when compared with non-responders (Figs. 3 and 4). The dot-plot data of key metabolites and ECTP in MDD subjects (both responders

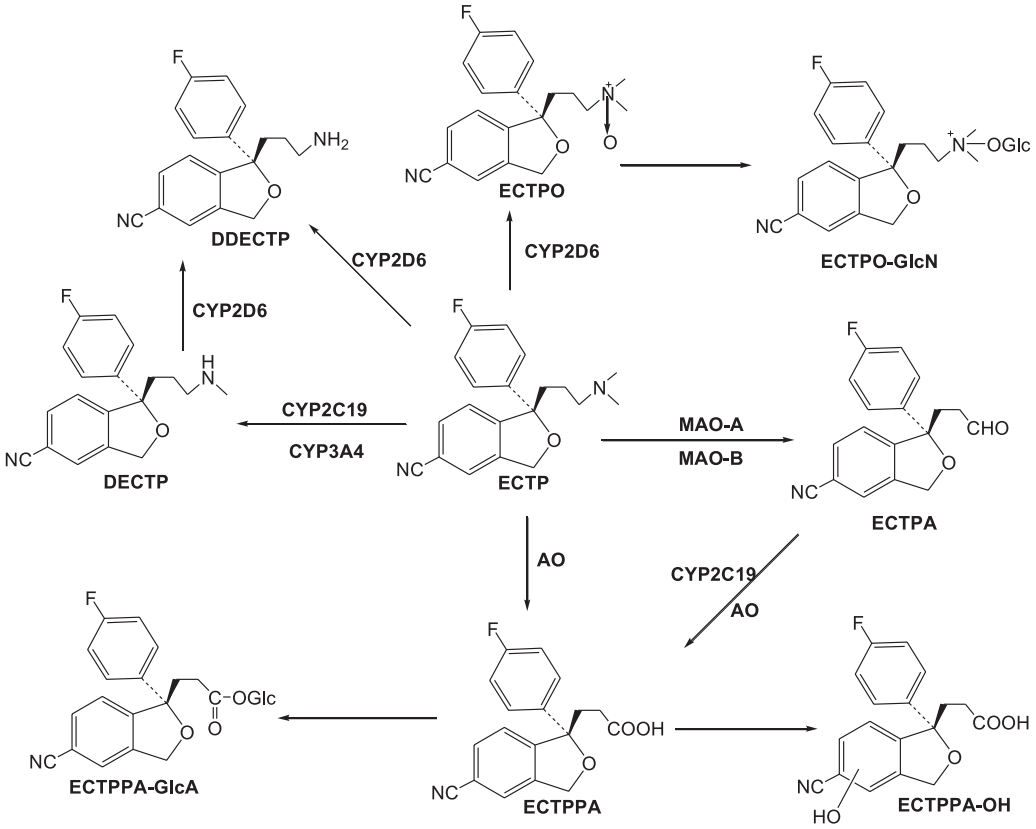

Fig. 2. Key metabolic pathway of ECTP in MDD subjects. DDECTP, *N*, *N*-didesmethylated escitalopram; DECTP, *N*-desmethylated escitalopram; ECTP, escitalopram; ECTPO, escitalopram *N*-oxide; ECTPO-GlcN, ECTPO-glucuronide; ECTPPA, escitalopram propionic acid; ECTPPA-GlcA, ECTPPA-acylglucuronide; ECTPPA-OH, hydroxylated-ECTPPA; MDD, major depressive disorder.

and non-responders) at different time points indicate that responders had relatively higher concentrations of key metabolites and ECTP when compared with non-responders who had lower plasma levels (Fig. 4). This indicates that MDD subjects with greater plasma levels of key metabolites and ECTP on day 7 are significantly associated with clinical improvement after final treatment (Figs. 4 and 5). We observed similar HAMD scores (from the viewpoint of depression) in both responders and non-responder group subjects on day 0, but significantly lower HAMD scores were observed when the plasma levels of key metabolites and ECTP were greater on day 7 (Tables 2 and 3, and Figs. 4 and 5). More than 50% clinical improvement was observed in responder group subjects, whereas less than 50% improvement was observed in non-responders due to lower plasma levels of key metabolites and ECTP on day 7 (Figs. 4 and 5). Greater treatment response and a reduction in HAMD score of more than 50% after 42 days were observed in responders (clinically improved) compared with non-responders (clinically non-improved) (Table 3, and Figs. 4 and 5). Mean plasma levels on day 7 and early improvement on day 14 as determined by HAMD scores indicated the differences in overall clinical response of MDD subjects on day 42 (Fig. 5). This suggests that mean plasma levels of key metabolites and ECTP can contribute to individual clinical outcomes in MDD.[25] Principal component analysis of key metabolites and ECTP concentrations at different time points clearly distinguished responder subjects from non-responders (Fig. 6).

Out of seven participants, four had greater plasma levels of key metabolites and ECTP on day 7 and showed early improvement (responders), while three had lower amounts and did not show any early improvement (non-responders) (Figs. 4–6). This observation is consistent with a previous study reported by Reis and co-workers,[26] who found that the participants had serum levels less than 50 ng/mL even when treated with daily doses of 10–60 mg ECTP. In another study reported by Preskorn *et al.*, more than half of participating MDD subjects did not respond or failed to respond adequately to treatment with ECTP.[27] Individual discrepancies in non-responders may be due to variation in the disposition of ECTP and metabolites as well as the complex nature of MDD. With regard to ECTP concentrations and clinical improvement, our findings are consistent with previous investigations[28,29] even though our sample size was relatively small. Because no other researchers have used a similar metabolomics approach, no comparable data on specific metabolic profiles and treatment response are available in the literature. Other investigations of ECTP concentrations[25,30,31] have reported similar plasma concentrations of ECTP, but with differences in multiple factors, such as compliance rates, dosage, co-medication, age, and gender. Existing reports focused merely on concentrations of ECTP and drug response but not metabolites. Furthermore, some investigators have shown that most patients with low ECTP concentrations became non-responders, or only 11% of patients became responders.[12,13] Our results clearly indicate that there is a significant correlation between mean plasma levels of key metabolites and ECTP on day 7 and percent improvement (or decrease) in HAMD scores on day 14, as well as the overall response after final treatment (Table 3

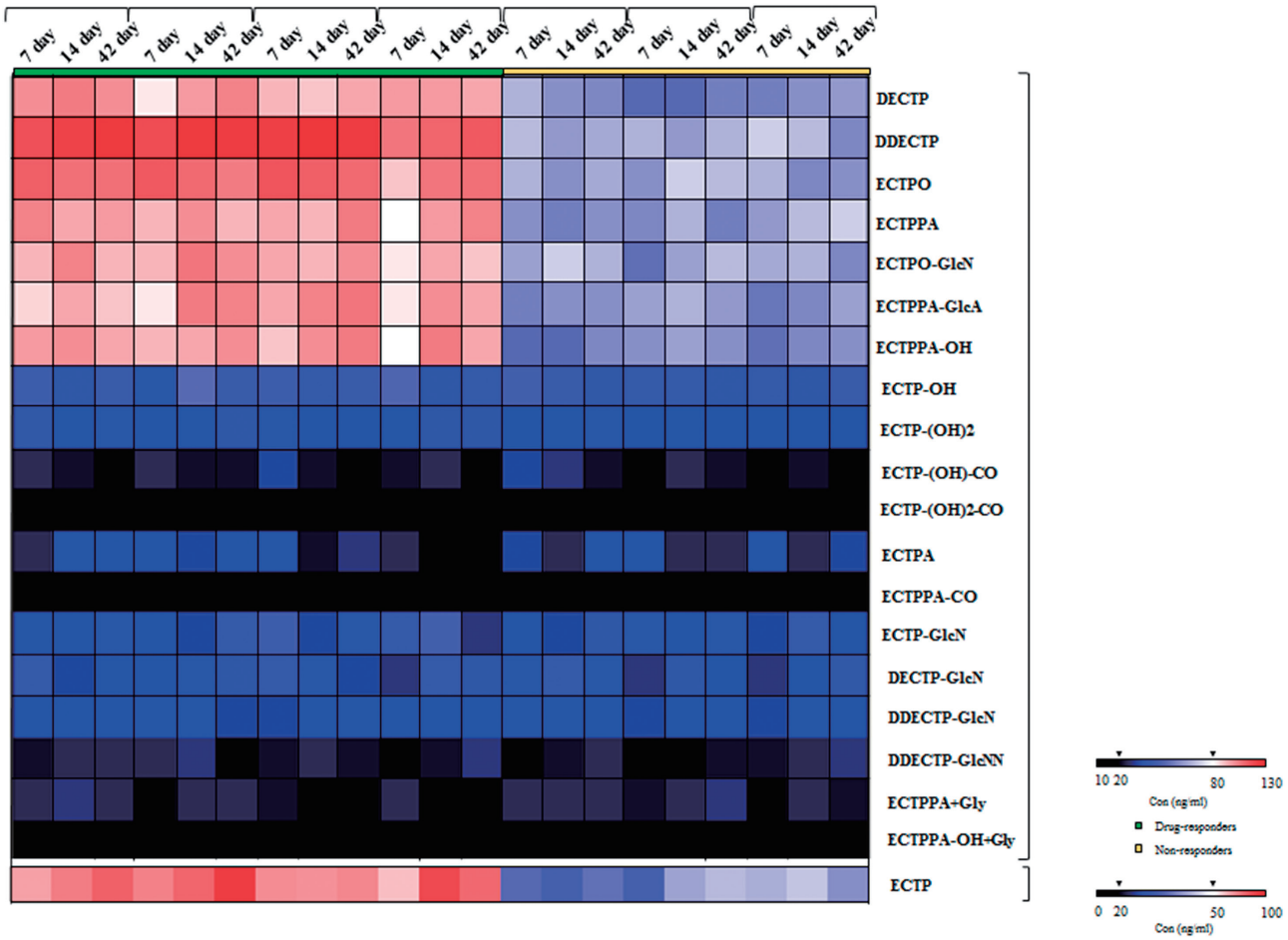

Fig. 3. Quantified levels (ng/mL) of metabolites and ECTP in different time-point plasma samples of MDD subjects. DDECTP, *N*, *N*-didesmethylated escitalopram; DDECTP-GlcN, *N*, *N*-di-glucuronide metabolite of DDECTP; DDECTP-GlcNN, DDECTP-glucuronide; DECTP, *N*-desmethylated escitalopram; DECTP-GlcN, DECTP-glucuronide; ECTP, escitalopram; ECTP-GlcN, ECTP-glucuronide; ECTP-(OH)-CO, carbonylated metabolites of hydroxylated-ECTP; ECTPO, escitalopram *N*-oxide; ECTPO-GlcN, ECTPO-glucuronide; ECTPPA, escitalopram propionic acid; ECTPPA-CO, di-hydroxylated ECTPPA; ECTPPA-GlcA, ECTPPA-acylglucuronide; ECTPPA-Gly, glycine-conjugated metabolite of ECTPPA; ECTPPA-OH, hydroxylated-ECTPPA; ECTPPA-OH-Gly, glycine-conjugated metabolite of ECTPPA-OH; MDD, major depressive disorder.

and Fig. 5). Subjects exhibiting early clinical improvement on day 14 did not show any significant differences when compared with non-improved subjects with regard to clinical characteristics on day 0. HAMD score changes between day 0 and day 14 correlate significantly with the response and percent improvement changes between day 14 and day 42. The superior efficacy of ECTP treatment in subjects with higher plasma levels suggests that dose adjustment based on therapeutic drug monitoring can be useful in the early phase of treatment. The increased difference in treatment effect of ECTP in moderate or severe depression is one of the major findings of this study and provides additional support for a study reported by Montgomery *et al.*,[32] in which they demonstrated superior efficacy among antidepressants.

For clinical practice, it can be concluded that measuring psychopathology and metabolite concentrations in blood plasma in the early phase can help guide antidepressant drug treatment. Drug and metabolite concentrations may predict treatment response far earlier (at 7 days) than clinical observation at 2 weeks. However, before implementation of the proposed schedule in clinical practice, it must be shown that the strategy of routine objective symptom rating and drug metabolite level measurements are important to achieving treatment response, because clinical response is the ultimate goal of antidepressant drug treatment.

Despite the promising results presented, this explorative study has some limitations. First, its small sample size (n=7) means its results should be confirmed by an independent study that includes more subjects. Nevertheless, a relationship between metabolites and clinical response was readily evident. Second, we obtained no pharmacogenetics information regarding the CYP450 status of each patient. A future study on MDD would ideally address these issues.

Our conclusions are limited to the early phase of antidepressant drug treatment and response prediction, as well as clinical improvement. Further studies to determine if these findings are applicable in continuation or maintenance treatment need to be performed. The results of our study are in agreement with those of previously reported analyses of depressed patients' samples, although we performed the analyses in a limited number of patients. The therapeutically recommended dose of 5–20 mg ECTP elicited key metabolite levels below 80 ng/mL and ECTP levels below 50 ng/mL levels in a significant number of subjects, suggesting that therapeutic drug monitoring should be optimized in the early phase of treatment to choose ECTP dose.

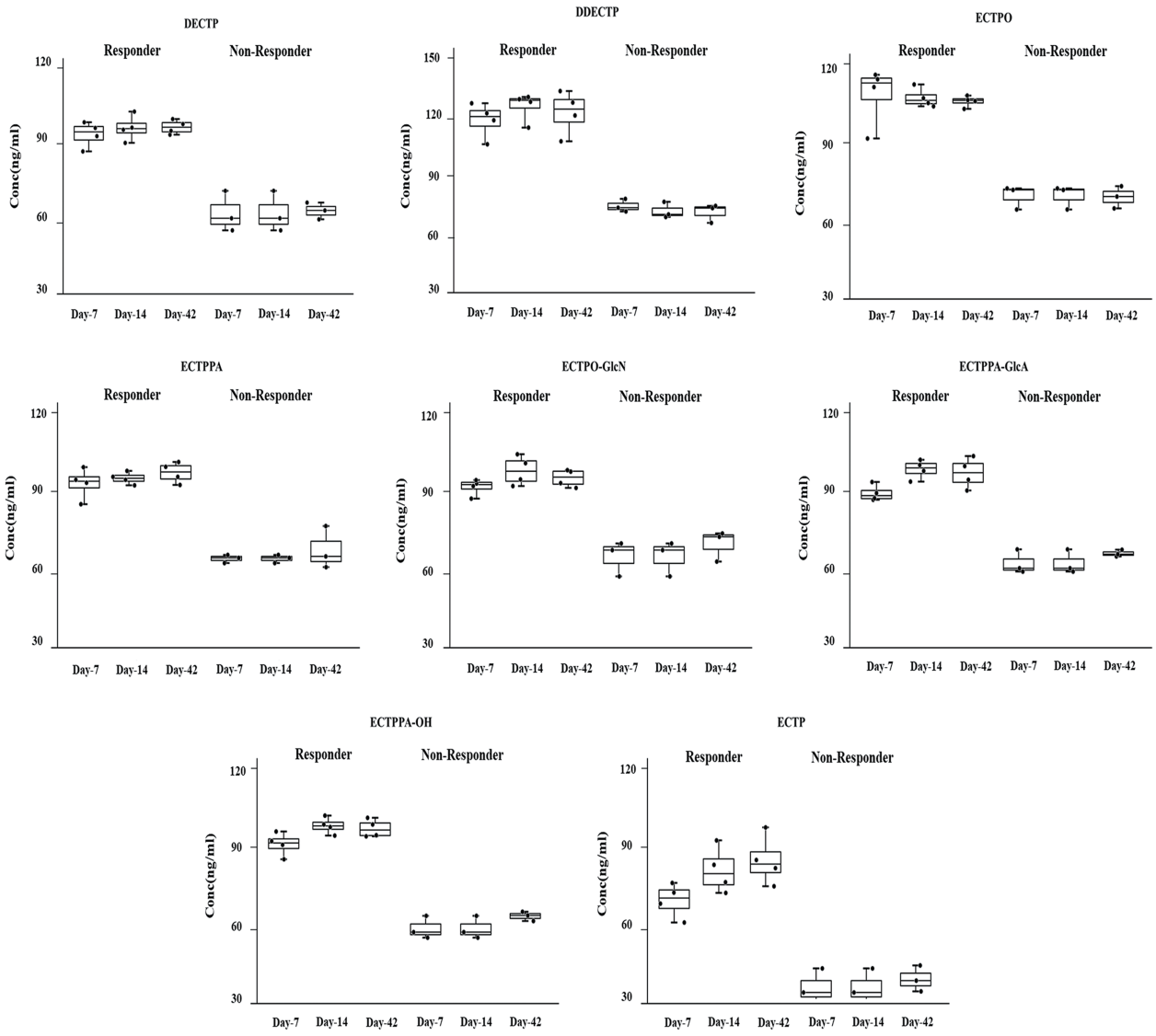

Fig. 4. Mean plasma levels of key metabolites and ECTP levels (ng/mL) in different time-point plasma samples of MDD subjects. DDECTP, *N*, *N*-didesmethylated escitalopram; DECTP, *N*-desmethylated escitalopram; ECTP, escitalopram; ECTPO, escitalopram *N*-oxide; ECTPO-GlcN, ECTPO-glucuronide; ECTPPA, escitalopram propionic acid; ECTPPA-GlcA, ECTPPA-acylglucuronide; ECTPPA-OH, hydroxylated-ECTPPA; MDD, major depressive disorder.

CONCLUSIONS

In this study, a LC/MS-based plasma metabolomics approach was shown to distinguish clinical responders and non-responder MDD subjects. Our results indicated that subjects with higher plasma levels of key metabolites and ECTP on day 7 became responders and subjects with lower plasma levels of key metabolites and ECTP on day 7 became non-responders. Plasma levels on day 7 as well as early improvement on day 14 explained the variations in response on day 42. Four MDD subjects out of seven had key metabolites of 80 ng/mL or greater and ECTP levels of 50 ng/mL or greater on day 7, and three subjects did not reach those thresholds. A significant correlation between mean plasma levels of key metabolites and ECTP on day 7 and a decrease in HAMD scores between day 0 and day 42 was observed. These findings indicate that this approach has potential as a dependable laboratory-based treatment tool for MDD. Given the functional and transient nature of the metabolome, further studies should examine whether this approach can be applied to assess disease progression as well as differentiate responsive subgroups within larger numbers of depressed patients. The results of metabolic profiles in posttreated MDD subjects suggest that those observations can play a major role in variation in plasma levels of ECTP or its key metabolites during the treatment of MDD subjects with SSRIs. The present study examined the potential of therapeutic drug monitoring and symptom rating to predict responses after drug treatment. These observations improve our understanding of response and individual variation in MDD in the biotransformation of widely prescribed SSRI antidepressant medication. Our study provides evidence that identification of changes in ECTP metabolism can be crucial for predicting treatment, drug and key metabolites' plasma levels, and patient responses to treatment. Further confirmation of these findings is required and the obtained results will need to be further validated in other larger patient cohorts for longer observation periods.

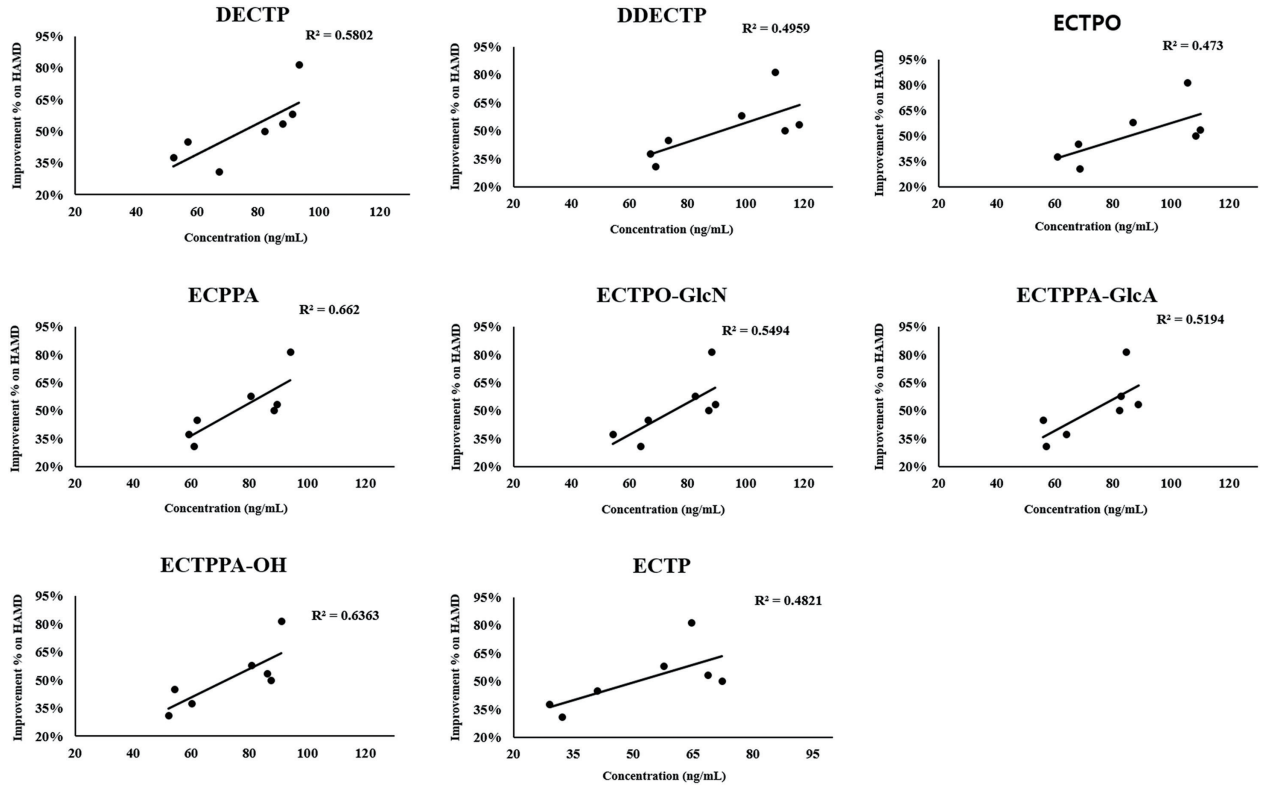

Fig. 5. Correlation between the plasma levels of key metabolites and ECTP (ng/mL) on day 7 and % improvement on day 14 in MDD subjects (both responders and non-responders). DDECTP, *N*, *N*-didesmethylated escitalopram; DECTP, *N*-desmethylated escitalopram; ECTP, escitalopram; ECTPO, escitalopram *N*-oxide; ECTPO-GlcN, ECTPO-glucuronide; ECTPPA, escitalopram propionic acid; ECTPPA-GlcA, ECTPPA-acylglucuronide; ECTPPA-OH, hydroxylated-ECTPPA; HAMD, Hamilton Depression Rating Scale; MDD, major depressive disorder.

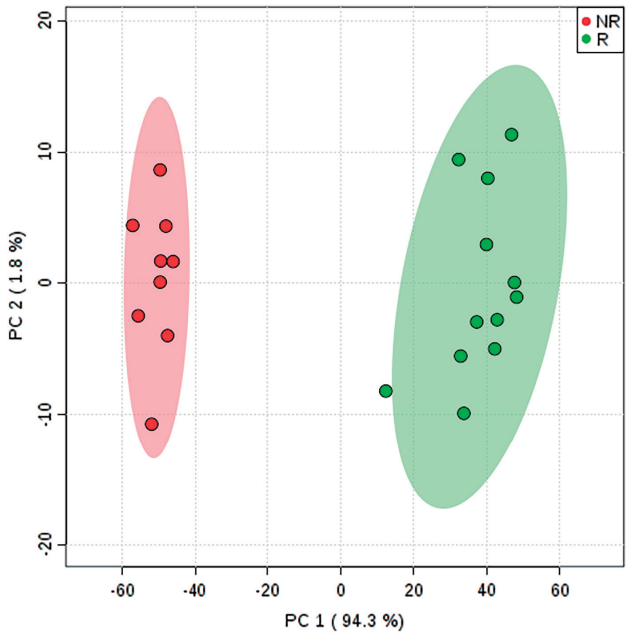

Fig. 6. Principal component analysis of both responders and non-responders.

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