

Original Article

Diagnostic accuracy for drug detection using liquid chromatography/mass spectroscopy in overdose patients

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Aim: Information about the causative drugs is essential for appropriate treatment for drug overdose, but patients sometimes cannot provide information about overdosed drugs owing to disturbed consciousness or an unwillingness to cooperate with treatment. The purpose of this study was to decide whether liquid chromatography/mass spectroscopy (LC/MS) is useful as a detection method for overdosed drugs.

Methods: Overdose patients ($n = 279$) treated in our facility were retrospectively studied. Specimens from gastric lavage, blood serum, and urine were tested using LC/MS. The matching rates between drugs overdosed and those detected by LC/MS were evaluated; LC/MS and Triage DOA^R were also compared. Data are shown as means.

Results: Patients overdosed on 3.2 kinds of drugs and were transferred to our hospital 4.6 h after. Overall 3.5 kinds of drugs were detected by LC/MS, and 2.4, 1.9, and 2.2 kinds were from the stomach, blood, and urine, respectively. Matching rate among the ingested drugs (kinds of drugs matched/ones ingested) was the highest in the gastric samples (0.56), and the lowest in the urine samples (0.46) ($P < 0.01$). In addition, the matching rates among the detected drugs (kinds of drugs matched/ones detected) were as high as 0.74 and 0.78 in the gastric and blood samples, respectively. Comparing the sensitivity and specificity of detection of benzodiazepines and tricyclic antidepressants between LC/MS and Triage DOA^R, we found that these two methods were comparable.

Conclusion: Liquid chromatography/mass spectroscopy was proven to be an effective method to detect overdosed drugs, especially when there was not enough information about the drugs ingested.

Key words: Detection, drug overdose, liquid chromatography, mass spectroscopy, poisoning, screening, toxicology

INTRODUCTION

DRUG OVERDOSE IS frequently observed among patients admitted to intensive care units worldwide.^{1–3} Overdose patients are frequently found to have ingested many tablets, sometimes more than 100 at a time, and have often simultaneously abused several kinds of drugs to harm themselves owing to the effects of various psychiatric diseases. Information about the overdosed drugs is essential for appropriate treatment, because clinical symptoms and complications vary among drugs, and antidotes are available for

some drugs.^{4,5} The availability of information about causative drugs permits the use of “drug-specific treatments” in addition to general medical care. However, patients sometimes cannot provide information about overdosed drugs due to disturbed consciousness or an unwillingness to cooperate with treatment because of their desire for self-harm, and witnesses are unavailable in many cases.

For the detection of abused drugs, the Triage DOA^R system, which uses the immunoassay method, is commercially available for routine clinical use.^{6,7} Although the system is convenient and easy to handle for the detection of seven predetermined drugs, including benzodiazepines, barbiturates, and tricyclic antidepressants, other drugs cannot be detected. Furthermore, Triage DOA^R can analyze only urine samples and cannot be used for gastric lavage or blood samples. Other point-of-care detection kits, such as Monitect-9^R or Triage-Tox^R are also subject to the same limitations.⁸ There is a definite need for alternative methods of detecting ingested drugs from various human

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specimens, candidates for which include high performance liquid chromatography (HPLC), gas chromatography/mass spectroscopy, and liquid chromatography/mass spectroscopy (LC/MS).^{9–11}

Liquid chromatography/mass spectroscopy sorts chemicals using HPLC and analyzes them using mass spectroscopy. Although it has been in clinical use for years, only limited clinical data are available regarding its clinical utility in drug detection.^{10,11} To the best of our knowledge, no reliable data are currently available regarding whether LC/MS can detect overdosed drugs, the reliability of these results, or the optimal clinical specimens for analysis. In the present study, we undertook a series of analyses to assess the feasibility and reliability of the use of LC/MS for drug detection in overdose patients.

METHODS

Study design and patients

WE RETROSPECTIVELY ANALYZED consecutive 279 drug overdose patients, in whose cases LC/MS was used for drug detection, among the 5590 intensive care unit admissions in Kyorin University Hospital (Tokyo, Japan), between January 2011 and December 2014.

Medical record review

Medical records were reviewed to obtain general patient data (age, gender, and medical history) and information on the overdosed drugs. Complete or partial information on the overdosed drugs was available in 254 patients, including empty blister packages or remaining pills from the scene and/or patients' declarations after recovery of consciousness. The types of overdosed drugs were counted repeatedly for each person. In 170 patients, information on the time interval between drug ingestion and hospital admission was also available.

Measurement

Specimens from gastric lavage ($n = 202$), blood serum ($n = 249$), and urine ($n = 240$) were tested for drug screening using a LC/MS system (HPLC: LC-10AD, Shimadzu, Kyoto, Japan; MS: Waters Quattro Micro API, Nihon Waters, Tokyo, Japan). The drug detection libraries consisted of 183 drugs, accounting for more than 95% of the drugs used clinically over a period of 8 years in our hospital. In 234 patients, urine specimens were also analyzed using Triage DOA^R screening kits (Sysmex, Kobe, Japan). All the measurements using LC/MS were carried out by a single medical technologist.

Number of drugs detected by LC/MS and its matching rates

The numbers of drugs detected by LC/MS were tallied and compared among three sites from where specimens were obtained. The correlation between the interval from time of ingestion to treatment and the number of drugs detected by LC/MS was also evaluated. To evaluate the performance of LC/MS for drug detection, two types of matching rates were calculated in patients with available information about overdosed drugs: "matching rate-1 (MR-1)" was defined as (the number of types of drugs detected by LC/MS and matched to overdosed drugs) / (the number of types of overdosed drugs), whereas "matching rate-2 (MR-2)" was defined as (the number of types of drugs detected by LC/MS and matched to overdosed drugs) / (the number of types of drugs detected by LC/MS).

Sensitivity and specificity of drug detection using LC/MS and Triage DOA^R

The sensitivity and specificity of detection of benzodiazepines and tricyclic antidepressants were also compared between LC/MS and Triage DOA^R in 234 patients where information on overdosed drugs was available. True positive was defined as the accurate detection of overdosed drugs. These two types of drugs were selected for comparison because overdose incidents involving these drugs were relatively more frequent than those of the other drugs detected by Triage DOA^R. In addition, although selective serotonin reuptake inhibitors (SSRIs) and risperidone cannot be detected by Triage DOA^R, the sensitivity and specificity of detecting these drugs utilizing LC/MS were also checked for comparison.

Statistical analysis

Results were expressed as mean \pm standard error of the mean, unless mentioned otherwise. Univariate analyses were carried out using Student's *t* test or the Mann–Whitney *U*-test, depending on their distributions. Fisher's exact test or the χ^2 -test was used for comparisons of proportion. Differences among three groups were analyzed using one-way analysis of variance, followed by the post hoc test, if applicable. All analyses were undertaken using StatFlex version 6 (Artec, Osaka, Japan). A *P*-value <0.05 was considered statistically significant.

RESULTS

Patient characteristics and overdosed drugs

OVERDOSE PATIENTS WERE 37 ± 1 years old and predominantly female (78.5%), and most (94.4%) had

a history of psychiatric disease including depression (42.9%), schizophrenia (16.8%), and personality disorder (12.7%). The number of different types of drugs ingested was 3.2 ± 0.1 , and the time elapsed between ingestion and admission was 4.6 ± 0.4 h. With respect to the types of overdosed drugs, benzodiazepines were the most frequently overdosed ($n = 339$, 42.4%), followed by antipsychotic ($n = 161$, 20.2%), non-benzodiazepine anxiolytic drug ($n = 64$, 8.1%), and SSRIs/serotonin and norepinephrine reuptake inhibitors ($n = 61$, 7.6%).

Number of drugs detected by LC/MS and matching rates

Table 1 shows the comparisons of the number of drugs detected by LC/MS, number of drugs matched to overdosed drugs, MR-1, and MR-2 between three study sites. Overall, 3.5 types of drugs were detected from any of the three sites, and 2.4, 1.9, and 2.2 types of drugs were from the stomach, blood, and urine, respectively. The number of drugs detected from blood was significantly lower than that from the stomach or urine ($P < 0.01$). Figure 1 shows the comparison of the number of drugs detected by LC/MS in three study sites correlated with the interval from ingestion to admission. When the interval was more than 4 h, there was no difference among the three sites. However, if the interval was less than 4 h, drugs were more frequently detected in gastric samples than in the blood and urine ($P < 0.01$).

Average MR-1 was the highest in gastric samples (0.56 ± 0.03) and the lowest in urine samples (0.46 ± 0.03) ($P < 0.01$). In addition, the average MR-2

values were 0.74 and 0.78 in gastric and blood samples, respectively, whereas it was the lowest in the urine (0.59) in all three sites ($P < 0.01$). Furthermore, in samples from patients without any information about overdosed drugs ($n = 25$), LC/MS detected 3.8 types of drugs overall from any of the three sites (Table 2).

Sensitivity and specificity of drug detection using LC/MS and Triage DOA^R

Table 3 shows the sensitivity and specificity of drug detection using LC/MS (in gastric, blood, and urine samples) and Triage DOA^R (in urine samples). For benzodiazepines, Triage DOA^R was relatively superior to LC/MS regarding sensitivity, whereas LC/MS showed much better performance than Triage DOA^R regarding specificity. These two methods were almost comparable for the detection of tricyclic antidepressants. In addition, LC/MS could detect SSRIs and risperidone, which were undetectable by Triage DOA^R, with sensitivity and specificity comparable to those for benzodiazepines and tricyclic antidepressants.

DISCUSSION

THE PRESENT STUDY revealed that LC/MS is an effective method to detect drugs among overdose patients in critical care settings. We also clarified several conditions where LC/MS is especially useful with respect to sample sites, time interval, types of overdosed drugs, and information availability on overdose drugs. Even for patients without any information about overdosed drugs, LC/MS

Table 1. Comparisons of number of drugs detected and matched, and matching rates in three specimen sites of overdose patients by liquid chromatography/mass spectroscopy

Variables	Total	Stomach	Blood	Urine	P-value
Number of patients	254	202	249	240	–
Drugs overdosed (kinds in each patient)	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	NS
Number of patients with drugs detected	242 (95.3)	170 (84.2)	218 (87.6)	206 (85.8)	NS
Drugs detected (kinds in each patient)	3.5 ± 0.1	2.4 ± 0.1	$1.9 \pm 0.1^{\dagger}$	$2.2 \pm 0.1^{\dagger\dagger}$	<0.001
Drugs matched [†] (kinds in each patient)	1.9 ± 0.1	1.7 ± 0.1	$1.4 \pm 0.1^{\dagger}$	$1.3 \pm 0.1^{\dagger,\dagger\dagger}$	<0.001
Matching rate-1 [‡]	0.66 ± 0.02	0.56 ± 0.03	$0.51 \pm 0.02^{\dagger\dagger}$	$0.46 \pm 0.03^{\dagger}$	<0.010
Matching rate-2 [§]	0.60 ± 0.02	0.74 ± 0.02	0.78 ± 0.02	$0.59 \pm 0.03^{\dagger,\dagger\dagger}$	<0.001

Data are expressed as counts (proportions) or mean \pm standard error of the mean.

–, not applicable; NS, not significant.

[†]Drugs detected and matched to overdosed ones.

[‡]Drugs matched / drugs overdosed.

[§]Drugs matched / drugs detected.

[†]<0.01 versus stomach.

^{††}<0.01 versus blood.

^{†††}<0.05 versus stomach.

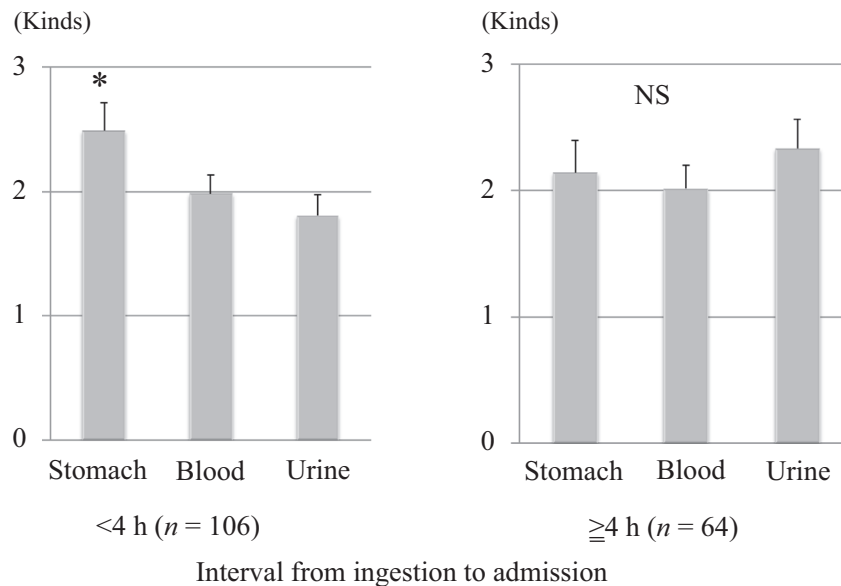


Fig. 1. Comparisons of number of drugs detected in three specimen sites by liquid chromatography/mass spectroscopy in overdose patients with respect to the interval from ingestion to admission ($n = 170$). Data are expressed as mean \pm standard error of the mean. * $P < 0.01$ versus blood and urine.

could detect several drugs and showed possibilities for drug-specific treatments.

The numbers of detected drugs varied among three specimen sites, with the stomach being the site with the most number of drugs and the blood being the least (Table 1). Particularly, in patients who were hospitalized within 4 h after overdose, gastric specimen was significantly better than blood or urine specimens with respect to drug detection (Fig. 1). The data seem to be reasonable considering the time taken for drugs to reside, to be metabolized, and to be stored at each site. For maximizing the efficacy of detection, we can carefully consider the sites for collecting specimens depending on the interval from overdose and can also combine the results of LC/MS using specimens from different sites.

We evaluated two different matching rates, MR-1 and MR-2. MR-1 indicates the detection capability of the method, whereas MR-2 indicates the detection reliability of the method. For example, the average MR-1 of the stomach was significantly higher than those of blood and urine, suggesting that LC/MS using specimens from stomach is most sensitive for the detection of overdosed drugs. In contrast, the average MR-2 of urine sample was lower than those of the other sites, suggesting that urine specimens are less reliable for the detection of overdosed drugs compared to other sites. Urine specimens are not so reliable for the detection of overdosed drugs, especially in the early stage after overdose, because there exists an interval between ingestion of drugs

and their secretion into urine. Taken together, we concluded that stomach was the best site to detect overdosed drugs using LC/MS.

Liquid chromatography/mass spectrometry was almost comparable to and sometimes even better than Triage DOA^R for drug detection in the current study. This might be partly because Triage DOA^R is applicable only for urine specimens, which was the worst site for drug detection by LC/MS. Furthermore, Triage DOA^R can detect the predetermined seven drugs only,^{6,7} whereas LC/MS can analyze any drugs as long as libraries for them are equipped. We can renew the library to cover certain targeted drugs or new drugs as required. Yamamoto *et al.* studied the clinical utility of Triage DOA^R compared to quantitative analyses such as gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry (LC-MS/MS), and reported several limitations of Triage DOA^R including relatively higher detection limits.⁷ Thus, LC/MS could have better potential than Triage DOA^R for care of overdose patients.

Nevertheless, when utilizing LC/MS in clinical settings, several factors should be taken into account, including the availability of LC/MS. In most countries, only a few hospitals are equipped with the instrument and medical technologists to operate it. Therefore, screening by point-of-care testing such as Triage DOA^R is of course more feasible in most institutions. In our facility, medical technologists operate LC/MS measurements on request and the results will be

Table 2. Comparisons of number of drugs detected in three specimen sites by liquid chromatography/mass spectroscopy in patients without any information about overdosed drugs

Variable	Total	Stomach	Blood	Urine	P-value
Number of patients	25	22	25	23	–
Number of patients with drugs detected	23 (92.0)	21 (95.5)	20 (80.0)	18 (78.3)	NS
Drugs detected (kinds)	94	62	43	56	–
Drugs detected (kinds in each patient)	3.8 ± 0.4	2.8 ± 0.3	1.7 ± 0.3	2.4 ± 0.4	NS (0.07)

Data are expressed as counts (proportions) or mean ± standard error of the mean. –, not applicable; NS, not significant.

Table 3. Comparisons of sensitivity and specificity for drug detection in overdose patients in three specimen sites by liquid chromatography/mass spectroscopy (LC/MS) and Triage DOA^R

Drug		LC/MS Total (n = 234)	LC/MS Stomach (n = 184)	LC/MS Blood (n = 231)	LC/MS Urine (n = 220)	Triage DOA ^R (n = 234)
Benzodiazepine (n = 158)	Sensitivity	70.9	58.3	41.4	50.0	81.6
	Specificity	78.9	92.2	97.3	80.6	59.2
Tricyclic antidepressant (n = 22)	Sensitivity	90.5	88.2	81.8	78.9	77.3
	Specificity	82.5	92.8	94.3	84.6	90.6
SSRI (n = 40)	Sensitivity	74.4	60.0	36.8	50.0	NA
	Specificity	93.3	93.3	97.9	96.7	NA
Risperidone (n = 19)	Sensitivity	93.8	92.3	75.0	86.7	NA
	Specificity	95.0	98.8	99.5	95.1	NA

NA, not available; SSRI, selective serotonin reuptake inhibitor.

returned to clinicians in a timely manner, within several hours. The average running cost of LC/MS per analysis is estimated to be approximately \$20 in daytime and \$60 at night-time, including extra labor costs, whereas that of commercially available Triage DOA^R is approximately \$30–\$40 per test. In everyday practice, we first screen all overdose patients with Triage DOA^R and add LC/MS analysis when it is necessary. The present study suggests that the most ideal candidates for LC/MS are critical patients with suspicion of drug overdose with the following conditions: (i) no information is available regarding overdosed drugs, (ii) suspicious overdosed drugs cannot be detected by point-of-care testing. For treating those patients, clinicians should utilize LC/MS if available, or consider getting the patients transferred to higher-level facilities where LC/MS measurements are available. Of note, as a new and improved version of LC/MS, LC-MS/MS has been in routine clinical use in some institutions, including ours. Although promising data regarding drug detection for overdose patients by LC-MS/MS have been obtained recently, most of them are from in vitro studies or forensic studies.^{7,12–14} To the best of our knowledge,

there have been no published studies in which the abilities of LC/MS or LC-MS/MS to detect overdosed drugs were evaluated with a large sample size in clinical settings. More data should be gathered to reliably utilize these methods in clinical care for overdose patients.

There are several limitations in the present study. First, due to the nature of the study, some information about overdosed drugs was missing or could not be proved to be completely correct, which might affect the results of matching rates, sensitivities, and specificities. Because we cannot always get complete truth in the clinical setting, especially in the treatment of patients who self-harm, careful consideration should be paid for interpreting the data. For example, low sensitivities do not always mean low detection ability of the methods because of the possibilities of patients' false statements, and low specificities do not always mean low credibility due to missing information of ingestion or patients' misdeclaration. Although we cannot correctly calculate those sensitivities or specificities without true positive data, we used the drug information collected by the way shown in the "Methods" section as the best possible option

we could use in this study. By introducing these assumptions in the analysis, we could compare the performance among detection methods, and we believe those findings to be still useful in clinical practice. Second, our libraries for drug detection with LC/MS might not be sufficient for the study because there are no standard sets of libraries for overdose patient care. While treating various overdose cases, we have to keep revising our set of libraries for clinical use in the future. Finally, because of high sensitivity of drug detection and qualitative presentation of the results by LC/MS, the positive results do not always mean that overdose of drugs occurred. There is the possibility that detected drugs are just regular medications and need not to be treated. Therefore, we must be careful in applying the results of the present study in clinical use. The clinical usefulness of LC/MS for overdose patients should be revisited prospectively in future studies.

CONCLUSION

IN CONCLUSION, LC/MS was proven to be an effective method to detect drugs for overdose patients, especially for drugs that were not detected by other screening methods, such as Triage DOA^R, or when there was no information about overdosed drugs. For treating critical patients with a suspicion of drug overdose under such conditions, clinicians should utilize LC/MS if available, or decide to transfer the patients to higher-level facilities where LC/MS is available.

DISCLOSURE

Approval of the research protocol: The present study was approved by the ethics committee of our institution.

Informed consent: The requirement of informed consent was waived because of the anonymous nature of the data.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

Conflict of interest: None.

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