Screening of Antihistamine Agents (Diphenhydramine) with Blood and Urine Samples by REMEDi-HS® System

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ABSTRACT

Diphenhydramine (Dip) is one of the antihistamine agents (Anti-His) which are often compounded in cold remedies in Japan. Because of its sedative effects, it is advisable for drivers not to take it. However, as cold remedies can be obtained without a doctor’s prescription, Dip is frequently taken by all age groups in Japan. We sometimes experience the traffic accidents’ autopsy cases in which the urine specimens of drivers were Anti-His positive. The noninvasive screening for Anti-His on drivers as well as alcohol seems, therefore, necessary in order to examine whether etiologically Anti-His was directly involved in the occurrence of traffic accident or not.

Urine samples were collected from 5 male volunteers at 0, 1, 2, 3, 6, 9, 24, 36 and 50 hours after Dip intake (a single 30 or 60mg oral dose), and 1 ml of each urine sample was used for the analysis by REMEDi-HS® based on HPLC system. Dip and its 3 different kinds of metabolites, which reached at their maximum concentrations at 3 hours, were detected until at 24 to 50 hours. Maximum concentration of Dip ranged from 0.53 to 4.15 µg/ml (mean value = 1.93 µg/ml. SD= 1.53). At 1 hour though Dip was often undetected probably because of its very low concentration, one specific metabolite, on the other hand, was detected in the urine.

These results show that REMEDi-HS® is useful for the Dip screening in human urine samples and that the metabolite is an early index suitable for revealing Dip intake.

INTRODUCTION

Diphenhydramine is one of the antihistamine agents which are often compounded in antiphlogistics and in cold remedies in Japan. Because of its sedative effects, it is advisable for automobile drivers not to take it. However, diphenhydramine can be obtained without a doctor’s prescription, it is easily taken by all age groups, nevertheless before driving, in Japan. The traffic accidents’ autopsy cases, in which the serum and/or urine samples of drivers were antihistamine agents positive, have been sometimes experienced. Therefore, the non-invasive and/or very slightly invasive screening for antihistamine agents as well as alcohol seems to be important in order to examine to what extent antihistamine agent was etiologically involved in the occurrence of traffic accident. Screening of antihistamine agent (diphenhydramine) with blood and urine samples by REMEDi-HS® system (BIO-RAD, Hercules, CA, USA), which has recently begun to be used in Japan, is performed, and the
results were compared with those by gas chromatography-mass spectrometry (GC-MS) method.

MATERIALS AND METHODS

Samples

Blood and urine samples were collected from the 17 male volunteers (from 18 to 27 in age) at 0, 1, 2, 3, 6, 9 and 24 hours after the oral ingestion of 3 to 12 pills (10 mg diphenhydramine hydrochloride was included in a pill). Urine samples were, moreover, collected at 36 and 48 hours. Each blood sample was centrifuged 3000 revolutions per minute (rpm) for 5 minutes (min), and the serum was extracted. These serum and urine samples were then analyzed by REMEDI-HS system. Before each sample collection every volunteer's consent was obtained in writing.

REMEDI-HS

REMEDI-HS consists of high performance liquid chromatography (HPLC) system with computer and scanning ultraviolet (UV) detector. It is able to analyze about 500 kinds of basic and neutral drugs for about 20 min by the same analytical procedure. Four cartridges are used in this system; two cartridges initially separate drugs from proteins, salts and so on, and the other two cartridges produce an analytical differentiation of drugs, based on their characteristic retention times. Sample preparing procedures for this system were as follows; one ml serum or urine samples were mixed with 0.2 ml internal standard solution (IS) in a microfuge tube and were centrifuged 9500 rpm for 1 min. Serum samples were, moreover, centrifuged at 5000 rpm for 5 min in the centrifugal filter which was for REMEDI-HS's exclusive use. And then they were placed in the automated sampler. After necessary information was inputted, the analyzing can be started. IS contains 2 µg/ml N-ethyl-nordiazepam and 3 µg/ml chlorpheniramine and is for exclusive use of REMEDI-HS.

GC-MS

Moreover, to evaluate the quantitation value measured by REMEDI-HS, randomly selected samples were also analyzed by GC-MS. Extraction procedure for GC-MS analysis was as follows; two µg chlorpheniramine maleate was added as IS to 1 ml serum or urine samples. The samples were alkalized to pH 9.0 with 0.2 M NaOH. Thereafter, the samples were applied to an Extrelut column® and eluted with 20ml mixed solution of dichloromethane and isopropyl alcohol in the ratio of 85:15 (volume). The eluent was evaporated to dryness by nitrogen gas and the residue was reconstituted in 40 µl ethanol. One µl of the ethanol solution was analyzed by GC-MS. The conditions of GC-MS were as follows; A QP-1000 instrument (Shimadzu, Japan) equipped with a DB-1 column (15 m x 0.53 mm i.d., 1.5 µm film thickness, J & W Scientific, Folsom, CA, USA) was used. The column temperature was programmed from 180˚C to 250˚C with the increase at 10˚C/min. The injection temperature was at 250˚C, and helium flow rate was 15 ml/min. The mass spectrometer was operated in the positive electron impact mode by selected ion monitoring. The ionization energy and emission current were 70eV and 60µA, respectively.
RESULTS

Diphenhydramine and N-desmethyldiphenhydramine began to be detectable by REMEDI-HS system in the serum samples 1 or 2 hours after the drug ingestion, and other two metabolites as well as them were detected in the urine samples (Figure 1). N-desmethyldiphenhydramine was detected in 50 samples out of 66 diphenhydramine positive serum samples, and diphenhydramine metabolites were detected in every diphenhydramine positive urine sample (104 samples). Moreover, diphenhydramine metabolites were detected in 20 diphenhydramine
negative urine samples. The detection of its metabolites in addition to diphenhydramine confirmed the intake of the drug.

**Figure 2**

Calibration Curves for Diphenhydramine in the Serum (open square) and Urine Samples (closed circle) by REMEDI-HS System

The curves showed excellent linearity in the range of 0.04 µg/ml to 3 µg/ml, and the lower detection limit of diphenhydramine by this system was 0.04 µg/ml in the both samples.

Regression line:
the serum samples; \( Y = 0.746X - 0.002 \) (\( r = 1.000 \))
the urine samples; \( Y = 0.697X + 0.011 \) (\( r = 1.000 \))

\( Y \): peak height ratio of diphenhydramine to chlorpheniramine.
\( X \): diphenhydramine concentration. \( r \), correlation coefficient.

For quantitative analysis, the calibration curves for diphenhydramine in the serum and urine samples were prepared, and the curves showed excellent linearity between the peak height ratio of diphenhydramine to chlorpheniramine, and diphenhydramine concentration was determined in our experiment in the range of 0.04 to 3 µg/ml in the both samples (Figure 2). The lower detection limit of diphenhydramine by REMEDI-HS was about 0.04 µg/ml in the both samples. Average maximum concentrations of the serum and the urine samples, following each oral diphenhydramine hydrochloride dose, were as follows. The oral administration of 30 mg (\( n=1 \)) resulted in maximum serum concentration of 0.12 µg/ml at 3 hours after the ingestion and in maximum urine concentration of 0.82 µg/ml at 6 hours. A 60 mg dose intake resulted in average maximum serum concentration of 0.07 µg/ml (\( SD = 0.02, n = 6 \)) at the point of 1, 2 or 3 hours and in average maximum urine concentration of 1.73 µg/ml (\( SD = 1.77, n = 6 \)) at the point of 2, 3 or 6 hours. A 100 mg dose intake resulted in
0.21 µg/ml in the serum (SD = 0.09, n = 8) at 2 or 3 hours’ samples and in 4.94 µg/ml in the urine (SD = 3.35, n = 7) at 2 or 3 hours’ samples. In the case of 120 mg oral dose (n = 1), diphenhydramine reached at maximum concentrations at 2 hours, which were 0.20 µg/ml in the serum sample and 5.12 µg/ml in the urine sample. Diphenhydramine was not detectable in every serum sample collected at 24 hours (n = 16) and urine sample at 48 hours (n = 17) (Figure 3).

Figure 3
Time Course of Diphenhydramine Concentrations in the Serum and Urine Samples of a 22-year-old Male Who Ingested Orally 100 mg of Diphenhydramine Hydrochloride.

Moreover, by the use of GC-MS, 14 randomly selected samples (5 serum samples and 9 urine samples) were quantified with the peak height ratio of diphenhydramine (m/z 165) to chlorpheniramine maleate (m/z 203). There were good correlations between the results measured by conventional GC-MS and those by REMEDi-HS (Y = 0.946 X + 0.037, r = 0.941, n = 14, Y; quantitation value by GC-MS method, X; quantitation value by REMEDi-HS).

DISCUSSION
Rapid screening of drugs and poisons is very important not only in emergency medicine but also in forensic practice. In Japan, however, the analyzing system of drugs and poisons, especially for the cases without toxicological information, has not been well established. As REMEDi-HS, consisting of HPLC system, is able to analyze about 500 kinds of basic and neutral drugs for about 20 min by the same analytical procedure. There are over 60 REMEDi-HS units placed in USA, and their usefulness has been established in routine
clinical and emergency room toxicology as well as forensic autopsy cases. In this study, the availability of REMEDI-HS system for practical use was experimentally examined through the trial for screening of antihistamine agent in the blood and urine samples. In fact, not only diphenhydramine but also chlorpheniramine is often used as antihistamine agent in Japan. However, chlorpheniramine was contained in IS2 for exclusive use of REMEDI-HS, and only diphenhydramine was, therefore, examined as a target in this experiment.

Diphenhydramine and its metabolites were detectable by REMEDI-HS system in the samples 1 or 2 hours after the ingestion of the drug. Since REMEDI-HS is based on HPLC system, drug metabolites as well as the parent drug could be identified simultaneously. Metabolites of diphenhydramine were also detected in this experiment. Though the structure of diphenhydramine metabolites except of N-desmethyldiphenhydramine could not be determined by this system, these results showed that the simultaneous detection of the drug metabolites confirmed the intake of the parent drug.

Quantitative analysis of diphenhydramine was also performed by the use of peak height ratio of the drug to chlorpheniramine in IS2. The calibration curves for the drug in the serum and urine samples, which showed excellent linearity in the range of 0.04 to 3 µg/ml, could be made. Quantitation values by use of these calibration curves were approximately corresponded to previous reports (Blizer et al, 1973; Blyden et al, 1986; Glazko et al, 1974), and the changes of diphenhydramine concentrations in serum and urine samples generally showed the very similar pattern of time course. However, its lower detection limit was 0.04 µg/ml, while its therapeutic serum concentration was 0.01 to 0.10 µg/ml (Winek, 1985). Therefore, under our experimental conditions, diphenhydramine may be sometimes undetectable from the serum samples in case of normal dose ingestion.

Moreover, randomly selected samples were quantified by conventional GC-MS method, and the quantitation values by this method well corresponded with those by REMEDI-HS. The reliability of the quantitative analysis by REMEDI-HS system was thus shown by these results, and the usefulness of REMEDI-HS system on the screening and analysis of diphenhydramine was confirmed.

REFERENCES


