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Studies on Radioimmunoassay for Methamphetamine Excreted in Human Urine

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Methamphetamine excreted in urine of habitual users was analyzed by radioimmunoassay using specific antiserum prepared by the method previously described.[1] As a labeled compound, ^{3}H-methamphetamine was employed instead of the ^{125}I-methamphetamine derivative used in previous studies,[1b,c] on grounds of sensitivity. The results obtained by this radioimmunoassay were compared with those obtained by conventional methods, and good agreement was found.

Keywords——radioimmunoassay; methamphetamine; *N*-carboxymethylmethamphetamine; 1-^{3}H-methamphetamine; antiserum; human urine

In recent years, a striking increase of habitual users of methamphetamine (MA) has become a serious social problem in Japan. As the most common method for discrimination of habitual users of MA, examination of MA excreted in urine has been widely carried out. Therefore, the number of urine samples submitted to forensic laboratories for examination of MA has markedly increased and the development of a simple, rapid and reliable detection procedure for MA in urine is desirable.

In previous papers, specific and highly sensitive antisera against MA were prepared, and a radioimmunoassay (RIA)[1b,c] and hemagglutination inhibition test[1d] for MA in urine were developed.

In this study, RIA was applied to the estimation of MA in urine samples obtained from suspected habitual users by utilizing specific antiserum prepared by immunization of rabbits with bovine serum albumin conjugate of *N*-carboxymethylmethamphetamine[1a,c] and ^{3}H-MA. The results obtained by this RIA were compared with those obtained by conventional methods such as thin-layer chromatography (TLC), gas chromatography (GC) and GC-mass spectrometry (GC-MS).

Materials and Methods

Preparation of Antigen and Antiserum——*dl*-*N*-Carboxymethylmethamphetamine was synthesized from *dl*-ephedrine and conjugated with bovine serum albumin by the mixed anhydride method as described previously.[1a,c] The antiserum was prepared in rabbits by immunization with the antigen in the same manner as mentioned in previous papers.[1a,c]

Synthesis of *d*-1-^{3}H-MA——^{3}H-MA was prepared from *d*-1-phenyl-1-chloro-2-methylaminopropane by catalytic reduction with $^{3}H_2$ and Pd·$BaSO_4$ according to the method of Emde.[2] Purification of the product was carried out by preparative TLC using Silica gel 60 F-254 (E. Merck) plates and chloroform–ethanol (95 : 5, v/v) saturated with 28% aqueous ammonia as a developing solvent. The radiochemical purity of the product assayed by TLC using an Aloka JTC-203 radio thin-layer chromatoscanner was >99%. The specific radioactivity of the product was 170

mCi/mmol.

RIA Procedure——For dilution of antiserum and normal rabbit serum, 0.02 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer (pH 7.2) was used. Aliquots (100 μl each) of antiserum diluted 100 times, normal rabbit serum diluted 5 times, 0.02 M Tris-HCl buffer, ^{3}H-MA solution (*ca.* 0.01 μCi) and an unknown or standard solution were mixed, and incubated at 25 °C for 1 h. After the incubation, 500 μl of saturated ammonium sulfate solution was added, and the mixture was centrifuged at 3000 rpm for 15 min. The pellet was dissolved in 100 μl of the buffer, 100 μl of saturated ammonium sulfate solution was added, and the mixture was centrifuged. The pellet was dissolved in 300 μl of Soluene 350 (Packard Instrument Co.), 10 ml of scintillation cocktail containing *ca.* 5 μl of acetic acid was added, and the bound radioactivity was counted in a Beckman LS-9000 liquid scintillation counter.

Analytical Procedures——The extraction procedure, TLC and GC for MA in urine were carried out using a 5 ml sample by the methods described in "Standard Methods of Chemical Analysis in Poisoning."[3] GC-MS was performed on a Hitachi M-80 double-focussing mass spectrometer. The glass column (1 m × 3 mm i.d.) was packed with 3% OV-17 on Chromosorb W AW DMCS (100—120 mesh). The column, injector and separator temperatures were 125, 165 and 180 °C, respectively. The carrier gas was helium at 50 ml/min. The ionization voltage was 100 eV and the ionization current was 110 μA. Isobutane was used as a reagent gas.

Results and Discussion

Sensitivity and Specificity of RIA

In the RIA reported previously, the ^{125}I-labeled MA derivative was used. Although MA was determined in the range of 1—10^3 ng when ^{125}I-MA derivative having *ca.* 100 Ci/mmol specific radioactivity was used,[1c] the limit for determination of MA was 8 ng when ^{125}I-MA derivative having *ca.* 17 Ci/mmol specific radioactivity was used.[1b] Since it was difficult to ensure a high specific radioactivity of the ^{125}I-labeled compound for highly sensitive RIA, ^{3}H-MA was used in this study. The percent inhibition of binding of ^{3}H-MA to antiserum by various amounts of MA is shown in Fig. 1. As little as 1 ng of MA could be detected by this RIA and the standard curve was linear up to 20 ng.

The specificity of the antibody directed toward MA was examined previously with several compounds related to MA.[1c] Additional compounds including metabolites of MA were used for evaluation of the specificity of the present RIA (Table I).

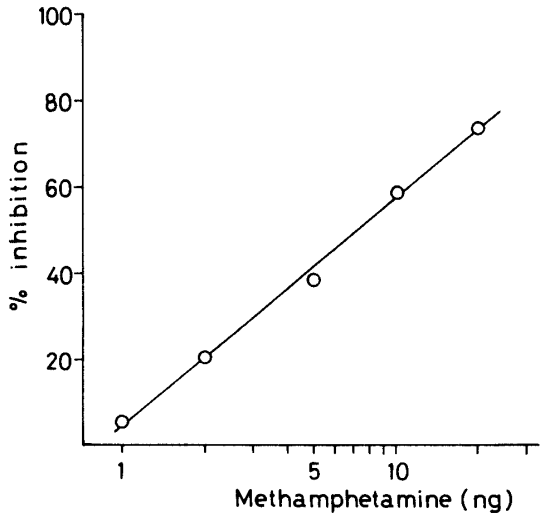

Fig. 1. Inhibition of Antiserum–^{3}H-Methamphetamine Binding by Various Amounts of Methamphetamine

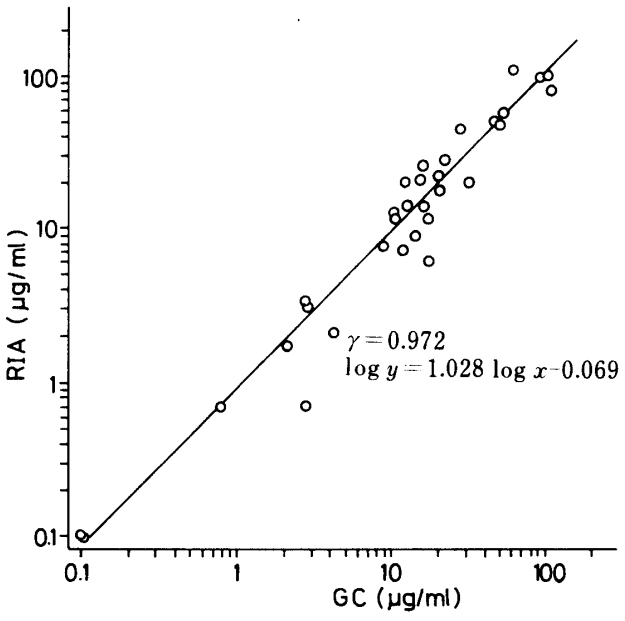

Fig. 2. Correlation of Concentrations of Methamphetamine in Urine Determined by Radioimmunoassay and by Gas Chromatography

Table I. Percent Cross-Reaction[4] of Methamphetamine Analogs with Antiserum against Methamphetamine

Compound	% cross-reaction
Methamphetamine	100
Amphetamine	3.5
Dimethylamphetamine	175
Ephedrine	7.8
Norephedrine	0.3
Methylephedrine	30
Mephentermine	64
Phentermine	8.8
p-Hydroxymethamphetamine	0.8
Methoxyphenamine	2.5
p-Hydroxyephedrine	<0.1

Analogs possessing substituents on an aromatic ring showed remarkably decreased affinity for the antibody. Primary amines exhibited very low affinity for the antibody, but the affinity of tertiary amines for the antibody was higher than that of primary or secondary amines. *p*-Hydroxymethamphetamine and amphetamine, the major metabolites of MA excreted in human urine,[5] showed negligible cross-reactivity, and this result suggested that the presence of metabolites in urine did not interfere with determination of MA.

Determination of MA in Human Urine by RIA and Comparison of the RIA Results with the Results of Conventional Analytical Methods

In order to examine nonspecific inhibition of binding by human urine, blank urine samples obtained from 10 healthy men and 5 healthy women with no experience of MA use were diluted up to 10-fold with water and assayed by RIA. Non-diluted urine samples showed 18—33% nonspecific inhibition but no inhibition was observed in more than 7-times-diluted urine samples. Therefore, urine samples were diluted 10 times with water for analysis of MA in human urine by RIA.

The recovery of MA, which was added to 10-times-diluted blank urine at concentrations of 10, 20, 50, 100 and 200 ng/ml as the hydrochloride, were in the range of 95—100%. The coefficient of variation was less than 8% ($n=6$) at each concentration.

Human urine samples obtained from 50 suspected habitual users were examined by RIA, TLC, GC and GC-MS. In 12 urine samples of the samples tested, MA was not detected by RIA, TLC, GC and GC-MS. In 6 samples found to be positive for MA by RIA, MA was not detected by TLC, GC and GC-MS. This was considered to be a result of the lower sensitivity in GC than in RIA, as the MA concentrations in the 6 samples were 0.1—0.3 μg/ml by RIA. For 32 samples in which MA was detected by RIA, TLC, GC and GC-MS, MA was determined by RIA and GC. The concentrations of MA determined by RIA were in good agreement with those obtained by GC, as shown in Fig. 2.

It is evident that the present RIA is useful not only as a screening test but also as a determination procedure for MA in multiple urine samples.

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