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DETERMINATION OF THE HERBICIDE MECOPROP (MCPP) IN THE SERUM AND URINE OF A PATIENT WITH MCPP POISONING

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Abstract

In this study, we developed a simple and sensitive method for determining mecoprop (MCPP) by HPLC and LCMS. We determined the MCPP levels in serum and urine samples from a patient with MCPP poisoning. The detection limit of MCPP in both serum and urine were 0.1 mg/l. The volume of MCPP ingested was estimated to be 50 g. The patient's serum and urine MCPP concentrations were 522 mg/l (1.5 hours after ingestion) and 385 mg/l (16 hours after ingestion), respectively. The MCPP level in the stocked urine sample (1.5–5 hours after ingestion) was very high at 1,047 mg/l. Serum and urine levels decreased rapidly in the first 32 hours, and thereafter the levels decreased slowly. The biological half-life ($T_{1/2}$) of MCPP in serum and urine were calculated to be 3.9 and 3.8 hours in the first 32 hours. Other than MCPP, an unknown peak (X) was found in the patient's urine that showed a molecular base peak of m/z 229. Peak X appears to be a substance derived from a metabolite of MCPP, to which is adducted one oxygen atom, namely hydroxy-MCPP.

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—**Key words**—
Mecoprop, Serum, Urine

Introduction

2-(4-chloro-2-methylphenoxy) propanoic acid (mecoprop, MCPP) is a chlorophenoxy herbicide that is widely used in Japan for the control of broadleaf weeds, especially in rice fields and golf courses[1)]. MCPP is an irritant to the skin, eyes, and airways[2)]. Several documented cases of oral ingestion of MCPP for the purpose of committing suicide have been reported. These cases of MCPP poisoning have resulted in death, as well as various clinical symptoms such as hyperthermia, coma, involvement of pulmonary complications, rhabdomyolysis, myoglobinuria, and renal failure[3)~8)].

MCPP levels have been determined in plasma and urine samples from patients who had ingested MCPP[3)~8)] and workers exposed to MCPP[9)], using gas chromatography (GC)[3)9)] and high-performance liquid chromatography (HPLC)[4)]. In this paper, we describe the analysis of MCPP by HPLC and by liquid chromatograph-mass spectrometry (LC-MS) of both urine and serum samples collected from a patient who had ingested MCPP. We also found hydroxylated MCPP, which is probably derived from a metabolite of MCPP.

Materials and methods

Case

A 30-year-old male ingested MCPP solution with the purpose of committing suicide. The volume of MCPP ingested was estimated to be 50 g, close to the LD_{50} level[1)2)]. The patient was discovered twenty-five minutes later. Vomiting was induced by having the patient immediately drink one liter of water. Next, the patient was transferred to an emergency hospital within one hour of the MCPP ingestion. The patient's level of consciousness had not sig-

nificantly altered (Japan Coma scale I-1). Electrocardiograph and chest radiograph revealed no abnormalities. Gastric lavage, treatment with activated charcoal, infusion, and forced alkaline diuresis were performed immediately after the patient was admitted. C-reactive protein (CRP) level on the 2nd day was elevated to 7.8 mg/dl. The patient's body temperature rose to over 38°C during the first 4 days of treatment. He subsequently made a full recovery without additional complications, and was discharged from the hospital on the 8th day after admission.

The serum and urine samples from the patient were collected and stored during his 5-day hospital admission. These samples were sent to our Occupational Poisoning Center for the determination of MCPP concentrations.

Chemicals

Mecoprop was obtained from Kanto Chemical Co. (Tokyo, Japan). Acetonitrile (AcCN, HPLC grade), acetic acid (AA), and sodium dihydrogenphosphate were purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). Hydroxy-MCPP (assumed to be a metabolite of MCPP) was custom-synthesized by Hayashi Pure Chemicals (Tokyo, Japan).

Sample preparation

Serum and urine samples from the patient were stored at –80°C until analysis. Urine and serum from healthy subjects as controls were also used in the comparison. For the preparation of serum, 900 μl of AcCN was added to 100 μl of serum and vigorously agitated using a vortex mixer. The urine samples were diluted 50-fold with distilled water. After centrifuging the serum and urine samples at 15,000 rpm for 10 minutes in a microcentrifuge, 50 μl of the supernatant was injected into the HPLC or LC-MS.

HPLC analysis

The HPLC utilized was an LC-10 Series model (Shimadzu Co., Kyoto, Japan) consisting of a pump (LC-10AD), an automatic sample injector (SIL-10A), and a column oven (CTO-10A). A Pegasil ODS column (250 × 6 mm i.d., 5 μl, Senshu Scientific Co. Ltd., Tokyo, Japan) was used for the analytical column. Flow rate and column oven temperature were set at 1 ml/min and 40°C. A spectrophotometer (SPD-10A) and a data processor (C-R4A) were used for quantitative analysis of MCPP. The wavelength was set at 290 nm and the mobile phase was 40% AcCN containing 0.1% AA.

LC-MS analysis

Apparatus used for LC-MS consisted of an HPLC (HP1100 series, Hewlett-Packard, USA) and MS (LCQ, Finnigan, USA). The analytical column used was a Pegasil ODS (250 × 6 mm i.d., 5 μl, Senshu, Japan). The mobile phase was 40% AcCN containing 0.1% AA. Flow rate and oven temperature were set at 0.8 ml/min and 40°C. Atmospheric pressure chemical ionization (APCI) mode with negative-ion detection was used for LC-MS detection.

Results

Fig. 1 shows the HPLC separation of urine (a) and serum (c) from the patient. MCPP in both serum and urine was eluted after about 20 minutes, the same as the retention time of MCPP in distilled water (b). Other than the MCPP peak, an unknown peak (X), an MCPP-related compound, was found in the urine. The "Peak X", which eluted in 6.8 minutes, was initially relatively high but gradually decreased during the patient's hospitalization period. Peak X was also found in the serum of the patient (but only in the sample taken on admission), but at a very low level. Neither MCPP nor Peak X was found in the control subjects.

Fig. 2 shows the LC-MS determination of MCPP in urine from the patient. Fig. 2 (a), (b) and (c) show the total-ion chromatograms (TIC), and the selected-ion monitoring (SIM) of MCPP and Peak X, respectively. Fig. 2 (d) shows the mass spectrum of MCPP, indicating the base peak of a molecular ion, m/z 213 ([M-H]-). The mass spectrum was confirmed by standard MCPP. The ion with m/z 273 is thought to be MCPP adducted to the AA used in the mobile phase. Fig. 2 (e) shows the mass spectrum of Peak X (a) with a molecular base peak of 229.

Fig. 3 shows the LC-MS determination of Peak X in urine and hydroxy-MCPP in distilled water. Fig. 3 (a) and (b) show the TIC of Peak X and hydroxy-MCPP. Fig. 3 (c) and (d) show the mass spectrum of Peak X and hydroxy-MCPP, respectively. These two peaks have same mass spectrum and were eluted in the same time.

The calibration curves of MCPP added to distilled water, urine and serum showed linear relationships up to 1,200 mg/l of MCPP. The detection limit and recovery rate of serum were 0.1 mg/l and 87.5% (n=7), respectively. In urine, the detection limit and the recovery rate were 0.1 mg/l and 99.8%, respectively. MCPP concentrations de-

termined by HPLC (x) and LCMS (y) were closely correlated in the patient's serum (y=−1.89+0.822x (r=0.990, n=6)) and urine (y=17.2+1.13x (r=0.993, n=7)).

Fig. 4 shows the time course of MCPP concentrations in the patient's serum and urine. The MCPP level of serum on admission (1.5 hours after ingestion) was 522 mg/l. Sixteen hours had elapsed when the first spot urine sample was collected for determination of MCPP, and that level was 385 mg/l. The MCPP level in the stocked urine sample (1.5–5 hours after ingestion) was very high at 1,047 mg/l. MCPP concentrations in both serum and urine had declined to trace levels on the 5th day. Serum and urine levels decreased rapidly in the first 32 hours (the rapid phase), and thereafter the levels decreased slowly (the slow phase). During the rapid phase (≈32 hours), the re-

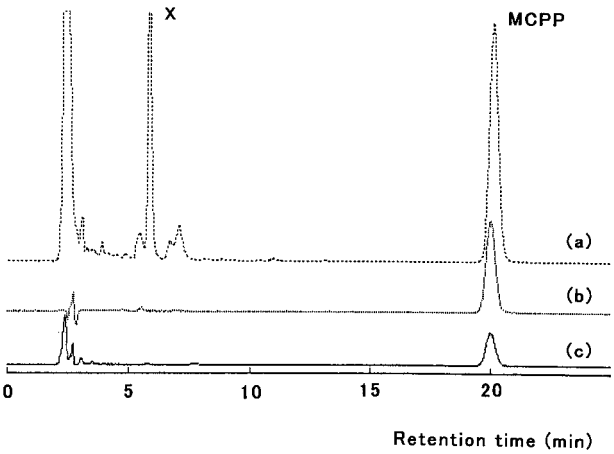

Fig. 1 Chromatographic separation of MCPP and its related compound (X).

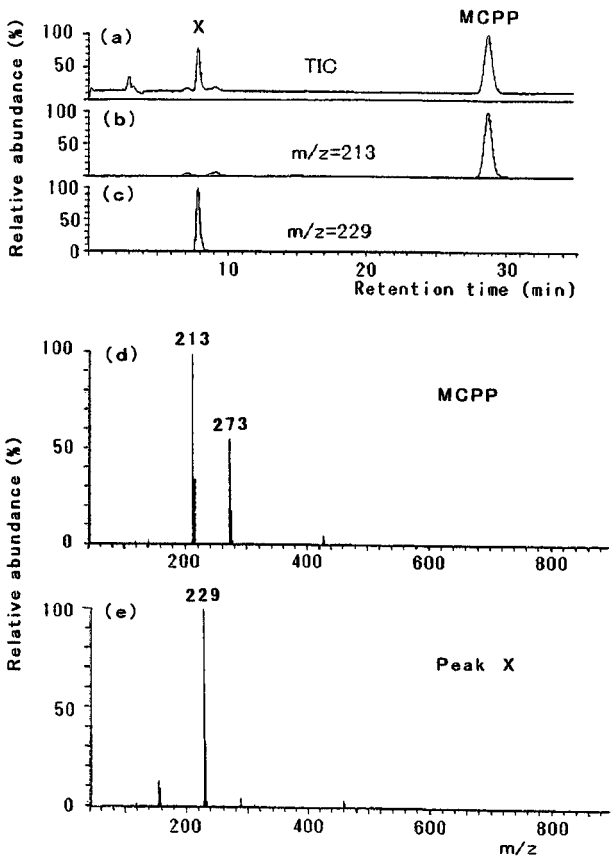

Fig. 2 Typical mass chromatograms and mass spectra of MCPP and its related compound (X) in a urine sample from the patient.

gression curves for serum and urine were $y=860.4e^{-0.179x}$ (r=0.993) and $y=7{,}637.2e^{-0.182x}$ (r=0.993), respectively. For the slow phase (from 32 hours onwards), the regression curves for serum and urine were $y=12.3e^{-0.048x}$ (r=0.991) and $y=71.2e^{-0.034x}$ (r=0.972), respectively. The biological half-life ($T_{1/2}$) of MCPP in serum and urine were calculated to be 3.9 and 3.8 hours in the rapid phase, and 14.4 and 20.4 hours in the slow phase, respectively.

Discussion

MCPP in biological fluids is usually determined by GC[3)9)] and HPLC[4)]. Gas chromatography requires the complicated procedure of double extraction and derivatization[3)]. In HPLC[4)], homogenized samples are mixed with an equal volume of AcCN/methanol (2:1) and the supernatant is used for the analysis. The detection limit is reported to be 1 mg/l[4)]. In the present study, MCPP can be determined by simple precipitation of serum protein with AcCN or after dilution of urine with water. In the present HPLC analysis, MCPP was effectively separated from the other components in the urine or serum (Fig. 1). The detection limit of MCPP was 0.1 mg/l, lower than the previously reported 1 mg/l[4)].

Other than the MCPP peak, an unknown peak (X) was detected in the patient's urine (Fig. 1). Peak X was also found in the patient's serum (only in the sample taken on admission), but the level was very low. Since MCPP and Peak X gradually decreased over the time course of the patient's hospitalization, and were not detected in control subjects, we believe Peak X to be related to the MCPP ingested, either as a metabolite of MCPP or as a low-level impurity in the herbicide.

In the present investigation we also developed the LC-MS method for MCPP analysis. MCPP levels measured by LC-MS determination correlated with those by HPLC. For the identification of Peak X, we can attempt to speculate on the chemical structure from the mass spectrum obtained by LC-MS (Fig. 2 (e)). The molecular base peak (m/z 229) suggests that the substance is derived from MCPP with the addition of one oxygen atom, i.e., hydroxy-

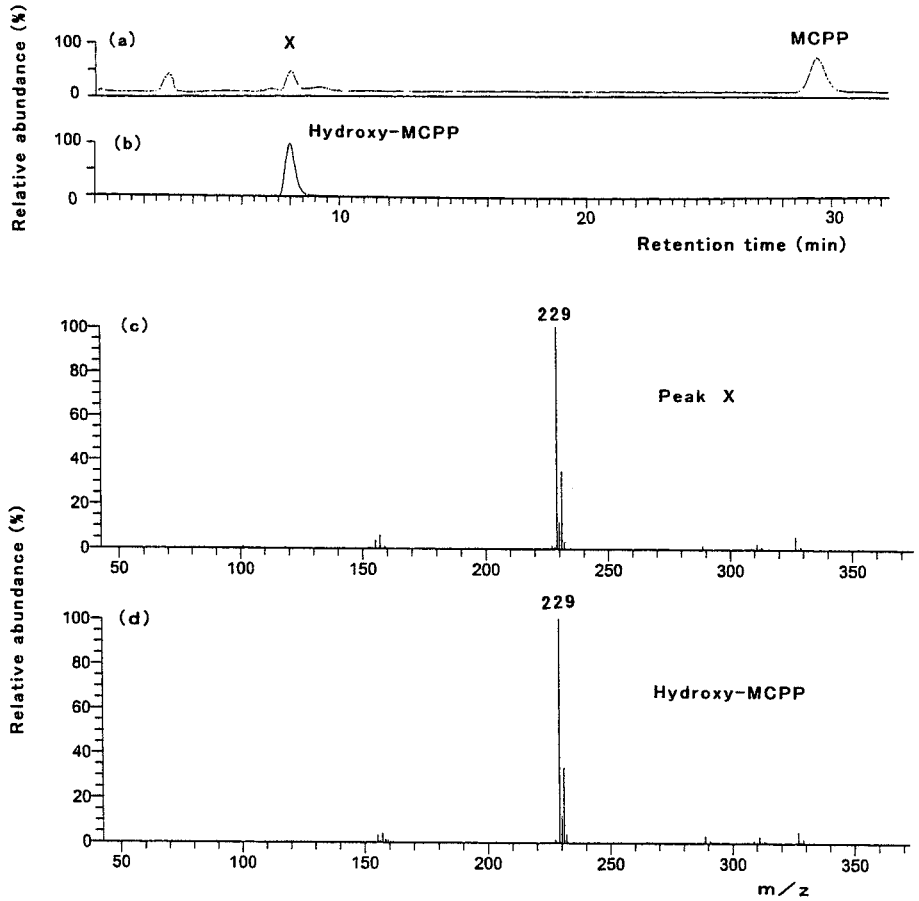

Fig. 3 Typical mass chromatogram (TIC) and mass spectrum of Peak X and hydroxy-MCPP.

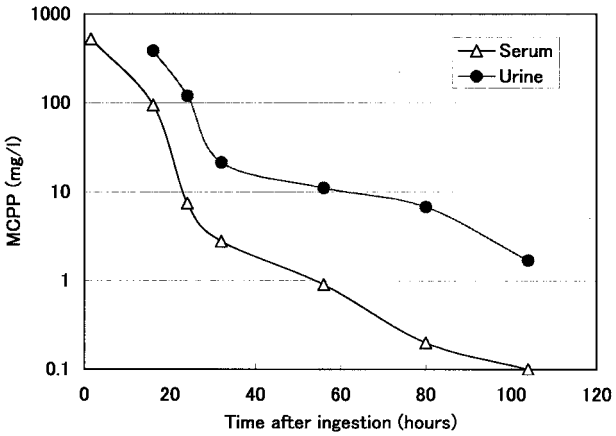

Fig. 4 Decay curves for MCPP in serum and urine during the patient's hospitalization.

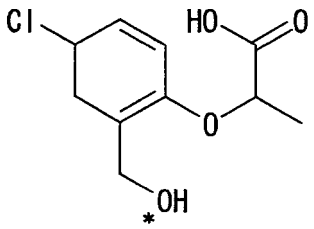

Fig. 5 Chemical formula of hydroxy-MCPP

lated MCPP. For confirmation of its structure, we prepared this substance by custom synthesis. Our tests revealed that Peak X and hydroxy-MCPP have the same mass spectrum and retention time (Fig. 3). The structure of hydroxy-MCPP is shown in Fig. 5 and * is the position of oxygen adducted. Peak X is likely to be a substance derived from an MCPP metabolite, to which is adducted one oxygen atom, namely hydroxy-MCPP.

Decreases in MCPP levels after receiving medical treatment showed a two-phase decline. The half-lives of MCPP in serum and urine were 3.9 and 3.8 hours for the rapid phase, and 14.4 and 20.4 hours for the slow phase, respectively. The serum half-life (rapid phase) in the present study was the shortest of those previously reported[3)5)6)]. Meulenbelt *et al.*[5)] reported a 17-hour half-life for MCPP elimination from the plasma. The plasma MCPP level was 298 mg/l at 3–4 hours after ingestion and elimination was slower than during the rapid phase in the present case. The faster elimination, especially in the rapid phase of the present case, may be due to treatment by forced alkaline diuresis. The patient of Meulenbelt *et al.*[5)] was not treated by forced alkaline diuresis.

In the case of Prescott *et al.*[3)], the plasma MCPP levels on admission were 751 mg/l, which then declined slowly with a half-life of about 40 hours, even with treatment by forced alkaline diuresis. The very slow elimination of MCPP in this case seems to be due to the ingestion of a mixture of herbicides comprising MCPP (20%) and 2,4-D (10%). In their case, renal clearance of 2,4-D gradually increased with forced alkaline diuresis, and a rapid fall in plasma 2,4-D levels ($T_{1/2}$=3.7 hours) was observed during the treatment. In another case of ingestion of mixed herbicide (MCPP and 2,4-D), treated with forced alkaline diuresis[6)], apparently slower elimination than ours was found. Therefore, MCPP elimination from blood seems to be slow, even with alkaline treatment, if a mixed herbicide is ingested.

Acknowledgments

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除草剤メコプロップ（MCPP）中毒患者の血清・尿中MCPP濃度の測定

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—キーワード—
メコプロップ，血清，尿

高速液体クロマトグラフィー（HPLC）および液体クロマトグラフ質量分析計（LCMS）による除草剤メコプロップ（MCPP）の簡便・高感度な測定法を開発し，MCPP中毒患者の血清・尿中MCPP濃度の測定に応用した．血清および尿中MCPPの検出限界は0.1mg/lである．患者が摂取したMCPPはおよそ50gであった．摂取から1.5時間後の血清中MCPP濃度は385mg/l，16時間後の尿中MCPPは522mg/l，摂取後1.5～5時間の蓄尿中MCPPは1,047mg/lであった．血清・尿中のMCPP濃度は摂取後32時間までに急速に減少し，その後はゆるやかに低下した．32時間までのMCPP濃度の半減期は，血清では3.9時間，尿は3.8時間であった．患者の尿中にはMCPPの他に分子イオンm/z = 229のピークが認められた．このピークはMCPPの代謝物の1つであり，酸素原子が1個付加した水酸化MCPPであると推定された．