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Fate of Hydralazine in Man. II. Formation of Tetrazolo[5,1-*a*]phthalazine *in Vivo* and Inhibition of Monoamine Oxidase by This Metabolite[1]

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Tetrazolo[5,1-*a*]phthalazine (Tetra-P) was detected in the urine of a patient on hydralazine treatment. Tetra-P is a new type of monoamine oxidase (MAO) inhibitor.

Keywords——fate of hydralazine; tetrazolo[5,1-*a*]phthalazine formation *in vivo*; urinary extract from patient on hydralazine treatment; HPLC; new type of MAO inhibitor

In the previous paper,[2] we reported that tetrazolo[5,1-*a*]phthalazine (Tetra-P) was detected by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) in the extracts of incubation mixtures of hydralazine (HP) in human saliva with simulated gastric juice (SGJ), and Tetra-P was also found in rabbit urine when an aqueous solution of $NaNO_2$ was given before and after the oral administration of HP.

In the present work, we succeeded in the detection of Tetra-P in the urine of a patient on HP treatment, by using a high-performance liquid chromatography (HPLC) procedure established by us.[3] Furthermore, we have found that Tetra-P is a new type of monoamine oxidase (MAO) inhibitor: a sensitive fluorometric (FL) assay with human platelet MAO[4] clearly revealed the MAO inhibition by Tetra-P.

Materials and Methods

Chemicals——HP-HCl, Tetra-P, 3-methyl-*s*-triazolo[3,4-*a*]phthalazine (MTP) and *s*-triazolo[3,4-*a*]-phthalazine (Tri-P) were the same compounds as described in the previous paper.[2]

Apparatus and HPLC Conditions——A high-performance liquid chromatograph, HLC-803A (Toyo Soda), equipped with a TSK GEL LS-410 ODS column (15 cm × 4 mm) and UV-8 and FS-970 detectors, was used. A 20% solution of acetonitrile in 0.05 M KH_2PO_4 was used as a mobile phase. The solvent was degassed by ultrasonication prior to HPLC. The flow rate was 1.0 ml/min. The column eluate was monitored at 228 nm by a UV detector (0.02 a.u.f.s.) and at 228 nm (excitation) and above 370 nm (emission) by an FL detector (1.0 μA-f.s.). The intensities were recorded by means of a two-pen recorder (Rikadenki Kogyo Ltd.) and a Chromatopac C-RIA chromatography integrator (Shimadzu Seisakusho Ltd.).

Patient——A female patient of 74 years of age who was suffering from hypertension participated in this study. One tablet containing 25 mg of HP-HCl was taken three times a day after meals, *i.e.*, 75 mg of HP-HCl a day was administered to the patient. She is an out-patient taking trichloromethiazide, clonidine-HCl, pindorol, dicyclomine-HCl, nitrazepam and thyroid, simultaneously. The urine was collected for 24 h from 8 A.M. till the same time next morning.

Sample Preparation for HPLC——The total volume of 24-h urine was used for preparing the sample for HPLC. The urine was extracted three times with CH_2Cl_2. The extract was dried over anhydrous sodium sulfate and the solvent was removed. Next 50 ml of MeOH was added to the residue and 10 μl of the methanolic solution was injected into the column.

FL Assay with Human Platelet MAO——The MAO inhibitory effect of Tetra-P *in vitro* was examined by the method previously described.[4] The results are shown in Fig. 2.

Results and Discussion

It is the first report to describe the detection of Tetra-P in a patient on HP treatment. HP–HCl (75 mg/d) was orally administered to the patient three times after meals. The urine collected over 24 h was extracted with CH_2Cl_2. The residue from the extract was injected into the HPLC column to give the chromatograms shown in Fig. 1. The peak of Tetra-P (retention time: 6.9 min) was observed between the peak of Tri-P (t_R: 4.4 min) and that of MTP (t_R: 8.6 min). Tri-P and Tetra-P are well-known as the major metabolites of HP.[2,5] The patient was taking many drugs simultaneously, but these should have had no effect on Tetra-P formation, because they are not thought to produce any materials which might affect the nitrosation of HP. When 200 mg/body weight of HP–HCl was orally administered to a male albino rabbit, Tetra-P was detected qualitatively in the urine. Tetra-P formation takes place easily under acidic conditions following the reaction of HP with the nitrosonium ion liberated from nitrite ion.[5] Tetra-P formation did not occur when HP was kept for three days in the blank urine of man or rabbit at room temperature.

The amount of Tetra-P excreted was examined twice in the same patient, and 43—52 μg of Tetra-P was detected in the 24-h urine of the patient. The values shown in Table I were rough estimates, because the excreted amount of Tetra-P was calculated from the peak height

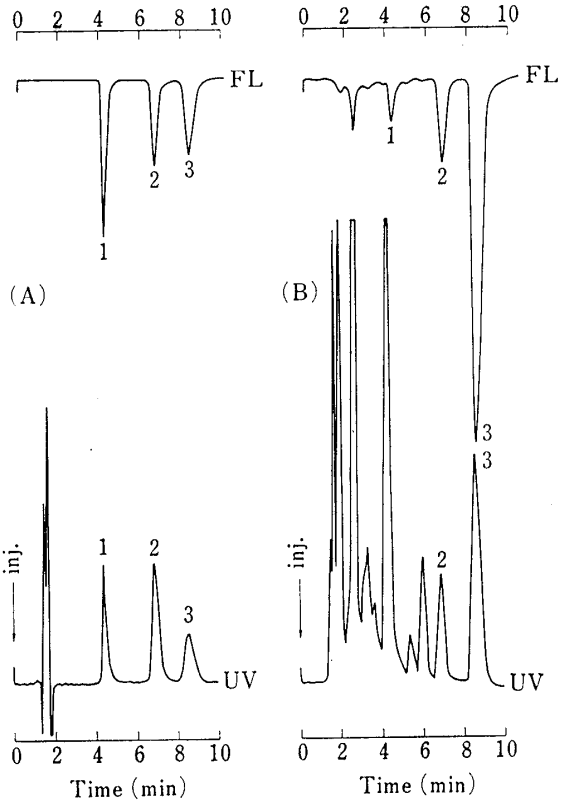

Fig. 1. HPLC Chromatograms of Authentic Samples and the Urinary Extract of a Patient on Hydralazine Treatment (HP–HCl: 75 mg, t.i.d.p.c.)

HPLC conditions:
Column: TSK-GEL LS-410 ODS, 15cm×4 mm i.d.
Mobile phase: 20% acetonitrile in 0.05 M KH_2PO_4.
Flow rate: 1.0 ml/min.
Detection: UV 228 nm, 0.02 a.u.f.s.; fluorescence, excitation: 228 nm, emission: >370 nm, 1.0 μA-f.s.
(A): authentic samples (1: Tri-P, 2: Tetra-P, 3: MTP).
(B): extract from the patient's urine.

Table I. Amount of Tetra-P excreated in 24-h Urine of a Patient on HP Treatment[a]

Total volume of 24-h urine (ml)	Detector	Excreted amount of Tetra-P (μg)
(1) 1275 (pH 7.0)	FL	49
	UV	43
(2) 1120 (pH 7.0)	FL	51
	UV	52

a) 75 mg of HP–HCl/d was administered in three times after meals.

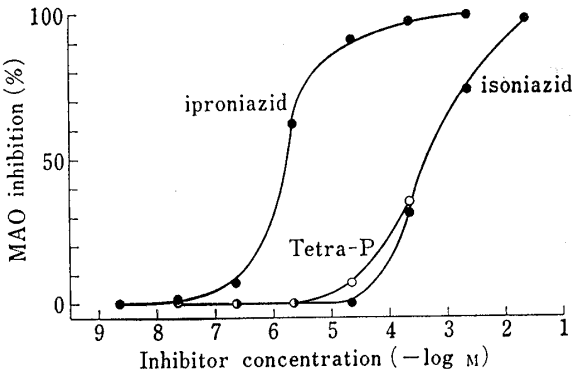

Fig. 2. Inhibition of Human Platelet MAO by Tetra-P, Iproniazid and Isoniazid

ratio of the urine sample to a fixed amount of the authentic sample without a calibration curve. The value obtained by the UV detector coincided well with that obtained by the FL detector.

We had already reported an effect of Tetra-P analogous to that of an MAO inhibitor, nialamid, on the basis of behabioral tests in pharmacology.[6] In order to confirm the effect of Tetra-P, its inhibitory effect on MAO was examined in an FL assay with human platelet MAO.[4] In this method, benzylamine and human platelets are used as the substrate and the enzyme fraction. The results in comparison with those for well-known MAO inhibitors, iproniazid and isoniazid, are shown in Fig. 2. Tetra-P showed MAO inhibitory potency at concentrations of more than 2×10^{-6} M. The potency was almost identical with that of isoniazid and lower than that of iproniazid. Unfortunately, the potency of Tetra-P at concentrations of more than 5×10^{-4} M could not be measured because of the poor solubilityof the compound. However, it is of interest that Tetra-P is a new type of MAO inhibitor. We are examining the MAO inhibitory effects of other phthalazine derivatives which are more hydrophilic. The details will be reported soon.

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References and Notes

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