Determination and Properties of Acetyl Conjugate of N-Desisopropylpropranolol, AcNDP

Atsuko NODA,*a Yoko ONO,a Xiuzhong WU,a Keiko KUDO,b Narumi JITSUFUCHI,b Seiji ETO, and Hiroshi NODAa

Faculty of Pharmaceutical Sciences, Kyushu Universitya and Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan, and Department of Hospital Pharmacy, School of Medicine, University of Occupational and Environmental Health, Japan (Sangyo Ika-Daigaku), 1-1 Iseigaoka, Yatabanishi-ku, Kitakyushu 807, Japan.

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1-Acetamino-3-(1-naphthoxy)-2-propanol (AcNDP) detected in human urine was formed as a metabolite of propranolol (PL) via 1-acetamino-3-(1-naphthoxy)-2-propanol (N-desisopropylpropranolol, NDP). The excreted amount of AcNDP was determined by GC-MS using an isotope dilution method. More than 40% of total AcNDP in 24 h urine was detected 10 h after the oral administration of PL to two volunteers, and the total amounts during 24 h urine were at least 1.9–3.9% of the PL dose. As AcNDP is an intermediate metabolite of PL, its urinary amount cannot be determined exactly. Incidentally, AcNDP was chemically stable and was not formed from NDP when acetyl CoA was added to the inactivated hepatocyte system. Thus, the acetylation of NDP, an aliphatic primary amine, was confirmed to be catalyzed by N-acetyltransferase, and interestingly, the acetyl conjugation was inhibited not by sulfamethazine but by p-amino benzoic acid.

Key words: GC-MS determination; propranolol metabolite; aliphatic primary amine; acetyl conjugation

Since propranolol (PL) is frequently employed as a nonselective β-adrenergic blocking agent, its metabolism has been studied by many researchers to explore the ring oxidation of PL to 4-, 5- or 7-hydroxypropranolol, the side chain oxidation to 1-amino-3-(1-naphthoxy)-2-propanol (NDP), 3-(1-naphthoxy)-1,2-propanediol (PG1), 3-(1-naphthoxy)-2-propionic acid (NLA) and 1-naphthoxy acetic acid (NAA), some glucuronidations and sulfate conjugations. 1-Acetamino-3-(1-naphthoxy)-2-propanol (AcNDP) is a metabolite of NDP, which is an aliphatic primary amine derived from PL, and was found not only in isolated rat hepatocytes but also in the urine of a patient and a healthy man using LC-MS and GC-MS.1 The detection is very valuable, since the study about an acetyl conjugation of an aliphatic primary amine in human subjects has not been reported except for one case of doxylamine.2 In this paper, the determination, the stability and the formation mechanism of AcNDP were described.

MATERIALS AND METHODS

Chemicals Racemic PL·HCl was purchased from Aldrich Chem. Co. (Milwaukee, WI, U.S.A.). NDP and AcNDP were synthesized by the methods described.1) d3-AcNDP was prepared by the modified method of AcNDP using d3-acetic anhydride instead of d4-acetic anhydride.

Sample Preparation from Human Urine Nineteen ml portions of 0–10 and 10–24 h urine collected from two healthy volunteers (male, 33 years old; female, 59 years old) after the oral administration of racemic PL·HCl (20 mg) were diluted twice with water. After pH of each portion was adjusted to 9 with 1.9 ml of 1N Na2CO3, 0.5 ml of 80 μM methanolic solution of d3-AcNDP (I.S.) was added. The solution containing AcNDP and d3-AcNDP was extracted twice with 20 ml of chloroform. The combined extracts were dried over using nitrogen gas, and the samples were stored at −20°C until GC-MS assay.

Incubation of NDP in Isolated Rat Hepatocyte System and Sample Preparation for HPLC Assay Incubation, sample preparation and HPLC assay were performed under the conditions described.1) Five-tenths mM of NDP was employed to explore the interaction with 1 eq mol of p-aminobenzoic acid (PABA) or sulfamethazine (SMZ). To examine the mechanism of acetylation, 0.5 mM of NDP was incubated at 37°C for 40 min with 1 eq mol of acetyl CoA.

GC-MS Apparatus and Analytical Conditions A gas chromatograph HP-5890 equipped with a HP-5989A mass spectrometer (Hewlett-Packard, Waldbronn, Germany) was employed. The samples were separated on a HP-1 capillary column (12 m × 0.2 mm i.d. × 0.33 μm). The oven temperature was programmed to increase from 90°C to 260°C at a rate of 10°C/min. The injector temperature was 260°C, and the carrier gas (helium) velocity was set to 1.0 ml/min. The ions of m/z 116 and 119 were monitored at 15.93 min (average) in the selected-ion monitoring mode.

RESULTS AND DISCUSSION

The amount of AcNDP was determined by the isotope dilution method of GC-MS. The peak of authentic AcNDP was directly detected without any derivatization. The EI-MS spectra of authentic samples of AcNDP and d3-AcNDP from GC-MS are shown in Fig. 1; their base ion peaks appear at m/z 116 and 119, respectively. These peaks were employed to determine the amounts of AcNDP in human urine, since their molecular ion peaks, m/z 259 and 262, were very small and not adequate to estimate a small quantity of AcNDP.

As shown in Fig. 2, a linear standard curve ranging from 1 to 5 μl with the relative coefficient 0.996 was obtained. The amount of AcNDP in human urine during

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0—10 and 10—24 h after the oral administration of 20 mg of racemic PL·HCl to the two subjects was calculated by this curve (Table 1). More than 40% of total AcNDP in 24 h urine was detected 10 h after the oral administration of PL, and the total during 24 h urine was 1.9—3.9% of PL dose. Actually, the amount of AcNDP formed in patients under PL therapy is speculated to be much greater, since AcNDP is an intermediate metabolite of PL as described later.

The mechanism governing formation of AcNDP was examined in the isolated rat hepatocyte system using NDP as a substrate, which is only a plausible precursor of AcNDP among the metabolites of PL. The results are shown in Fig. 3. The amount of AcNDP formed initially increased, then decreased time-dependently. The formation was markedly inhibited by PABA, while it was little inhibited by SMZ and did not take place at all after incubation for 40 min at 37 °C with acetyl CoA in the inactivated hepatocyte system containing NDP. Simultaneously, the stability was examined by the incubation of NDP or AcNDP at 37 °C for 1 h in medium for the hepatocyte system (pH 7.4), in human urine (pH ca. 6.2), in McIlvain buffer solution (pH 3 and 5), and in Sörensen buffer solution (pH 9), respectively. Under these conditions, AcNDP as well as NDP was non-enzymatically stable and did not change chemically. AcNDP might therefore be an intermediate metabolite which is bio-transformed into other metabolites.

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REFERENCES