DETECTION OF 1-ACETAMINO-3-(1-NAPHTHYLOXY)-2-PROPANOL AS A NEW METABOLITE OF PROPRANOLOL

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1-Acetamino-3-(1-naphthoxy)-2-propanol which is an acetyl conjugate of N-desisopropylpropranolol (AcNPD) was detected as a new metabolite of propranolol (PL) from the incubation medium of isolated rat hepatocyte system and from the urinary extracts of a patient under PL therapy as well as a healthy volunteer given PL. In the case of the hepatocyte systems, the optical selectivity of PL elimination and the metabolite formation were discussed by HPLC determination, and the effect of pretreatment by phenobarbital (PB) or 3-methylcholanthrene (3-MC) on the metabolism was also clarified.

KEYWORDS propranolol metabolite; acetyl conjugate; rat hepatocyte; human urine

Propranolol (PL) is a nonselective β-adrenergic blocking agent still widely used in the treatment of a variety of cardiovascular disorders. The metabolism of propranolol in animals and humans has been extensively explored by many groups, and is well known as the aromatic ring oxidation to 4-, 5- or 7- hydroxypropranolol (4-, 5-, or 7-OH-PL), the side chain oxidation to 1-amino-3-(1-naphthoxy)-2-propanol (NPD), 3-(1-naphthoxy)-1,2-propanediol (PGL), 3-(1-naphthoxy)-2-propionic acid (NLA) and 1-naphthoxyacetic acid (NAA), some glucuronidations and sulfate conjugations. In the present study, we report the detection and identification of an acetyl conjugate of NPD (AcNPD), a new metabolite of PL, separated from three kinds of samples, which are the extracts from an incubation medium of isolated rat hepatocyte system and from the urinary extracts of a patient under PL therapy and a healthy volunteer given PL.

MATERIALS AND METHODS

Chemicals (R,S)-, R- and S- PL hydrochlorides were purchased from Aldrich Chem. Co. (Milwaukee, WI, USA). Sodium phenobarbital (Na-PB) and 3-methylcholanthrene (3-MC) were from Tokyo-Kasei Kogyo (Tokyo, Japan) and Sigma Chemical Co. (St. Louis, MO, USA), respectively. Tetraclo[5,1-a] phthalazine (Tetra-P, I.S.) and ethyl-s-triazolo[3,4-a] phthalazine (ETP, I.S.) were prepared by the method described in the previous paper. Other chemicals were of reagent grade, and the solvents for elution were of HPLC grade. NPD and its N-acetyl derivative (AcNPD) were synthesized as follows.

1) NPD hydrochloride was obtained from 1-naphthol as a starting material via 1,2-epoxy-3-(1-naphthoxy) propane and then 3-(1-naphthoxy)-2-hydroxypropy-N-phthalimide, mp. 208-209 °C (ethanol-ethyl acetate). MS m/z : 217 (M+). Anal. Calcd C13H13NO2·HCl : C, 61.50; H, 6.50; N, 5.52. Found : C, 61.60; H, 6.31; N, 5.49.

2) NPD was treated with acetic anhydride in pyridine, and the obtained diacetate was hydrolyzed with 1 N HCl at 70 °C for 15 min to give N-monoacetylacetate(AcNPD), mp. 101-102 °C (methanol-isopropyl ether). MS m/z : 259 (M+). Anal. Calcd C15H17NO3 ; C, 69.45; H, 6.60; N, 5.47. Found : C, 69.48; H, 6.61; N, 5.40. IR : 1640 cm⁻¹ (C=O), 1540 cm⁻¹ (NCOCH3) NMR (270 MHz, CD3OD) : 2.43 ppm (NCOCH3)

Animals Male Wistar rats (Kuroda Pure Animals Co., Fukuoka, Japan) weighing 200-260 g were used for the study. The animals were divided into three groups, i.e. one group is the control group (intact) and the others are the pretreated groups with Na-PB (80 mg/kg, i.p., in saline) and 3-MC (20 mg/kg, i.p., in corn oil), respectively, once
a day for three days.

Incubation of Propranolol in Isolated Rat Hepatocyte Systems and Sample Preparation for Assay Isolated rat hepatocytes were prepared according to the method described by Moldes et al. The cell concentration of the hepatocytes was adjusted to 8x10⁶ cells/ml using a Krebs-Henseleit buffer, pH 7.4, and supplemented with 13 mm HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid) and 10 mm glucose.

After the addition of 500 μM of (R,S)-, R- or S-PL hydrochloride, the hepatocyte suspensions were incubated at 37 °C in rotating round-bottomed flasks under a 95%O₂ - 5%CO₂ atmosphere. After incubation for 0, 10, 20, 30 and 40 min, each 1 ml of sample solution was added to the mixed solution of 1 ml of 1 n sodium hydroxide containing 25 mg of sodium bisulfate and 0.1 ml of 1 mm methanolic solution of Tetra-P (I.S.), and shaken with 5 ml of ethyl acetate for 15 min at pH 9. The upper organic layer was dried with anhydrous sodium sulfate and evaporated to dryness. After the addition of 0.1 ml of methanol, the sample was used for HPLC analysis. On LC/MS, the extract was purified by TLC before injection. Methanolic extract from the silica gel plate at Rf value around 0.88 (CHCl₃:CH₃OH =3:1), which corresponds to that of authentic AcNDP, was employed for assay.

Sample Preparation from Human Urine for HPLC, LC/MS or GC/MS Assay Each 30 ml portion of 24 hr urine (2150 ml) collected from a healthy volunteer (Female, 58 years old) after the oral administration of (R,S)-PL (20 mg) and a patient under Inderal therapy (Female, 75 years old) was diluted twice with water, and extracted with chloroform at pH 9. In the case of human subjects, the urinary extracts were purified by preparative TLC before HPLC or GC injection. Methanolic extract from the silica gel plate at Rf value around 0.88 (CHCl₃:CH₃OH =3:1), which corresponds to the value of authentic AcNDP, was collected and employed for LC/MS or GC/MS.

HPLC Apparatus and Analytical Conditions A Shimadzu LC-9A high performance chromatograph (Kyoto, Japan) equipped with a spectrofluorometric detector (Shimadzu RF-550) and a reversed phase column (TSK gel ODS-80TM, 25 cm x 4.6 mm I.D., Tosoh Co., Japan) was employed. The column temperature was 37 °C and the flow rate was 0.8 ml/min. The mobile phase was a mixture of acetonitril : methanol : water : acetic acid (Phase A =18:12:70:1, Phase B =36:20:44:1). The column eluate was monitored at 310 nm (excitation) and 380 nm (emission). The retention time (min) is shown in parenthesis: Phase A : 5-OH-PL(5.3), 4-OH-PL(6.2), 7-OH-PL(8.2), ND(12.9), Tetra-P(12.9; I.S.), PL(15.6), PGL(48.0) and AcNDP(52.0). Phase B : ETP(6.4; I.S.), NLA(7.6) and NAA(10.6).

LC/MS Apparatus A Hewlett-Packard (HP)-1090 high performance chromatograph (Waldbronn, Germany) equipped with HP-5989A mass spectrometer and HP-59980B interface (California, USA) was employed.

GC/MS Apparatus JEOL JMS-DX 300 (Tokyo, Japan) equipped with GC column NB-1 (30 cm x 0.25 mm fused silica) was employed.

RESULTS AND DISCUSSION

While studying PL metabolism in isolated rat hepatocyte systems using HPLC, a peak derived from an unknown metabolite of PL was detected at a retention time of 52 min, just after the peak of PGL at 48 min. It was assumed to be a peak of acetyl conjugate of NDP, because it was derived from the incubation medium of isolated rat hepatocytes using not only PL but also NDP as a substrate, while the peak could not be detected from that of hepatic microsomal fraction. Thus, we synthesized AcNDP as an authentic sample and ascertained the metabolite as AcNDP using LC/MS. Furthermore, the new metabolite AcNDP was detected from the urinary extracts of a patient and a healthy volunteer given PL using LC/MS and GC/MS, respectively. The MS charts are shown in Fig. 1.

The formation amount of AcNDP from (R,S)-, R- or S-PL in isolated rat hepatocyte systems was estimated by HPLC. Although the amounts of PL and the other 8 metabolites indicated each retention time above were determined, the data of PL, NDP and a new metabolite AcNDP are summarized in Table I. The other data will be soon reported as a full paper.

According to Suzuki et al., major metabolites of PL in rat microsomal fractions are 4-, 5-, 7-OH-PL and NDP.
Fig. 1. FAB-MS Charts from LC/MS (a,b) and EL-MS Charts from GC/MS (c,d) of AcNDP and PL Metabolite
Authentic AcNDP(a,c), PL Metabolite obtained from the urinary extract of a patient(b) or a volunteer(d) given PL.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Isomeric forms</th>
<th>Concentration of PL metabolites (µM)</th>
<th>AcNDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>RS</td>
<td>467.7</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>415.1</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>480.3</td>
<td>9.9</td>
</tr>
<tr>
<td>PB</td>
<td>RS</td>
<td>350.0</td>
<td>35.1</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>315.5</td>
<td>53.2</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>383.2</td>
<td>32.0</td>
</tr>
<tr>
<td>3-MC</td>
<td>RS</td>
<td>290.7</td>
<td>58.8</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>174.2</td>
<td>62.5</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>367.3</td>
<td>18.4</td>
</tr>
</tbody>
</table>

Each value is expressed as the mean of three samples.

However, in isolated rat hepatocyte systems, NDP is a main metabolite of (RS)−, R− and S− PL. In general R− PL was eliminated more rapidly than S− isomer, and the amount of NDP derived from R− PL is larger than that from S−PL. Thus the rate of (RS)−, R− or S− PL elimination is dependent on that of successive formation of NDP.

Furthermore, the effect of PB or 3-MC pretreatment on PL metabolism was examined using isolated rat hepatocytes pretreated with PB or 3-MC. As shown in Table I, the hepatocytes pretreated by 3-MC and PB indicated some remarkable changes both in PL elimination and in NDP formation in comparison with the intact cells. However, in the case of acetyl conjugation of NDP, the formation of AcNDP is independent of PB- and 3-MC pretreatment, since the formation amounts of AcNDP are 2 - 5 % of those of NDP in all cases.

Then the excreted amount of AcNDP in the 24 hr urine of a healthy volunteer was determined by HPLC. About 3 % of dose was excreted in the urine and 67 % of AcNDP was detected in 10 hr urine.

Thanks to in vivo evaluation of drug metabolism using isolated rat hepatocytes, AcNDP was detected as a new metabolite of PL. It is an important and valuable observation for PL therapy from the viewpoint of acetylator phenotype and drug interaction, since the amounts of AcNDP derived from PL is significant. Further data containing other metabolites will be reported as a full paper in the near future.

REFERENCES

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