Estimation of Chemical Structure of Notopterol Metabolites

Atsushi HASEGAWA, a, b Hiroyoshi NAKAMURA, a Toshiko WATANABE, b Emi OKUYAMA, b Shigeru OIMORI, a Tsutomu ISHIKAWA, a and Mitsukazu KITADA a

Division of Pharmacy, Chiba University Hospital, a 1–8–1 Inohana, Chuo-ku, Chiba 260–8677, Japan and Faculty of Pharmaceutical Science, Chiba University, b 1–33 Yayoicho, Inage-ku, Chiba 263–8522, Japan.
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Notopterol, 5(1E,5)-hydroxy-3,7-dimethyl-2,6-octadienylxylporalen showed an inhibitory effect on aminopyrine N-demethylase activity in liver microsomes. In addition, notopterol has been found to be metabolized by cytochrome P450, and two kinds of metabolites were formed from notopterol. Furthermore, specific cytochrome P450 3A4 inhibitors which were isolated from grapefruit juice had the same furanocoumarin structure as notopterol. Two metabolites of notopterol were separated by HPLC, and the chemical structures of the hydroxylated metabolites were estimated by 1H-NMR spectra and liquid chromatography–mass spectrometry.

Key words notopterol; furanocoumarin structure; grapefruit juice; 1H-NMR; cytochrome P450

It has been reported that notopterol is one of the major components of Kyokhatsu, belonging to the Umbelliferae family.1) Notopterol has anti-inflammatory activity, and has also been demonstrated to prolong pentobarbital-induced hypnosis.2) Besides, notopterol has shown an inhibitory effect on aminopyrine N-demethylation activity in liver microsomes comparable to that observed with SKF-525A. It is, therefore, likely that the prolongation of pentobarbital sleeping time caused by notopterol is due to the inhibition of pentobarbital metabolism in vivo.2) In our preliminary experiments, notopterol was found to be metabolized to at least two kinds of compounds by cytochrome P450 in rat liver microsomes. However, the chemical structure of these metabolites remains to be elucidated. Furthermore, it is also unclear whether the inhibitory effect of notopterol on cytochrome P450 catalytic activity is due to the effects of notopterol itself and/or its metabolites. Therefore, in the present study, we estimated the chemical structure of two notopterol metabolites.

MATERIALS AND METHODS

Preparation of Notopterol Notopterol was isolated from Tou-kyoikatsu as previously described.2)

Preparation of Rat Liver Microsomes Sodium phenobarbital dissolved in saline (80 mg/kg, i.p.) was administered to male Sprague Dawley rats (8 weeks old) once a day for 3 d. The rats were deprived of food for 20 h after the final administration, and after that time were used in this study. Microsomes were prepared by differential centrifugations as previously described.4) After the concentration of protein was determined according to the method of Lowery et al.,5) the microsomes were frozen and kept at -80 °C until use.

Preparation of Notopterol Metabolites A reaction mixture consisted of 0.1 M potassium phosphate buffer (pH 7.4), 0.1 mM EDTA, an NADPH-generating system (0.33 mM NADP, 8 mM glucose 6-phosphate, 0.1 unit of glucose 6-phosphate dehydrogenase and 6 mM MgCl2), liver microsomes of phenobarbital pretreated rats (30.02 mg/ml), and 10 mM notopterol in a final volume of 100 ml. After the reaction mixture was incubated at 37 °C for 2 h, the reaction was terminated by the addition of ethyl acetate (300 ml), and metabolites were extracted. Then, the ethyl acetate extract was evaporated to dryness under reduced pressure at room temperature. The residue was dissolved in isopropyl alcohol, and the solution was used for HPLC analysis, as described below.

HPLC Systems HPLC analysis was performed with a Senshu pack SSC-silica 1251-N (4.6×250 mm Senshu Kagaku Co., Ltd., Japan). A Hitachi Model L-6000 pump was used for the analysis. Metabolites were detected by the monitoring of absorbance at 240 nm using a Hitachi Model L-4000 variable wavelength UV detector at room temperature. The mobile phase consisted of hexane (67%), dichloromethane (30%), and ethanol (3%). The flow rate was set at 2.30 ml/min.

The isolated metabolites were subjected to the measurement of 1H-NMR spectra, which were recorded on a JEOL Model JNM-GSX 500A spectrometer (Tokyo, Japan). The sample was dissolved in CDCl3 with tetramethylsilane as an internal standard. Atmospheric pressure chemical ionization-liquid chromatography/tandem mass spectrometry (APCI-LC/MS) were carried out using the negative ion mode with ThermoQuest Co., LCQ ion trap-type LC/MS.

RESULTS AND DISCUSSION

A typical HPLC chromatogram is shown in Fig. 1. The chromatograms showed mainly two metabolites, M1 (0.6%) and M2 (1.3%), as the retention times were 10.3 and 15.5 min, respectively. Each peak fraction was pooled separately, and was subjected to 1H-NMR analysis. Figure 2 showed the chemical structure estimated for the two metabolites. (Detailed chemical shifts are shown in references.1,6–8) Chemical shifts of the methyl groups at the position of C-8', C-9' and C-10' of notopterol were observed at 1.72, 1.77 and 1.70 ppm, respectively. On the other hand, 1H-NMR spectra of M1 and M2 showed that both metabolites are compounds having only two methyl groups. Signals for C-10' of M1 and C-8' of M2 were identified at about 4.03–4.22 ppm, indicating that biotransformation at the positions of C-10' and C-8' of notopterol are responsible for the formation of M1 and M2, respectively. Furthermore, judging from the H-6' chemical shift observed against the methylene proton of M1 and M2, both M1 and M2 were determined to be geometric isomers. The APCI/MS (negative mode) spectrum of M1 and M2 showed m/z 369 [M–H]−. From these results, we presumed

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inhibitors, have been isolated from grapefruit juice.\textsuperscript{25} Interestingly, these compounds have the same furoucoumarin structure as notopterol. Two of these furocoumarins in grapefruit juice showed a strong inhibition of cytochrome P450 3A activity \textit{in vitro}. These results may also imply that we must fully consider the drug interaction between herbal medicines containing the components of the furocoumarin structure. The inhibitory effect of M1 and M2 on cytochrome P450 is now under investigation.

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\textbf{REFERENCES AND NOTES}

6) \textsuperscript{1}H-NMR of notopterol (CDCl\textsubscript{3}) \(\delta: 8.15 \ (1 \ H, d, J=0.5, 9.8 \ Hz, H-4), 7.60 \ (1 \ H, d, J=2.5 \ Hz, H-10), 7.17 \ (1 \ H, s, H-8), 6.96 \ (1 \ H, dd, J=1.0, 2.2 \ Hz, H-9), 6.28 \ (1 \ H, d, J=9.8 \ Hz, H-3), 5.65 \ (1 \ H, dt-like, J=1.2, 6.7 \ Hz, H-2'), 5.18 \ (1 \ H, dt-like, J=3.1, 8.6 \ Hz, H-6'), 4.98 \ (2 \ H, d, J=6.8 \ Hz, H-1'), 4.51 \ (1 \ H, dt, J=5.0, 8.4 \ Hz, H-5'), 2.30 \ (1 \ H, dd, J=8.6, 13.7 \ Hz, H-4'), 2.22 \ (1 \ H, dd, J=4.6, 13.7 \ Hz, H-4'), 1.77 \ (3 \ H, s, H-9'), 1.72 \ (3 \ H, d, J=1.2 \ Hz, H-8'), 1.70 \ (3 \ H, d, J=1.2 \ Hz, H-10').
7) \textsuperscript{1}H-NMR of M1 (CDCl\textsubscript{3}) \(\delta: 8.16 \ (1 \ H, d, J=10.0 \ Hz, H-4), 7.61 \ (1 \ H, d, J=2.4 \ Hz, H-10), 7.18 \ (1 \ H, s, H-8), 6.95 \ (1 \ H, m, H-9), 6.29 \ (1 \ H, dd, J=9.8 \ Hz, H-3), 5.66 \ (1 \ H, m, H-2'), 5.35 \ (1 \ H, m, H-6'), 4.97 \ (2 \ H, d, J=6.1 \ Hz, H-1'), 4.60 \ (1 \ H, m, H-5'), 4.22 \ (1 \ H, dd, J=12.4 \ Hz, H-10'), 4.11 \ (1 \ H, dd, J=12.2 \ Hz, H-10'), 2.33 \ (1 \ H, dd, J=1.1, 8.8 \ Hz, H-4'), 2.27 \ (1 \ H, dd, J=1.1, 4.8 \ Hz, H-4'), 1.82 \ (3 \ H, s, H-8'), 1.77 \ (3 \ H, s, H-9').
8) \textsuperscript{1}H-NMR of M2 (CDCl\textsubscript{3}) \(\delta: 8.16 \ (1 \ H, d, J=9.8 \ Hz, H-4), 7.61 \ (1 \ H, d, J=2.2 \ Hz, H-10), 7.18 \ (1 \ H, s, H-8), 6.96 \ (1 \ H, m, H-9), 6.29 \ (1 \ H, d, J=9.8 \ Hz, H-3), 5.67 \ (1 \ H, m, H-2'), 5.48 \ (1 \ H, d, d-like, J=10.0 \ Hz, H-6'), 4.98 \ (2 \ H, d, J=7.1 \ Hz, H-1'), 4.58 \ (1 \ H, m, H-5'), 4.03 \ (2 \ H, s, H-8'), 2.32 \ (1 \ H, d, J=8.6 \ Hz, H-4'), 2.27 \ (1 \ H, d, J=4.9 \ Hz, H-4'), 1.78 \ (3 \ H, s, H-9'), 1.73 \ (3 \ H, s, H-10').