XENOBIOTICA, 2000, VOL. 30, NO. 6, 643–653

Metabolism of a novel hypnotic, N^3-phenacyluridine, and hypnotic and sedative activities of its enantiomer metabolites in mouse

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Received 19 November 1999

1. The metabolism of N^3-phenacyluridine (3-phenacyl-1-β-D-ribofuranosyluracil), a potent hypnotic nucleoside derivative, was studied in mouse.

2. Of the radioactivity, 65% was excreted in urine within 48 h after intraperitoneal (i.p.) administration of [^{3}H]N^3-phenacyluridine. The urinary metabolites N^3-phenacyluracil and N^3-α-hydroxy-β-phenethyluridine were extracted, isolated and analyzed by mass spectrometry.

3. Racemates of N^3-α-hydroxy-β-phenethyluridine were synthesized and both isomers were separated as N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine and N^3-(*R*)-(−)-α-hydroxy-β-phenethyluridine by hplc (CHIRALCEL-OJ column) with retentions of 13.8 and 17.9 min respectively. The reduction process took place with high stereo-selectivity, which gave an alcohol product in the urine with the same retention (17.9 min) as one of the synthetic isomers separated by hplc.

4. One of urinary metabolites was identified as N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine. N^3-phenacyluridine was predominantly converted to an alcoholic metabolite of (*S*)-(+)-configuration.

5. N^3-phenacyluracil and uridine were also identified as minor metabolites.

6. The pharmacological effects of the metabolites and related compounds were also evaluated in mouse. N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine, but not N^3-(*R*)-(−)-α-hydroxy-β-phenethyluridine, possessed hypnotic activity and potentiated pentobarbital-induced sleeping time with a similar potency to the parent compound, N^3-phenacyluridine. N^3-α-hydroxy-β-phenethyluridine (racemate) had almost two thirds of the hypnotic activity of N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine. No other metabolites exhibited hypnotic activities.

7. The present study indicates that N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine, a major metabolite of N^3-phenacyluridine, is an active metabolite and contributes a significant CNS depressant effect.

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Introduction

Uridine possesses central nervous system (CNS) depressant effects such as decreasing spontaneous activity in the mouse (Krooth *et al*. 1978) and protection of metrazol- and penicillin-induced seizures in frog (Roberts 1973). In addition, uridine was isolated as a sleep-promoting substance from 24 h sleep-deprived brainstems and promoted natural sleep by intracerebroventricular (i.c.v.) infusion in rat (Komoda *et al*. 1983, Honda *et al*. 1984, Inoue *et al*. 1984). However, unlike barbiturates and benzodiazepines, uridine does not possess any hypnotic action. Reported for the first time was that the N^3-benzyl substituted uridine possessed

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Xenobiotica ISSN 0049–8254 print/ISSN 1366–5928 online © 2000 Taylor &
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hypnotic activity when the compound is administered by i.c.v. injection to mouse at 2.0 μmol/mouse (Yamamoto *et al*. 1985). N^3-benzyluridine, but not its metabolites, possessed hypnotic activity (Kimura *et al*. 1991). In addition, it was found that N^3-phenacyluridine is the most potent hypnotic agent in mouse (i.c.v. injection) among all the oxopyrimidine nucleoside derivatives synthesized to date (Yamamoto *et al*. 1986, 1987a,b, 1990, 1994, Koshigami *et al*. 1991, Kimura *et al*. 1993, 1996). However, the metabolite of N^3-phenacyluridine is different from that of N^3-benzyluridine because it has a ketone moiety in the N^3-substituent group. It is known that certain drugs having a ketone moiety are reduced to the corresponding hydroxy group by reductases in the cytosol to produce the active metabolite. Therefore, it is anticipated that N^3-phenacyluridine is also metabolized by reduction to form an alcohol product. Acetohexamide is widely used as an orally antidiabetic drug and has a ketone group in the structure. In man, acetohexamide was reported to be mainly biotransformed to (−)-hydroxyhexamide, which is a pharmacologically active metabolite (Imamura *et al*. 1985). Furthermore, McMahon *et al*. (1965) reported that (−)-hydroxyhexamide is 2.4 times as potent as acetohexamide with respect to its pharmacological effect. Therefore, in the present study, the metabolites of N^3-phenacyluridine were identified and their pharmacological effects on the CNS evaluated in mouse.

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Materials and methods

Materials

[^{3}H]uridine (42.7 Ci/mmol, 97.8% purity) was from Daiichi Pure Chemical Co. Ltd/NEN (Tokyo, Japan) and other synthetic reagents were from Wako Pure Chemical Co. Ltd (Osaka, Japan). Male ddY mice (body weight 23–28 g) were from the Sankyo Laboratory Co. Ltd (Toyama, Japan).

Preparation of N^3*-phenacyluridine and* [^3H]N^3*-phenacyluridine*

N^3-phenacyluridine and [^{3}H]N^3-phenacyluridine were prepared according to Yamamoto *et al*. (1994) from uridine and [^{3}H]uridine respectively. [^{3}H]N^3-phenacyluridine was developed on thin layer chromatography (tlc) (solvent chloroform/methanol, 9/1 v/v), and the radioactivity of the spot was measured by a Radiochromanizer (Aloka, Japan). The purity of [^{3}H]N^3-phenacyluridine was $>98\%$.

Preparation of N^3*-phenacyluracil*

N^3-phenacyluracil was prepared by the method of Kimura *et al*. (1991) with slight modifications. Sodium hydride (60% purity), 1.07 g (120 mmol), was washed with petroleum ether and dissolved in 133 ml *N*,*N*-dimethylformamide (DMF). Uracil 7.5 g (67 mmol) was added to the mixture and stirred at room temperature for 30 min. Chloromethylether, 7.5 g (94 mmol), was added drop-wise to the mixture and stirred at room temperature for 90 min. After the reaction was completed, the solvent was evaporated to dryness and the residue applied to a silica gel column and eluted with chloroform/isopropanol (9/1 v/v) as the solvent. N^1-methoxymethyluracil was isolated and purified from the reaction mixture. The N^1-methoxymethyluracil obtained was recrystallized from chloroform and ligroin to yield 2.79 g (27.2%) of the compound with a m.p. = 153–156 °C, ^{1}H-nmr ($CDCl_3$) δ: 3.20 (3H, s, OCH_3), 5.13 (2H, s, NCH_2), 5.79 (1H, d, $\mathcal{J}$ = 8 Hz, 5-H), 7.32 (1H, d, $\mathcal{J}$ = 8 Hz, 6-H), 8.91 (1H, broad s, NH), MS, m/z = 156 [M^+]. N^1-methoxymethyl-N^3-phenacyluracil was prepared by N^3-phenacylation of N^1-methoxymethyluracil according to Kimura *et al*. (1991). The compound yielded 30% as an oil with ^{1}H-nmr ($CDCl_3$) δ: 3.30 (3H, s, OCH_3), 5.15 (2H, s, NCH_2), 5.34 (2H, s, NCH_2-CO), 5.75 (1H, d, $\mathcal{J}$ = 8 Hz, 5-H), 7.30–8.08 (6H, m, 6-H, C_6H_5), MS, m/z = 274 [M^+]. N^3-phenacyluracil was then from N^1-methoxymethyl-N^3-phenacyluracil by deprotection at N^1 position. N^1-methoxymethyl-N^3-phenacyluracil 210 mg (0.77 mmol) was refluxed in a mixture of 7 ml methanol, 8 ml HCl, 8 ml H_2SO_4 and 3 ml water at 100 °C for 24 h. The reaction mixture was neutralized with 0.1 N sodium hydroxide, extracted with chloroform, and the solvent evaporated followed by purification by a silica gel column chromatography with chloroform/isopropanol (9/1 v/v) as solvent. Finally, N^3-phenacyluracil was recrystallized from water to yield 110 mg (62.1%) of the compound with a m.p. = 195–199 °C, ^{1}H-nmr ($CDCl_3$) δ: 5.20 (2H, s, NCH_2), 5.69 (1H, d, $\mathcal{J}$ = 8 Hz, 5-H), 7.25–8.10 (6H, m, 6-H, C_6H_5). MS, m/z = 230 [M^+].

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Synthesis of N^3*-α-hydroxy-β-phenethyluridine (racemate)*

N^3-phenacyluridine, 362 mg (1 mmol), was dissolved in anhydrous methanol and sodium borohydride, 47 mg (1 mmol), was added. The reaction mixture was stirred at room temperature for 30 min, followed by evaporation. The residue was applied to Amberlite XAD-2 and eluted with 250 ml ethanol after washing with 100 ml water. N^3-α-hydroxy-β-phenethyluridine was separated by silica gel column chromatography with chloroform/methanol (9/1 v/v). N^3-α-hydroxy-β-phenethyluridine was recrystallized from acetone and *n*-hexane, yielding 60.4 % of the compound. M.p. = 143–147 °C, ^{1}H-nmr (DMSO-d_6) δ: 3.22–3.63 (2H, m, 5′-H_2), 3.66–3.80 (1H, m, 4′-H), 3.90–4.12 (3H, m, 2′-H, 3′-H, -CH-), 5.07–5.81 (3H, m, NCH_2, 1′-H), 7.24–7.41 (5H, m, C_6H_5), 8.14–8.16 (1H, d, J = 8 Hz, 6-H). FAB-MS (magic bullet) m/z = 365 $[M+H]^+$.

Hplc separation of the racemic metabolites, N^3*-(R)-(−)-α-hydroxy-β-phenethyluridine and* N^3*-(S)-(+)-α-hydroxy-β-phenethyluridine*

Racemic mixtures of N^3-α-hydroxy-β-phenethyluridine (racemate) were separated by hplc (Hitachi 655–21, Japan) using a CHIRALCEL-OJ column (0.46 mm × 25 cm, Daisel Chemical Co. Ltd, Japan), eluent: *n*-hexane/isopropanol (7/3 v/v), flow rate = 1.0 ml/min, and detection at 254 nm.

Administration of N^3*-phenacyluridine and* $[^3H]N^3$*-phenacyluridine to mouse*

Unlabelled N^3-phenacyluridine (1.0 mmol/kg) or $[^3H]N^3$-phenacyluridine (1.0 mmol/kg, 1.7 MBq/kg) was suspended in 1% Tween-80 saline solution and administered by intraperitoneal (i.p.) injection to the male ddY mouse (weight 23–28 g). Each group consisted of three mice.

Excretion of $[^3H]N^3$*-phenacyluridine to urine and faeces*

Urine and faeces were collected 1, 3, 6, 12, 24, 48 h after the i.p. administration of $[^3H]N^3$-phenacyluridine to mouse. An aliquot of urine was added to Clear-sol I (Nacalai-tesque, Japan) and radioactivity was counted by a liquid scintillation counter (Aloka LSC-3000, Japan). An aliquot of faeces was subjected to Automatic Sample Combustion System (Aloka, Japan). The recovered radioactivity from the faeces was counted by a liquid scintillation counter.

Determination of metabolites

A 24-h urine sample from mouse was collected after the administration of $[^3H]N^3$-phenacyluridine. Of urine, 40 ml was pretreated with Bond Elut (Si) (Varian, CA, USA) with methanol as eluant, and was subsequently stirred with 40 ml Amberlite XAD-2 for 2 h. The residue was applied to a column, eluted with 250 ml methanol after washing with 100 ml water, and each fraction was evaporated and dissolved in ethanol. The fraction was developed on tlc (SIL-G25, Macherey-Nagel, Germany) (solvent chloroform/methanol/acetic acid/water, 80/20/6/4 v/v/v/v). The scraped band was added to Clear-sol I, and radioactivity was counted by a liquid scintillation counter. After administration of noneradioactive N^3-phenacyluridine, each tlc extract was analyzed by mass spectrometry for the determination of the metabolites. Twenty-four-hour urine samples from mouse were collected after the administration and were handled in a similar manner as mentioned above. The metabolite fraction was analyzed by hplc, optical rotation and FAB-MS. Hplc was carried out using a Hitachi hplc apparatus equipped with a CHIRALCEL-OJ column and a Hitachi UV monitor (254 nm). *n*-Hexane/isopropanol (7/3 v/v) was employed as a mobile phase at 1.0 ml/min. The optical rotation and FAB-MS were performed by using Nippon-bunko DIP-370 (Tokyo, Japan) and JEOL JMX-DX-300 instruments (Tokyo, Japan) respectively.

Hypnotic activity

Compounds tested (2.0 μmol/mouse) were injected i.c.v. to the male ddY mouse (body weight 23–28 g) according to Haley and McCormick (1957). Hypnotic activity was expressed as sleeping time (min) and was considered as the time between the loss of righting reflex of animals and the time they righted themselves. Each group had eight mice.

Pentobarbital-induced sleep prolongation

Compounds tested (0.5 μmol/mouse) were injected (i.c.v.) in the animals and pentobarbital (40 mg/kg) was injected (i.p.) 15 min after the i.c.v. administration of the tested compound. Sleeping time was measured as the time between the loss and recovery of righting reflex in the mouse, and the data were expressed as percentage of that of the controls. Each group had eight mice. Statistical significance of difference was calculated using Bonferroni test.

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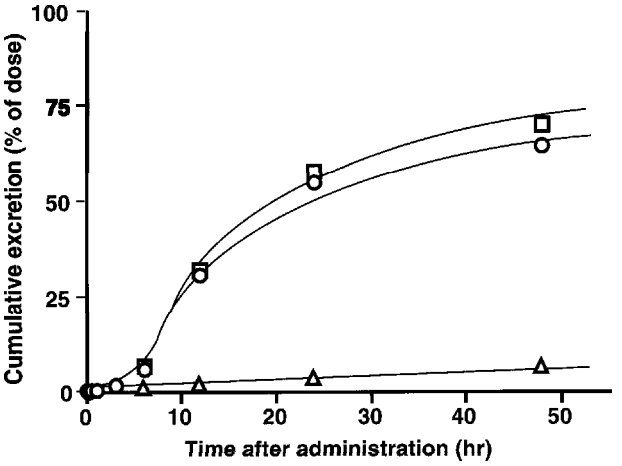

Figure 1. Excretion of radioactivity after the i.p. administration of [^{3}H]N^3-phenacyluridine to mouse. [^{3}H]N^3-phenacyluridine, 1.0 mmol/kg, was administered by i.p. injection. Total, □, urine, ○, faeces, △. Data are the average of three mice.

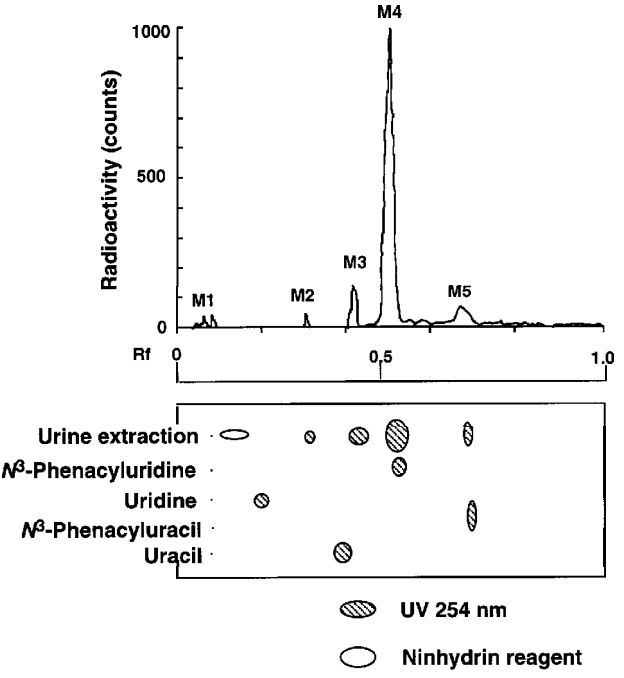

Figure 2. Analysis of [^{3}H]N^3-phenacyluridine and its urinary metabolites by radiochromanyzer analysis. Urinary extracts were developed on tlc (solvent chloroform/methanol/acetic acid/water, 80/20/6/4 v/v/v/v), and the radioactivity of the fractions were counted by liquid scintillation spectrometry.

Results

N^3-phenacyluridine and its metabolites were mainly excreted in the urine and by 48 h after [^{3}H]N^3-phenacyluridine administration (1.0 mmol/kg, i.p.), 65 and 7% of the radioactivity administered to mouse was found in the urine and faeces respectively (figure 1).

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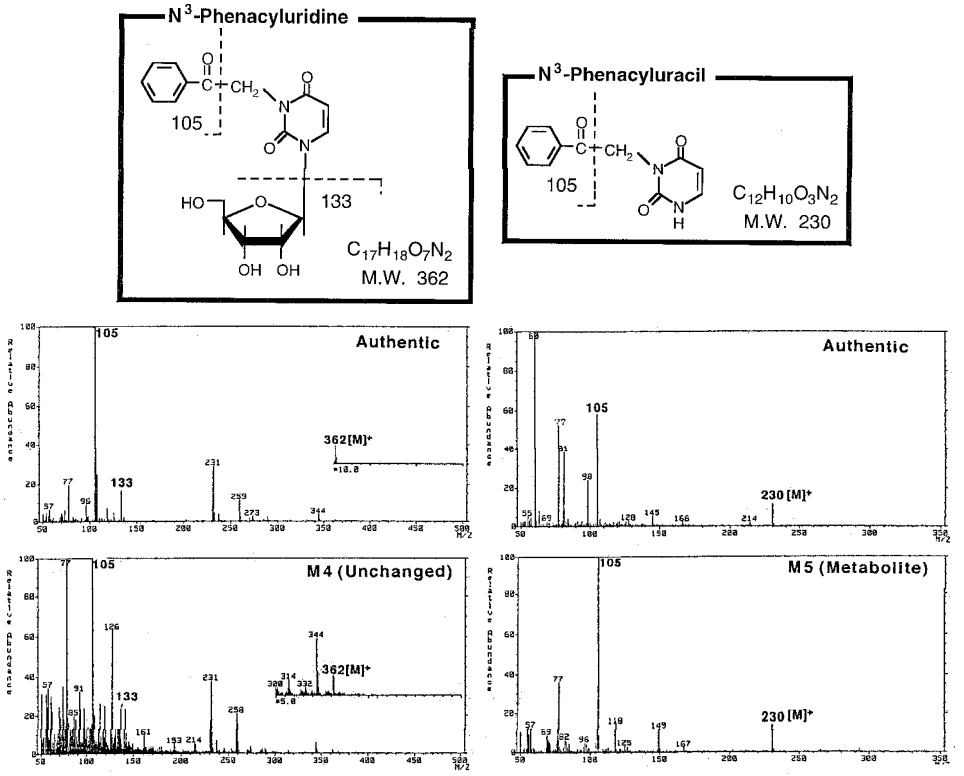

Figure 3. Mass spectra of M4 and M5 fractions after the i.p. administration of N^3-phenacyluridine to mouse.

The extracted sample from urine was developed on tlc and the radioactivity was analyzed. The amount of radioactivity in fractions of M1–5 were 13.0, 8.3, 11.6, 51.5 and 15.6% of that excreted in the urine respectively (figure 2). Each fraction was also extracted and the metabolite structures identified by mass spectrometry and compared with that of authentic samples.

Figure 3 shows mass spectra of M4 and M5 and the corresponding authentic samples. Since the mass spectra of M4 and M5 indicated molecular weights of 362 (m/z = 362 $[M]^+$) and 230 (m/z = 230$[M]^+$) respectively, the fractions were identified as N^3-phenacyluridine and the corresponding deribosylated compound, N^3-phenacyluracil respectively. The mass spectrum (FAB mass, magic bullet: Na) of M3 showed ions at 387 $[M+Na]^+$ and 365 (m/z = 364 $[M+H]^+$). The mass fragment of m/z = 107 was confirmed, whereas m/z = 105, derived from the phenacyl moiety disappeared, suggesting metabolic alteration of the phenacyl moiety (figure 4).

Additional data were also from authentic samples. The mass spectrum of M3 was compared with that of an authentic sample, indicating the reduction of the ketone moiety to an alcohol in the phenacyl group of N^3-phenacyluridine.

Figure 5 shows the urinary excretion of N^3-phenacyluridine, N^3-α-hydroxy-β-phenethyluridine and N^3-phenacyluracil. By 48 h after administration, 40% of N^3-phenacyluridine remained unchanged, whereas N^3-α-hydroxy-β-phenethyluridine and N^3-phenacyluracil accounted for 9 and 11% of the total amount of urinary excretion respectively.

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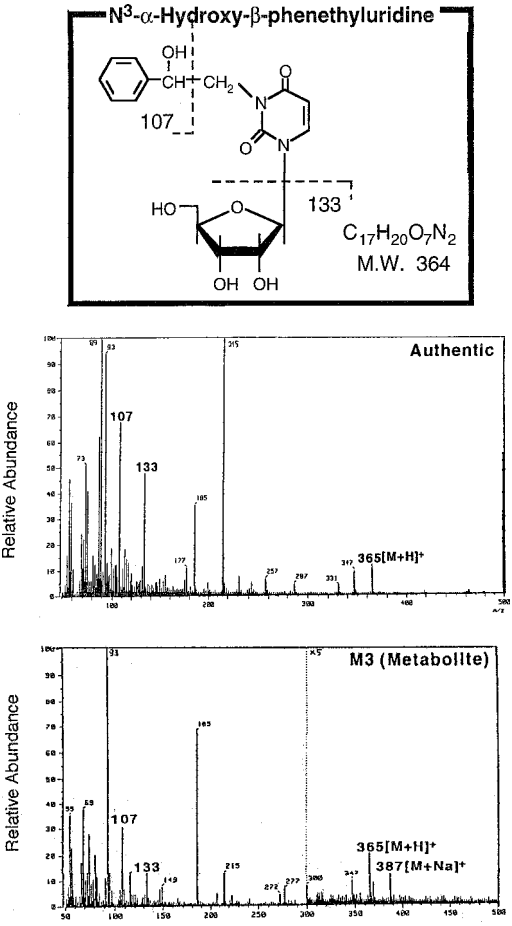

Figure 4. Mass spectra of the M3 fraction after the i.p. administration of N^3-phenacyluridine to mouse.

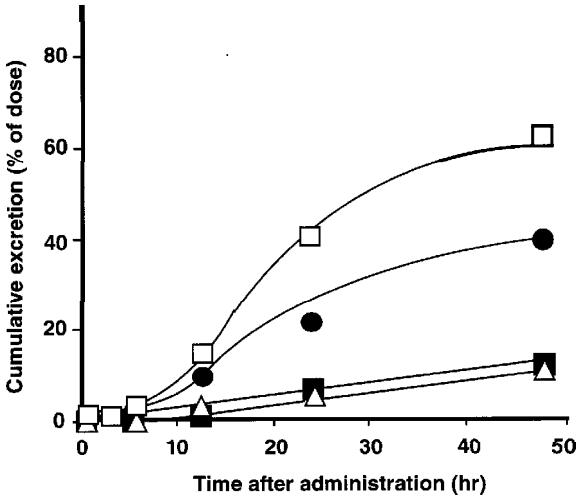

Figure 5. Urinary excretion of metabolites after the i.p. administration of [^{3}H]N^3-phenacyluridine. [^{3}H]N^3-phenacyluridine, 1.0 mmol/kg, was administered by i.p. injection. Total, □; N^3-phenacyluridine, ●; N^3-(*S*)-(+)-α-hydroxy-*β*-phenethyluridine, △; N^3-phenacyluracil, ■.

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Table 1. Analytical data for the (*R*)- and (*S*)-enantiomers of N^3-α-hydroxy-β-phenethyluridine.

Compound	Hplc (min	Optical rotation $[\alpha]_D$	m.p. (°C)	FAB-MS (*m/z*)
N^3-α-hydroxy-β-phenethyluridine (racemate)	13·8, 17·9	+2·9	143–147	107 ($C_6H_5CH(OH)$) 365 ($[M+H]^+$) 387 ($[M+Na]^+$)
N^3-(*R*)-(−)-α-hydroxy-β-phenethyluridine	13·8	−82·5	145–147	107 ($C_6H_5CH(OH)$) 365 ($[M+H]^+$)
N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine	17·9	+81·3	145–148	107 ($C_6H_5CH(OH)$) 365 ($[M+H]^+$) 387 ($[M+Na]^+$)
Metabolite	17·9	+83·9	144–146	107 ($C_6H_5CH(OH)$) 365 ($[M+H]^+$) 387 ($[M+Na]^+$)

Compounds were injected onto an hplc (Hitachi 655-21, Japan) using a CHIRALCEL-OJ column (0·46 mm × 25 cm; Daisel Chemical Co. Ltd, Japan). Eluent: *n*-hexane/isopropanol (7/3 v/v). Flow rate = 1·0 ml/min. Detection = UV 254 nm.

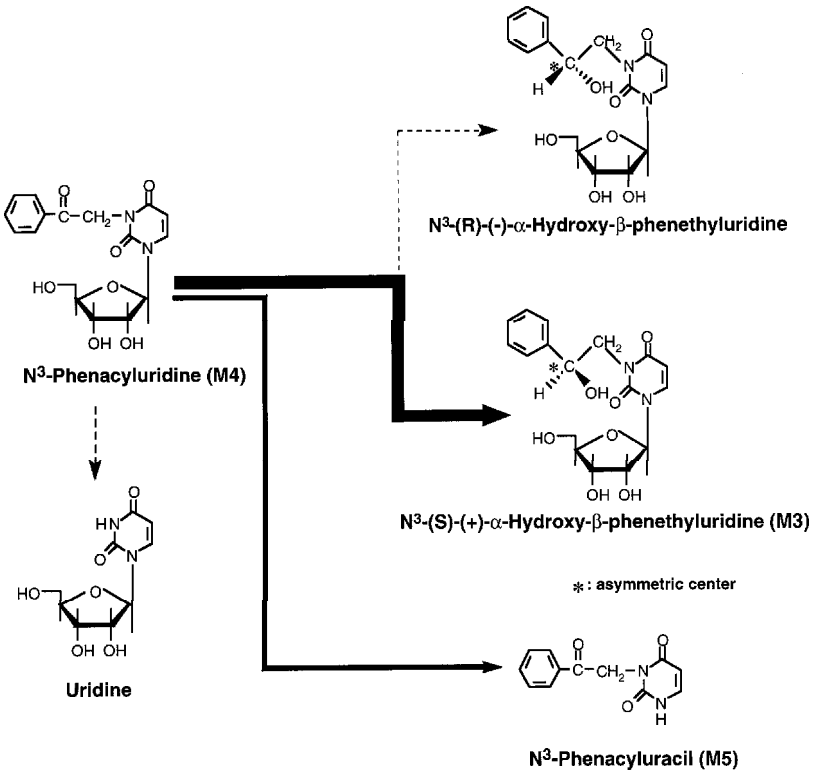

Figure 6. Proposed metabolic pathways of N^3-phenacyluridine in mouse.

N^3-α-hydroxy-β-phenethyluridine theoretically has either the (*R*)- or (*S*)-enantiomer configuration, derived from the asymmetric carbon of the phenacyl moiety. These isomers were successfully separated from the synthetic racemates by hplc using a CHIRACEL-OJ column, with retentions of 13.8 (PI) and 17.9 min (PII) for the (*R*)- and (*S*)-enantiomers respectively (table 1).

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Table 2. Hypnotic activities of N^3-phenacyluridine, N^3-(*R*)-(−)- and N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine, and related compounds in mouse.

Compound	Hypnotic activity (min)	Pentobarbital-induced sleep (% of control)
N^3-phenacyluridine	487±47	423±31*
N^3-α-hydroxy-β-phenethyluridine (racemate)	279±24	447±26*
N^3-(*R*)-(−)-α-hydroxy-β-phenylethyluridine	27±7	259±54*
N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine	409±21	690±47*
N^3-phenacyluracil	none	105±12
Uridine	none	128±26
Uracil	none	136±21
D-Ribose	none	94±9

Compounds were administered i.c.v to the male ddY mouse (body weight 23–28 g) at 2·0 μmol/mouse. Hypnotic activity was evaluated as the time between loss and recovery of the mouse righting reflex. 'none' indicates that the compound did not show hypnotic activity. Pentobarbital (40 mg/kg, i.p.) was injected 15 min after the i.c.v. injection of the compound tested at 0·5 μmol/mouse. Data are the mean % of control sleeping time (53±9 min)±SEM, where $n = 8$.

* Significantly different from control ($P < 0.01$).

The analytical data for both enantiomers were compared with that of (*R*)- or (*S*)-*sec*-phenethylalcohol. The optical rotations of (*R*)- and (*S*)-*sec*-phenethylalcohol are +73.9 and −78.7 respectively. The (*R*)- and (*S*)-*sec*-phenethylalcohol were injected to hplc using the CHIRALCEL-OJ column, showing retentions of 35.1 and 31.8 min respectively. According to the Baumann and Prelog rule (1958), (*R*)- and (*S*)-*sec*-phenethylalcohol correspond to N^3-(*S*)-(+)- and N^3-(*R*)-(−)-α-hydroxy-β-phenethyluridine respectively. For N^3-α-hydroxy-β-phenethyluridine, the optical rotation of the racemate is +2.9, whereas compounds separated hplc (PI and PII) show −82.5 and +81.3 for the optical rotations respectively. One of the urinary metabolites showed a retention of 17.9 min on hplc and the optical rotation of +83.9. Furthermore, the data from measurement of m.p., FAB-MS and ^{1}H-nmr (data not shown) support the view that this metabolite is N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine (figure 6).

M1 and M2 have not been identified to date because of their trace amounts in urine. Since M1 showed a positive reaction with the ninhydrin reagent (data not shown), ring opening to an amino acid derivative may be possible.

The pharmacological properties of N^3-phenacyluridine, N^3-α-hydroxy-β-phenethyluridine (racemate) and the enantiomers were evaluated by their hypnotic activity and synergistic effects with pentobarbital in mouse.

As shown table 2, in mouse receiving an i.c.v. injection of 2.0 μmol/mouse N^3-phenacyluridine, the sleeping time was 487 min. The same dose of N^3-α-hydroxy-β-phenethyluridine (racemate) induced a sleeping time of 279 min, suggesting that there is an active metabolite of N^3-phenacyluridine. In the case of N^3-(*R*)-(−)-α-hydroxy-β-phenethyluridine and N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine, N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine induced a sleeping time of 409 min, whereas N^3-(*R*)-(−)-α-hydroxy-β-phenethyluridine only resulted in a sleeping time of 27 min at the same dose. With respect to the effects on the pentobarbital-induced sleeping time, N^3-phenacyluridine, N^3-α-hydroxy-β-phenethyluridine (racemate), N^3-(*R*)-(−)- and N^3-(*S*)-(+)-enantiomers significantly prolonged to 423, 447, 259 and 690% of the vehicle controlled group (53±9 min) respectively. N^3-phenacyluracil, uridine, uracil, and D-ribose did not show any significant changes in the pentobarbital-induced sleeping time.

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Discussion

In a previous study, N^3-benzyluridine was biotransformed to N^3-benzyluracil and trace amounts of uridine in mouse (Kimura *et al*. 1991). Since these metabolites do not show any CNS depressant effects in mouse, the parent compound, N^3-benzyluridine, but not the metabolites possessed a hypnotic activity. In contrast, the structure of N^3-phenacyluridine differs from that of N^3-benzyluridine in that there is a carbonyl group in the phenacyl moiety. In the current study, urinary metabolites were analyzed by mass spectrometry and compared with authentic samples. M4 was identified as unchanged compound, N^3-phenacyluridine. Since M5 showed a molecular weight of 230 (m/z = 230 $[M]^+$), it was identified as N^3-phenacyluracil, a deribosylated product of N^3-phenacyluridine similar to that previously reported for N^3-benzyluridine (Kimura *et al*. 1991). R_f of M3 was smaller than that of N^3-phenacyluridine, suggesting a polar metabolite. The mass spectrum (FAB-MS, magic bullet: Na) of M3 showed ions at 387 $[M+Na]^+$ and 365 (m/z = 364 $[M+H]^+$). Mass fragmentation of m/z = 105 was derived from phenacyl moiety. Therefore, a fragment ion of m/z = 107 instead of 105 indicated an addition of two hydrogens to the phenacyl moiety. Since the mass spectrum of an authentic sample of N^3-α-hydroxy-β-phenethyluridine showed the same mass fragmentation of M3, N^3-α-hydroxy-β-phenethyluridine is one of metabolites of N^3-phenacyluridine.

In man, acetohexamide has been reported to be mainly biotransformed to (−)-hydroxyhexamide, a pharmacologically active metabolite (Imamura *et al*. 1985). In addition, McMahon *et al*. (1965) reported that (−)-hydroxyhexamide is 2.4 times as potent as acetohexamide. In addition, the carbonyl moiety of the anti-inflammatory agent, sodium 2-[4-(2-oxocyclopentylmethyl)-phenyl]propionate dihydrate (loxoprofen sodium), has been shown to be metabolized to the correspond hydroxy group (Tanaka *et al*. 1983). The *trans*-OH form was a pharmacologically active compound and has an anti-inflammatory effect. In the present study, the metabolite theoretically has (*R*)- and (*S*)-isomers derived from the asymmetric carbon of the phenacyl moiety. These isomers were separated by hplc and compared the synthetic racemates. In general, carbonyl groups are metabolized according to the Baumann–Prelog rule. In the cases of acetophenone (McMahon *et al*. 1965), warfaline (Moreland and Hewick 1975), nabilone (Billings *et al*. 1980) and 2-octanone (Gal *et al*. 1981), essentially one enantiomer, (*S*)-alcohols, is mainly formed in mammals. Optical rotation of the isomers indicated that one of the urinary metabolites is N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine. The metabolic pathways of N^3-phenacyluridine are proposed in figure 6 and the reactions are stereo-specific in that only the (*S*)-(+)-enantiomer is produced.

The CNS depressant effects of N^3-phenacyluridine, its metabolites and related compounds were also evaluated for hypnotic activity and synergistic effects with pentobarbital in mouse by i.c.v. injection. Since N^3-α-hydroxy-β-phenethyluridine (racemate) exhibited a sleeping time of 279 min in mouse at 2.0 μmol/mouse by i.c.v. injection, it is suggested that the metabolite retained depressant activity. For the pharmacological effects of both enantiomers, N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine exhibited a sleeping time of 409 min, whereas N^3-(*R*)-(−)-α-hydroxy-β-phenethyluridine had only ~ 6% of the effect as compared with the (*S*)-isomer at the same dose, indicating that the enantiomers possess stereo-selective pharmacological activity. N^3-phenacyluracil, uridine, uracil, and D-ribose did not exhibit any hypnotic action. N^3-phenacyluridine, N^3-α-hydroxy-β-phenethyluridine (racemate), (*R*)- and (*S*)-enantiomers significantly prolonged the

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pentobarbital-induced sleeping time to 423, 447, 259 and 690% of the control. N^3-(*S*)-(+)-α-hydroxy-β-phenethyluridine possesses a strong synergistic effect but N^3-phenacyluracil, uridine, uracil and D-ribose did not show significant changes in the pentobarbital-induced sleeping time.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan, and by the Special Fund of Hokuriku University.

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