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Determination of naproxen and its metabolite O-desmethylnaproxen with their acyl glucuronides in human plasma and urine by means of direct gradient high-performance liquid chromatography

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ABSTRACT

Naproxen is metabolized in humans by O-demethylation, and by acyl glucuronidation to the 1-O-glucuronide. Naproxen, its metabolite and the conjugates can be measured directly by gradient high-performance liquid chromatographic analysis without enzymic deglucuronidation. The glucuronide conjugates were isolated by preparative chromatography from human urine samples. Mild acidic hydrolysis of one urinary conjugate resulted in naproxen. This conjugate was also formed by alkaline isomerization of isolated naproxen acyl glucuronide, indicating that the structure of this urinary conjugate must have been naproxen isoglucuronide (4-O-glucuronide). Mild acidic hydrolysis of another urinary conjugate resulted in O-desmethylnaproxen. This conjugate was also formed by alkaline isomerisation of isolated O-desmethylnaproxen acyl glucuronide, indicating that the structure of this urinary conjugate must have been O-desmethylnaproxen isoglucuronide (4-O-glucuronide). Calibriation curves were constructed by enzymic deconjugation of samples containing different concentrations of isolated naproxen acyl glucuronide, O-desmethylnaproxen acyl glucuronide, and the isoglucuronides of naproxen and O-desmethylnaproxen by mild acidic hydrolysis. The limit of quantitation of naproxen in plasma is 1.5 μg/ml. The limits of quantitation in urine are: naproxen, O-desmethylnaproxen, naproxen acyl glucuronide and O-desmethylnaproxen acyl glucuronide, 1 μg/ml; the isoglucuronide of naproxen and O-desmethylnaproxen, 1.5 μg/ml. A pharmacokinetic profile of naproxen is shown, and some preliminary pharmacokinetic parameters of naproxen obtained from two human volunteers are given.

INTRODUCTION

Naproxen is a non-steroidal anti-inflammatory drug used in painful and inflammatory rheumatic and non-rheumatic conditions. It is the dextrorotary isomer of 6-methoxy-α-methyl-2-naphthalene acetic acid. The anti-inflammatory effects are related to the inhibition of cyclooxygenase and consequent decrease in prostaglandin concentrations in various fluids and tissues. Inhibition of the prostaglandin synthesis may result in gastrointestinal microbleeding and in a reduction of kidney functioning [1].

In humans, naproxen is metabolized by phase I metabolism to 6-O-desmethylnaproxen and both compounds are conjugated by phase II metabo-

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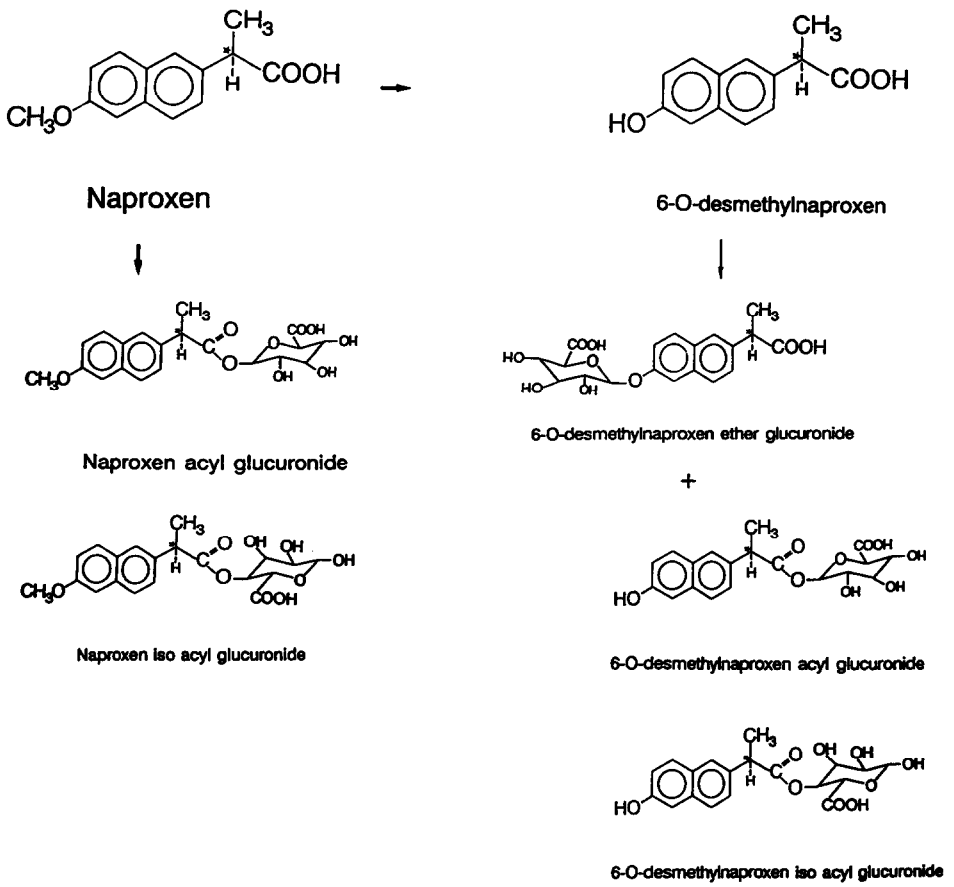

Fig. 1. Structures of naproxen and its possible metabolites.

lism into naproxen acyl glucuronide (1-O-glucuronide) and 6-O-desmethylnaproxen acyl glucuronide (1-O-glucuronide), as shown in Fig. 1 [1]. The possible presence of isoglucuronide (4-O-glucuronide) in the urine must be anticipated as the result of isomerization of the acyl glucuronides in blood of pH 7.4.

Naproxen concentration analysis in plasma and urine has been performed by fluorimetric methods [2–4], gas chromatography [4,6–13], thin-layer chromatography [14] and mass fragmentography [15,16]. High-performance liquid chromatographic (HPLC) analysis of naproxen and O-desmethylnaproxen is now the most common method of analysis, however, the conjugates being determined after enzymic or alkaline hydrolysis [17–22]. Direct HPLC analyses of other similar drugs, such as carprofen [23], zomepirac [24] and the salicylic acid derivative diflunisal, with their acyl glucuronide and sulphate conjugates, has been reported [19,25]. A direct isocratic HPLC analysis of naproxen with its acyl glucuronide enantiomers was reported by El Moehli and co-workers [26–28].

Acyl glucuronides are unstable in alkaline media (pH > 6.0). Therefore urine (human or animal urine) must be kept acidic at pH 5.0 (already inside the body) in order to prevent hydrolysis of acyl glucuronides [29].

The aim of this investigation was to develop a simple and direct gradient HPLC analysis of the acyl glucuronide conjugates of naproxen and its metabolite O-desmethylnaproxen in human plasma and urine.

EXPERIMENTAL

Chemicals

Naproxen and its metabolite O-desmethylnaproxen were obtained from Sarva-Syntex (Palo Alto, CA, USA). Naprosyne was obtained from our hospital pharmacy. All other reagents were of p.a. quality and obtained from Merck (Darmstadt, Germany). Naproxen acyl glucuronide, O-desmethylnaproxen acyl glucuronide, naproxen isoglucuronide and O-desmethylnaproxen isoglucuronide were identified in human urine after intake of 500 mg of naproxen. β-Glucuronidases were obtained from Sigma (St. Louis, MO, USA).

Gradient HPLC analysis

The HPLC system consisted of a Spectra Physics SP 8775 autosampler (Spectra Physics, Eindhoven, Netherlands), a Spectra Physics SP 8800 ternary HPLC pump, a Kratos Spectroflow 757 UV detector (Separations, Hendrik Ido Ambacht, Netherlands), a Schoeffel FS 970 LC fluorimeter (Spectra Physics) and a Spectra Physics SP 4290 integrator. The column was packed with Cp Spherisorb 50DS (250 mm × 4.6 mm I.D., Chrompack, Bergen op Zoom, Netherlands) with a guard column (75 mm × 2.1 mm I.D.) packed with pellicular reversed phase (Chrompack). The mobile phase was a mixture of acetonitrile buffer (5 g of orthophosphoric acid in 1000 ml of water) and N,N-dimethylformamide (DMF). At $t = 0$, the mobile phase consisted of 10% acetonitrile, 10% DMF and 80% buffer. During the following 20 min the mobile phase was changed linearly until it attained a composition of 15% DMF, 45% acetonitrile and 40% buffer. At 25 min ($t = 25$) the mobile phase was changed within 5 min to the initial composition, followed by equilibration for 2 min. The flow-rate was 1.2 ml/min. The UV detection wavelength was 330 nm. Fluorimetric detection was achieved by excitation at 330 nm and emission at >389 nm (cut-off filter).

The capacity factors of naproxen, O-desmethylnaproxen with their acyl glucuronides are given in Table I.

TABLE I

RETENTION TIMES AND CAPACITY FACTORS OF NAPROXEN, ITS METABOLITE AND CONJUGATES

Acyl glucuronide = 1-O-glucuronide; isoglucuronide = 4-O-glucuronide.

Compound	t_r (min)	k'
t_0	2	
O-Desmethylnaproxen isoglucuronide	10.57	3.74
O-Desmethylnaproxen acyl glucuronide	11.32	4.07
O-Desmethylnaproxen	14.28	5.40
Naproxen isoglucuronide	15.27	5.97
Naproxen acyl glucuronide	15.75	6.19
Naproxen	19.68	7.98

Sample treatment

Plasma samples (100 μl) were deproteinized with 0.4 ml of 0.20 *M* perchloric acid and centrifuged at 3000 *g*, and 20 μl of the supernatant were injected onto the column. During the first day of the human experiment, 100 μl of plasma sample were processed immediately upon receipt in order to detect the presence of acyl glucuronides of naproxen or O-desmethylnaproxen.

Urine samples were diluted ten-fold with 0.01 *M* H_3PO_4, and 20 μl were injected onto the column.

Isolation of the acyl glucuronides

The peaks in the chromatogram that showed the pharmacokinetic behaviour of metabolites of naproxen were isolated by preparative HPLC.

The preparative Gilson HPLC System consisted of a Gilson 302 sample pump (Gilson, Meyvis, Bergen op Zoom, Netherlands), two 305 Gilson gradient pumps, an 811 B Dynamic mixer, a Kratos 757 UV detector (Separations), an LKB 2211 superrac (LKB, Woerden, The Netherlands), and a BD7 recorder (Kipp & Zonen, Delft, Netherlands). The column was a C_8 Rainin Dynamax 60 Å (250 mm × 10 mm I.D., particle size 8 μm) from Meyvis.

The mobile phase consisted of 1% acetic acid in water–acetonitrile (80:20, v/v). This was run initially for 1 min, and then changed linearly over 15 min to 65:35 (v/v). The retention times were:

naproxen isoglucuronide, 12 min; naproxen acyl glucuronide, 14 min; O-desmethylnaproxen acyl glucuronide, 6.7 min; and O-desmethylnaproxen isoglucuronide, 5.8 min.

Concentration of the trapped sample was carried out by a IKA rotavapor (Janke and Kunkel, Staufen, Germany) equipped with a Trivac vacuumpump (Leybold-Heraeus, Woerden, Netherlands).

The urine was concentrated as follows. To urine (pH 5) was added 20 g/l Celite 545 and the mixture was filtered. The compound was extracted on an XAD-2 column at pH 5 and eluted with methanol. The fractions that contained the acyl glucuronides and the isoglucuronide conjugates were evaporated to dryness by a gentle stream of nitrogen, dissolved in water, filtered and injected onto the column.

Deconjugation of the acyl glucuronides

Deglucuronidation was carried out with 100 μl of human urine containing naproxen and O-desmethylnaproxen acyl glucuronide, 100 μl of β-glucuronidase and 800 μl of 0.2 *M* (Na_2H–KH_2) phosphate buffer at 37°C for 2 h.

Four different β-glucuronidase enzymes were tested: (A) 100 000 U/ml β-glucuronidase type B1 (bovine liver, Sigma, Cat. No. G-0251) and phosphate buffer pH 5.0; (B) 107 200 U/ml β-glucuronidase type H2 (*Helix pomatia,* Sigma, Cat. No. G-0876) and phospate buffer pH 5.0; (C) 100 000 U/ml β-glucuronidase type LII (lyophilized powder from limpets *Patella vulgata,* Sigma, Cat. No. G-8132) and phosphate buffer pH 3.8; (D) 20 000 U/ml β-glucuronidase type VIIA (*Escherichia coli,* Sigma, Cat. No. G-7646) and phosphate buffer pH 6.8.

Calibration curves

Samples containing different concentrations of naproxen acyl glucuronide isolated from human urine by preparative HPLC were deconjugated by system D. Samples containing different concentration of naproxen and O-desmethylnaproxen isoglucuronide isolated from human urine by preparative HPLC were deconjugated by mild acidic hydrolysis.

The increase in the concentration of naproxen (aglycon) represented the concentration of the conjugate naproxen acyl glucuronide. A calibration curve was constructed with the help of the formula $[\text{N-gluc}] = d[\text{N}] \times M_{\text{N-gluc}}/M_{\text{N}}$, where $d[\text{N}]$ is the difference in concentration of naproxen before and after deconjugation and M is relative molecular mass (all $r = 0.999$).

Calibration curves for naproxen, the phase I metabolite O-desmethylnaproxen and (isolated) phase II acyl (iso)glucuronides were constructed by spiking water samples with known concentrations of the compounds ($r > 0.995$).

Stability

The stability of naproxen acyl glucuronide and O-desmethylnaproxen acyl glucuronide in urine/water was tested as follows. Three samples of 2 ml of urine were brought to pH 5.0, 6.0 and 8.0 and incubated at 37°C for 6 h. Each hour a 100-μl sample was taken, and the reaction stopped with 900 μl of 0.01 *M* H_3PO_4. From this mixture, 20 μl were injected onto the column.

The stability of naproxen and O-desmethylnaproxen acyl glucuronide, naproxen isoglucuronide and O-desmethylnaproxen isoglucuronide in the autosampler in water and 0.01 *M* H_3PO_4 was tested during 24 h. Samples were taken every 0.5 h and injected onto the column.

Isomerization of the acyl glucuronides

Isolated naproxen acyl glucuronide and O-desmethylnaproxen acyl glucuronide were subjected to hydrolysis and isomerization in a phosphate buffer of pH 5.0, 6.0 and 7.4 during 24 h at 37°C. The formation of isoglucuronides was followed by taking and analysing a sample every hour.

Deconjugation of naproxen isoglucuronide

Mild acidic hydrolysis was performed by adding 400 μl of a solution of isolated naproxen isoglucuronide (4-O-glucuronide) to 200 μl of 0.5 *M* $BaCl_2$ and 600 μl of 2.5 *M* HCl. The mixture was vortex-mixed and kept at 50°C for 20 h.

Limits of quantitation

At a signal-to-noise ratio of 3, the limit of

quantitation of naproxen in human plasma was 1.5 μg/ml with UV detection and 5 μg/ml with fluorimetric detections. The limits of quantitation with UV and fluorimetric detection, at a signal-to-noise ratio of 3, of naproxen, its metabolite and conjugates in urine were naproxen, O-desmethylnaproxen, naproxen acyl glucuronide and O-desmethylnaproxen acyl glucuronide, 1.0 μg/ml; naproxen isoglucuronide and O-desmethylnaproxen isoglucuronide, 1.5 μg/ml.

Subjects

Two human subjects (I, male 45 years, 82 kg; II, female 45 years, 65 kg) took 500 mg of naproxen orally (Naprosyne). One subject (I) took 100 mg of O-desmethylnaproxen for identification of its acyl glucuronide. The study had the approval of the hospital ethics committee, and informed consent was obtained from the volunteers.

Sampling

Blood samples were drawn at regular time intervals after administration during five days by means of fingertip puncture with Monolet lancets (Monoject, St. Louis, MO, USA). After centrifugation, plasma samples were stored at −20°C pending analysis. Urine was collected upon spontaneous voiding. The total time of sample collection was 120 h (six times the expected $t_{1/2}$ of 20 h). Urinary pH was kept acidic (pH 5.0–5.5) by the oral intake of 1 g ammonium chloride q.i.d. (Ammonchlor, Südmedica, Munich, Germany). Four urine samples of 5 ml of each void were stored at −20°C pending analysis.

RESULTS AND DISCUSSION

Fig. 2 shows the chromatograms (UV detection) of a human urine sample after oral administration of 500 mg of naproxen, before and after β-glucuronidase treatment. This sample shows the presence of the acyl glucuronides of naproxen and O-desmethylnaproxen, and of β-glucuronidase-resistant isoglucuronides of naproxen and O-desmethylnaproxen. In plasma only naproxen could be detected. Compound X is an endogenous compound that is secreted with a circadian rhythm. This means that when X is absent in the blank urine or secreted at a minimal rate, O-desmethylnaproxen isoglucuronide can be detected and recognized as a metabolite/conjugate; however, X disturbs the renal excretion rate–time profile. When X is present or excreted at a maximal rate, the presence of the isoglucuronide must be denied. The interference of X with O-desmethylnaproxen isoglucuronide depends on the type of column and on the gradient. Compound X had no fluorescence properties, as shown in Fig. 3. When X and the isoglucuronide cannot be sep-

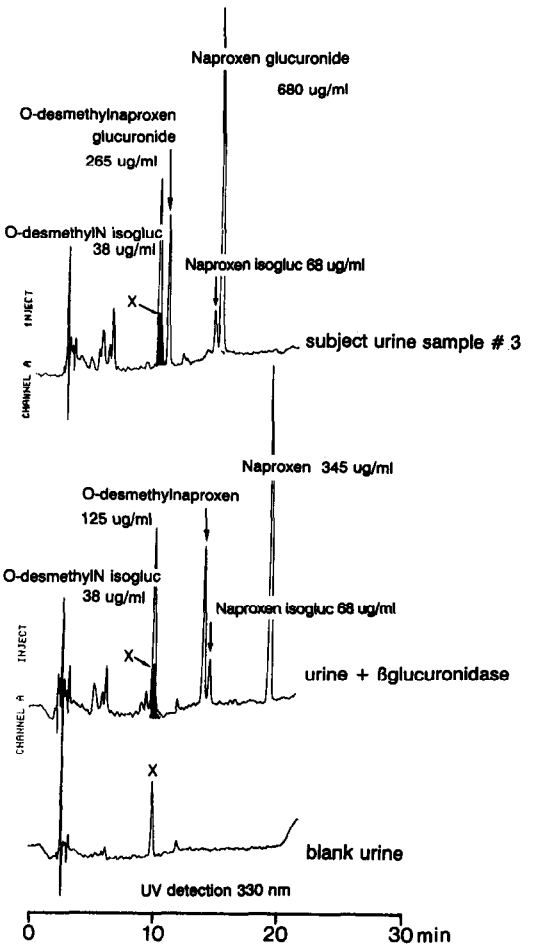

Fig. 2. Chromatograms of human urine containing naproxen and its metabolites before and after β-glucuronidase treatment. UV detection at 330 nm. Compound X is an endogenous compound, secreted with a circadian rhythm.

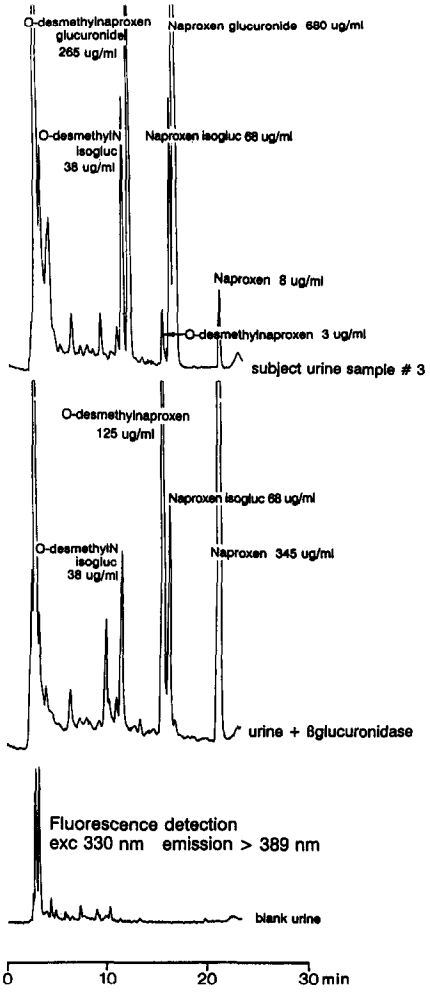

Fig. 3. Chromatograms of human urine containing naproxen and its metabolites before and after β-glucuronidase treatment. Fluorimetric detection with excitation at 330 nm and emission at >389 nm (cut-off filter). The endogenous compound X is not visible anymore.

arated in the chromatographic system used, fluorimetric detection solves this problem.

Fig. 4 shows that the acyl glucuronides of naproxen and O-desmethylnaproxen were stable at pH 5, and unstable at pH 7.4 and 8. The slightly acidic samples were stable in the autosampler of the HLPC system. In a similar way it was demonstrated that the isoglucuronide of naproxen and O-desmethylnaproxen were stable at pH 5, and unstable at pH 7 and 8. Naproxen and O-desmethylnaproxen isoglucuronide were stable at pH 5 during 24 h in the autosampler.

Fig. 5 shows the chromatogram and the rate of isomerization of isolated naproxen acyl glucuronide (1-O-glucuronide) into naproxen isoglucuronides (2-O-, 3-O- and 4-O-glucuronide) and naproxen (UV detection). This reaction does not proceed between pH 5 and 6. The stable naproxen isoglucuronide (4-O-glucuronide), which is the most abundant compound in Fig. 5, is also present in human urine.

Fig. 6 shows similar data for the isomerization and hydrolysis of isolated O-desmethylnaproxen acyl glucuronide (UV detection). The retention time of the stable O-desmethylnaproxen isoglucuronide may coincide with an endogenous compound X in the human urine, as shown in Fig. 2. For the detection of O-desmethylnaproxen isoglucuronide in human urine, fluorescence detection is recommended when X and this particular isoglucuronide cannot be separated.

Hydrolysis of the isoglucuronides with β-glucuronidase system D did not result in the formation of naproxen or O-desmethylnaproxen.

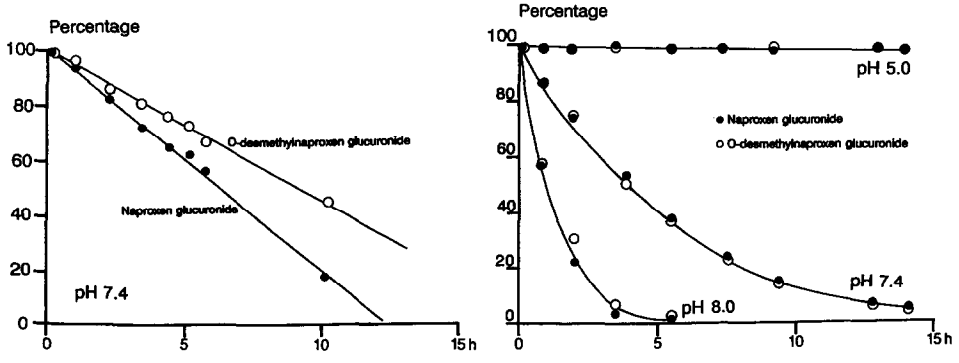

Fig. 4. Stability of naproxen acyl glucuronide and O-desmethylnaproxen acyl glucuronide at 37°C at different pH values in plasma (left panel) and urine (right panel) (UV detection).

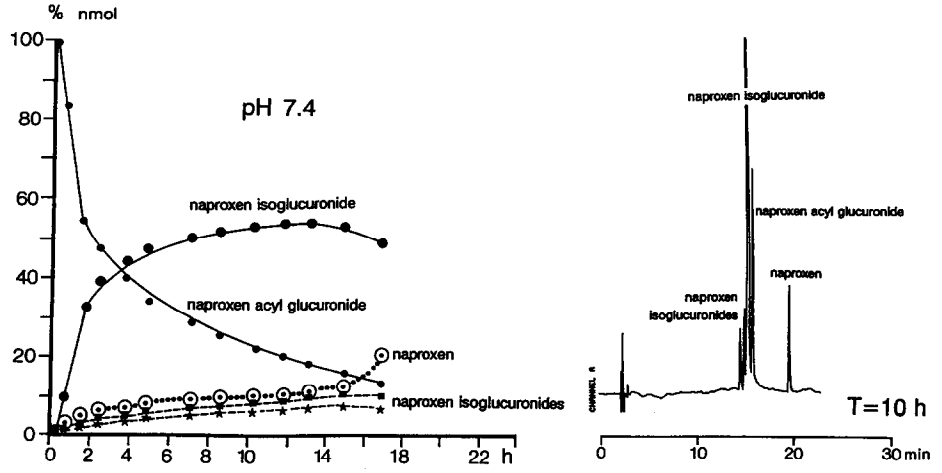

Fig. 5. Isomerization of isolated naproxen acyl glucuronide (1-O-glucuronide) into naproxen isoglucuronides (2-O-, 3-O- and 4-O-glucuronide) and into naproxen (left panel). The right panel shows the chromatogram of the reaction mixture at t = 10 h (UV detection).

Fig. 7 shows the chromatograms (UV detection) and Fig. 8 the rate of the mild acidic hydrolysis of the naproxen isoglucuronide into naproxen. Hydrolysis of naproxen with this system did not result in a decrease of naproxen or in the formation of O-desmethylnaproxen. When naproxen is subjected to acidic hydrolysis by stronger acid (5 *M* HCl at 80°C), it is hydrolysed to O-desmethylnaproxen (Fig. 9). O-Desmethylnaproxen can be synthesized by refluxing naproxen in 5 *M* HCl; the reaction is completed within 1 h. Urine hydrolysed by 5 *M* HCl at 37°C results in the final presence of O-desmethylnaproxen.

Tables II and III show the intra- and inter-day variations of naproxen and its metabolites.

The presence of ethereal sulphate of O-des-

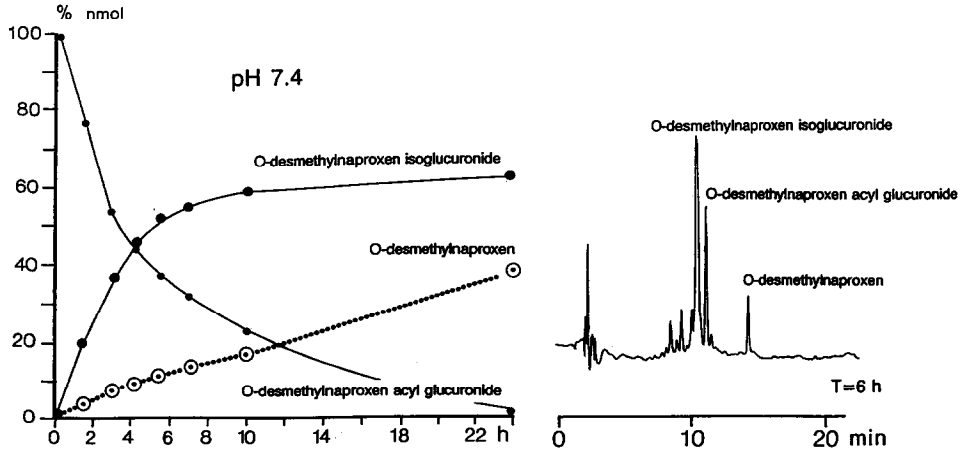

Fig. 6. Isomerization of isolated O-desmethylnaproxen acyl glucuronide (1-O-glucuronide) into isoglucuronides (mainly 4-O-glucuronide) and into O-desmethylnaproxen (left panel). The right panel shows the chromatogram of the reaction mixture at t = 6 h (UV detection).

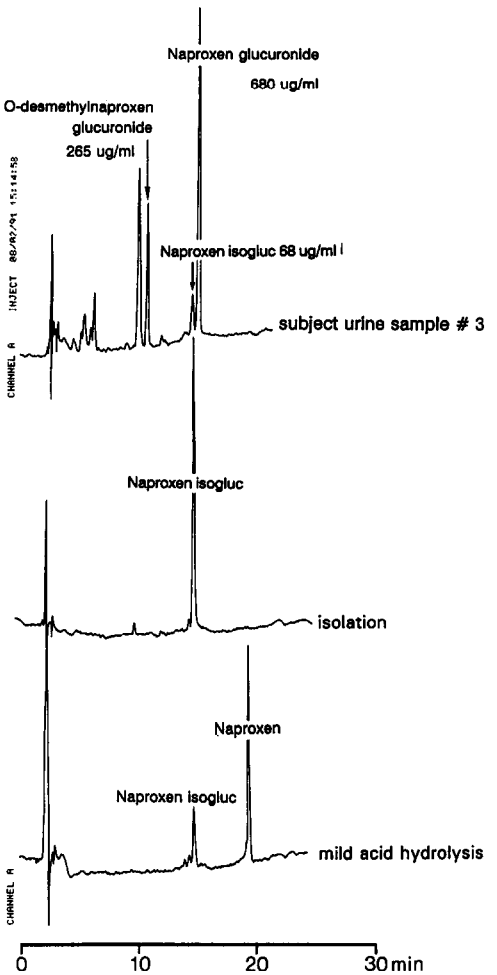

Fig. 7. Chromatograms of isolated naproxen isoglucuronide in human urine before and during mild acidic hydrolysis with 2.5 *M* HCl at 50°C (UV detection).

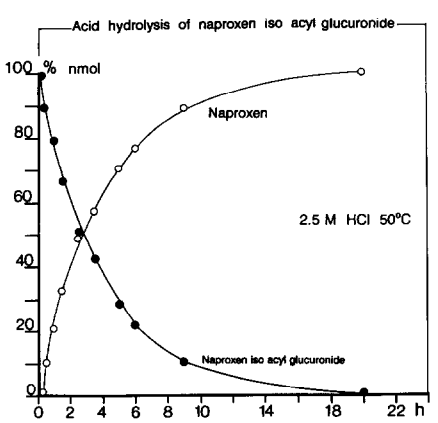

Fig. 8. Time course of the acidic hydrolysis with 2.5 *M* HCl at 50°C of isolated naproxen isoglucuronide into naproxen (UV detection).

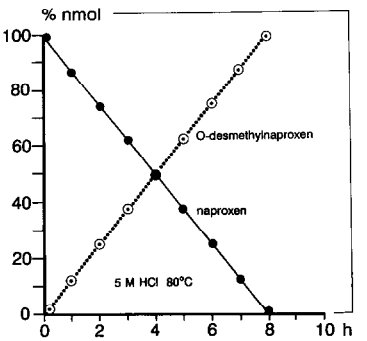

Fig. 9. Hydrolysis of naproxen by strong acid (5 *M* HCl at 80°C) into O-desmethylnaproxen. The reaction proceeds within 1 h under reflux (UV detection).

methylnaproxen in plasma of patients with impaired renal function of patients [30] and in a 24-h urine sample was reported [31]. The tentative identification of the glycine conjugate of naproxen in urine of rats and mice was reported by Sugarawa *et al.* [31] but, as a substituted phenylacetic acid, the molecular structure of naproxen is too big for conjugation with glycine or glutamic acid [32]. These structures might have been present and correctly assigned when the isoglucuronides of naproxen and O-desmethylnaproxen were also present and identified in the total metabolic profile of naproxen. Other metabolites, such as the ethereal glucuronide of O-desmethylnaproxen, were not found in plasma or urine. An

TABLE II

INTER-DAY AND INTRA-DAY COEFFICIENT OF VARIATION OF SPIKED NAPROXEN IN HUMAN PLASMA

UV detection; fluorimetric quantitation of naproxen in plasma was not performed owing to the high limit of quantitation; n = 4, *in vitro*.

Concentration (μg/ml)	Coefficient of variation (%)	
	Inter-day	Intra-day
71	2.3	1.6
56	4.7	4.8
28	10.3	6.6
6	7.5	6.6

TABLE III

INTER-DAY AND INTRA-DAY COEFFICIENT OF VARIATION OF NAPROXEN AND ITS METABOLITE AND CONJUGATES IN HUMAN URINE

Acyl glucuronide = 1-O-glucuronide; isoglucuronide = 4-O-glucuronide; $n = 4$, *in vivo*.

Concentration (μg/ml)	C.V. (%)			
	Inter-day[a]	Intra-day[a]	Inter-day[b]	Intra-day[b]
Naproxen				
20	3.6	2.1	3.2	1.1
3	12.6	8.5	4.5	1.9
Naproxen acyl glucuronide				
1800	2.7	1.1	1.1	0.3
520	1.7	4.2	0.5	0.9
85	4.0	7.2	2.5	0.5
12	7.8	6.9	2.1	3.0
Naproxen isoglucuronide				
170	2.0	1.1	1.1	1.1
75	3.9	2.1	4.0	2.8
8	2.8	3.7	3.1	3.2
2	10.3	9.3	4.2	3.8
O-Desmethylnaproxen				
5	4.7	8.2	4.9	9.5
3	10.3	5.6	9.5	4.6
O-Desmethylnaproxen acyl glucuronide				
350	1.9	1.1	1.3	1.9
120	3.1	0.5	0.5	0.4
19	8.1	3.1	3.3	3.1
4	7.1	7.7	4.3	4.1
O-Desmethylnaproxen isoglucuronide				
90	–[c]	–[c]	0.8	0.8
40	–[c]	–[c]	1.2	2.5
20	–[c]	–[c]	4.0	2.8

[a] UV detection.

[b] Fluorimetric detection.

[c] Not possible due to interfering endogenous compound.

ethereal glucuronide of 7-hydroxymethylnalidixic acid was present in lower amounts in human urine than the acyl glucuronide of this metabolite and could be hydrolysed with β-glucuronidase [33]. Ethereal glucuronides are present in more phenolic compounds, such as salicylic acid [34] and diflunisal [25].

The presence of the isoglucuronide of naproxen and of O-desmethylnaproxen in human urine, despite the precaution of keeping the urine acidic, can be explained as follows. With the urine kept at pH 5, the acyl glucuronides of naproxen and O-desmethylnaproxen were found to be stable. So, naproxen isoglucuronide could not have been formed during the passage through the kidney and storage in the bladder. It must have been formed in the blood of pH 7.4 during the passage of hepatically formed naproxen acyl glucuronide from the liver to the kidney. The stable isoglucuronide of naproxen and of O-desmethylnaproxen, after being formed in the blood, is excreted by the kidney as if it were a stable metabolite formed by the liver.

Fig. 10 shows the plasma concentration–time

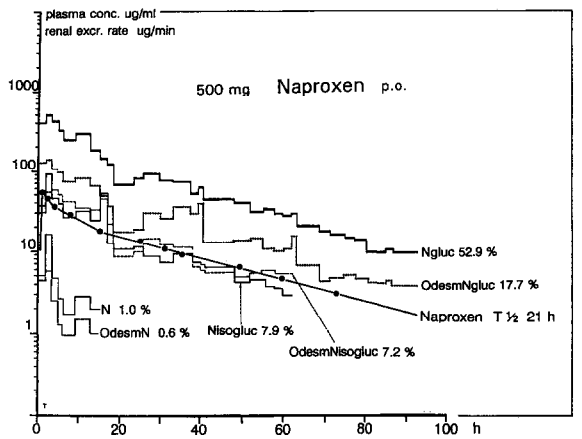

Fig. 10. Plasma concentration–time curve of naproxen (N) and renal excretion rate–time profile of naproxen (N), O-desmethylnaproxen (OdesmN), naproxen acyl glucuronide (Ngluc), naproxen isoglucuronide (Nisogluc), O-desmethylnaproxen acyl glucuronide (OdesmNgluc) and O-desmethylnaproxen isoglucuronide (OdesmNisogluc) is a human volunteer after an oral dose of 500 mg of naproxen (subject I, plasma UV detection, urine fluorimetric detection).

curve of naproxen and renal excretion rate-time profiles of naproxen acyl glucuronide and naproxen isoglucuronide, and that of the metabolite O-desmethylnaproxen acyl glucuronide in one male volunteer (fluorescence detection). No acyl glucuronides of naproxen or O-desmethylnaproxen were detectable in plasma. Table IV shows some pharmacokinetic parameters of naproxen in the two human volunteers.

The main elimination pathway in humans is similar to that in animals, namely renal excretion of the conjugates of naproxen and its metabolite O-desmethylnaproxen. Naproxen itself shows low renal clearance values, owing to extensive tubular reabsorption [22,35].

As no naproxen acyl glucuronide is found in plasma, presumably because of high renal clearance and hydrolysis in an alkaline medium (pH 7.4), the high concentration of naproxen acyl glucuronide found in controlled acidic urine may therefore in part be formed by the kidney.

In conclusion, the analysis of naproxen and its metabolites can be performed by gradient HPLC with UV detection. When the endogenous compound X cannot be separated from O-desmethylnaproxen isoglucuronide, fluorimetric detection will solve this problem and make this isoglucuronide visible in urine.

TABLE IV

SOME PHARMACOKINETIC PARAMETERS OF NAPROXEN, ITS METABOLITE AND CONJUGATES IN HUMANS

Parameter	Subject I	Subject II
Gender	Male	Female
Dose (mg)	500	500
C_{max} (μg/ml)	53.7	71.3
T_{max} (h)	1.0	1.0
Elimination half-life (h)	21.0	16.0
Total body clearance (ml/min)	7.7	8.4
Volume of distribution (l)	13.7	10.3
Percentage of the dose excreted (%)		
Naproxen	1.0	1.6
O-Desmethylnaproxen	0.6	1.1
Naproxen acyl glucuronide	52.9	51.8
Naproxen isoglucuronide	7.9	7.6
O-Desmethylnaproxen acyl glucuronide	17.7	12.6
O-Desmethylnaproxen isoglucuronide	7.2	4.9
Urinary pH acidic (between pH 5.0 and 5.5).		

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