Journal of Chromatography, 341 (1985) 105—113
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2542

DETERMINATION OF PROQUAZONE AND ITS *m*-HYDROXY METABOLITE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

CLINICAL APPLICATION: PHARMACOKINETICS OF PROQUAZONE IN CHILDREN WITH JUVENILE RHEUMATOID ARTHRITIS

MATTI LEMPIÄINEN

Department of Clinical Chemistry, University Central Hospital, Turku (Finland)

and

ANNA-LIISA MÄKELÄ*

Department of Paediatrics, University Central Hospital, Turku (Finland)

(First received August 24th, 1984; revised manuscript received January 11th, 1985)

SUMMARY

A method for the determination of proquazone and its *m*-hydroxy metabolite in serum and urine by reversed-phase high-performance liquid chromatography is described. The technique is based on a single extraction of the unchanged drug, its metabolite and an internal standard from serum or urine with chloroform. The column was packed with μBondapak C_{18} and the mobile phase was acetonitrile—water (50:50) (pH 3). The detection limits for proquazone and its metabolite were 0.02 μmol/l using 500 μl of sample. For the determination of the total *m*-hydroxy metabolite only 100 μl of sample are needed. The method described is suitable for routine clinical and pharmacokinetic studies. The clinical application of this method suggests that the pharmacokinetics of proquazone in adults and children are similar.

INTRODUCTION

Proquazone [1-isopropyl-7-methyl-4-phenyl-2(1*H*)-quinazolinone] is a non-acidic, synthetic non-steroid anti-inflammatory drug (NSAID), which has been shown to have significant anti-inflammatory and analgesic activity in patients with rheumatoid arthritis and other rheumatic disorders [1—16]. Proquazone

0378-4347/85/$03.30 © 1985 Elsevier Science Publishers B.V.

has also been given to child patients for the treatment of juvenile rheumatoid arthritis [17]. The recommended daily doses for children range from 10 to 20 mg/kg, corresponding to a dose of about 400—800 mg/m^2 of body surface area; the total daily dose should not exceed 1000 mg [18].

Pharmacokinetic studies of proquazone have, however, been carried out only in adults [19] and no information is available about the pharmacokinetics of proquazone in children. The purpose of this study was to provide a preliminary pharmacokinetic profile of proquazone in children and to find a simple and relatively reliable means of controlling the levels of proquazone and/or its metabolites in serum and urine.

No suitable method for the measurement of proquazone was found in the literature. Therefore, a high-performance liquid chromatographic (HPLC) method for the determination of proquazone and its metabolites was developed. The technique is based on a single extraction of the unchanged drug, its metabolite and an internal standard from serum or urine with chloroform (Fig. 1).

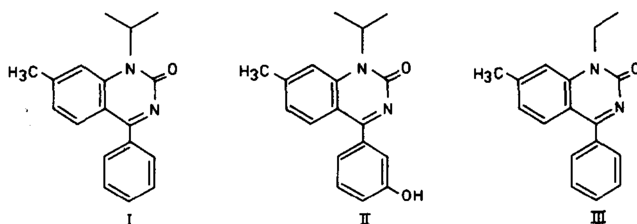


Fig. 1. Structures of proquazone (I), its *m*-hydroxy metabolite (II) and the internal standard (III).

EXPERIMENTAL

Reagents and standards

Pure samples of proquazone [1-isopropyl-7-methyl-4-phenyl-2(1*H*)-quinazolinone; Biarison], its *m*-hydroxymetabolite [1-isopropyl-7-methyl-4-(*m*-hydroxyphenyl)-2(1*H*)-quinazolinone] and the internal standard [1-ethyl-7-methyl-4-phenyl-2(1*H*)-quinazolinone] were supplied by Sandoz (Basle, Switzerland). The acetonitrile used was Baker Analyzed HPLC Reagent (J.T. Baker, Deventer, The Netherlands). Other reagents and solvents were of analytical-reagent grade obtained from E. Merck (Darmstadt, F.R.G.).

Stock solutions of 1000 μmol/l were prepared by dissolving 27.8 mg of proquazone or 29.4 mg of *m*-hydroxy metabolite in 100 ml of absolute ethanol. Working standard solutions were then prepared by diluting the appropriate volume of stock solution with drug-free serum to give final concentrations of proquazone and its metabolite of 0.10, 0.25, 0.50, 1.00 and 2.00 μmol/l. Standard solutions for the determination of the total *m*-hydroxy metabolite were made in the same manner to give final concentrations of 1.0, 5.0, 10.0, 20.0 and 30.0 μmol/l. A stock solution of the internal standard of 1000 μmol/l was prepared by dissolving 26.4 mg in absolute ethanol. A working standard solution of the internal standard of 20.0 μmol/l was prepared from the stock solution by dilution with distilled water. All standards were stored at −20°C.

Sample preparation

Serum and urine samples were stored at −20°C until assayed.

Chromatographic conditions

A Pye Unicam liquid chromatograph equipped with a variable-wavelength UV detector and a column (300 × 4 mm I.D.) packed with μBondapak C_{18}, 10 μm (Waters Assoc., Milford, MA, U.S.A.), was used with acetonitrile—water (50:50), adjusted to pH 3.0 with orthophosphoric acid, as the mobile phase. Isocratic elution was carried out at a flow-rate of 2.0 ml/min. The liquid chromatograph was connected to a Hewlett-Packard 3380A integrator.

Procedure

To 0.5 ml of serum or urine were added 0.5 ml of sodium acetate buffer (0.2 mol/l, pH 5.0) and 50 μl of internal standard solution (20 μmol/l). When the total amount of *m*-hydroxy metabolite in serum or urine was determined, any conjugated metabolite was first hydrolysed enzymatically. Sample (100 μl), acetate buffer (0.5 ml) and *Suc d'hélix pomatia* (50 μl) (β-glucuronidase activity, 100 000 U/ml; sulphatase activity, 1 000 000 U/ml; Pharmindustrie, l'Industrie Biologique Française, Clichy, France) were mixed in stoppered glass tubes and incubated at 37°C for 16 h. The tubes were cooled to room temperature and 100 μl of internal standard solution were added.

Proteins were precipitated by sulphuric acid—sodium tungstate precipitation and the mixture was extracted with 7 ml of chloroform. The sample was centrifuged and the aqueous layer and protein precipitate were aspirated. The chloroform extract was evaporated to dryness under a stream of air, the residue dissolved in 50 μl of methanol and 10 μl were injected into the liquid chromatograph.

A calibration graph was prepared by subjecting standards to the above procedure. The ratios of the peak area of proquazone or metabolite to that of the internal standard were plotted as a function of concentration. The peak-height ratio also gave sufficient accuracy with a linear relationship between peak-height ratio and concentration up to 2 μmol/l. A calibration graph for total *m*-hydroxy metabolite was constructed in the same manner and the linear range of the assay was 0—30 μmol/l (Fig. 2).

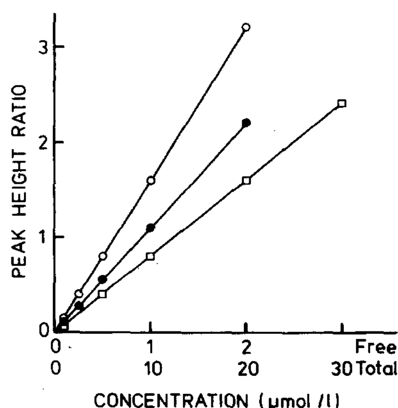


Fig. 2. Calibration graphs for proquazone (•), the free *m*-hydroxy metabolite (○) and the total *m*-hydroxy metabolite (□).

RESULTS

Selectivity

Representative chromatograms of serum specimens extracted according to our procedure are shown in Fig. 3. Chromatogram A is a typical pattern for serum from a normal individual not receiving drugs. Chromatogram B is obtained from the serum of an individual receiving proquazone. The concentrations of proquazone and its *m*-hydroxy metabolite in this sample were 0.59 and 0.14 μmol/l, respectively. Chromatogram C was obtained from the same serum sample as B after hydrolysis, with a total concentration of metabolite of 12.5 μmol/l. The retention times of proquazone, its *m*-hydroxy metabolite and internal standard were 4.9, 3.1 and 3.8 min, respectively. No interfering peaks in the same region were encountered from constituents of lipaemic or haemolytic sera. Paracetamol, salicylic acid, ketoprofen, indometacin, naproxen, flurbiprofen, ibuprofen and mefenamic acid do extract and chromatograph under these conditions, but they have retention times of 1.6, 2.2, 3.4, 3.5, 3.6, 5.2, 6.2 and 8.4 min.

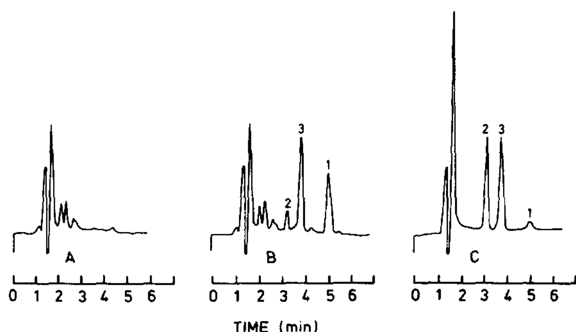


Fig. 3. Chromatograms of (A) serum blank (attenuation 0.005), (B) serum from a patient treated with proquazone (the serum was found to contain 0.59 μmol/l proquazone and 0.14 μmol/l *m*-hydroxy metabolite) (attenuation 0.005) and (C) the same serum as in B after hydrolysis (the serum was found to contain 12.5 μmol/l *m*-hydroxy metabolite) (attenuation 0.02). Peaks: 1 = proquazone; 2 = *m*-hydroxy metabolite; 3 = internal standard.

Sensitivity

In sensitivity studies, drug-free serum samples were spiked with proquazone and its *m*-hydroxy metabolite to a concentration of 0.02 μmol/l and processed as described above. The mean peak-area ratios were found to be 0.024 (n = 6, C.V. 11.2%) for proquazone and 0.021 (n = 6, C.V. = 11.5%) for the metabolite, indicating that 0.02 μmol/l of proquazone and its metabolite in serum can be determined with acceptable precision in 0.5-ml serum samples.

Recovery

Absolute recoveries of proquazone, its *m*-hydroxy metabolite and the internal standard were studied by adding known amounts of these compounds to a serum known to be drug-free. These samples were extracted as described above. A second set of standards were prepared in methanol at the same concentration. Volumes of 10 μl of each sample were chromatographed. Absolute

recoveries from serum were calculated by comparing the peak heights of the extracted serum samples with those of the non-extracted standards. The recoveries of proquazone and metabolite were 92.1 and 92.8%, respectively, at a concentration of 0.50 μmol/l. The recovery of internal standard was 92.6%. The relative recoveries of proquazone and metabolite were also determined. Known amounts of these compounds in ethanol were added to pooled serum to achieve the concentrations shown in Table I. These samples were processed as described above and at least ten samples were analysed. The relative recoveries are shown in Table I.

TABLE I

RELATIVE RECOVERIES OF PROQUAZONE AND ITS *m*-HYDROXY METABOLITE FROM SERUM ($n = 10$)

Compound	Initial concentration (μmol/l)	Calculated concentration (μmol/l)	Measured concentration (μmol/l)	Recovery (%)
Proquazone	0.42	0.82	0.80	97.6
Metabolite	0.22	0.62	0.61	98.4

TABLE II

PRECISION OF ASSAY FOR PROQUAZONE AND ITS *m*-HYDROXY METABOLITE ($n = 20$)

Parameter	Within-run		Day-to-day	
	Proquazone	Metabolite	Proquazone	Metabolite
Mean (μmol/l)	0.421	0.222	0.504	0.502
S.D. (μmol/l)	0.0133	0.0101	0.0206	0.0271
C.V. (%)	3.2	4.5	4.1	5.4

Precision

The within-run precision was evaluated by processing aliquots of pooled sera containing proquazone and the metabolite at the concentrations shown in Table II. The day-to-day precision was calculated from data obtained on samples analysed over a period of four months.

Clinical study

Subjects and procedure. Nineteen children with juvenile rheumatoid arthritis (JRA) were treated with daily doses of proquazone (Biarison) ranging from 9.4 to 21.4 mg/kg (274–600 mg/m^2). An initial pharmacokinetic short-term clinical study was performed by giving proquazone to eleven patients (eight girls and three boys) of age range 4–15 years (mean age 11.5 years). No other NSAIDs were allowed during the study.

Proquazone was administered to the patients as capsules containing 25, 50 or 200 mg of the drug. On the morning of day 1 (acute period) one single dose

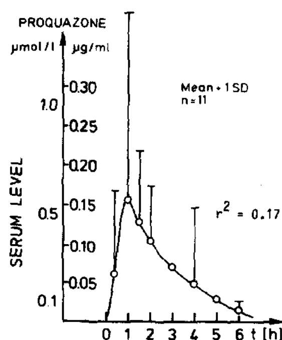
of about 5 mg/kg (range 4.7—7.5 mg/kg) was given to fasting subjects and the fasting was continued for a further 2 h. Blood samples were taken before (0 h) and 0.5, 1, 1.5, 2, 4 and 6 h after the administration of the drug. The duration of the constant repeated treatment period was fourteen days (days 2—15) and the total daily dose of about 10 mg/kg (range 9.4—10.8 mg/kg) was divided into three doses. All capsules were taken with food or a snack. During the elimination period (days 16—18) the patients took the last dose of proquazone in the morning of day 16 on an empty stomach and the fasting was continued for a further 2 h. On day 16 a blood sample was drawn at 8.00 a.m. before the administration of the last dose and thereafter at 0.5, 1, 1.5, 2, 4, 6, 24 and 48 h; 24-h urine samples were collected on days 11, 17 and 18.

Later, eight additional patients with JRA (three girls and five boys of age range 5—15 years (mean age 10.4 years) were included in the study. In order to achieve a better clinical effect, the daily dosage of proquazone was increased (range 13.0—21.4 mg/kg). In these patients the metabolism of proquazone was followed by collecting serum samples during a constant repeated dosing of the drug. In addition to the unchanged drug, its *m*-hydroxy metabolites (free and total) were also determined in the serum samples.

All patients fulfilled the diagnostic criteria of Brewer *et al.* [20]. The design and purpose of the study were explained orally to the patient (if old enough to understand) and to the parents in a simple and understandable way. The treatment was started only after written consent from the parent(s). The study was approved by the ethical committee of Turku University Central Hospital.

Results. Figs. 4 and 5 show the mean levels of unchanged proquazone in serum after a single dose and after the constant repeated dosing. Peak levels of unchanged proquazone in serum were attained 1—2 h after dosing. There was no unusual accumulation of unchanged proquazone in serum after two weeks of treatment.

The levels of the free *m*-hydroxy metabolite of proquazone in serum were generally very low. Peak serum levels of the total *m*-hydroxy metabolite



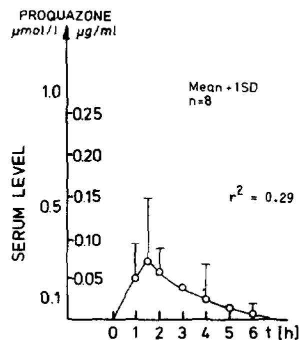
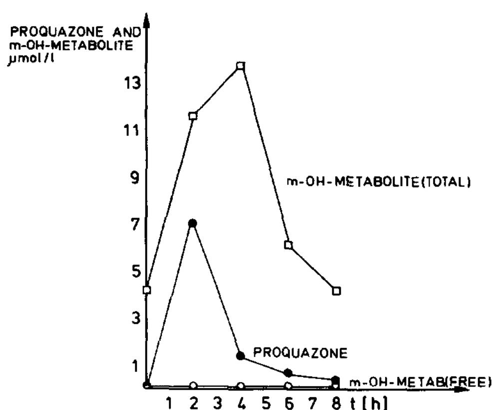


Fig. 4. Elimination profile after a single dose. Eleven patients (eight girls and three boys), age range 4—15 years (mean age 11.5 years). Mean dose of proquazone 5.3 mg/kg (range 4.7—7.5 mg/kg).

Fig. 5. Elimination profile after repeated dosing. Eight patients (six girls and two boys), age 10—15 years (mean age 12.6 years). Mean daily dose of proquazone 10.2 mg/kg (range 9.4—10.8 mg/kg/day).



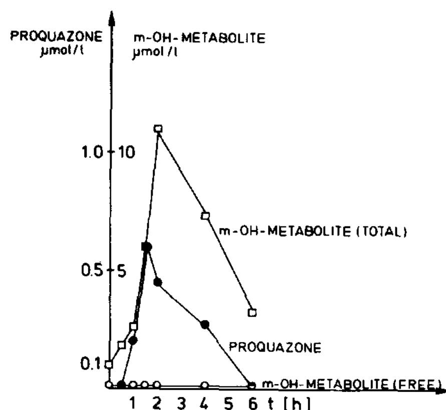


Fig. 6. Levels of proquazone and its *m*-hydroxy metabolite (free and total) during repeated dosing of proquazone. Dose: 13 mg/kg per day. Patient 15-year-old girl.

Fig. 7. Levels of proquazone and its *m*-hydroxy metabolite (free and total) during repeated dosing of proquazone. Dose: 17 mg/kg per day. Patient: 10-year-old boy.

(conjugated and unconjugated) were reached 2—3 h after dosing (Figs. 6 and 7). The peak values of 20—25 µmol/l (6—7 µg/ml of total *m*-hydroxy metabolite) were achieved with the repeated dosing of 15 mg/kg of body weight daily (divided into three equal doses).

The amounts of unchanged proquazone and of free *m*-hydroxy metabolite excreted in urine were low, being 0.01% and 0.06% of the total daily dose of the drug, respectively. During fourteen days' repeated dosing the amount of the total *m*-hydroxy metabolite in the urine ranged from 3.5% to 20.9% of the total daily dose. After the discontinuation of treatment on days 17 and 18 the amount of the total *m*-hydroxy metabolite excreted into urine decreased very quickly, being only 0.2—3% 24—48 h after the last dose.

DISCUSSION

Pharmacokinetic studies on adult healthy volunteers have shown that proquazone is rapidly absorbed from the gastrointestinal tract and before reaching serum nearly totally converted into its metabolites. About 90% of the orally administered dose is metabolized in the first liver passage. Owing to this high first-pass metabolism, the unchanged drug itself is very unstable in serum and gives large biological and inter-subject variations in the blood levels. In the liver of man proquazone is mainly hydroxylated to two active metabolites [19]. These two basic pathways are hydroxylation of the *m*-position in the phenyl ring and hydroxylation of the 7-methyl group, the former being by far the predominant one in man. Correspondingly, the *m*-hydroxy metabolite is the major metabolite in the blood and urine. It is present mainly in its conjugated form, with the proportion of unconjugated *m*-hydroxy metabolite accounting for only 5% of the total metabolite (Fig. 8).

According to the results of our study, the pharmacokinetics of proquazone in children correspond to the pharmacokinetics seen in adults. The levels of unchanged proquazone in serum and urine correlate very poorly with the daily

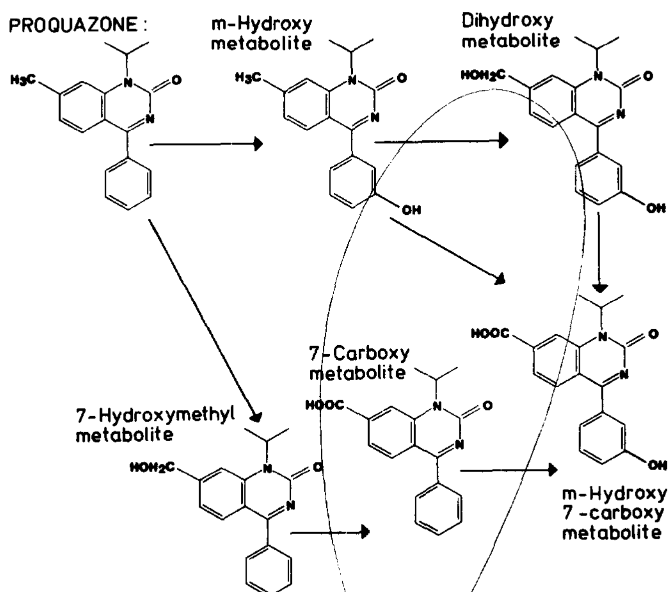


Fig. 8. The metabolism of proquazone.

dose of the drug. However, our study shows that the amount of total *m*-hydroxy metabolite correlates fairly well with the dosage of the drug and may offer a reliable basis for adjusting the dosage and following the compliance of the patients.

A dose of 15 mg/kg per day (500 mg/m^2 per day) gives *m*-hydroxy metabolite values of 20—25 μmol/l (6—7 μg/ml). In adults, when given proquazone in a dosage of 300 mg thrice daily, the corresponding value is about 5 μg/ml [21]. For those laboratories in which a high-performance liquid chromatograph with a UV detector is available, the present method is suitable for routine use. The advantages of the method are its simplicity and the small serum sample volume, combined with good accuracy and precision.

REFERENCES

1 R.Allan and M. Bleicher, J. Int. Med. Res., 5 (1977) 253.
2 G.L. Bach, Egypt. Rheumatol., 1 (Suppl.) (1980) 55.
3 G.L. Bach and P. Fotiades, Med. Klin. (Munich), 73 (1978) 1517.
4 F. Bachmann, Therapiewoche, 28 (1978) 6552.
5 R.V. Coombs, R.P. Danna, M. Denzer, G.E. Hardtmann, B. Huegi, G. Koletar, J. Koletar, H. Ott, E. Jukniewicz, J.W. Perrine, E.I. Takesue, J.H. Trapold, J. Med. Chem., 16 (1973) 1237.
6 M. Karakaya, Curr. Ther. Res., 22 (1977) 127.
7 G. Kupfrian and A. Zöller, Z. Allg. Med., 55 (1979) 1891.
8 M. Nissilä, and A. Kajander, Scand. J. Rheumatol., 21 (Suppl.) (1978) 36.
9 H. Ott and M. Denzer, J. Org. Chem., 33 (1968) 4263.
10 J. Puranen and H. Rönty, Scand. J. Rheumatol., 21 (1978) 21.
11 A. Ruotsi and B. Skrifvars, Scand. J. Rheumatol., 21 (Suppl.) (1978) 28.
12 A. Ruotsi and U. Vainio, Scand. J. Rheumatol., 21 (Suppl.) (1978) 15.
13 B. Skrifvars, Scand. J. Rheumatol., 21 (Suppl.) (1978) 40.

14 B. Skrifvars and M. Nissilä, Scand. J. Rheumatol., 9 (1980) 33.
15 J.T. Vainio and P.V. Lepistö, Scand. J. Rheumatol., 21 (Suppl.) (1978) 25.
16 H.-G. Velcovsky, R.G. Bretzel, H. Michels and K. Federlin, Therapiewoche, 29 (1979) 7103.
17 E.J. Brewer, E.H. Giannini, J. Baum, C.W. Fink, V. Hansen, J.C. Jacobs and J. Schaller, J. Rheumatol., 9 (1982) 135.
18 J.C. Jacobs, Pediatric Rheumatology for the Practitioner, Springer-Verlag, New York, 1982, p. 234.
19 H. Ott and J. Meier, Scand. J. Rheumatol., 21 (Suppl.) (1978) 12.
20 E.J. Brewer, J. Bass, J. Baum, J.T. Cassidy, Ch. Fink, J. Jacobs, V. Hanson, J.E. Levinson, J. Schaller and J.S. Stillman, Arthritis Rheum., 20 (1977) 195.
21 Sandoz, Basle, unpublished data.