Journal of Chromatography, 424 (1988) 440–444
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3987

Note

High-performance liquid chromatography and preliminary pharmacokinetics of articaine and its 2-carboxy metabolite in human serum and urine

T.B. VREE*

Department of Clinical Pharmacy and Department of Anesthesiology, Sint Radboud Hospital, University of Nijmegen, Nijmegen (The Netherlands)

A.M. BAARS

Department of Clinical Pharmacy, Sint Radboud Hospital, University of Nijmegen, Nijmegen (The Netherlands)

G.E.C.J.M. VAN OSS

Department of Anesthesiology, Hervormd Diaconessen Huis, Arnhem (The Netherlands)

and

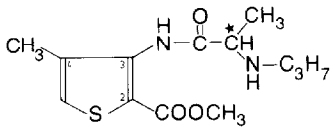
L.H.D.J. BOOIJ

Department of Anesthesiology, Free University Hospital, Amsterdam (The Netherlands)

(First received September 1st, 1987; revised manuscript received September 29th, 1987)

Articaine (3-*n*-propylamino-α-propionylamino-2-carbomethoxy-4-methylthiophen hydrochloride, MW 320.9) is a new local anesthetic drug [1,2], which is currently under clinical investigation. For the pharmacokinetic evaluation of the drug a gas chromatographic method was developed [3] with a detection limit of 0.05 μg/ml. For a full kinetic evaluation, the parent drug and its 2-carboxy metabolite (Fig. 1) must be taken into account. The aims of this investigation were to develop a high-performance liquid chromatographic (HPLC) analysis and to present preliminary pharmacokinetics for articaine and its 2-carboxy metabolite in humans.

0378-4347/88/$03.50



articaine

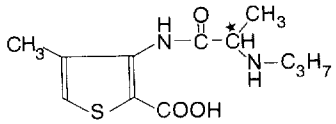


Fig. 1. Structures of articaine and its 2-carboxy metabolite. The centre of asymmetry is marked with a star.

EXPERIMENTAL

Chromatography

A Spectra-Physics 3500B liquid chromatograph (Spectra-Physics, Eindhoven, The Netherlands) was used, equipped with a variable-wavelength spectrophotometer (Spectroflow 757, Kratos U.S.A., Rotterdam, The Netherlands). The detector was connected to a 10-mV recorder (BD7, Kipp and Zonen, Delft, The Netherlands); the chart speed was 1 cm/min.

The analytical stainless-steel column (25 cm×4.6 mm I.D.) was prepacked with Spherisorb 5 ODS (Chrompack, Middelburg, The Netherlands). A guard column (7.5 cm×2.1 mm I.D.), tap-filled with pellicular reversed-phase material (Chrompack), was placed between the sampling valve and the analytical column. Articaine and its 2-carboxy metabolite were detected at 275 nm.

The mobile phase consisted of 400 ml of water, 1 g of phosphoric acid, 0.15 g of tetramethylammonium chloride and 600 ml of acetonitrile. The solvent flow-rate was 2 ml/min at a pressure of 22.5 MPa. The injection volume was 100 μl. The chromatographic analysis was carried out at room temperature.

Drugs

Articaine and its 2-carboxy metabolite were a gift of Hoechst Pharma (Amsterdam, The Netherlands).

Chemicals

Acetonitrile was of HPLC grade from Fisons (Loughborough, U.K.). The other chemicals were obtained from Merck (Darmstadt, F.R.G.) and were of analytical grade.

Patients

Patients (ASA I-II) undergoing surgery gave their informed consent to participate in the study, which was approved by the Diaconessen Huis ethics committee.

Sample preparation

To 0.2 ml of serum in an Eppendorf reaction vessel, 0.3 ml of acetonitrile was added and mixed thoroughly on a vortex mixer. The mixture was centrifuged in a Biofuge A (Heraus Christ, F.R.G.) at 11 000 *g* for 5 min. A 100-μl volume of the clear supernatant was injected onto the column. Urine was diluted 100 times with the mobile phase, the solution was mixed and 100 μl were injected onto the column.

Testing of the analytical procedure

The accuracy, precision and linearity of the method were determined using spiked samples of human serum and urine analysed at random. Total recovery of articaine and its 2-carboxy metabolite was determined as the response from serum standards relative to standards in water–acetonitrile (20:30, v/v), injected directly onto the column.

Total recovery from the urine was determined as the response from urine standards relative to standard solutions in water diluted 100 times with the mobile phase, injected directly onto the column.

RESULTS

Chromatography

Articaine and its 2-carboxy metabolite were well separated from endogenous human serum and urine components with UV detection at 275 nm (Fig. 2). The recovery of articaine added to human serum in the concentration range 0.04–8.0 μg/ml was $64.5 \pm 2.8\%$ (mean $\pm$ S.D., $n=8$). The recovery for the metabolite was $82.9 \pm 3.7\%$ in the concentration range 0.1–20.0 μg/ml. The recovery for both compounds added to urine was $99.5 \pm 1.5\%$.

The precision, expressed as the relative standard deviation (R.S.D., $n=10$) was 1.21% for articaine in serum and 2.20% in urine. The precision for the 2-carboxy metabolite was 0.73% for serum and 1.15% for urine. The accuracy for articaine was 3.5% in serum and 3.3% in urine. For the metabolite these figures were 3.3 and 3.1%, respectively. Calibration curves for articaine ($r=0.9999$) and its 2-carboxy metabolite ($r=0.9999$) were linear in serum and urine over the concentration range 0.05–100 μg/ml. The minimum detectable concentration for both compounds in serum was 0.02 μg/ml and in urine 0.5 μg/ml, at a signal-to-noise ratio 3:1.

Stability

Stability experiments in which articaine was added to serum and whole blood at 22, 4 and 0°C, showed that 40, 80 and 98%, respectively, of the compound was recovered after a 4-h interval. Articaine was hydrolysed by esterase activity to its 2-carboxy derivative (Fig. 3). The half-life of degradation was 3 h at 22°C and 16 h at 4°C, respectively. The half-life in serum–acetonitrile (20:30, v/v) at 22°C was similar to the half-life in serum at 4°C (16 h). Patient serum samples stored at −20°C were stable for at least one month.

Standard solutions of articaine were freshly prepared every day in ice-cold serum

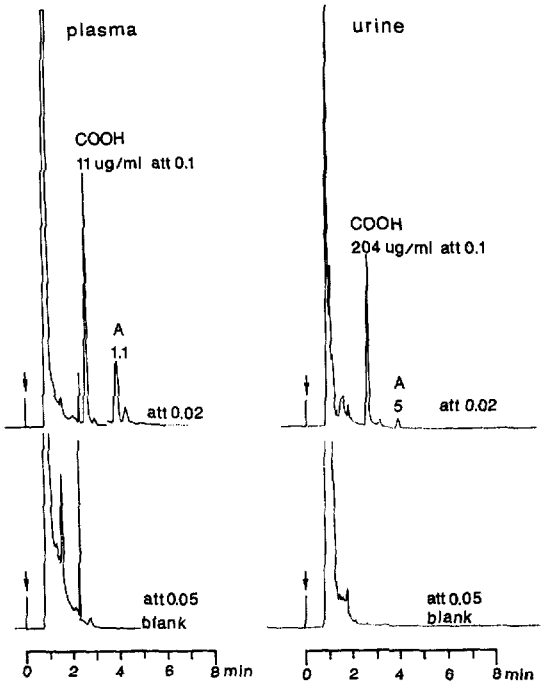


Fig. 2. HPLC profiles of articaine (A) and its 2-carboxy metabolite (COOH) in plasma (left) and urine (right) of a patient in different concentrations (11 μg/ml, etc.).

before analysis. Articaine added to human urine (pH 6.6) and phosphate buffer of pH 4.5, 6, 7 and 8 at room temperature did not show any form of degradation within 48 h. The 2-carboxy metabolite of articaine did not show any instability.

Pharmacokinetics

Fig. 4 shows the plasma concentration–time curve of articaine and its 2-carboxy metabolite in a patient after an epidural administration of 600 mg of arti-

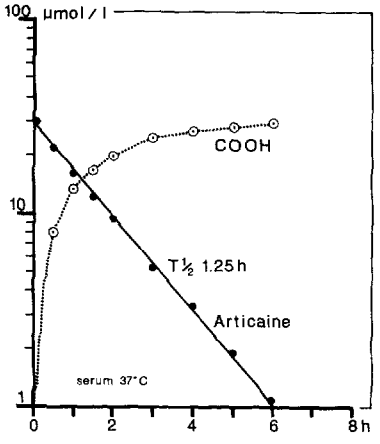


Fig. 3. Hydrolysis of articaine to its 2-carboxy metabolite (COOH) by serum esterase activity.

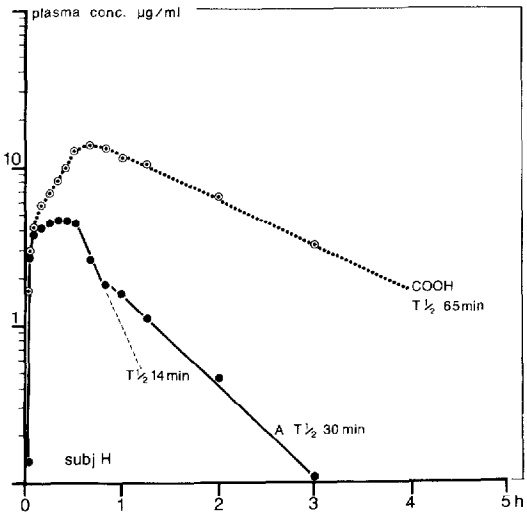


Fig. 4. Plasma concentration-time curves of articaine (A) and its 2-carboxy metabolite (COOH) in a patient after an epidural administration of 600 mg of articaine.

caine. Articaine shows a biphasic elimination with half-lives of 14 and 30 min, while the metabolite shows a half-life of 65 min. Articaine is excreted in small amounts in the urine (1–5%), and mainly as its 2-carboxy metabolite (60–95%). The total recovery of the dose in the urine varies between 80 and 100%. Full details of the pharmacokinetic study of articaine in patients will be published elsewhere.

DISCUSSION

This HPLC method enables the measurement of plasma and urine concentrations of articaine and its 2-carboxy metabolite and can be used for the determination in patients. The previously reported method of analysis of articaine did not include analysis of the 2-carboxy metabolite [3]. Care must be taken with the stability of articaine during storage or the analysis, as it is readily hydrolysed by esterases [3,4]. The reported half-life of articaine is in agreement with those reported in the literature [3,4].

REFERENCES

1 W. Kirch, N. Kitteringham, G. Lambers, P. Hajdu and E.E. Ohnhaus, Schweiz. Monatsschr. Zahnheilkd., 93 (1983) 714–719.
2 R. Muschawek and R. Rippel, Prakt. Anästh., 9 (1974) 135–140.
3 M. Uihlein, Prakt. Anästh., 9 (1974) 152–157.
4 I. Hornke, H.G. Eckert and W. Rupp, Dtsch. Z. Mund. Kiefer. GesichtsChir., 8 (1984) 67–71.