Journal of Chromatography, 121 (1976) 243–250

CHROM. 8978

A RAPID GAS CHROMATOGRAPHIC DETERMINATION OF THIAMPHENICOL IN PLASMA AND AMNIOTIC FLUID

T. A. PLOMP*, and R. A. A. MAES
Centre for Human Toxicology, University of Utrecht, Utrecht (The Netherlands)
and
M. THIERY
Department of Obstetrics, University of Ghent, Ghent (Belgium)
(Received December 1st, 1975)

SUMMARY

A rapid gas chromatographic method for the determination of thiamphenicol in plasma and amniotic fluid is described. The antibiotic is extracted from biological fluids with ethyl acetate and, after concentration of the extract, the trimethylsilyl derivative of the drug is determined by electron-capture gas chromatography using a ^{63}Ni detector. After the intravenous administration of a single dose of 500 mg of thiamphenicol during the first stage of spontaneous labour to clinically normal gravidae at term, transmission of the drug across the placenta was demonstrated by a combination of gas chromatography and mass spectrometry.

INTRODUCTION

Thiamphenicol (TAP**) (Fig. 1) is a synthetic[1] broad-spectrum antibiotic[2], in which the *para*-NO_2 group on the benzene ring of chloramphenicol (CAP) is replaced

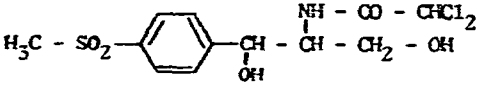

Fig. 1. Thiamphenicol or D(+)-*threo*-1-(4-methylsulphonylphenyl)-2-dichloroacetamido-1,3-propanediol; molecular weight 356.2.

with a CH_3SO_2 group. In spite of their chemical analogy, the two antibiotics differ in kinetics and toxicity. TAP, unlike CAP, is not inactivated in the body: it is always present in the active form, reaching particularly high and sustained levels in the liver–bile and kidney–urine[3,4].

In man, CAP is extensively glucuroconjugated in the liver before being excreted by the kidneys; TAP, on the contrary, is eliminated by the kidney in the unmodified form. The quotas of CAP and TAP eliminated in the unmodified form by the kidney within 24 h are 5–10% and 50–70%, respectively[5,6].

* To whom correspondence should be addressed.
** Synonyms: dextrosulphenicol, thiophenicol, Win 5063-2, CB 8053, Urfamycine, Glitisol.

The serum albumin-bound fraction of CAP is 24–52% and that of TAP is 0–10%[5,6]. In renal insufficiency, excretion of TAP is delayed, whereas that of unmodified CAP remains unaltered, although accumulation of monoglucuronides and arylamines in the blood is observed[5]. In hepatic insufficiency and particularly in liver cirrhosis, elimination of CAP is delayed, whereas there is no delay in the excretion of TAP, thus confirming the conclusion that TAP is not biotransformed by the liver[6].

In contrast to chloramphenicol, the toxicity of thiamphenicol is very low and consists mainly of a weak dose-related depression of erythropoiesis and, in some cases, also of the leukopoiesis[7]. These haematological alterations, reported for only 2.7% and 777 patients[8], were dependent on the duration of treatment and were found to be entirely reversible.

As a result of these characteristics, TAP has proved to be valuable for the treatment of both general and local infections. Nothing has previously been reported on the transmission of TAP across the human placenta or its passage into the amniotic fluid. Information on this aspect would be particularly useful, because TAP is used as a broad-spectrum antibiotic in obstetric patients and more specifically for the treatment of intrauterine infections[9].

Although we will subsequently report preliminary data on TAP levels in maternal and foetal blood and in amniotic fluid after the administration of a single i.v. dose of the drug to women in the first stage of labour, the purpose of this paper is to present a new analytical method used in the present study.

The methods usually applied for the determination of TAP in biological material are bacteriological assay[3], colorimetry based on alkaline hydrolysis[10] and gas chromatography[11–13].

The microbiological assay, described by Kunin and Finland[3], in which a cup-plate method is used with *Pasteurella bovisepticus* and *Sarcina lutea* as test organisms, is inaccurate when other antibacterial agents are administered together with TAP. The colorimetric method according to McChesney *et al.*[10] consists in alkaline hydrolysis of TAP, oxidation with periodate at pH 7.5 and determination of *p*-methylsulphonylbenzaldehyde as an alkali salt of its *p*-nitrophenylhydrazone. This method is time consuming and lacks sensitivity. Nakagawa *et al.*[13] described a gas chromatographic procedure for the determination of thiamphenicol, but their method is applicable only to urine samples. Gazzaniga *et al.*[12] and Aoyama and Iguchi[11] described gas chromatographic procedures for the determination of TAP as the trimethylsilyl (TMS) ether in biological fluids; these workers used a flame-ionization and an electron-capture detector, respectively. In our opinion, the use of flame-ionization detection[12] has several disadvantages inherent in the characteristics of the detector, including limited sensitivity, especially for organic compounds that contain S, O, Cl and Si (TAP TMS ethers), lack of specificity resulting in the detection of many interfering substances co-extracted from biological material and contamination of the detector caused by the SiO_2 from the TMS derivatives. As the TAP molecule (Fig. 1) contains two chlorine atoms and an aromatic methylsulphonyl group, all of which have a high electron affinity, it is obvious that the above difficulties can best be overcome by the use of a suitable electron-capture detector, which has the advantages of high specificity and high sensitivity. Aoyama and Iguchi[11] reported results obtained with a ^3H electron-capture detector. In spite of its high sensitivity, this type of detector is not entirely satisfactory for the determination of TAP in biological

material, however, because the detector temperature is limited to 225°C and contamination occurs easily when substances with a high boiling point are used. In order to solve this problem, we chose a ^{63}Ni electron-capture detector on the basis of its high sensitivity and specificity, its applicability up to 400°C, its relative immunity to contamination and, in the event of contamination, the ease with which the apparatus can be cleaned.

EXPERIMENTAL

Reagents

All reagents were of analytical grade: ethyl acetate, pyridine dried over potassium hydroxide pellets before use, anhydrous sodium sulphate and TRI-SIL/BSA in pyridine (Formula P) (Pierce, Rockford, Ill., U.S.A.). The following stock solutions were used: a 1000 ppm solution of TAP (A), obtained by dissolving 100 mg of thiamphenicol in 100 ml of pyridine; a 100 ppm solution of TAP (B), obtained by diluting 10 ml of A to 100 ml with pyridine; and a 1000 ppm internal standard (I.S.) solution, obtained by dissolving 100 mg of D(+)-*threo*-1-(4-methylsulphonylphenyl)-2-acetamido-1,3-propanediol in 100 ml of pyridine. Under refrigeration, these solutions remain stable for several weeks.

Gas chromatographic conditions

A Varian Model 2100 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a Varian Model A25 1-mV recorder were used. The ^{63}Ni detector (8.5 mCi ^{63}Ni beta source) was operated with a constant polarizing voltage (d.c. mode).

A U-shaped glass column (1.5 m × 2 mm I.D.) packed with 3% OV-17 on Gas-Chrom Q (60–80 mesh) was conditioned at 250°C for 2 days with a nitrogen flow-rate of 30 ml/min. The injection port was maintained at 250°C, the column oven at 200°C and the detector at 280°C. Pre-purified nitrogen was used as the carrier gas, at a flow-rate of 15 ml/min.

For structural confirmation of the TAP di-TMS ether obtained, a Finnigan 1015–6000 GC–MS computer system operated in the electron impact mode was used.

Biological material

All samples were collected from individuals belonging to the "clinically normal" category[14]. During the first stage of term labour (except for the two patients undergoing primary caesarean section), a single dose of 500 mg of thiamphenicol was administered intravenously in the form of its water-soluble and easily hydrolyzable glycinate (Glitisol, Urfamycine). Labour was monitored throughout via an intra-amniotic fluid-filled open-tipped cathether inserted through the cervical canal and connected with a strain gauge. The foetal heart rate was registered and calculated from the FECG signal obtained via a scalp electrode. Samples of maternal venous blood (antecubital vein) and amniotic fluid were collected before and at various times after the administration of TAP. At delivery, maternal (antecubital vein, femoral artery) and foetal (umbilical vein and artery) blood samples were collected. Plasma was separated immediately and stored at −20°C until the TAP assay was performed.

All amniotic fluid specimens assayed were free from meconium and blood; they were stored at −20°C and centrifuged before analysis.

Extraction procedure

To 0.1–0.5 ml of each sample of plasma or amniotic fluid, 10 μl of 1000 ppm I.S. solution (10 μg of I.S.) were added. The resulting solution was then mixed and allowed to equilibrate for 15 min, after which 10 ml of ethyl acetate were added. Extraction was carried out in 20-ml PTFE-stoppered glass centrifuge tubes by swirling on a Vortex–Genie mixer (Scientific Industries, U.S.A.) for 10 min, after which 0.5 g of anhydrous sodium sulphate was added to the mixture for dehydration. After swirling, followed by centrifugation at 2000 *g* for 10 min, the ethyl acetate solution was transferred to a 25-ml round-bottomed flask and evaporated under reduced pressure in a Büchi Rotavapor at room temperature.

Next, 0.20 ml of the TRI-SIL/BSA reagent was added to the residue and the round-bottomed flask was stoppered and allowed to equilibrate at room temperature for 10 min. Finally, the solution was made up to 1.00 ml with pyridine and transferred to 1-ml Reacti-vials (Pierce, Cat. No. 13221). Of this solution, 1 μl was injected into the gas chromatograph. The solution was stable for at least 3 weeks when stored in a refrigerator.

RESULTS

Using these conditions, the internal standard and the TAP di-TMS ethers had retention times of approximately 12 and 22 min, respectively. Fig. 2 shows the chro-

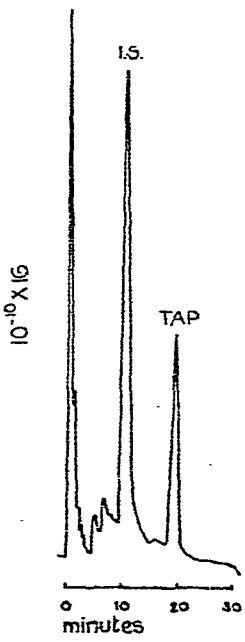

Fig. 2. Gas chromatogram of a standard mixture of the di-TMS ether of TAP and the internal standard (I.S.). Amounts injected: 10 ng of I.S. and 5 ng of TAP.

matogram of a standard mixture of di-TMS ether derivatives of TAP and the internal standard in pyridine.

Figs. 3 and 4 show chromatograms of blank and sample (after the intravenous administration of 500 mg of TAP) extracts of plasma and amniotic fluid. No interfering peaks were observed in the chromatograms of several blank samples.

The presence of the TAP di-TMS ether in plasma and amniotic fluid extracts was confirmed with a combination of gas chromatography, mass spectrometry, and computer analysis. Two of the most characteristic peaks (at *m/e* 242 and *m/e* 257) were selected from the mass spectrum of TAP di-TMS ether for multiple ion detection. After each chromatographic run, a base-line was drawn and peak heights were recorded for both the TAP and the internal standard peak. The ratio of the peak height of TAP to that of the internal standard was calculated. Quantitation was carried out by referring to a standard graph constructed from values of known amounts (1–12 μg) added to 1 ml of TAP free-sample (plasma, amniotic fluid) and then treated as described under *Extraction procedure*.

The ratios of the peak heights were plotted against the concentration of TAP; Fig. 5 shows the linearity for peak ratios between 0 and 0.8. The chromatographic response per microgram decreased, as expected, when concentrations sufficient to saturate the detector were injected (Fig. 5). When the calculated peak ratio did not lie between 0 and 0.8, the sample volume was changed. Losses due to extraction or any other manipulation were automatically compensated for on the basis of the internal standard.

One of the mixtures of the TAP and the I.S. used for the calibration graph was chromatographed several times daily prior to the determination of the unknown

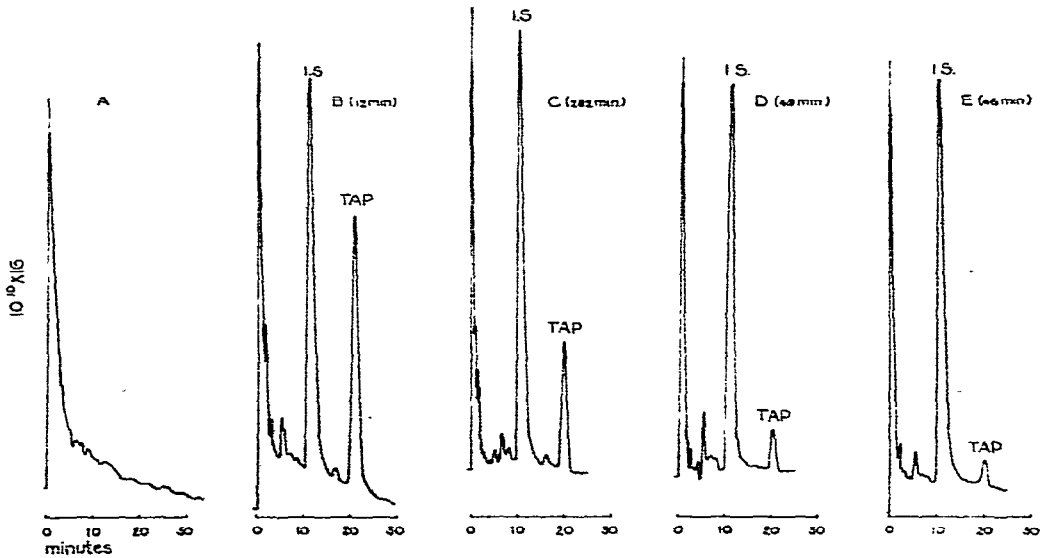

Fig. 3. Gas chromatograms of 0.2-ml extracts of plasma. A = blank sample; B–E = samples obtained at various times after a single i.v. injection of 500 mg of TAP to the mother (12 min, maternal femoral artery; 282 min, maternal antecubital vein; 48 min, umbilical artery; 46 min, umbilical vein). Volume of extracts injected, 1 μl.

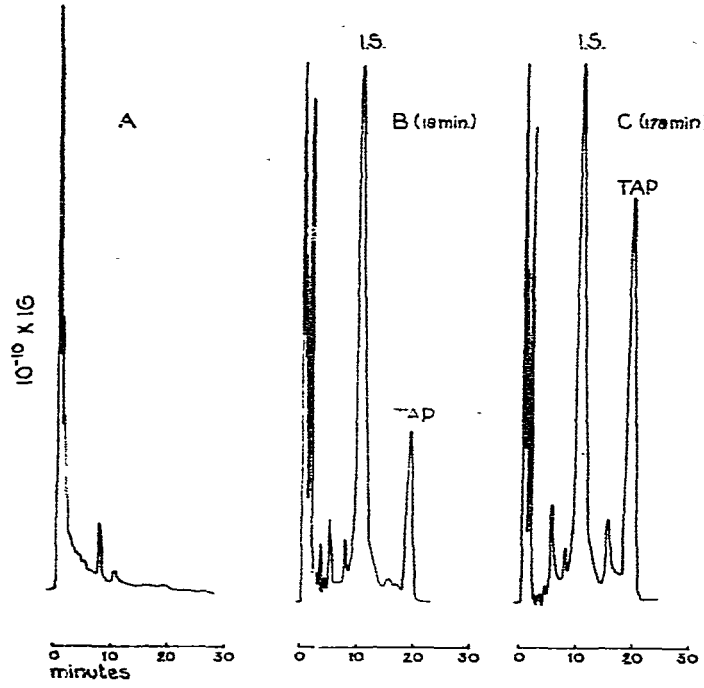

Fig. 4. Gas chromatograms of extracts of 0.5 ml of amniotic fluid. A = blank; B and C = 18 and 178 min, respectively, after maternal i.v. injection of 500 mg of thiamphenicol (1 μl of extract injected).

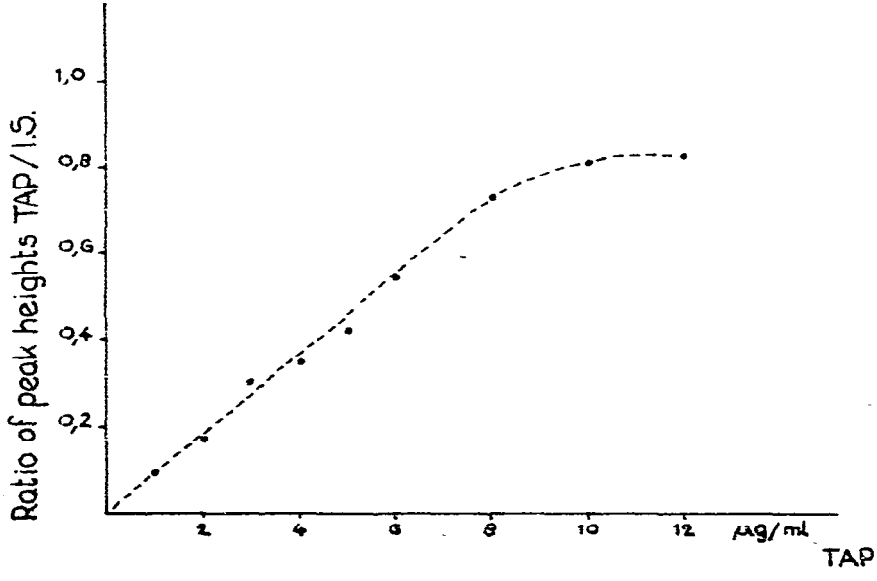

Fig. 5. Calibration graph for the determination of TAP in plasma and amniotic fluid (10 μg/ml of internal standard added).

TABLE I

RELATIVE RETENTION TIMES ON 3% OV-17 OF THE TRIMETHYLSILYL ETHERS OF THIAMPHENICOL, THE INTERNAL STANDARD AND THE PEAKS OBTAINED FROM PLASMA AND AMNIOTIC FLUID OF INDIVIDUALS GIVEN THIAMPHENICOL

Compound	*Relative retention times*
Internal standard	1.00
Thiamphenicol	1.88
Peak from plasma	1.88
Peak from amniotic fluid	1.87

samples; the peak heights were measured and the ratio was compared with the calibration graph to check the condition of the column and the detector.

The limit of sensitivity for the TAP di-TMS ether was 0.1 ng/μl. When thiamphenicol was added to plasma in concentrations of 1, 5 and 10 μg/ml, its mean recovery in nine experiments was 91.3% (standard deviation 5.1%). When thiamphenicol was added to amniotic fluid in amounts of 1, 5 and 10 μg/ml, its mean recovery in nine experiments was 90.3% (standard deviation 6.2%). The mean recovery of the internal standard when added to plasma or amniotic fluid in amounts of 10 μg/ml was 97% (standard deviation 6%).

All of the women (29) were delivered within 5 h after the administration of an intravenous dose of 500 mg of thiamphenicol; over this 5-h period, the mean maternal plasma concentration decreased from 30 to 4 μg/ml, the mean cord plasma concentration decreased from 11.5 to 3.8 μg/ml and the mean amniotic fluid concentration increased from 1.2 to 9.5 μg/ml.

DISCUSSION

The procedure described here is more specific and sensitive than the widely used colorimetric[10] and microbiological[3] methods, the lowest measurable concentration of TAP being of about 0.1 μg/ml. The method is also less time consuming, as the assay of thiamphenicol in plasma or amniotic fluid can be performed within 80 min.

The results obtained by gas chromatography with a ^{63}Ni detector alone or in combination with mass spectrometry clearly demonstrate that in the human subject thiamphenicol diffuses across the placenta and reaches the foetus. Analysis of extracts of maternal and cord plasma and amniotic fluid showed that the compound determined is the free antibiotic and that no significant amounts of TAP metabolites were detected. The levels of TAP found in cord plasma and amniotic fluid after a single maternal intravenous injection of 500 mg suggest that in these biological fluids the antibiotic reaches concentrations likely to be bactericidal against most pathogenic organisms. Therefore, thiamphenicol, a broad-spectrum antibiotic with low toxicity, may ultimately prove to be the antibiotic of choice for both the prevention and the treatment of intrauterine infections.

ACKNOWLEDGEMENT

The authors acknowledge the technical expertise of Mrs. I. D. M. Wagemaker-Engels.

REFERENCES

1 R. A. Cutler, R. J. Stenger and C. M. Suter, *J. Amer. Chem. Soc.*, 74 (1952) 5475.
2 N. W. Blacow and A. Wade (Editors), *Martindale: The Extra Pharmacopoeia*, The Pharmaceutical Press, London, 26th ed., 1973, p. 1440.
3 C. M. Kunin and M. Finland, *Proc. Soc. Exp. Biol. Med.*, 103 (1960) 246.
4 E. W. McChesney, R. F. Koss, J. M. Shekosky and W. H. Deitz, *J. Amer. Pharm. Ass.*, 49 (1960) 762.
5 F. Azzollini, A. Gazzaniga and E. Lodola, *Int. J. Clin. Pharmacol.*, 3 (1970) 303.
6 F. Azzollini, A. Gazzaniga, E. Lodola and R. Natangelo, *Int. J. Clin. Pharmacol.*, 6 (1972) 130.
7 M. Janbon, A. Bertrand, D. Brunel and F. Michel, *Presse Méd.*, 74 (1966) 151.
8 Zambon Research Laboratory, Bresso, Italy, personal communication 1969.
9 D. Charles and M. MacAulay, *Clin. Obstet. Gynecol.*, 13 (1970) 255.
10 E. W. McChesney, J. M. Shekosky, H. W. Eckert and R. F. Koss, *J. Amer. Pharm. Ass.*, 49 (1960) 28.
11 T. Aoyama and S. Iguchi, *J. Chromatogr.*, 43 (1969) 253.
12 A. Gazzaniga, E. Pezzotti and A. Cotta Ramusino, *J. Chromatogr.*, 81 (1973) 71.
13 T. Nakagawa, M. Masada and T. Uno, *J. Chromatogr.*, 111 (1975) 355.
14 M. Thiery, R. Derom, E. Lybeer, R. Serreyn and P. van Damme, *Biol. Neonat.*, 18 (1971) 203.