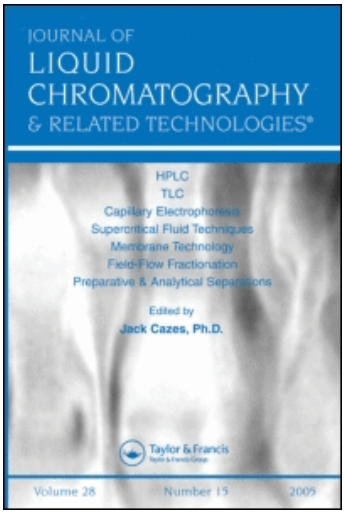

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Determination of Thiamphenicol in Serum and Cerebrospinal Fluid with High-Pressure Liquid Chromatography

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DETERMINATION OF THIAMPHENICOL IN SERUM AND CEREBROSPINAL FLUID WITH HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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ABSTRACT

A procedure for quantitation of thiamphenicol in serum and cerebrospinal fluid was developed using high-pressure liquid chromatography. The drug was extracted from biological samples with methanol and separated by reverse-phase high-pressure liquid chromatography. Detection and subsequent quantitation were performed at 254 nm by on-line ultraviolet spectrophotometry. After the intramuscular administration of a single dose of 1 g of thiamphenicol to a patient, a poor transmission of the drug across the hematoencephalic barrier was demonstrated by this assay.

INTRODUCTION

The properties of thiamphenicol (TAP), a broad spectrum antibiotic with an antimicrobial spectrum similar to that of chloramphenicol, would be particulary useful nowadays. This antibiotic is a more soluble weak base (pKa $\pm$ 7.2) than chloramphenicol, it is only slightly bound to plasma's proteins ($\pm$ 10 %), and is not inactivated in the body by metabolic processes (1) (2) (3) (4) (5).

The difference in toxicity of these two products would be an important indication for the use of thiamphenicol. Only a few

results have previously been reported concerning the plasma kinetics and on the crossing of thiamphenicol to the cerebrospinal fluid (6) (7) (8) (9) (10) (11) (12). Several methods have been described for the determination of TAP in biological materials, including microbiological assay, colorimetry and gas chromatography with various detectors (13) (14) (15) (16).

The microbiological assay, in which a cup-plate method is used with pasteurella bovisepticus and sarcinea lutea as test organisms, is inaccurate when other antibacterial agents are administered together and when thiamphenicol is metabolized in active compounds. The colorimetric procedure is time consuming and lacks sensivity. The gaz chromatographic assay with different modes of detection seems to be time consuming due to the extraction and derivatization procedures that are required. High-pressure liquid chromatography (HPLC) has been largely used for determination of chloramphenicol (17) (18) (19). The chemical analogy of these two antibiotics, which differ only by a group on the benzene ring and allowsa more lipophilic comportment for chloramphenicol, indicates by their partition coefficient, the possibility of a similar behavior in high-pressure liquid chromatography (20) (21) (22). In the present study, this technique offers an opportunity to detect thiamphenicol in plasma and cerebrospinal fluid. This method is as rapid as the methods for chloramphenicol, and has the same specificity and accuracy. A concentration of 0.5 µg thiamphenicol per ml could be measured on sample as small as 100 µl. Thiamphenicol glycinate is totally hydrolysed in thiamphenicol alcohol in plasma and could not be determinated in this case.

MATERIAL AND METHODS

a) Chromatographic equipment

A Waters ALC/GPC 204 liquid chromatograph was used (Waters Associates, Paris, France). It consisted of a model 6000 solvent delivery system, and U6K universal injector and a model 440 U.V. absorbance detector. Absorbance was recorded on a 10 mV chart recorder.

b) Solvents and standards

Freshly distilled deionized water used throughout the procedure. Methanol was analytical grade (Merck, Darmstadt, Germany). Thiamphenicol alcohol was kindly donated by CLIN-MIDY, Paris, France.

c) Chromatographic eluent

The mobile phase consisted of a mixture of water and methanol (80 : 20, V/V), passed through a 0.6 µm filter (Millipore Corp., Bedford, Mass., U.S.A.) and deaerated by ultrasonics.

d) In vitro samples

Serum Thiamphenicol alcohol stock solution (100 $\mu g.ml^{-1}$) was directly prepared in pooled human plasma and congealed in aliquots at - 80°C. An aliquot was decongealed and diluted in pooled human plasma before use.

CSF Thiamphenicol alcohol stock solution (10 $\mu g.ml^{-1}$) was directly prepared in pooled human CSF and congealed in aliquots at - 80°C. It was decongealed before and diluted with CSF for standardisation.

e) In vivo samples

One patient was given thiamphenicol glycinate in physiological saline (equivalent to 1 g of thiamphenicol) intramuscularly. Blood and CSF were drawn prior to injection and at various times up to 12 hours. All the more different hospitalized patients were taking of blood for a test one hour after intramusculary injection of one g of thiamphenicol glycinate. An aliquot of each sample was dosed one day after experiment and others aliquots were stored at -30°C and - 80°C up to three months before analysis. No signs of decomposition were observed and identical determinations were found.

f) Chemical assay

- Extraction

100 µl of methanol was added to 100 µl of plasma or CSF and standard solutions. The samples were mixed, then centrifuged for 10 minutes (at 2000 g) and the supernatant

passed through 0.22 µm filters and injected into the chromatograph.

- Chromatography

A reverse phase system was chosen to quantitate thiamphenicol. 100 µl of the extract was injected on to a µ-Bondapack C-18 (Waters) and eluent pumped through at 1 ml/min. The absorbance detector was set at 254 nm at a sensivity of 0.005 absorbance units full scale. Similar results was obtained at 268 nm at a sensivity of 0.01 absorbance units full scale. Quantitation was based on peak heights recorded. Two standard curves were used : One on plasma and the other for CSF.

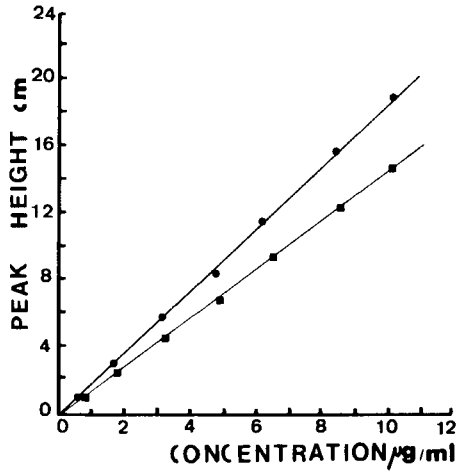

FIGURE 1

Standard curve for serum ■ and CSF ● thiamphenicol determinations. Chromatographic peak height expressed as a function of concentration in serum.

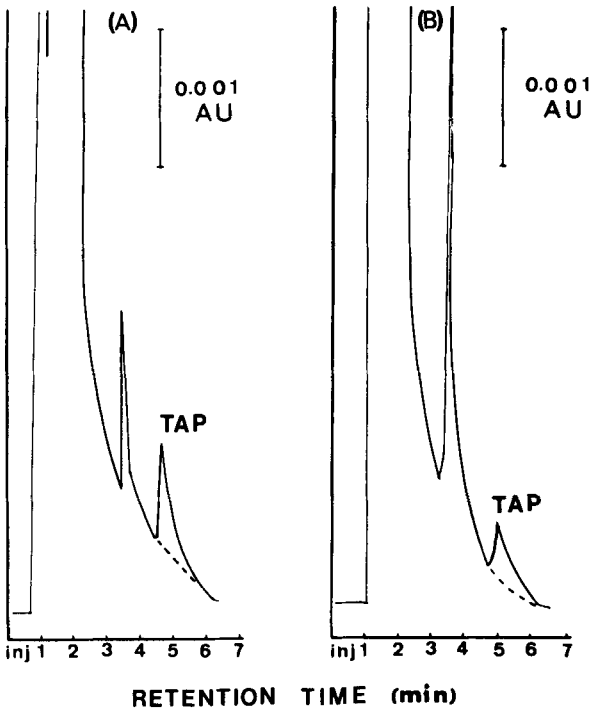

FIGURE 2

Chromatographic separation of thiamphenicol (TAP) in extract of serum (A) and of cerebrospinal fluid (CSF-B). The dashed lines are for the blanks of serum and CSF.
Colum : μ bondapak C 18, 30 cm x 4 mm I.d (Waters Associates).
Mobile phase : (water : methanol, 80 : 20, V/V).
Flow rate : 1 ml/min. Sample volume : 100 μl for serum extract and for CSF extract.

RESULTS

Chromatographic separation

With the conditions described, thiamphenicol had a retention time of 5 minutes and chloramphenicol had a retention time of 17.5 minutes in the same system. Thiamphenicol in plasma and CSF extract had the same retention time ; it was the same in aqueous solutions

of the drug. No interfering peaks were observed in chromatogramms of plasma or CSF. The more polar metabolized forms of thiamphenicol were eluted in the first peaks due to plasma or CSF and were undetectable for this reason.

Recovery

By assaying in vitro samples of known concentrations against standard curves obtained from pool control samples, recovery from serum and CSF was calculated and found to be quantitative.

By assaying in vivo samples of elevated concentration obtained from patients at the first hour after injection, recovery was also found to be quantitative for serum.

Sensitivity

A thiamphenicol concentration of 0.5 µg/ml serum of CSF could be accurately determined (peak height 5 mm).

TABLE I

Amount added to serum (µg/ml)	Amount added to CSF (µg/ml)	Amount measured* (µg/ml)	Recovery
1		0.97	97
2		1.95	97.6
5		4.9	98
8		7.83	97.9
10		10.15	101.5
	1	0.98	98
	2	1.97	98.7
	5	4.95	98.9
	8	7.92	99
	10	10.10	101

Recovery of Thiamphenicol from Serum and CSF

*Each value represents the mean of duplicate analyses.

TABLE II

Recovery of thiamphenicol from serum by dilution

	Serum of patients (µg/ml)	Dilution	Amount measured* (µg/ml)	Recovery
Serum A	5.4	0	5.4	100.0
		1/2	2.6	96.2
		1/4	1.2	80.9
Serum B	6.3	0	6.9	100.0
		1/2	3.0	95.2
		1/4	1.5	95.2
Serum C	10.4	0	10.4	100.0
		1/2	5.1	98.0
		1/4	2.5	96.2

*Each value represents the mean of duplicate analyses.

Precision

Intra-assay variation was determined by assaying a serum sample in two separate runs on the same day. For a concentration of 5 µg/ml the variation coefficient was 2.8 % and for 8 µg/ml it was 3 % and 0.9 % for 12 µg/ml. Interassay variation was calculated by dividing a serum sample into 5 portions which were stored at - 30°C and - 80°C and assayed at different weeks during a five weeks period. For a concentration of 5 µg/ml, the variation coefficient (with one standard deviation) was 3.1 % and at a concentration of 12 µg/ml it was 1.5 %.

Patient values

The kinetics of thiamphenicol in serum showed a period of elimination of 5 1/2 hours if we used a monoexponential representation for one patient ; his intramuscular injection (1 gram of thiamphenicol) gives a maximum value of 5.4 µg/ml ; in cerebros-

pinal fluid the values of the concentration were very low (0.6 to 1.2 µg/ml). Two other patients with therapeutic doses (1 g, IM) didn't show thiamphenicol in cerebrospinal fluid.

DISCUSSION

The procedure described above is more specific and sensitive than the widely used colorimetric and microbiological methods ; it is easier to perform than the gas chromatographic assay. This method is also less time consuming ; the largest step was the centrifugation following extraction. The limit of sensivity could be decreased by means of two processes : First, a larger sampling volume (1 ml serum) and ethyl-acetate extraction (2 ml) followed by evaporation and redissolution in mobile phase (50 µl) before injection ; secondly, the wavelenght must be set at 268 nm at

TABLE III

Analysis of clinical samples from three patients receiving thiamphenicol therapy (1 g, I.M. at t = 0).

	Time of prelevment after I.M. injection (hours)	Amount* measured in serum	Amount* measured in CSF
Patient A	1	5.28	0.7
	3	4.32	0.6
	6	2.40	0.9
	9	1.76	0.8
	12	1.52	0.8
Patient B	1	10.40	-
Patient C	1	6.30	-

*Each value is the mean of duplicate analyses. For patient B and C the limit of detection was lowed to 0.1 µg/ml in CSF sample and no thiamphenicol was measured.

0.01 full scale sensitivity ; this procedure was not useful for our clinical samples where serum or cerebrospinal concentrations of thiamphenicol were above the limit of sensivity. The metabolism of thiamphenicol as previously quoted is not significant and direct determination of thiamphenicol seems to be sufficient. In spite of this fact, samples could be dansylated or silylated and separated in a reverse phase system and its metabolites (glucuroconjugated and acylamine) quantitated. The first results obtained by this method, show a poor transfer of the drug into the CSF and, for two patients, thiamphenicol was respectively 6.3 and 10.4 µg/ml in serum after (1 gram intramusculary injection) one hour ; no thiamphenicol in CSF was detectable at the same time. These facts could be explained by the polarity of this molecule in comparison of the polarity of chloramphenicol which enters investigate into CSF in a significant proportion. Indeed, we need to further investigate this subject. This simple method seems to be very useful for such studies.

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