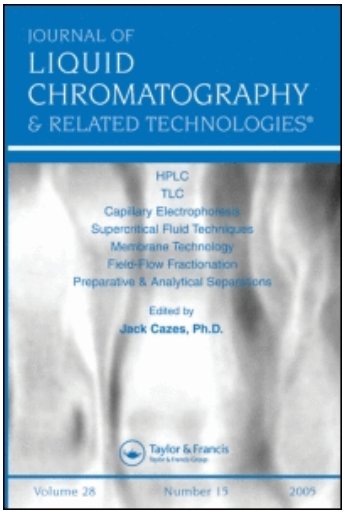

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Determination of Piperacillin in Plasma and Cerebrospinal Fluid by High Performance Liquid Chromatography

A. Meulemans[a]; J. Mohler[a]; J. M. Decazes[b]; I. Dousset[c]; A. Modai[d]

[a] Département de Biophysique, U. E. R. Xavier Bichat Université Paris VII, Paris [b] Département de Pharmacologie, Clinique des Antibiotiques-U 13 INSERM-Hôpital Claude Bernard, Paris [c] Clinique des maladies infectieuses A., Hôpital Claude Bernard, Paris [d] Clinique des Maladies Infectieuses A., Hôpital Hôpital Claude Bernard, Paris

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DETERMINATION OF PIPERACILLIN IN PLASMA AND CEREBROSPINAL FLUID BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

MEULEMANS A. Département de Biophysique, U.E.R. Xavier Bichat
Université Paris VII, 75018 PARIS.
MOHLER J. Département de Biophysique, U.E.R. Xavier Bichat
Université Paris VII, 75018 PARIS.
DECAZES J.M. Département de Pharmacologie Clinique des Antibiotiques-
U 13 INSERM - Hôpital Claude Bernard, 75019 PARIS.
DOUSSET I. Clinique des maladies infectieuses A.,
Hôpital Claude Bernard, 75019 PARIS.
MODAI A. Clinique des Maladies Infectieuses A., Hôpital
Hôpital Claude Bernard, 75019 PARIS.

ABSTRACT

Reversed-phase high performance liquid chromatography was applied to the quantitative determination of a new β lactam antibiotic piperacillin. The procedure was applied to human plasma and cerebrospinal fluid samples. After an extraction of plasma or cerebrospinal fluid by dimethyl formamide, chromatographic separation was effected on a bonded hydrophobic stationary phase with one mobile phase : methanol-phosphate buffer. This assay was applied to study human pharmacokinetics on ten patients with purulent meningitis during their therapy. Simultaneous determination of piperacillin in plasma and cerebrospinal fluid one and two hours after piperacillin perfusion showed a good penetration of this antibiotic in human cerebrospinal fluid.

INTRODUCTION

Piperacillin sodium is a new semi-synthetic penicillin that possesses a broad spectrum of in vitro activity against gram-negative and gram-positive bacteria including anaerobes (1 - 2). The pharmacokinetics of this drug was described after bolus intravenous injection into healthy volunteers in which serum and urine concentrations were determined by a microbiological agar diffusion method using Sarcina lutea ATCC 9341 as the test organism (3).

The pharmacokinetic parameter of piperacillin sodium form indicates that piperacillin sodium possesses a small half-life (64 minutes), relatively large volume of distribution (22 liters) and low degree of protein binding (21 %). In animals (rats and mice)

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tissue distribution demonstrated highest concentrations in the liver and kidney, almost no drug penetrated to the brain. In man piperacillin sodium is excreted mainly in the urine and is unmetabolised (4-5-6). Evans (7) studies serum levels in eight adult volunteers who received a 15 mg per Kg dose intravenously over 3 minutes and found that two hours later the serum level was down to 12 µg per ml and six hours later to 1.5 µg per ml ; the same piperacillin dose was also given as a two hour infusion ; this level falls to an undetectable level six hours later. So it is clear that the limit of sensitivity of the bioassay (about 1 µg per ml) and its applicability don't permit it to be followed rapidly and during a long time for patient therapy. The penetration of the drug in human cerebrospinal fluid has not yet been investigated and it is important to know if piperacillin penetrates in front of its broad spectrum of activity.

MATERIAL AND METHODS

a) Chromatographic equipment

Analyses were performed on Waters Associates Liquid Chromatograph (Waters Assoc., Paris, France) equipped with a model 440 absorbance detector, a model 6000 A pump, a U6K universal injector and a 10 mV recorder.

b) Solvents and standards

Freshly distrilled deionized water was used thoughout the procedure. Methanol was analytical grade (Merck, Darmstadt, Germany). Piperacillin sodium was kindly given by Lederle (Lederle, Rungis, France). Phosphoric acid, di-natriumhydrogen phosphate and dimethyformamide were analytical grade (Merck, Darmstadt, Germany).

c) Chromatographic eluent

To 400 ml of methanol was added 500 ml of water, then 94 ml of a di-natrium hydrogenphosphat solution ($0.2\ M.l^{-1}$). The pH of the solution was ajusted to 6.5 with approximately 6 ml of 1 M. phosphoric acid and the volume of the solution was completed to one liter with water. The mobile phase was filtered through a 0.6 µm filter (Millipore Corp., Paris, France) and degassed using ultrasonics.

d) In vitro samples

Plasma Piperacillin sodium stock solution ($1\ mg.ml^{-1}$) was directly prepared in pooled human plasma and congeled in aliquots

at -80°C. Each day of analysis an aliquot was decongeled and diluted in pooled human plasma before use.

CSF Piperacillin sodium stock solution (100 $\mu g.ml^{-1}$) was directely prepared in pooled human CSF and congealed in aliquots at - 80°C. It was decongealed before and diluted with CSF for standardization.

e) In vivo samples

Ten patients with purulent meningitis were given 4 g of piperacillin sodium in 30 minutes'infusion each eight hours ; before the third infusion a blood was drawn, and two or one hour after the infusions' stop blood and CSF were drawn off. Each sample was immediatly centrifuged. Plasma and CSF were congealed in three aliquots at -80°C with the stock standard solution. Other patients with different therapy were also included for their monitoring in this study.

f) Chemical assay

- Extraction

500 µl of dimethylformamide (DMF) were added to 500 µl of plasma or CSF. The samples have been mixed, heated at 60°C for twenty minutes, then centrifuged for 10 minutes (at 2 000 g) and the supernatant passed through 0.22 µm filters and injected into the chromatograph.

- Chromatography

Piperacillin was quantitated on a reverse phase system. 25 µl of the extract was injected on to a µ- Bondapack C 18 (Waters) and eluent pumped through at 1.5 $ml.min^{-1}$. The absorbance detector was set at 254 nm at a sensitivity of 0.1 absorbance units full scale. Quantitation was based on peak heights recorded.Two standard curves were used : one on plasma and the other one for CSF.

RESULTS

Chromatographic separation

The retention time of piperacillin was 3.6 minutes in the described conditions. Piperacillin had the same retention time in plasma and CSF (FIG. 1). No interfering peaks were observed in our patients'plasma for aminoglycosides, thiamphenicol and chloramphenicol,

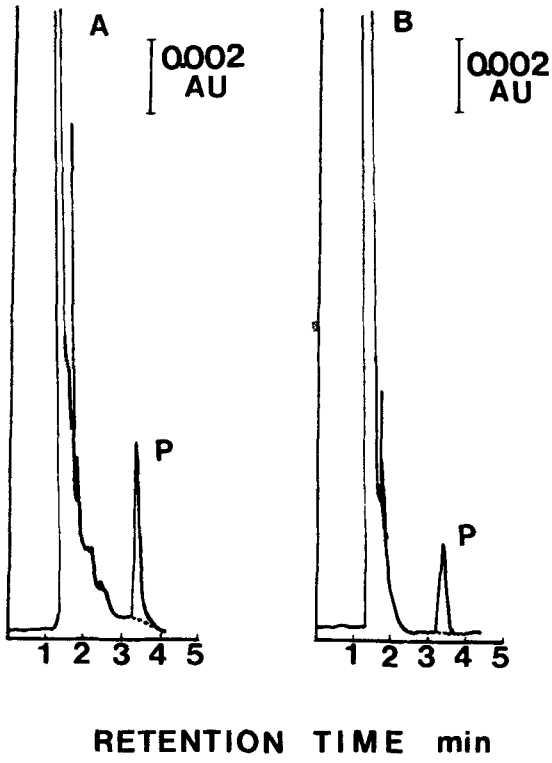

FIGURE 1

Chromatographic separation of Piperacillin in extracts of plasma (A) and cerebrospinal fluid (B). The dashed lines are for the blanks of plasma and CSF.
Column : μ Bondapak C_{18} (Waters)
Mobile phase : (Phosphate buffer : methanol, 60:40 V/V)
Flow rate : 1.5 ml/min. Sample volume : 25 μl for plasma or CSF extract.
Detector : 254 nm

vancomycin, ampicillin (drugs which were in plasma for patients'therapy). In these conditions we have not seen metabolites of piperacillin in human plasma or CSF. Also when we moved the retention time to 14 minutes by changing the mobile phase, we have not found metabolites more polar than the parent drug ; it was described in the literature for animals (8).

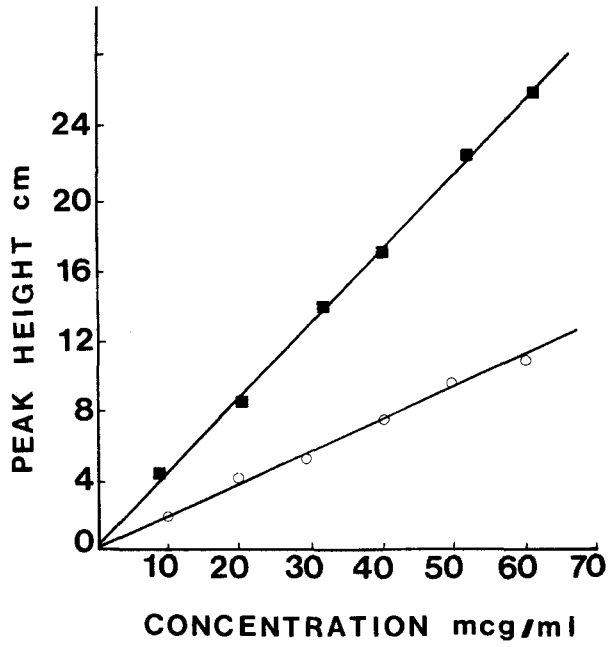

FIGURE 2

Standard curve for plasma (■) and CSF (o) piperacillin determinations. Chromatographic peak height expressed as a function of concentration in plasma.

Recovery

The two standard curves showed good linearity in the range studied (FIG. 2) recovery of the assay was tested using two procedures : by adding known amounts of drug to plasma or CSF of patients, and by dilution of elevated plasma and CSF of patients.

Sensitivity

The limit of sensitivity of the method was determined on pool plasma and CSF, and by dilution of known samples. The limit of detection could be evaluated to 0.5 µg/ml in the described method (peak height 4 mm). But if we slightly change the volume of injection (100 µl) and the sensitivity of the detector (0.05 U.A.) we were able to detect 0.1 $\mu g.ml^{-1}$ which is largely sufficient for clinical purposes.

TABLE I

Recovery of piperacillin from plasma and CSF

Amount added to plasma (µg/ml)	Amount added to CSF (µg/ml)	Amount measured* (µg/ml)	Recovery
2		1.9	95
5		5.5	90
10		9.5	95
20		22	90
30		31	97
	2	1.8	90
	5	5	100
	10	8.9	89
	20	21	95
	30	30.5	98

* Each value represents the mean of duplicate analyses.

TABLE II

Recovery of piperacillin from plasma and CSF by dilution

Plasma of patients ($\mu g.ml^{-1}$)		Dilution	Amount * measured ($\mu g.ml^{-1}$)	Recovery %
Plasma A	54	0	53	98
		1/2	26	96
		1/4	14	104
Plasma B	32	0	32	100
		1/2	15	94
		1/4	7	88
Plasma C	11	0	11	100
		1/2	5.5	100
		1/4	2.5	90
CSF of patients ($\mu g.ml^{-1}$)				
CSF A	30	0	30.5	101
		1/2	15	100
		1/4	7	93
CSF B	18	0	17	95
		1/2	9	100
		1/4	5	111
CSF C	12.8	0	12.5	98
		1/2	6.5	101
		1/4	3.5	109

*Each value represents the mean of duplicate analyses.

Precision

Intra-assay variation was studied on three plasmas and CSF which where determined ten fold on the same day. In plasma for a concentration of 34 $\mu g.ml^{-1}$ the coefficient of variation was 2.5 % and for 18 $\mu g.ml^{-1}$ it was 4 % and 5.3 % for 8 $\mu g.ml^{-1}$. In CSF for a concentration of 22 $\mu g.ml^{-1}$ the coefficient of variation was 3.5 % and 4.2 % for 10 $\mu g.ml^{-1}$ it was and 6 % for 5.4 $\mu g.ml^{-1}$.

Inter-assay variation was calculated on the same plasmas and CSF which were stored at -80°C and assayed ten times at different days. For plasma of 34, 18, 8 $\mu g.ml^{-1}$ the interassay variation was respectively 5.1 %, 6 %, 7.2 % and for CSF of 22, 10, 5.4 $\mu g.ml^{-1}$ the interassay variation was respectively 4.5 %, 5 %, 3.5 %. (The coefficient of variation was defined with one standard deviation).

TABLE III

Piperacillin values in plasma and CSF after an infusion (30 minutes, 4 grammes)

Patient	Residual value in in plasma before the infusion ($\mu g.ml^{-1}$)	Time of prelevment (after 30 minutes' infusion of 4 gr. piperacillin) ($\mu g.ml^{-1}$)	Amount measured in plasma ($\mu g.ml^{-1}$)	Amount measured in CSF ($\mu g.ml^{-1}$)
1	4	1 Hour	54	30
2	3	1 Hour	38	22
3	4	1 Hour	32	5
4	9 8	1 hour First prelevment 7 days later	43 72	2.5 2.5
5	17 4	1 hour First prelevment 10 days later	73 40	4 4
6	18 - 16	2 hours First prelevment 2 days later 12 days later	30 74 62	27 18 10
7	7 -	2 hours First prelevment 15 days later	40 30	4 12
8	4 8	2 hours First prelevment 12 days later	24 60	14.8 12.8
9	3	2 hours	3	0.8
10	3 4 3	2 hours First prelevment 2 days later 12 days later	30 16 11	6 6 4

Patient values

Each patient received 4 gr of piperacillin in 30 minutes'infusion, each eight hours during their therapy which lasted a few weeks. At the beginning of the treatment after two infusions and before the beginning of the third infusion a residual plasma was drawn off ; then one or two hours later the infusions' stopped and a plasma and a CSF sample were drawn off. For a few patients their clinical evolution needed after several days another control of plasma and CSF (see table III)

DISCUSSION

The HPLC analysis for the determination of piperacillin is a simple, sensitive and reliable method. Its advantages over microbiological assays are evident because of its ability to differentiate between the various antibacterial active species in a sample : a situation which is very usual in clinical treatment of meningitis (9). The other advantage is the rapidity of the assay which could be done in less than five minutes for the chromatographic process and thirty minutes for the samples' extraction step. Also the good limit of sensitivity permits to monitor patients which have a low value in plasma and CSF. The dimethylformamide extraction of piperacillin from plasma and CSF is the most practical, althought for CSF the determination of piperacillin could be made directly on the sample ; other liquid-liquid extractions could be performed with a good recovery ethyl-acetate and chloroform-pentanol (3 : 1) give a good extraction coefficient which is>80 % which is similar for other ß-Lactamines (10-11-12).

Aqueous solution of piperacillin, plasma or CSF lose their activity rapidly between pH 6 to 8 (from 10 to 30 %) ; heating plasma or CSF at 60°C with dimethylformamide doesn't alter the stability of the drug and the surpernatant can stay at ambient temperature for at least a day.

The pharmacokinetics'parameters that we calculated with the values of table III, give an half-life of 2 hours 24 minutes, a volume of distribution of 66 liters ; these parameters are in agreement with those of the literature (3-4-7). The mean value in CSF was the same at one or two hours (10 and 10.5 $\mu g.ml^{-1}$) and showed a good penetration of the drug in the cerebrospinal fluid. This preliminary study showed important individual variation that explained the necessity of determination of piperacillin in plasma and CSF for a correct clinical monitoring of patients with meningitis. This assay permitted such studies.

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