J. Pharm. Pharmac., 1974, **26**, 1–8 *Received June* 11, 1973

Plasma and cerebrospinal fluid concentrations of pentazocine in patients: assay by mass fragmentography

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Mass fragmentography has been used for the determination of low concentrations of pentazocine in blood plasma and cerebrospinal fluid (csf) after intravenous administration of a 30 mg dose to eight patients undergoing neurosurgery under general anaesthesia. A pharmacokinetic analysis based upon mean plasma levels indicated a half-life of 134 min. Lumbar csf levels of pentazocine increased rapidly with mean values from about 3 ng ml^{-1} at 5 min to 10 ng ml^{-1} at 30 min and to about 15 ng ml^{-1} at 90–120 min. The possibility of repeated analyses of drug concentrations in the csf represents an important step towards the correlation of chemical data with clinical effects for centrally acting drugs.

Blood concentrations of pentazocine in man after oral, rectal and parenteral administration have been studied in volunteers (Beckett, Taylor & Kourounakis, 1970b; Beckett, Kourounakis & others, 1970a; Berkowitz & Way, 1971; Burt & Beckett, 1971) and in post-operative patients (Berkowitz, Asling & others, 1969). None of these reports provides data on concentrations in the cerebrospinal fluid (csf). Since one of the main routes for access to the central nervous system from the blood is via the csf, we have measured the concentration of pentazocine in patients during neurosurgery using the technique of mass fragmentography which has recently been applied to the analysis of endogenous compounds and of drugs (see Holmstedt & Palmer, 1973). Blood plasma concentrations of the drug were simultaneously measured by the same technique.

MATERIALS AND METHODS

Source. Csf and blood plasma were taken from eight patients undergoing neurosurgery. Premedication and induction drugs and the anaesthestics used are listed with other patient data in Table 1. All patients were moderately hyperventilated. For patients VI–VIII the operation was performed under moderate hypothermia.

Pentazocine was given as an intravenous injection in a single dose of 30 mg as soon as sampling of the csf could be started which was about 3 h after induction. Blood samples were collected in heparinized glass tubes from an indwelling catheter in the radial artery immediately before the administration of pentazocine, and then if possible 5, 10, 15, 30, 60, 90, 120, 180 and sometimes 240 and 300 min after the pentazocine dose (Fig. 3). Samples of csf were obtained and taken simultaneously with

Table 1. *Patient data.*

	Patient			Blood transfusion administered between	Mannitol infusion† started at	Pentazoine c30 mg Administered at	Sampling of blood and csf between	Other drugs	
	Sex	Age	kg					Premedication	Anaesthesia
I	F	47	47	13·15–15·00 (1000 ml)		10·15	10·10–13·10	Atropine, droperidol, diazepam, dexamethasone	Enibomal, pancuronium, fentanyl
II	F	51	64	09·30–17·00 (2500 ml)	10·30	11·10	11·00–16·00	Atropine, droperidol, diazepam, dexamethasone	Enibomal, pancuronium, fentanyl
III	F	47	59		10·20	10·40	10·37–13·10	Atropine, droperidol, diazepam, phenytoin, dexamethasone	Phenoperidine, pancuronium, halothane
IV	M	63	59	11·00–13·00 (500 ml)		11·27	11·22–14·22	Atropine, droperidol, diazepam, hydrocortisone	Enibomal, pancuronium, phenoperidine
V	F	29	60		09·25	11·30	11·15–13·30	Atropine, droperidol, diazepam	Enibomal, pancuronium, halothane
VI‡	M	32	65	11·10–12·30 (1000 ml)	11·10	12·05	12·00–14·10	Atropine, chlorpromazine, phenytoin, dexamethasone	Thiopentalum, pancuronium, halothane
VII‡	F	26	70	11·15–11·45 (1000 ml)	10·45	11·45	11·43–14·45	Atropine, chlorpromazine, dexamethasone	Enibomal, pancuronium, fentanyl
VIII‡	F	38	55	11·20–12·55 (500 ml)	09·45	10·45	10·10–13·15	Atropine chlorpromazine	Enibomal, pancuronium, fentanyl halothane

† Infused 1–1·5 g kg^{-1} during 2 h.

‡ Hypothermia 28–30°.

the blood samples through an indwelling catheter placed percutaneously into the lumbar subarachnoid space before the operation. Plasma and csf samples were stored at −20° until analysed.

Mass fragmentography. An LKB model 9000 gas chromatograph-mass spectrometer (LKB-Produkter, Bromma, Sweden) was used. The separations were made on a 1 m × 3 mm (i.d.) silanized glass column, packed with 3% SE-30/100–120 mesh Gas-Chrom Q, maintained at a temperature of 210°. The flow rate of helium carrier gas was 25 ml min^{-1}. The ionizing potential and trap current were 50 eV and 60 μA, respectively. The temperature of the flash heater was 250° and of the ion source 250°. The instrument was used to obtain conventional spectra of reference compounds (Fig. 1), but was modified (Hammar & Hessling, 1971) by adding a new multiple ion detector (MID) to permit quantitation of nanogram amounts of pentazocine.

For quantitative mass spectrometry—mass fragmentography—the MID served as an ion specific detector for the gas chromatograph and was adjusted to record (Fig. 2) exclusively the intensity of three of the ions generated by the mass spectrometer, *viz.* *m/e* 202 and 217 (base peak) from pentazocine and *m/e* 230 (base peak) from cyclazocine (internal standard). Focusing was by adjusting the magnetic field to record *m/e* 217 with the accelerating voltage set at 3·5 kV; *m/e* 202 and 230 were brought into focus by a computer (PDP-12, Digital Equipment Corp., Maynard, Ma., USA) interfaced with the MID. The entrance and collector slits of the spectrometer were adjusted to 0·2 and 0·6 mm, respectively.

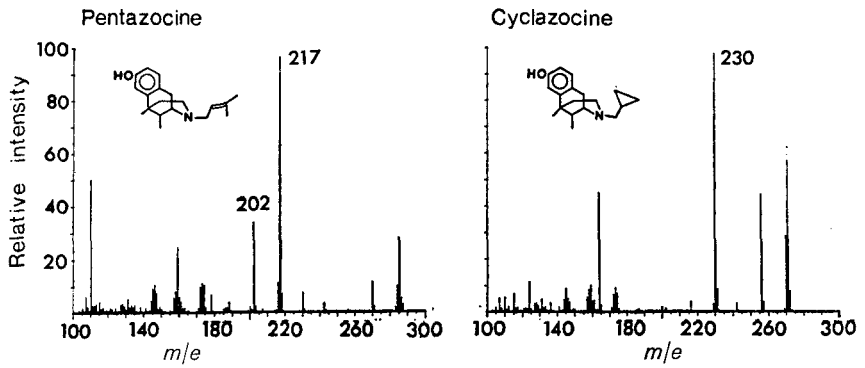

FIG. 1. Mass spectra and formulae of pentazocine and cyclazocine.

Procedure. To a 1·00 ml sample of csf or blood plasma in a glass stoppered tube was added 25 μl of methanol containing 10 ng (for csf) or 100 ng (for plasma) of cyclazocine as internal standard. After addition of 0·10 ml sodium bicarbonate-carbonate buffer pH 10·5, ionic strength 1·0, the sample was extracted (Borg & Mikaelsson, 1970) with 6·0 ml redistilled benzene (Fisher Spectroanalysed Benzene B-411, Fisher Scientific Co., Fair Lawn, New Jersey, USA). The recovery of pentazocine in the extraction was determined with [^{3}H]pentazocine. Five ml of the benzene phase was transferred to a silanized tube and the benzene was evaporated under a stream of nitrogen. The residue was dissolved in 0·1 ml methanol using an ultra-mixer. This solution was evaporated under nitrogen to a volume of 10–20 μl and an aliquot of this sample was analysed by mass fragmentography (Fig. 2).

Standard curves for the determination of pentazocine in csf and plasma were prepared by adding known amounts of pentazocine to samples of plasma and csf containing no pentazocine. The peak height ratio: pentazocine *m/e* 217, internal standard *m/e* 230 was plotted *vs* the pentazocine concentration.

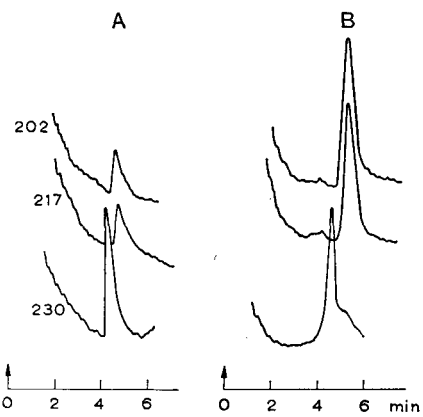

FIG. 2. Mass fragmentogram of pentazocine (*m/e* 202, 217) in csf from patient II. Cyclazocine (*m/e* 230) used as internal standard. Csf levels: A, 3·3 ng ml^{-1}; B, 8·2 ng ml^{-1}.

Calculation of pharmacokinetic parameters. The mean plasma concentrations (C_1) of pentazocine *vs* time following the rapid intravenous injection may be described by the biexponential equation:

$$C_1 = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$$

which can be interpreted in terms of a two compartment open model assessed by among others Riegelman, Loo & Rowland (1968). The rate constants of the model were calculated according to these authors as $k_e = 1/(A^1/\alpha + B^1/\beta)$; $k_{21} = A^1 \cdot \beta + B^1 \cdot \alpha$; $k_{12} = A^1B^1\ (\beta-\alpha)^2/k_{21}$; where $A^1 = A/(A + B)$ and $B^1 = B/(A + B)$. The parameters A, α, B, β were determined by a digital computer program based on non-linear least squares estimates by weighting amounts. The apparent volumes of the central compartment (V_1) and the peripheral compartment (V_2) were estimated by the following expressions (Wagner, Novak & others, 1968):

$$V_1 = \frac{D}{A + B} \quad V_2 = \frac{V_1 \cdot k_{12}}{k_{21}}$$

where D is the intravenous dose administered.

RESULTS AND DISCUSSION

Analytical method. Available gas chromatographic (Beckett & others, 1970) and combined ion-pair separation-fluorometric procedures (Borg & Mikaelsson, 1970) for the determination of pentazocine in body fluids are limited to levels above 20 ng ml^{-1}. Previous results (Gordon, Agurell & others, to be published) on csf levels of pentazocine in patients indicated that concentrations below this level could be expected. Measurement of these levels by the sensitive techniques of mass fragmentography was therefore investigated.

Analysis of interfering factors showed that a 1·00 ml csf or blood plasma samples with redistilled benzene gave negligible interference in chromatograms of control samples at the retention times of pentazocine and cyclazocine (int. standard) when focusing upon fragments *m/e* 202, 217 and 230. Standard curves, using peak height ratio pentazocine *m/e* 217/cyclazocine *m/e* 230, were linear in the range 2–30 ng ml^{-1} for csf samples and in the range 20–500 ng ml^{-1} for plasma samples. The specificity of the method was increased by the simultaneous recording of the *m/e* 202 fragment of pentazocine since the two fragments *m/e* 202 and 217 are formed in a certain ratio if derived only from pentazocine (Fig. 2).

Cyclazocine was selected as internal standard since it is structurally similar to pentazocine and has a suitable retention time. As little as 0·4–0·6 ng of underivatized pentazocine could be quantitated by mass fragmentography. Csf levels were estimated from 2 ng ml^{-1} with a standard deviation (n = 4) of $\pm 12\%$ in the low range 2–5 ng ml^{-1}, and $\pm 7\%$ (n = 4) above 10 ng ml^{-1}. Plasma samples were estimated with a standard deviation of $<7\%$. The recovery of pentazocine in 6·00 ml benzene was 92% from 1·00 ml plasma and 94% from 1·00 ml csf. The alcohol metabolites which have much longer retention times than pentazocine do not interfere with the method. This is also supported by work with an ion-pair fluorimetric procedure (Paalzow & Arbin, 1972), in which metabolites do not interfere and which gave results congruent with the gc-ms findings.

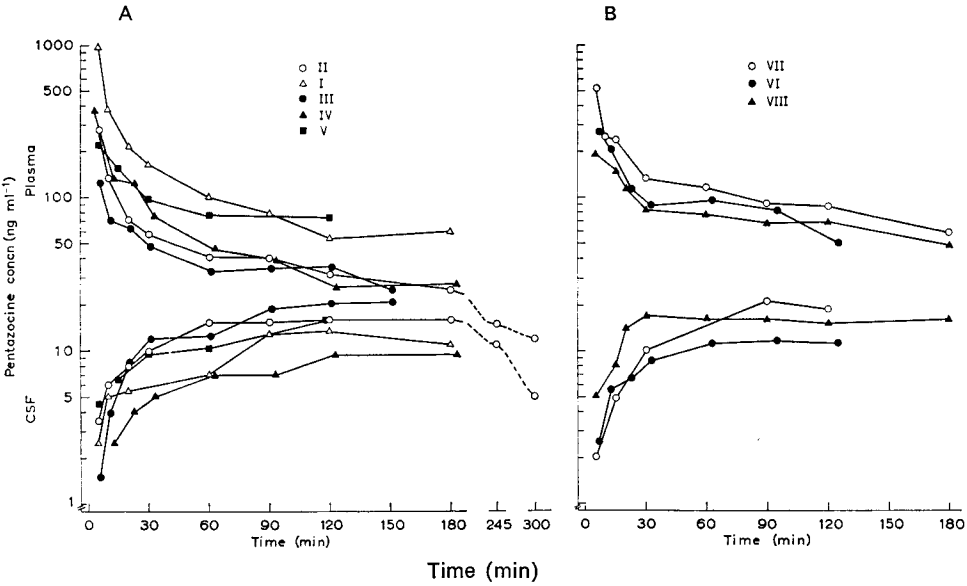

FIG. 3. Plasma and csf concentrations of pentazocine after i.v. administration of a 30 mg dose. A. Patients under normothermia. B. Patients under controlled hypothermia (rectal temp. 28–30°).

Plasma levels. Fig. 3 A–B shows the plasma and csf concentrations of pentazocine in the eight patients following intravenous injection of a 30 mg dose. Although it is known that the effect of a drug can be prolonged due to a decreased rate of its biotransformation at low temperatures (for review see Fuhrman & Fuhrman, 1961), the data do not suggest a difference in pentazocine levels between the five patients (Fig. 3A) who were operated upon under normothermia and the three (Fig. 3B) under controlled hypothermia with a rectal temperature of 28–30°.

The mean plasma and csf values are similar to those found in a much larger patient survey (Gordon, Agurell & others, to be published). Similar pentazocine plasma concentrations after i.v. administration (20–25 mg/70 kg) have been reported earlier by Berkowitz & others (1969).

Blood concentrations have also been investigated after administration of pentazocine by intravenous injection and other routes by Beckett & others (1970a, b), Mitchard (1971) and Burt & Beckett (1971) with volunteers, in whom the urinary pH was maintained acid by ammonium chloride.

Although in these latter studies *blood* levels rather than *plasma* levels were assayed, the present results are comparable since Ehrnebo, Agurell & others (to be published) have shown that pentazocine distributes about evenly between plasma and blood cells. Our results are in general agreement with these studies except that the fluctuations in pentazocine levels are less in our study in spite of administration of i.v. fluids and other drugs (Table 1). Burt & Beckett (1971) have suggested that the fluctuations may be due to biliary recirculation. This factor is probably of less importance in anaesthetized patients than in young healthy volunteers.

Pharmacokinetic interpretation. Preferably, pharmacokinetic analysis should be carried out using data from individual subjects. However, average plasma concentrations have been used (*e.g.* Wagner, Aghajanian & Bing, 1968; Dittert, Griffen & others, 1969) with satisfactory results. In the present study, which covers a 3 h period after administration, mean values were better accommodated to the digital computer pro-

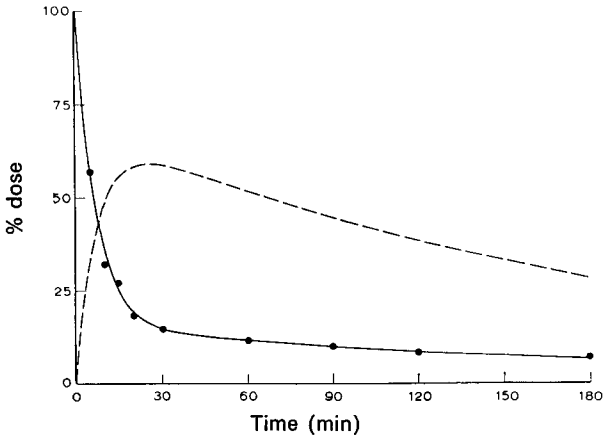

FIG. 4. Analogue computer curves of amounts of pentazocine in central (———) and peripheral (- - - -) compartment following i.v. administration (k_{12} = 0·0969 min^{-1}, k_{21} = 0·0273 min^{-1} and k_e = 0·0278 min^{-1}). The mean experimental values are indicated by ●.

gram than the individual data. By using these mean plasma values it was possible to estimate the parameters in the bi-exponential equation as follows (t in min):

$$C_1(\text{ng ml}^{-1}) = 553 \cdot e^{-0.147 \cdot t} + 102 \cdot e^{-0.00157 \cdot t}$$

for which there was good correlation between computed and experimental data (Fig. 4). The mean rate constants in the two-compartment open model were:

$$k_{12} = 0{\cdot}0969 \text{ min}^{-1},\ k_{21} = 0{\cdot}0273 \text{ min}^{-1} \text{ and } k_e = 0{\cdot}0278 \text{ min}^{-1}.$$

Apparently, k_{12} was greater than k_{21} and k_e which were almost in the same order. The average volume of the central compartment was 45·8 litres and the volume of the peripheral 162·6 litres, giving a total volume of distribution of 208 litres. The β-phase half-life (0·693/β) was 134 min which was in good agreement with the half-life reported (126 min) by Berkowitz & others (1969).

The maximum "tissue" concentration shown in Fig. 4 is obtained at 25 min and it is interesting to find that Berkowitz & others (1969) have reported that the maximum analgesia after intravenous pentazocine injections occurs after about 30 min. This suggests that the target for the pentazocine effect is situated in the peripheral rather than in the central compartment. We conclude that the two-compartment model may be a good starting point in the examination of pentazocine pharmacokinetics. Also, the first pass effect can be accounted for in the two-compartment model (Rowland, 1972).

Csf levels. By means of the present assay procedure it was possible to follow both the csf and plasma levels in eight patients. Fig. 3 shows that the csf levels increase rapidly, with mean values from about 3 ng ml^{-1} at 5 min to 10 ng ml^{-1} at 30 min, and then slowly to 15 ng ml^{-1} at 90–120 min. In general, the individual csf levels of pentazocine remain in the range of 7–18 ng ml^{-1} from 30–180 min. In one patient (II, Fig. 3A) the csf concentration was 15 ng ml^{-1} at 3 h, 11 ng ml^{-1} at 4 h and 5 ng ml^{-1} after 5 h. The csf curves showed only minor fluctuations in spite of the surgical procedures, the administration of intravenous fluids and the presence of other drugs (Table 1).

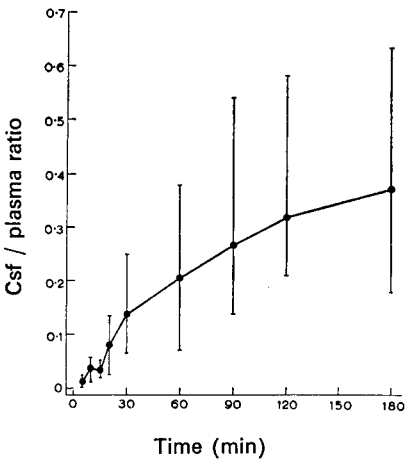

FIG. 5. Mean values and ranges of ratio csf/plasma concentration of pentazocine.

Ratio: csf levels/plasma levels. In the apparent distribution equilibrium of the β-phase, one would expect the same csf/plasma ratio as the ratio unbound/total pentazocine concentration in plasma. In fact, this was also found. As seen in Fig. 5, the csf/plasma ratio, which was initially very low, increases rapidly to about 0·14 after 30 min. Between 2–3 h the mean ratio is somewhat above 0·3 and within a rather narrow range (0·2–0·6). This agrees well with the value 0·3–0·4 for unbound/total pentazocine in plasma found by Ehrnebo & others (to be published).

The patients I, IV, VI, VII, who achieved the highest plasma levels, in general showed low and more slowly increasing csf levels. Conversely, the patients who also initially displayed low plasma levels showed rapidly increasing and high csf levels.

Drugs may penetrate from the blood into the csf through the secretion of the chloroid plexus, through a direct diffusion from the brain parenchyma or from the vessels of the pia. If the substance is highly lipid-soluble it will probably diffuse into the subarachnoid fluid more rapidly than into the ventricles (Brodie, Kurz & Schanker, 1960).

In the present study, csf was obtained from the lumbar subarachnoid space and pentazocine was already present in the sample taken 5 min after injection of the drug. It is reasonable to assume that the pentazcoine found in these csf samples entered the lumbar subarachnoid space directly from the blood perfusing the spinal canal, since pH and PCO_2 changes following abrupt alterations of ventilation occur in cisternal fluid at least 10–20 min before lumbal fluid changes (Fisher & Christensson, 1963).

Acknowledgements

The mass spectrometric work was supported by the following grants to the Department of Toxicology: The Swedish Medical Research Council (70E-3743, 14X-4041, 40Y-2375), the Bank of Sweden Tercentenary Fund (68/53), the Wallenberg Foundation and the National Institute of Mental Health (MH 12007). The authors wish to thank Dr. S. O. Nilsson who assisted in programming the computers.

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