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Letter to the Editor

High-performance liquid chromatographic determination of dexamethasone in cerebrospinal fluid and plasma in the rabbit

Sir,

Little information concerning the pharmacokinetics of dexamethasone (DXM) is available in the recent literature [1,2]. High-performance liquid chromatographic and radioimmunological methods have been reported for measuring DXM in plasma samples [3–7]. This paper describes a method for the investigation of the penetration of DXM into the central nervous system in the rabbit after a single intravenous administration.

EXPERIMENTAL

Materials

Acetonitrile, dichloromethane and methanol, all of analytical-reagent grade, and acetic acid were from Merck (Darmstadt, F.R.G.). Water was double-distilled. A 0.002 *M* acetate buffer (pH 4.8) was used. DXM and 11-deoxycortisol (11-DOC) were from Steraloïds (Wilton, NY, U.S.A.).

Apparatus

A Varian Model 5000 liquid chromatograph (Varian, Sunnyvale, CA, U.S.A.) equipped with a Kratos Spectroflow 783 variable-wavelength UV detector (Kratos, Ramsey, NJ, U.S.A.) was used in a reversed-phase system with a C_8 column (LiChrospher 100 CH-8 II, 5 μm, 250×4 mm I.D., Merck) linked to a C_8 precolumn (Hibar LiChroCART, 25×4 mm I.D., Merck). The injection volume was 20 μl. The flow-rate of the mobile phase, acetate buffer–acetonitrile (58:42), was 1.2 ml/min. The UV detection wavelength was 246 nm, and the sensitivity scale was 0–0.01 or 0–0.002 a.u.f.s.

Preparation of reagents and standard

Standard solutions (1 mg of drug per litre of methanol) were made of DXM alcohol and 11-DOC (internal standard). Dilutions were made up in drug-free

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plasma and cerebrospinal fluid (CSF) to provide concentrations of 1250–10 000 μg/l (plasma) and 125–500 μg/l (CSF) for DXM.

Extraction of DXM from CSF and plasma samples

DXM was determined by an adaptation of our method [4]. In a 1.5-ml cone-shaped polystyrene tube, 11-DOC (10 μl for CSF and 50 μl for plasma of the standard solution) was added to the drug-containing CSF (50 μl) or plasma (20

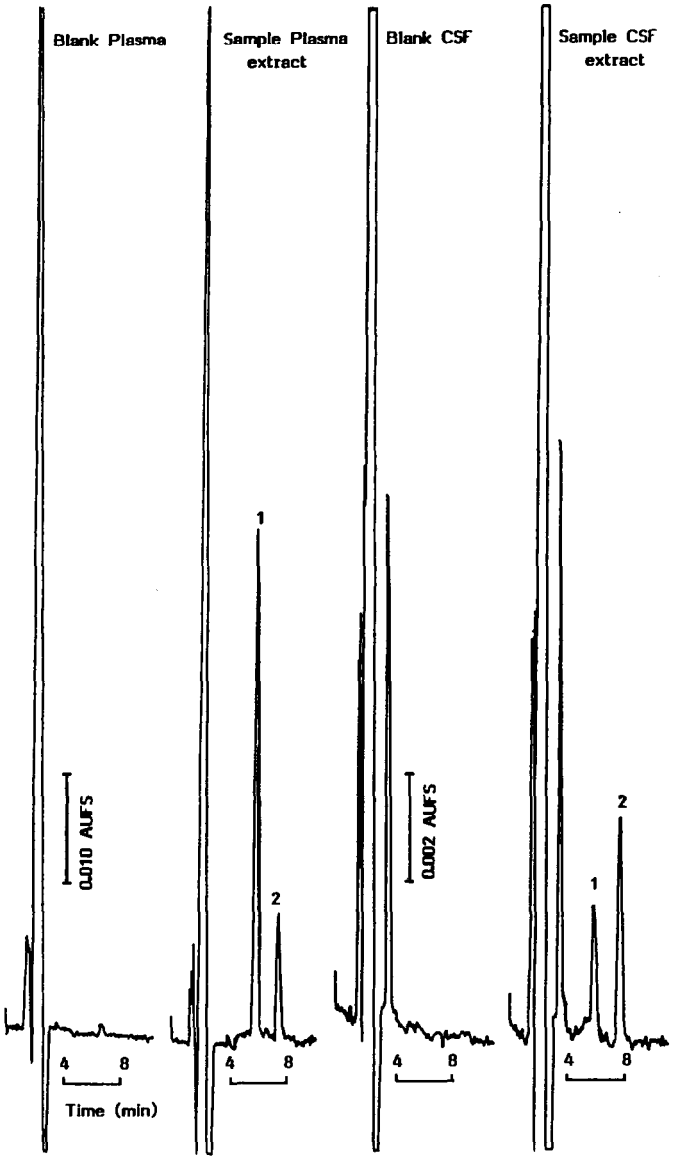

Fig. 1. Chromatograms of extracts from drug-free plasma and CSF samples and simultaneous plasma and CSF samples after intravenous administration of DXM to rabbits. Peaks: 1 = DXM (9265 ng/ml in plasma, 114.7 ng/ml in CSF); 2 = 11-DOC (internal standard).

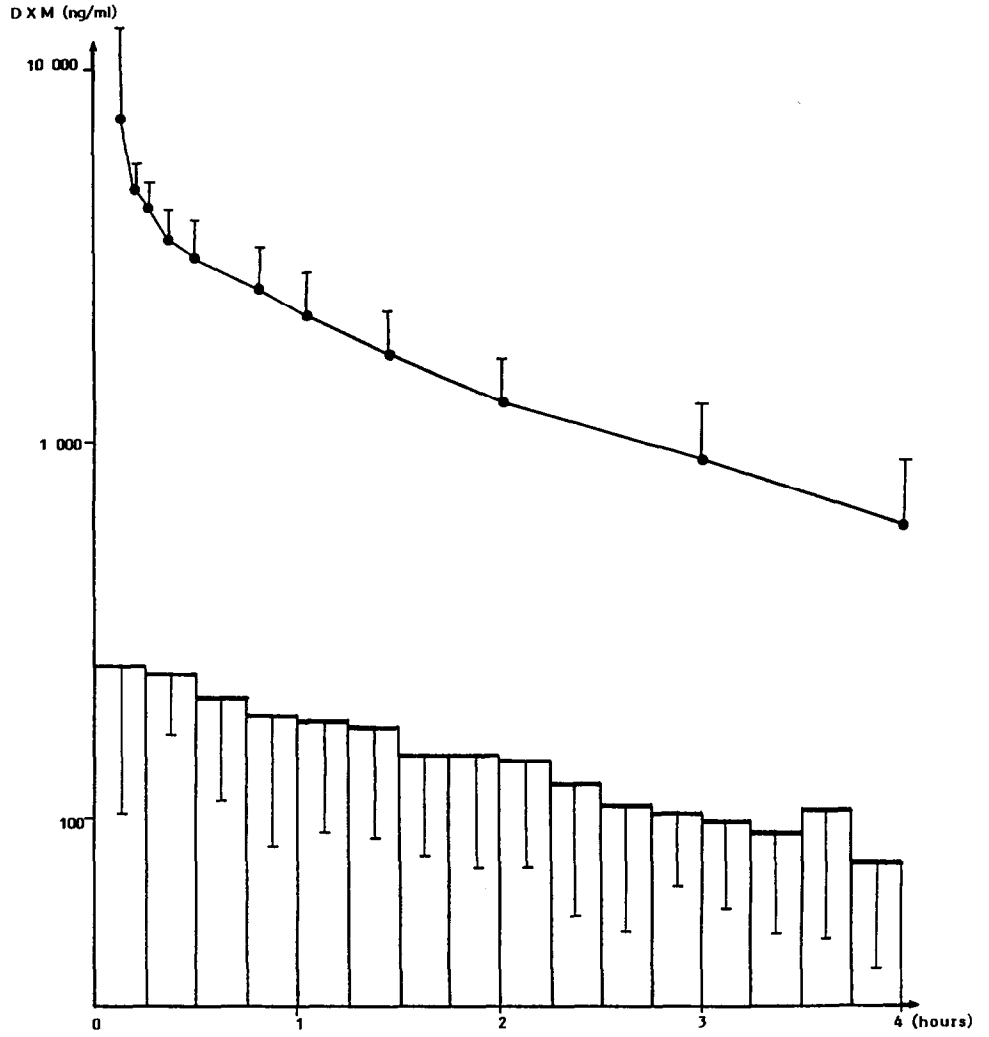

Fig. 2. Total (●) and CSF (bars) concentrations (mean ± S.D.) in six rabbits after intravenous injection of DXM (2 mg/kg).

μl) for assay. The mixtures were vortex-mixed for 30 s with 1000 μl of dichloromethane and centrifuged 5 min at 2600 *g*.

The organic extracts were evaporated to dryness in glass evaporation tubes at 60 °C under a stream of nitrogen, reconstituted with methanol (30 μl for CSF and 100 μl for plasma) and vortex-mixed for 30 s before injection.

Disposition of DXM in the rabbit

We have studied the pharmacokinetics of DXM in six curarized rabbits (Fauve de Bourgogne). CSF samples were continuously taken from the third ventricule and collected every 0.25 h for 4 h [8]. Blood samples (0.5 ml) were collected at 0, 0.083, 0.166, 0.250, 0.333, 0.5, 0.75, 1, 1.5, 2, 3 and 4 h with a femoral arterial catheter.

RESULTS AND DISCUSSION

As previously described [4], the optimal UV absorbance was set at 246 nm for DXM and was also approximately the maximum for 11-DOC. The retention times of DXM and 11-DOC were 5.8 and 7.2 min, respectively, and their resolution factor was greater than 1.0 (3.2). The peak symmetry was satisfactory. Fig. 1 illustrates chromatograms of extracts from drug-free plasma and CSF samples and biological samples from rabbit after intravenous administration of DXM. No interfering endogenous substances (i.e. cortisol and corticosterone) were expected in the drug-free plasma and CSF samples.

The extraction efficiency for DXM was always greater than 80% in the range 125–10 000 ng/ml. The relation between DXM concentration and peak-height ratio (vs. internal standard) was linear over the calibration curves for CSF and plasma. Correlation coefficients were always greater than 0.99.

The between-day variability, calculated by doing five replicate analyses of known standards, was 0.6% (relative standard deviation) at 125 ng/ml, 1.1% at 250 ng/ml, 1.2% at 500 ng/ml for CSF, and 3.9% at 1250 ng/ml, 1% at 5000 ng/ml, 2.2% at 10 000 ng/ml for plasma.

Fig. 2 shows CSF and plasma concentrations after intravenous administration of 2 mg/kg DXM (alcohol form) via the marginal ear vein. The mean concentration ranged from 7334 to 587 ng/ml in plasma and from 245 to 75 ng/ml in CSF.

CONCLUSION

The assay uses only a small amount of biological fluid (50 μl of CSF or 20 μl of plasma) so is particularly useful in animal experimentation. In the case of CSF, the secretion flow is always weak (10 μl/min on average for continuous sampling), and for plasma it is possible to obtain many samples without drastically changing the total blood volume.

The speedy preparation of the sample, suitable for analysis in series, allows sufficient purification of the extract and an extraction efficiency greater than 80%. With a repeatability of less than 5%, this technique is useful for pharmacokinetic studies of DXM in animals.

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