ENTRY OF LORAZEPAM INTO CEREBROSPINAL FLUID

Lorazepam is a 3-hydroxy-1,4-benzodiazepine derivative extensively used as a premedicant and induction agent in anaesthetic practice (Greenblatt & Shader, 1974). The rate and extent of lorazepam entry into brain tissue probably is an important determinant of its clinical action. The present study assessed concentrations of lorazepam in serum and cerebrospinal fluid (CSF) of patients receiving this drug as a pre-anaesthetic medication.

Seven patients scheduled for elective lower extremity orthopedic surgical procedures participated. They received a single 4 mg oral dose of lorazepam with a small amount of water 2 h before installation of regional lumbar spinal anaesthesia. Three ml of CSF was obtained at the time of lumbar puncture, together with a single venous blood sample drawn into an additive-free tube. Blood samples were allowed to clot, and the serum was separated. Serum and CSF samples were frozen until the time of assay.

Lorazepam concentrations in serum and CSF were determined by electron-capture gas-liquid chromatography after addition of oxazepam as the internal standard (Greenblatt, Franke & Shader, 1978). When sufficient serum remained, the extent of lorazepam binding to serum protein was determined by equilibrium dialysis (Woo & Greenblatt, 1979) after samples were spiked to contain 200 ng lorazepam ml⁻¹.

Lorazepam produced clinically satisfactory sedation in all patients. One elderly individual developed respiratory insufficiency approximately 1 h after lorazepam dosage, and required assisted ventilation. This emphasizes the need for close monitoring of cardiopulmonary function in the elderly following administration of central depressant drugs.

Table 1 shows lorazepam concentrations in serum

and CSF and the extent of lorazepam protein binding. CSF concentrations ranged from 3–8 ng/ml, and in all cases were considerably less than those in serum (range: 16–45 ng/ml). The unbound fraction in serum ranged from 10 to 16% and in four of six cases was nearly identical to the CSF: serum concentration ratio. In two patients, the free fraction underestimated the CSF: serum ratio, possibly due to contamination of CSF with small amounts of blood.

Our assessment of lorazepam entry into CSF was based on single samples taken 2 h after an oral dose. The results may not reflect steady-state conditions, since diffusion of lorazepam from serum into CSF could have been incomplete (Aaltonen, Karto & Salo, 1980). A previous animal study of diazepam entry into CSF indicated that equilibration between plasma and CSF following a single intravenous dose of diazepam was almost instantaneous (Greenblatt, Ochs & Lloyd, 1980). Rapid CSF uptake has also been observed with quinidine, another lipophilic basic drug (Ochs, Greenblatt, Lloyd, Woo, Sonntag & Smith, 1980).

As in the case of other benzodiazepines, CSF concentrations of lorazepam were considerably lower than those in serum. The CSF to serum concentration ratio approximated the unbound fraction in serum, suggesting that CSF uptake occurs by passive diffusion and is limited by the unbound fraction in serum. If brain concentrations are proportional to those in CSF (Ramsay, Hammond, Perchalski & Wilder, 1979), unbound rather than total serum concentrations are more likely to reflect levels at the site of action.

We are grateful for the assistance of Elke Moritz, Monika Linden, Ann Locniskar, and Lawrence J. Moschitto.

Table 1 Serum and CSF lorazepam concentrations

Patient	Serum concentration (ng/ml)	CSF concentration (ng/ml)	CSF/serum ratio	% unbound in serum
2	29.0	3.5	0.120	11.0
3	36.2	6.8	0.188	16.4
4	15.5	4.4	0.284	10.2
5	37.0	7.0	0.189	9.8
8	28.8	2.8	0.098	11.6
19	45.2	8.1	0.179	—
20	26.0	4.0	0.154	12.9
Mean	31.0	5.2	0.173	12.0
(±s.d.)	(±9.6)	(±2.0)	(±06)	(±2.4)

This work was supported in part by Grant Oc 10/3 from Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, West Germany, and by Grant MH-12279 from the United States Public Health Service.

HERMANN R. OCHS, JORG BUSSE, DAVID J. GREENBLATT & MARCIA DIVOLL ALLEN

The Medizinische Universitätsklinik, Bonn; the Department of Anaesthesia, University of Koln, West Germany; and the Division of Clinical Pharmacology, Tufts-New England Medical Center, Boston, MA.

Received April 15, 1980

References

- AALTONEN, L., KANTO, J. & SALO, M. (1980). Cerebrospinal fluid concentrations and serum protein binding of lorazepam and its conjugate. *Acta Pharmac. Toxi.* **46**, 156–158.
- GREENBLATT, D.J., FRANKE, K. & SHADER, R.I. (1978). Analysis of lorazepam and its glucuronide metabolite by electron-capture gas-liquid chromatography: use in pharmacokinetic studies of lorazepam. *J. Chromatog.* **146**, 311–320.
- GREENBLATT, D.J., OCHS, H.R. & LLOYD, B.L. (1980). Entry of diazepam and its major metabolite into cerebrospinal fluid. *Psychopharmacology*, (in press).
- GREENBLATT, D.J. & SHADER, R.I. (1974). *Benzodiazepines in Clinical Practice*. New York: Raven Press.
- OCHS, H.R., GREENBLATT, D.J., LLOYD, B.L., WOO, E., SONNTAG, M. & SMITH, T.W. (1980). Entry of quinidine into cerebrospinal fluid. *Am. Heart J.*, (in press).
- RAMSAY, R.E., HAMMOND, E.J., PERCHALSKI, R.J. & WILDER, B.J. (1979). Brain uptake of phenytoin, phenobarbital, and diazepam. *Arch. Neurol.*, **36**, 535–539.
- WOO, E. & GREENBLATT, D.J. (1979). Pharmacokinetic and clinical implications of quinidine protein binding. *J. pharm. Sci.*, **68**, 466–470.

AN IMPROVED EXTRACTION PROCEDURE FOR THE LIQUID CHROMATOGRAPHIC DETERMINATION OF PROPRANOLOL AND 4-HYDROXYPROPRANOLOL IN PLASMA

The β -adrenergic receptor blocking agent propranolol and its active metabolite, 4-hydroxypropranolol, have been determined by direct fluorimetry, electron capture GLC, mass fragmentometry, radioimmunoassay and more recently by HPLC, mostly using fluorimetric detection. Table 1 reviews the different extraction procedures, column types and detection specifications used for the quantification of propranolol. As shown only two of the author-teams proceed to the estimation of 4-hydroxypropranolol: Mason, Amick & Weddle (1977) and Taburet, Taylor, Mitchell, Rollins & Pool (1979). Krol, Mannan, Pickering, Amato, Kho & Sonnenschein (1977) report, yet without any reference to plasma extraction, the much higher sensitivity for the propranolol and 4-hydroxypropranolol determination utilizing a fluorimeter set at 210 nm excitation-wavelength. In our experiments we tried out these different methods but none permitted us to produce, with our HPLC system, chromatograms free from interfering peaks. However, by using the different extraction procedures for propranolol and 4-hydroxypropranolol described below, such chromatograms can be readily obtained.

Our separations were performed on a RSIL C 18 HL 10 μ m reversed phase column (150 mm $\times$ 3.2 mm). As mobile phase a mixture of 60

volumes of methanol, 39 volumes of water and 1 volume of glacial acetic acid containing 0.005 M heptane sulfonic acid sodium salt was used. The high performance liquid chromatograph used consisted of a Waters Model 6000 A solvent delivering system, a Waters fluorescence detector with mercury lamp and a Valco syringe loading sample injector, Model CV-6-UHPa-N60. The flow is set at 1.3 ml/min.

For both the compounds an interference filter of 254 nm was used for excitation. A type 360 filter was selected for the analysis of propranolol and a type 425 filter for determination of the 4-hydroxymetabolite. For the quantification of propranolol this procedure was followed: To 1 ml of plasma in a conical centrifuge tube 0.64 μ g internal standard (4-methylpropranolol) was added and 400 μ l phosphate buffer solution (0.1 M, pH 7.5). After extraction with 7.8 ml of a mixture of 10 volumes *n*-butanol and 90 volumes chloroform the tube was centrifuged for 10 min at 4000 rev/min. The aqueous phase was withdrawn and the organic phase transferred as completely as possible to a clean conical test tube and evaporated to dryness under a gentle current of nitrogen. The residue is dissolved in 100 μ l of 0.2% sulphuric acid and 100 μ l methylethylketone were added. After intense mixing for 5 s, the mixture was centrifuged for 2 min at 4000 rev/min, and 50 μ l of