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## Note

# Determination of ranitidine in rat plasma and brain by high-performance liquid chromatography

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Increasing clinical experience with ranitidine indicates that alteration of the central nervous system (CNS) function may be detectable with this recent $H_2$-receptor antagonist [1–5] as with cimetidine [6–8]. Clinically, this action may result in mental confusion and hallucinations by mechanisms that are only partly understood. Attempts to clarify these adverse effects may be facilitated by information on the disposition of ranitidine, particularly its distribution in the CNS. Kinetic investigations in man have been obviously limited to the determination of the plasma-to-cerebrospinal fluid (CSF) concentration ratio of the drug [9], which provides only a partial picture of the drug's ability to penetrate and diffuse into the CNS. Preliminary reports on tissue distribution of radiolabelled ranitidine in animals have appeared [10], but no information is available about this aspect of the drug disposition.

Development of the high-performance liquid chromatographic (HPLC) procedure described here for the quantitation of the drug in biological samples has permitted the investigation of the relationships between plasma concentrations and brain distribution of ranitidine in the rat.

## EXPERIMENTAL

### *Chemicals*

Ranitidine was kindly suplied by Glaxo (Verona, Italy). The internal standard (AH 20480) [11] was synthesized by the Chemistry Division of Glaxo Group Research (Ware, U.K.). Acetone, chloroform, formic acid and *n*-heptane (Carlo Erba, Milan, Italy) were of analytical-reagent grade.

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*Apparatus*

HPLC was carried out on a Waters system (Waters Assoc., Milford, MA, U.S.A.) equipped with a Model U6K universal liquid injector, a Model 6000A solvent delivery system and a reversed-phase column ($\mu$Bondapak $C_{18}$, 30 cm $\times$ 3.9 mm I.D.) at room temperature. The mobile phase was 0.075 *M* phosphate buffer–acetonitrile (85:15) at a flow-rate of 1.5 ml/min. Compounds eluted from the column were detected with a Model 440 UV detector monitor at 313 nm.

*Animals and treatments*

Male CD-COBS rats (Charles River, Como, Italy), average weight 200 g, were used. They were dosed with ranitine hydrochloride (10 mg/kg) and subsequently killed by decapitation. Each group consisted of four rats. Blood samples were collected in heparinized tubes at various times (1–240 min) after drug administration, centrifuged, and the plasma was stored at $-20°C$. Brains and spinal cords were removed immediately and quickly frozen in dry-ice after blotting with paper to remove excess surface blood. Brain areas were dissected according to Glowinski and Iversen [12].

*Procedure*

To 0.05–2 ml of blood or heparin-treated plasma, 0.1 ml of a solution of AH 20480 (4 $\mu$g/ml) and 0.5 ml of 0.1 *M* sodium hydroxide solution were added. The samples were then extracted twice with 10 ml of chloroform by shaking for 15 min on an automatic shaker. After centrifugation, the organic phase was separated and evaporated to dryness. The residues were dissolved in 0.1 ml of the mobile phase and 25–75 $\mu$l were injected into the HPLC instrument.

Brains, brain areas and spinal cord were homogenized (6 ml/g) in cold acetone–1 *M* formic acid (85:15, v/v) and centrifuged at 10 000 *g* for 15 min. The supernatant was shaken twice with *n*-hepane–chloroform (4:1, v/v), the organic phase was discarded and the aqueous phase was used for drug extraction as described for plasma.

*Internal standard calibration graph*

Drug-free plasma, blood and brain homogenate samples containing known amounts of ranitidine were analysed concurrently with each set of unknown samples. The range of the standards was chosen to cover the concentrations of ranitidine likely to be present in plasma and brain (determined in a pilot study) after an oral and an intravenous (i.v.) dose of 10 mg/kg of ranitidine hydrochloride. The concentrations of ranitidine in blood, plasma and brain tissue were determined from a graph where the peak-height ratios of the drug to the internal standard were plotted against the concentration of known standards. Percentage recoveries were calculated by comparing the peak-height ratios of ranitidine after plasma and brain extraction with the peak-height ratios obtained by direct injection of standard solutions of the drug.

*Kinetic analysis*

The terminal elimination rate constants ($\beta$) were calculated by linear least-squares regression of the log (plasma and brain concentration) versus time graph

utilizing the data points in the terminal log-linear region of the plasma and brain concentration–time curves. The plasma and brain elimination half-lives ($t_{1/2}$) were determined from $\beta$ by the relationship $t_{1/2}=0.693/\beta$. The areas under the plasma and brain concentration–time curves (AUC) were calculated by the trapezoidal rule from the last measured concentration, then extrapolated to infinity [13].

The concentration of ranitidine in the brain homogenate had to be corrected for the drug contribution from the residual blood. By using a value of 11 $\mu$l as the residual blood per gram of brain and determining the blood-to-plasma ratio ($0.9 \pm 0.1$, $n=8$), it was possible to determine the amount of ranitidine that had to be subtracted from the amount determined in the brain [14].

## RESULTS AND DISCUSSION

Several methods based on high-voltage paper electrophoresis [15], radioimmunoassay [16] and $^{14}C$-labelled compound procedures [10] and HPLC techniques [11,17,18] have been developed for the determination of ranitidine in biological samples. However, most of these methods, including the HPLC procedures, have only been applied to body fluids and there appear to have been no reports on the brain tissue analysis of ranitidine using a specific procedure with relatively simple sample preparation. We have standardized an HPLC procedure for the measurement of ranitidine in either plasma or brain tissue and have simultaneously evaluated the kinetics of ranitidine in plasma and brain tissue of animals.

The method was selective for ranitidine; there were never any interfering peaks in the rat plasma and brain samples that we investigated, with either ranitidine or the internal standard. Fig. 1 shows typical chromatograms of extracts from drug-free (A) plasma and (D) homogenized brain, (B) plasma and (E) brain spiked samples and (C) plasma and (F) brain of a rat treated with ranitidine hydrochloride. The retention times were 7.2 and 12.5 min for ranitidine and the internal standard, respectively.

The chloroform extraction procedure with the evaporation, centrifugation and sample transfer steps resulted in recoveries of 73–91% and 70–85% of ranitidine from plasma samples and brain homogenates, respectively. Calibration graphs for each biological sample were prepared daily using known concentrations of ranitidine; inter-assay coefficients of variation (C.V.) were routinely between 3 and 10% utilizing either plasma or brain homogenate samples. The extracted drug concentration was linear over a range of 10–500 ng/ml in plasma and 25–500 ng/g in brain. The limit of detection was 10 ng/ml or better for plasma and 25 ng/ml for brain, extracting 2 ml of plasma and approximately 1 g of brain tissue. The limit of detection of the drug required a detector range setting of 0.01 a.u.f.s. The signal-to-noise ratio was 3 or more under these experimental conditions.

The plasma concentration–time curves of ranitidine after i.v. and oral doses of 10 mg/kg ranitidine hydrochloride to rats are shown in Fig. 2. The data at each time are the means for four rats. After i.v. injection the drug disappeared rapidly from plasma with an overall $t_{1/2}$ of 52 min. When given orally ranitidine reached

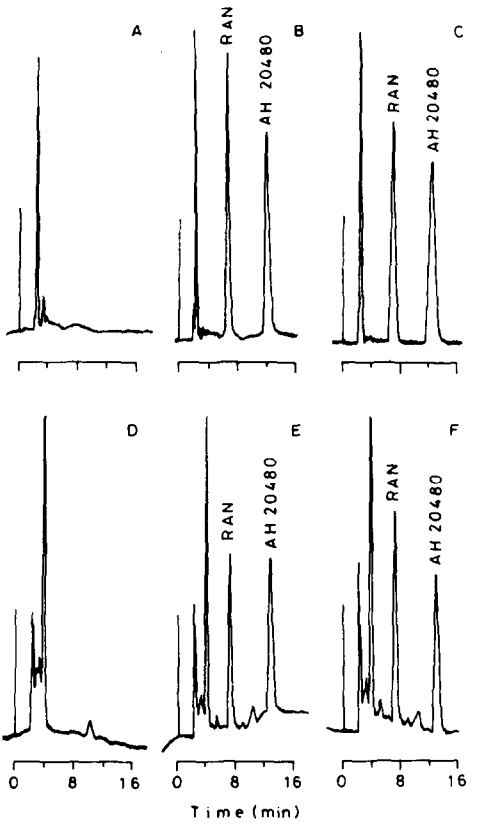


Fig. 1. Typical chromatograms of extract from drug-free (A) plasma and (D) brain, (B) plasma and (E) brain samples spiked with ranitidine (RAN) and (C) plasma and (F) brain of ranitidine-treated (10 mg/kg, i.v.) rats. AH 20480 = internal standard.

peak plasma concentrations ($C_{max}$) at 60 min ($0.42 \pm 0.05$ $\mu$g/ml) and then declined with a $t_{1/2}$ (65 min) comparable to that after i.v. injection. The bioavailability of the unchanged drug, calculated as the ratio of the AUC values after oral (56 $\mu$g/ml·min) and i.v. (144 $\mu$g/ml·min) ranitidine hydrochloride (10 mg/kg) was about 39%. This was apparently due to large-scale pre-systemic metabolism of this drug, as previous studies with $^{14}$C-labelled ranitidine have shown that it is well absorbed from the gastrointestinal tract of rats and other animal species [10].

Brain concentrations of ranitidine after i.v. and oral dosing are compared in Table I. The concentrations were corrected for the ranitidine contributed by the residual blood, as specified earlier. After i.v. injection ranitidine rapidly entered and equilibrated with plasma. Brain $C_{max}$ levels were reached 1 min after injection, representing about 2% of the plasma concentrations. Hence the brain-to-plasma concentration ratios showed a small increase over the first 30 min, then remained relatively constant at approximately 0.1. The brain $t_{1/2}$ (49 min) was comparable to the plasma $t_{1/2}$, at least in relation to the whole-brain concentra-

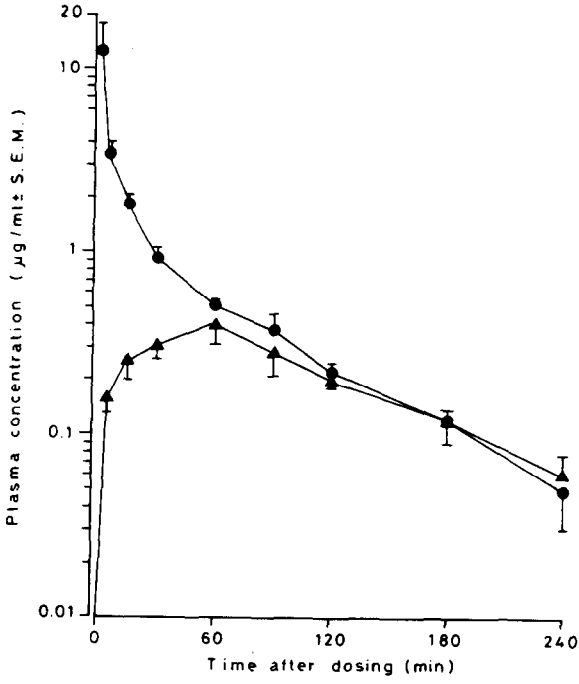


Fig. 2. Plasma concentration versus time curves for ranitidine after i.v. (●) and oral (▲) administration of ranitidine hydrochloride (10 mg/kg) to rats. Each value is the mean ± standard error of the mean for four rats.

TABLE I

BRAIN CONCENTRATIONS OF RANITIDINE AFTER INTRAVENOUS AND ORAL DOSES OF RANITIDINE HYDROCHLORIDE TO RATS

Each value is the mean ± standard error of the mean for four rats. The brain-to-plasma ratios are shown in parentheses. At 10 mg/kg (p.o.) the drug was not determinable (N.D.) in rat brain within the limits of sensitivity of the method.

| Time after administration (min) | Brain concentration (μg/g) | |
|---|---|---|
| | 10 mg/kg (i.v.) | 10 mg/kg (p.o.) |
| 1 | 0.29 ± 0.05 (0.02) | <0.025 (N.D.) |
| 5 | 0.22 ± 0.01 (0.06) | |
| 15 | 0.14 ± 0.02 (0.08) | |
| 30 | 0.11 ± 0.01 (0.12) | |
| 60 | 0.07 ± 0.01 (0.14) | |
| 90 | 0.05 ± 0.01 (0.14) | |
| 120 | 0.03 ± 0.01 (0.14) | |
| Area under the curve (μg/g·min) | 12.5 (0.09) | N.D. |

TABLE II

DISTRIBUTION OF INTRAVENOUS RANITIDINE (10 mg/kg) IN BRAIN REGIONS AND SPINAL CORD OF RATS

Rats were killed 30 min after injection of the drug (10 mg/kg). Each value is the mean ± standard error of the mean of three to five determinations (pool of three rats). The tissue-to-plasma ratios are shown in parentheses.

| Tissue | Concentration (μg/g) |
|---|---|
| Spinal cord | 0.12 ± 0.03 (0.13) |
| Cortex | 0.11 ± 0.04 (0.12) |
| Striatum | 0.11 ± 0.04 (0.12) |
| Brainstem | 0.08 ± 0.01 (0.08) |
| Hippocampus | 0.22 ± 0.06 (0.23) |
| Hypothalamus | 0.15 ± 0.02 (0.16) |
| Cerebellum | 0.05 ± 0.01 (0.05) |
| Pituitary gland | 3.27 ± 0.81 (3.44) |
| Pineal gland | 13.15 ± 3.02 (13.84) |

tion profile. Thus, the mean ratio of brain AUC (12.5 μg/g·min) to plasma AUC (144 μg/ml·min) was close to 0.09.

After oral administration of the same dose the drug was undetectable in rat brain within the limits of sensitivity of the method (0.025 μg/g). This probably reflects the large first-pass effect of this drug in the rat [10]; this effect markedly reduces the extent of entry into the general circulation of ranitidine after oral administration and consequently lowers the brain drug concentrations of the drug compared with the i.v. route.

The distribution of ranitidine in brain regions and spinal cord was investigated 30 min after i.v. injection (10 mg/kg). The concentrations of ranitidine in the various brain regions are presented in Table II. Under these experimental conditions the drug was almost evenly distributed in brain regions beyond the blood–brain barrier. The spinal cord showed concentrations similar to those in whole brain, whereas in regions "outside" the blood–brain barrier, such as the pineal and pituitary glands, the drug reached concentrations several times those in whole brain. Further, the pituitary and pineal gland-to-plasma ratios were ca. 3 and 14, respectively, indicating preferential concentration of ranitidine in these brain structures.

## CONCLUSION

We have developed a reliable and relatively rapid method for the determination of ranitidine in biological samples, including brain and brain regions. The procedure consists in a simple basic extraction, evaporation of the organic phase and injection of the reconstituted residue on to the chromatograph.

Studies of the CNS distribution of ranitidine confirmed that ranitidine enters the brain from the bloodstream. After i.v. injection the drug reaches brain concentrations amounting to ca. 9% of the plasma concentrations in the rat, concentrating more in some brain regions than in others. The drug disappeared from

whole brain almost in parallel with its disappearance from plasma, the whole brain-to-plasma ratio being essentially constant for as long as the drug remained measurable.

The route of administration appears important in determining the drug brain concentrations and, probably because of large-scale pre-systemic metabolism, a relatively high oral dose of ranitidine hydrochloride (10 mg/kg) produces much lower drug concentrations in rat brain than the same dose given i.v.

REFERENCES


1 D.W. Piper, Med. J. Aust., 2 (1983) 477.
2 J.D. Hughes, W.D. Reed and C.S. Serjeant, Med. J. Aust., 2 (1983) 12.
3 P.H. Silverstone, Lancet, i (1984) 1071.
4 C.M. Epstein, Lancet, i (1984) 1971.
5 W. Price, L. Coli, R.D. Brandstetter and V.P. Gotz, Eur. J. Clin. Pharmacol., 29 (1985) 375.
6 J.J. Schentag, F.B. Cerra, G. Calleri, E. DeGlopper, J.Q. Rose and H. Bernhard, Lancet, i (1979) 177.
7 J. Totte, S. Scharpe, R. Verkerk, H. Neels, M. Vanhaeverbeek, S. Smitz and J.J. Rousseau, Lancet, i (1981) 1047.
8 B.J. Kimelblatt, F.B. Cerra, G. Calleri, M.J. Berg, M.A. McMillen and J.J. Schentag, Gastroenterology, 78 (1980) 791.
9 R.P. Walt, S.J. LaBrooy, A. Avgerinos, T. Oehr, A. Riley and J.J. Misiewicz, Scand. J. Gastroenterol., 16 (Suppl. 69) (1981) 19.
10 L.E. Martin, J.A. Bell, P.F. Carey, F.A.A. Dallas, G.T. Dixon and W.N. Jenner, in The Clinical Use of Ranitidine, Medicine Publishing Foundation, Oxford, 1982, p. 23.
11 P.F. Carey and L.E. Martin, J. Liq. Chromatogr., 2 (1979) 1291.
12 J. Glowinski and L.L. Iversen, J. Neurochem, 13 (1966) 655.
13 L.Z. Benet and R.L. Galeazzi, J. Pharm. Sci., 68 (1979) 1071.
14 M.A. Gonzalez, T.N. Tozer and D.T.T. Chang, J. Pharm. Sci., 64 (1975) 99.
15 J.A. Bell, A. Bradbury, L.E. Martin and R.J.N. Tanner, Xenobiotica, 11 (1981) 841.
16 W. Jenner, L.E. Martin, B.A. Willoughby and I. Fellows, Life Sci., 28 (1981) 1323.
17 G.W. Mihaly, O.H. Drummer, A. Marshall, R.A. Smallwood and W.J. Louis, J. Pharm. Sci., 69 (1980) 1155.
18 P.F. Carey, L.E. Martin and P.E. Owen, J. Chromatogr., 225 (1981) 161.