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CARBAMAZEPINE PLASMA AND TISSUE LEVELS IN THE RAT

P. L. MORSELLI, M. GERNA and S. GARATTINI

Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea 62,20157 Milan, Italy

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Abstract—A procedure for determining carbamazepine in biological specimens both from animals and humans is described. Carbamazepine was found to enter the brain relatively rapidly and its distribution is uniform through the body. In animals absorption and tissue distribution was found to be influenced to a certain extent by the vehicle employed. A clear correlation between the brain levels and the protection towards maximal electroshock was observed.

Preliminary data on humans suggest a relatively slow absorption. An oral dose of 6 mg/kg gives plasma levels comparable to the ones obtained after an administration of 400 mg/kg in rats.

CARBAMAZEPINE, an iminostilbene derivative, shows marked anticonvulsant activity in the experimental animals together with a mild sedative and tranquilizing effect.[1,2]

In humans the compound has been found effective in grand mal seizures and psychomotor epilepsy with daily dosage ranging from 200 up to 1200 mg.[3–7]

No data are available up to now on the metabolic degradation of carbamazepine nor on its physiological disposition either in the experimental animals or in man.

Preliminary date on blood, urine and CSF levels of carbamazepine in humans have been reported[8,9] but no information exists on the pharmacological or therapeutical effective levels in plasma and brain. The present study deals with the distribution of carbamazepine in various tissues of the rat either after a single or a repeated administration and with the significance of brain levels in respect to the anticonvulsant activity. Preliminary data on carbamazepine plasma levels in humans are also reported.

MATERIALS AND METHODS

Female Sprague–Dawley rats (body wt. 200 ± 10 g) kept in makrolon cages at standardized temperature (21 ± 2°) and humidity (60%) were used. Plasma and tissues concentration of carbamazepine (Tegretol kindly supplied by Geigy, Milano) were determined at various times after single or repeated dosing. Immediately before sacrifice protection against maximal electroshock was tested with a Basile device for small animals electroshock (parameters: 100 mA, 0·2 p.w., 100 pulse/sec, shock duration 0·4 sec).

Carbamazepine plasma levels were also tested in two healthy volunteers 1–2 and 6 hr after an oral administration of 400 mg.

The determination of carbamazepine in biological specimens was performed by a modification of the procedure described by Führ.[8]

Determination from plasma

Two to three ml of plasma, alkalinized with 1 N NaOH to about pH 9, are shaken with 6 ml of *n*-heptane in a 50-ml glass-stoppered centrifuge tube. After centrifugation the organic phase is discarded and 3 ml of 1 N HCl and 15 ml of ethylene-chloride are added to the aqueous phase. The mixture is shaken gently for 30 min and, after centrifugation, 12 ml of the organic phase are transferred to another centrifuge tube and after two washings with 10 ml of 1 N NaOH treated with anhydrous Na_2SO_4. The organic phase is brought to dryness using a rotating evaporator. The residue is then dissolved in 3 ml of ethylene-chloride and read to 288 nm on a Beckman DU spectrophotometer in a quartz cell of 10 mm path length.

Determination from tissues

Tissues are homogenized in 1·15% KCl (3 ml for 1 g of tissue), alkalinized and extracted twice with 10 ml of *n*-heptane.

The organic phases are discarded and the aqueous phases processed as for plasma.

The washing with *n*-heptane allowed a significant reduction of blank values, without any appreciable interference on the recovery.

The absorbance–concentration ratio at 288 nm, was 0·068 $\pm$ 0·003 (S.E.). Blank values did not exceed an absorbance of 0·010 for plasma and of 0·030 for tissues. The sensitivity of the procedure was linear between 0·5 and 60 μg of carbamazepine per ml of plasma.

The average recovery was of 83 per cent from plasma and 66 per cent from tissues homogenates. Metabolites of carbamazepine do not interfere with the determination of carbamazepine. Preliminary observations on pooled extracts from plasma and tissues of chronically treated rats show on thin layer chromatography the evidence of at least four unknown ethyl-chloride extractable derivatives. (R_f = 0·53; 0·60; 0·68 and 0·74 in a CCl_4–MeOH 95:5 system). The R_f 0·53 spot eluted with ethanol showed a maximum of absorbance at 251 nm with minimal absorbance at 260 and 240 nm. The absorbance of the other three unknown metabolites was minimal (0·010) at 288 nm.

Also diphenylhydantoin and phenobarbital were found not to interfere in the determination of carbamazepine.

Reagents

Ethylene-chloride puriss. (Fluka), *n*-heptane RCG (Rudipont) hydrochloric acid 37% (Merck), sodium hydroxyde (Merck).

RESULTS

Single administration in rats

Rats plasma and brain levels of carbamazepine were determined 2, 4, 6, 8 and 10 hr after a single oral administration of 400 mg/kg.

Three different vehicles (0·5 ml/rat) were used: (a) arabic gum 4%; (b) *N,N*-diethylacetamide–tween–water (2:3:20, by vol.); (c) propylen-glycol.

The compound seems to be absorbed relatively slowly: the plasma and brain maximal concentrations were in fact reached 4 or 6 hr after dosing (Table 1).

TABLE 1. BRAIN (μg/g $\pm$ S.E.) AND PLASMA (μg/ml $\pm$ S.E.) LEVELS OF CARBAMAZEPINE IN FEMALE SPRAGUE–DAWLEY RATS AFTER ORAL ADMINISTRATION OF 400 mg/kg OF THE DRUG IN THREE DIFFERENT VEHICLES

		Time after administration (hr)				
Vehicle	Tissue	2	4	6	8	10
(a)	Brain (B)	11·1 ± 1·3	11·2 ± 1·2	14·1 ± 1·6		
	Plasma (P)	9·8 ± 0·6	8·9 ± 1·5	10·9 ± 1·3		
	B/P ratio	1·13	1·26	1·29		
(b)	Brain (B)	22·6 ± 1·5	20·7 ± 2·7	24·1 ± 2·0		
	Plasma (P)	14·2 ± 1·9	14·8 ± 2·1	8·9 ± 1·7		
	B/P ratio	1·59	1·39	2·70		
(c)	Brain (B)	15·6 ± 4·2	21·6 ± 3·8	28·0 ± 0·3	23·7 ± 3·5	19·9 ± 3·4
	Plasma (P)	9·4 ± 2·0	10·3 ± 2·7	17·9 ± 1·2	14·5 ± 1·9	11·9 ± 1·7
	B/P ratio	1·65	2·09	1·56	1·63	1·67

Each point represents the mean of five animals.
(a) = arabic gum; (b) = *N,N*-dimethylacetamide–tween 80–water–(2:3:20, by vol.); (c) = propylen glycol.

In all the experimental groups the brain concentrations exceeded the plasma ones.

It should be noticed that the vehicle employed for the administration had a marked influence both on the absorption and the distribution of carbamazepine.

With vehicle (a) the plasma and brain levels were in fact lower when compared to the two other groups and the brain–plasma ratio was relatively constant. With vehicle (b) a sharp increase of brain–plasma ratio was evident 6 hr after the administration and in this case the plasma levels were already at maximal values at 2 hr after dosing.

Highest plasma and brain concentrations were reached with vehicle (c) but in this case the brain–plasma ratio was relatively constant. All the animals were protected towards maximal electroshock.

Repeated administration to rats

Carbamazepine was administered orally in arabic gum 4% (0·5 ml/rat) at the dose of 100 mg/kg twice a day (every 12 hr) for 4 consecutive days and at 9.00 a.m. of the fifth day.

Animals were sacrificed 4, 6, 8 and 10 hr after the last dose and carbamazepine levels determined in plasma, brain liver, kidney, heart and lungs.

Protection towards maximal electroshock was tested immediately before sacrifice. The drug was quite uniformly distributed in the various organs, with highest concentrations in the liver (Table 2).

In most of the organs peak levels were attained 6–8 hr after dosing with the exception of the kidney.

The tissue–plasma ratios for the various organs were always > 1, liver and heart showing the highest values.

As shown in Table 3 a clear correlation between the brain concentration of carbamazepine and the pharmacologic effect was present, the minimal effective brain level being of about 4 μg/g.

TABLE 2. PLASMA (μg/ml ± S.E.) AND TISSUE (μg/g + S.E) LEVELS OF CARBAMAZEPINE AFTER AN ORAL REPEATED TREATMENT IN RATS (100 mg/kg EVERY 12 hr FOR 9 TIMES)

	Time after administration (hr)			
Tissue	4	6	8	10
Plasma	3·91 ± 0·18	4·07 ± 0·49	5·78 ± 0·88	3·13 ± 0·19
Brain	5·28 ± 0·61	10·00 ± 0·51	8·90 ± 2·04	2·66 ± 0·31
T/P ratio	1·35	2·45	1·53	0·84
Liver	11·33 ± 1·57	11·78 ± 0·38	16·04 ± 0·52	11·32 ± 2·08
T/P ratio	2·89	2·89	2·77	3·61
Kidney	8·64 ± 0·76	8·16 ± 0·85	6·77 ± 1·22	3·21 ± 0·72
T/P ratio	2·20	2·00	1·17	1·02
Heart	5·60 ± 0·94	6·18 ± 1·00	7·39 ± 1·60	7·80 ± 0·48
T/P ratio	1·43	1·51	1·27	2·49
Lungs	3·41 ± 1·35	6·87 ± 0·83	10·87 ± 2·02	6·73 ± 0·80
T/P ratio	0·87	1·68	1·88	2·15

Rats were sacrificed 4–6–8–10 hr after the last dosing. Each group is the mean of four to six animals. Arabic gum (0·3 ml/rat) was used as a vehicle.
T = tissue, P = plasma.

TABLE 3. RELATIONSHIP BETWEEN CARBAMAZEPINE BRAIN CONCENTRATION AND PROTECTION TOWARDS MAXIMAL ELECTROSHOCK IN RATS AFTER REPEATED TREATMENT WITH CARBAMAZEPINE (SEE TABLE 2)

Carbamazepine brain concentration	No. of rats protected	No. of rats not protected
>4	12 (85·7%)	2 (14·3%)
<4	1 (12·5%)	7 (87·5%)

$\chi^2 = 8{\cdot}41$. $P < 0{\cdot}01$.

Observations in humans

Plasma levels of carbamazepine determined in two healthy volunteers 1, 2 and 6 hr after oral administration of 400 mg were found to be of about 8–10 μg/ml of plasma (Table 4). The data suggest that also in humans the drug is absorbed relatively slowly, the maximal values, for the length of time considered, being reached at the sixth hour.

TABLE 4. PLASMA LEVELS (μg/ml) OF CARBAMAZEPINE IN TWO HEALTHY VOLUNTEERS AFTER ORAL ADMINISTRATION OF 400 mg OF CARBAMAZEPINE

				Time after administration (hr)		
Subject	Sex	Age	Body wt.	1	2	6
T.G.	♂	28	65	8·6	8·2	11·2
B.P.	♂	21	68	9·7	8·7	10·5

COMMENTS

The described analytical procedure seems to fit the requirements for the determination of carbamazepine in biological specimens both in animals and in humans. Carbamazepine is absorbed relatively slowly but enters rapidly the brain where high concentrations are maintained for a relatively long period of time. Our data are, in this respect, in good agreement with the pharmacological observation of Theobald and Kuntz[1] who described a persistance of the anticonvulsant effect for several hours in the rat. The choice of the vehicle for the oral administration seemed to have an important influence in determining both the rate of absorption and the tissue distribution. This is a point very seldom taken in consideration but which may play a certain role in determining the drugs effect.

After repeated administration the drug is widely distributed in the body with tissues/plasma ratio ranging from 1·0 to 3·6.

An oral dose of about 6 mg/kg of carbamazepine in man gives plasma levels comparable to a dose of 400 mg/kg in rats.

Although the pharmacological and clinical effect of carbamazepine have been known for many years[1,2,5,6], no attempts have been made to correlate plasma and brain levels with the drug activity. A clear correlation between brain concentrations and the protection towards maximal electroshock has been observed, with a minimal protective brain concentration of 4 μg/g.

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