PRAZIQUANTEL IN THE CEREBROSPINAL FLUID IN NEUROCYSTICERCOSIS

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The administration of praziquantel (PZQ) to patients with neurocysticercosis (NC) has been reported [2, 4, 7, 10, 11, 14, 16, 18, 20, 23-25, 27-30], and critical analyses on this subject have been published [1, 12, 19]. Variagated dose schedules adopted results from the lack of knowledge about the passage of PZQ through the blood-brain barrier (BBB) and therefore about the concentration attained in the central nervous system (CNS). Data on its concentration in the cerebral extracellular fluid (CEF) would be useful. However few reports have contributed to a further knowledge on this subject [11, 21] and no consistent data are yet available in man. An indirect rate allowing to evaluate the concentration of a given drug in the CEF is given by determining the respective concentration in the cerebrospinal fluid (CSF) particularly when samples are collected from the cisterna magna [3]. In addition, knowing the ratio between CSF and serum concentrations as well as the time elapsed between the drug administration and sample withdrawals it is feasible to achieve further elucidation about the readiness of the BBB crossing for a drug [3].

This investigation was carried out in order to provide data that contribute to analyze the aspects of PZQ passage through the BBB and the relationship between CSF and serum PZQ concentrations in patients with NC.

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MATERIAL AND METHODS

PZQ concentrations in the CSF and serum of 10 patients with NC were determined. Identification and clinical data and skull computerized tomography (CT) findings are summarized in table 1. For each case the following PZQ administration schedule was established: 50 mg orally per kg of body weight/day for 21 consecutive days, in association with dexametasone (12 mg/day orally). The total daily dosage of PZQ was distributed into three equal parts, each intake being 1/3 of the total daily dosage. The interval between administrations was 4 hours. For cases 6 through 10, on the last day of PZQ administration the total daily dosage was given on a single intake. Owing to reasons outside the established schedule, patient 3 was given 25 mg of PZQ per kg per day during the first 6 days of treatment and in cases 5 and 6 a 48 hours' interval in PZQ administration occurred between the 16 th. and 18 th. days for both cases. Samples of the cisterna magna CSF and of blood were collected from each patient on the same occasion and in four instances: before starting with PZQ (Day-0 samples), on the first day of administration (Day-1 samples), on the 7 th. day (Day-7 samples) and on the 21 st. day (Day-21 samples) of PZQ administration. Day-1 and day-21 samples were collected three hours after the last daily intake. Day-7 samples were collected at non specified period of time after the last daily PZQ administration (2 to 6 hours). For all blood samples the serum was separated, and maximum care was tkaen to avoid hemolysis. Each CSF sample was divided into two portions: one was intended for the analysis of the general characteristics and the other was centrifuged. The latter as well as the serum samples were frozen and kept at —20ºC in order to establish the PZQ concentration.

The general characteristics of the CSF samples were determined according to the methodology in use at CIN. PZQ concentrations in the CSF and in the serum were determined by one of us (H.W.D.), using gas-liquid chromatography according to the method previously described (8). Concentration in the serum was determined without deproteinization of the sample on a first stage, and then in the protein-free sample. The deproteinization was carried out in the remaining portion of frozen samples, after defrosting and reaching room temperature (+ 20º). Each one of the samples was deproteinized by ultrafiltration using the Amicon MPS 1 microfiltration system: YMT diaflow membranes 14 mm in diameter were used and the serum was forced centrifugally against the diaphragm at 400 rpm ($\cong$ 2000 g) on a Labofuge II (Heraeus Christ) centrifuge. The ultrafiltrate obtained was tested for proteins with Merckgnost test bands in order to exclude the presence of proteins owing to defective membranes. No proteins were found on any of the ultrafiltrates tested. Data were analyzed from a statistical standpoint.

RESULTS

General characteristics of CSF samples considered in this study are shown in table 2. Results of PZQ concentrations on serum and CSF are also shown in table 2. Statistical characteristics of PZQ concentration are shown in table 3. The distribution of PZQ concentrations and of CSF variables values have made it impossible to establish an appropriate model for variables changes that could result in normal distribution values. Thus, the Mann-Whitney test was used to establish significance levels (Table 4). Since many dependent variables were taken into account, the comparative study was carried out through simple and multiple linear regression tests, mainly considering the slope of the regression line (regression coefficient), its hypothesis tests (student t test referring to each one of the dependent variables), the overall correlation levels according to Pearson and the study of its significance levels through covariance analysis (Table 5). In conceptualizing the test values, an alpha of 0.05 was acknowledged.

Case	Name	Sex	Age	Clinical form	Diagnosis CSF	Diagnosis CT	CT findings	Date
01	MC	M	18	H	+		N	3/29/83
02	DJP	F	23	E	+		N	3/29/83
03	DR	M	47	E	+	+	VCPI	3/29/83
04	JSS	M	33	E		+	VCP	4/08/83
05	RAB	M	29	E		+	VCPEI	4/14/83
06	MCP	F	24	E		+	VPEI	4/14/83
07	LT	F	36	E	+	+	CP	5/04/83
08	WM	M	52	E	+	+	VCP	5/04/83
09	MAAB	F	18	H	+		D	5/04/83
10	PS	M	22	H	+		N	5/04/83

Table 1 — Patients with neurocysticercosis: case number, name, sex (M, male; F, female), age (years), clinical form (H, intracranial hypertension; E, epilepsy), diagnosis established by (+) cerebrospinal fluid examination (CSF) and/or computerized tomography (CT), CT findings (N, normal; V, nodular vesicles; C, nodular calcifications; P, localization in the parenchyma; D, ventricular dilatation; E, perinodular edema; I, perinodular inflammation), date (beginning of PZQ administration: month/day/year).

Case	Sample	CSF							PZQ			
	Day	Cyt	Eos	Prot	g	b/A	CFT	IFT	S	Sf	CSF	CSF/Sf
01	0	6,0	2	22	11.3	2.32	2	R	0		0	
	1	3.0	0	21	11.3	2.40	0	nr	612	42	142	3.38
	7	77	2	24	10.7	3.09	0	nr	108	22	12	0.54
	21	40	0	23	11.7	2.25	2	R	283	37	35	0.95
02	0	8.0	8	29	18.0	2.63	4	R	0		0	
	1	4.0	21	27	18.6	1.78	4	R	100	6	9	1.50
	7	11	20	35	18.2	2.76	4	R	25	3	1	0.33
	21	15	3	25	17.1	1.91	4	R	107	—	11	
03	0	10	4	50	19.3	2.29	2	R	0		0	
	1	10	12	52	21.0	2.31	2	R	51	5	5	1.00
	7	19	13	46	19.2	2.50	4	R	152	23	22	0.96
	21	8.0	0	44	17.7	1.86	0	R	431	45	67	1.24
04	0	0.3	0	21	15.3	2.50	0	nr	0		0	
	1	2.0	1	21	12.4	1.87	0	nr	16	4	4	1.00
	7	24	0	23	12.3	1.98	0	nr	33	6	4	0.67
	21	10	0	19	13.1	2.67	0	nr	148	26	25	0.96
05	0	1.0	0	17	12.6	3.08	0	nr	0		0	
	1	1.0	0	20	10.2	1.98	0	nr	40	6	4	0.67
	7	0.3	0	12	10.2	1.77	0	nr	25	4	3	0.75
	21	1.0	0	18	12.8	1.55	0	nr	244	30	31	1.03
06	0	1.7	0	36	11.0	2.02	0	nr	0		0	
	1	0.3	0	38	12.5	2.18	0	R	23	4	3	0.75
	7	1.0	0	31	9.0	1.34	0	nr	27	17	5	0.29
	21	2.0	0	45	9.4	1.39	0	nr	744	140	93	0.66

Table 2 — Continues in the next page.

Case	Sample	CSF							PZQ			
	Day	Cyt	Eos	Prot	g	b/A	CFT	IFT	S	Sf	CSF	CSF/Sf
07	0	33	0	73	23.0	1.58	64	R	0		0	
	1	43	1	57	33.1	1.83	64	R	7	—	3	
	7	41	5	41	36.2	2.48	64	R	86	16	10	0.63
	21	33	0	67	26.7	1.98	64	R	376	12	55	4.58
08	0	4.0	1	37	15.2	2.05	2	R	0		0	
	1	6.0	1	45	16.6	2.74	2	R	54	—	20	
	7	44	8	33	19.6	4.86	4	R	609	58	19	0.33
	21	14	0	40	23.0	3.50	4	R	126	—	14	
09	0	5.7	8	38	15.3	2.07	2	R	0		0	
	1	345	7	46	15.5	2.42	4	R	156	18	11	0.61
	7	117	9	38	22.4	2.92	2	R	32	3	22	7.33
	21	77	3	29	30.1	3.84	2	R	76	12	9	0.75
10	0	17	1	36	18.7	2.30	16	R	0		0	
	1	20	5	33	19.0	2.74	8	R	284	32	24	0.75
	7	9.0	0	22	15.2	2.45	4	R	29	4	4	1.00
	21	5.0	0	21	13.7	2.98	0	R	565	67	79	1.18

Table 2 — Characteristics of the CSF samples; concentrations of PZQ (ng/ml) in non-deproteinized serum (S), in protein free serum (Sf) and in the CSF and the relationship of PZQ CSF/Sf concentration for each case and each sample (days 0, 1, 7 and 21). For Sf, a (—) sign indicates samples where PZQ concentration could not be determined. Legend: Cyt, number of cells/cumm; Eos, eosinophil cells (%) in the cytomorphological profile; Prot, total proteins (mg/dl); g, gammaglobulins (%) in the electrophoretic profile (cellulose acetate gel); b/A, beta + tau globulins/albumin ratio in the electrophoretic profile; CFT, cysticercosis complement fixation test (results in Kolmer unities); IFT, immunofluorescence test for cysticercosis (R, reagent; nr, non-reagent).

Samples	Values	S	Sf	CSF	Sf/S	CSF/S	CSF/Sf
Day-1	mean	134.3	14.6	22.5	0.129	0.185	1.207
	s.d.	187.71	5.23	42.63	0.0620	0.0410	0.9229
	median	52.5	6.0	7.0	0.114	0.114	0.875
	range	7-612	4-42	3-142	0.06-0.25	0.071-0.250	0.611-3.38
Day-7	mean	112.6	15.6	10.2	0.196	0.170	1.283
	s.d.	179.87	16.91	8.16	0.1569	0.1877	0.6768
	median	32.5	11.0	7.5	0.156	0.121	0.646
	range	25-609	3-58	1-22	0.094-0.630	0.031-0.688	0.294-7.333
Day-21	mean	310.0	46.1	41.8	0.129	0.131	1.450
	s.d.	69.40	14.84	30.04	0.0488	0.0221	1.2910
	median	263.5	33.5	33.0	0.127	0.133	0.997
	range	76-744	12-140	9-93	0.032-0.188	0.093-0.169	0.664-4.583
For samples day-21:							
Cases 1-5	mean	242.6	34.5	33.6	0.133	0.144	1.107
	s.d.	126.95	8.35	20.95	0.030	0.022	0.2571
	median	244.0	33.5	31.0	0.127	0.141	0.998
	range	107-431	26-45	10-67	0.104-0.176	0.124-0.169	0.946-1.489
Cases 6-10	mean	377.4	57.8	50.0	0.124	0.132	1.794
	s.d.	284.44	60.65	37.72	0.0678	0.0129	1.8730
	median	376.0	39.5	55.0	0.139	0.133	0.965
	range	76-744	12-140	9-93	0.032-0.188	0.118-0.146	0.664-4.583

Table 3 — Mean, standard deviation (s.d.), median and range of praziquantel (PZQ) concentrations (ng/ml) in serum (S), protein free serum (Sf) and cerebrospinal fluid (CSF) and their relationships (Sf/S, CSF/S, CSF/Sf) for all patients in days 1, 7 and 21 after the beginning of PZQ administration. For samples of day 21 data regarding cases 1 to 5 and cases 6 to 10 are also shown separetedly.

Comparison					Result		
Variable	Day	x	Variable	Day	U test	zU	pU
For all 10 cases:							
CSF	1	x	S	1	86	2.72*	
CSF	1	x	Sf	1	45.5	0.40	
CSF	7	x	S	7	100	3.78*	
CSF	7	x	Sf	7	57	0.53	
CSF	21	x	S	21	98	3.63*	
CSF	21	x	Sf	21	41.5	0.13	
S	1	x	Sf	1	71	2.76*	
S	7	x	Sf	7	94	3.33*	
S	21	x	Sf	21	77	3.29*	
CSF	1	x	CSF	7	52.5	0.19	
CSF	1	x	CSF	21	81.5	2.38*	
CSF	7	x	CSF	21	81	2.34*	
CSF/S	1	x	CSF/S	7	46	0.30	
CSF/S	1	x	CSF/S	21	44	0.45	
CSF/S	7	x	CSF/S	21	53	0.23	
CSF/S	1	x	Sf/S	1	46.5	0.58	
CSF/S	7	x	Sf/S	7	66	1.21	
CSF/S	21	x	Sf/S	21	51	0.98	
CSF/Sf	1	x	CSF/Sf	7	57.5	2.00*	
CSF/Sf	1	x	CSF/Sf	21	38	0.63	
CSF/Sf	7	x	CSF/Sf	21	62.5	2.00*	
For cases 1-5		x	cases 6-10:				
S	1	x	S	1	13		< 0.004
S	7	x	S	7	14		< 0.004
S	21	x	S	21	15		~ 0.004
Sf	1	x	Sf	1	8.5		~ 0.08
Sf	7	x	Sf	7	13		< 0.004
Sf	21	x	Sf	21	8		$= 0$
CSF	1	x	CSF	1	13		< 0.004
CSF	7	x	CSF	7	20.5		~ 0.08
CSF	21	x	CSF	21	15		~ 0.24

Table 4 — Results of comparisons between praziquantel concentrations in cerebrospinal fluid (CSF), serum (S), protein free serum (Sf) and their relationships (CSF/S, CSF/Sf, Sf/S) through the Mann-Whitney U test. Significant values for alpha = 0.05 are indicated (*).

Fixed variable	Day		Free variable	Day	B	t	r	F
S	1	x	Sf	1	7.34	7.67*	0.99	333.55*
		x	CSF	1	2.16	7.28*		
S	7	x	Sf	7	9.99	6.90*	0.95	33.55*
		x	CSF	7	0.52	0.17		
S	21	x	Sf	21	1.21	2.86*	0.99	208.70*
		x	CSF	21	6.08	9.92*		
S	1	x	S	7	—0.09	—0.23	0.09	0.027
		x	S	21	—0.02	—0.06		
Sf	1	x	Sf	7	0.31	0.35	0.24	0.12
		x	Sf	21	0.08	—0.45		
CSF	1	x	CSF	7	0.46	0.23	0.13	0.06
		x	CSF	21	—0.11	—0.20		
Prot	1	x	CSF	1	—0.12	—1.15	0.38	1.31
Prot	7	x	CSF	7	0.78	2.24	0.62	5.03
Prot	21	x	CSF	21	0.19	1.11	0.37	1.23
b/A	1	x	CSF	1	0.03	0.92	0.31	0.84
b/A	7	x	CSF	7	0.07	1.95	0.56	3.65
b/A	21	x	CSF	21	—0.01	—1.52	0.47	2.31
g	1	x	CSF	1	—0.05	—0.88	0.30	0.78
g	7	x	CSF	7	0.36	1.11	0.37	1.24
g	21	x	CSF	21	—0.10	—1.38	0.44	1.91
Prot	1	x	CSF/S	1	33.13	0.93	0.31	0.87
Prot	7	x	CSF/S	7	12.44	0.66	0.23	0.44
Prot	21	x	CSF/S	21	95.95	0.39	0.14	0.15
b/A	1	x	CSF/S	1	—0.21	—0.22	0.08	0.05
b/A	7	x	CSF/S	7	—0.41	—0.23	0.08	0.05
b/A	21	x	CSF/S	21	—3.48	—0.26	0.09	0.07
g	1	x	CSF/S	1	23.81	1.46	0.46	2.13
g	7	x	CSF/S	7	6.52	0.43	0.15	0.19
g	21	x	CSF/S	21	—44.87	—0.41	0.14	0.17
Sf/S	1	x	CSF/S	1	—0.39	1.17	0.43	1.38
Sf/S	7	x	CSF/S	7	—0.09	0.18	0.06	0.03
Sf/S	21	x	CSF/S	21	—0.50	—0.45	0.18	0.20
CSF/Sf	1	x	CSF/Sf	7	—0.11	—0.74	0.29	0.54
CSF/Sf	7	x	CSF/Sf	21	—0.39	—0.54	0.21	0.29
CSF/Sf	1	x	CSF/Sf	21	—0.016	—0.099	0.04	0.0009
CSF/Sf	1	x	CSF/Sf	7	—0.12	—0.60	0.29	0.18
		x	CSF/Sf	21	—0.37	—0.20		
Sf/S	1	x	Sf/S	7	—0.07	—0.50	0.70	1.96
		x	Sf/S	21	1.56	1.81		
CSF/S	1	x	CSF/S	7	—0.26	—1.08	0.41	0.73
		x	CSF/S	21	0.98	0.49		

Table 5 — Linear regression analyses of praziquantel concentrations in cerebrospinal fluid (CSF), serum (S), protein free serum (Sf) and their relationships (CSF/S, CSF/Sf, Sf/S), and in relation to CSF components (Prot, total protein concentration; g, gammaglobulins; b/A, beta + tau globulins/albumin ratio): B, regression coefficient rate; t, student t test value; r, correlation value and its significance test (F). Significant values for alpha = 0.05 are indicated (*).

COMMENTS

Serum — PZQ is a lipid soluble isoquinoline-pyrazine derivative. Human pharmacokinetic studies showed that the extent of PZQ absorption after p.o. administration ranges from 80 to 100%. The drug upon reaching the liver is rapidly and extensively converted into hydroxylation derivatives. This first-pass effect shows distinct inter-individual variation. Consequently the serum level of unchanged PZQ is a function of time and dose and differs considerably in each case. Anyhow the plasma concentration of the biologically active principle is only about 5% of its total value (unmetabolized PZQ plus its metabolites). The peak serum level, in the average 1.3μg/ml following a single oral dose of 50mg/kg, already is achieved within 60 minutes. In the plasma protein-binding capacity of PZQ is a reversible process and varies from 70 to 90% and its half-life is 1.5 hours for the unchanged drug. The kidneys are the predominant elimination pathway accounting for about 80%, whilst the remaining portion is excreted through the bile and the intestinal wall. Only metabolites, a variety of very polar glucoronide and sulfate derivatives, are found in the excretion products. The renal elimination is very fast, 40 to 65% in the first 8 hours and more than 90% within 24 hours [1, 5, 13, 22].

These data are useful to understand some findings reported in this investigation. Serum levels of PZQ have greatly varied from patient to patient, which confirms previous observations in volunteers [13]. These were also carried out three hours after the drug administration and have shown similar results as the ones presented here either when the results of day-1 and day-21 samples (also collected three hours after drug administration) are considered, when the daily dosage was distributed into three intakes or, especially, when the total daily dosage was given in a single administration. The case-by-case variation may be responsible by the fact that significant differences could not be detected concerning the day-7 sample populations, and for day-21 samples between the cases 1-5 and 6-10. Among the causes for this observed dispersion of results, the following should be taken into account: different rates of absorption and hepatic conversion; inadequate dosage administration when the set schedule was not duly followed, as happened with case 3. However, the observed concentrations are quite close to those recorded on the volunteers previously mentioned, who were given a single dose of 50mg/kg/weight (average: 347ng/ml; range: 108-783) as compared to the day-21 samples of cases 6-10 in this series (average: 377.4; range: 76-744). Less dispersion was seen regarding the PZQ concentration relationship between protein-free serum/non-deproteinized serum. This is represented by the mean value of 0.129 taking into account the values obtained for day-1 and day-21 samples. Therefore, it can be considered that three hours after per os administration nearly 15% of PZQ present in the serum is found in the free form. This value comes very close to those already registered in the literature [1].

CNS and CSF — The activity of PZQ against *Cysticercus cellulosae* has been demonstrated in animal experiments and in vitro [1, 6, 9, 31-34]. In rats the drug is taken up by the tissues at a very high rate; 30 minutes after admi-

nistering 10mg/kg p.o. as single dose, the concentration in the plasma was 2.9μg/g and in the brain 0.3μg/g. Following the i.v. administration of 2mg/kg, these concentrations within 5 minutes were 10.3 and 5.7μg/g respectively. Such findings demonstrate that the unmetabolized PZQ is able to permeate the BBB whereas the more polar metabolites cannot pass through this barrier. Furthermore, comparing repeated doses 10mg/kg once daily during 4 days with one single dose of 10mg/kg, both given p.o., it was observed with the former administration regimen a slight increase of its concentration in the brain, 0.08μg/g as compared with 0.07μg/g, 24 hours after the last dose. In vitro studies pointed out that the isolated strobilicercus take up PZQ readly but this uptaking is markedly slower in the encysted parasite. Therefore the cystwall acts as a barrier to the penetration of the drug. In fact, a concentration factor of 1 or higher was reached within 10 minutes in the cystwall but in the larval tissue inside the cyst the concentration was 58% lower after 60 minutes incubation. On the other hand, the concentration in the cystwall and in the cyst fluid were higher than in the medium, showing that the parasite accumulates the drug up to a factor 3. This accumulation is a function of time since the concentration within the encysted larva was 0.1, 0.24 and 0.42μg/g after 10, 20 and 60 minutes respectively. In these studies it also became evident that the minimal effective concentration to severely damage the isolated strobilicerci is 0.01μg/ml following an exposure during 30 to 90 minutes [33].

Data regarding PZQ concentrations in the CSF in animals were registered: the CSF/plasma concentration relationship found is 1/5 to 1/7 in rats and 1/10 in rabbits [1]. There are no records for normal subjects. In subjects who volunteered for an oral administration of the drug, no CSF concentrations were monitored [13]. PZQ concentrations in the CSF have been recorded in few instances, in patients with NC. In one patient with brain and dermal cysticercosis treated with PZQ 3x25mg/kg/day for 3 weeks, serial plasma and CSF samples were obtained and subcutaneous cysticerci were excised after the first daily dose intake at four different days during the treatment period. It was demonstrated that the unbound-protein fraction enters rapidly (10 minutes) into the CSF whilst the penetration into cysticercus is slower (40 minutes) but still faster than its plasma half-life (60 to 90 minutes). The highest concentrations were found in the last day of treatment: 1.28μg/ml in serum, 0.075μg/ml in CSF and 0.234μg/ml in the cyst fluid, respectively 1.5, 2 and 2.5 hours after the drug intake [21]. In another patient with NC treatment with PZQ 50mg/kg daily for 15 days, one brain cysticercus was taken out during surgery performed to release intracranial hypertension 96 hours after finishing the treatment course. The PZQ concentration within the cyst fluid was 0.004μg/ml. This cysticercus was surrounded by a thick fibrous tissue like a capsule that might have acted as additional obstacle to the PZQ penetration. This fact plus the long time elapsed since the drug administration may explain the relatively low concentration observed in this case [11]. However, appropriate studies on the PZQ concentrations reached in the CSF according to the administration dosage, serum levels and time elapsed between administration and the CSF sample collection for concentration assessments, have not yet been recorded in man.

This study is based on patients with NC having an indication for CSF collection in order to control the effects of medication. Local inflammation conditions as well as the BBB conditions varied from one patient to the other. These facts are highlighted by the difference shown in CSF composition before PZQ administration (Day-0 samples) and in CT aspects. On the other hand, BBB conditions in each case were not properly known prior to the drug administration. An indirect rate of BBB conditions is given by albumin concentration in the CSF samples and is best expressed by the beta/albumin relationship: the lower the values, the greatest impairment of the BBB is to be expected. Dealing with pathological samples the interpretation of data should bear this in mind. Among the 10 cases, two had normal CSF before starting the drug administration (cases 4 and 5); the others showed changes (even if these were discreet) which in 7 of the cases characterized the CSF syndrome in NC. Exacerbation of this syndrome during the administration period was seen in at least 8 cases. Such exacerbation may be ascribed to the action of PZQ in cysticerci housed in the CNS [26]. In this exacerbation of the CSF syndrome in NC the following should be pointed out: an increase in cell number; an increase in the eosinophilic cells number in the cytomorphological profile; and, sometimes, a decrease in the beta/albumin relationship. Those three phenomena may be linked to the acute phase of the neuroimmunological phenomena triggered by heteroantigens release within the CNS [15]. These are likely to result from cysticerci whose metabolic processes were impaired owing to PZQ action [27]. Similar phenomena were seen in animals infested with schistosomes and cysticerci, and subjected to PZQ action: in the areas where a lacunar vacuolization in their external sincytial layer occurs, there is an invasion of neurotrophilic polynuclears. This is followed by intense invasion of eosinophilic cells and phagocytes, thus starting the local immunological reaction [1, 17]. These data show that new factors may be introduced during the administration of PZQ to patients with NC. The extent of their role on BBB changes cannot be evaluated, and they can vary from one case to the other. However, the results obtained in this study show that PZQ levels in the CSF are close to levels found in the protein-free serum. This is emphasized by the fact that case-to-case concentration differences found for samples day-1, day-7 and day-21 are not significative. The distribution between CSF and serum levels showed a rather constant ratio of about 1:7 amongst the different patients. With only a few exceptions the CSF concentration was equivalent to the protein-free serum fraction, i.e. a ratio of 1:1. Unbound PZQ crosses the BBB easily and quickly becoming evenly distributed between plasma and CSF. This distribution is displayed in figure 1.

Pharmacokinetics — The range of variations found in determining the PZQ levels derives from variations found in subjects, rather than from the aleatory dispersion of values obtained in each case. Actually, when looking at this study day-by-day it is found a correlation ranging from 95 to 99% between levels recorded case-by-case in the serum, in the protein-free serum and in the CSF. When seen as a whole, the values recorded for serum differ significantly from those obtained for protein-free serum in the 1st., 7th. and 21st. days. This

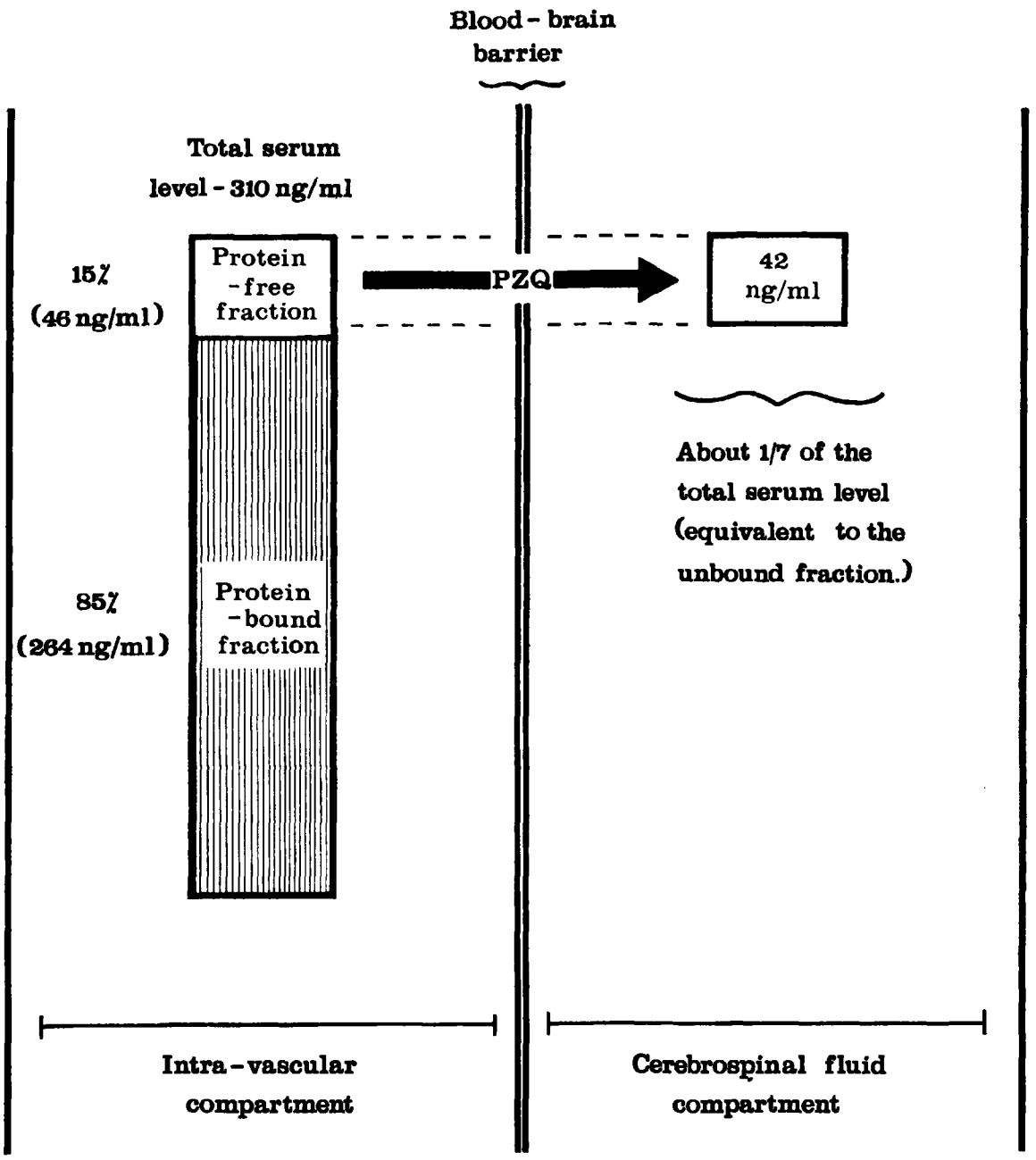

Fig. 1 — Mean praziquantel (PZQ) concentration in serum and in cerebrospinal fluid three hours after the drug administration on the 21st. day of treatment of patients with neurocysticercosis.

is an evidence of a marked predominance of the fraction linked to proteins over the free fraction throughout the study. The free fraction presents no significative difference from the fraction which was present in the CSF, and no discernible difference was found between value obtained for the 1st., 7th. and 21st. days. These data suggest that a pharmacokinetic balance might have taken place as early as on the first day between the serum compartment not linked to proteins and the CSF. This balance was maintained on 7th. and 21st. days but at significantly different levels. This is an evidence of changes in the system as a whole between the 7th. and 21st. days. Possibly it is due to variables interaction (PZQ level in the protein-free serum and the CSF) with some other factor whose dynamics in relation to the drug has varied during the study. A similar conclusion is reached when serum levels taken case-by-case

and in whole as seen in the 1st., 7th. and 21st. days presented a mere 9% correlation. An identical behavior was seen when studying the values recorded for protein-free serum and CSF on the same occasions, where correlation rates of 24% and 23% respectively were found. They have no valoration from the the statistical viewpoint. In pharmacokinetic studies and under standardized conditions value variations for the same subject during his exposure to the drug and under balanced conditions are restrict. When values for CSF obtained in the first day are compared to those recorded on the 7th. day no significant difference was seen; the pharmacodynamic balance has already taken place. Difference is characterized when levels obtained in the 7th. and in the 21st. days are compared. They suggest a certain delay in stabilization of the PZQ levels in the CSF, as compared to the levels recorded for protein-free serum. This fact is confirmed by the study of CSF/protein-free serum relationships where a difference was noted when comparing the values obtained in the 1st. and 7th. days and the 7th. and 21st. days. As there was no noticeable difference between the 1st. and 21st. days, this may suggest that on the 21st. day of treatment a stabilization in the variation factors on PZQ serum and CSF levels may have occurred, and as a consequence, on the CNS. This fact may be explained if it is taken into account: (a) dynamic aspects of CSF displacement; (b) the impact that variables dependent on the pathological conditions might have over the system; (c) the specific characteristics of each drug reflecting on CSF/serum concentrations in the presence of released substances that trigger inflammatory activity; (d) the possibility of retaining drugs in lipid-rich structures, as is the case with the CNS. To the extent that each one of these factors whether alone or together is variably expressed in each subject, this data dispersion as a whole can be explained. However, the variation recorded for each subject presents fairly constant characteristics, as was shown by the recorded correlations which have already been discussed.

Thus, results suggest that a non-casual variation factor or factors may be present during the period. These factors may include the biological changes of the parasite and/or an interaction of inflammatory nature with the CNS and the BBB as is suggested by the exacerbation of the CSF syndrome in NC throughout the period. If signs indicating the drug is acting on cysticerci housed in the CNS are considered, one should know its concentration on the CSF, since this is the method available to know about its concentration in the CEF, which is the nearest place where parasites may be housed in the intimacy of the CNS parenchyma. For cysticerci present in ventricles or in the subarachnoid space the concentration found in the CSF would give a direct measure of the possibility of drug contact. Provided we acknowledge that a minimum is needed for the PZQ to act on cysticerci and for a minimum delay of time [33], this is a truly important fact when therapeutical aspects are considered. It should not suffice to know that in most cases concentrations in the CSF are close to those of the free serum fraction of drug. The diversity of factors involved leads to assume that ideally an individual dose should be pursued for every patient, perhaps based on monitoring levels. On the other hand, the availability of an injectable preparate for i.v. use would be advantageous be-

cause the inter-individual variation due to different absorption rate and first-pass effect would be bypassed. This would allow more consistent data on the CSF concentration of PZQ, and consequently to a more uniform establishment of an effective dose regimen.

NC and PZQ — As a conclusion, some facts can be considered. 1) The individual variation seen in PZQ dosage is significant. 2) For each subject, comparative evaluations made on the same day have shown a highly significant correlation in recorded PZQ levels in the serum, protein-free serum and CSF. 3) There is no longitudinal correlation for PZQ levels on each subject, considering the 1st., 7th. and 21st. days. 4) Recorded values of CSF and protein free serum do not differ from one another on the 1st., 7th. and 21st. days of the study, which is an evidence of pharmacodynamic balance. 5) Although they were kept in balance, recorded values of protein free serum and CSF have significantly varied between the 7th. and 21st. days. This may suggest that during this period changes may have occurred in drug interaction with an element, or elements, capable of modifying PZQ balance levels, possibly an active type of inflammatory reaction in the presence of released parasite antigens. 6) Such interaction stops interfering in a significant way in the PZQ levels recorded on the 21st. day and which no longer differ from those recorded on the 1st. day.

On a case-by-case basis, one is faced with the question of whether the concentration reached at the CNS by the drug will allow it to act on cysticerci. In this regard, it should be recalled that: (a) a minimum of drug in the given environment is needed [33]; (b) a direct relationship between concentration and time is necessary in order to have a noticeable effect [1]; (c) younger cysticerci are less sensitive to the drug's action [35]; (d) there are no data on *Cysticercus racemosus*. Nevertheless, the mean CSF concentrations of PZQ on the 21st. day for instance should be enough to act on the parasite. In fact, 42% of this mean concentration supposedly would be able to penetrate inside the cyst thus reaching a higher level than the minimal effective dose (10ng/ml) required to severely damage the cysticercus in vitro [33].

SUMMARY

In 10 patients with neurocysticercosis (NC), an assessment was made of the praziquantel (PZQ) concentration in the cerebrospinal fluid (CSF), in non-deproteinized serum and in protein-free serum: before administration of the drug and the 1st., 7th. and 21st. days of oral administration (50mg/kg/day during 21 days). Samples of CSF and blood were collected three hours after the last administration of the daily total dosage, on the 1st. and 21st. days; and from 2 to 6 hours after drug administration on the 7th. day. The total daily dosage was distributed into three equal parts of 1/3 each, with a 4 hours' interval between intakes, except in the last 5 cases, who on the 21st. day only were given the total daily dosage on a single administration. Results have shown dispersion in serum concentrations, which are similar to those seen in normal subjects as recorded in literature. There is a correlation between PZQ

levels in the CSF and in the serum, the latter being very close to those found in protein-free serum fraction. The statistical treatment of results allowed the following considerations: PZQ concentrations in the CSF and in the protein free serum are in balance from the pharmacodynamic standpoint on the first day; this balance is maintained up to the 21st. day although at different levels from those seen on the 7th. day; on the 21st. day PZQ contents in CSF goes back to its similar values as recorded on the 1st. day, and this suggests that the participation of drug interaction factors has been reduced to non-significant levels. However, several factors can influence PZQ concentration in CSF, as absorption rate, liver first-pass effect and blood-brain barrier changes, and individual dose should be established for each patient based on drug concentration monitoring in the serum and/or in the CSF.

RESUMO

Praziquantel no líquido cefalorraqueano na neurocisticercose.

Em 10 pacientes com neurocisticercose (NC) foi determinada a concentração de praziquantel (PZQ) no líquido cefalorraqueano (LCR), no soro não desproteinizado e no soro desproteinizado: antes do início do emprego da droga e nos dias primeiro, sétimo e 21º de administração oral da droga (50mg/kg/dia durante 21 dias). As amostras de LCR e de sangue foram colhidas três horas após a administração da dose diária nos dias 1 e 21 e de duas a 6 horas após no dia 7; a dose total diária foi repartida em três porções semelhantes de 1/3 cada, com intervalo de 4 horas entre cada tomada, exceto nos 5 últimos casos que apenas no dia 21 receberam a dose total diária em uma só tomada. Os resultados mostraram haver dispersão das concentrações séricas e que estas são semelhantes às observadas em pessoas normais, fato já registrado na literatura. Há correlação entre os níveis de PZQ no LCR e no soro, sendo estes próximos àqueles observados no soro desproteinizado, isto é, próximo à fração sérica livre. O tratamento estatístico dos resultados permite considerar que: as concentrações de PZQ no LCR e no soro desproteinizado estão em equilíbrio, do ponto de vista farmacodinâmico, desde o primeiro dia; este equilíbrio persiste até o 21º dia, conquanto em níveis diferentes dos registrados no sétimo dia; no dia 21º os teores de PZQ no LCR retornam a valores semelhantes aos registrados para o primeiro dia, sugerindo haver sido reduzida a níveis não significativos a participação de elementos de interação com a droga. Contudo, fatores que influenciam na concentração de PZQ no LCR, como aqueles ligados à absorção da droga, sua metabolização no fígado e a sua passagem através da barreira hêmato-encefálica, que pode estar alterada pela doença, devem ser levados em conta e permitem recomendar o estabelecimento de dose individual para cada paciente mediante monitorização da concentração real alcançada no soro e/ou no LCR.

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