Journal of Neurology, Neurosurgery, and Psychiatry 1989;**52**:254–257

Humoral immune response in patients with cerebral parenchymal cysticercosis treated with praziquantel

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SUMMARY The humoral immune response to treatment with praziquantel (PZQ) was studied in eight patients with parenchymal cerebral cysticercosis (CC). In the serum and in the cerebrospinal fluid (CSF) before, during and after the administration of the drug, the following were quantitated (*a*) levels of specific anticysticercous antibodies measured in optical densities by the ELISA method; (*b*) levels of IgG, IgM, IgA and IgE; (*c*) levels of complement fraction C_3, C_4; (*d*) presence of immune complexes; (*e*) total number of white blood cells in the CSF. It was found that after treatment with PZQ, the level of specific anticysticercous antibodies and the level of IgG rose significantly in the CSF but not in the blood. The levels of the fractions of the complement and the immunoglobulins IgM, IgA and IgE did not change significantly either in the serum or in the CSF. The blood-brain barrier was found ruptured in three patients before therapy and in five patients after the therapy as measured by the albumin index. Nevertheless, the IgG index showed that there was local production of IgG in five patients before treatment and in seven after the end of it. The relative specific antibody index was greater than 1·0 in five patients before therapy and in seven after therapy. This data strongly supports the idea that the specific antibodies are produced intrathecally and are not derived from the serum pool through a ruptured blood-brain barrier. It was concluded that patients with parenchymal CC have an elevation of specific anticysticercous probably due to a combination of a ruptured blood-brain barrier and intrathecal synthesis. The relatively small rupture of the blood-brain barrier and the high IgG and relative specific antibody index suggest that intrathecal synthesis is the most important mechanism. The humoral immune response may be of importance not only in the elimination of the parasite but also in the genesis of the illness.

It is known that praziquantel (PZQ) is effective for parenchymal cerebral cysticercosis.[2-7] We also know that during the administration of the drug we can find: (1) cerebrospinal fluid (CSF) pleocytosis; (2) worsening of symptoms such as headache and seizures suggestive of cerebral parenchymal inflmmation; these symptoms fare better with the simultaneous administration of steroids; (3) computed tomography (CT) may show inflammation around the cysts; (4) some patients with parenchymatous cysticercosis while taking PZQ have developed hydrocephalus from arachnoiditis and obstruction of the subarachnoid space.[2-7] These findings suggest that: (1) the exacerbation of clinical symptoms and the presence of an inflammatory CSF and cerebral parenchymal inflammation are due to parasite necrosis and lysis, induced by the drug with a great antigenic stimulus to the immune system with the consequent presence of antibodies and cells at the site of inflammation; (2) the immune and inflammatory responses are probably related to the elimination of the parasite from the CNS. We postulated that the adequate explanation for these phenomena would probably involve increases in IgG, IgM, IgA or IgE; complement decrease and the possible appearance of immune complexes. If the immune reaction were confined to the CNS, the changes should be more conspicuous in CSF than in blood. On the other hand, if the immune reaction was generalised the changes should be detected in CSF and in blood.

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Received 6 July 1987 and in final revised form 8 August 1988. Accepted 26 August 1988

Materials and methods

We prospectively studied eight patients with proven cerebral cysticercosis. All of them had parenchymal cysts and some had calcification and/or granulomas. The mean age of the patients was 37 years with a range from 25 to 61 years; six

were male and two were female. All the patients underwent a complete neurological examination and clinical history. The patients who had concomitant disorders were excluded. We performed laboratory tests including complete haematological examination, glucose, blood urea nitrogen, creatinine, serum electrolytes and urinalysis. We obtained samples of CSF and serum in order to quantify immunoglobulins by nephelometry: IgG, IgM, IgA, IgE; complement: C_3, C_4; immune complexes. These were obtained before treatment, after 8 days of treatment, and after 15 days when the treatment was completed; cerebrospinal fluid WBC count was performed as well as detection of anticysticercous antibodies in serum and in CSF with the ELISA test, before treatment, after 8 days and 15 days after, when treatment was completed. The integrity of the blood-brain barrier was calculated by the ratio CSF albumin/serum albumin and by the total CSF protein.[12] The intrathecal synthesis of anticysticercous antibodies was calculated by the IgG index[12,13]:

$$\frac{\text{IgG CSF}}{\text{IgG serum}} + \frac{\text{Alb CSF}}{\text{Alb serum}}$$

The relative specific antibody index was calculated with the following formula:

$$\frac{\text{Titres ELISA CSF}}{\text{Titres ELISA serum}} + \frac{\text{IgG CSF}}{\text{IgG serum}}$$

The relative specific antibody index is significant at values greater than 1·0.[15-17] All the patients received PZQ to a dosage of 50 mg per kilogram of body weight daily during 15 days. They also received prednisone, 50 mg daily during the treatment with PZQ. The paired Student's *t* test and Wilcoxon test were used to compare the diverse results.

Antigen preparation: We followed the method of Kagan.[11] The cysticerci were obtained from parasitised pig's meat. The parasites were washed in saline solution, frozen and unfrozen three times; afterwards they were sonicated in an ice bath, then they were lyophilised and crushed in a mortar; thereafter, they were hydrated in a coca solution and centrifugated for 30 minutes at 3,500 RPM (centrifuge WIFV6). The supernatant was dialysed against saline solution. The dyalisate was again centrifuged and the proteins were quantified. Finally it was lyophilised again and stored.

Elisa test: The indirect ELISA test was used and the technique was as follows: in Dynatech Immulon plates with removable wells were added 200 μl of cysticercous cellulose antigen in a concentration of 5 μg/ml for the serum test and 3 μg/ml for the CSF test for each well; each was kept at 4°C for 12 hours. To it was added 200 μl of serum or CSF diluted 1:200 for the serum in PBs tween and 1:10 for the CSF also in PBs tween. Then, it was incubated for 30 minutes at 37°C and then washed for 5 minutes three times. Anti IgG (200 mcl) was added to each well. After washing for 5 minutes each well received 200 mcl of substrate solution and was incubated for 30 minutes at 37°C. Finally the reaction was stopped with 50 mcl of NaOH solution in a normal concentration and the reading was made in optic densities in a microlector for ELISA 810-C Titertek-Multiskar. A detailed description of the ELISA test used is given elsewhere.[10]

Nephelometry: The technique was as follows: the antiserum dilution was prepared from filtrated saline solution from each protein that was going to be determined. The dilution of the standard serum LN protein standard was done for the reference curve. The reference curves and the patients curves were placed together with IgG controls in their respective cell. AntilgG antiserum was added and afterward was left to settle for 60 minutes. The reading was done on a Hoechst nephelometer. The same procedure was performed for the IgM, IgA, C_3, and C_4. The immune complexes determination was done as follows: a standard curve was obtained; the lipids of the patients serum were removed and were diluted with phosphate buffers. 100 μl of patients serum were mixed with 200 μl of P.E.G. (3%). It was incubated for one hour at ambient temperature. The lecture was done on a Hoechst nephelometer and the standard curve was interpolated.

Results

Six of the eight patients with parenchymal cysticercosis had seizures, four of them partial and two of the generalised type. Three had headache (table 1). The CT findings are shown in table 2. None of them had hydrocephalus. The mean duration of the illness was 11 months.

A statistically significant increase of the titres of anticistercercous antibodies in CSF was found when we compared the titres before treatment with those titres at the end of it ($p < 0{\cdot}01$) and after eight days of it ($p < 0{\cdot}01$). There was no significant difference in serum concentrations of antibodies before and after treatment. IgG was increased in CSF at the end of the treatment with PZQ, and this was significant ($p < 0{\cdot}02$). We did not find significant difference in blood. The rest of immunoglobulins did not show significant changes on their titres, in serum or in CSF. The complement did not show significant changes. In addition there was no significant appearance of immune complexes. The CSF white blood cells increased significantly after eight days of treatment with PZQ ($p < 0{\cdot}01$) (table 3), but not at the end of it. The total CSF protein was elevated in three and normal in five patients before treatment but was significantly elevated after the administration of the drug ($p < 0{\cdot}05$) (table 3). All patients had a serum albumin within normal limits.

The CSF albumin/serum albumin ratio was normal

Table 1 *Symptoms of patients with parenchymatous cerebral cysticercosis*

Patients	*Symptoms*
1	Headache
2	Partial seizures, headache
3	Partial seizures
4	Partial seizures
5	Headache
6	Partial seizures
7	Generalised seizures
8	Generalised seizures

Table 2 *Findings in patients with cerebral cysticercosis*

Patients	*Findings*
1	Multiple parenchymatous cysts
2	Multiple paranchymatous cysts and calcifications
3	Multiple parenchymatous cysts
4	Parietal cyst with edema
5	Parenchymatous cysts and multiple granulomas
6	Parenchymatous cyst
7	Parenchymatous cysts and calcifications
8	Multiple parenchymatous cysts and granulomas

in five of the eight patients before therapy but after therapy only one patient had a normal ratio (table 3). The IgG index was abnormally high (>0·65) in five patients before therapy and in seven patients after the treatment was given. Before therapy in five patients it could be demonstrated that there was de novo synthesis of anticysticercous antibodies intrathecally. The IgG index rose significantly after therapy ($p < 0·01$) and in seven patients intrathecal synthesis of antibody could be demonstrated.

The relative specific antibody index was greater than 1·0 in five patients before therapy but rose to seven after therapy.

No significant correlation was found between the albumin index and the CSF IgG using the Pearson correlation coefficient ($p > 0·05$). We did not find a significant correlation between the IgG serum and the IgG of the CSF ($p > 0·05$).

Discussion

Our findings suggest that the central nervous system responds to the antigenic stimulus produced by parasite lysis, increasing the titres of antibodies.[1 8–10] These antibodies may play an important role in the genesis of inflammation in the subarachnoid space and cerebral parenchyma in those patients treated with PZQ.[2 3 6] Likewise, it is probable that the immunological reaction and the concomitant inflammatory reaction may play an important role in the disappearance of the parasites or of the lysed parasitic antigen as a consequence of the treatment with PZQ. It is likely that the number of cysts, that is to say, the amount of parasitic antigen, is important for the genesis of the disease, since it is probable that the central nervous system may not be able to eliminate a great amount of parasitic antigen, thus leading to disease due to antigen excess. On the other hand, if the parasite is located in the subarachoid space, the parasitic lysis may induce an inflammatory reaction that produces arachnoiditis and subarachnoid block of CSF and under these circumstances the administration of PZQ can produce a deleterious affect. The presence of inflammatory reaction in CSF and the rise of IgG type antibodies may depend on the contiguity, or the distance from the parasite to the subarachnoid space. We have shown that the sensitivity of the ELISA test is greater in patients with parasites in the subarachnoid space than in those cases in which the parasite is in the cerebral parenchyma.[10] Therefore, in those cases of subarachnoid cysticercosis or in those in which the cyst is near or inside the subarachnoid space, there is likely to be a considerable increase of antibodies titres[10] in the CSF. It is somewhat perplexing that we did not find immune complexes in these patients, because we thought it was likely that the parasitic lysis with a major antigenic stimulus should have produced them. This may be due to the techniques used, to the low sensitivity of our method, or to the distance of the parasite from the subarachnoid space. We did not find immune complexes either in the CSF or in the blood. The complement fractions did not change significantly, suggesting that there is no consumption of com-

Table 3 *CSF findings in the patients*

Total CSF protein mg (normal values 30–60 mg)		*CSF Alb/serum Alb (normal values: 0·0037–0·0052)*[12]	*IgG index (normal value > 0·65)*[13]	*Number of WBC in CSF per mm³*	*Relative specific antibody index abnormal > 1.0.5*
First sample:					
1	22	0·004	0·9	2	2·6
2	94	0·016	1·12	3	1·3
3	28	0·0048	0·52	4	0·48
4	30	0·0054	0·9	11	0·82
5	30	0·0054	0·79	20	2·2
6	78	0·014	0·69	34	1·4
7	144	0·025	0·452	20	0·86
8	18	0·003	0·8	16	1·08
Second sample:					
$p < 0·05$		$p < 0·05$	$p < 0·01$	$p < 0·01$	$p < 0·02$
1	34	0·006	0·98	70	8·7
2	132	0·02	2·4	210	2·0
3	54	0·0094	0·72	800	2·0
4	34	0·006	1·65	110	1·45
5	45	0·008	1·5	290	2·4
6	84	0·014	1·57	40	1·2
7	140	0·024	0·45	220	0·9
8	16	0·0028	0·82	110	2·0

plement fractions in CSF; it suggests that an immune complex mediated disease is an unlikely cause of the inflammatory reaction. Again, this may be different in patients with subarachnoid cysts in whom the parasite is in direct contact with the CSFF. The immunoglobulins levels, IgM, IgA and IgE, did not change either in CSF or in blood, suggesting that these immunoglobulins do not take part importantly on the immune reaction that is seen during the treatment with PZQ.

Further studies are necessary to determine the immune response in patients with other types of cerebral cysticercosis. Apparently, the antibody increase take at least 15 days to be produced, and we do not know precisely its temporal course. All of our patients had high antibodies titres in CSF before treatment, but they rose significantly after it. Two important questions arise: is the blood brain barrier ruptured in these patients or does it rupture during treatment? Are the anticysticercous antibodies produced de novo intrathecally or they come from the blood? The total CSF protein was elevated in only three patients before and after treatment, although the mean protein level rose significantly after treatment. The CSF albumin/serum albumin ratio was significantly elevated in the three patients who had a high CSF protein level (patients 2, 6 and 7) and slightly elevated in three other patients. In two patients in whom the total CSF protein was normal and the CSF albumin/serum albumin ratio was also normal, it could be demonstrated that de novo synthesis of IgG took place. Treatment with PZQ ruptured the blood-brain barrier in several patients.

The very high synthesis of intrathecal IgG and the presence of a high relative specific antibody index, along with the relatively small rupture of the blood-brain barrier, as measured by the albumin index, suggests that intrathecal synthesis is the most important mechanism.

The role of the humoral immune response in the production of the disease remains controversial but this study suggests that antibody increase may be responsible for the antigen parasite elimination after the lysis produced by PZQ and, paradoxically, it may also enhance the associated inflammatory reaction. It is likely that it is also of importance in the genesis of the clinical symptoms observed during treatment with PZQ. The relative specific antibody index was significantly elevated ($>0{\cdot}01$) in five patients before treatment and in seven patients after the end of the medication. This index is the best indication that specific antibodies are produced intrathecally.[15–17] This study suggests that most of the IgG produced intrathecally is specific. Specific antibody production inside the blood-brain barrier has been demonstrated in several infective processes of the nervous system, including mumps, meningitis[17] and herpes simplex infection of the nervous system.[15] In patients with measles encephalomyelitis, in whom no viral particles are found inside the central nervous system, no specific intrathecal antibody production has been demonstrated.[16] It is likely that specific intrathecal antibody production plays a role in several infective processes of the SNC including bacterial, viral and parasitic infections.[12]

References

1 Flisser A. Inmunologia de la cisticercosis humana. *Bol Estud Méd Biol* 1983;**suplemento Vol. 32**:143–76.

2 Sotelo J, Escobedo F, Rodríguez C, Torres B, Rubio F. Therapy of paranchymal brain cysticercosis with praziquantel. *N Engl J Med* 1984;**310**:1001–7.

3 Lombardo L, Vasconcelos D, Cruz H. Tratamiento de la cisticercosis con praziquantel. Informe preliminar de 10 casos. *Gac Med Mex* 1983;**119**:17.

4 Thomas H, Andrews P, Melhorn H. New results of the effect of praziquantel in experimental cysticercosis. *Am J Trop Med Hyg* 1982;**31**:903.

5 De Ghetaldi L, Norman RM, Dorville AW. Praziquantel for cysticercosis of the brain parenchyma. *N Engl J Med* 1984;**311**:732–3.

6 Botero D, Castaño S. Treatment of cysticercosis with praziquantel in Colombia. *Am J Trop Med Hyg* 1982; **31**:811.

7 Ci Meri. Praziquantel for cysticercosis of the brain parenchyma. *N Engl J Med* 1984;**311**:733.

8 Miller B, Myers A, Goldberg M. Cerebral cysticercosis diagnosis by a specific IgG antibody. *Neurology* 1982;**32**:79.

9 Mohammad I, Heiner D, Fuller B, Goldberg M, Kagan T. Enzyme linked immunosorbent assay for the diagnosis of cerebral cysticercosis. *J Clin Microbiol* 1984;**20**:777.

10 Corona T, Pascoe D, González-Barranco D, Abad P, Landa L, Estañol B. Anticysticercous antibodies in serum and cerebrospinal fluid in patients with proven cerebral cysticercosis. *J Neurol Neurosurg Psychiatry* 1986;**49**:1044.

11 Kagan IG. A review of serological tests for the diagnosis of hydatidic disease. *Bull WHO* 1968;**61**:154.

12 Leibowitz S, Hughes RAC. Immunology of the Nervous System. London: Edward Arnold, 1983.

13 Lefvert AK, Link H. IgG production within the central nervous system: A critical review of proposed formulae. *Ann Neurol* 1985;**17**:13.

14 Tourtellote WW, Potuin AR, Fleming JO, *et al.* Multiple sclerosis: measurement and validation of central nervous system IgG synthesis rate. *Neurology* 1980; **30**:240–4.

15 Felgenhaver K, Nekic M, Ackermann. The demonstration of locally synthesized herpes simplex IgG antibodies in CSF by sepharose 4B linked enzyme immunoassay. *J Neuroimunol* 1982;**3**:149.

16 Johnson RT, Griffin DE, Hirsch RL, Wolinsky JS, Roedenbeck S, De Soriano IL, Vaisberg A. Measles encephalomyelitis clinical and immunological studies. *N Engl J Med* 1984;**310**:137.

17 Ukkonen P, Granstom ML, Rasanen J, Salomen EM, Penttinen K. Local production of mumps IgG and IgM antibodies in the cerebrospinal fluid of meningitis patients. *J Med Virol* 1981;**8**:252.