FOLLOW-UP OF NEUROCYSTICERCOSIS PATIENTS AFTER TREATMENT USING AN ANTIGEN DETECTION ELISA

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Summary:
Seven patients with active neurocysticercosis (NCC) received an eight days treatment with albendazole and were followed up using computed tomography (CT-scan) and a monoclonal antibody based ELISA for the detection of circulating antigen (Ag-ELISA). Only three patients were cured as was shown by CT-scan and by the disappearance of circulating antigens one month after treatment. After a second course of albendazole therapy, two other patients became seronegative. CT-scan showed the disappearance of viable cysts in all persons who became seronegative whereas patients who were not cured remained seropositive. These preliminary results show that this Ag-ELISA is a promising technique for monitoring the success of treatment of NCC patients because of the excellent correlation between the presence of circulating antigens and of viable brain cysts.

KEY WORDS : Taenia solium, CT-scan, ELISA, circulating antigen, neurocysticercosis, albendazole.

Neurocysticercosis (NCC) is an infection of the central nervous system by the larval stage of Taenia solium. It is recognized as a common cause of neurological disease in developing countries (Schantz et al., 1998). The presence of T. solium metacestodes in the nervous system leads to a variety of clinical manifestations depending upon the number, size, viability and location within the brain and the host inflammatory reaction (White, 2000). Albendazole and praziquantel are the drugs of choice for the treatment of NCC. Follow-up after treatment is usually done using computed tomography (CT-scan), which is an expensive technique for the people of developing countries (Tsang & Wilson, 1995). Considering the importance of NCC and the increasing poverty in these countries, it is necessary to investigate alternative techniques, which could reduce at least the cost of evaluation of the efficacy of the treatment. Since an antigen detection ELISA (Ag-ELISA) has been developed, which has a high sensitivity for the detection of human cysticercosis (Erhart et al., 2002) and which allows to distinguish cattle and pigs carrying living cysts from those harbouring only dead cysts (Brandt et al., 1992; Nguekam et al., in press), it was decided to evaluate its use in NCC patients. The purpose of this study was to compare the Ag-ELISA test with CT-scan to evaluate the efficacy of an albendazole treatment of NCC.

MATERIALS AND METHODS

Patients
Seven patients with active lesions of NCC from the West province of Cameroon identified by serology (Ag-ELISA) and confirmed by brain CT-scan (in Yaounde Central Hospital) were included in this study. NCC was asymptomatic in all but two of them, who were epileptics (Batoula 94 and KE). Five of them were women and two men. Their age ranged between seven and 73 years (with a mean of 39.9 ± 24.9 years). The patients harboured an average of five viable cysts (range: 1 to 12) and of 7.6 calcified cysts (range: 0 to 25) in the
TREATMENT PROTOCOL

The patients were treated with albendazole (Alben® 400 mg, Smithkline Beecham) at a dosage of 15 mg/kg body weight/day for eight days as described by Del Brutto et al. (1999). To prevent adverse reactions, prednisolone (Solupred® 5 mg, Laboratoire Houdé) at 10 mg per person thrice a day was given from one day prior to the albendazole treatment until at least four days after the end of it (Groll, 1982). The two epileptic patients continued to receive anti-epileptic treatment (Gardenal®).

Six to nine months after the end of the first treatment, the patients who remained seropositive were treated again with the same dose of albendazole for a period of one month. In one patient (KE) the first treatment was not a course of eight days, but of one month.

ANTIGEN DETECTION ELISA FOR CYSTICERCOSIS (AG-ELISA)

The patients were sampled before the beginning of the treatment, one and three months after the first treatment and in those patients who received a second treatment, one month later. The serum samples were tested using a monoclonal antibody based antigen detection ELISA as described by Brandt et al. (1992) but slightly modified according to Pouedet et al. (2002). The sera were pre-treated using trichloroacetic acid and used in ELISA at a final dilution of 1/4. Two monoclonal antibodies (MoAb) were used in a sandwich ELISA. MoAb B158C11A10 was used for coating and a biotinylated MoAb B60H8A4 was included as detector antibody. Orthophenylene diamine and H$_2$O$_2$ were used as chromogen-substrate solution. After arresting the reaction with 4 N H$_2$SO$_4$ the plates were read using an ELISA reader (Labsystem Multiskan RC) at 492 nm.

Eight negative reference control sera from local people of the region of Dschang (without any history of taeniasis or cysticercosis in the family) and one reference positive serum from a Cameroonian patient with confirmed cysticercosis (by CT-scan) were included in each ELISA run. The optical density (OD) of each serum sample was compared with the mean of the eight negative reference sera at a probability level of $P=0.001$ to determine the result using a modified Student test (Sokal & Rohlf, 1981). The ELISA values were expressed as a ratio by dividing the OD of the test sample by the OD of the cut-off value. An ELISA ratio $>1$ was considered as positive.

RESULTS

The serological results and brain CT-scan status of each patient before and after albendazole therapy are presented in Table I and Figures 1 and 2.

AG-ELISA RESULTS

One month after the end of the first course of albendazole three patients became negative in the Ag-ELISA
**FOLLOW-UP OF NEUROCYSTICERCOSIS USING ELISA**

**CT-SCAN RESULTS**

CT-scan of the brain after one or two courses of albendazole therapy showed that viable cysts had completely disappeared in five out of seven (71.4%) patients. In the two others, either a reduction of the number of viable cysts (Batoula 94) or the presence of ring enhanced cysts indicating a process of degeneration (KE) was observed after treatment (Table I). In all patients where the viable cysts disappeared after treatment a negative ELISA result (ratio < 1) was obtained whereas a persistence of even few living or degenerating cysts resulted in positive ELISA values.

**COMPARATIVE COSTS OF ELISA AND CT-SCAN TECHNIQUES IN THE FOLLOW-UP OF NCC PATIENTS AFTER TREATMENT**

Up to now, CT-scan in Cameroon can only be carried out in Yaoundé or Douala. Besides the costs of scanning and the contrast agent, the total cost of a scan includes therefore also the travel and food expenses (for two days). This cost was estimated for each patient involved in the present study to approximately 152.2 € (100,000 CFA) whereas the cost of a test of an Ag-ELISA (for 40 samples or one plate) was about 17 €, i.e. 0.425 € per patient. The salaries of the medical doctor or the laboratory technicians are not included in this calculation.

**DISCUSSION**

Computed tomography is an useful imaging technique for the diagnosis of human neurocysticercosis and the assessment of the efficacy of anthelminthic drugs in the treatment of this disease (Padma et al., 1994; Garcia et al., 1997; White, 2000). Its high cost, however, and the fact that it is often unavailable in rural regions of developing countries, where the prevalence of NCC is high (Tsang & Wilson, 1995), constitute a limitation for its wide use. In this study, we compared this technique with an Ag-ELISA as an alternative method for the follow-up of neurocysticercosis patients after cysticidal treatment. The Ag-ELISA has been shown to detect the excretory-secretory products of viable cysticerci in cattle (Brandt et al., 1992), pigs (Nguekam et al., in press) and man (Erhart et al., 2002). These latter authors reported a sensitivity of 94.4% and the absence of cross-reactions with sera from human patients infected with *Schistosoma*, hydatid cysts, *Ascaris*, *Trichuris*, filaria, *Entamoeba*, *Plasmodium* and *Trypanosoma*.

Although the follow-up period was not the same for the two techniques, the results obtained in this study were very promising. There was 100% agreement between the CT-scan and the Ag-ELISA results. The five patients, who became seronegative one month after one or two albendazole courses, showed a complete disappearance of the viable cysts in the brain whereas the two remaining seropositive patients (Batoula 94 and KE) showed respectively two living cysts and four cysts with ring enhancement.

The sensitivity of our Ag-ELISA was better than that of another monoclonal antibody-based Elisa used by Garcia et al. (2000) in monitoring neurocysticercosis patients after treatment. This latter Ag-ELISA could not detect patients with only one viable cyst and/or enhancing lesion whereas in this study two patients with one single living cysticercus in the brain could be identified.

These preliminary results clearly show that this monoclonal antibody-based Ag-ELISA is a promising technique to monitor neurocysticercosis patients after treatment. In addition, it is much cheaper than CT-scan in particular for patients of developing countries where poverty is an increasing reality. Currently, studies on a larger number of NCC patients are going on in order to validate the Ag-ELISA as an alternative for CT-scan.

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