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Effects of Mel B Arsobal and Alpha-Difluoromethylornithine on the Awakening Electroencephalogram of Humans With Gambiense Trypanosomiasis Disease: Preliminary Report

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HAMON, J. F., B. SERI, F. DOUA, P. CAMARA AND L. ABA. *Effects of Mel B Arsobal and alpha-difluoromethylornithine on the awakening electroencephalogram of humans with gambiense trypanosomiasis disease: Preliminary report.* PHARMACOL BIOCHEM BEHAV **36**(4) 831–835, 1990.—Five males and three females, at the encephalic stage of sleeping sickness, were submitted to trypanocide therapies. Three of the patients were treated with the Mel B Arsobal drug, the five others with difluoromethylornithine, using different protocols. Awakening electroencephalographic data were obtained prior to treatment and, at regular intervals, during and after treatment. Prior to treatment the awakening tracings showed important abnormalities (slow delta waves were superimposed on theta background rhythms). During treatment (except in one patient treated with Arsobal) recordings returned gradually to fast rhythms and several days after therapy, tracings returned to the normal awakening patterns. The use of the awakening electroencephalogram as a tool to test effects of curative drugs in the sleeping sickness syndrome is discussed.

Sleeping sickness Electroencephalogram Mel B Arsobal DFMO

SLEEPING sickness is an important African tropical disease; it is caused by the infection of humans with *Trypanosoma gambiense* or *Trypanosoma rodesiense*.

Two stages have been described in its pathology: a blood-lymph and an encephalitic stage (9). Only drugs which cross the blood-brain barrier are effective against the infectious parasite at the stage of central nervous system involvement. Derivatives of trivalent arsenic salts, which have been used in treatment for more than 25 years (6), can induce arsenical encephalopathy (2, 3, 12), an acute cerebral edema, characterized by a sudden onset of coma associated with convulsions. This complication causes the death of 2–5 percent of patients treated with arsenicals (7,20).

New therapeutic drugs, which meet the criteria of efficacy and tolerance, have been developed with the aim of improving the treatment of human trypanosomiasis. Thus, DL alpha-difluoromethylornithine (DFMO) has been shown recently to be effective in curing humans affected by sleeping sickness (8,19).

In order to throw more light on the effectiveness of DFMO in treatment, we compared the effects of treatment with arsenicals and DFMO on the awakening electroencephalograms of humans at the stages of cerebral involvement of sleeping sickness.

METHOD

The study was conducted in the trypanosomiasis clinic in the city of Daloa (Côte d'Ivoire). Eight patients (five males and three females, age range 16 to 46) with abnormal cerebrospinal fluid (second stage of gambiense trypanosomiasis) participated in the experiment. In all the patients, trypanosomes were found in peripheral blood and lymph-nodes (8).

Three subjects (patients 1, 2, and 3) were treated with Mel B Arsobal (Mel B Arsobal is a compound of the trivalent arsenical melarsen oxide and dimercaptol: BAL). Arsobal treatment courses consisted of three series of four daily intravenous injections (3 and 6 mg/kg); the series were separated by an interval of ten days (8).

The five other subjects were treated with DL alpha-difluoromethylornithine hydrochloride monohydrate (DFMO), an inhibitor of ornithine decarboxylase. DFMO inhibits nuclei acid synthesis and cytokinesis, and alters trypanosomal morphology (5). Treatments were conducted according to the following two protocols:

(A) In three subjects (patients 4, 5, and 6), the drug was administered by intravenous perfusion during 14 days at 100 mg/kg every six hours. For the subsequent 21 days DFMO was

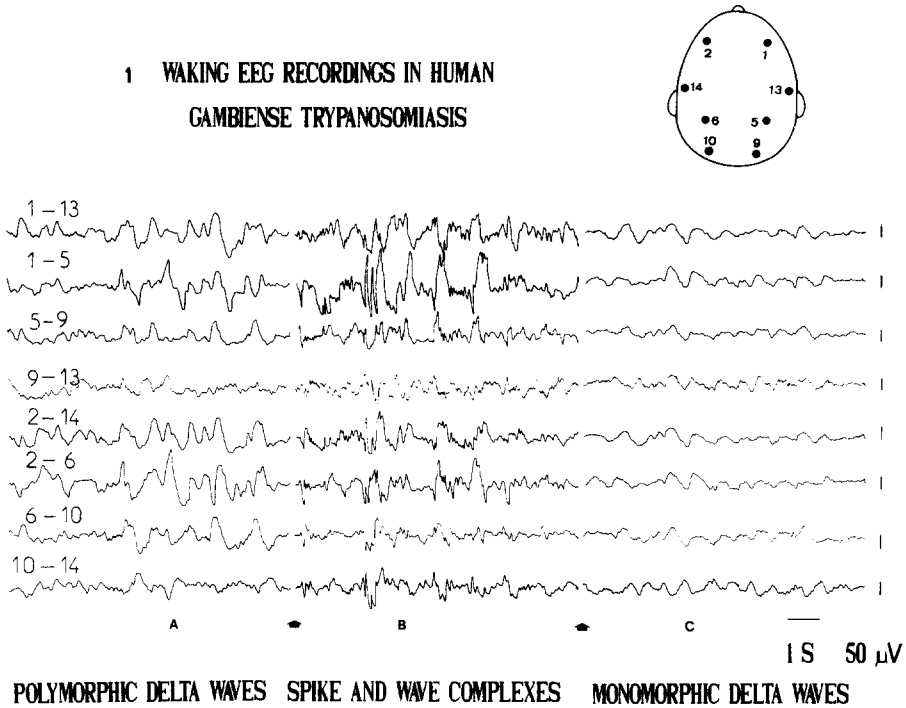

FIG. 1. Awakening recordings from sleeping sickness encephalic stage patients: The plate shows the presence on a theta background rhythm of (A) polymorphic delta waves, (B) spike and wave complexes, (C) monomorphic delta waves.

administered orally at a dose of 75 mg/kg every six hours (19).

(B) In the last two patients (subjects 7 and 8), DFMO was administered by intravenous perfusion during 14 days, at a dose of 400 mg/kg/day.

Electroencephalograms were recorded on an 8-channel Alvar polygraph (Reega Minihuit TR) operating at a chart speed of 15 mm/sec, with a time constant of 0.3 second and without filter. Bilateral, longitudinal mountings were used.

Awakening electroencephalographic recordings were performed before instituting treatments, at regular intervals during treatment and after the end of the treatments.

RESULTS

Before Treatment

Awakening tracings in the eight individuals with sleeping sickness in the phase of cerebral involvement were performed. On a theta background rhythm, slow bilateral polymorphic delta waves of 2 to 4 c/sec or monomorphic delta waves of 1 c/sec were frequently superimposed (see right and left recordings of Fig. 1). Spike and wave complexes are obvious in the recordings of the third patient (see central recordings of Fig. 1).

Subjects 1, 2, 5, and 8 presented the symptoms consistent with a cerebromeningitis. Slow bilateral burst of delta waves of 1 to 2 c/sec were superimposed on an irregular transcient theta rhythm of 5 to 7 c/sec. The five other patients had severe and diffuse cerebral involvement. Slow bilateral and diffuse theta and delta activities predominated in the electroencephalograms of these subjects.

Arsobal Therapy

Figure 2 shows recordings from patient 2, before, during and after Arsobal therapy. Five days after the first series of Arsobal injections (except for the third patient), the electroencephalogram recordings showed improvement. On fast background activity, delta waves, which had lost their burst features, were superimposed. Five days after the second series of Arsobal injections, the recordings revealed a return to a fast rhythm of 10 to 13 c/sec, still interrupted by slow waves. Five days after the last course of Arsobal injections, the tracings were characterized by a fast bilateral and irregular background activity. Tracings of the third patient remained unchanged after the end of Arsobal therapy.

DFMO (A) Therapy

Figure 3 shows recordings from patient 6 (a 46-year-old male), before, during and after DFMO (A) treatment. In the three treated subjects, five days after the start of DFMO (A) treatment, the overall amplitude of the tracings was reduced. Patient 6 showed a short periodic activity of 1 c/sec, which predominated on fronto rolandic areas. This periodic activity is consistent with a paroxysmal attack. Twenty days after the onset of the treatment, a symmetric and irregular background activity of 3 to 10 c/sec mixed with faster rhythm became visible in all the treated subjects. Twelve days after the end of the treatment, the tracings returned to normal awakening patterns against an irregular and bilateral background activity.

DFMO (B) Therapy

Figure 4 shows tracings from patient 7 (a 20-year-old male), before, during and after DFMO (B) therapy. The recordings of both patient 7 and a second subject showed essentially a bilateral diffuse monomorphic delta activity of 1 c/sec. Three days after

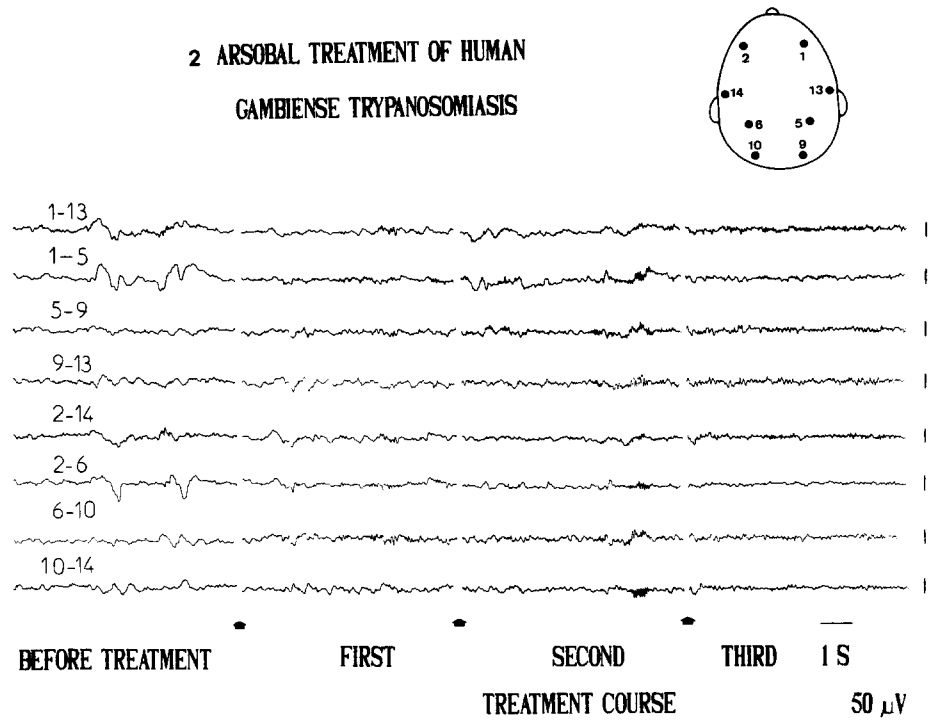

FIG. 2. Recordings from patient 2, a 21-year-old male with background disease consistent with a cerebromeningitis. The recordings made at regular intervals during Arsobal treatment show recovery of normal awakening EEG patterns.

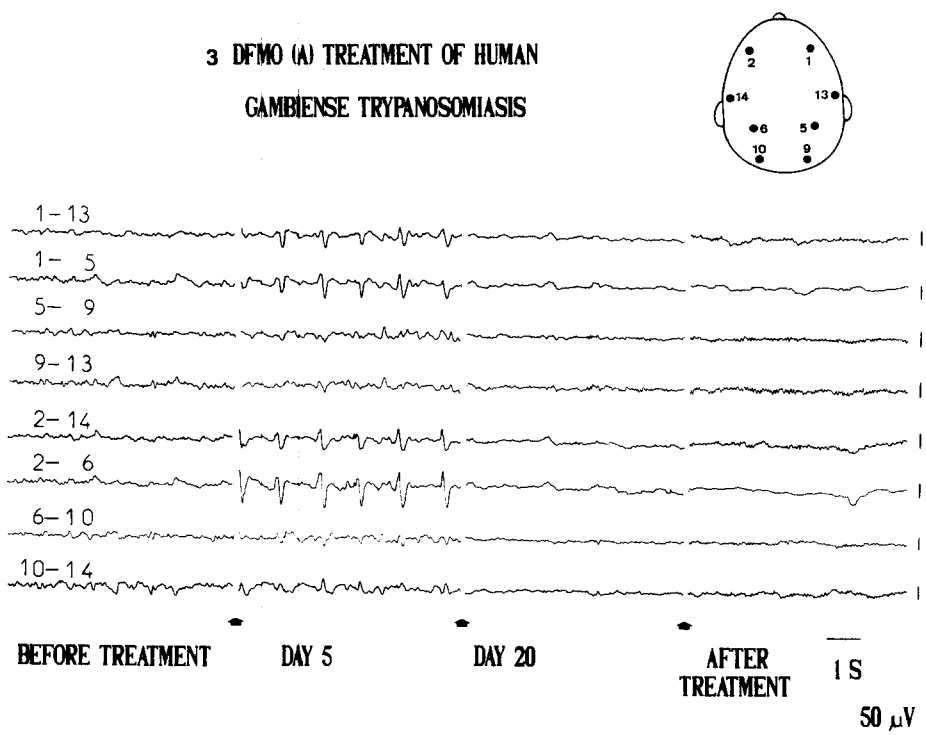

FIG. 3. Recordings from patient 6, a 46-year-old male with sleeping sickness who suffered from severe and diffuse cerebral pain. The tracing shows recovery of normal awakening EEG patterns, during DFMO (A) therapy. Note the presence, five days after instituting DFMO (A) treatment, of a periodic activity, which predominated on fronto rolandic areas.

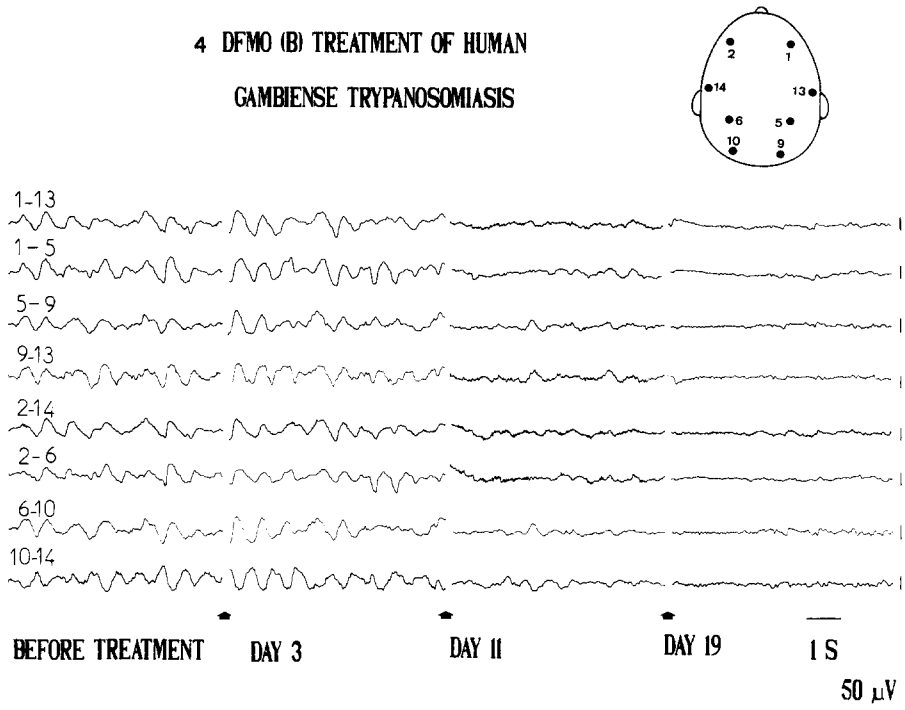

FIG. 4. Recordings from patient 7, a 20-year-old sleeping sickness, who suffered from diffuse cerebral pain. The recordings show amelioration and partial recovery of normal awakening EEG patterns during DFMO (B) treatment.

instituting the treatment the electroencephalogram remained unchanged. Twelve days later, faster rhythms were superimposed on a slow wave background. The nineteenth day tracings showed improvement compared to the earlier tracings. A noticeable reversal to slow monomorphic delta waves was obvious in patient 7. An irregular theta rhythm of 4 to 7 c/sec and faster rhythms predominated in the recordings.

DISCUSSION

In seven of the eight patients, normal electroencephalographic awakening tracings, in spite of important initial abnormalities, were restored in the days which followed the end of the treatments. Although a third subject was unresponsive to arsenical therapy, the overall results demonstrate the efficacy of both arsenical and difluoromethylornithine therapies on the recovery of normal awakening electroencephalogram patterns in humans affected with severe sleeping sickness.

Our results on the recovery of awakening electroencephalograms in subjects treated with Arsobal are consistent with the literature concerning the trypanocidal activity of Mel B Arsobal (9). Recordings from a third patient, which remained unchanged after Arsobal treatment, agree with cases of refractoriness to organic arsenical therapy (14, 16, 10), and encephalopathy induced by arsenic toxicity (2, 11, 12, 15, 17) reported by many authors. Thus, Arsobal therapy appears to represent a significant risk for patients hypersensitive to arsenicals. Several reports have emphasized the curative effects of DFMO (14, 13, 18) on *Trypanosoma gambiense*-caused sleeping sickness (8,19). Despite its largely reversible side effects (1,18), DFMO seems to be an effective and nontoxic drug for the treatment of patients at the encephalic stage of sleeping sickness. Insofar as DFMO is effective on subjects refractory to arsenical therapy (4), its use seems more effective than that of organic arsenical therapy.

To conclude, electroencephalograms appear to be a good clue for studying recovery of normal awakening patterns during therapy, in humans with sleeping sickness at the encephalic stage. It is well known that arsenical encephalopathy tends to occur around day 10 after initiation of Arsobal treatment (7). EEG recordings associated with biological measurements, such as the determination of the occurrence of trypanosomes in peripheral blood and the determination of cerebrospinal fluid proteins, prior and during treatments, will serve as a good clinical test to monitor the effects of therapy, with the possibility to interrupt or decrease drug administration immediately after the first intolerance symptoms.

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REFERENCES

1. Abeloff, M. D.; Slavik, M.; Luck, G. D.; Griiffin, C. A.; Herman, J.; Blanc, O.; Sjoersdma, A.; Baylin, S. B. Phase 1 trial and pharmacokinetic studies of difluoromethylornithine—an inhibition of polyamine biosynthesis. J. Clin. Oncol. 2:124–130; 1984.
2. Adams, J. H.; Haller, L.; Boa, F. Y.; Doua, F.; Dago, A.; Konian, K. Human African trypanosomiasis (T.b. gambiense): A study of 16 fatal cases of sleeping sickness with some observations on acute reactive arsenical encephalopathy. Neuropathol. Appl. Neurobiol. 12:81–94; 1986.
3. Antoine, P. H. Etude neurologique et psychologique de malades trypanosomés et leur évolution. Ann. Soc. Belge. Med. Trop. 57(4–5):227–247; 1977.

4. Bacchi, C. J.; Nathan, C. H.; Hutner, G. H.; McCann, P. P.; Sjoerdsma, A. Polyamine metabolism a potential therapeutic target in trypanosomes. Science 210:332–334; 1980.
5. Bacchi, C. J.; Garofalo, J.; Mockenhaupt, D.; McCann, P. P.; Dicklema, K. A.; Pegg, A. E.; Nathan, H. C.; Mullaney, E. A.; Chunosoff, L.; Sjoerdsma, A.; Hutner, S. A. In vivo effect of DL difluoromethylornithine on the metabolism and morphology of Trypanosoma brucei brucei. Mol. Biochem. Parasitol. 7:209–225; 1983.
6. Collomb, H.; Ayats, H.; Martino, P.; Lariviere, M. Les incidents et accidents du traitement de la trypanosomiase par le Mel W (deuxième note à propos de quarante nouvelles observations). Bull. Soc. Med. Afr. Noire Langue Francaise. 9:325–329; 1964.
7. De Raadt, P.; Seed, J. R. Trypanosomes causing disease of man in Africa. Parasitic Protozo. 1:175–237; 1977.
8. Doua, F.; Boa, F. Y.; Schechter, P. J.; Miezan, T. W.; Diai, D.; Sanon, S. R.; Deraadt, P.; Haegele, K. D.; Sjoerdsma, A.; Konian, K. Treatment of human late stage gambiense trypanosomiasis with difluoromethylornithine (eflornithine): efficacy and tolerance. Am. J. Trop. Med. Hyg. 37(3):525–533; 1987.
9. Dumas, M.; Girard, P. H. Human African trypanosomiasis. In: Vinken, P. J.; Debruyn, G. W., eds. Handbook of clinical neurology. vol. 35, part 3. 1978:67–82.
10. Ginoux, F. Y.; Lancien, P.; Frezil, J. L.; Bisadidie, N. Les échecs du traitement de la trypanosomiase à T. gambiense au Congo. Med. Trop. 44:149–154; 1984.
11. Haller, L.; Adams, J. H.; Merouze, F.; Dago, A. Clinical and pathological aspects of human African trypanosomiasis (T. gambiense) with particular reference to reactive arsenical encephalopathy.Am. J. Trop. Med. Hyg. 79:550–571; 1985.
12. Hurst, E. W. The lesions produced in the central nervous system by certain organic arsenical compounds. J. Physiol. Bacteriol. 77: 523–533; 1959.
13. McCann, P. P.; Bacchi, C. J.; Clarckson, A. B., Jr.; Seed, J. R.; Nathan, C. H.; Amole, B. O.; Hutner, S. H.; Sjoerdsma, A. Further studies on difluoromethylornithine in African trypanosomes. Med. Biol. 59:433–440; 1981.
14. Ogada, T. Clinical Mel B resistance in Rhodesian sleeping sickness. E. Afr. Med. J. 51:56–59; 1974.
15. Rive, J.; Serie, F.; Kone, L.; Bertrand, E. Considérations sur le traitement de la trypanosomiase humaine à Trypanosoma gambiense (méthodes, résultats, encéphalopathie arsénicale, orage liquidien). Med. Afr. Noire. 20:379–387; 1973.
16. Ruppol, J. F.; Burke, J. Folow-up des traitements contre la trypanosomiase expérimentés à Kimpangu (Rép du Zaire). Ann. Soc. Belge. Med. Trop. 57:481–491; 1977.
17. Sina, G.; Triolo, N.; Trova, P.; Clabaut, J. M. L'encéphalopathie ardénicale lors due traitement de la trypanosomiase africaine à T. gambiense (à propos de 164 cas) Ann. Soc. Belge. Med. Trop. 57:67–74; 1977.
18. Sjoerdsma, A.; Schechter, P. J. Chemotherapeutic implications of polyamine biosynthesis inhibition. Clin. Pharmacol. Ter. 35:287–300; 1984.
19. Van Nieuwenhove, S.; Schechter, P. J.; De Clercq, J.; Bone, G.; Burke, J.; Sjoerdsma, A. Treatment of gambiense sleeping sickness in the Sudan with DFMO an inhibitor of ornithine decarboxylase, first field trial. Trans. R. Soc. Med. Hyg. 79:692–698; 1985.
20. WHO. The African trypanosomiasis. Technical report series 635. World Health Organization. Geneva; 1979.