the number of PEs required for simulating a particular activity estimated from modelling, cannot be equal to the number of neurons in the brain taking part in the activity. But, the ratio of PEs estimated by modelling for two activities may be an indication of the ratio of active neurons in the brain during two activities. Although another neural network model, namely back propagation model, was not able to fit these EEG patterns, other neural network models could also be attempted.

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Received 13 May 1996, revised accepted 21 January 1997

Response of IgG sub-classes to diethylcarbamazine therapy in bancroftian filarial patients

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The response of IgG subclasses to diethylcarbamazine (DEC) treatment was studied in bancroftian filariasis patients. On the basis of clinical signs and parasitological examination, a total of 22 patients were categorized into asymptomatic microfilaraemics (AS-Mfmic; $n = 12$) and symptomatic amicrofilaraemics (S-AMfmic; $n = 10$). The subjects were treated with DEC (300 mg/day) for 21 days. Before treatment, AS-Mfmic cases showed higher levels of IgG_1 and IgG_4 than the S-AMfmics whereas IgG_2 was higher in S-AMfmics than in AS-Mfmics. DEC caused more than 90% reduction in microfilaraemia by day 30 since the start of treatment in AS-Mfmics, while S-AMfmics remained amicrofilaraemic throughout the study period. In AS-Mfmics, DEC treatment enhanced IgG_4 and decreased IgG_1 levels while IgG_2 and IgG_3 remained unaffected. In S-AMfmics, DEC treatment caused decrease in IgG_1, IgG_3 and IgG_4, while IgG_2 level remained unchanged. We report that DEC therapy brings about changes in specific IgG_1 and IgG_4 in AS-Mfmics and IgG_1, IgG_3 and IgG_4 in S-AMfmics.

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FILARIAL parasite initiates immune response in its host at both cellular and humoral levels. Clinical expression of filariasis, therefore, reflects not only the duration and intensity of infection but also the degree and character of different types of immunologic responses. Asymptomatic microfilaria (mf) carriers have depressed antibody- and cell-mediated immune responses while acute manifestations and chronicity are associated respectively with an intermediate and hyper-immune response[1–4].

The major immunoglobulins involved in the antifilarial antibody responses in human host are IgG, IgM, and IgE[3,5,6]. IgG is the major immunoglobulin detectable in all categories of filarial subjects and the clinical severity of the infection is directly related to this isotype[7]. Recent studies have also shown that different categories of filarial subjects have different IgG subclass profiles. Specific IgG_1 and IgG_3 are predominant in chronic lymphatic filariasis whereas IgG_4 is elevated in mf carriers and tropical pulmonary eosinophilia cases[8,9]. As IgG_4 was suggested to indicate the presence of parasites[8,10], Wamae *et al.*[11] used it as an indicator of adulticidal efficacy of diethylcarbamazine (DEC) or ivermectin in microfilaraemic (bancroftian) human subjects. However, whether DEC can also bring about alteration in other subclass responses in bancroftian filarial patients is not known. We report here the response of IgG subclasses to DEC treatment (shortly after cessation of the treatment) in symptomatic amicrofilaraemic and asymptomatic microfilaraemic bancroftian patients.

Patients reporting to the outdoor clinic of King George's Medical College, Lucknow for treatment of various ailments, were examined for filariasis. The patients were from Lucknow and its adjoining areas which are known to be endemic to bancroftian filariasis. A

total of 22 patients diagnosed clinically and parasitologically positive for filariasis were included in the study. Of these, 12 subjects were asymptomatic with microfilaraemia (AS-Mfmic) while 10 had symptoms of 3–4 years duration (elephantiasis of legs and/or hydrocele) but amicrofilaraemic (S-AMfmic). Microfilariae (mf) in venous blood (1 ml; collected between 9 and 11 pm) were assessed using the membrane filtration technique[12]. For the assay of IgG and its subclasses, serum was separated from blood and stored at –20°C till use.

All the AS-Mfmic and S-AMfmic subjects received diethylcarbamazine (DEC) at 300 mg/day for 21 days. Venous blood was collected before therapy and one week after the last dose. Treated patients were examined daily during and after the therapy for any adverse reactions.

Brugia malayi adult worms were harvested from intraperitoneal cavity of jirds infected intraperitoneally with *B. malayi* L_3. Soluble somatic extract of the worms was prepared as described by Lammie *et al.*[13] and used as the antigen source. The protein content of the extract was determined by the method of Lowry *et al.*[14].

Circulating antifilarial IgG subclasses were assessed by ELISA as described by Hussain *et al.*[15], with some modifications. Briefly, polystyrene plates were coated overnight at 4°C with the antigen (10 μg/ml) in carbonate buffer. Unsaturated sites of the surface were blocked with 1% gelatin in phosphate buffered saline (G-PBS) for 1 h at 37°C, and the plates were incubated with sera diluted (1:25) in G-PBS containing 0.01% Tween-20 (G-PBS-T) for 90 min at 37°C. The plates were washed with PBS-T and incubated with optimally diluted mouse monoclonal anti-human IgG_1 (1:7,500), IgG_2 (1:5000), IgG_3 (1:5000) and IgG_4 (1:7500) for 90 min at 37°C. The plates were again washed and incubated for 90 min at 37°C with 1:1000 dilution of anti-mouse IgG-peroxidase conjugate. The plates were washed and incubated in the substrate medium consisting of 0.08% each of *o*-phenylenediamine and H_2O_2 in citrate buffer (pH 5.0). After stopping the reaction with 2.5N H_2SO_4, the optical density was read at 492 nm using an automated ELISA reader (Multiscan). All the antibodies and conjugates were from Sigma Chemical Co., St. Louis.

The values of the IgG subclasses in the two categories of the patients were compared by Student's *t* test. Paired *t* test was applied for the analysis of effects of DEC treatment on the responses. Differences were considered significant if $p \leq 0.05$.

The concentration of specific IgG subclasses (IgG_1, IgG_2, IgG_3 and IgG_4) in sera of AS-Mfmic and S-AMfmic cases before and after DEC treatment is shown in Figure 1.

Amongst the four subclasses of IgG, the levels of IgG_4 alone and IgG_2 and IgG_4 were significantly elevated ($p < 0.01$) respectively in AS-Mfmic and S-AMfmic

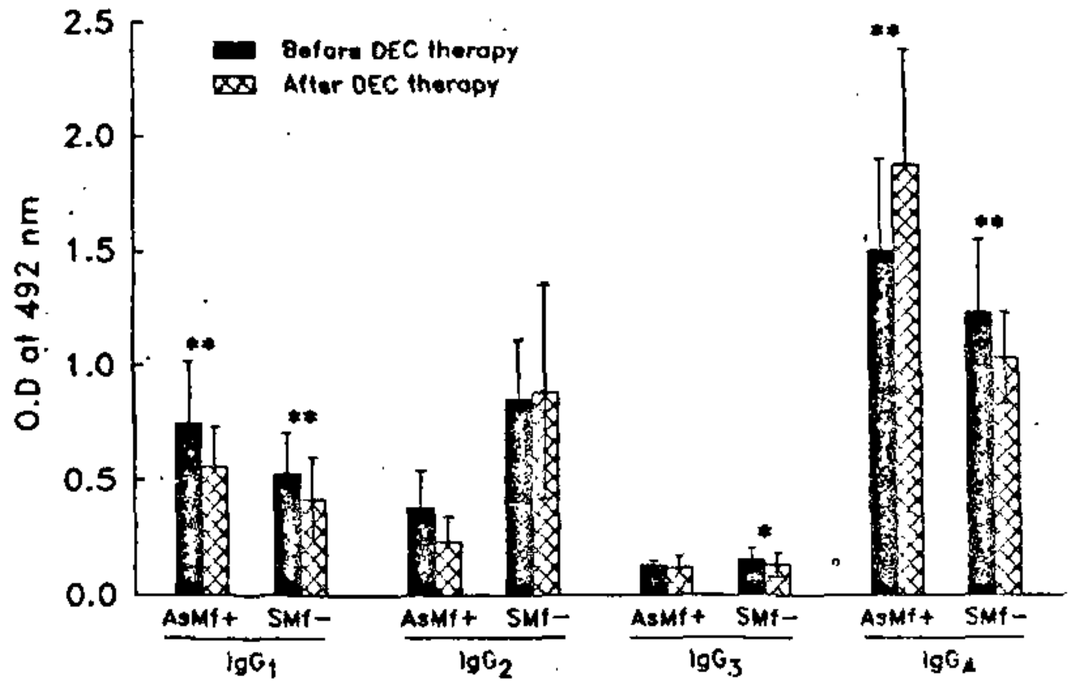

Figure 1. IgG subclasses in asymptomatic microfilaraemics and symptomatic amicrfilaraemics before and after DEC therapy (*$p < 0.05$; **$p < 0.01$).

cases. When the values of the 4 IgG sub-classes were compared between the two groups, the responses of IgG_1 and IgG_4 were significantly higher ($p < 0.01$) in AS-Mfmic while IgG_2 was greater in S-AMfmic cases ($p < 0.05$).

By day 30 post-DEC treatment, there was more than 90% reduction (88.29–98.33%) in microfilaraemia in AS-Mfmic cases while the S-AMfmic patients remained amicrofilaraemic (data not shown). There was no amelioration of manifestations in S-AMfmic cases nor any appearance/development of tenderness in any part of the body in AS-Mfmic cases during the course of treatment.

In AS-Mfmic subjects, DEC treatment decreased the antifilarial IgG_1 response ($p < 0.01$) but increased specific IgG_4 response ($p < 0.01$). IgG_2 and IgG_3 responses were not affected by DEC therapy.

In S-AMfmic cases, DEC treatment resulted in decrease in IgG_1 ($p < 0.01$) and IgG_4 ($p < 0.01$), and IgG_3 ($p < 0.05$) whereas IgG_2 remained unaffected.

Responses of IgG subclasses (IgG_1, IgG_2, IgG_3 and IgG_4) have been reported for chronic helminthic diseases, namely, filariasis, schistosomiasis and cysticercosis; IgG_4 was found to be the most dominant subclass in these diseases[16–18] though it accounts for only 3–4% of the total IgG in normal human serum[19]. The present study revealed that in the untreated subjects the concentration of subclass IgG_4 was maximum in AS-Mfmics while IgG_2 and IgG_4 concentrations were raised in S-AMfmic subjects. Inter-category comparison showed that IgG_1 and IgG_4 were higher in AS-Mfmics than in S-AMfmics, whereas IgG_2 was higher in S-AMfmics than in AS-Mfmics.

A significantly high level of IgG_1, as found in AS-Mfmics in this study, was also reported by Mak *et al.*[20] in brugian microfilaraemic patients but not in ban-

croftian microfilaraemics. S-AMfmic cases had high IgG_2 compared to AS-Mfmic patients which supports the findings of Rahmah *et al.*[21] in malayan filariasis. These authors even suggested that IgG_2 may be used as diagnostic tool for *B. malayi*-induced chronic elephantiasis. On the other hand, Ottesen *et al.*[8] reported elevated antifilarial IgG_1 and IgG_4 but not IgG_2 in patients with chronic elephantiasis due to *W. bancrofti* infection.

IgG_3 was lower than other subclasses in both the categories of subjects. This is in contrast to the findings of Hussain *et al.*[15] who found low IgG_3 levels only in AS-Mfmic bancroftian filarial cases. AS-Mfmic brugian cases, on the other hand show elevated levels of IgG_3 (ref. 20).

We found elevated IgG_4 levels in both AS-Mfmics and S-AMfmic patients. Ottesen *et al.*[8], Hussain *et al.*[15], Lal and Ottesen[9] and Kwan-Lim *et al.*[22] reported predominance of IgG_4 antibody response not only in mf (*W. bancrofti*) carriers but also in tropical pulmonary eosinophilia cases.

Significant variations have been reported in the levels of IgG subclasses in filarial subjects. Although no explanation is forthcoming for this disparity, the strain of parasite prevalent, the immune status of population studied and many other associated factors might be responsible for these variations.

DEC treatment resulted in significant decrease in IgG_1 levels in AS-Mfmics and IgG_1, IgG_3 and IgG_4 levels in S-AMfmics. While the present studies were in progress, Atmadja *et al.*[23] reported a decrease in IgG_1 and IgG_4 levels 12 months after DEC therapy in *malayan* microfilaraemic and elephantiasis patients. However, they reported a fall in IgG_2 as well as IgG_3 in elephantiasis patients while our present study showed no change in IgG_2 response in S-AMfmics by day 30 since the initiation of treatment. In view of the known role of IgG_1 and IgG_3 in hypersensitivity/allergic (Type III) reactions[24] which lead to tissue damage and the recent report of Casley-Smith *et al.*[25] that DEC reduced lymphoedema in chronic symptomatics, these two IgG sub-classes may be involved in the responses to DEC treatment in symptomatic amicrofilaraemic bancroftian subjects. In AS-Mfmics on the other hand, IgG_1 is evidently involved in the responses to DEC treatment. It is interesting to note that recently, using lymphoscintigraphy, lymphatic tissue pathology was demonstrated in asymptomatic microfilaraemics also[26]. If IgG_1 is involved in tissue pathology seen in asymptomatic microfilaraemics, as suggested by the above reports, our results indicate that this subclass in combination with IgG_4 (discussed below) may be useful as a convenient tool for assessing the response of microfilaraemics to DEC therapy.

In AS-Mfmic subjects, IgG_4 levels were increased by DEC treatment by day 30 since the initiation of therapy. This confirms the recent finding of Wamae *et al.*[11] who found increased IgG_4 levels at day 30 since start of a twelve-day course of DEC therapy in bancroftian microfilaraemics which decreased significantly to below pretreatment levels by day 180. As suggested by these authors, the increase in IgG_4 levels in our microfilaraemic subjects is related to sudden release of microfilarial antigen(s). The significant reduction (more than 90%) in microfilaraemia found in our subjects supports this suggestion.

In conclusion, the DEC therapy brings about changes in specific IgG_1 and IgG_4 in bancroftian AS-Mfmics and in IgG_1, IgG_3 and IgG_4 sub-classes in S-AMfmics. Assessment of IgG subclass levels in AS-Mfmics and S-AMfmics may possibly be used as a convenient tool for monitoring the infection and especially the clinical status of the disease following antifilarial therapy.

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ACKNOWLEDGEMENTS. We are grateful to Dr V. P. Kamboj, Director, Central Drug Research Institute, Lucknow, for providing the necessary facilities and to Mr S. K. Mandal for statistical analysis. Thanks are also due to Dr P. S. R. Murthy for his valuable suggestions and critical review of the manuscript.

Received 21 September 1996; revised accepted 20 January 1997

Synthesis and antitumour activity of new derivatives of podophyllotoxin

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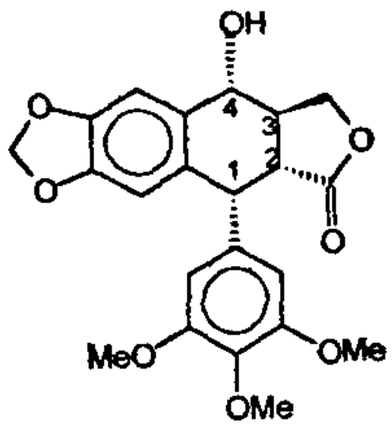
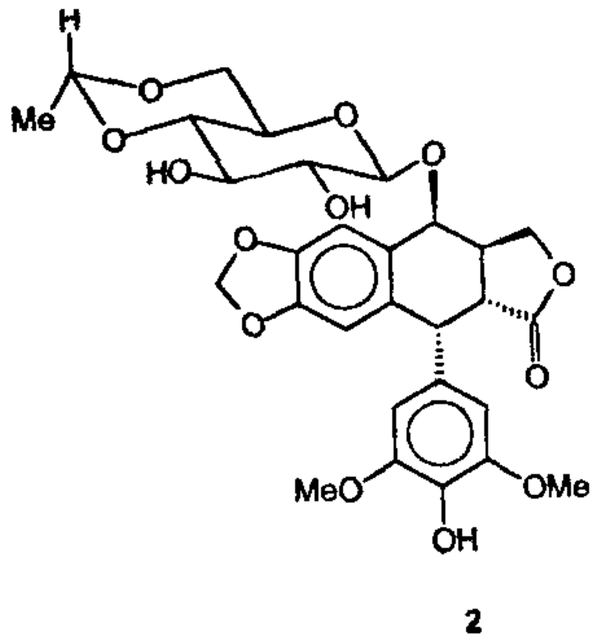
A series of new podophyllotoxin derivatives 3–8 have been synthesized and evaluated for their antitumour activity *in vitro*. Compounds 3 and 8 exhibited comparable or superior activity to clinically used etoposide (VP-16, 2) in their inhibition of human stomach carcinoma SGC-7901, lung cancer A 549, and mouse leukemia P388 cells.

SEMISYNTHETIC analogues of the naturally-occurring podophyllotoxin (**1**) have drawn much renewed interest in recent years as a result of the development of etoposide (VP-16, **2**) and teniposide (VM-26) as anticancer drugs[1,2]. It is believed that analogues of 4′-demethylepipodophyllotoxin exert their antitumour activity through stabilization of a cleavable complex between DNA and type II DNA topoisomerase. This leads ultimately to inhibition of DNA catenation activity and produces single and double strand breaks[3,4].

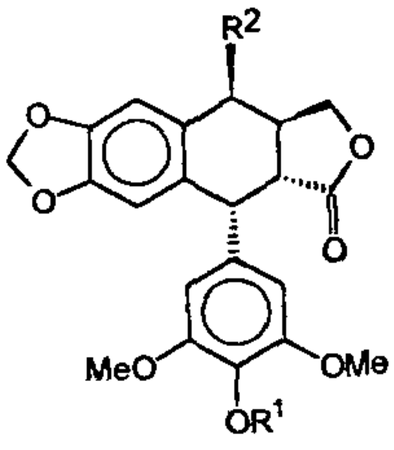
In our previous studies[5–8], we found that substitution of the glycosidic moiety in **2** by a configurationally similar nitrogen-containing group led to some compounds which have comparable or superior antitumour activity to **2**. The results suggested that the β-anomeric configuration at C-4 was indispensable for the antitumour activity. Changes in the 4β-glycosyl group are also of interest for simplified structure which might retain the activity of **2**, and be accessible to practical industrialization. Here we wish to present the synthesis of a series of new analogues of epipodophyllotoxin **3–8** and their biological activities *in vitro*.



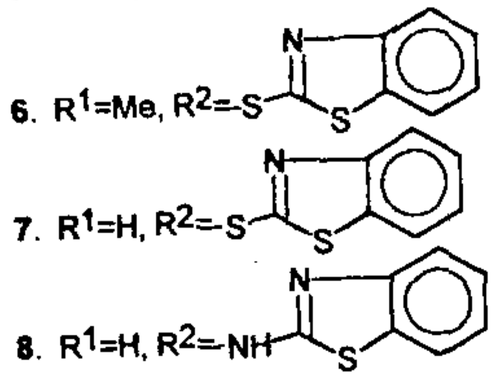
1



2



3. R^1=H, R^2=-NHCO-C_6H_4-OAc(o)
4. R^1=Me, R^2=-OCO-C_6H_4-OAc(o)
5. R^1=H, R^2=-OCO-C_6H_4-OAc(o)



Scheme 1.

The synthesis of target compounds started from **1** as shown in Scheme 2. 4β-bromo-4′-demethyl-4-deoxypodophyllotoxin (**9**) and 4′-demethylepipodophyllotoxin (**10**) were prepared from **1** by our previous procedure[5,9]. **10** was treated with HN_3 to yield 4β-azido-4′-demethyl-4-deoxypodophyllotoxin (**11**) as the major product, which was accompanied by the C-4 isomer product, 4α-azido-4′-demethyl-4-deoxypodophyllotoxin, **11** can be purified by crystallization. Further reduction of **11** led to 4β-amino-4′-demythyl-4-deoxypodophyllotoxin (**12**). Condensation of **12** with aromatic acid **13** in the presence of DCC gave compound **3**. Compounds **4** and **5** were synthesized by the reaction of **13** with **1** and **10**, respectively. Thio-etherification of **1** and **10** with 2-mercaptobenzothiazole (**14**) yielded compounds **6** and **7**, respectively. Compound **8** was synthesized by direct substitution of 2-amino-benzothiazole (**15**) with 4β-bromo-4′-demethyl-4-deoxypodophyllotoxin (**9**).

All new target compounds were characterized by m.p., ^{1}H NMR, MS and IR spectral analysis, as well as elemental analysis. The assignment of the configuration at C-4 for compounds **3–8**, **11** and **12** was based on the difference of $J_{3,4}$ coupling constants. The C-4β-substituted compounds **3–8**, **11** and **12**, have a $J_{3,4} \approx 4.0$ Hz as seen in **2** and **10** (ref. 10), due to a *cis*