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Immunological Abnormalities and Hydantoins

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Summary

Various immunological features were measured in 20 patients with epilepsy who had received prolonged treatment with hydantoins. Immunoglobulin A (IgA) was shown to be absent or low in five patients, β_1C/A was low in 10 patients. Five patients showed negative reactions to skin tests, and two could not be sensitized to dinitrochlorobenzene. The corresponding features were normal in 14 control patients with epilepsy but without hydantoins. It is suggested that the hydantoins influence humoral immunity, whereas other immunosuppressant agents have been found to affect cellular immunity.

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Introduction

Hydantoins and their derivatives are a mainstay of antiepileptic and lately of antiarrhythmic therapy. In the course of the long history of their therapeutic use a broad spectrum of side effects has been attributed to these drugs (Sparberg, 1963), most frequently consisting of visual disturbances, gingival hyperplasia (King, 1952; Sparberg, 1963; Goodman and Gilman, 1970), and various exanthemas (Mandelbaum and Kane, 1941; Loscalzo, 1948; Martin *et al.*, 1954; Watts, 1962). Other side effects described have been haematological abnormalities (Benhamon and Albon, 1953; Best and Paul, 1950; Sparberg, 1963), benign (Siegal and Berkovitz, 1961; Harrington *et al.*, 1962; Holland and Mauer, 1965; Goodman and Gilman, 1970) and, rarely, malignant lymphadenopathies (Hyman and Sommers, 1966), hepatitis (Chaiken *et al.*, 1950; Gropper, 1956; Bajoghli, 1961; Pezzimenti and Andrew, 1970), disease states resembling serum sickness (Bravermann and Levin, 1963), lupus erythematosus (Miescher and Delacrétaz, 1953; Lindquist, 1957; Rallison *et al.*, 1961) and periarteritis nodosa (Van Wyk and Hoffman, 1948), and endocrine disorders such as hirsutism (Sparberg, 1963; Kniper, 1969).

Our attention was focused on the immunological system by an epileptic patient undergoing prolonged treatment with phenytoin (diphenylhydantoin; Antisacer) and showing lymphadenopathy, recurrent infections, and absence of immunoglobulin A (IgA). We therefore started a study to assess the immunological status of 20 patients receiving high doses of phenytoin, and here report the various immunological abnormalities found in them.

Material and Methods

The 20 patients (7 men and 13 women aged 24–48 years) were in hospital because of idiopathic or symptomatic epilepsy. All had received more than 400 g (in daily doses of 0·2–0·3 g) of phenytoin (Dilantin; Antisacer) and were still under treatment at the time of testing. All showed various degrees of gingival hyperplasia, two also had lymphadenopathies, two showed hirsutism, and six had eosinophilia.

Fourteen patients (nine women and five men) with epilepsy served as controls. They were matched for age, sex, and duration of the disease. Seven of these patients had never been treated with hydantoins, and in the other seven such treatment had been abandoned for at least two years. Other antiepileptic drugs, such as derivatives of barbiturates and succimide and oxazolidine or carbamazepine (Tegretol), were not considered in analysing the results since they had been used by both groups of patients. All the subjects studied were selected at random out of 650 epileptic patients from three hospitals. (In these hospitals the average epileptic has a 60% chance of being treated with hydantoins.)

The following tests were performed on sera from all the patients: quantitative determination of immunoglobulins IgG, IgA, and IgM and of β_1C/A (an antigen related to C'3, the third component of complement) by the radial immunodiffusion technique (Mancini *et al.*, 1965) according to the methods described elsewhere (Grob and Jemelka, 1971), immunoelectrophoresis, indirect immunofluorescence (Coons and Kaplan, 1950) for antinuclear antibodies and anti-muscle antibodies, passive haemagglutination and complement fixation for antithyroid antibodies (Doniach and Roitt, 1967), and the Coombs test. The isohaemagglutinins and antibodies against cytomegalovirus (Krech *et al.*, 1968) and rubella (Krech *et al.*, 1969) were also measured. In addition the patients received 1 ml of a polyvalent vaccine against influenza viruses (Inflexal: 2,500 U of HA A2 Hong Kong 12/64; 2,500 U of HA A2 England 1/68; 3,000 U of HA B England 939/54) injected twice intramuscularly at two-weekly intervals. Haemagglutination inhibition tests against A2 Hong Kong virus were performed before the first injection and two weeks after the second one. To assess the cellular immune response the patients were skin-tested with five antigens—staphylotoxin, tuberculin, trichophytin, oidiomycin, and streptokinase-streptodornase. A 0·05-ml injection of each solution was given intradermally, the skin reactions being measured 24 and 48 hours later. Sensitization was also attempted with dinitrochlorobenzene (4%), the skin reactions being assessed 24 and 48 hours after the second application. Epicutaneous skin tests were performed with a solution of phenytoin (50 mg/ml).

Results

The results in the 20 patients under treatment with phenytoin are given in the Table. The most striking features were the total absence of measurable IgA in the sera of three patients and a reduced IgA level in the sera of another two patients. IgA was increased in five patients. In contrast all but one of the controls had normal IgA concentrations in their sera, the remaining subject having a slightly reduced level of 0·5 mg/ml. In one patient (Case 1) the IgG level was also reduced and IgM was at the lower limit of normal. In 12 patients a slight increase of IgG was found in the sera. Similar findings were seen in the control group. No patient, including the controls, had a decreased IgM level apart from two (Cases 1 and 3) whose isohaemagglutinins were also low. Five of the 20 patients (25%) were positive for antibodies against cytomegalovirus, while in this area the percentage of positive reactors in healthy individuals is 60% (Krech and Jung, 1971). Twelve patients (60%) were positive for antibodies against rubella virus, the corresponding percentage in healthy people being 85% (Krech and Jung, 1971). No antibodies against rubella or cytomegalovirus combined with little or no antibody response to the vaccination were found in three patients (Cases 8, 12, and 13). In all other patients tested at least one of these measurements gave positive results.

β_1C/A levels were clearly reduced in 10 of the 20 patients, while they were normal in the control subjects. Positive skin reactions with each antigen used occurred in 30–40% of the patients tested, the percentage corresponding to that obtained in healthy subjects. But negative skin tests with all five antigens used were found in five of the 20 patients. They were also negative, except for one weak reaction, in two further individuals (of these seven patients, however, five were successfully sensitized to dinitrochlorobenzene). In all other patients at least one antigen, but generally two or three, provoked positive skin

Immunological Features in Patients receiving Hydantoins

Case No.	Immunoglobulins (in mg/ml)			Antibodies Against:			β_1C/A (in mg/100 ml) (110–180*)	No. of Positive Skin Reactions against Total No. of Tests	Sensitization with Dinitrochlorobenzene
	IgA (0·6–4·0*)	IgG (7·1–15·4*)	IgM (0·4–2·0*)	Rubella (Reciprocal Titre)	Cytomegalovirus (Reciprocal Titre)	A2 Hong Kong (Increase of Titre after Vaccination)			
1	N.M.	3·6	0·3	40	10	–	90	(1)/5	–
2	0·9	9·5	0·7		–	+ + + +	70	5/5	Toxic reaction
3	1·5	20·0	0·3	320	–	+ +	80	0/5	+ +
4	6·5	24·5	1·2	–	30	Not done	140	4/5	+
5	1·9	20·0	0·9	80	40	Not done	110	0/5	+
6	4·0	15·0	5·0	40	–	+ + + +	130	5/5	+ +
7	6·0	15·0	3·4	40	30	+ + + +	120	4/5	Toxic reaction
8	6·0	16·5	1·1		–	+ +	130	0/5	Toxic reaction
9	2·2	13·0	0·5	40	120	+ +	90	(1)/5	+
10	0·4	18·5	1·6	640	–	+ + + +	70	2/5	+ +
11	N.M.	26·0	1·5		–	+ + + +	120	0/5	+
12	9·0	12·0	1·2		–	–	160	2/5	–
13	5·2	24·0	0·9	–	–	–	130	Not done	–
14	3·9	18·5	1·8	160	–	Not done	105	1/5	+
15	0·3	14·5	0·4	640	–	+ + + +	95	3/5	+ +
16	4·2	20·0	0·95	80	–	+ + + +	90	1/5	+
17	3·2	16·0	1·1	1,280	–	+ + + +	85	2/5	–
18	N.M.	17·0	0·95	–	–	+ + + +	140	3/5	+
19	3·0	9·5	1·1	–	–	+ + + +	85	0/5	+
20	4·2	19·0	0·8	160	–	+ + + +	75	2/5	+ +

*Normal values obtained in 88 to 100 sera from blood-bank donors.
N.M. = Not measurable (<0·05 mg/ml).

reactions. Sensitization against dinitrochlorobenzene failed to occur in four patients. The results were difficult to assess in three others because of toxic reactions. None of the patients tested gave positive skin reactions with phenytoin.

Antithyroid antibodies were found in seven of the 20 patients receiving hydantoins but also in five of the 14 control patients. Antinuclear antibodies were found in three patients receiving hydantoins but never in controls. None of the patients had anti-muscle antibodies or a positive Coombs test.

Discussion

Abnormal or at least unusual immunological findings were obtained in many of the 20 patients with epilepsy who had had prolonged treatment with phenytoin. The findings seem to point to drug-induced immunological defects of various degrees and qualities, the most profound being in a woman whose IgA was unmeasurable and who had reduced IgG, low IgM, and low β_1A levels in her serum, lack of antibody response after vaccination, a negative skin test (except for one weak reaction against oidiomycin), and was not sensitized to dinitrochlorobenzene. This patient showed recurrent infections probably due to the hypogammaglobulinaemic state, requiring therapy with gammaglobulins.

The most striking feature in the 20 patients tested was the IgA abnormalities in 10 of them, including total lack or diminution of this immunoglobulin in five. The results seem to be significant since IgA was normal in all but one of the control patients, in whom treatment with large doses of phenytoin sodium had been abandoned only two years earlier. According to Bachmann (1965) low IgA is found in one out of 700 healthy persons. It was never found in 500 sera tested in our laboratory except in the case of seven patients with myeloma, lymphatic leukaemia, and ataxia telangiectasia (Louis-Barr syndrome). Low IgA is also known to occur in sprue syndrome, cirrhosis of the liver, and Still's disease (West *et al.*, 1962; Bull and Tomasi, 1968). None of the present patients seemed to be affected by any of these ailments. West *et al.* (1962) described 13 patients with low IgA levels of unknown origin, four of whom had diseases of the nervous system. Unfortunately no detail was given of the treatment of these patients. Absence of common specific antibodies and lack of antibody formation after vaccination in three patients pointed to an impaired humoral immune response. Similarly, the results of the skin test might indicate an impaired cellular immune response in five of the 20 patients. The results gain in significance since positive reactions for antibodies and skin tests should be higher in patients with long periods of hospital stay compared with healthy individuals.

Another finding deserves comment. Low levels of β_1C/A were found in 10 of the 20 patients undergoing treatment but not in the controls. Low levels of β_1C/A are known to occur in sera of patients with autoimmune diseases, certain forms of glomerulonephritis, hepatitis, and disorders of the lymphatic system (Grob and Jemelka, 1971). To our knowledge they have never been found in connexion with drugs. Consumption of complement due to immune complexes could explain the findings but other mechanisms might be responsible, such as deceased synthesis or increased catabolism (Grob and Jemelka, 1971). No obvious correlation seems to exist between the immunological abnormalities and other side effects of the drug. IgA deficiency was associated with lymphadenopathy in one case but not in the others.

The pathogenesis of these immunological complications is not known. This is also true of the other side effects of hydantoins. A toxic effect on the bone marrow has been observed by various authors (Poynton and Schlesinger, 1929; Benhamon and Albon, 1953; Best and Paul, 1950). It was suggested that an enzymatic defect leading to toxic compounds in the course of drug catabolism might play a part (Bravermann and Levin, 1963; Pezzimenti and Andrew, 1970). An allergic reaction towards the drug has also been discussed, but neither corresponding antibodies nor positive skin tests could be detected (Schick *et al.*, 1933; Olmer *et al.*, 1952; Bravermann and Levin, 1963).

Our findings are probably of little consequence for the current therapeutic use of these drugs. They could, however, encourage one to study other derivatives of hydantoins for their immunosuppressive properties. Interestingly, hydantoins seem rather to influence the humoral system, whereas the immunosuppressants so far available act mainly on the cellular immune system.

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