Clin. exp. Immunol. (1978) 33, 437–440.

Reduced immunoglobulin concentration and impaired macrophage function in mice due to diphenylhydantoin

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(*Received* 28 *November* 1977)

SUMMARY

Diphenylhydantoin (DPH) depresses serum IgA and IgM concentrations and polyvinyl pyrrolidone (PVP) clearance in mice.

INTRODUCTION

Selective deficiency of serum IgA has been demonstrated in man after treatment with diphenylhydantoin (DPH) (Sorrell & Forbes, 1975, Seager *et al.*, 1975). Similar effects have been ascribed to penicillamine (Proesmans, Jacken & Eeckels, 1976) and sodium aurothiomalate (Stanworth *et al.*, 1977). No comparable information exists on the effect of DPH on immunoglobulin concentrations in animals. Development of IgA is particularly dependent on B and T cell cooperation (Clough, Mims & Strober, 1971) and might be related to macrophage activity. We have investigated immunoglobulin concentrations in young CBA mice before and after DPH and have used the polyvinyl pyrrolidone (PVP) clearance test (Morgan & Soothill, 1975) to measure the effect of DPH on their macrophage function.

MATERIALS AND METHODS

The animals used were 9 week old male CBA mice; average weight 19 g. DPH or a control solution was given orally as a daily dose for 19 days to mice in each of three groups. The control group (twelve mice) received 0·15 ml of saline adjusted to pH 11 with sodium hydroxide. A constant dose group (twelve mice) received approximately 80 mg/kg/day of DPH sodium (0·15 mg/day) in 0·15 ml of solution (phenytoin injection BPC in de-ionised water, pH 11), and an increasing dose group (twenty-four mice) received DPH sodium in a dose which was slowly increased to the limit of their tolerance (see Results section for details). DPH and alkalinised saline were administered by oral gavage using a piece of soft bevelled polythene tube attached to a 1 ml syringe. The mice were weighed twice weekly.

Immunoglobulins were measured on serum from each mouse at the start of the experiment and on day 18 by radial immunodiffusion (Fahey & McKelvey, 1965) using specific rabbit antisera against purified mouse IgG_1, IgA and IgM kindly supplied by Dr F. C. Hay of the Middlesex Hospital Medical School. Sera were stored at −20°C and thawed once before examination. Internal standards were included with each batch of immunoglobulin estimations. Concentrations were expressed as a percentage of the activity of a reference serum prepared by pooling serum from twenty 8–10 week old CBA mice.

The PVP test was done on day 18 on all mice in control and constant dose groups and the fifteen which survived in the increasing dose group. Mice were injected by the tail vein with 0·1 ml of a solution containing approximately 50 μg of ^{125}I-labelled PVP in saline. Three specimens of blood were taken between 24 and 48 hr later. The exponential rate constant K (hr^{-1}) for PVP clearance was calculated from the slope of the regression line of $\log_e$ blood level against time.

All mice were killed on day 20 and DPH was measured in pooled serum from treated groups by a gas chromatographic method (Goudie & Burnett, 1973). A preliminary study of CBA mice showed that their immunoglobulin concentrations conformed to a log normal distribution, so results were analysed by the Student's *t*-test after logarithmic transformation.

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0099-9104/78/0900-0437$02.00

RESULTS

All mice in control and constant dose groups survived and appeared healthy. In the increasing dose group, DPH was increased to approximately 160 mg/kg/day by day 6, when several mice became unsteady. This dose was maintained for 2 days and then the drug was withdrawn for 3 days (days 8–10), being restarted on day 11 and kept at 160 mg/kg/day for the rest of the experiment. Nine mice in this group died but the remaining animals tolerated the regimen well.

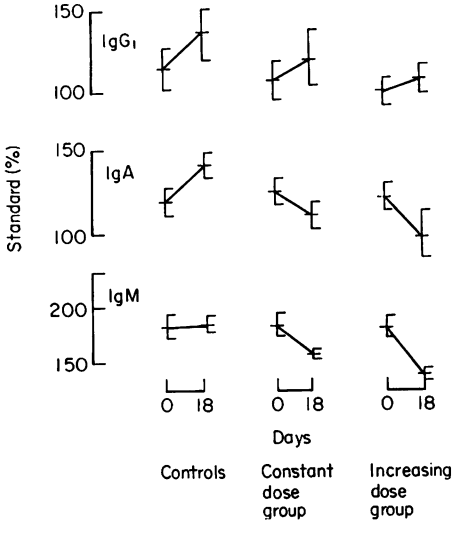

FIG. 1. Immunoglobulins before and after 18 days DPH. Controls, $n = 12$; constant dose group, $n = 12$; and increasing dose group, $n = 24-9 = 15$.

Serum immunoglobulin concentrations at the start of the experiment and on day 18 in mice which survived are shown in Fig. 1. Levels of the three immunoglobulin classes did not differ significantly between pre-treatment samples in any of the three groups of mice, but there was a trend for IgG_1 values to be higher in the control group. All immunoglobulins tended to rise in the control animals as part of normal development with age. As observed in humans, values of IgA at the end of the experiment in both of the treated groups were significantly lower than in the controls (Student's t-test, one-tailed $t = 1{\cdot}9$ $P < 0{\cdot}05$ and $t = 3{\cdot}3$ $P < 0{\cdot}0025$ for constant and increasing dose groups respectively). There was an apparent dose effect though this was not statistically significant. Unlike human studies, there was an even bigger fall of IgM ($t = 2{\cdot}2$ $P < 0{\cdot}05$ for constant dose and $t = 4{\cdot}7$ $P < 0{\cdot}001$ for increasing dose group, two-tailed test). Again, there was a trend for a dose effect. There was no significant difference between levels of IgG_1 in treated and control mice—the non-significant differences paralleled those in the pre-treatment values.

DPH concentration in pooled serum was 29 μg/ml in the constant dose group and 32·6 μg/ml in the increasing dose group. Weight of control mice at day 18 was $19{\cdot}9 \pm 3{\cdot}7$ g (mean$\pm$s.d.). In the constant dose group it was $19{\cdot}3 \pm 5{\cdot}2$ g and in the increasing dose group $19{\cdot}5 \pm 3{\cdot}9$ g.

There was a significant depression (Fig. 2) of PVP clearance in both the constant and the increasing dose groups ($t = 2{\cdot}1$, $P < 0{\cdot}05$ and $t = 2{\cdot}28$ $P < 0{\cdot}05$, respectively, two-tailed t-test), as compared with controls. Surprisingly, there was a negative correlation between serum IgA and PVP clearance in the constant dose group ($r = 0{\cdot}73$ $P < 0{\cdot}01$) and between serum IgM and PVP clearance in the increasing dose group ($r = 0{\cdot}51$ $P < 0{\cdot}05$), but there was no other significant correlation between PVP clearance and either immunoglobulin concentration or fall in immunoglobulin concentration.

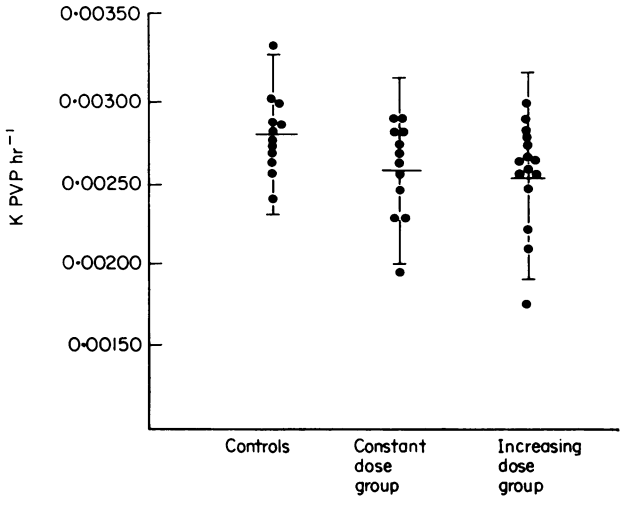

FIG. 2. PVP clearance in control and DPH-treated mice.

DISCUSSION

The most common immunological abnormality found during DPH treatment in man has been selective deficiency of serum IgA (Sorrell & Forbes, 1975, Seager *et al.*, 1975, Aarli, 1976) and this seems to be more common in children than in adults (Aarli & Tonder, 1975). This is partly due to an association of some childhood epilepsy with primary IgA deficiency, but prospective studies show that the drug depresses IgA. Our finding of diminished IgA in mice accords with the human data. The fall in IgM is consistent with the observation of Levo, Markowitz & Trainin, (1975) that spleen cells from DPH-treated mice had an impaired response to sheep red blood cells, as measured by the IgM-dependent direct Jerne plaque technique. The apparent sparing of IgG_1 could be related to its half life which is longer than that of IgA or IgM (Spiegelberg, 1974) and might indicate that the fall in these immunoglobulins is due to a failure of synthesis rather than an increase in their catabolic rate.

Macrophages are involved in the afferent limb of the immune response, where they perform a cooperative role together with the T cells in presenting antigen to the antibody-producing B cell line (Feldmann, 1972), and there is evidence that an IgA antibody response is particularly dependent on efficient cooperation (Clough *et al.*, 1971). In the immature animal, inhibition of cooperation by a drug might well suppress production of this class, and the low IgA concentrations found in nude mice support this possibility (Pritchard, Riddaway & Micklem, 1973). We therefore sought and found evidence of suppression of macrophage function by DPH, but the lack of a positive correlation between K PVP and either IgA or IgM, together with the suppression of IgM, the least cooperation-dependent class, and the sparing of IgG_1, which is highly T cell dependent in the mouse (Torrigiani, 1972), makes it unlikely that the immunoglobulin depression was the result of macrophage dysfunction.

DPH affects many enzyme systems so it is difficult to speculate on the mechanism of these effects. The impaired macrophage function may well be of importance in other immunological abnormalities seen in DPH-treated patients, such as lymphoid hyperplasia and systemic lupus erythematosus.

We thank Mr A. A. Weir, Chief Animal Technician, Institute of Child Health for help with the mice, Dr D. Burnett of the Biochemistry Unit, St. Albans for measurement of diphenylhydantoin levels, Mrs M. Diment for preparing the manuscript and the National Fund for Research into Crippling Diseases for financial support.

REFERENCES

AARLI, J.A. (1976) Drug-induced IgA deficiency in epileptic patients. *Archs. Neurol.* **33**, 296.

AARLI, J.A. & TÖNDER, O. (1975) Effect of antiepileptic drugs on serum and salivary IgA. *Scand. J. Immunol.* **4**, 391.

CLOUGH, J.D., MIMS, L.H. & STROBER, W. (1971) Deficient IgA antibody responses to arsanilic acid bovine serum albumin (BSA) in neonatally thymectomised rabbits. *J. Immunol.* **106**, 1624.

FAHEY, J.L. & MCKELVEY, E.M. (1965) Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.* **94**, 84.

FELDMANN, M. (1972) Cell interactions in the immune response *in vitro*. II. The requirement for macrophages in lymphoid cell collaboration. *J. exp. Med.* **135**, 1049.

GOUDIE, J.H. & BURNETT, D. (1973) A gas-chromatographic method for the simultaneous determination of phenobarbitone, primidone and phenytoin in serum using a nitrogen detector. *Clin. Chim. Act.* **43**, 423.

LEVO, Y., MARKOWITZ, O. & TRAININ, N. (1975) Hydantoin immunosuppression and carcinogenesis. *Clin. exp. Immunol.* **19**, 521.

MORGAN, A.G. & SOOTHILL, J.F. (1975) Measurement of the clearance function of macrophages with ^{125}I-labelled polyvinyl pyrrolidone. *Clin. exp. Immunol.* **20**, 489.

PRITCHARD, H., RIDDAWAY, J. & MICKLEM, H.S. (1973) Immune responses in congenitally thymus-less mice. II. Quantitative studies of serum immunoglobulins, the antibody response to sheep erythrocytes and the effect of thymus allografting. *Clin. exp. Immunol.* **13**, 125.

PROESMANS, W., JAEKEN, J. & EECKELS, R. (1976) D-Penicillamine-induced IgA deficiency in Wilson's disease. *Lancet*, **ii**, 804.

SEAGER, J., JAMISON, D.L., WILSON, J., HAYWARD, A.R. & SOOTHILL, J.F. (1975) IgA deficiency, epilepsy, and phenytoin treatment. *Lancet*, **ii**, 632.

SORRELL, T.C. & FORBES, I.J. (1975) Depression of immune competence by phenytoin and carbamazepine. *Clin. exp. Immunol.* **20**, 273.

SPIEGELBERG, H.L. (1974) Biological activities of immunoglobulins of different classes and subclasses. *Advan. Immunol.* **19**, 259.

STANWORTH, D.R., JOHNS, P., WILLIAMSON, N., SHADFORTH, M., FELIX-DAVIES, D. & THOMPSON, R. (1977) Drug-induced IgA deficiency in rheumatoid arthritis. *Lancet*, **i**, 1001.

TORRIGIANI, G. (1972) Quantitative estimation of antibody in the immunoglobulin classes of the mouse. II. Thymic dependence of the different classes. *J. Immunol.* **108**, 161.