The effects of phenytoin on T-lymphocyte enumeration

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Although generally acknowledged as a safe and effective drug in the treatment of epilepsy several reports have associated phenytoin with altered immune status (MacKinney & Booker, 1972; Sorrell & Forbes, 1975; Seager, Jamison, Wilson, Haywood & Soothill, 1975; Bluming, Homer & Khiroya, 1976).

Two populations of human T-lymphocytes (designated 'active' and 'total') can be determined by their capacity to form rosettes with sheep erythrocytes (SRBCs) (Fudenberg, Wybran & Robbins, 1975; Woody & Sell, 1975). There is evidence that changes in the active (those counted immediately after resuspending a centrifuged lymphocyte-SRBC mixture), rather than the total, T-cell population reflect certain pathological changes (Fudenberg, Wybran & Robbins, 1975). Preliminary results describing the effects of phenytoin and its major metabolite, 5-(p-hydroxyphenyl)-5-phenylhydantoin (HPPH) on the enumeration of both active and total T-lymphocytes are presented here.

All studies were carried out on peripheral venous blood specimens from healthy volunteer subjects (mean age 29 years). Lymphocytes were isolated on Ficoll-Triosil (Harris & Ukaejiofo, 1969), washed ($\times 2$) with phosphate buffered saline (pH 7.2), resuspended in Eagle's basal medium and counted on a haemocytometer slide. Equal volumes (50 µl) of lymphocyte (8×10^6 cells/ml) suspension and medium containing phenytoin or HPPH (controls contained 0.25% v/v ethanol) were incubated for 30 min at 37°C prior to the addition of 50 µl of foetal calf serum (FCS). After a further 1 h incubation 100 µl of a SRBCs suspension (0.5% for 'actives'; 1.0% for 'totals') in medium containing 33.3% FCS were added just before centrifugation (200 g for 5 min). To determine active rosette forming cells the lymphocyte-SRBC pellet was immediately resuspended and counted. 'Total rosettes' were counted after an 18 h incubation at 4°C. Individual results (mean of triplicates) were tabulated as the percentage of lymphocytes forming rosettes (3 or more adhering SRBCs).

In an initial survey ($n = 30$) phenytoin (10 mg/l), but not HPPH, caused a relatively slight (18.7%) but significant (paired t test, $P < 0.01$) reduction in the percentage of active rosettes (percentages $\pm$ s.e. percentage in control and phenytoin treated cells were 27.8 ± 2.6 and 22.6 ± 2.8, respectively) although neither agent affected total rosettes (control = 57.0%). In a separate study ($n = 6$) total rosette formation was unaffected by both phenytoin and HPPH (2.5, 10, 20 and 50 mg/l). 'Active' percentages, however, were reduced to approximately 0.78 of the control values by each of the 3 higher phenytoin concentrations. Time course studies (15, 30, 45, 60 and 120 min pre-incubations) indicated that maximal inhibition of active rosettes by phenytoin (10 mg/ml) occurred at 45 min but were complicated by the fact that the controls also decreased with time. Preincubating SRBCs with phenytoin (10 mg/l) did not alter subsequent active rosette formation.

The results suggest that phenytoin (at concentrations > 2.5 mg/l) may have an effect on a separate T-lymphocyte population. The clinical significance of these findings, however, remains to be established.

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