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Detrimental effect of propylene glycol on natural killer cell and neutrophil function

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Propylene glycol (PG) in a concentration of 0·5–1·0% inhibits natural cytotoxicity and neutrophil chemiluminescence. This is the first reported effect of PG on defence mechanisms in man. PG is frequently used as a vehicle in high concentrations by the pharmaceutical industry and it would be wise to consider its potential immunosuppressive effects in the evaluation of drug formulations.

During the course of some experiments testing the effect of aflatoxin on natural killer (NK) cell function, propylene glycol (PG) was used as a solvent. Thought to be relatively harmless, it is a commonly used solvent in the pharmaceutical industry for a wide variety of preparations and is included as a solvent in some spray solutions to stabilize droplet size. It is also used by the food industry as a solvent for various flavourings (Reynolds 1982). No effects on the immune system have previously been recorded.

Materials and methods

NK cytotoxicity assay

Preparation of peripheral mononuclear cells. Blood was obtained from a resting healthy volunteer and collected into a syringe containing heparin 3 units mL^{-1}. Whole blood was carefully layered onto Ficoll-Hypaque (Pharmacia) and mononuclear cells separated by centrifugation at 300*g* for 25 min. The interface cells were removed and washed twice in L15 medium and resuspended in 5 mL growth medium, bicarbonate-buffered RPMI 1640 supplemented with L-glutamine, penicillin, streptomycin and 10% foetal calf serum. Plastic adherent cells were removed by incubation for 90 min at 37 °C in a large sealed nunc flask. Non-adherent cells were washed off and resuspended at concentrations of 4, 2 and 1 × 10^6 mL^{-1}.

Preparation of target cells. Cultured cells from the K562 erythroleukaemia line (kindly suplied by J. Armitage of Rayne Institute, University College Hospital, London) grown in growth medium were used. The cells were incubated in 100 μL of the medium together with 3·7 MBq of sodium ^{51}chromate (Amersham International, UK, Code CJS4) for 1 h at 37 °C with mixing at 10 min intervals. Target cells were gently washed three times by centrifugation at 50 *g*. Viability was assessed after the third wash by trypan blue exclusion and the viable cells resuspended at a concentration of 5 × 10^4 cells mL^{-1}.

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Cytotoxicity assay (Onwubalili & Scott 1985). Propylene glycol (Sigma) was diluted with phosphate-buffered saline (PBS) to concentrations of 10, 1 and 0·1%. Into each well of a U-well microtitre plate, 20 μL of PG or PBS (as control) was pipetted. Effector cells were then added, in the three different concentrations, in a volume of 100 μL. Finally target cells were added in a volume of 100 μL. Wells were prepared in triplicate. Minimum lysis was assessed by the addition of PG 10% 20 μL and growth medium 100 μL to 100 μL target cells. Maximum lysis was assessed by the addition of 100 μL of 10% Triton X-100 to 100 μL of target cells. The cultures were incubated at 37 °C for 4 h in humidified sandwich boxes. The microtitre plate was then centrifuged at 70*g* for 10 min and 100 μL of supernatant collected and placed in LP2 tubes. Radioactivity was determined in a gamma counter (Wallac GTL 300/1000). The whole experiment was done twice.

The percentage of specific ^{51}Cr release (% cytotoxicity) was calculated as (E-S)/(W/2-S) × 100 where E is mean of three counts min^{-1} in experimental tubes, S is counts min^{-1} spontaneous isotope release (minimum lysis) and W is counts min^{-1} maximal isotope release effected by Triton X-100 lysis. Dose-response values were plotted for concentration of PG as per cent cytotoxicity against effector : target (E : T) cell ratio.

Neutrophil chemiluminescence

Preparation of mononuclear cells. Blood was collected from a healthy volunteer after exercise into a plastic Universal tube containing heparin 3 units mL^{-1}. Blood was mixed in a ratio of 5 : 2 with dextran 100 in 0·9% NaCl, transferred to a clean universal tube and the red cells allowed to sediment at room temperature (20 °C) for 45 min. The supernatant was aspirated and the cells collected by centrifugation at 150*g* for 10 min. Contaminating red cells were removed by hypotonic lysis and the cells recollected by centrifugation. They were resuspended in 5 mL Hank's buffered salt solution containing HEPES 2%, penicillin 1% and streptomycin 1%, but no phenol red, adjusted to pH 7·4 with NaOH together with heparin 10 units mL^{-1} and 10 μL luminol (5-amino-2,3-dihydro-1,4-phthalazimedione in DMSO 10^{-2} M). Neutrophils were then counted in a counting chamber and adjusted to 1·25 × 10^6 mL^{-1}.

Chemiluminescence (Lever et al 1985). Aliquots of cells (1 mL) were placed in specially designed cuvettes and

covered with plastic clingfilm. After a 30 min resting period the first cuvette was transferred to the hot room (37 °C) for a further 10 min (as was each subsequent tube). Dilutions of PG were made with PBS to 20, 10, 2 and 0·2%. To the first tube 50 μL of PBS was added and the tube shaken once followed 30 s later by latex particles coated with IgG without shaking. The cuvette was immediately placed in the luminometer (1250 LKB Wallac) which rotated the cuvette and recorded the luminescence produced every 10 s for 8 min. Each concentration of PG was then tested. This experiment was done on two occasions.

Results

NK cytotoxicity in the presence of propylene glycol is shown in Fig. 1. There is an obvious depression in NK cytotoxicity by 1% propylene glycol. This effect reaches significance on a paired *t*-test at a level of $P < 0{\cdot}002$. Concentrations of 0·1 and 0·01% PG are not significantly different from the control.

There was no effect of 10% PG alone on the release of radiochromium from the target cells in the two experiments when this was tested. For example, in one experiment the maximal isotope release with Triton X-100 was 11 700 counts min^{-1}, the mean spontaneous release in medium was 401 counts min^{-1}, and with 10% PG was 373 counts min^{-1}.

Fig. 2 shows the pattern of chemiluminescence in the presence of propylene glycol at final concentrations of 1, 0·5 and 0·1%. There is a marked reduction of light production in the presence of both 1 and 0·5% concentrations and some reduction at the lower concentration of 0·1%. The duplicate experiments of both 1 and 0·5% PG gave a statistically significant result with a *P* value of 0·023.

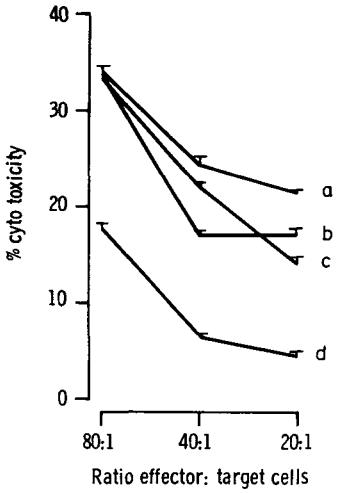

FIG. 1. Natural killer cell cytotoxicity in the presence of propylene glycol at given concentration. Key: a, PBS control; b, 0·01%; c, 0·1%; d, 1% PG.

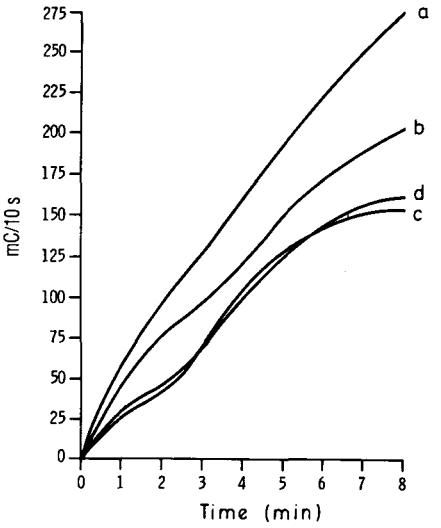

FIG. 2. Neutrophil chemiluminescence in the presence of propylene glycol at given concentrations. Key: a, PBS control; b, 0·1%; c, 0·5%; d, 1% PG.

Discussion

Some toxic effects of PG have been noted. Toxic effects are related to overdose or chronic ingestion and manifest as central nervous system depression and fits (Martin & Finberg 1970; Arulanantham & Genl 1978; Sawchuck et al 1982). Sensitization has also been described in both topical (Huriez et al 1966; Angelini & Meneghini 1981) and oral preparations (Hannuksela & Forstrom 1978). Effects on rat hepatic microsomal metabolism have previously been noted (Dean & Stock 1974; Yamamoto & Adachi 1978) and changes in lipid metabolism of the rat have also been reported (Amma et al 1978; Hoeing & Werner 1980). Assuming equal distribution of PG through all water compartments, full absorption and slow metabolism in these experimental rats, these changes occurred at predicted tissue levels of 1·1% (Dean & Stock 1974), 1·4% (Yamamoto & Adachi 1978), and 0·57% (Hoeing & Werner 1980). The effect on chromosomes has been studied and PG inhibits the normal development of polyploidy in mouse bladder epithelium (Farsund 1981), and although negative in the Ames test, does generate frequent chromosomal aberrations in-vitro with a hamster cell line at a maximum dose of 3·5% (Ishidate et al 1984).

These observations, together with our own here, suggest that PG is a cytotoxin and should not be regarded as harmless. Central nervous system depression has occurred with the ingestion of as little as 60 mL (Gosselin et al 1976) which would give a total body concentration of approximately 0·15% if uniformly distributed in cells. If only distributed in the extracellular fluid space of about 15 L (Talbot et al 1959) a

concentration of 0·4% is attained. A defect in the immune mechanisms in-vivo would be anticipated from our results if an adult consumed this amount of PG.

Some intravenous infusion preparations contain considerable amounts of propylene glycol. Use of Septrin paediatric infusion (co-trimoxazole) would administer 150 mg kg^{-1}/24 h which might attain levels of 0·06% in the extracellular space, and Tridil infusion (glyceryl trinitrate) could deliver 15 g in 24 h, which in a 50 kg woman would give extracellular levels of 0·12%. High local concentrations of PG may also be attained by intranasal and topical preparations. For example Metosyn ointment contains 65% PG, Dermovate contains 47·5% PG and several other preparations 20% PG. Usually PG is included in corticosteroid preparations and it is interesting to speculate about the relative role of PG in these anti-inflammatory preparations.

We have made no attempt to unravel the mechanism of the effects described here. It should be noted that 2% PG is iso-osmotic with serum and therefore the final concentration used in these experiments did not upset osmotic balance. Recent work has thrown light on the previously poorly understood mechanism of NK cytotoxicity (Schmidt et al 1985). We would argue that the effects described here are not specific to the specialist functions of NK cells and neutrophils but reflect more general toxicity. A reappraisal of the use of propylene glycol by the pharmaceutical and food industries may be appropriate.

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