Notes and miscellanea

Induction of sister chromatid exchanges in human lymphocytes by DTPA

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The chelating agent, diethylenetriaminepentaacetic acid (DTPA) is a non-registered drug used in the treatment of over-exposure to heavy metals such as iron, zinc and lead (Fahey *et al.*, 1961; Brugsch *et al.*, 1965). DTPA has also been used on a few occasions in the United Kingdom when individuals have inhaled plutonium dust or received contaminated wounds (Schofield and Dolphin, 1974). These patients received a series of intravenous injections of 0.25 g of calcium-DTPA in 20 ml of physiological saline.

At concentrations of 6–30 times the therapeutic dose DTPA produces transient toxic effects in the liver and kidneys of mice (Morgan and Smith, 1974). It may cause the release of proteins from cell membranes (Stack and Fox, 1972) and there is some evidence to suggest that chelating agents may induce chromosome aberrations in *Vicia faba* (Cohn, 1961; Michaelis *et al.*, 1962). It is possible, therefore, that DTPA may be hazardous to humans; this study investigates the risk involved in the use of this drug.

Sister chromatid exchange (SCE) induction is one of many short-term screening tests which are likely to be used in the initial testing of new drugs for possible mutagenic side effects (Bridges, 1976). Although the procedure is reliable, quick and relatively simple it does possess several shortcomings. In common with other *in vitro* mutagenicity tests it is not sensitive to certain known mutagens, such as chloramphenicol, whose metabolites are the agents causing damage. False negative results may be avoided in some instances by including metabolically active liver microsomes in the cell culture (Stetka and Wolff, 1976). The high background incidence of SCEs, presumably caused by the necessary addition of a base analogue, and the possibility of synergism between the test drug and

the base analogue, are further criticisms of the technique. Hence for full mutagenicity testing of any chemical a large number of *in vitro* and *in vivo* test systems must be adopted.

The ability of DTPA to induce SCEs in human lymphocytes was investigated using a differential staining procedure, in which the base analogue bromodeoxyuridine (BrdU) is incorporated into the deoxyribonucleic acid (DNA) of cultured human blood lymphocytes. Bifilarly substituted DNA chains stain more lightly and therefore after two mitotic cycles in the presence of BrdU any SCEs can be readily observed under the microscope as exchanges between the light and dark staining chromatids (Perry and Wolff, 1974) (see Figure).

A derivative of DTPA, developed recently in the National Radiological Protection Board laboratories, and which possesses lipophilic fatty acid chains (Bulman *et al.*, 1976) was also tested for SCE induction. It is anticipated that the lipophilic portion of the molecule will enable it to enter cells and hence to remove intracellular metal contaminants, which calcium-DTPA cannot do; as an alternative intravenous treatment this new derivative shows very great promise. It might also be expected that, once inside the cell, the molecule will exert any toxic effects directly on the cell constituents, particularly on nuclear material and the internal cell membranes. It would not be unexpected therefore for the DTPA derivative to possess a greater mutagenic potential than that of calcium-DTPA.

The staining method used was that of Perry and Wolff (1974). In brief, separated human lymphocyte cultures were incubated at 37°C with 10⁻⁴ M BrdU, plus the chelating agent to be tested, for 72 h in darkness. The culture medium consisted of 4 ml Eagles' medium (Wellcome), 1.5 ml calf serum (Difco) and 0.1 ml reconstituted phytohaemagglutinin (Wellcome) containing approximately 9 mg of reagent per ml. For the final 3 h of incubation, 2.5 µg colcemid (Ciba) was added to each culture. After fixation (Purrott and Lloyd, 1972) the slides were treated with 0.5 µg/ml Hoechst 33258 in phosphate buffered saline (PBS), pH 7.0 for 14–30 min, rinsed in distilled water and then PBS, and exposed to a bright light for 3 h. Slides were then placed in 2× standard saline citrate solution (0.3M sodium chloride in 0.03M sodium citrate) at 60°C for 2 h, rinsed and stained in 2% Giemsa for 30 min.

Calcium-DTPA was added to the test cultures as 0.1 ml of solution in distilled water at three concentrations, 0.77, 0.077 and 0.0077 mg/ml,

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Figure 'Harlequin' chromosomes showing sister chromatid exchanges.

giving a final concentration in the culture medium of 13.51, 1.351 and 0.135 $\mu\text{g/ml}$ respectively. The 1.351 $\mu\text{g/ml}$ concentration corresponds approximately to that in blood after a single intravenous administration. The yields of SCEs are shown in Table 1.

Table 1 SCE induction by calcium-DTPA

Concentration ($\mu\text{g/ml}$ of culture medium)	Mean no. of SCEs per cell $\pm$ SE*	No. of cells examined
Control	9.17 $\pm$ 0.50	150
0.135	10.14 $\pm$ 0.61	35
1.351	12.41 $\pm$ 0.63	100
13.51	No mitoses observed	

*Standard errors were calculated according to the method of sums of squares and Student's *t* test applied to determine the significance of differences between the means

It was consistently observed that the highest concentration of DTPA (13.51 $\mu\text{g/ml}$ of culture medium) inhibited the production of metaphase

figures on the final microscope slide preparation. At the other two DTPA concentrations an increase in the number of SCEs above the control value of 9.17 per cell was observed. For the lower concentration of DTPA (0.135 $\mu\text{g/ml}$) this increase is not significant, but for the higher concentration (1.3 $\mu\text{g/ml}$) the SCE yield is significantly greater than that of the control ($p < 0.001$).

The number of SCEs induced by various concentrations of DTPA-derivative, tested in the same way as calcium-DTPA, are shown in Table 2.

Table 2 SCE induction by lipophilic DTPA derivative

Concentration ($\mu\text{g/ml}$ of culture medium)	Mean no. of SCEs per cell $\pm$ SE*	No. of cells examined
Control	8.83 $\pm$ 0.20	50
0.270	9.90 $\pm$ 0.45	50
2.70	11.44 $\pm$ 0.53	50
27.0	No mitoses observed	

*Standard errors and significance of differences between means calculated as in Table 1.

A small increase in the yield of SCEs was found at both of the concentrations which permitted metaphase production ($P < 0.05$ for the concentration of 0.270 $\mu\text{g/ml}$ and $P < 0.001$ at 2.70 $\mu\text{g/ml}$). No stable aberrations (fragments, rings or dicentric) were observed in any of the lymphocyte cultures examined.

The values given in Tables 1 and 2 may be compared with data from Perry and Evans (1975) for SCE induction by various known mutagens, many of which produce large numbers of exchanges. Quinacrine mustard, for example, gave an SCE value of 121.1 per cell at a concentration of 10^{-6}M . The present data therefore suggest that the limited ability to induce gross structural abnormalities in chromosomes is probably not a major hazard of DTPA or of its lipophilic derivative. However, the absence of mitotic figures at higher concentrations of both materials suggests some further toxic effect, possibly associated with the removal of metal ions from the culture medium or even from the cell membranes.

DTPA and its lipophilic derivative were kindly provided by Dr Robert Bulman.

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Erratum

A mortality study of coke oven workers in two South Wales integrated steelworks

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It is regretted that there was an error in this paper (*British Journal of Industrial Medicine* (1977) **34**, 291-297) which affected the estimate of the total man-years of survival. The study was based on 610 men, all of whom were employed in 1954 and 100% of whom were traced to death or survival in June, 1965. The age-specific man-years were calculated from a double decrement table in single years of age for each calendar year and deaths were assumed to occur at the mid-year. This allowed 5-year age groups to be obtained and the 'expected' deaths were calculated from the national age-specific rates. Unfortunately in this calculation the first and last years were considered as full years whereas, in fact, the deaths were registered for only half of these two years. This resulted in an incorrect estimate of 6837 man-years which was 9% above

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the true total of 6261.5 man-years. The numbers of 'expected' deaths are reduced almost proportionally causing a related increase in the SMRs. The recalculated SMRs are:- Works A, 107%; Works B, 78%; Works A + B, 92%; as compared with the published SMRs for Works A, 98%; Works B, 73%; Works A + B, 85%.

The general findings of this paper, however, are not affected; in particular, in the population studied, the observed and expected deaths were not very different for respiratory disease (14 observed, 12.6 expected), cancer of the lung (8 observed, 8.94 expected) and cancer of the bladder and kidney (3 observed, 1.09 expected). The marked deficiency of cardiovascular disease is still apparent (29 observed, 41.36 expected).

A corrected paper can be obtained from the author, British Steel Corporation, Welsh Laboratory and Strip Mills Products, Port Talbot, W. Glam.