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Discussion of Dr. Zimmermann's paper

ALKYLATION of HeLa cell DNA with sulphur mustard or half sulphur mustard has been shown to result in the inhibition of DNA synthesis which results in a delay in mitosis and eventually cell death (see discussion of Dr. Farber's paper). These studies also suggested that cells were able to recover to some extent from alkylation damage and it was subsequently shown that, following treatment with mustard gas, cells exhibit non-semiconservative DNA replication.[1] This may be associated with "repair" synthesis possibly following excision of an alkylated moiety on the DNA and degradation of a portion of the DNA template. More recent work has been designed to answer the following questions.

How important is the nature of the alkyl substituent on the DNA in exhibiting these various effects? Is the level of alkylation of DNA the only factor determining toxicity or does reaction with other cell constituents influence the "repairability" of this damage? Do all cell types respond equally to a similar level of alkylation or are there differences in the ability of cells to repair DNA damage? Accordingly, the effect of various concentrations of the methylating agents, *N*-methyl *N*-nitrosourea (NMU) *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and methyl methanesulphonate (MMS) on the survival of hamster (V79) and HeLa cells has been determined. A striking feature of the results was the wide range of concentrations required to produce equivalent effects on survival. Thus the concentrations of MNNG, NMU and MMS which, when applied to V79 cells for 1 hr, reduce survival to 10 per cent were 0·009, 1·05 and 1·8 mM, respectively, a range of 200-fold. When, however, the binding of these three methyl-labelled compounds to the DNA of V79 cells was determined at concentrations which reduced survival to similar levels it was found that, despite these differences in concentrations, the levels of reaction were virtually the same. At concentrations of drugs reducing survival to 10 per cent the levels of reaction were between 1 and 1·2 μmole of agent per gram of DNA. The corresponding concentration of these three compounds, MNNG, NMU and MMS, which reduced HeLa cell survival to 10 per cent were 0·08, 20 and 180 μM respectively. This represents a 2000-fold difference in the concentrations required to produce equivalent effects. A second feature of these results is the vast difference between the concentrations of any particular compound which produced equivalent effects on the survival of the two cell types. In the case of MNNG there was a 100-fold difference in the concentrations which reduced the survival of V79 and HeLa cells to 10 per cent. The levels of reaction of these three agents to the DNA of HeLa cells was again very similar at doses producing comparable cell survival (10 per cent), and were within the range 0·02–0·8 μmoles/g DNA. It follows from these results that the external concentration of a particular methylating agent required to produce an effect on cells may not be a true indication of the actual interactions occurring with cell constituents. In the situations described here, with these three compounds and two cell types, equivalent effects on survival were obtained with concentrations of agents ranging from 0·08 μM to 1·8 mM, a difference of more than 20,000. However, the levels of reaction with DNA did not differ by more than 60-fold.

The similar level of reaction of the three compounds with V79 cell DNA suggests that these agents are acting similarly on these cells and in all probability produce their toxic effects by reaction with DNA, and the same is probably true for HeLa cells, despite a somewhat greater range in the levels of reaction with DNA with these cells. (Other studies, to be reported elsewhere, indicate comparable effects on the inhibition of DNA synthesis at concentrations producing comparable effects on cell survival.) However, the difference in the binding of methylating agents to the DNA of V79 cells compared with that to the DNA of HeLa cells does suggest a difference in the response of these two cell types to DNA alkylation by NMU and MNNG.

The possibility that the generally higher level of alkylation of DNA of V79 cells is associated with a greater ability to repair alkylation lesions is being further investigated.

These unpublished results were obtained in collaboration with Dr. A. R. Crathorn, Mrs. J. Pascoe, Miss J. Plant and Mrs. J. Sturrock.

Chester Beatty Research Institute,
Pollards Wood Research Station, Bucks.

J. J. ROBERTS

REFERENCE

1. J. J. ROBERTS, A. R. CRATHORN and T. P. BRENT, *Nature, Lond.* **218**, 970 (1968).