MITOZANTRONE INDUCED DNA STRAND BREAKS IN THE PRESENCE OF CYTOCHROME P450 REDUCTASE

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Mitozantrone is a bis(alkylamino)anthraquinone antitumour agent which can be one electron reduced to a free radical in liver subcellular fractions (Patterson et al, 1983). Furthermore, in the presence of liver cyt. P450 reductase (EC 1.6.2.3) mitozantrone will redox cycle and generate superoxide anions (Kharasch and Novak, 1983). At present intercalative binding of mitozantrone to DNA has been considered responsible for its cytotoxic action. However it is important to determine whether or not metabolically activated mitozantrone can contribute to DNA damage. We have investigated this using plasmid DNA as a model system to determine drug induced DNA single and double strand breaks. Plasmid DNA can exist in three forms, the covalently closed superhelical form (I), the 'nicked' relaxed open circular form (II) produced by DNA single strand breaks, and the linear form (III) produced by DNA double strand breaks. These forms can be separated and quantitated using gel electrophoresis.

Plasmid DNA (pBR322) was isolated according to the procedure of Birnboim and Doly (1979). Mitozantrone (5nmol) was incubated at 37° for 20 min in the presence of pBR322 DNA (0.8μg), purified rat liver cyt.P450 reductase (2.5μg) and NADPH (400nmol) with tris-HCl buffer (pH7.4) in a final volume of 100ul. In some experiments mitozantrone and/or reductase were absent. The incubation mixture was extracted with butan-1-ol (4x100μl) to remove drug, stained with ethidium bromide and assayed for the presence of DNA single and double strand breaks using agarose gel electrophoresis essentially as described by Boon et al,(1984). The data obtained are presented in Table 1.

Table 1. Effect of mitozantrone on plasmid DNA

	% plasmid DNA (pBR322)		
incubation	Form I	Form II	Form III
plasmid DNA	76.2	23.8	0
DNA+mitozantrone	76.1	23.9	0
DNA+P450 reductase	72.0	28.0	0
DNA+P450 reductase + mitozantrone	38.9	61.1	0

results are the mean of duplicate experiments

The results show that incubation of mitozantrone in the presence of cyt.P450 reductase causes a 2-fold increase in the amount of plasmid DNA containing single strand breaks (Form II) compared to incubations where drug or reductase were absent. Hence metabolically activated mitozantrone can damage double stranded DNA. It is likely that such a mechanism involves reactive oxygen generation.

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