

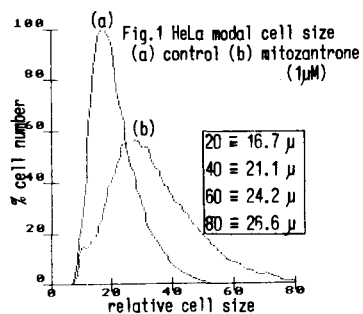
EFFECT OF FREE RADICAL SCAVENGERS ON THE IN-VITRO CYTOTOXICITY OF MITOZANTRONE

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Mitozantrone (Novantrone) is a bis(alkylamino)anthraquinone antitumour agent broadly based on the anthracyclines such as doxorubicin. Intercalative binding of mitozantrone and the associated effects on DNA and RNA synthesis and replication may be a factor in its cytotoxic activity (Cheng and Zee-Cheng, 1983). However mitozantrone can be metabolically reduced to a free radical species (Patterson et al, 1984). Since mitozantrone free radical may contribute to the overall antitumour activity of the parent compound we have investigated the effect of free radical scavengers on the cytotoxicity of mitozantrone against cultured HeLa cells.

HeLa cells were grown as monolayers and passaged using standard techniques essentially as described by Freshney (1983). The method of quantitating cytotoxicity was based on that of Uyeki et al (1981) with electronic enumeration and sizing of harvested monolayers using a coulter counter. In some experiments HeLa cells were incubated with free radical scavengers (100 μ M) 24h prior to the addition of mitozantrone.

Mitozantrone (1 μ M) incubated with exponentially growing cells for 24h caused a 49 \pm 5% decrease in cell numbers compared to monolayers not treated with drug. In addition, the modal cell size distribution was increased by 22% (fig. 1).



Although 24h drug contact time was routinely used it was also observed that a 30min drug contact time produced the same effect as shown in figure 1. Incorporation of free radical scavengers did not diminish the antiproliferative effect nor cell size distribution resulting from mitozantrone treatment (table 1).

Table 1 Effect of free radical scavengers and mitozantrone (MTZ) on HeLa cells.

Treatment	% cell number	modal cell size (μ)
no drug	100	15.7 \pm 0.5
MTZ (1 μ M)	51 \pm 5	19.1 \pm 0.7
MTZ+N-acetylcysteine	44 \pm 4	18.5 \pm 0.2
MTZ+vitamin C	41 \pm 7	19.0 \pm 0.5
MTZ+glutathione	44 \pm 4	19.3 \pm 0.4

results are the mean \pm S.D. of at least 3 experiments

The results show that mitozantrone is cytotoxic to HeLa cells producing an antiproliferative effect and inhibition of cell cycle progression. The inability of free radical scavengers to diminish this cytotoxicity suggests that the mechanism of action of mitozantrone in HeLa cells does not depend on mitozantrone free radical formation.

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Freshney, R.I. (1983) In Culture of Animal Cells Pub. Alan R. Liss (N.Y.) pp 119-128

Uyeki et al (1981) J. Pharm. Sci. 70:1011-1014