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SENSITIZING AND TOXICITY PROPERTIES OF MISONIDAZOLE AND ITS DERIVATIVES

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IN the early search for radiation sensitizers specific for hypoxic cells it was hoped that such compounds would mimic a number of critical features of oxygen, that they would produce a dose modification type of radiation sensitization, be without effect on aerobic cells, be slowly metabolized and would be non-toxic. Our most recent studies with misonidazole indicate that under certain conditions it does produce a dose-modifying effect specific for hypoxic cells, but that under conditions of more prolonged exposure hypoxic cells produce derivatives of the compound, at least one of which will reduce the shoulder of both aerobic and hypoxic cell survival curves (Wong, Whitmore and Gulyas, 1978). It also appears that this compound, or compounds, may be responsible for the toxicity seen in hypoxic cells and that once formed in hypoxic cells will also produce toxicity in aerobic cells.

Figure 1 illustrates the response of hypoxic Chinese hamster ovary (CHO) cells to radiation after a 15-min exposure to various concentrations of the drug under hypoxic conditions at 20°C. Under these conditions the effect of the drug is essentially dose-modifying and in all instances the survival curves extrapolate to a common point.

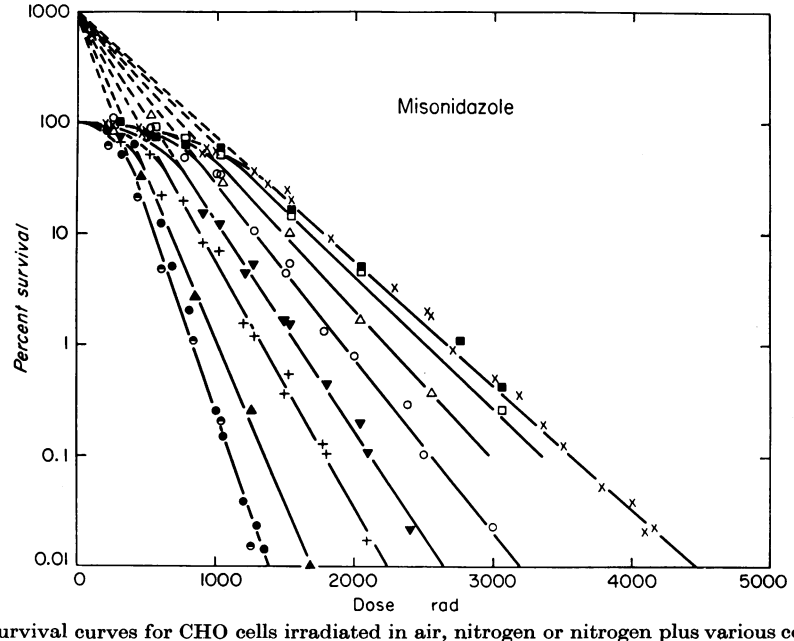

FIG. 1.—Survival curves for CHO cells irradiated in air, nitrogen or nitrogen plus various concentrations of misonidazole following a 15-min exposure to the drug at 20°C under hypoxic conditions. The symbols ●, ◓, ▲, +, ▼, ○, △, □, ■, ×, refer respectively to: air control. 51·4 mM, 17·1 mM, 2·0 mM, 1·0 mM, 0·5 mM, 0·25 mM, 0·12 mM, 0·06 mM and N_2 control.

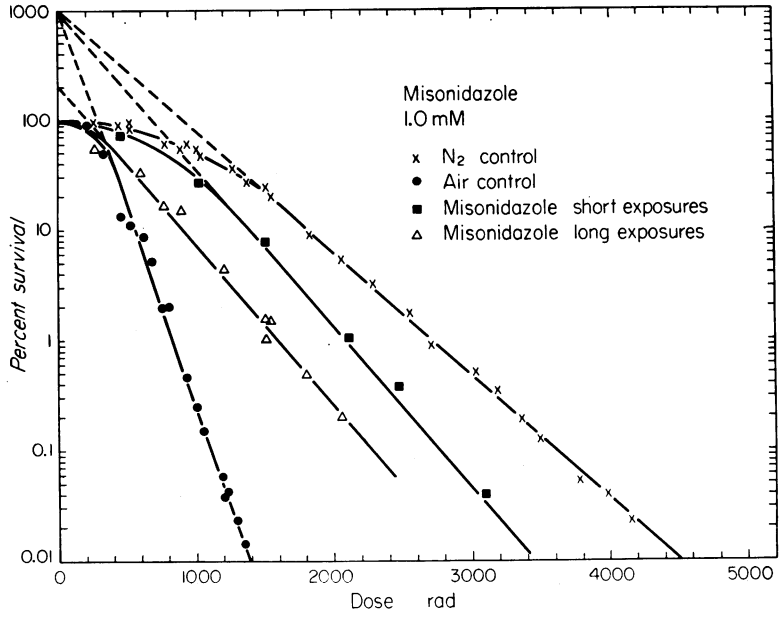

FIG. 2.—Survival curves for CHO cells irradiated in air (●), N_2 (×) or N_2 plus 1·0 mM misonidazole after a 15-min exposure at 20°C (■) or a 3·0-h exposure at 37°C (△).

Figure 2 shows the response of hypoxic CHO cells after a 3-h exposure to the drug under hypoxic conditions at 37°C. Once again there is pronounced sensitization but under these conditions of prolonged exposure at physiological temperatures not only is there a progressive reduction of Do with increasing drug concentration but also a marked reduction in the extrapolation number, indicating that the drug is no longer acting as a dose-modifying agent and that a new mechanism of sensitization is coming into play. Further indication that there are two mechanisms of sensitization comes from experiments in which hypoxic cells are exposed to misonidazole (0·4 or 1·0 mM) at either 0°C or 37°C and then exposed to a single dose of radiation after various time intervals. These experiments indicate that at both concentrations immediately upon exposure to the drug hypoxic cells show an immediate dose-modification type of sensitization. At 0°C this sensitization remains unchanged with time but at 37°C is coupled with a further shoulder-reduction type of sensitization which increases in magnitude over a period of several hours, suggesting that it may arise from metabolites of the drug.

Having seen the development of two types of sensitization, we have attempted to correlate their appearance with the presence of various metabolites in cells exposed to the drug. For these studies we have used misonidazole containing ^{14}C in the 2-position of the imidazole ring.

Figure 3 shows chromatograms obtained under a variety of conditions. The panels in sequence are: (1) misonidazole; (2) a cell homogenate obtained after a 3-h exposure of hypoxic cells at 37°C; (3) a cell homogenate obtained after a 3-h exposure of aerobic cells at 37°C; (4) misonidazole after extended chemical reduction in the presence of zinc and ammonium chloride and (5) misonidazole after limited chemical reduction.

A comparison of Panels 1, 2 and 3 indicates that under hypoxic conditions cells do produce at least 3 and possibly more derivatives of the parent compound but that under aerobic conditions the production of these derivatives is markedly

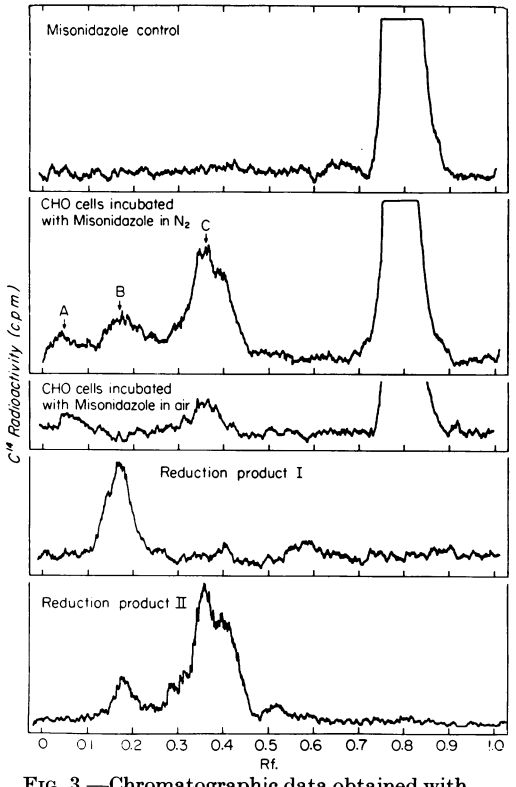

FIG. 3.—Chromatographic data obtained with ^{14}C-misonidazole treated under a variety of conditions described in the text. All chromatograms used Whatman 3 MM paper, developed for 18 h in a solvent consisting of water-saturated secondary butanol.

reduced. The small amount of material produced under aerobic conditions may have been due to a failure to maintain aerobic conditions throughout the exposure. Comparison of Panels 2, 4 and 5 suggests that at least some of the material produced during incubation of the drug in hypoxic cells is identical to that produced by chemical reduction. Limited analysis suggests that the product in Panel 4 contains a fully reduced NO_2 group, *i.e.*, an amine, and that the other products seen in Panel 5 are probably partially reduced intermediates, either nitroso compounds or hydroxylamines.

Having demonstrated the formation of a series of derivatives in both cell homogenates and chemical systems, we have attempted to look at the biological properties of Products I and II. Figure 4 shows the results of a toxicity experiment to compare the effects of misonidazole and the two reduction products under aerobic and hypoxic conditions. The data were obtained by measuring cell outgrowth over 48 h after a 3·5-h exposure to the appropriate agent at 37°C. In agreement with earlier observations (Hall and Biaglow, 1977; Hall and Roizin-Towle, 1975; Moore, Palcic and Skarsgard, 1976; Wong, *et al.*, 1978), misonidazole is appreciably more toxic under hypoxic than aerobic conditions. Product I, which is formed only under hypoxic conditions, once formed is equally toxic to both aerobic and hypoxic cells. Product II, which also is formed only under hypoxic conditions, is even more toxic than Product I, under both aerobic and hypoxic conditions. It should be pointed out that the toxicity seen with Product I may be due to contamination with Product II since adequate methods of purifying the two compounds have not been developed.

Figure 5 shows radiation survival data obtained with the two reduction products under hypoxic and aerobic conditions From the data it is apparent that Product I (0·5 MM) has little or no effect on the survival curves of either aerobic or hypoxic cells but that even at a concentration of 0·04 MM Product II totally removes the shoulder from the survival curve but has little or no effect on the ultimate slope.

Up to the moment it has not been possible adequately to purify or to determine the chemical nature of the intermediates of misonidazole responsible for toxicity and the shoulder reduction in the radiation survival curve. Likely candidates would appear to be either nitroso or hydroxylamine derivatives. What is apparent, however, is that these compounds, while formed only under hypoxic conditions, are capable of producing toxicity and sensitization in both aerobic and hypoxic cells. One might, therefore, predict that in the presence of hypoxic tumour cells sensitizers may be metabolized to

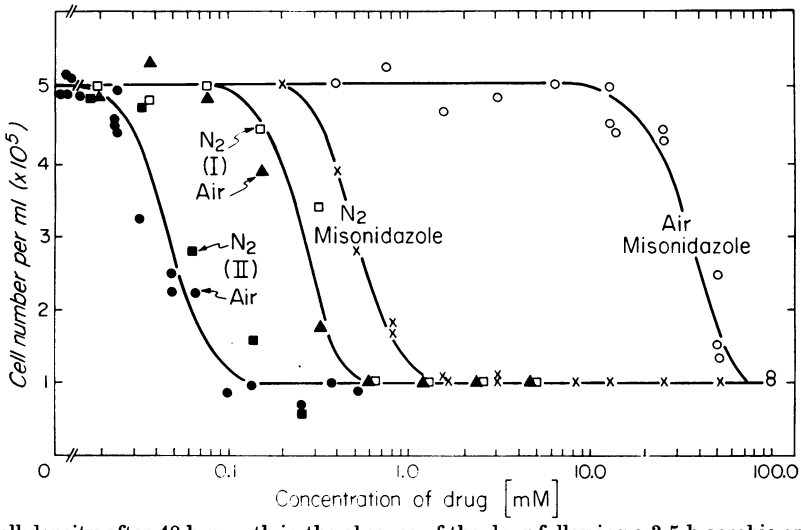

FIG. 4.—Cell density after 48 h growth in the absence of the drug following a 3·5-h aerobic or hypoxic exposure to various concentrations of misonidazole or Products I and II having chromatographic patterns similar to those shown in Fig. 3.

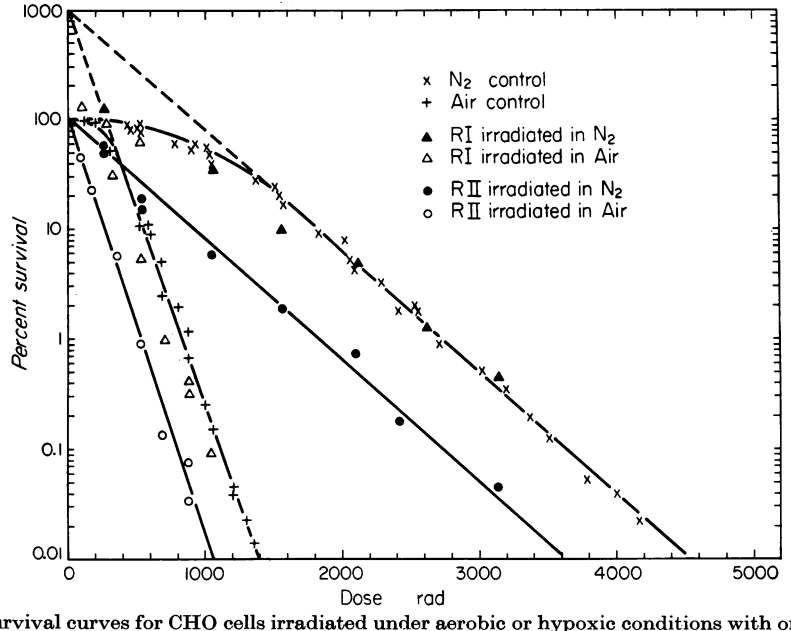

FIG. 5.—Survival curves for CHO cells irradiated under aerobic or hypoxic conditions with or without prior exposure to Product I (0·5 mM) or Product II (0·04 mM) for 2·5 h at 37°C under aerobic or hypoxic conditions.

form compounds which will not only have effects on the cells in which they are produced but might also kill or sensitize surrounding tumour or normal tissues. While such effects might be therapeutically beneficial, it is also possible that they will necessitate reductions in radiation dose below those used in conventional fractionation regimes in order to prevent excessive damage to critical normal tissues.

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