TOXICITY OF METRONIDAZOLE AND SODIUM ASCORBATE TO HYPOXIC SV_{40}-TRANSFORMED 3T3 CELLS

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Metronidazole is an electron-affinic nitroimidazole which has been used primarily as a trichomonacide. Recently it has been shown to be effective as a radiosensitiser of hypoxic tumour cells both *in vivo* and *in vitro* (Begg, Sheldon & Foster, 1974). Furthermore it is considerably more toxic to hypoxic mammalian cells *in vitro* than to similar cells grown in aerobic conditions (Mohindra & Rauth, 1976). We report that the toxicity of metronidazole to hypoxic transformed mouse cells in culture is enhanced by the addition of sodium ascorbate. Sodium ascorbate has previously been shown to be toxic to aerobic cells (Cope & Dawson, 1978) but anaerobic conditions protect cells from the effects of ascorbate, presumably by preventing the production of hydrogen peroxide during ascorbate oxidation. The experiment described demonstrates that, in anaerobic conditions, concentrations of ascorbate and metronidazole which are ineffective when applied to SV_{40} - transformed 3T3 cells alone, are toxic when both substances are applied together.

The cells, seeded in 30 mm vented Petri dishes, were treated with 2mM ascorbate or 15mM metronidazole or both in media rendered hypoxic by passing 5% CO_2/95% N_2 over the magnetically stirred solutions. The cells were exposed to the treatments for 3 hours during which they were kept in an anaerobic jar, in which the air was replaced by 5% CO_2/95% N_2, and incubated at 37°C. The media were then substituted with normal aerobic medium and the CO_2/N_2 atmosphere replaced with 5% CO_2/95% air. The cells were photographed and counted 15 hours later. Cells treated with either ascorbate or metronidazole alone exhibited a normal epithelial-like morphology and were similar in number to the untreated control group. Cells treated with both ascorbate and metronidazole were rounded in shape and showed a 40% reduction in number when compared with the untreated control group. Sodium ascorbate rapidly removes dissolved oxygen from a solution and in a 1mM solution, the oxygen tension is reduced to zero in approximately 20 minutes. Although the experiments were carried out in as near to anaerobic conditions as possible, it seemed likely that the ascorbate could be removing any traces of oxygen left in solution and thus increasing the hypoxic toxicity of metronidazole. To test this hypothesis, one group of cells was treated with glucose oxidase and catalase, a combination which results in removal of oxygen with the production of water. This had no effect on the toxicity of metronidazole suggesting that sodium ascorbate acts in some other way.

A similar interaction has been reported between misonidazole and sodium ascorbate (Josephy, Palcic & Skarkgard, 1978) and the enhanced misonidazole toxicity was attributed to its reduction to more toxic species. A similar effect may be occurring with metronidazole. While it is possible that ascorbate treatment could increase the effectiveness of metronidazole as a tumour radiosensitiser, high dose ascorbate therapy might compound the side effects of metronidazole treatment.

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