EFFECTS OF SODIUM ASCORBATE AND COPPER IONS ON SOME CULTURED CELLS

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In the course of investigating fundamental differences between normal and malignant cells we have previously reported a greater sensitivity of the latter to sodium ascorbate, and have suggested that this might be caused by differences in catalase levels (Cope 1978). Also, Bram et al (1980) reported ascorbate to be preferentially toxic to melanoma cells compared with various other malignant and normal cells. They suggested this was caused by the high copper content of the melanoma cells. There are many references to the instability of ascorbic acid in the presence of copper (Martindale 1977). We now report that copper increases the already high toxicity of ascorbate to a malignant cell line while having little effect on its normal counterpart. A possible mechanism for this is discussed.

The cells used were 3T3 cells, a fibroblastic cell of mouse origin, and SV_{40}-3T3 cells, the same cells malignantly transformed by Simian Virus 40, i.e. a matched pair of cells differing only in transformation and a truer basis for comparison than random tumour material. The cells were seeded out at 5×10^4/ml into Petri dishes and treated 3 hours later with 1mM sodium ascorbate or 5μM copper sulphate or both or both + catalase(0.2μM). Eight Petri dishes of each were set up. After 2 days the cells were counted by Coulter Counter, counting 3 samples per dish. Only in the case of the SV_{40}-3T3 cells was the ascorbate toxicity augmented by the copper sulphate, which at the concentration used was not toxic per se. Catalase protected both cell types from the toxicity of both ascorbate and ascorbate + copper. The experiment was repeated using five days' contact time instead of two. The 3T3 cells were now unaffected by any of the treatments but the SV_{40}-3T3 cells treated with ascorbate + copper showed no significant recovery compared with the 2 day contact time. Thus the effect on the normal cells was less and was reversible while the effect on the transformed cells was greater and was not reversed. Also since catalase protected both cell types from ascorbate and ascorbate + copper, hydrogen peroxide must play a key role in ascorbate toxicity.

Hydrogen peroxide (60μM) produced similar effects to ascorbate (1mM) (Cope 1978). However in virus work Peloux et al (1962) claimed that ascorbate + copper did not produce enough hydrogen peroxide to account for their viricidal effects and that these latter were caused by free hydroxyl radical formation. Nevertheless one effect of hydroxyl radicals generated by ascorbate + copper is to inhibit catalase (Orr 1967). A possible mechanism for the selective toxicity of ascorbate + copper to SV_{40}-3T3 cells would be therefore that an already low level of catalase is readily inhibited by free hydroxyl radicals, and hydrogen peroxide, formed by the auto-oxidation of ascorbate, becomes therefore extremely toxic to these cells. Beneficial effects of ascorbic acid have been reported, in some cancer patients but not in others (Cameron & Campbell 1974). Thus if there were different levels of catalase and copper in a patient's tumour, then that patient might benefit from ascorbate treatment.

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