Ribonucleotide Reductase Overproduction in Hydroxyurea Resistant Mouse Cells

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Hydroxyurea is a specific inhibitor of DNA synthesis. The intracellular target is the enzyme ribonucleotide reductase which provides the cell with a balanced supply of the four deoxyribonucleotides.[1] Altered ribonucleotide reductase activities have been shown in mammalian cells selected for resistance to hydroxyurea.[2] The levels of ribonucleotide reductase activity were studied in hydroxyurea resistant 3T6 mouse fibroblast subcultures which were grown in 1 mM drug concentration.

Ribonucleotide reductase activity (^{3}H-CDP reduction[3]) was measured in permeabilized cells. In contrast to cell extracts reductase activity in permeabilized cells was reported to increase linearly with increasing protein concentration even at low protein concentrations.[2] The method reported by Lewis *et al.*[2] was adapted to 3T6 cells.

Experimental. Cells (10^7 cells/ml buffer) were incubated at 37 °C in a permeabilizing buffer consisting of 1.0 % TWEEN-80, 0.25 M sucrose, 0.01 M 4-(2-hydroxyethyl)-1-piperazinesulfonic acid buffer, pH 7.5, and 2 mM dithiothreitol. With this treatment about 95 % of the cells were permeabilized after 50 min of incubation as judged by Trypan Blue uptake. Furthermore, the highest values of ribonucleotide reductase activity were obtained in cells after 50 min of permeabilization.

Results and discussion. It is known that the activity of ribonucleotide reductase varies during the cell cycle.[1] In the case of the 3T6 cells the reductase activity increased to a maximum two days after inoculation when 10^6 cells were plated on a 150 mm in diameter tissue culture plate. At this point about 50 % of the cells were in the S-phase. Both the activity of ribonucleotide reductase and the rate of DNA synthesis (as measured by [^{3}H]thymidine incorporation) decreased during further incubation.

Ribonucleotide reductase activity was 3 – 15 times higher in the hydroxyurea resistant cell sublines than the activity measured in the parental, hydroxyurea sensitive cells (Table 1). In all cases the activities were measured when about 50 % of the cells were in the S-phase.

Table 1. Comparison of ribonucleotide reductase activities in parental 3T6 cells and in two hydroxyurea resistant sublines.

Cell line	Ribonucleotide reductase activity (nmol dCDP/30′ × 10^7 cells)
Non resistant	0.3 – 1.0
Resistant 1 [a]	4.4
Resistant 2 [a]	3.1

[a] The resistant cell lines are two separate subcultures of 3T6 cells selected for resistance to 1 mM hydroxyurea.

Resistance to a drug can arise by a number of mechanisms, *e.g.* alterations in the structure of a target enzyme or changes in the intracellular levels of a drug-sensitive enzyme. To distinguish between these two alternatives the sensitivity of ribonucleotide reductase to hydroxyurea was measured in permeabilized hydroxyurea resistant cells and in the parental, sensitive cells. The reductase in resistant cells did not show any decreased *in vitro* sensitivity to hydroxyurea inhibition; enzyme activity both in parental and resistant cell lines was inhibited to 50 % at a hydroxyurea concentration of 0.25 mM.

The results indicate that the resistance to hydroxyurea shown by the 3T6 cells is due to an overproduction of the target enzyme, ribonucleotide reductase. This may be due to gene amplification as in the well-studied case of the methotrexate resistant mouse cells which overproduce dihydrofolate reductase.[4] The hydroxyurea resistant ribonucleotide reductase overproducing cell lines will be used in further studies of ribonucleotide reductase and DNA synthesis in mammalian cells.

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