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Short Communication

Selective Sensitivity of Malignant Cells to Adriamycin*

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Summary. *The relationship of Adriamycin sensitivity to oxygen utilization by the cell is unclear. We examined the oxygen uptake and Adriamycin sensitivity of seven human colon carcinoma cell lines under normal aerobic conditions. Adriamycin sensitivity was inversely related to oxygen consumption and lactate dehydrogenase-specific activity.*

Introduction

Adriamycin is thought to exert its predominant cytotoxic effects by intercalation into nuclear DNA. The drug's potent cardiotoxicity has restricted its usefulness as a chemotherapeutic agent and has led to studies on the non-nuclear effects of Adriamycin exposure. The mitochondria have been implicated as an additional target for Adriamycin toxicity due to reports of inhibited mitochondrial respiration and mitochondrial degeneration following drug treatment. It has been suggested that Adriamycin sensitivity is dependent upon oxygen utilization [4, 6, 7]. In this report the oxygen uptake of several malignant cell lines under normal aerated conditions was inversely correlated with their susceptibility to Adriamycin.

Materials and Methods

Cell Lines. Human colonic carcinoma cell lines, obtained from M. Brattain, were maintained in McCoy's enriched medium (Grand Island Biological Co., NY, USA) containing 10% fetal calf serum [2, 5].

Oxygen Uptake. Harvested cells were suspended in warm medium at a concentration of 2×10^6 cells/ml and oxygen uptake measured with an oxygen-measuring electrode for 20 min. Oxygen uptake was corrected for exogenous uptake by the media and was linear during the timed interval.

Drug Sensitivity. Harvested cells were suspended in 1 ml medium containing Adriamycin (Batch 776) at a concentration of 1×10^6 cells/ml. Cells were exposed to various concentrations of the drug for 1 h at 37° C in subdued light. Cells were subsequently cultured in fresh medium for 5 days then harvested and counted with hemocytometer chambers. The Adriamycin concentration (μM) which caused a 50% reduction in cell number (LD_{50}) was determined.

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Table 1. Summary of data[a]

Cell line	Oxygen uptake (nmoles)	Adriamycin LD_{50} (μM)	Predominant LDH isozymes	LDH specific activity (U/mg protein)
A	86	10	3, 4	8.9
B	78	9	3, 4, 5	11.0
C	70	3	3, 4, 5	8.5
D	54	0.2	2, 3, 4	7.8
E	56	1	2, 3	3.3
F	46	0.9	3, 4	5.8
G	38	0.6	2, 3	6.0

[a] Oxygen uptake was measured over a 20-min period. LD_{50}, concentration of Adriamycin required to reduce cell number by 50%. Isozymes comprising 50% of the enzymatic activity obtained by gel electrophoresis. Specific activity equals absorption units at 520 nm/mg protein

Lactate Dehydrogenase Activity. LDH activity was determined as previously described [2]. Protein content was measured by the technique of Hartree [3]. Specific activity is expressed as units of absorbance at 520 nm per mg protein. Isoenzymes were separated and stained as described by Acharya et al. [1]. Gels were scanned with a densitometer containing a strip-chart recorder. The area under each peak was calculated and the percentage of each isozyme determined.

Results

The cell lines demonstrated inherent differences in oxygen uptake which was independent of the degree of differentiation or the growth rate of the individual cell line. Cell lines which consumed less than 70 nmoles of oxygen demonstrated a 3- to 50-fold increase in Adriamycin sensitivity (Table 1). Predominant LDH isozymes did not correlate as expected with oxygen uptake. Cell lines with LDH activity less than 8 U/mg had decreased oxygen consumption and increased drug sensitivity relative to those with LDH activity greater than 8 U/mg.

Discussion

The cardiomyopathy induced by clinical exposure to Adriamycin has led to the suggestion that the drug affects a

secondary cellular site in addition to the nucleus. Modulation of mitochondrial function has been implicated because of reported abnormalities in mitochondrial morphology and respiration following Adriamycin treatment. The suggestion that Adriamycin cytotoxicity is dependent upon mitochondrial respiration has been typically explored by the determination of cellular sensitivity to the drug under aerobic and hypoxic conditions. Results have been contradictory [4, 6, 7]. We compared drug sensitivity to the oxygen uptake of seven malignant cell lines under aerobic conditions. Cell lines with an oxygen uptake exceeding 70 nmoles were less sensitive to Adriamycin than those with lower oxygen uptake values. The occurrence of 'muscle' LDH isozymes with the cell lines demonstrating the greatest oxygen uptake suggested that oxygen uptake may not be an accurate index for oxygen utilization by mitochondrial respiration. However, one must wonder whether the determination of oxygen uptake from primary tumor specimens could be clinically useful in the prediction of Adriamycin sensitivity.

Cardiac tissue, with its high respiratory quotient, is radically affected by clinical exposure to Adriamycin. The malignant cells used in this experimental system demonstrated an inverse relationship between oxygen uptake and drug sensitivity. Teicher et al. [7] suggested the existence of different mechanisms of Adriamycin cytotoxicity which were dependent on the presence or absence of oxygen. We suggest that the mechanisms of cellular sensitivity or resistance to Adriamycin may differ between normal and malignant tissue. Additionally, malignant cells may be heterogeneous with respect to the mechanisms of drug sensitivity or may possess different abilities for the conversion of Adriamycin to more or less toxic forms.

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