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EFFECTS OF ADRIAMYCIN ON SURFACE PROPERTIES OF SARCOMA 180 ASCITES CELLS

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Adriamycin is an important therapeutic agent in the treatment of a variety of neoplastic diseases. The biochemical mechanism of action of this agent, as well as for the related anthracycline antibiotic daunorubicin, has been equated with intercalation with DNA and consequent inhibition of DNA and RNA biosynthesis (1-7). While intercalative binding of the anthracycline antibiotics to DNA and blockade of DNA and/or RNA synthesis might well be sufficient to account for their cytotoxic action, Silvestrini et al. (8,9) and Kitaura et al. (10) reported significant mitotic inhibition by adriamycin and daunorubicin under conditions in which nucleic acid synthesis was unaffected. Similarly, we have observed in the present study essentially complete inhibition of the growth of Sarcoma 180 cells in culture by adriamycin with no decrease in the rate of incorporation of ^{3}H-thymidine or ^{3}H-uridine into nucleic acids. Furthermore, N-acetyldaunorubicin, which is a weak inhibitor of nucleic acid synthesis, has significant antimitotic activity (1).

Several investigators have reported that chromosomal aberrations were produced by adriamycin and daunorubicin (11-13), as well as extensive DNA fragmentation, at relatively low concentrations of drug (2). DNA breakage did not appear to be simply the result of the formation of a drug-DNA complex, since isolated DNA was not degraded when mixed in vitro with daunorubicin (14). Other effects have been noted for the anthracycline antibiotics that appear to be unrelated to their actions on the nucleic acids. Drug induced decreases in the respiration of isolated mitochondria (15) and of the coenzyme Q mediated enzyme activities succinoxidase and NADH oxidase (16) have been documented; however, relatively high concentrations of drug are required to produce these inhibitions. We have reported in abstract form (17), an adriamycin induced alteration of the concanavalin A (Con A) mediated agglutination of Sarcoma 180 ascites cells. This communication extends these findings and implicates cell surface phenomena in the growth inhibitory properties of adriamycin.

Sarcoma 180 ascites cells were removed from the peritoneal cavities of mice and incubated with adriamycin in Fischer's medium containing 10% horse serum for 2 to 3 hours. Under these conditions, the rate of agglutination of cells by Con A, as measured by the procedure of Hwang et al. (18), was enhanced 2- to 3-fold. ^{3}H-Acetyl Con A (New England Nuclear Corp., Sp. Act. 2 μCi/mg) binding to Sarcoma 180 cells was determined after exposure of cells to 7 x 10^{-5} M or 7 x 10^{-6} M adriamycin for 3 hours. This was accomplished by washing cells with Ca^{++}-Mg^{++} free phosphate buffered saline (18) and incubating untreated and adriamycin treated cells (1.6 x 10^{6} cells) in 0.25 ml of reaction mixture containing various concentrations of ^{3}H-Con A in Ca^{++}-Mg^{++} free phosphate buffered saline. After 0 to 30 min at 23° in a shaking water bath, the reactions were terminated by the addition of 5 ml of ice-cold Ca^{++}-Mg^{++} free phosphate buffered saline, and cells were collected by centrifugation, washed twice with 10 ml of Ca^{++}-Mg^{++} free phosphate buffered saline, dissolved in 0.3 ml of 0.4 N NaOH, and radioactivity was determined in a Packard scintillation spectrometer using a toluene-liquifluor based scintillation fluid.

Neither the rate nor the extent of binding of ^{3}H-Con A to Sarcoma 180 cells was affected by exposure to adriamycin, suggesting that the number of sites available for binding by the plant lectin and the rate of their occupancy by Con A were unaffected by the treatment with this agent. The adriamycin induced increase in the rate of cellular agglutination by Con A in the absence of effects by the anthracycline on the binding of the plant lectin suggests that the antineoplastic agent may be causing an acceleration of the clustering of the Con A occupied receptors, a phenomenon necessary for cellular agglutination. Thus, an action of adriamycin would appear to be at the membrane level affecting the movement of molecules in the membrane itself. In keeping with a possible effect of the anthracyclines on processes involved with both movement of cellular components and mitosis, Dano (19,20) has suggested that adriamycin and daunorubicin might interfere with the function of microtubules; this hypothesis was based upon the cross-resistance between the anthracycline antibiotics and the vinca alkaloids expressed by neoplastic cells.

To equate the actions of adriamycin on surface phenomena with its growth inhibitory capabilities, we have measured the effects of a range of concentrations on the growth of Sarcoma 180 cells in culture.

The results shown in Table 1 demonstrate that adriamycin at a concentration of 10^{-8}M was not inhibitory to the growth of Sarcoma 180 cells at either 24 or 72 hours after continuous exposure to this agent. Adriamycin, at a level of 10^{-7}M, however, caused a marked interference with the proliferation of these cells. Higher concentrations of this agent were even more inhibitory to the growth of Sarcoma 180 cells.

Table 1. Effects of Adriamycin on the Growth of Sarcoma 180 Cells*

Concentration of adriamycin (M)	Cells/ml (24 hours)	% Control growth	Cells/ml (72 hours)	% Control growth
0	2.6×10^4	100	2.5×10^5	100
10^{-8}	3.2×10^4	123	2.2×10^5	88
10^{-7}	1.5×10^4	58	4.3×10^4	17
10^{-6}	9.1×10^3	35	1.1×10^4	4.4
10^{-5}	7.8×10^3	30	4.5×10^3	1.8
10^{-4}	6.7×10^3	26	3.9×10^3	1.6

*Log phase Sarcoma 180 cells were incubated in the presence and absence of adriamycin in Fischer's medium supplemented with 10% horse serum at an initial concentration of 10^4 cells/ml. Tubes were incubated at 37° and at various times thereafter, the cell number was determined using a Model B Coulter Counter.

The rate of agglutination of Sarcoma 180 cells was measured after 24 hours of continuous exposure of cells in culture to adriamycin employing a micromodification of the method of Hwang et al. (18). This involved the addition of 0.25 ml of a cell suspension (10^7 cells/ml) and 0.05 ml of Con A (0.2 mg/ml) to a 0.5 ml microcuvet, as described earlier (18). The contents of the cuvet were gently mixed by inversion, the cuvet was placed into the heated (37°C) chamber of a Gilford recording spectrophotometer and the absorbancy at 546 nm was measured. Figure 1 shows that a concentration of 10^{-8}M adriamycin, which was not inhibitory to the growth of Sarcoma 180 cells under these conditions, did not alter the rate of agglutination by Con A; however, the growth inhibitory level of 10^{-7}M adriamycin caused an increase in the rate of plant lectin induced cellular agglutination. A higher concentration of the anthracycline (10^{-6}M), which was even more toxic to Sarcoma 180 cells, not only increased the rate of agglutination, but also shortened the time required for the agglutination process to begin.

The findings indicate a correspondence in the concentrations of adriamycin required for both cytotoxicity and effects on surface phenomena, suggesting that the changes in the cell surface induced by adriamycin may be involved, at least in part, in the antineoplastic actions of this drug.

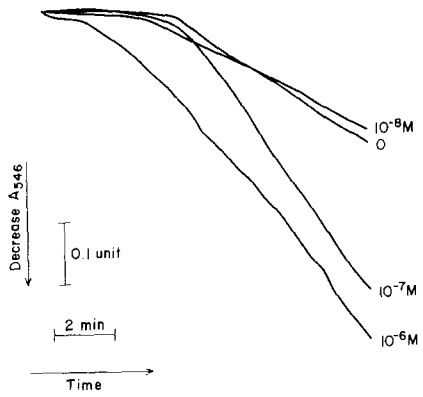

Fig. 1. The rate of agglutination of Sarcoma 180 cells by Concanavalin A (10 μg/0.3 ml) after exposure to various concentrations of adriamycin for 24 hours in culture.

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