Cell Surface Effects of a Phthalanilide Derivative

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Using the technique of cell electrophoresis to estimate surface charge density of mammalian tumor cells, a phthalanilide derivative (NSC 38280) caused a significant fall in cell electrophoretic mobility. These results suggest that the drug forms an irreversible complex with cell surface components, thereby lowering their zeta potential. The cell aggregation may account for the thromboembolism noted clinically.

A VARIETY of substituted phthalanilide derivatives possess antileukemic activity (1, 2). In view of the possibility that these compounds may form complexes with cell surface components, the present experiments were devised to study this phenomenon *in vitro*. The derivative used was the compound 2-chloro-4',4"-di(2-imidazolin-2-yl) terephthalanilide dihydrochloride (NSC 38280).[1]

The experimental system used was based on the sensitivity of cell electrophoresis to detect changes in charge density of cell surfaces following surface binding of drugs (3, 4). The cell electrophoresis equipment and technique have been described previously in detail (5). Briefly, the rectangular cell, maintained at 25°, was in the lateral position, and readings were taken at the first stationary level using a current of 4.0 ma. The phosphate-sucrose electrophoretic buffer had a relative viscosity of 1.14, pH 7.0, ionic strength 0.083, and osmolarity of 290 mosmoles. The tumor cells studied were Ehrlich ascites tumor cells, leukemic cells (buffy coat) from a patient with acute granulocytic leukemia, and cells from a patient with chronic lymphatic leukemia.

The washed cells were suspended in Hanks balanced salt solution containing NSC 38280 ($1.8 \times 10^{-4} M$, 100 mcg./ml.) for 30 minutes at room temperature; the cell concentration was 4×10^6/ml. The cells were then washed in phosphate-sucrose buffer and their electrophoretic mobilities determined. Control experiments, in the absence of NSC 38280, were run in parallel. Each experiment was repeated on three separate occasions. The results were highly reproducible, the figures in Table I represent the means of the experiments.

The results, summarized in Table I, show that exposure of tumor cells to NSC 38280 resulted in a highly significant ($P < .001$) drop in surface charge density. This finding suggests that the drug forms an irreversible complex with cell surface proteins and blocks surface anionic groups. In all instances, it is significant that tumor cells exposed to the drug underwent considerable aggregation presumably related to lowering of zeta potential. The mechanism of aggregation is probably complex, possibly initiated by London dispersion forces between small islands of adsorbed drug. These findings support the suggestion of York *et al.* (6) that phthalanilide-induced thromboembolism may be due to binding of the drug to cell surface components. The actual binding sites of the molecule include electrostatic forces of the imidazolin end-groups and hydrogen bonding at the amide bridges, although hydrophobic attractions have been suggested also (7).

During the course of these experiments an unexpected result emerged—namely, drug-treated red blood cells showed a slight increase in electrophoretic mobility: this effect was abolished if the red cells were pretreated with sialidase. At present the significance of these finding is obscure.

The possible relationship between NSC 38280 cytotoxicity and the cell surface effect is unknown. Phthalanilide derivatives have been shown to suppress protein synthesis (8), to complex with nucleic acids (7), and to be retained in leukemic ascitic fluid (9). In the present experiments a plausible explanation for the data may be some degree of denaturation of cell surface protein by the drug. It is of interest that another cytotoxic drug (chlorambucil) has been shown recently to affect cell surfaces (10).

TABLE I.—CELL SURFACE EFFECTS OF NSC 38280[a]

Tumor Specimen	Control u./sec./v./cm.	Control ESU/cm.$^{-2}$	NSC 38280 Treated u./sec./v./cm.	NSC 38280 Treated ESU/cm.$^{-2}$
Ehrlich's ascites tumor	1.33	2.66×10^3	1.05	2.10×10^3
Chronic lymphatic leukemia	1.45	2.90×10^3	1.18	2.36×10^3
Acute granulocytic leukemia	1.41	2.82×10^3	1.16	2.32×10^3

[a] Mean negative electrophoretic mobilities, corrected to viscosity of water at 25°, are expressed as u./sec./v./cm. Mobility values represent the average of 30 or more readings, with S.D.'s $\leq \pm 0.05$.

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