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USE OF PLANT LECTIN-INDUCED AGGLUTINATION TO DETECT ALTERATIONS IN SURFACE ARCHITECTURE OF SARCOMA 180 CAUSED BY ANTINEOPLASTIC AGENTS

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Considerable evidence exists suggesting differences in plasma membranes between normal cells and cells that have been subject to transformation by oncogenic viruses and chemical carcinogens (1,2). Little effort is being expended to determine whether (a) these differences can be exploited by chemotherapeutic attack and (b) existing clinically useful antineoplastic agents create, either directly or indirectly, changes in the plasma membrane of susceptible cancer cells. The plant lectins, which possess the capacity to bind to specific cell surface receptor sites and to cause agglutination, are important reagents to probe the surface architecture of eukaryotic cells. The utility of these agglutinins to assess: the structure, function and movement of the components of the cell surface of normal and neoplastic cells; the changes in constituents of plasma membranes that occur during the cell cycle, differentiation and transformation; the role of surface macromolecules in cell recognition phenomena; and the location of cellular transport sites, has been summarized (3). This communication reports the use of concanavalin A (Con A) and wheat germ agglutinin (WGA) to detect drug-induced alterations in the surface membrane of Sarcoma 180 ascites cells.

Tumor cells were isolated from the ascitic fluid of female CD-1 mice 7 or 8 days after the intraperitoneal injection of 6×10^6 neoplastic cells. The cells were washed 2 to 3 times with 10-15 volumes of 5 mM Tris-HCl (pH 7.2)-0.9% NaCl per ml of packed cells to remove contaminating erythrocytes. Cells were suspended in Fischer's medium + 10% horse serum at a concentration of $3\text{-}5 \times 10^6$ cells/ml. Antineoplastic agents were added to the cell suspension at various concentrations, and sealed reaction vessels were incubated at 37° in a gyrorotatory shaking water bath for various periods of time. Control Sarcoma 180 cells were treated identically in the absence of drugs. Following incubation, both untreated and drug-treated cells were washed with Ca^{++}, Mg^{++}-free phosphate buffered-0.9% NaCl, suspended in this solution at a concentration of 1×10^7 cells/ml, and agglutination using either Con A or WGA was performed as previously described (3). The methodology employed allowed an assessment of the rate and extent of cell agglutination by

Table 1. The Effect of Antineoplastic Agents on the Agglutination of Sarcoma 180 Ascites Cells by Concanavalin A (Con A) and Wheat Germ Agglutinin (WGA)

Antineoplastic agent	Concentration uM	Incubation time (h)	Plant lectin (ug/1.2ml)		Agglutination Process: Lag period (min)		Agglutination Process: Rate (ΔA_{546}/2 min)	
					Control	Treated	Control	Treated
6-Thioguanine	60	2	Con A	200	2.0	2.0	0.13	0.13
		7	Con A	200	2.5	4.7	0.13	0.08
Bleomycin*	50	2.5	Con A	120	2.5	2.2	0.54	0.52
			WGA	3.5	1.9	2.4	0.30	0.14
		7.5	Con A	120	2.4	3.1	0.35	0.17
			WGA	3.5	1.9	2.5	0.42	0.16
Chromomycin A_3	60	2	Con A	75	4.4	4.4	0.08	0.08
			WGA	2.5	3.1	3.1	0.05	0.05
		7	Con A	50	4.0	4.0	0.11	0.16
			WGA	2.5	3.1	2.5	0.05	0.10
Adriamycin+	7	2	Con A	150	2.2	1.5	0.14	0.20
	70	2	Con A	150	2.2	1.2	0.14	0.30
Methotrexate	60	2	Con A	45	2.2	2.0	0.16	0.24
			WGA	3.2	1.8	1.6	0.21	0.34
			WGA	6.0	1.6	1.4	0.58	0.74
1,3-Bis(2-chloroethyl-amine)-1-nitrosourea (BCNU)	60	2	Con A	100	2.4	2.0	0.40	0.47

Con A was employed in concentrations ranging from 35 to 300 ug/1.2 ml and WGA at levels of 2.5 to 6 ug/1.2 ml with each agent; for simplicity, only selected concentrations of agglutinin are listed.

+The stimulatory effect of adriamycin on agglutination of Sarcoma 180 ascites cells was reported in abstract form by Murphree et al. (11).

*Bleomycin was obtained from Bristol Laboratories in vials containing 15 units (equivalent to 8 mg) of a mixture of bleomycins with an average molecular weight of 1321.

two parameters: a characteristic lag phase preceding agglutination, created by initiating the process at 0° and allowing programmed warming to 37°, and a decrease in absorbance at 546 nm resulting from cell aggregation and sedimentation.

The effects of various chemotherapeutic agents on this process are summarized in Table 1. 6-Thioguanine and bleomycin inhibited the plant lectin-induced agglutination of Sarcoma 180 cells. The alteration of the agglutination process by the purine antimetabolite required relatively prolonged incubation (7 hr), suggesting that the effect was secondary to other cellular lesions; however, it is conceivable that the drug induced changes in the cell surface may be a factor in the expression of ultimate cytotoxicity by this agent. In contrast, changes in the plasma membrane caused by bleomycin were found as early as 2.5 hr after exposure to drug using WGA. Progressive changes in the cell surface induced by this antibiotic were detected by Con A, which agglutinated cells normally after 2.5 hr exposure to bleomycin, but at a decreased rate with cells exposed to this agent for 7 hr. In contrast, chromomycin A_3, adriamycin, BCNU and methotrexate enhanced the rate of plant lectin-induced agglutination of Sarcoma 180 ascites cells. The changes produced by chromomycin A_3 required incubation for 7 hr; whereas, those created by adriamycin and methotrexate occurred as early as 2 hr after exposure to drug. Differences in the apparent rate of agglutination of untreated cells in different experiments were the result of differences in the potencies of various batches of plant lectin and cellular preparations employed. Other agents tested and not listed in the table (i.e. arabinosyl cytosine, 5-fluorouracil and 5-fluorodeoxyuridine) did not significantly affect the rate and extent of agglutination of Sarcoma 180 cells by Con A under the conditions employed.

The present findings and those of other investigators (4-11) indicate that alterations in the plasma membrane, due either to direct or indirect action, may play a role in the cytotoxic mechanism of action of certain antineoplastic agents.

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