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Characterization of cell-surface alterations produced by NSC 208642 (Lymphosarcin)

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The poylene antibiotic Lymphosarcin, NSC 208642, has been found active* against a wide spectrum of experimental animal tumors, including the P388 lymphocytic leukemia, B16 melanoma, colon 38 and Danny Martin mammary carcinoma. The studies described here were designed to assess the effects of this agent on some cell-surface and membrane properties of the L1210 murine leukemia which, unlike P388, can readily be grown in culture. Properties of new antitumor agents are usually characterized with regard to inhibition of RNA, DNA and protein synthesis. We report here a different type of drug toxicity resulting from selective inhibition of certain membrane functions.

NSC 208642 was provided by the Drug Development Branch, National Cancer Institute; aqueous drug solutions were stored at −20°. Methods for maintenance of L1210 cells in culture, together with procedures for partitioning, transport and cell viability studies, have been described [1, 2]. Two-phase partitioning systems contained 5% (w/v) Dextran T500, lot 7839 (Pharmacia, Piscataway, NJ) and 3.6% polyethylene glycol (PEG), mol. wt 6000 (Pierce Chemical Co., Rockland, IL), as described by Walter [3]. A phosphate-rich mixture was made up in 70 mM NaCl + 60 mM NaH_2PO_4 at pH 7.0. A phosphate-poor mixture was made up in 140 mM NaCl + 10 mM NaH_2PO_4 at pH 7.0, and contained 1 μg/ml of PEG-palmitate (PEG-p) prepared as described in Ref. 4; 55–60 per cent of the total OH groups were esterified.

Suspensions of 7×10^6 cells/ml were treated with specified levels of drug for 10 min at 37°. To minimize drug–substrate interactions, the cells were suspended in fresh medium (5×10^6/ml) for further studies. Viability was determined, as described in Ref. 1, at 24- and 48-hr intervals after drug treatment; cell number was measured with a model Z_F Coulter Electronic Cell Counter. Accumulation of labeled actinomycin D and cycloleucine was measured over 5-min intervals at 37°; uridine accumulation was measured during a 1-min interval at 10° (to minimize subsequent incorporation of label into RNA). In one series of experiments, the non-metabolized nucleoside 5′-deoxyadenosine [5] was substituted for uridine, without altering any result. Partitioning studies were carried out in 10-ml portions (5 ml of each phase) containing 10^6 cells. After the phases had separated, the cell density in the upper phase was measured, and the partition coefficient was expressed as per cent of total cells found in the upper phase. When control cells were partitioned, 40 per cent were found in the upper phase of the phosphate-rich mixture and 10 per cent in the upper phase of the system containing PEG-p.

Transport data are reported in terms of per cent control rates; partitioning results are shown as per cent control values. In general, these data are reproducible to ± 10 per cent of indicated numbers.

A summary of the results is shown in Fig. 1, and is described below. Treatment of L1210 cells with NSC 208642 caused an initial increase in the number of cells partitioning into the upper phase of the phosphate-rich system (Ph), followed by a fall in partition coefficient at higher drug levels. In contrast, the number of cells partitioning into the upper phase of the low-phosphate mixture containing PEG-p increased with increasing drug concentration.

No inhibition of rate of uptake of cycloleucine or uridine was found at drug levels of 3 μg/ml or less; at higher drug concentrations, impaired uptake of both cycloleucine and uridine was not significantly different in this study. The rate of actinomycin D uptake was in-

*John Douros, National Cancer Institute, personal communication.

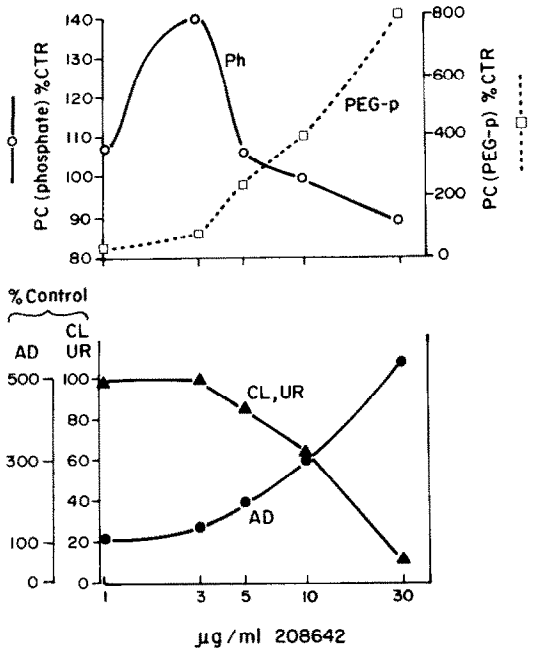

Fig. 1. Effect of graded levels of NSC 208642 on membrane properties of L1210 cells. Cells were exposed to specified levels of drug for 10 min at 37° and then suspended in fresh medium. The relative number of cells partitioning into the upper phase of a phosphate-rich (Ph) and a phosphate-poor two-phase system containing PEG-palmitate (PEG-p) is shown as per cent control values. The relative rates of uptake of actinomycin V.C. (AD) and of cycloleucine (CL) and uridine (UR), over 5-min intervals, are shown as per cent control rates. Since the experimental values obtained using the latter two substrates did not significantly differ, the data are shown as single points.

creased to 140 per cent of control at a drug level of 3 µg/ml, a concentration which did not affect uridine or cycloleucine transport, but did markedly promote partition of cells into the upper phase of the phosphate-rich system. Viability studies (not shown) indicated that cell death was correlated with amino acid and nucleoside transport inhibition. No effect was found at the 3 µg/ml drug level; cell viability was decreased by 50 per cent at 10 µg/ml and by >95 per cent at 30 µg/ml.

Treatment of L1210 cells with NSC 208642 is associated, therefore, with alterations in both biologic (transport) and biophysical (partitioning) properties of the cell membrane, and with a decrease in capacity for subsequent proliferation. These effects mimic the action of the photo-activated porphyrins [1], but we cannot conclude that similar mechanisms of action are involved. Evidence has been reported to indicate permeability alterations produced by other polyenes [6, 7], perhaps via interaction with phospholipid membrane bilayers [8]. In contrast, the photo-activated porphyrins are believed to exert their disruptive effects via protein cross-linking [9] or through oxidation of membrane components, e.g. cholesterol [10]. The major emphasis of this report is the detection of drug-induced alterations of membrane properties by sensitive test procedures.

The nature of the permeability barrier to uptake of actinomycin D is unknown. Selection for cells resistant to the drug generally results in mutants with altered membrane properties, usually containing elevated levels of cell-surface glycoprotein [11–15]. Disruption of the membrane with surface-active agents [15–17] promotes actinomycin D uptake. Therefore, we have used measurements of relative rates of actinomycin D permeation as an index of drug effects, presumably at the outer, glycoprotein-rich region of the membrane.

Deeper within the membrane is a lipid-rich region believed to contain specific transport systems involved in uptake of hydrophilic substrates, e.g. amino acids and nucleosides [18, 19]. Disorganization of this deeper membrane section would result, therefore, in inhibition of other transport processes. We cannot, at this point, distinguish between drug interactions with components of transport systems and the environment of such systems.

Other information is provided by partitioning studies. There is evidence that the net membrane charge is a major determinant of the partition coefficient of cells in the phosphate-rich system [3, 20]. We interpret the data shown in Fig. 1 to indicate a cell-surface alteration, induced by low drug levels, resulting in increased exposure of electro-negative groups to the environment. Unmasking of sialic acid or anionic phospholipid moieties may be involved. At higher drug levels, loss of such components or unmasking of cationic groups may occur.

A second partitioning system incorporates a 0.001% concentration of PEG-palmitate into a phosphate-poor mixture [21, 22]. The palmitate is believed to interact with hydrophobic membrane regions, bringing cells containing such regions into the upper phase of the system [23]. We interpret enhanced partition of cells into the PEG-rich upper phase to indicate increased cell-surface hydrophobicity, although there are alternative explanations involving unknown affinity relationships. The data shown in Fig. 1 are consistent with the hypothesis of a drug-induced increase in membrane sites with affinity for a palmitate residue; corroborative evidence to indicate enhanced membrane hydrophobicity has not yet been obtained.

Treatment of L1210 cells, therefore, has been shown to result in a progressive loss of cell viability, disruption of barriers to actinomycin D accumulation, and inhibition of transport of a model amino acid, and both a non-metabolized and a metabolized nucleoside. The drug also causes membrane perturbations resulting in altered cell behavior in two-phase systems designed to detect both charge-associated and "hydrophobic" membrane properties. Whether the apparent selective toxicity of NSC 208642 toward neoplastic cell types is related to the surface-active properties of the drug remains to be established.

Note added in proof: Contamination of NSC 208642 with Actinomycin D has been reported to us by the Division of Cancer Treatment, NCI. The level of contamination was not sufficient to account for any result reported in this communication.

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Effects of indomethacin administration on hepatic steroid and drug metabolism in male and female rats

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The broad spectrum of biological effects of prostaglandins has made them the subject of intensive investigation in many laboratories. Inhibitors of prostaglandin synthesis, such as indomethacin, represent powerful tools for studying the actions of prostaglandins [1, 2]. However, care must be taken when administering prostaglandin inhibitors to experimental animals because of their toxic effects at high doses [3, 4]. The liver, for example, has been shown to undergo degenerative changes as a result of long-term indomethacin administration to rats [3]. Nonetheless, relatively large amounts of indomethacin have been given to animals in attempts to determine the role of prostaglandins in hormone action [5–10] and in other types of physiological and pharmacological research. Since the liver is a major site of hormone metabolism, changes in hepatic function after indomethacin administration may complicate interpretation of changes in plasma hormone concentrations and/or hormone action. Furthermore, since hepatic microsomal enzymes are of principal importance in oxidative drug metabolism, effects of indomethacin on the liver might alter the half-life of, and consequently the response to, other substances simultaneously administered. For these reasons, it is important to know what effects, if any, indomethacin has on hepatic metabolism. The data presented in this report indicate that indomethacin administration can have profound effects on both drug and steroid metabolism by hepatic microsomal enzymes.

METHODS

Male and female Sprague–Dawley rats, 50 to 60-days-old, were obtained from Zivic–Miller Laboratories, Pittsburgh, PA, and maintained under standardized conditions of light (6:00 a.m. to 6:00 p.m.) and temperature (22°) on a diet of Purina Laboratory Chow and water *ad lib.* Indomethacin (5 mg/kg) was administered as an intraperitoneal injection in 0.1 M phosphate buffer (pH 8.0) twice a day for 1, 2 or 3 days. The start of treatment was staggered, permitting control and experimental animals in each group to be killed on the same day. All animals received the same number of injections of indomethacin or vehicle only, as appropriate.

Animals were killed by decapitation between 9:00 and 10:00 a.m. and livers were removed quickly and homogenized in cold 1.15% potassium chloride. Homogenates were centrifuged at 9000*g* for 20 min in a Sorvall refrigerated centrifuge. Aliquots of the supernatant fraction were removed for enzyme assays and the remainder was centrifuged at 105,000*g* for 60 min in a Beckman preparative ultracentrifuge. All steps in the preparation of microsomes were performed with the tissue kept at 0–4°. Microsomal pellets were resuspended immediately prior to use in 1.15% potassium chloride containing 0.05 M-Tris–HCl (pH 7.4) at a concentration of 3–4 mg protein/ml. Microsomal cytochrome P-450 was measured as described by Omura and Sato [11] and protein was measured by the method of Lowry *et al.* [12]. The demethylation of ethylmorphine and the hydroxylation of aniline were assayed as the rates of formation of formaldehyde [13] or *para*-aminophenol [14], respectively, by 0.5 ml liver 9000 *g* supernatant (equivalent to 200 mg/ml) incubated with glucose 6-phosphate (9.0 μmoles), $MgSO_4$ (24.2 μmoles), Tris–HCl (0.05 M, pH 7.4) and ethylmorphine–HCl (12 μmoles) or aniline–HCl (6 μmoles) in a final volume of 3.0 ml. Semicarbazide–HCl (25 μmoles) served as a trapping agent for formaldehyde produced from ethylmorphine.

Hepatic Δ^4-steroid hydrogenase activity was measured as described by Tompkins [15] using the 9000 *g* supernatant fraction as enzyme source and corticosterone as substrate. Hepatic supernatant fraction equivalent to 10 mg tissue was incubated with 150 ηmoles corticos-