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PRELIMINARY COMMUNICATIONS

INHIBITION OF URIDINE TRANSPORT THROUGH SARCOMA-180 CELL MEMBRANE BY ANTHRACYCLINE ANTIBIOTICS

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The anthracycline aminoglycoside antitumour antibiotics are active against certain animal and human neoplasm (1,2) by interacting with DNA (3-6). The mechanism of the antimitotic activity of these anthracyclines, based exclusively on interaction with DNA and subsequent inhibition of nucleic acid synthesis, has recently been questioned. A direct interaction of adriamycin with negatively charged phospholipid has been reported (7-9), and a number of observations have indicated a direct interaction of adriamycin with cell membrane (10) and the cytoskeleton (11). An alteration in the fluidity of the model liposome by adriamycin has also been reported (12). These group of antibiotics showed inhibition in protein synthesis in tumour cells (13) and relative degree of inhibition in RNA synthesis by these drugs could not be fully correlated with the DNA binding parameters (14). Recent studies with nogalamycin derivative, 7-con-O-methyl nogarol (7-OMEN), and daunomycin-conjugate clearly indicate that cytotoxicity of anthracyclines does not mediate only through nucleic acid synthesis (15,16). Plasma membrane has been suggested to be a possible target of these drugs (12,16). However, to our knowledge there is not report on the effect of these drugs on the transport of small precursor molecules across the plasma membrane. In this communication we present evidence to show that these drugs alter the active transport of small molecules through sarcoma-180 ascites tumour cell membrane.

5-^{3}H-Uridine (13,800mCi/m mole) was obtained from Bhabha Atomic Research Centre, India, Nogalamycin and 7-OMEN were kind gifts from Upjohn Company, USA. Adriamycin was a product of Farmitalia Research Laboratories, Italy. Other chemicals were of analytical grade. Sarcoma-180 cells were maintained in ascitic form and harvested according to the method described earlier (17).

To study the uptake of uridine into sarcoma-180 cells, the cells (4×10^{6} cells/ml) were incubated at 20°C with gentle shaking in Medium-199 with Earle's salt, pH-7.4 with ^{3}H-Uridine (0.15μCi/ml). After desired period of incubation, aliquots of 0.25ml each were filtered through millipore filters, followed by washing with 20ml of ice cold isotonic saline containing unlabelled uridine (50μM). The filters were finally dried and radioactivity was measured in liquid scintillation spectrometer using tolune based fluor. Zero minute incubation was always performed in each

experiment. Three aliquots were processed for each period of incubation; variability of uptake in each set was always within 5%. The results presented are mean of these three values.

The conversion of ^{3}H-Uridine to different nucleotides was measured according to the method described by Plagemann (18).

It is known that, at low temperature, cells take up nucleosides from medium and metabolise them to nucleotides, with a much reduced incorporation rate into nucleic acid (19,20). As shown in Fig.1, nogalamycin inhibit the uptake of ^{3}H-Uridine by sarcoma-180 cells. Under the experimental conditions described here uptake of ^{3}H-uridine by sarcoma-180 cells was almost linear upto one hour, and the incorporation of uridine into acid insoluble fraction was less than 5% of that into whole cell at 20°C. Similar results were also obtained with adriamycin. As there is a lag period before any inhibition in uridine uptake is apparent, subsequent experiments have been done by pre-incubating the cells with drugs for one hour prior to the addition of uridine. The effect of nogalamycin and

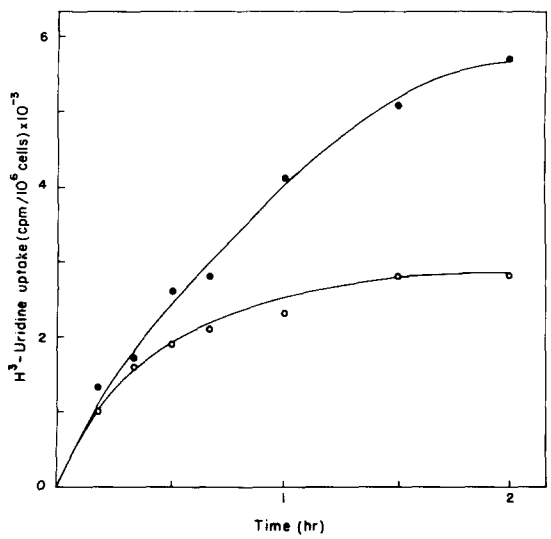

Fig. 1

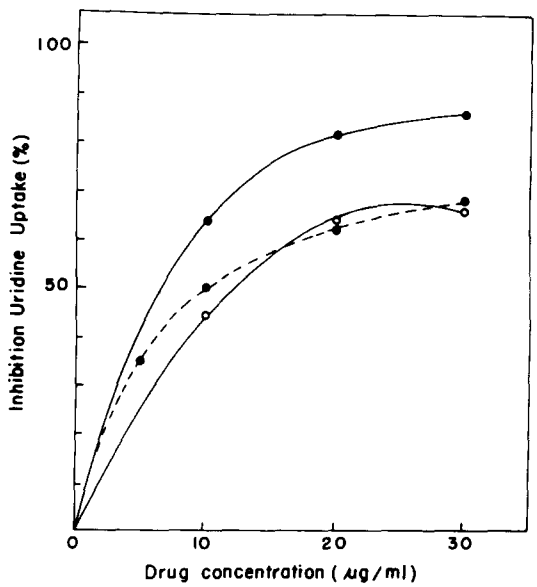

Fig. 2

Fig. 1. Uptake of ^{3}H-Uridine by sarcoma-180 ascites tumour cells : ●——● -control; O——O -Treated with nogalamycin (20µg/ml).

Fig. 2. Inhibition of ^{3}H-Uridine uptake by anthracycline antibiotics (a dose response study) : Sarcoma-180 cells were pre-incubated with the drugs for one hour prior to addition of ^{3}H-Uridine. The cells were incubated for 20 min. after addition of uridine. Inhibition of uridine uptake by Nogalamycin (●——●), Adriamycin (O——O) and 7-OMEN (●---●).

adriamycin on ^{3}H-Uridine uptake has been compared with 7-OMEN, a non-DNA binding antitumour anthracycline (Fig. 2). The data show that all the three antibiotics exhibit dose dependent inhibition in uridine uptake. It is apparent that the effect of nogalamycin is more pronounced than adriamycin and 7-OMEN, but the later two drugs show similar effect.

Acid soluble extracts from sarcoma-180 cells labelled with ^{3}H--Uridine at 20°C in presence and absence of nogalamycin were chromatographed to separate different uridine nucleotides. No difference in the relative distribution of radioactivity was observed among various nucleo-

tides between control and drug treated cells.

To study the mechanism of inhibition, the kinetics of ^{3}H-Uridine uptake by sarcoma-180 cells was studied in presence and absence of the drugs. A double reciprocal plot of these data, according to Lineweaver and Burke (21) suggested that the inhibition by nogalamycin and adriamycin was non-competitive type, having ki values of 32μm and 59μm respectively (Fig.3).

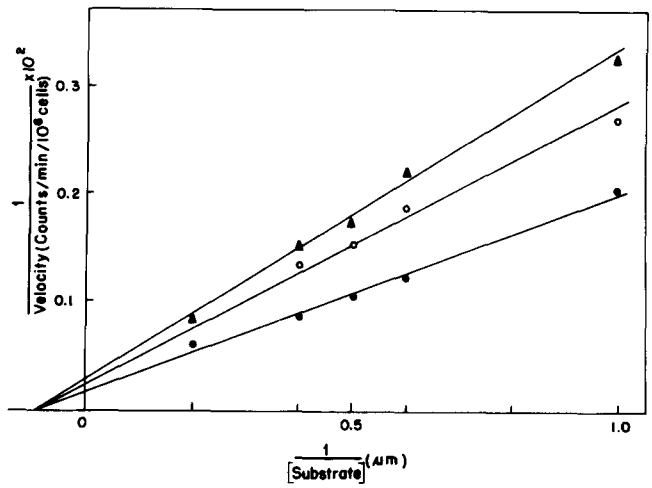

Fig. 3

Fig. 3. Kinetics of inhibition of ^{3}H-Uridine uptake by Nogalamycin (Lineweaver and Burke plot) : The cells were pre-incubated for one hour with nogalamycin followed by incubation for 20 min. after addition of varying concentrations of ^{3}H-Uridine. ●——● -Control; O——O - nogalamycin (10μg/ml) and ▲——▲ -nogalamycin (20μg/ml).

Mammalian cells are thought to take up nucleosides from the environment both by simple diffusion and a carrier-mediated transport system (18,19). It is reported that simple diffusion is significant only at relatively high concentrations of nucleosides, while at low concentrations carrier-mediated transport is the main pathway (18-20,22-24). At the concentration range used in our studies uridine is expected to enter the cells mainly through carrier-mediated transport, which is also evident from straight lines in Lineweaver-Burke plot (Fig.3). This plot also indicate a non-competitive inhibition of uridine transport by both nogalamycin and adriamycin. Therefore, these drugs seem to act by binding at a site different from uridine binding site (active site) of the carrier protein. It has recently been reported that adriamycin can bring about a long range disorder in the plasma membrane structure by binding with its lipid components (25,26). Conformational changes in the membrane proteins induced by drugs are known to inhibit physiologically significant functions of erythrocyte membranes, such as glucose and ion transport (27-29). We also observed inhibition in 2-deoxy glucose uptake by sarcoma-180 cells in presence of nogalamycin and adriamycin.

Finally, we suggest that though DNA-intercalation and subsequent inhibition of transcription is the primary mode of action of these antibiotics, the cellular toxicity is partly contributed by altering the transport properties of cellular plasma membrane.

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