Inhibition of Nucleoside Incorporation into HeLa Cells by Streptovaricin

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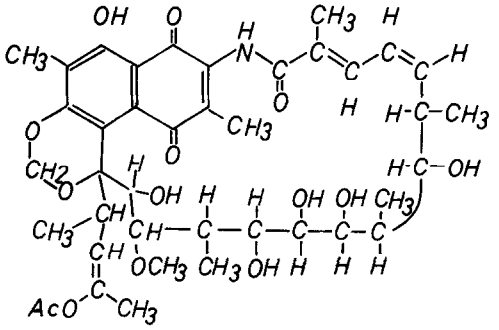
The ansamycin streptovaricin D selectively inhibits incorporation of nucleoside into HeLa cells. This inhibition is rapid and maximal and probably reflects inhibition of transport of nucleoside into cells.

The ansamycin complex streptovaricin consists of a mixture of seven streptovaricins (assigned the letters A through G). The structure of streptovaricin D was provided by Dr. K.L. Rinehart, University of Illinois (Fig. 1). In our investigations on the inhibition of virus replication by streptovaricins, we found that some streptovaricins inhibit the incorporation of uridine into cellular RNA and viral mRNA (Tan and McAuslan, 1970). This is in part due to a streptovaricin depression of uridine transport into the cell. This communications presents data on the effect of streptovaricins on the incorporation of uridine, adenosine and thymidine into HeLa cells.



STREPTOVARICIN D

Fig. 1. Structure of streptovaricin D.

MATERIALS AND METHODS

HeLa S_3 cells were grown in suspension at 37° at a concentration of 5 x 10^5 cells/ml in Eagle's medium supplemented with 5% heated fetal calf serum.

The acid soluble and acid insoluble fractions of cells incubated in the presence of ^{3}H adenosine (sp. activity 5-15 C/mM, New England Nuclear), ^{3}H uridine (sp. activity > 20 C/mM, New England Nuclear) or ^{3}H thymidine (sp. activity 6.7 C/mM, New England Nuclear), were obtained as follows: cell sample (5 ml) was chilled with an equal volume of ice cold PBS and immediately centrifuged (800 x g/3 mins/0^{o}). The cell pellet was suspended in 1 ml 5% TCA and kept in ice for 15 mins., then centrifuged (2000 x g/10 min./0^{o}). A 100 µl aliquot of the clear supernatant (acid soluble fraction) was mixed with 10 ml of a dioxan base scintillation fluid (Butler, 1961) and radioactivity determined in a Beckman LS-250 Liquid scintillation counter. The acid insoluble pellet was washed twice with cold 5% TCA, centrifuged, dissolved in 1 ml 0.5 N NaOH and a 100 µl aliquot taken for radioactivity determination as described above. Quenching caused by NaOH was corrected for in all cases.

Streptovaricins and rifampicin were obtained from Dr. K.L. Rinehart, University of Illinois and Dr. J. Gelzer, Ciba Pharmaceutical Co., respectively. Both antibiotics were dissolved in a small volume of dimethyl sulfoxide and then diluted with water to yield stock solutions of 1 mg/ml which were diluted to the final concentrations desired. Dimethyl sulfoxide at this final concentration had no effect on nucleoside incorporation by cells.

Table I. Inhibition of ^{3}H uridine incorporation into HeLa RNA in the presence of streptovaricin*

RNA	Streptovaricin Complex	A	B	D	F
45 S	75	5	3	39	0
4 S	69	9	12	44	36

* Results presented as % inhibition of ^{3}H uridine incorporation into RNA determined by the methods described by Tan and McAuslan, 1970.

RESULTS

Effect of Streptovaricins on Nucleoside Incorporation

The effects of purified streptovaricin species on the incorporation of ^{3}H uridine into HeLa RNA are shown in Table I. Both the streptovaricin complex and streptovaricin D markedly inhibited incorporation of uridine. Streptovaricin D (strep. D) was selected for further investigation on the incorporation of nucleosides into HeLa cells.

The effects of various concentrations of strep. D on incorporation of nucleosides is shown in Fig. 2. The incorporation of nucleoside into both acid soluble and acid insoluble fractions was markedly reduced with increasing concentrations of streptovaricin. Incorporation of adenosine and uridine into the acid soluble materials was inhibited to the same extent (50% inhibition at 16 μg strep. D/ml) whereas thymidine incorporation was less inhibited (50% inhibition at 34 μg strep. D/ml). We have previously shown that both streptovaricin A and rifampicin had no significant effect on RNA or DNA synthesis (McAuslan, 1969; Tan and McAuslan, 1970).

Incorporation of nucleosides into cells in the presence of various concentrations of streptovaricin A or rifampicin was investigated as described

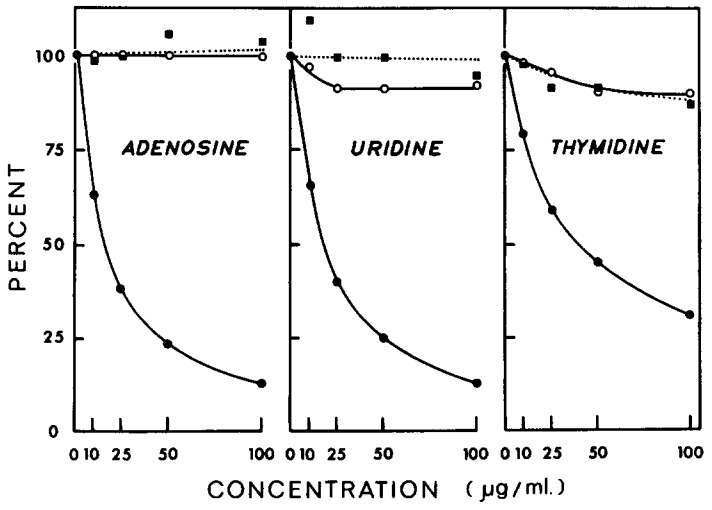


Fig. 2. Cells incubated with different concentrations of strep. D (●—●), Strep A (o—o) or rifampicin (■—■) for 30 min. were pulsed 15 min. with 1 μc ^{3}H nucleoside/ml. Radioactivity in the acid soluble fraction was determined and expressed as % of that of cells receiving no antibiotics.

above. The results, presented in Fig. 2 for comparison with strep. D show that neither streptovaricin A nor rifampicin caused any significant inhibition of nucleoside incorporation.

Time Course of Nucleoside Incorporation: The time course of nucleoside incorporation into the acid soluble fraction of strep. D (10 μg/ml) treated cells was determined by processing samples at various times after nucleoside addition. The observed rate of nucleoside incorporation (Fig. 3) was depressed by strep. D. Maximum inhibition was observed at the earliest time sample taken and this inhibition remained unchanged for up to at least 8 hours (unpublished data). Thymidine incorporation was inhibited 25 - 40%, adenosine 50 - 60%, and uridine 40 - 50% by 10 μg strep. D/ml. Aliquots of samples taken at each time point in the experiment depicted in Fig. 3 were

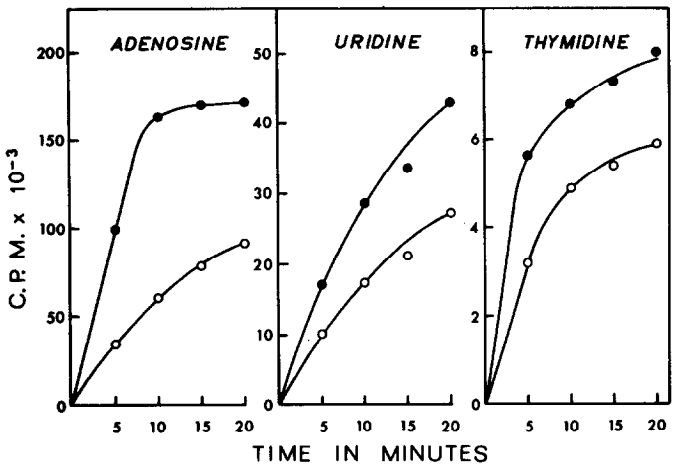


Fig. 3. Cells not treated (●—●) or treated(o—o) with 10 μg strep. D/ml for 30 min. were incubated with 1 μc ^{3}H nucleoside/ml. At indicated times after nucleoside addition, acid soluble radioactivity was determined.

subjected to chromatography on DEAE cellulose paper (Morrison, 1968) to separate the nucleoside phosphates. Tri-, di- and monophosphates of all 3 nucleosides were formed; the depressed rate probably reflects the streptovaricin depression of nucleoside uptake (Fig. 4).

Rapidity of Inhibition of Nucleoside Incorporation: The rapidity of inhibition of nucleoside incorporation by strep. D (10 μg/ml) was investigated by pulse labeling cells at various times after streptovaricin addition. In-

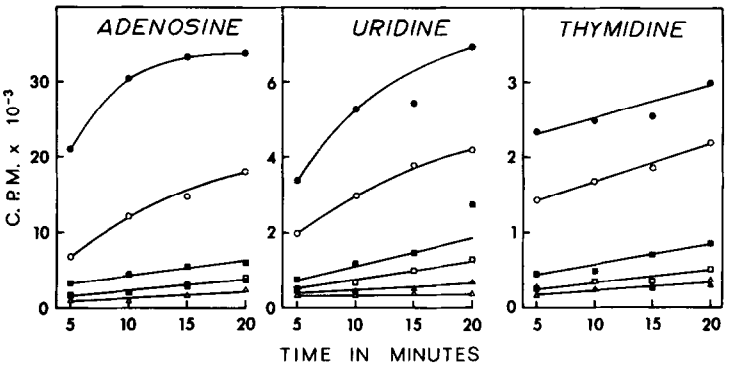


Fig. 4. Incorporation of nucleosides into nucleoside phosphates in the acid soluble fraction of cells treated with 10 µg strep.D/ml (o = tri-, □ = di- and Δ = monophosphate) or in untreated cells (● = tri-, ■ = di- and ▲ = monophosphate). For details see Fig. 3.

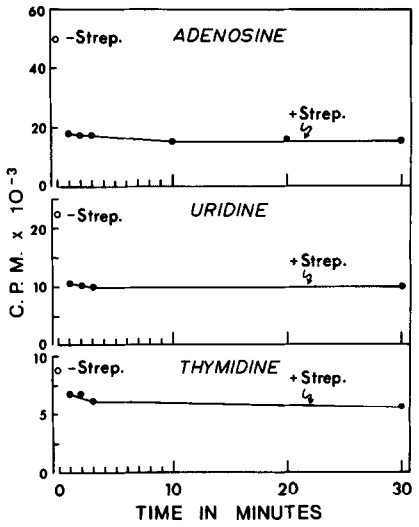


Fig. 5. At indicated times after strep. D addition (10 µg/ml), cells were pulsed 5 min. with 1 µc ^{3}H nucleoside/ml and acid soluble radioactivity determined.

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hibition of nucleoside uptake was immediate and maximal (Fig. 5.). About the same degree of inhibition was obtained with 1 min., 30 min. or longer treatment with strep. D. A further refined experiment using very brief pulses (20 secs) of ^{3}H uridine shows that maximum inhibition of uridine incorporation was observed when both uridine and strep. D were simultaneously added or when uridine was added immediately after streptovaricin addition.

Selective Inhibition of Nucleoside Incorporation by Streptovaricin D:

Strep. D at 10 μg/ml inhibited the incorporation of uridine more severely than that of thymidine (Fig. 2). This difference in the degree of inhibition may reflect differences in specific activities of the nucleosides (thymidine 1 μc/0.036 μg and uridine 1 μc/0.009 μg). However, this is unlikely because decreasing the specific activity of the ^{3}H uridine used to 1 μc/0.3 μg did not alter the degree of inhibition by strep. D (Fig. 6). Thus strep. D selectively inhibits uridine uptake when compared with thymidine uptake.

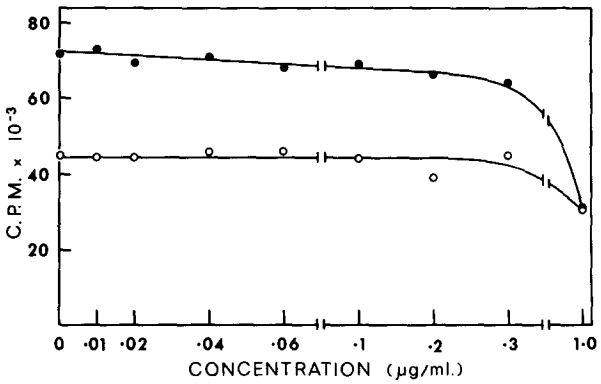

Fig. 6. Cells not treated (●—●) or treated with 10 μg strep. D/ml (o—o) for 30 min. were pulsed 15 min. with 1 μc ^{3}H uridine/ml in the presence of different concentrations of unlabeled uridine. Acid soluble radioactivity was determined as described in methods.

DISCUSSION

Our results demonstrate that inhibition of nucleoside incorporation into cells by strep. D occurs rapidly; the degree of inhibition is not a function of time. This inhibition of nucleoside incorporation is competitive and probably affects the active transport of nucleosides into cells as is suggested by the speed of inhibition. Alternatively, strep. D inhibits phosphorylation of nucleosides and thus their incorporation into cells. Phosphorylation of nucleosides at the cell surface has been suggested as a mechanism for uptake of nucleosides (Piatigorsky and Whiteley, 1965).

Streptovaricin is a reversible inhibitor of nucleoside incorporation, reversal of inhibition being complete and rapid (Tan and McAuslan, 1970). The

rapidity and reversibility of inhibition suggest that streptovaricin is closely but loosely associated with the cell membrane. Inhibition of nucleoside incorporation is probably not a result of complexing of nucleoside to streptovaricin (Rinehart, personal communications). Amino acid incorporation into cells was not affected by streptovaricin (Tan and McAuslan, unpublished data). Selective inhibition of incorporation of substrate has also been reported for acridines with a substituted amino group (Scholtissek and Becht, 1966) which inhibited cellular uptake of nucleosides but not amino acids, actinomycin or cycloheximide. In addition, streptovaricin shows specificity of structure, streptovaricin D is a potent inhibitor but streptovaricins A and B are not. In view of the findings that streptovaricin (Tan and McAuslan, 1970; present communication), some benzimidazoles (Bucknall, 1967; Skehel et al. 1967) and some acridines (Scholtissek and Becht, 1966) are reversible inhibitors of nucleoside incorporation into cells, caution should be exercised in interpreting data in which isotopic nucleosides are used to monitor nucleic acid synthesis in the presence of inhibitors.

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