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Preferential incorporation of sangivamycin into ribonucleic acid in Sarcoma 180 cells *in vitro**

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Sangivamycin is a pyrrolopyrimidine antibiotic with antitumor activity against a variety of experimental tumors†. As an adenosine analog, it undergoes phosphorylation [1] and is incorporated into RNA and DNA of normal tissues [2]. Sangivamycin has a variety of effects on nucleic acid synthesis such as inhibition of nuclear RNA synthesis [3], *de novo* purine synthesis [4], tRNA acylation *in vitro* [5], and *Escherichia coli* RNA polymerase activity *in vitro* [6]. However, none of these studies attempted to correlate the cytotoxicity produced by sangivamycin with a specific biochemical lesion. In a previous study, we reported that sangivamycin produced a pronounced effect on the viability of Sarcoma 180 cells in culture which was highly dependent on the duration of drug exposure [7]. Incorporation of [^{3}H]thymidine or [^{3}H]uridine into nucleic acids was proportionally reduced, although DNA synthesis was inhibited more rapidly than RNA synthesis [7]. Flow cytometry indicated a time-dependent accumulation of cells in the late S and G_2M region of the DNA histogram [7]. In this communication, we report that [^{3}H]sangivamycin is preferentially incorporated into cellular RNA in log and plateau phase Sarcoma 180 cells. Moreover, the incorporation of this drug into total poly(A)RNA shows the same time-dependency as its effects on cell viability and inhibition of nucleic acid synthesis.

Sangivamycin was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD. [^{3}H]Sangivamycin was prepared from [^{3}H]toyocamycin by hydrolysis in 2 N HCl at 100° for 4 hr [8]. Following lyophilization, [^{3}H]sangivamycin was purified to a specific activity of 485 mCi/mole by high performance liquid chromatography using a Partisil SCX column and isocratic elution with 25 mM KH_2PO_4 (pH 3.65)–5% acetonitrile at a flow rate of 1 ml/min. By this procedure, toyocamycin eluted in 5 min and sangivamycin eluted in 9 min.

Sarcoma 180 cells were grown in Earle's medium 199 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin [7]. Replicate flasks of 2 day log phase cells and 4 day early plateau phase cells were incubated with 1×10^{-6} M [^{3}H]sangivamycin (485 mCi/mmole) for 1, 3 and 6 hr. At the end of this period, 2–4 × 10^7 cells from pooled flasks were rinsed three times with Hanks' balanced salt solution, harvested by trypsinization, washed once in phosphate-buffered saline, and frozen immediately in dry ice. Total nucleic acids were extracted with 3 ml of 1% sodium dodecylsulfate–0.1 M Tris–HCl (pH 8.0)–0.01 M EDTA and mixed with 1.5 ml of phenol mixture (phenol–cresol–water), 7:2:2, by vol containing 0.1% 8-hydroxyquinoline) and 1.5 ml of chloroform. The emulsion was separated by centrifugation at 10,000 *g*, and the aqueous phase containing DNA and RNA was precipitated with 3 vol, of 2% potassium acetate in 95% ethanol at −20° overnight. Samples were centrifuged at 10,000 *g* for 20 min, and the precipitate was washed once with 2% potassium acetate in 95% ethanol. The pellet was dissolved in 0.2 ml water and divided into two equal samples. To obtain DNA, a 0.1-ml sample was adjusted to 0.01 M Tris–HCl (pH 7.4)–0.2 M NaCl–0.01 M EDTA and incubated with 20 μg RNase A and 20 units of RNase T_1 for 2 hr at 37°. To obtain RNA, 0.1-ml sample was adjusted to 0.01 M Tris–HCl (pH 7.2)–0.5 M NaCl–0.01 M $MgCl_2$ and incubated with 10 μg DNase for 2 hr at 37°. Each sample was then precipitated with 3 vol. of 2% potassium acetate in 95% ethanol at −20° overnight. To obtain non-poly(A)- and poly(A)RNA, total RNA was fractionated by poly(U)Sepharose chromatography as previously described [9]. All experiments were repeated three times, and the reported results are the means ± S.E. of replicate studies at each time point.

The time-dependent incorporation of 1×10^{-6} M [^{3}H]sangivamycin into total cellular DNA and RNA of log phase and plateau phase Sarcoma 180 cells is shown in Fig. 1. The rate of incorporation of [^{3}H]sangivamycin into RNA and DNA was three to four times greater in log phase cells than in plateau phase cells. Under both conditions of growth, approximately ten times more drug was incorporated into RNA than DNA.

To determine if sangivamycin was preferentially incorporated into a particular class of RNA, total cellular RNA was fractionated into non-poly(A)RNA (rRNA and tRNA) and poly(A)RNA (mRNA) by poly(U)Sepharose chromatography (Fig. 2). Incorporation of drug into non-poly(A)RNA was about six times greater in log phase cells than in plateau phase cells during the initial 3 hr of drug

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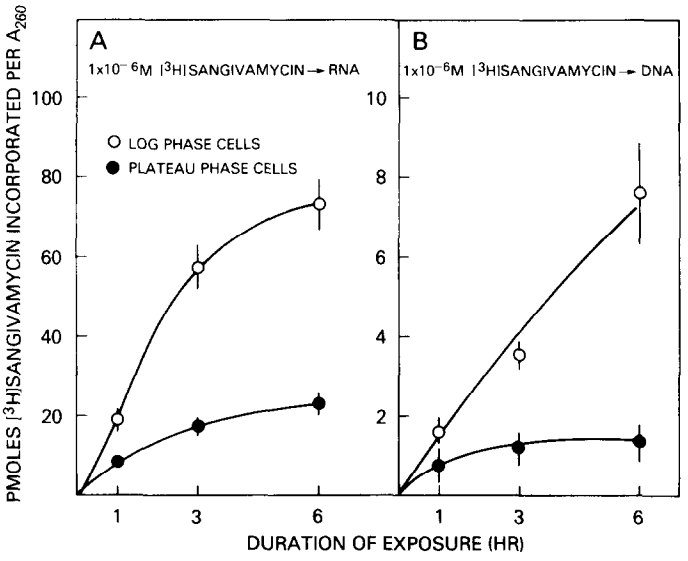

Fig. 1. Incorporation of [^{3}H]sangivamycin into RNA and DNA of Sarcoma 180 cells.

exposure (Fig. 2A); however, at 6 hr of drug exposure, log phase cells incorporated only 3-fold more drug than plateau phase cells. In contrast, log phase cells progressively incorporated 6-, 11- and 14-fold more [^{3}H]sangivamycin into poly(A)RNA than plateau phase cells after 1-, 3- and 6-hr intervals of drug exposure respectively (Fig. 2B).

Previously, we found that, under identical experimental conditions, exposure of Sarcoma 180 cells to 1×10^{-6} M sangivamycin for 1 and 6 hr reduced cell viability (as determined by soft agar cloning) by 40 and 92%, respectively, in log phase cells, and by 35 and 55%, respectively, in plateau phase cells [7]. In log phase cells following 1 and 6 hr of exposure to 1×10^{-6} M sangivamycin, RNA synthesis was reduced by 17 and 58%, respectively, while DNA synthesis was inhibited 11 and 78%, respectively. Thus, the progressive cell lethality produced by 1×10^{-6} M sangivamycin correlated more closely with inhibition of DNA synthesis. That the latter effect may be RNA-dependent is suggested in the present experiments by the 10-fold greater level of drug incorporation into RNA than DNA, and the 3- to 4-fold greater amount of drug incorporated into DNA and RNA in log phase cells than in plateau phase cells.

We have shown previously that log phase cells are more susceptible to the lethal effects of sangivamycin than plateau phase cells, and that this differential sensitivity is more pronounced at prolonged intervals of drug exposure. Our present studies demonstrate that the time-dependent incor-

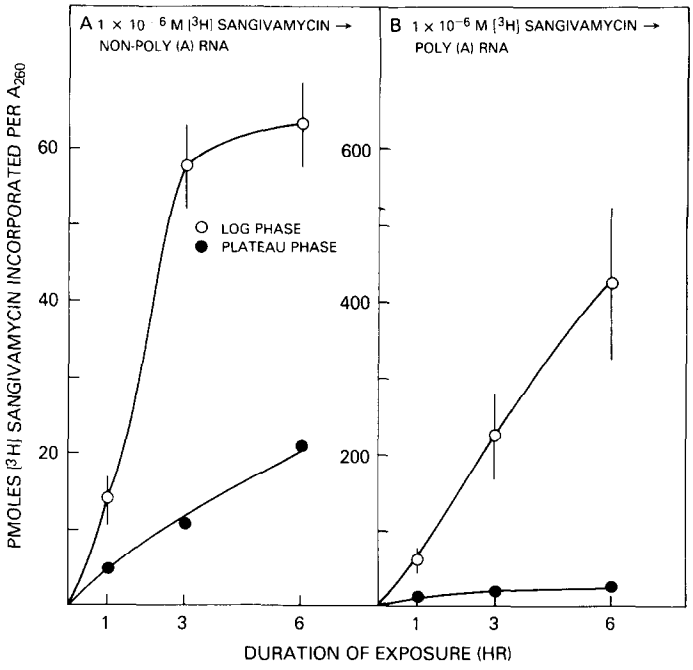

Fig. 2. Incorporation of [^{3}H]sangivamycin into non-poly(A)- and poly(A)RNA of Sarcoma 180 cells.

poration of sangivamycin into poly(A)RNA is also more pronounced in log phase cells, i.e. the proliferation rate-dependence of sangivamycin incorporation into poly(A)RNA increases with the duration of drug exposure and closely reflects the lethality characteristics of the drug. These data suggest that incorporation of drug into poly(A)RNA may be directly responsible for the effect of sangivamycin on DNA synthesis and cell viability, and is related to its antitumor activity.

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