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LANDOMYCIN A INHIBITS DNA SYNTHESIS AND G_1/S CELL CYCLE PROGRESSION

Robert T. Crow,* Betty Rosenbaum, Roger Smith, III, Yu Guo, Kenneth S. Ramos, and Gary A. Sulikowski*

Departments of Chemistry, Veterinary Pathobiology, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77842, U.S.A.

**Current Address: St. Louis College of Pharmacy, Division of Pharmaceutical Sciences, 4588 Parkview Place, St. Louis, MO 63110, U.S.A.*

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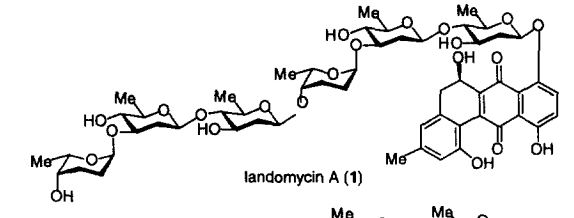
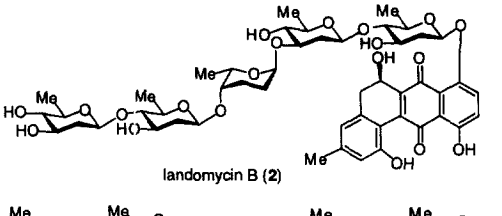
Abstract: Landomycin A was found to inhibit the uptake of [^{3}H]thymidine into DNA in murine smooth muscle cells indicating decreased DNA synthesis. Subsequent studies showed that landomycin A inhibits cell cycle progression.

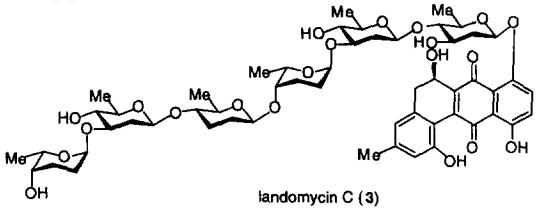
The landomycin antibiotics are a subgroup of the angucycline family of natural products that are of microbrial origin and number over one hundred.[1,2] Many of these quinone containing natural products possess antitumor activity, with landomycin A showing the most interesting spectrum of activity.[3,4] Structurally, landomycin A (**1**) is the largest member of the angucycline family of antibiotics with a molecular weight of 1,087. Landomycins B–E (**2–5**) differ from landomycin A (**1**) principally in oligosaccharide chain length.5 Interestingly, differences in the cytostatic activity of landomycins A–E have been noted, suggesting a dependence on the length of the oligosaccharide sidechain. This pattern of activity suggests the oligosaccharide may play a critical role in binding to a cellular target and/or in the regulation of bioavailability. The observation that landomycins A–E differ in cytotoxicity, their undefined mode of action and the potential for preparing structural analogs with reduced cytotoxicity has encouraged us to study the biological activity of landomycin A (**1**).



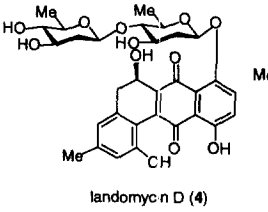
landomycin A (**1**)



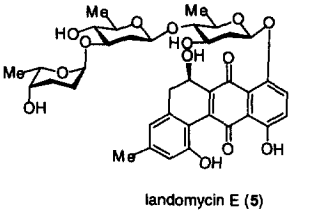
landomycin B (**2**)



landomycin C (**3**)



landomycin D (**4**)



landomycin E (**5**)

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Our first experiment was to evaluate the effect of landomycin A on DNA synthesis by examining its effect on the uptake of [^{3}H]thymidine into DNA using G_0-synchronized smooth muscle cells in culture.[6] Marked inhibition of cell cycle transit following mitogenic stimulation with 10% serum was observed at concentrations of 80 and 200 nM of landomycin A (Figure 1). The inhibition of thymidine uptake suggested either direct inhibition of DNA synthesis or interference of cellular processes critical to DNA synthesis. It should be noted that minimal effects were observed in the first twelve hours of incubation with landomycin A, which was independently verified by flow cytometry of both treated and untreated cells (data not shown). This pattern of inhibition should be expected since few cells enter the DNA synthesis phase of the cell cycle in the first twelve hours, regardless of the presence or absence of landomycin A.

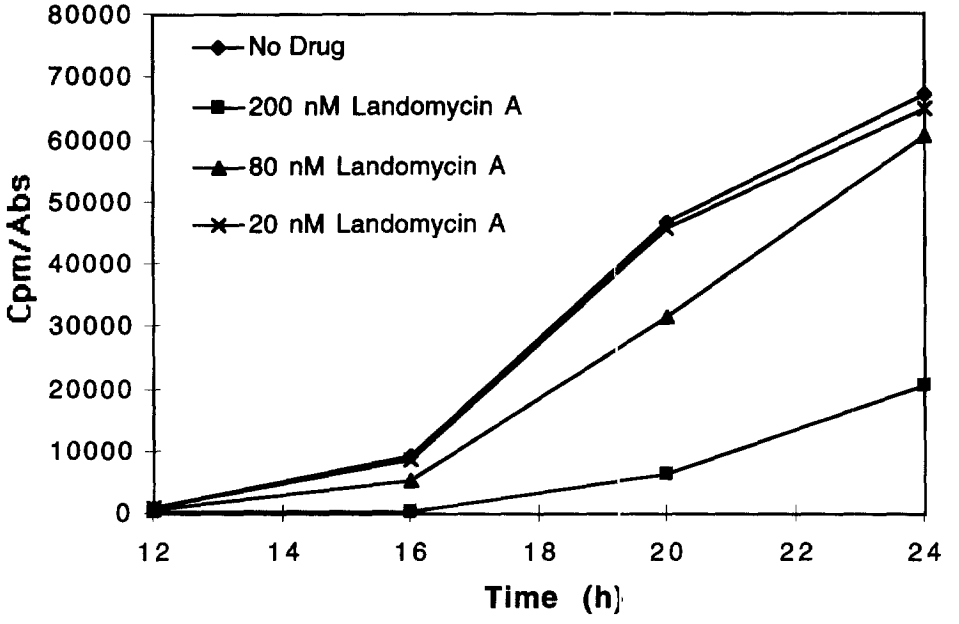

Figure 1. Inhibition of uptake of [^{3}H]thymidine by murine smooth muscle cells by landomycin A (**1**) at concentrations of 20, 80, and 200 nm.

A valuable approach to elucidating the mode of action of a cytotoxic agent, such as a natural product, is to determine the effect of the agent on the progression of the cell cycle.[7] The cell cycle is divided into four sequential phases consisting of the first gap phase (G_1), the DNA synthesis phase (S), the second gap phase (G_2) and the mitosis phase (M). Knowledge of the timing of cell cycle events provides an opportunity to identify the cellular target (or mode of action) of a cytotoxic agent by identifying the point of inhibition. For instance, antimetabolites that interfere with DNA synthesis such as fluorouracil cause arrest at S phase while the antimitotic agent taxol induces G_2/M phase arrest.[8] Flow cytometry is one method of following the progression of the cell cycle using sychronized cells in culture.

We examined the effect of landomcyin A on the cell cycle progression of synchronized mammalian cells using flow cytometry. As shown in Figure 2, we observed cell cycle inhibition in the G_1 phase and delayed entry into S phase in chemically treated cultures. As a result, the percentage of landomycin A treated cells in G_0/G_1 at 24 h is increased in comparison to the control sample. This correlates with a proportional decrease in the number of cells entering S phase. A second set of experiments was designed in which cells were treated with landomycin A after 24 h to provide sufficient time for the cells to exit G_1 phase. In this set of experiments, no effect on cell cycle progression was observed relative to the control sample suggesting that the landomycin A effect is G_1 specific (data not shown).

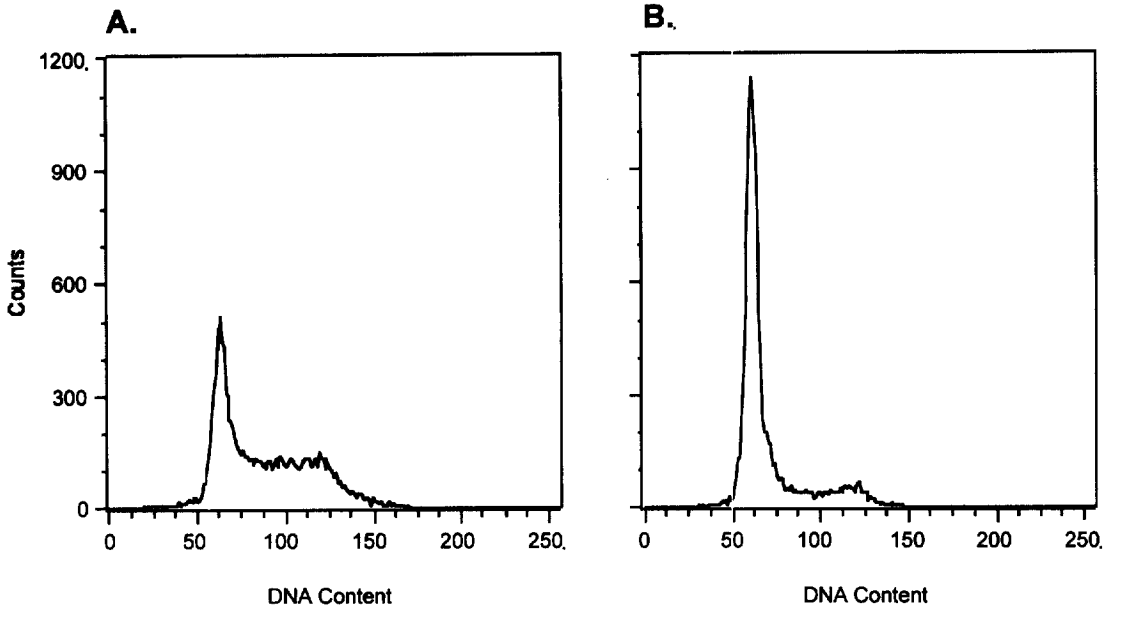

Figure 2. The influence of landomycin A on the cell cycle progression of synchronized murine smooth muscle cells. (**A**) Synchronized cells cultured at 37 °C for 24 h without landomycin A. (**B**) Synchronized cells cultured at 37 °C in the presence of 200 nM landomcyin A. Note that the retention of cells in G_0/G_1 coincides with a diminished number of cells in S phase in (**B**).

In summary, landomycin A is an antitumor antibiotic that demonstrated an interesting spectrum of cytotoxicity when evaluated by the National Cancer Institute using a standard in vitro screening protocol. The molecular structure of landomycin A consists of an angular tetracyclic quinone conjugated to a linear hexasaccharide. We have shown that landomycin A inhibits cell cycle progression from the G_0/G_1 phase to S phase of the cell cycle using flow cytometry. For comparison purposes we note a class of chemotherapeutic agents cause DNA damage leading to G_1 (or G_2) checkpoint-mediated cell cycle arrest. These include bleomycin A_2, mitomycin C and necarzinostatin.[7] Experiments aimed at determining whether landomycin A inhibits cell cycle progression by a similar mechanism are currently under investigation.[9]

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