Cell Kill Kinetics of Several Nogalamycin Analogs and Adriamycin for Chinese Hamster Ovary, L1210 Leukemia, and B16 Melanoma Cells in Culture

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ABSTRACT

Nogalamycin is an anthracycline antibiotic which was markedly cytotoxic in vitro and was active against several tumor systems in vivo. We compare here the lethality of several nogalamycin analogs against Chinese hamster ovary (CHO), mouse leukemia (L1210), and mouse melanoma (B16) cells in culture. 7-con-O-Methylnogalarol (7-con-OMEN) was the most lethal of all the analogs tested. Thus, for CHO cells exposed for two hr to the drug, the 50% lethal doses of 7-con-OMEN, nogalamycin, and dis-nogamycin were 0.25, 2.7, and 5.8 µg/ml, respectively. In general, CHO cells were less sensitive than B16 or L1210 cells to most compounds. All compounds gave dose-survival curves which consisted of a shoulder region followed by a region of exponential decline in survival.

The nogalamycin analogs nogalamycin, dis-nogamycin, 7-con-O-methylnogalarol, and 7-con-OMEN were selected for further study because of their greater lethality in vitro and antitumor activity in vivo. The lethality of these compounds was compared to that of Adriamycin. 7-con-OMEN was more toxic to CHO cells than was Adriamycin but was less toxic to B16 and L1210 cells. All of these compounds (except 7-con-O-methylnogalarol which was not tested) were more lethal to exponentially growing cells than to plateau-phase cells.

The survival response after different periods of exposure to these drugs was compared. In order to make valid comparisons of the time-survival response to different drugs, the drug concentrations chosen were such that they were equitoxic after a two-hr exposure. Under these conditions, the order of lethality after long-term exposure (8 hr to 24 hr) was nogalamycin > dis-nogamycin > 7-con-OMEN, Adriamycin > 7-con-O-methylnogalarol. With all the drugs, the rate of cell death increased with increasing drug concentrations.

INTRODUCTION

Nogalamycin, an antibiotic of the anthracycline series, was markedly cytotoxic in vitro and was active against several tumor systems in vivo (5). Biochemically, nogalamycin was interesting since it bound preferentially to the adenine-thymine region of the DNA resulting in inhibition of RNA synthesis (10). However, the renal toxicity, venous occlusion, and pulmonary thrombosis caused by nogalamycin during toxicity testing in animals precluded its clinical trial. Recently, Wiley et al. (20) prepared several nogalamycin analogs with the hope of reducing their toxicity while increasing their antitumor activity.

When the in vivo antitumor activity of these analogs was determined, the following 4 compounds were found to be the most active: 7-con-OMEN,^3^ dis-nogamycin, nogalamycin, and 7-con-O-methylnogalarol (15). Therefore, we decided to compare in detail the cytotoxicity and cell kill kinetics in vitro of the above 4 analogs. The cytotoxicity of the other analogs are also reported. Since Adriamycin is another anthracycline antibiotic with potent antitumor activity in animals and in the clinic, its effects in vitro were compared to those of the nogalamycin analogs.

The 2 tumor cell lines, L1210 leukemia and B16 melanoma, were chosen for the in vitro study because the compounds were active against these tumors in vivo. The CHO cell line was chosen because it is pseudodiploid, has high cloning efficiency, and can be easily synchronized.

MATERIALS AND METHODS

L1210 Cell Culture. L1210 cells were maintained in culture in Roswell Park Memorial Institute Medium 1634 supplemented with fetal calf serum (5%) NaHCO3 (0.75 mg/ml), penicillin (0.1 mg/ml), and streptomycin (0.05 mg/ml) as described previously (7). In this medium, the cells grew exponentially with a generation time of about 12 hr. Exponential growth was maintained until a cell density of 4 x 10^5 cells/ml was reached; stationary cultures had about 8 x 10^5 to 10^6 cells/ml. These cell densities were obtained by planting cells at 5 x 10^4/ml and using them after 24 (exponential) and 72 hr (stationary) of growth (7). Under these conditions, cells in exponential and stationary growth had cloning efficiencies of about 70 and 35%, respectively.

CHO Cell Culture. CHO cells were maintained in Ham's F-10 medium supplemented with 15% fetal calf serum. CHO cells grow exponentially up to a cell density of about 5 x 10^6/75 sq cm.

To obtain plateau-phase CHO populations, the cells were grown to confluency (10^7 to 2 x 10^7 cells/75 sq cm) and maintained there without medium change for 36 hr (16). Both exponential and plateau-phase CHO cells had cloning efficiencies ranging between 70 and 90%. Plateau-phase CHO cells accumulated predominantly in the G2 phase (90 to 95%) with the rest (5 to 10%) being in G1 (Ref. 16 and our flow cytometry results).

B16 Melanoma Cell Culture. The B16 (clone F-10) cell line was obtained from Dr. I. J. Fidler (Frederick Cancer Research Center, Frederick, Md.). The cells were grown as a monolayer

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^3^The abbreviations used are: 7-con-OMEN, 7-con-O-methylnogalarol; CHO, Chinese hamster ovary; D_50, the dose which reduces survival by a factor 1/e in the straight exponential portion of the dose-survival curve; LD_50, 50% lethal dose; LD_90, 90% lethal dose.

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in Eagle's minimum essential medium supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, 2 mM l-glutamine, twice the recommended concentration of vitamins, 0.1 mM concentration of each nonessential amino acid, penicillin (60 μg/ml), and streptomycin (10 μg/ml). The origin of the highly metastatic F-10 line has been described (13).

Drug Exposure and Cell-Survival Determination. B16 and CHO cells were planted as monolayer and L1210 cells in suspension culture 24 hr before an experiment in order to assure exponential growth during drug exposure. Cells were exposed to drug at 37° in their respective growth medium. For each drug concentration tested, 2 separate cultures were used.

To determine percentage of survival after drug exposure, L1210 cells were centrifuged, and the cell pellet was washed with medium to remove drug and then resuspended and serially diluted in medium. L1210 cells were then planted in a soft agar medium (7) to determine cloning efficiency. Each sample was pipetted into 4 tubes, and the colonies were counted after about 10 days of incubation at 37° in a humid 8% CO2 atmosphere.

After drug exposure, the B16 or CHO cell monolayer was harvested with trypsin and the cells were centrifuged and washed to remove drug. The cells were diluted in medium, and 2-ml CHO cells were planted (to give about 20 to 100 colonies) in plastic multiwell Linbro plates with 3.5-cm-diameter wells. For B16, 5-ml cells were planted in multiwell Linbro plates with 6-cm diameter wells. Four wells were used per sample. The plates were then incubated in a humid 5% CO2 atmosphere for 8 days after which the colonies were fixed, stained with 0.2% methylene blue in 70% ethanol, and counted.

Cloning efficiency of exponentially growing CHO, L1210, and B16 cells ranged between 50 and 90%. In all cases, the cloning efficiency of the untreated (control) cells was normalized to 100%, and the cloning efficiency of the treated cells was expressed as a percentage of control survival. The coefficient of variation (standard deviation expressed as a percentage of the mean) in determining cell survival was about 15% within each experiment. All experiments were repeated at least once.

Drugs Tested. Nogalamycin and its analogs were prepared by Wiley et al. (19, 20) at The Upjohn Company. The structures of these compounds are shown in Chart 1 (18–20) and are described in detail under “Discussion.” All compounds were dissolved in 0.1 M glucuronic acid at 1 mg/ml and further diluted in medium prior to adding to the cells. Adriamycin (NSC 123127) was obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

RESULTS
Lethality of Nogalamycin Analogs and Adriamycin for CHO, L1210, and B16 Cells in Culture

Exponentially growing cultures of the 3 cell lines were exposed to drugs for 2 hr, and cell survival was determined. Representative dose-survival curves obtained with CHO cells are shown in Chart 2. For every compound, a sigmoid survival curve was obtained which was characterized by an initial shoulder followed by an exponential (log-linear) decline in survival as the dose increased. These curves were used to determine the D0 (1/slope) of the exponential portion of the curve and the LD50 and the LD90 values for each drug (see Table 1).

We consider the LD50 and LD90 values to be a more functional
and useful measure of cell kill than is $D_0$ since they reflect the effect of both the threshold region and the slope of the exponential portion of the survival curve. It is possible that 2 drugs may have the same $D_0$ values but different $D_{50}$ values due to differences in threshold region.

In Table 1, the analogs are arranged in 2 groups, those derived from nogalamycin and those derived from nogamycin. These results show that: (a) 7-con-OMEN was the most cytotoxic of all the nogalamycin analogs against CHO and L1210 cells (see probit analysis in legend to Table 1), it had the lowest $D_0$, $LD_{50}$, and $LD_{90}$ values. For B16 cells, 7-con-OMEN and nogalamycin had the lowest $LD_{50}$ values (see legend to Table 1). However, the $LD_{50}$ values for 7-con-OMEN (95% confidence limit, 0.261 to 0.288 $\mu$g/ml) were lower than that of nogalamycin (95% confidence limit, 0.38 to 2.37 $\mu$g/ml); (b) for both 7-OMEN and 7-O-methylnogalarol, the con forms were about 10 times as lethal as the dis forms; (c) increasing the size of the substituent at position 7 of 7-con-OMEN (from O-methyl to O-isopropyl) resulted in lower lethality; (d) CHO cells were less sensitive than B16 or L1210 cells to most compounds except for 7-con-O-methylnogalarol. The 2 parent compounds, nogalamycin and dis-nogamycin, were about 7- to 15-fold more toxic to B16 than to CHO cells. Similar toxicity differences were also seen for Adriamycin. In contrast, 7-con-O-methylnogalarol was almost equally toxic to CHO and B16 cells. In general, B16 and L1210 cells respond similarly to these compounds; (e) Adriamycin, like the nogalamycin analogs, gave a dose-survival curve characterized by an initial shoulder followed by an exponential decline (log-linear) in survival as the drug concentration increased.

Response of Exponential and Plateau-Phase CHO and L1210 Cells

The lethality of nogalamycin analogs and Adriamycin for exponential and plateau-phase CHO and L1210 cells were determined. Representative dose-survival curves with nogalamycin and 7-con-OMEN are shown in Chart 3. Exponentially growing CHO and L1210 cells were much more sensitive to

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**Table 1**

Lethality of nogalamycin analogs and Adriamycin

Exponentially growing CHO, L1210, and B16 cells were exposed to drug for 2 hr after which the percentage of survival was determined by cloning assay. For selected compounds, after 2 hr of drug exposure the $LD_{50}$ and $LD_{90}$ values were analyzed by the probit procedure in which log dose was used. The 95% confidence limits for $LD_{50}$ are: CHO cells: nogalamycin, 2.61 to 2.87; 7-con-OMEN, 0.23 to 0.27. L1210 cells: nogalamycin, 0.23 to 0.27; 7-con-OMEN, 0.06 to 0.12. B16 cells: nogalamycin, 0.03 to 0.27; 7-con-O-methylnogalarol, 0.52 to 0.82; 7-con-OMEN, 0.11 to 0.128.

<table>
<thead>
<tr>
<th>CHO cell kill ((\mu g/ml))</th>
<th>L1210 cell kill ((\mu g/ml))</th>
<th>B16 cell kill ((\mu g/ml))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$LD_{50}$</td>
<td>$LD_{90}$</td>
</tr>
<tr>
<td>Nogalamycin</td>
<td>2.74</td>
<td>4.91</td>
</tr>
<tr>
<td>7-con-O-Methylnoagarol</td>
<td>0.78</td>
<td>1.21</td>
</tr>
<tr>
<td>7-dis-O-Methylnoagarol</td>
<td>10.7</td>
<td>14.0</td>
</tr>
<tr>
<td>dis-Nogalaminic acid</td>
<td>6</td>
<td>&gt;30</td>
</tr>
<tr>
<td>dis-nogamycin</td>
<td>5.77</td>
<td>95.1</td>
</tr>
<tr>
<td>7-con-OMEN</td>
<td>0.25</td>
<td>0.47</td>
</tr>
<tr>
<td>7-dis-O-Methylnoagarol</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7-con-O-Ethylnoagarol</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>7-con-O-isopropylnoagarol</td>
<td>0.8</td>
<td>1.9</td>
</tr>
<tr>
<td>7-con-O-Acetylnoagarol</td>
<td>3.2</td>
<td>9.6</td>
</tr>
<tr>
<td>7-con-Thiomethyldeoxynoagarol</td>
<td>0.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>0.5</td>
<td>1.3</td>
</tr>
</tbody>
</table>

- $D_0$, reciprocal of the slope of the straight exponential portion of the dose-survival curve. It is the dose which reduces survival by a factor $1/e = 0.37$ in the straight exponential portion of the curve.
- ND, not done. These compounds were not studied further in vitro because their antitumor activity in vivo against L1210 leukemia and B16 melanoma was not superior to those of nogalamycin, dis-nogamycin, or 7-con-OMEN.
the drugs than were plateau-phase cells. For example, the LD₅₀ value for plateau L1210 cells increased over that for exponential cells by 2.8-, 2.2-, 5.4-, and 7.5-fold for nogalamycin, disnogamycin, 7-con-OMEN, and Adriamycin, respectively. For nogalamycin and 7-con-OMEN, the LD₅₀ of plateau-phase CHO cells was 6.4- and 5.3-fold higher than that of exponential cells.

The difference between the LD₅₀ values for exponential and plateau-phase cells was due to a change in either or both the threshold region and the slope of the survival curve. For example, there was little change in the threshold, but there was marked difference in the slope of plateau-phase and exponential (CHO, L1210) cells exposed to 7-con-OMEN (Chart 3). In contrast, the threshold region of plateau-phase CHO cells exposed to nogalamycin was much greater than that of exponential cells (Chart 3).

We also wanted to compare the drug sensitivity of exponential and plateau-phase cells under conditions where the drug:cell ratio was similar in both populations. In order to achieve this, the stationary L1210 population was diluted with medium from stationary cultures to a cell density similar to that in exponential cultures and immediately exposed to the drug. In this experiment exponential cells exposed to 7-con-OMEN (0.2 µg/ml) gave 10.8% survival. In contrast, when stationary cells were diluted to a cell density similar to that of exponential cells and then exposed to 7-con-OMEN, 81.2% of the cells survived.

**Kinetics of CHO and L1210 Cell Kill**

**CHO Cells.** Representative curves for the time-survival response of these drugs are shown in Chart 4. For this experi-

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**Chart 3.** Lethality of nogalamycin and 7-con-OMEN for CHO and L1210 cells in exponential and plateau phase. The protocol for obtaining exponential and plateau-phase cells is described under "Materials and Methods." Cells were exposed to drug for 2 hr. In all cases, the lethality of the drug to the exponential and plateau-phase populations was determined in the same experiment. O, plateau; •, exponential; bars, S.D.
moment, the drug doses were grouped such that they killed about the same percentage of cells at 2 hr. This enabled us to make valid comparisons of the time-survival response of these drugs. Chart 4 shows detailed results for 7-con-OMEN and nogalamycin and representative curves for 7-con-O-methylnogalarol and dis-nogamycin. At the lowest equitoxic concentration (7-con-OMEN, 0.03 μg/ml; nogalamycin, 0.3 μg/ml), both drugs gave 100% survival after 8 hr of exposure to the drugs. After 48 hr, only 5.5% of the cells exposed to nogalamycin survived as compared to 40% of the cells exposed to 7-con-OMEN. At the next higher concentration (nogalamycin, 1.25 μg/ml; 7-con-OMEN, 0.1 μg/ml; 7-con-O-methylnogalarol, 0.4 μg/ml), all compounds gave 92 to 98% survival at 2 hr. However, with increasing time of exposure, the rate of cell death for the different compounds were markedly different. For example, after 24-hr exposure, the lethality of nogalamycin (0.07% survival) > dis-nogamycin (3.6% survival) > 7-con-OMEN (16% survival) 7-con-O-methylnogalarol (42.1% survival).

Similar results were obtained at the next 2 higher groups of drug concentrations. Thus, at the highest concentration tested, 2-hr exposure to nogalamycin, 7-con-OMEN, and 7-con-O-methylnogalarol (not shown) gave 32, 24, and 18% survival, respectively. At 8 hr, the order of lethality was nogalamycin > 7-con-OMEN > 7-con-O-methylnogalarol [3.3 + 0.3% (S.D.) survival]. In an experiment where Adriamycin was directly compared to 7-con-OMEN, the time-survival response for the 2 drugs were very similar. These results show that when the drugs were compared at concentrations which were equitoxic after 2 hr of exposure, the order of lethality after long-term (8- to 24-hr) exposure was nogalamycin > 7-con-OMEN, Adriamycin > 7-con-O-methylnogalarol. The results also suggest that the response of dis-nogamycin may lie between nogalamycin and 7-con-OMEN. With all the drugs, the rate of cell death increased as the drug concentration increased.

**L1210 Cells.** Time-survival studies with L1210 cells gave results similar to those with CHO cells. At the low dose of 7-con-OMEN (0.05 μg/ml) and nogalamycin (0.2 μg/ml), 80% of the cells survived 2-hr drug exposure. However, by 5 hr nogalamycin (23% survival) was clearly more lethal than was 7-con-OMEN (48% survival).

At the higher dose (nogalamycin, 0.3 μg/ml; 7-con-OMEN, 0.1 μg/ml), between 45 and 50% of the cells survived 2 hr of drug exposure. After 7.5 hr exposure to nogalamycin and 7-con-OMEN, respectively, 0.04 and 4% of the cells survived. The time-survival response of dis-nogamycin was similar to that of nogalamycin. Adriamycin gave a time-survival response similar to that of 7-con-OMEN. Therefore, the order of lethality for these drugs after long-term exposure was similar to that seen with CHO cells.

**DISCUSSION**

A brief discussion of the structural differences between these analogs is necessary in order to understand their different biological (15) and biochemical effects (14, 9).
Nogalamycin, dis-nogamycin, and dis-nogalamicin acid all contain the sugar nogalose but differ in the substituent at R1 (Chart 1). The “nogala” and the “noga” compounds originate from nogalamycin and nogamycin, respectively, and differ in that the nogala series contain a COOCH3 group at R1 whereas the noga series contain a hydrogen atom at R1. The compounds can exist as 2 possible isomers, namely con and dis configurations, which differ in the stereochemistry at C-7. In dis isomers, the C-9 hydroxy group and the C-7 nogalose or alkoxy substituents project from opposite sides of the A ring (Chart 1). In con isomers, the C-9 hydroxy group and the 7-substituent project from the same sides of the A ring. These isomers are not interchangeable under physiological conditions, and the con and dis isomers can be readily distinguished by circular dichroism measurements (14). Based on this measurement, nogalamycin exists in the dis configuration.

The biochemical effects of these analogs have been studied in detail by Li et al. (14). The 3 compounds containing nogalose, namely, nogalamycin, dis-nogamycin, and dis-nogalamicin acid, bound to DNA and inhibited RNA synthesis more than DNA synthesis. Similar drug concentrations were required to cause 50% inhibition of growth and of RNA synthesis. Collectively, the results suggested (14) that these 3 compounds acted by binding to the DNA template which led to inhibition of RNA synthesis and ultimately to cell death.

The picture was not so clear for the 7-O-alkyl analogs. Among the 7-O-alkyl analogs, the 7-con compounds inhibited L1210 cell growth much more than the 7-dis isomers (14). This may also be seen by comparing the LD50 of the con and dis isomers of 7-O-methylnogarol and 7-O-methylnogalarol (Table 1). However, biochemically, the 7-dis compounds had a stronger affinity for DNA and inhibited RNA synthesis more than did their con isomers. Li et al. (14) found that inhibition of RNA synthesis due to binding to the DNA template could partially account for the cytotoxic effects of the dis compounds. In contrast, the biochemical effects observed with the con isomers could not account for their cytotoxic effects. For example, 7-con-OMEN, which is the most cytotoxic of all the nogalamycin analogs, bound to DNA to a much lesser extent and inhibited DNA and RNA synthesis minimally at doses which were significantly lethal. Therefore, the lethality of 7-con-OMEN is probably mediated through some mechanism other than drug interaction with DNA.

The shape of the survival curve with all compounds and the 3 cell lines were of the “threshold exponential” type described by Drewinko et al. (12). These curves are characterized by a threshold where at low concentration minimum cell kill is seen but higher concentrations kill cells exponentially. The shape of our survival curve with Adriamycin differed from that reported by Barranco et al. (1). These authors obtained a survival curve that was linear until about 99.9% of the cells were killed after which the line assumed a shallower slope presumably due to the presence of resistant cells. We did not see such a response with Adriamycin or any of the nogalamycin analogs probably because our survival data were limited to less than 99.9% cell kill.

For most compounds, L1210 and B16 cells were more sensitive than were CHO cells (Table 1). This difference in the sensitivity of the different cell lines was investigated in detail for 7-con-OMEN and Adriamycin. We found that at similar extracellular concentrations B16 and L1210 cells accumulated much more drug intracellularly than did CHO cells (4). It is tempting to say that the greater intracellular drug concentration in B16 and L1210 cells as compared to CHO cells accounts for the greater sensitivity of the former cell lines. However, we know neither the subcellular sites at which the drug is localized nor the lethal effects of localization at any of the subcellular sites. Therefore, at this time it is not possible to explain the difference in sensitivity of the cell lines solely on the basis of the difference in total intracellular drug concentration.

All of the anthracyclines (nogalamycin and its analogs, Adriamycin, and daunomycin) that we have tested have been more cytotoxic to exponential than to plateau-phase CHO and L1210 cells (this report; Ref. 6). Similar results have been reported for Adriamycin by Twentyman and Bleehen (17) and Barranco et al. (2). The difference in drug sensitivity between exponential and plateau-phase cells could be due to either: (a) difference in intracellular concentration of the drug; or (b) difference in the cell cycle phase distribution between the 2 populations.

The uptake of 7-con-OMEN by exponential and plateau-phase CHO cells has been investigated in detail (4, 8). These experiments showed that exponential cells accumulated more drug than did plateau cells. It would be tempting to explain the greater sensitivity of the exponential population on the basis of greater intracellular drug concentration. However, when the results were expressed as percentage of survival per µg intracellular drug per cell it was found that exponential cells were inherently more sensitive than were plateau cells. We do not know whether similar conclusions will also be true for the other anthracyclines.

The cell cycle phase distribution of exponential cells is different from that of plateau populations. For CHO cells, the plateau population resides almost entirely (90 to 95%) in G1 phase (12). For L1210 cells, the percentage of cells in G1 + G2 increased from 30% (in exponential cells) to 54% (in plateau) with a corresponding decrease in S-phase cells. If G1 and G2 cells are less sensitive to drug than S-phase cells, then changes in phase distribution can explain the change in drug sensitivity. However, this is not likely to be the case for nogalamycin and its analogs. Our experiments showed that 99% of plateau-phase CHO cells (where 90 to 95% cells are in G1) survived exposure to nogalamycin (7 µg/ml). However, this was not due to the inherent insensitivity of cells in G1, to the drug. G1 cells in a synchronized population were killed (30% survival) by nogalamycin (7 µg/ml) (3). This indicates that cycling G1 cells present in the synchronized population were more sensitive than were the noncycling G1 or G0 cells in the plateau population. For purposes of this discussion, G0 cells are noncycling cells of G0 DNA content.

Our results clearly showed that even when the doses of different drugs were chosen to be equitoxic after a 2-hr exposure, the cell kill kinetics of the drugs after long-term exposure could be markedly different. For example, when CHO cells were exposed to equitoxic (after 2 hr) doses of nogalamycin and 7-con-OMEN, there was marked difference between the survival obtained after 24-hr exposure to these 2 drugs. Thus, after 24 hr, 0.7% survival was obtained with nogalamycin (1.25 µg/ml) compared to 16% survival with 7-con-OMEN (0.1 µg/ml). The survival kinetics of Adriamycin was similar to that of 7-con-OMEN. These results could be due to degradation or metabolism of 7-con-OMEN to a less cytotoxic species. We did...
not observe any change in 7-con-OMEN concentration or metabolism of the drug over at least an 8-hr period. Therefore, we do not have any good explanation for this effect.

Among the nogalamycin analogs, 7-con-OMEN was clearly the most cytotoxic against the 3 cell lines in culture and was significantly active against several tumor systems in vivo (15). For example, against L1210 leukemia it gave 140% increase in life span compared to 30 to 40% for nogalamycin, dis-nogamycin, and Adriamycin. Nogalamycin, dis-nogamycin, and 7-con-OMEN all gave more than 100% increase in life span with B16 melanoma (15). Therefore, detailed cell kinetic studies were done with several of the more active compounds, and the results were compared to those obtained with Adriamycin. The results are presented as LD90 values in Table 1. The LD90 of a compound may be a useful guide to the antitumor activity of that compound in vivo. Thus, in order to be active in vivo, the LD90 value of a compound should be lower than the attainable plasma level of the drug. Preliminary data on plasma level in mice after a single i.v. injection of 7-con-OMEN (10 mg/kg) are available. At 0.5 hr after injection, the drug plasma level was 0.5 to 1 µg/ml which decreased with a half-life of about 3.5 hr. Thus, the LD90 value of 7-con-OMEN for L1210 and B16 cells is lower than the attainable plasma level. Similarly, the plasma level (11) of Adriamycin after a single i.v. injection into mice ranged between 0.3 to 0.1 µg/ml which is much higher than the LD90 for L1210 and B16 cells. We do not have any data regarding plasma level of the other drugs. Neil et al. (15) observed some correlation between the cytotoxic activity in vitro to that seen in vivo. Analogs with low cytotoxicity (i.e. high 50% inhibiting dose for growth inhibition) were not very active in vivo; however, a low 50% inhibiting dose value did not necessarily predict high in vivo activity. Factors other than in vitro cytotoxicity, such as metabolism and pharmacokinetics, will influence the activity in vivo.

REFERENCES

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