Action of Camptothecin on Mammalian Cells in Culture

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SUMMARY

Camptothecin (CN) is active against several experimental tumors and has also been studied clinically. We report here the effect of CN on LI210 cells and asynchronous and synchronous DON cells in culture.

CN was toxic both to LI210 cells (0.06 μg/ml, 2-hr exposure) and DON cells (0.15 μg/ml, 1-hr exposure), and it inhibited DNA and RNA synthesis more than it inhibited protein synthesis. CN was more cytotoxic to DON cells in S phase than cells in G1 or G2, although it inhibited DNA and RNA synthesis of LI210 cells and asynchronous DON cells almost equally. When asynchronous DON cells were exposed to CN for 30 min and CN was then removed, RNA synthesis was no longer significantly inhibited, but the inhibition of DNA synthesis persisted. The survival patterns of synchronous DON cells were closely related to DNA synthesis inhibition but not to RNA synthesis inhibition. These results collectively suggested that the marked effect of CN on DNA synthesis appeared to be one of the primary determinants of its cytotoxicity. Since no significant effect was observed on the enzymes involved in DNA synthesis, DNA synthesis inhibition by CN may be in part due to its effect on the DNA template. The interaction between CN and DNA was detected by melting point determinations. CN did not block the progression of mitotic cells into S phase. At 100 μg/ml, CN prevented the progression of S phase cells into G2; at 1 μg/ml, some cells did leave S and proceeded into G2. Cells in G2 were blocked from moving into mitosis even at 0.01-μg/ml doses of CN. Thus, the progression of late S or early G2 cells into mitosis was most sensitive to the drug.

INTRODUCTION

CN2 (NSC 100880) is active against several experimental tumors (5, 7) and has also been studied clinically (6, 17). The drug inhibits nucleic acid synthesis in mammalian cells (2, 9–12, 21) and phytohemagglutinin-stimulated human lymphocytes (5). The 1st part of this report describes the biological effect of CN on LI210 cells and asynchronous and synchronous DON cells in culture, and the 2nd part attempts to describe the relationship between the biochemical effects and drug cytotoxicity.

MATERIALS AND METHODS

Tissue Culture Systems

LI210 cells were grown at 37° in basal medium (RPMI 1634 medium3: fetal calf serum, 100:5) developed by Moore et al. (16). A mixture of penicillin (0.1 mg/ml of medium) and streptomycin (50 μg/ml of medium) was added. Cells used in uptake studies were in the logarithmic phase of growth (ca. 5 X 10⁶ cells/ml).

DON cells, a Chinese hamster fibroblast line (ATCC No. CCL 16), were grown in McCoy’s Medium 5A modified by the addition of lactalbumin hydrolysate (0.8 g/liter) and fetal calf serum (200 ml/liter). The cell monolayer was detached from glass by treatment with a 0.1% trypsin solution. The cells were dispersed and grown in 8-oz bottles planted with about 2 X 10⁶ cells in 25 ml medium. The cells were maintained in the logarithmic growth phase by subculturing every 2 days.

Chemicals and Medium

CN was obtained from Drug Research and Development, National Cancer Institute, Bethesda, Md. TdR-3H (2 Ci/m mole), UdR-3H (2 Ci/m mole), and DL-valine-1-14C (9.1 mCi/m mole) were purchased from New England Nuclear, Boston, Mass. RPMI 1634 medium was supplied by Associated Biomedic Systems, Inc., Buffalo, N. Y. McCoy’s Medium 5A and supplements were purchased from Grand Island Biological Co., Grand Island, N. Y.

LI210 Cell Growth Study

Four ml of cells (ca. 5 X 10⁶ cells/ml) were pipetted into sterile plastic culture tubes with screw caps (16 x 125 mm; Falcon Plastics Co., Oxnard, Calif.) and incubated at 37°. The experiment was initiated by the addition of 1.0 ml of medium, metabolites, and/or CN, and cells were incubated for a given period (0 to 2 hr). At the termination of each incubation, drug was removed by centrifugation; cells were washed twice and then resuspended in fresh medium at about 5 X 10⁶ cells/ml. Five ml of these washed cells were pipetted into each culture tube and incubated at 37°. Cell number was determined twice a day for 3 days with a Coulter counter (Coulter Electronics, Chicago, Ill.) and was compared to control (no drug).

For determination of 50% and 90% cell-kill dose values, 5

1This investigation was supported in part by Contract PH 43-NCI-68-1023 with Drug Research and Development, National Cancer Institute, NIH, Bethesda, Md. 20014.

2The abbreviations used are: CN, sodium camptothecin; TdR, thymidine; UdR, uridine.

3RPMI 1634 medium is medium developed at Roswell Park Memorial Institute, Buffalo, N. Y., for growing human and mouse leukemic cells.
ml of L1210 cells (5 X 10^5 cells/ml) were incubated with the
drug at 37° for 3 days, and cell growth was determined (3).

Macromolecular Synthesis

**L1210 Cells.** Cells, 25 ml, were incubated simultaneously
with CN (0 to 20 μg/ml) and a labeled metabolite [TdR-3H or
UdR-3H (2.5 μCi/12 μmole of cells per ml) or
DL-valine-l-14C (0.5 μCi/200 μmole/ml)] for up to 4 hr
(37°) with gentle shaking. Aliquots (10 ml) removed at
different times (1, 2, and 4 hr) were then pipetted into a
centrifuge tube containing 1 ml of corresponding unlabeled
metabolite at high concentration (10 mg/ml) kept at 4°. DNA
and RNA were extracted with perchloric acid from the
trichloroacetic acid- and organic solvent-washed cell pellet,
according to a procedure described elsewhere (13). Protein
was extracted from the washed cell pellet with 4 ml 1 N NaOH for
2 hr at 37°. Two ml of aliquots were mixed with 10 ml of 10%
trichloroacetic acid; and the protein precipitant was collected
by centrifugation, washed with 10 ml of cold water, and then
dissolved in 4 ml of Hyamine (Packard Instrument Company,
Inc., Downers Grove, Ill.). An 0.4-ml aliquot was added to 12
ml Diol and radioactivity was determined. Protein content
were determined by the procedure of Lowry et al. (14).

**Asynchronous DON Cells.** Cells were planted at 10^6
cells/3-oz bottle in 10 ml of medium. After overnight
incubation, the cells were refed with fresh medium, and drug
and labeled precursors were added for 1 hr. To stop uptake of
radioactivity, cells were detached with 0.1% trypsin containing
unlabeled precursors (100 μg/ml), and the cells were
suspected in 0.9% NaCl solution. One aliquot was counted in
the Coulter counter to give the cell number, while another (1
ml) aliquot of cells was filtered through 0.45-μm Millipore
filters. The filter was washed 4 times with cold 10%
trichloroacetic acid and once with ethanol. The filter was then
incubated with 0.5 ml of 0.5 N perchloric acid at 70° for 20
min. Diol (15 ml) was added, and the filter was counted in a
scintillation counter.

**RESULTS**

Cytotoxicity. CN was cytotoxic to both DON cells and
L1210 cells in culture. The dose for 50% cell-kill were 0.15
μg/ml for DON cells (1 hr exposure to drug) and 0.06 μg/ml
for L1210 cells (2 hr exposure), respectively. The inhibition
was greater (50% cell kill, 0.008 μg/ml) when L1210 cells
were exposed continuously to CN for 3 days.

Inhibition of L1210 cell growth was an immediate
phenomenon as shown in Chart 1. The results indicate that
inhibition of cell growth could no longer be eliminated after
only 5 min contact between drug and cell, and inhibition
increased with contact time. Furthermore, the inhibition was
not affected by the simultaneous addition of drug (4.5 μM)
of various metabolites such as purine and pyrimidine mixture
(ca. 10 μM concentrations of each compound), tricarboxylic acid
cycle intermediates (ca. 40 μM concentrations of each
compound), vitamins and coenzymes (riboflavin, ca. 0.9 μM;
folie acid and thiamine, ca. 6.5 μM; biotin, pantothenic acid,
and pyridoxal, ca. 10 μM; choline, inositol, and nicotinamide,
Chart 1. Time-course study of the effect of CN on L1210 cell growth in culture. Cells (ca. 5 x 10⁶/ml) were exposed to CN (0.25 µg/ml) for 5 to 120 min (37°C), and drug was removed afterward by centrifugation and washings. Cells were then resuspended in fresh medium at a given concentration of 5 x 10⁴/ml, and cell growth was determined twice a day for 3 days. Percentage of inhibition was calculated from the growth of CN-treated cells as compared to growth of the control (no drug) cells.

ca. 20 µM concentrations of each), and amino acid mixtures (approximately 250 µM concentrations of each compound) (data not presented).

Survival of Asynchronous and Synchronous DON Cells. Chart 2A shows that when asynchronous DON cells were exposed to CN (0.25 to 20 µg/ml) for 1 hr (37°C), the percentage of cell kill reached a constant saturation value, indicating the presence of a subpopulation of cells resistant to CN. However, CN killed more than just the S-phase cells, as shown by the larger percentage of cell kill with CN as compared to high-specific-activity TdR-3H.

Chart 2B indicates that CN was much more toxic to synchronous DON cells in S phase than to cells in G₁ or G₂. The cell population in the 1st 2 hr after planting is composed of mitotic cells and G₁ cells; after 8 hr it is composed of slow S cells, G₂ cells, and M cells.

The survival of synchronous DON cells exposed to different concentrations of CN is shown in Chart 3. The doses for killing 50% of cells in M, G₁, S, and G₂ phase were >1000, 4, 0.04, and >1000 µg/ml, respectively. About 10% of the cells in S survived even with 50-µg/ml doses of the drug. This could be due to contamination with G₁ and/or G₂ cells. Since M cells continuously divide and enter G₁, 2 types of experiments were done in order to expose M cells to CN. Mitotic cells were harvested and exposed to Colcemid (which kept them in mitosis) and CN together for 1 hr; then cell survival was determined. In the 2nd type of experiment, Colcemid was added to asynchronous cells at 0 time. Two hr later, different levels of CN were added, and mitotic cells were harvested after 1 hr of exposure to CN. The survival of the harvested mitotic cells was determined. Both types of experiments gave similar results, namely, the 50% inhibiting dose for mitotic cells is over 1000 µg/ml.

Chart 2. Sensitivity of DON cells to CN and TdR-3H. In A, asynchronous cells were exposed to either CN (0.25 to 20 µg/ml) or TdR-3H (10 µCi/ml) for 1 hr. The drugs were removed, and cells were diluted and planted. Colonies were counted after 7 to 8 days of incubation. In B, at different times after planting, synchronous DON cells were exposed to CN (2 µg/ml) for 1 hr and percentage of cell survival was determined. ---, average of 3 experiments; - - - - - , percentage of control cells labeled by a 15-min pulse with TdR-3H (2 µCi/ml).

Chart 3. Survival of synchronous DON cells exposed to CN for 1 hr in M, G₁, S, and G₂ phases. Mitotic DON cells were planted and exposed to different levels of CN for 1 hr in G₁ (1 hr after planting), S (4 hr after planting), or G₂ (9 hr after planting). For exposure of mitotic cells, Colcemid was added to a monolayer of asynchronous cells at 0 hr, CN was added 2 hr later, and mitotic cells were harvested at 3 hr. The survival of the harvested mitotic cells was determined. Similar results were obtained when mitotic cells were harvested in the usual manner and were then exposed to Colcemid (0.06 µg/ml keeps them in mitosis) and CN for 1 hr. Bars, average deviation of 2 experiments.

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Effect on Progression of Cells through the Cell Cycle. The progression through the cell cycle of mitotic DON cells exposed continuously to CN is shown in Chart 4. The percentage of mitotic cells in the population exposed to CN, 100 μg/ml, was only 0.2% at 2.5 hr, indicating that CN did not affect the completion of mitotic division. The percentage of labeled cells in the control and the population exposed to CN were quite similar at 2.5 and 4 hr, indicating that CN had no appreciable effect on the progression of G₁ cells into S. DNA synthesis was inhibited 58 and 85% of the control at 1- and 100-μg/ml concentrations of CN, respectively. Therefore, after exposure to these concentrations of the drug, labeled cells had fewer grains per cell than the control. At 100 μg/ml, CN prevented the progression of cells out of S into G₂. At 1 μg/ml, some cells did leave S and proceed into G₂, as shown by the lower level of percentage of labeled cells at 9 and 11 hr.

Cells in early G₂ or at the G₂-S boundary were blocked from progressing into mitosis even when exposed to only 0.01 μg/ml doses of the drug. This effect of CN is further illustrated in Table 1. In this experiment, cells in G₁, S, or G₂ were exposed to the drug for 1 hr, and their ability to progress into mitosis was measured. Cells exposed to 1 μg/ml doses of drug were almost completely blocked from entry into mitosis.

Table 1

Effect of CN on progression of synchronous DON cells into mitosis

<table>
<thead>
<tr>
<th>CN (μg/ml)</th>
<th>Drug exposure in G₁</th>
<th>Drug exposure in S</th>
<th>Drug exposure in G₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>6.0</td>
<td>5.6</td>
<td>7.6</td>
</tr>
<tr>
<td>0.1</td>
<td>2.6</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>1</td>
<td>0.4</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0.2</td>
<td>0</td>
</tr>
</tbody>
</table>

Some of the cells in G₁ exposed to CN, 0.1 μg/ml, could enter mitosis. The effect of CN on the progression of asynchronous cells into mitosis is shown in Chart 5. The results indicate that CN (between 0.01 to 0.1 μg/ml) did not inhibit the flow of cells into mitosis during the 1st hour of exposure suggesting the block is probably in early G₂ or at the G₂-S boundary. Similar results were obtained when the cells were preincubated for 2 hr with Colcemid prior to the addition of CN. However, at higher concentration (0.3 μg/ml) a block was observed during the 1st hr of exposure suggesting that the block is in late G₂.

Effect of CN on Macromolecular Synthesis. As illustrated in Table 2, CN at 10 to 20 μg/ml did not markedly inhibit protein synthesis of either L1210 cells or asynchronous DON cells in culture. However, both DNA and RNA synthesis were inhibited by approximately 50% at much lower concentrations (1 to 2 μg/ml) of CN, and the inhibition was noted within 15 min following the addition of drug.

Effect of Removal of Drug on Subsequent DNA and RNA Synthesis. Asynchronous DON cells were exposed to different levels of CN for 1 to 12 hr. After different contact periods, the drug was removed and the cells were allowed to recover from drug effects for 0.5 or 4.5 hr. The cells were then pulse labeled with TdR-3H or UdR-3H for 0.5 hr. Therefore, the actual recovery periods after the removal of CN were 1 and 5 hr, respectively. The results shown in Chart 6 indicate the following. (a) For a 1-hr exposure to the drug and a 1-hr recovery period, DNA synthesis was inhibited about 70 to 95% (Chart 6B) while the inhibition of RNA synthesis was largely reversed at all levels of CN (Chart 6A). For a 1-hr exposure and a 5-hr recovery period, DNA-synthetic capacity was partially recovered (Chart 6D), while RNA synthesis was restored completely at all doses used (Chart 6C). (b) For a 4-hr exposure and a 5-hr recovery period, there was minimal recovery of DNA synthesis after such a long exposure to the drug. However, there was significant recovery of RNA synthesis (Chart 6, C and D). The degree of recovery of RNA synthesis appeared to be dose related, as was the recovery of DNA synthesis. (c) For an 8-hr exposure or longer and a 1-hr or 5-hr recovery period, no significant recovery of either DNA or RNA synthesis was observed at all the concentrations used (Chart 6). At the 100-μg/ml level, the RNA-synthetic capacity was further reduced 5 hr after the removal of drug (Chart 6C), indicating the extensive damage to the cells after such a long
exposure to CN. However, the overall recovery of RNA synthesis was a dose-related phenomenon.

Although DNA and RNA synthesis are equally inhibited by CN (Table 2), the recovery of synthetic capacity for DNA and RNA are different after the drug is removed. The degree of recovery was related to the drug concentration and the period

Chart 5. Effect of CN on the progression of asynchronous DON cells into mitosis. Bottom curves, CN and Colcemid (0.06 μg/ml) were added at zero time and samples were taken 1, 2, and 4 hr later; top curves, cells were preincubated with Colcemid for 2 hr, after which CN was added. Samples were taken 1 and 2 hr later.

Chart 6. Effect of CN on DNA and RNA synthesis (Syn.) of asynchronous DON cells in culture. Cells were exposed to CN (1, 10, and 100 μg/ml) for various times (1, 4, 8, and 12 hr), and drug was then removed. One-half or 4.5 hr later, cells were pulse labeled with either Tdr-3H or UdR-3H (4 μCi/1 μg of cells per ml) at 37°. Inhibition was determined by comparison to the controls.

Table 2
Effect of CN on macromolecular synthesis of mammalian cells in culture

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CN concentration (μg/ml)</th>
<th>DNA synthesis</th>
<th>RNA synthesis</th>
<th>Protein synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1210 cells</td>
<td>1</td>
<td>56.1</td>
<td>54.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>82.4</td>
<td>74.3</td>
<td>12.0</td>
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<tr>
<td></td>
<td>20</td>
<td>86.4</td>
<td>78.0</td>
<td>11.5</td>
</tr>
<tr>
<td>Asynchronous DON</td>
<td>1.85</td>
<td>60.0</td>
<td>53.0</td>
<td>0</td>
</tr>
<tr>
<td>DON cells</td>
<td>9.2</td>
<td>77.0</td>
<td>64.0</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated from specific activity (cpm/μg of metabolites or cpm/10⁶ cells).
of exposure to CN. A complete restoration of DNA synthesis was never achieved under our experimental conditions.

Similar results were obtained with L1210 cells. Inhibition of RNA synthesis was largely reversed when the drug (20 µg/ml) was removed after 2 hr contact with cells. However, DNA synthesis was still inhibited where CN (2 µg/ml) was removed after 15 min contact with the cells (data not presented). The inhibition pattern of DNA synthesis by the drug was closely related to that of cell growth (Chart 1).

Effect of CN on Polynucleotide Synthesis and Cell Survival of Synchronous DON Cells in Culture. The relationship between inhibition of DNA and RNA synthesis by CN and the cytotoxicity of CN was further evaluated with the use of synchronous DON cells.

At various times (0 to 7 hr) after mitotic cells were planted, CN (2 µg/ml) was added to the cells; CN was removed 30 min later. Ten min later, cells were pulse labeled with either TdR-3H or UdR-3H for 20 min. The results shown in Chart 7 clearly indicate that the effect on cell survival is closely related to inhibition of DNA synthesis rather than to that of RNA synthesis.

Effect of CN on Several Enzyme Systems of L1210 Cells in Culture. CN (250 µg/ml) did not cause significant inhibition of TdR kinase, UdR kinase, or DNA polymerase of L1210 cells, as shown in Table 3.

Interaction between CN and DNA. In an attempt to detect the possible interaction between DNA and CN, the effect of CN on the melting temperature (Tm) of DNA was studied. Although the Tm of DNA was not affected, CN suppressed the hyperchromicity seen on strand separation of DNA caused by heating.

The effect of CN concentration on the suppression of hyperchromicity is shown in Table 4. There was greater inhibition with sonically disrupted than in control DNA. Thus this inhibition appeared to be related to drug concentration and the structure of DNA.

The effect on CN on the Tm and the hyperchromicity of several DNA is shown in Table 5. The results indicate that in all cases there was no marked change in Tm on addition of CN, but there was significant suppression of hyperchromicity, which appeared to be inversely related to GC content of DNA.

DISCUSSION

CN was cytotoxic toward both L1210 cells and DON cells growing in culture, but L1210 cells were about 3 times more sensitive to this drug. Although the exposure time to CN for these 2 cell lines were different in this study (1 hr for DON cells and 2 hr for L1210 cells), cell kill by CN was closely related to the drug concentration and rather insensitive to exposure time after cells had been in contact with the drug for 30 min or so (Chart 1). However, the inhibition of CN on mammalian cells growing in culture was an immediate phenomenon. When cells were exposed to the drug for a very short time (15 min) and drug was then removed, the damage had been done and a lesion was demonstrated by following cell growth for 3 days. Approximately 40% of the cells could no longer function as compared to the controls (Chart 1). In an attempt to understand the nature of its activity, the effect of CN on macromolecular synthesis was studied. At a given concentration (1 to 2 µg/ml), DNA and RNA synthesis of both cell lines were almost equally inhibited (Table 2), but protein synthesis was not significantly affected in the same period of exposure (1 to 2 hr). We found that the dose-survival curves of asynchronous DON cells exposed to CN (Chart 2A) decreased to a constant saturation value at high doses (up to 20 µg/ml), suggesting that cells in a certain phase or phases of the cell cycle were more sensitive to CN. Results obtained with synchronous cells (Chart 2B) indicate that S-phase cells were more sensitive to CN. The results also show that CN killed more than just the S-phase cells. The cell kill with CN was greater than that with high-specific-activity TdR-3H (Chart 2A). High-specific-activity TdR-3H was used since it specifically kills S-phase cells subsequent to its incorporation into DNA. Dose-survival curves of M, G1, S, and G2 cells (Chart 3) showed that S-phase cells (50% inhibition dose, 0.040 µg/ml) were about 100 times more sensitive than G1 cells (50% inhibition dose, 4 µg/ml), while G2 and M cells 50% inhibition dose > 1000 µg/ml) were highly resistant to CN.

Our results (Table 2) clearly indicate that CN inhibited DNA and RNA synthesis almost equally. However, with both asynchronous DON (Chart 6) and L1210 cells, inhibition of RNA synthesis was largely reversed when CN was removed. However, inhibition of DNA synthesis was not reversible and this might be related to the rapid inhibition of cell growth by CN as discussed in the previous paragraph. The reversibility of RNA synthesis appeared to be both dose related and dependent on contact time (Chart 6). These observations are in agreement with those of Kessel (12) on L1210 cells and of Horwitz et al. (9) on HeLa cells, suggesting that this may be a common phenomenon for the action of CN on mammalian cells in culture.
Table 3  
**Effect of CN on the enzyme systems of crude cell-free extracts isolated from L1210 cells in culture**

<table>
<thead>
<tr>
<th>CN concentration (µg/ml)</th>
<th>TdR kinase</th>
<th>UdR kinase</th>
<th>DNA polymerase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate (%)</td>
<td>% inhibition</td>
<td>Rate (%)</td>
</tr>
<tr>
<td>0</td>
<td>38.1</td>
<td>27.5</td>
<td>28.1</td>
</tr>
<tr>
<td>10</td>
<td>34.8</td>
<td>8.7</td>
<td>32.5</td>
</tr>
<tr>
<td>50</td>
<td>35.7</td>
<td>6.3</td>
<td>30.5</td>
</tr>
<tr>
<td>250</td>
<td>36.7</td>
<td>5.2</td>
<td>28.9</td>
</tr>
</tbody>
</table>

a The assay conditions for nucleoside and deoxynucleoside kinases are essentially those described by Chu and Fischer (4) for deoxycytidine kinase. The complete reaction mixture in 0.2 ml final volume contained ATP, 2.25 x 10^{-3} M; MgCl₂, 3.5 x 10^{-3} M; the appropriate substrate (TdR³H, etc.), 0.0015 µmole; protein (crude cell-free extract), 0.2 mg; Tris buffer, 10.0 µmole (pH 7.95). The reaction system was incubated at 37° for up to 60 min, with continual gentle shaking, and terminated by heating for 2 min in a boiling water bath; the phosphorylated derivatives were separated by thin-layer chromatography (alica gel) with the solvent system methyl ethyl ketone:acetone:water (7:2:1). The plates were sectioned (1 X 5 sq cm) and scraped; radioactivity was then determined.

b The assay and work-up conditions for DNA polymerase were essentially those described by Magee (21). The reaction mixture contained (in µmoles): potassium phosphate (pH 7.4), 15; EDTA, 2; ATP, 0.05; Mg⁺⁺, 2.5; triphosphates of deoxycytidine, deoxyguanosine, and adenosine, 0.01 each; TTP³H, 0.01 (100 mCi/µmole); DNase-treated *Escherichia coli* DNA, 0.2 mg; and cell sonic extract, 0.2 mg protein in a total volume of 0.5 ml. After incubation at 37° for up to 60 min with continual gentle shaking, the reactions were stopped by the addition of carrier DNA (200 µg) and perchloric acid (to 0.5 N) containing 2% Celite. The pellet was washed with perchloric acid, dissolved in 1 N NaOH, reprecipitated, washed with perchloric acid, extracted with 0.15 ml 0.5 N perchloric acid (70°) for 20 min, and counted in 12 ml of Diotol. The enzyme rates (cpm/min) were derived from the slope of initial phosphorylation or incorporation rates calculated via a linear regression program on a Hewlett-Packard Model 9100 calculator. The Pearson coefficients (r) for those experiments were from 0.9912 to 1.000.

to detect any significant effect of CN on RNA polymerase.

Recently, Nahas (18) reported that efflux of drug from L1210 cells in drug-free medium was rapid in the beginning, although about 10% of the drug was left in the cells after 30 min. After that, however, the intracellular level did not decrease further. His results suggested that CN might be bound to certain cellular components. Since we were not able to demonstrate any significant effect on the enzymes involved in DNA synthesis, the possible interaction between CN and DNA was studied. CN inhibited the hyperchromicity that occurs when DNA strands separate ("DNA melting") on heating. The Tm of DNA was not affected by CN (Table 5). The inhibition of hyperchromicity by CN appears to be related both to the concentration of CN and to the composition and/or structure of DNA (Tables 4 and 5). When we mixed CN and DNA in the proportion (CN:DNA, 3:50) used by Gallo et al. (5), we also did not detect any significant effect of CN on the DNA. While these findings suggest interaction between CN and DNA, the nature of the interaction is not clear. Horwitz et al. (9) also reported that CN might bond to DNA under certain experimental conditions, although the conditions were not defined.

Recently, Horwitz and Horwitz (8) observed that DNA of uninfected HeLa cells and viral DNA of adenovirus-infected cells were degraded under the influence of CN, and they suggested that the degradation of template DNA could account for the inhibition of DNA synthesis. Horwitz et al. (9) also found that an intermediate in the chemical synthesis of CN was equal to CN in inhibiting RNA synthesis but was much less effective in inducing DNA degradation. This intermediate

ACKNOWLEDGMENTS

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REFERENCES

4. Chu, M. Y., and Fischer, G. A. Comparative Studies of Leukemic was inactive in vivo. We observed that CN was most cytotoxic to cells in S (Chart 2B) and that inhibition of DNA synthesis, rather than that of RNA synthesis, was closely related to the effect of CN on cell survival (Chart 7). These results collectively suggest that the marked effect on DNA synthesis is one of the primary determinants of CN cytotoxicity. However, at present, the reasons for the ready reversibility of the inhibition of RNA synthesis after the removal of CN remain unclear.

We also studied the effect of CN on progression of DON cells through the cycles and found that CN did not significantly affect mitotic cells moving through G1 phase or on the progression of G2 cells into S phase. At 100 μg/ml, CN prevented the movement of cells out of S phase, and this may be interpreted as the result of its effect on DNA synthesis (Chart 4). The most striking effect is that CN (even at 0.01 μg/ml) blocks the progression into mitosis of cells in early G2 phase or at the S-G2 boundary.

Similar results were obtained by Tobey (20), using synchronized CHO cells. We found that CN prevented the progression of G1, S, and G2 cells into mitosis when these cells were exposed to the drug for only 1 hr (Table 1). Gallo et al. (5) reported that, when "G0" lymphocytes exposed to CN were subsequently stimulated to enter the cell cycle, their diversion was still blocked. It has not been determined whether their G2 "lesion" is due to the effects of the drug on DNA synthesis or on specific RNA or protein synthesis.

Table 5

<table>
<thead>
<tr>
<th>DNA sedimentation</th>
<th>Control</th>
<th>CN, 50 μg/ml</th>
<th>Control</th>
<th>CN, 50 μg/ml</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium perfringens</td>
<td>30°</td>
<td>74.6°</td>
<td>73.2°</td>
<td>16.6</td>
<td>9.6</td>
</tr>
<tr>
<td>T4 phage</td>
<td>39</td>
<td>75.5</td>
<td>75.0</td>
<td>8.8</td>
<td>8.8</td>
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<tr>
<td>B. subtilis</td>
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<td>81.0</td>
<td>29.2</td>
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</tr>
</tbody>
</table>

a The final DNA concentration was from 0.220 to 0.250 A 260 unit.
b Arbitrary units.

The melting of DNA was determined in a buffer of NaCl and Tris-HCl, pH 7.4, 2 mM each, on a Gilford Model 2000 spectrophotometer.
Action of Camptothecin on Mammalian Cells in Culture

L. H. Li, T. J. Fraser, E. J. Olin, et al.


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