STUDIES ON CAMPTOTHECIN-INDUCED DEGRADATION AND APPARENT REAGGREGATION OF DNA FROM L1210 CELLS

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Summary Analysis of DNA from L1210 cells treated with camptothecin on alkaline sucrose gradients indicates that the cellular DNA is degraded within one minute at 37°. Degradation also occurs at 0°. Reaggregation of the DNA occurred rapidly at 37°, slowly at 0°. Acriflavine (1 µg/ml) has similar rapid effects on the sedimentation of DNA in alkaline sucrose gradients but does not rapidly inhibit RNA synthesis as does camptothecin.

Introduction The alkaloid camptothecin has antitumor activity against several rodent tumors (1,2) and its potential clinical use is currently under study. Preliminary results indicate that toxicity prevents its effective use in the treatment of human neoplasia (3,4).

Camptothecin's primary mode of action in mammalian cells appears to involve inhibition of nucleic acid synthesis. It rapidly and reversibly inhibits RNA synthesis in leukemia L1210 and HeLa cells (5-7). Inhibition and recovery of DNA synthesis is slower (8). However, camptothecin does not significantly inhibit DNA and RNA polymerases prepared from HeLa cells (6) nor nucleolar RNA polymerase prepared from rat liver (Spataro and Kessel, unpublished results). Protein synthesis is inhibited only after prolonged exposure to camptothecin (9). Nucleic acid and protein synthesis in isolated mitochondria and Escherichia coli are unaffected by the drug (10). Analysis on alkaline sucrose gradients indicates that treatment with camptothecin causes an apparent decrease in the molecular weight of HeLa cell DNA (6,11). Recently it has been shown that only low molecular weight nucleoplasmic and nucleolar RNA is made in HeLa cells in the presence of camptothecin (12). A possible explanation for the rapid inhibition of high molecular weight RNA

synthesis by camptothecin could involve drug-induced degradation of the DNA template, if this phenomenon occurred with sufficient speed. In the present study we have examined the rate of camptothecin induced degradation of DNA from L1210 cells, together with some factors involved in recovery of the DNA from such degradation.

Methods and Materials Exponentially growing L1210 cells, maintained in minimum essential Eagle's medium plus 10% fetal calf serum as previously described (9), were labeled for 24 hours with [methyl-^{14}C]-thymidine (54.7 mCi/mmole). The cells were collected by centrifugation, resuspended in fresh medium and incubated for 30 min at 37°. The cells were then incubated in minimum essential Eagle's medium plus Hepes buffer plus 10% fetal calf serum with camptothecin for varying amounts of time and under different conditions. Camptothecin (sodium salt, USC 100 880), provided by the National Cancer Institute Drug Development Branch, was freshly dissolved in distilled water for use. To terminate the incubations the cells were centrifuged at 1000 x g at 4° or at room temperature for recovery studies. According to the method of Horowitz (11), 1 mg (wet wt., approx. 5 x 10^5 cells) in 0.3 ml of 0.9% NaCl was layered on an alkaline sucrose gradient (5-20% sucrose, 1 M NaCl, 0.01 M EDTA and 0.19 M NaOH) which was overlaid with a 0.5 ml lysing layer (0.5% deoxycholate, 0.19 M NaOH, 0.01 M EDTA). Neutral gradients were similarly prepared,with the omission of NaOH and substitution of 1% deoxycholate in the lysing layer. Para-aminosalicylate (0.06 M) was added to the neutral gradients. The gradients were centrifuged in an SW41 rotor at 5° for 15 hours in a Beckman ultracentrifuge at 15,000 rpm. After centrifugation the gradients were fractionated from the top using a Buchler densiflow and a fraction collector. Fifteen fractions of 0.75 ml were collected. The last fraction was obtained by rinsing the bottom of the tube with 0.9% NaCl. The fourteenth fraction was a layer of CsCl (1.8g/ml). Trichloracetic acid was added to each fraction to a final concentration of 12.5%. The precipitates were

washed with 5% trichloracetic acid on Whatman GF/A glass fiber filters. Radioactivity on the filters was measured by liquid scintillation counting.

Results and Discussion The results shown in Figure 1 indicate that under the described conditions, 90% of the DNA from control cells sedimented into the CsCl layer and the bottom of the centrifuge tube. After treatment of L1210 cells for 10 min with 10 μg/ml camptothecin, 90% of the DNA was found on the gradient. This is in agreement with the earlier report of DNA degradation by camptothecin (11). 100 μg camptothecin/ml produced a shift in the sedimentation profile toward the top of the gradient. The duration of exposure to camptothecin did not appear to affect the amount of degradation; that is, at 37° with 10 μg camptothecin/ml degradation seemed to be complete within 1 min of incubation. The sedimentation patterns of DNA on alkaline sucrose gradients from cells incubated with 10 μg camptothecin/ml for 1 to 90 min were the same. Degradation also occurred after incubation of the cells with camptothecin at 0° for 10 min. When the DNA from camptothecin treated cells was analyzed on neutral gradients, degradation was not seen. This implies that camptothecin induces single strand breaks in DNA. However, the possibility that camptothecin makes DNA labile to alkaline hydrolysis in the gradient cannot be eliminated. The exact biological mechanism for the shift in molecular weight of camptothecin treated DNA is unclear. The possibility that camptothecin might influence the activity of an endonuclease has been suggested (11). The lack of temperature dependence and the fact that degradation is complete in 1 min may rule out enzymatic processes, but the evidence is not conclusive since many enzymes can function at low temperatures.

Figure 2 shows that DNA "recovers" rapidly from the effects of 10 μg/ml camptothecin. "Recovery" only implies that it was again possible to centrifuge the DNA to the bottom of the gradient under the described experimental conditions. A quick wash of the cells with warm medium allows some recovery. After 5 min at 37° most of the DNA again sedimented to the bottom of the

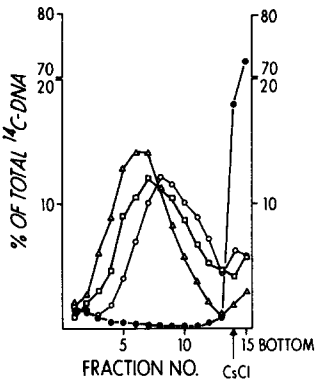

Fig. 1.

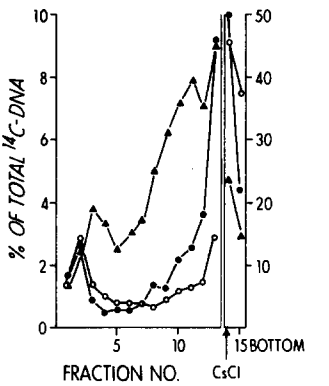

Fig. 2.

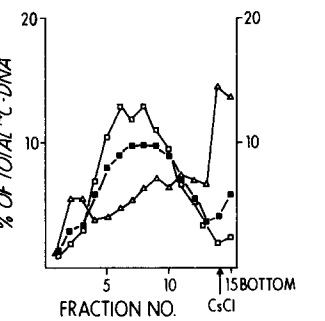

Fig. 3.

Fig. 1. Sedimentation of DNA from L1210 cells labeled with [methyl-^{14}C]-thymidine for 24 hours and incubated with: ●—●, no, ▫—▫, 10 µg/ml, or Δ—Δ, 100 µg/ml camptothecin for 10 min at 37°; or O—O, 1 µg/ml acriflavine for 10 min at 37°. Experiments were performed as described in Methods.

Fig. 2. Recovery at 37° of DNA from 10 µg/ml CN for 10 min. Δ—Δ, washed with warm medium; O—O, 5 min recovery; O—O, 30 min recovery. Experiments were performed as described in Methods.

Fig. 3. Recovery at 0° of DNA from 10 µg/ml at 37°: ▫—▫, camptothecin always present; ■—●, camptothecin for 10 min, cells spun down at 0° and immediately layered on gradient; Δ—Δ, camptothecin for 10 min, cells spun down at 0° and left on ice for 90 min before layering on gradients. Experiments were performed as described in Methods.

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centrifuge tube. Recovery beyond that observed at 30 min was not found even at 3 hours. After 3 hours of recovery at 37°, the camptothecin treated DNA did not sediment exactly as did the DNA from control cells. Approximately 70% of the DNA from control cells was always found in the bottom of the tube after centrifugation for 15 hours at 15,000 rpm. After treatment with 10 µg camptothecin/ml, about 40% of the DNA sedimented to the bottom of the centrifuge tube. The remainder was shifted into the CsCl layer. DNA from cells treated with 100 µg/ml camptothecin also recovers rapidly if the cells are washed.

In order to determine what metabolic processes were necessary for recovery,

attempts were made to prevent reformation of high molecular weight DNA after camptothecin treatment. Figure 3 illustrates that even at 0°, unless camptothecin is present, some recovery will occur; 90 min at 0° does not allow full recovery of DNA. Since a slow recovery does occur at 0°, the recovery, like the degradation, may not require an enzymatic process. Hydroxyurea (10 mM), an inhibitor of DNA synthesis, did not slow reaggregation of DNA. Cycloheximide (10 μg/ml), an inhibitor of protein synthesis, had variable effects; in some preparations, the drug slowed reaggregation of DNA from camptothecin treated cells.

The presence of acriflavine (1 μg/ml), which intercalates with DNA, prevented recovery. However, as illustrated in Figure 1, acriflavine itself alters the sedimentation pattern of DNA on alkaline sucrose gradients. Degradation by acriflavine (1 μg/ml) is similar to, although not as great as, that produced by camptothecin (10 μg/ml). Increasing the concentration of acriflavine to 10 μg/ml did not produce more degradation. The alteration is complete within 1 min at 37° and occurs at 0°. Recovery from 1 μg acriflavine/-ml occurs within 5 min at 37° after removal of the drug. Acriflavine (1 μg/ml) for 10 min at 37° does not inhibit [^{14}C]uridine incorporation into RNA (unpublished results). Labeling of total acellular RNA is rapidly and substantially inhibited by 1 μg/ml camptothecin (12).

Since the degradation and recovery of DNA from camptothecin treatment are both rapid, degradation of the template may be the basis for the inhibition of high molecular weight RNA synthesis. However, acriflavine under the described conditions produces a similar rapid alteration of the molecular weight of DNA without inhibiting RNA synthesis. This suggests that degradation of template is not a sufficient explanation for the inhibition of high molecular weight RNA synthesis.

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