INTRACELLULAR DEGRADATION OF HeLa AND ADENOVIRUS TYPE 2 DNA INDUCED BY CAMPTOTHECIN*

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SUMMARY. The addition of camptothecin, an inhibitor of nucleic acid synthesis, to HeLa cells or adenovirus-infected HeLa cells results in the partial degradation of cellular and viral DNA. There is no detectable release of acid soluble nucleotides.

Camptothecin, a cytotoxic plant alkaloid, has antitumor activity against leukemia L1210 in mice and Walker 256 tumors in rats (1). The alkaloid is under investigation as an antineoplastic agent in man (2). The structure of the compound isolated from the tree, Camptotheca acuminata, has been elucidated by Wall et al (3).

Camptothecin inhibits the synthesis of RNA and DNA but not protein in HeLa cells (4,5,6) and in L1210 cells (7). It inhibits the synthesis of adenovirus DNA but not viral protein in type 2 adenovirus-infected HeLa cells (8). However, camptothecin does not significantly inhibit the activity of DNA and RNA polymerases prepared from HeLa cell extracts (5). This report presents evidence that camptothecin promotes the partial degradation of both HeLa cell DNA and adenovirus DNA within the cell.

METHODS. Sedimentation techniques were used to study the effect of the sodium salt of camptothecin on the size of adenovirus DNA. Camptothecin was obtained from the United States Cancer Chemotherapy National Service

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Center, Bethesda, Maryland. A suspension culture of HeLa cells in Eagle's minimum essential medium (9) was infected with 1,000 adenovirus particles per cell. At 18 hours after infection, when greater than 85% of the newly made DNA is viral specific (10), 4×10^6 cells in 20 ml were labeled with ^{3}H-thymidine (1 μc/ml, 16 C/mM) for 1 hour at 37°. The cells were then washed once in Eagle's medium containing 20 μM unlabeled thymidine and incubated in 6 ml of medium. Thirty minutes later, camptothecin at a final concentration of 2×10^{-5} M was added to one half of the cells and both cultures were incubated for an additional 60 minutes. After incubation the cells were sedimented at 200 g and resuspended in 0.5 ml of 0.15 M NaCl. The intact cells were layered onto alkaline sucrose gradients (0.5 ml of CsCl, density 1.8 gms/ml, at the bottom of a 15 ml linear 5-20% sucrose gradient, prepared in 1.0 M NaCl, 0.01 M EDTA and 0.19 M NaOH, overlaid with 0.5 ml of 0.5% sodium deoxycholate in the same alkaline solution). The gradients were centrifuged for 15 hours at 24,000 rpm in the SW 27 rotor (Spinco). This method minimizes mechanical shearing of the DNA because the intact cells are placed on the gradient. Each gradient included purified ^{14}C-thymidine-labeled virus as a sedimentation marker. Fractions of 0.6-0.7 ml were collected beginning at a position 2.5 cm above the bottom of the tube. The solution remaining in the tube, called the "pellet", was disrupted by sonic oscillation for 20 seconds and all fractions including the sonicated material were adjusted to a final concentration of 12.5% trichloroacetic acid. After standing for 15 minutes at 4°C, precipitates were collected on Millipore membrane filters and the radioactivity determined by scintillation counting.

RESULTS AND DISCUSSION. The effect of camptothecin on the sedimentation of viral DNA is shown in Fig. 1. Viral DNA from untreated cells sedimented to fraction 8 (34s) but the viral DNA from camptothecin treated cells sedimented slower. The radioactivity in the pellet represents the small amount of HeLa cell DNA synthesized in the infected cell (10). There was no

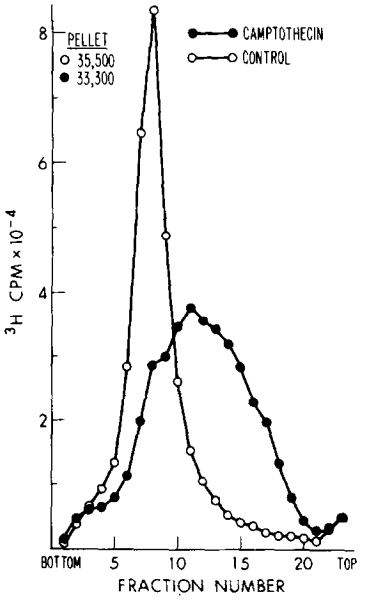

Fig. 1. Sedimentation of DNA from adenovirus infected HeLa cells (o——o) and camptothecin-treated infected cells (●——●) in alkaline sucrose gradients. The "pellet" represents the acid insoluble ^{3}H-thymidine in the 2.5 cm remaining at the bottom of the gradient.

detectable decrease of acid insoluble radioactivity after camptothecin treatment.

Adenovirus infected cells have recently been shown to have endonucleolytic activity within the penton, a structural component of the virus (11). Therefore, camptothecin was added to uninfected HeLa cells to examine the effect of the drug on DNA in the absence of the penton endonuclease.

The effect of camptothecin on the sedimentation of DNA from uninfected HeLa cells is shown in Fig. 2. The conditions of labeling, washing and camptothecin treatment were the same as for the infected cells except that the uninfected cells were incubated with the drug for 30 minutes and subsequently centrifuged for 15 hours at 15,000 rpm. Most of the DNA from untreated HeLa cells sedimented to the bottom of the gradient as high molecular weight DNA. After treatment of HeLa cells with 2×10^{-5} M camptothecin, approximately 90% of the DNA no longer sedimented to the bottom of the gradient. The 34s viral marker sedimented to fraction 10 on this gradient.

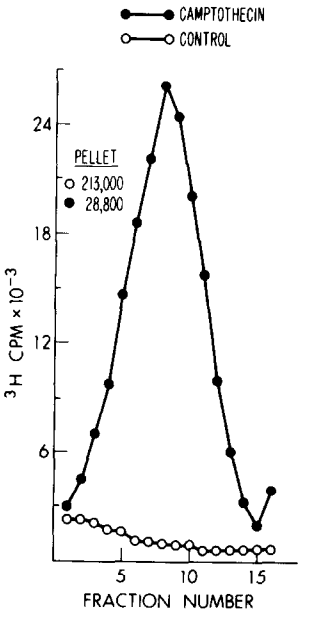

Fig. 2. Sedimentation of DNA from HeLa cells (o——o) and camptothecin-treated HeLa cells (•——•) in alkaline sucrose gradients. The "pellet" represents the acid insoluble ^{3}H-thymidine in the 2.5 cm remaining at the bottom of the gradient. Sedimentation is from right to left.

We interpret the change in sedimentation of both HeLa cell DNA and viral DNA to represent partial degradation of these molecules in the presence of camptothecin. Since camptothecin does not degrade purified adenovirus DNA (8), the compound most likely influences the activity of an endonuclease present in HeLa cells. This nuclease does not appear to be the adenovirus penton, since a change in the sedimentation of DNA is observed in uninfected cells.

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