Camptothecin cytotoxicity in mammalian cells is associated with the induction of persistent double strand breaks in replicating DNA

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ABSTRACT

Camptothecin is a specific topoisomerase I poison and is highly cytotoxic to eukaryotic cells. In the present study, we show, using a pulse field gel electrophoresis assay, that camptothecin induces DNA double strand breaks (DSBs) specifically in newly replicated DNA. Camptothecin induces these replication associated DNA DSBs in a dose-dependent manner. At levels of the drug which are toxic to the cell, these breaks are long-lived, and still measurable 24 hr after treatment. Both camptothecin induced DSBs and cytotoxicity are prevented by co-exposure with aphidicolin—a result which indicates that ongoing DNA synthesis is required for the production of DNA DSBs and cell killing. It has been proposed that camptothecin toxicity involves an interaction between the replication machinery and a drug-mediated topoisomerase I-DNA cleavable complex. The present work indicates, for the first time in mammalian cellular DNA, that one possible outcome of this interaction is a replication-associated DSB, a lesion which is likely to be highly cytotoxic.

INTRODUCTION

Topoisomerases play a central role in DNA replication, transcription and segregation (1). Certain antitumour drugs have been found to inhibit either topoisomerase I or II, and are widely used as tools for assessing the roles of each enzyme in vivo (2, 3). Camptothecin is a cytotoxic alkaloid with strong antitumour activity whose intracellular target is eukaryotic topoisomerase I (3). Camptothecin inhibits both RNA and DNA synthesis, and causes fragmentation of cellular DNA (4). The drug interferes with the topoisomerase I DNA breakage-reunion activity, stabilising a reaction intermediate known as a 'cleavable complex' (3, 5). Treatment of these cleavable complexes with SDS or alkali exposes a single-strand DNA break in which the topoisomerase is covalently linked to the 3′ end of the broken DNA strand (3, 6). Based on the localisation of camptothecin-induced topoisomerase I cleavage sites, it has been shown that the enzyme is involved in transcription and is also part of the DNA replication apparatus (7). Although there is strong evidence that camptothecin-mediated cell killing involves cleavable complex formation on chromosomal DNA, several studies have shown that camptothecin toxicity does not correlate with the frequency of induced cleavable complexes. For example, camptothecin has been shown to be specifically toxic to cells in S phase, though the levels of topoisomerase I and drug-induced cleavable complexes appear relatively constant throughout the cell cycle (4, 8, 9, 10). The S phase specificity of camptothecin suggests that some interaction between cleavable complexes and the replication machinery may be critical to the mechanism of cytotoxicity (1, 11). A model for camptothecin toxicity has been suggested from results obtained by the study of SV40 replication products (1, 12, 13, 14). It has been proposed that there is an interaction between moving replication forks and topoisomerase I cleavable complexes resulting in irreversible fork arrest, and the conversion of the cleavable complex to an irreversible enzyme-linked DNA strand-break. These breaks, being at the replication fork, will manifest as double-strand DNA breaks and might be expected to be highly toxic (11).

In the present work we show using a pulsed-field gel electrophoresis assay, that camptothecin specifically induces double strand breaks in replicating cellular DNA. Results obtained with aphidicolin, an inhibitor of DNA replication, indicate that ongoing DNA synthesis is required for both camptothecin toxicity and the induction of DNA double strand breaks. At toxic concentrations of camptothecin, double strand DNA breaks persist, and can be detected 24 hr after their induction. These persistent long-lived double strand breaks induced in nascent DNA, presumably by collision of replication forks with cleavable complexes, are postulated as the lethal lesions in camptothecin treated cells.

MATERIALS AND METHODS

Camptothecin (sodium salt, Sigma) was dissolved in dimethylsulphoxide and stored in small aliquots at concentrations of 1−10 mM at −20°C. The drug was protected from visible light and, immediately before use, diluted in growth medium. Eagles' minimal essential medium (MEM), vitamins and essential amino acids were purchased from Life Technologies Ltd. and foetal calf serum was from ICN Flow. Agarose (5510UB) for gel electrophoresis, and low melting point agarose (5517UB) for embedding cells, were obtained from Bethesda Research Laboratories Inc.

Cell culture and cytotoxicity measurements

Two human lines were used, SV40-transformed skin fibroblasts (SV40MRC5VI—a gift from MRC Cell Mutation Unit, Brighton) and a clone of a bladder carcinoma line (EJ30/8D). Cells were grown in MEM containing 5% foetal calf serum. The toxicity of drugs was assayed by cell proliferation. One day after seeding, cells were exposed for 60 min to different concentrations of freshly prepared camptothecin. After removal of the drug, cultures were washed in warm medium or in phosphate buffered saline (PBS), and incubated further in the appropriate growth medium. The rate of proliferation was determined by cell counting (Coulter Electronics Inc.) twice in the period 3–6 days after seeding, and is expressed as a relative cell number compared to untreated controls.

Camptothecin-induced DNA damage

2×10^5 cells were seeded in 60 mm dishes. After 4 hr, fresh medium containing [^{14}C]thymidine (0.1 μCi/ml, 57 mCi/mmol) was added. 48 hr later the label was removed and unlabelled medium added. After a further 16 hr the cells were exposed to various concentrations of camptothecin (0–2.5 μM) for a period of 50 min in fresh medium containing [^{3}H]thymidine (1 μCi/ml 42–50 Ci/mmol). The drugs and label were removed and the cells washed twice in PBS and thereafter embedded in agarose immediately (see below), or after further incubation in unlabelled medium for various lengths of time.

In order to examine the effect of aphidicolin cells were labelled with [^{14}C]thymidine as above, and then given a 5 min pulse of [^{3}H]thymidine (1.5 μCi/ml). Following this pulse cells were given a 5 min exposure to aphidicolin (0 or 2.5 μM), after which camptothecin was added (0 or 1 μM) and incubation continued for a further 50 min. The drugs were removed and the cells assayed for double strand DNA breaks immediately or after a 24 hr period in growth medium.

Asymmetric field inversion gel electrophoresis (AFIGE)

The gel electrophoresis system developed by Stamato and Denko was used (15, 16). Briefly cells were washed, detached with viokase, spun down, and resuspended at a concentration of $0.3-1.0\times10^7$ cells/ml in 0.8% low melting point agarose at 37°C. The agarose/cell mixture was taken up into 3 mm internal diameter tubing, solidified on ice, and cut into 5 mm pieces. These agarose plugs were incubated overnight at 50°C in 0.5 M EDTA pH 8.0, 1% sarkosyl (N-lauroyl sarcosinate) and 50 μg/ml proteinase K. Electrophoresis was carried out in 1.5% agarose gels using 45 mM Tris, 45 mM Boric acid, 1.5 mM EDTA pH 8.5, containing 0.025 μg/ml ethidium bromide. The pulse conditions were 5 V/cm for 125 sec in the direction of net DNA migration and 10 V/cm for 15 sec in the reverse direction, for a total run time of about 6 hr. The temperature during electrophoresis was maintained at 10–14°C using cooled recirculating buffer. Using these conditions DNA which enters the gel runs as a band which can be visualised under ultraviolet light. The fraction of radioactivity released (FAR) can be calculated by determining the radioactivity present in the gel band and that remaining in the plug. For all experimental conditions, the low FAR values of the untreated controls were deducted from the values of the drug treated cells.

Irradiation

Irradiations were performed with a Mainance 500C machine using a [^{137}Cs] gamma ray source at a dose rate of 1 Gy/min.

RESULTS

The AFIGE assay for DNA double-strand breaks

A method for detection of DNA double-strand breaks (DSB) using asymmetric field inversion gel electrophoresis (AFIGE) has been described (15, 16). In this method cells are first embedded in agarose and then DNA purified by incubation in a solution containing sarkosyl and proteinase K. Plugs of agarose, containing naked DNA, are subjected to AFIGE under conditions where: (i) only DNA below a certain size (<3 Mbp) enters the gel; (ii) DNA which has entered the gel then migrates in a size-independent manner (6 Kbp–3 Mbp) and can be visualised as a band if ethidium bromide is present; (iii) the amount of DNA which is released from the plug into the gel is a measure of DSBs in that DNA.

Single strand DNA breaks do not lead to release of DNA in the AFIGE assay (16). Using radiolabelled precursors, the extent of DSBs in a sample can be determined by excising both the band

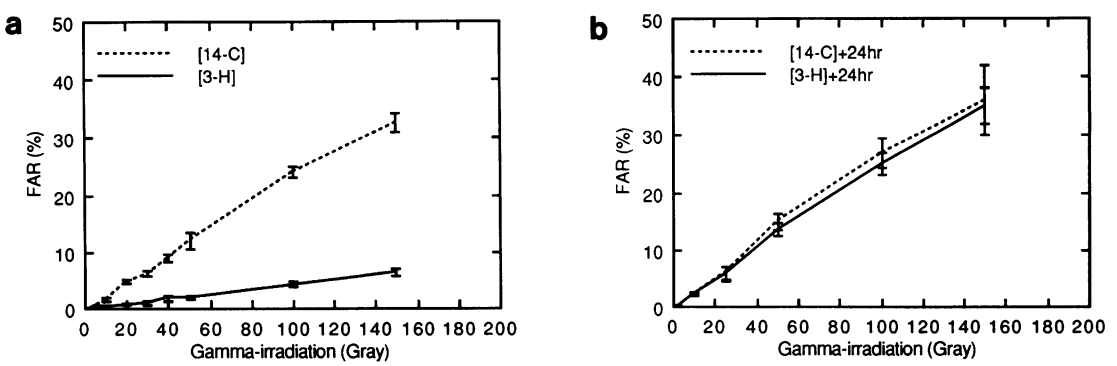

Figure 1. AFIGE assay of DSBs induced in bulk and newly replicated DNA by gamma irradiation. SV40MRC5 cells were labelled in bulk DNA with [^{14}C]thymidine and then labelled for 50 min with [^{3}H]thymidine, and DNA prepared in agarose plugs immediately (Figure 1a) or after a 24 hr chase period (Figure 1b). Agarose plugs were exposed to various doses of gamma-irradiation, and analysed by AFIGE. Double strand DNA breaks are expressed as fraction radioactivity released (FAR%) which is a measure of the proportion of DNA which leaves the agarose plug and enters the gel. The error bars represent ±S.E.M. for at least four plugs from two or more independent experiments.

of migrating DNA and the agarose plug from the gel following AFIGE, counting the radioactivity present in each, and expressing the results as fraction activity released (FAR%).

Exponentially growing SV40MRC5 cells, uniformly labelled with [^{14}C]thymidine, were pulse-labelled for 50 min with [^{3}H]thymidine to identify replicating and newly replicated DNA. Figure 1a shows the response of uniformly labelled and pulse-labelled DNA to gamma irradiation-induced DSBs measured in the AFIGE system. The dose-responses of both bulk and replicating DNA are approximately linear with increasing irradiation. Gamma irradiation of bulk DNA at less than 2 Gy, or replicating DNA at less than 4 Gy, does not produce a measurable release of radioactivity (FAR%) by AFIGE, and these values represent the limits of detection of this assay under these conditions. Significantly, the dose-response of replicating DNA is much reduced in comparison to uniformly labelled DNA. 100 Gy gives a FAR value of 24% for uniformly labelled DNA but only 4.3% (i.e. 5.6 fold less) for pulse-labelled DNA. Stamato and Denko (16) observed a 2.9 fold difference between bulk and replicating CHO hamster DNA, and in general the extent of the reduced response of replicating DNA in the AFIGE assay is likely to be dependent on cell-type. For example, we observe a 3.6 fold difference in EJ30, and a 2.9 fold difference in HeLa (results not shown). The reduced response of pulse labelled newly replicated DNA compared to bulk DNA in the AFIGE assay is not likely to be due to different amounts of radiation-induced damage in the two DNA classes, since a similar difference is maintained whether intact cells, or DNA which was purified from these cells are irradiated, suggesting the altered AFIGE response is the result of the physical nature of the replicating DNA, and not due to shielding by chromosomal proteins (16). The reduced response of pulse-labelled DNA is replication associated since a chase period of 24 hr elicits a similar AFIGE response in both [^{3}H] pulsed DNA and [^{14}C] bulk labelled DNA following gamma irradiation (Figure 1b). Retardation in agarose of DNA which contains forks or eyes may contribute to the unusual migration response to AFIGE of replicating gamma irradiated DNA. Another possibility is that replicating DNA may remain tightly bound to a residual, insoluble nuclear matrix which does not allow free migration into the agarose.

Camptothecin preferentially induces DSBs in replicating DNA

SV40MRC5 cells were uniformly labelled with [^{14}C]thymidine and subsequently pulse-labelled for 50 min with [^{3}H]thymidine in the presence of 2.5 μM camptothecin to label DNA which was synthesised during drug treatment. Although the presence of camptothecin reduced the incorporation of [^{3}H]thymidine into DNA when compared to control cells (for example, 1 μM and 2.5 μM camptothecin gave 35% and 30% of control [^{3}H] incorporation respectively), sufficient label was incorporated to allow further analysis . Both drug and label were washed away and agarose plugs of purified DNA prepared for AFIGE analysis after a 0, 2, 5 or 24 hr chase period. Figure 2 shows that immediately after camptothecin exposure only a very low level of DSBs could be detected in [^{14}C] labelled DNA (FAR = 0.25 $\pm$ 0.05%). In contrast camptothecin induced a much greater release of pulse-labelled DNA (FAR = 2.1 $\pm$ 0.1%) indicating a preferential induction of DSBs in DNA replicating at the time of drug exposure. The specific release of nascent DNA is more marked considering the experiments using ionising radiation (Figure 1a) which show that the nature of [^{3}H] pulse-labelled DNA leads to a 5.6 fold reduction in FAR compared to bulk DNA, for a given amount of DNA damage.

Gamma irradiation of agarose plugs prepared from camptothecin treated SV40MRC5 gave a similar AFIGE response to the control SV40MRC5 shown in Figure 1. Immediately after a 50 min exposure to camptothecin and [^{3}H]thymidine, (after subtracting FAR due to camptothecin alone) the pulse of [^{3}H] label was released about 5.6 fold less per Gy irradiation than [^{14}C]-labelled bulk DNA. Following a 24 hr chase period both [^{14}C] and [^{3}H] were released equally (data not shown). This indicates that camptothecin does not alter the nature of [^{3}H]-labelled replication-associated DNA such that it is released from agarose plugs in the AFIGE assay with greater efficacy than normal. Rather the evidence suggests that DNA which is pulse-labelled during camptothecin treatment is of a similar nature to DNA replicating in untreated cells in that it is released to a reduced extent compared to bulk DNA in the AFIGE assay.

Following camptothecin exposure, the release of replicating DNA by AFIGE reflects a highly specific induction of DSBs by the drug at, or close to, sites of DNA synthesis. Some repair or processing of the camptothecin-induced DSBs is indicated in

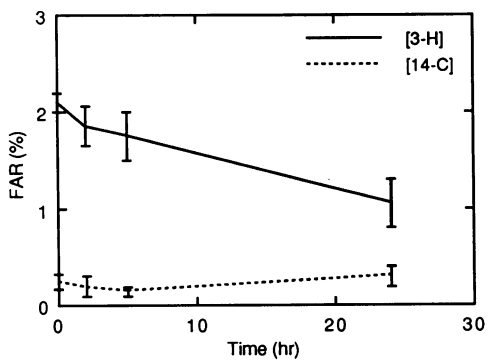

Figure 2. Camptothecin induced DSBs preferentially in newly replicated DNA. SV40MRC5 cells were labelled as in Figure 1 except that during the 50 min exposure to [^{3}H]thymidine cells were co-incubated with camptothecin (2.5 μM). Cells were embedded in agarose immediately or after chasing for various periods of time in unlabelled medium without camptothecin. DNA was then analysed for DSBs by AFIGE. The error bars represent $\pm$ S.E.M. for at least four plugs from two or more independent experiments.

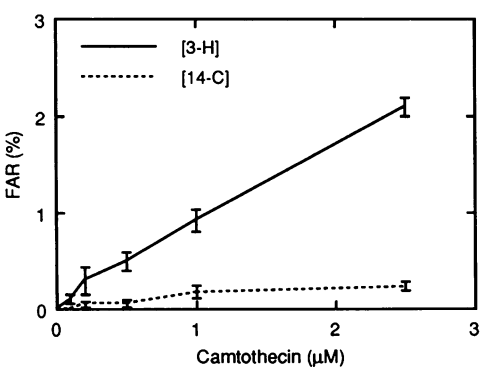

Figure 3. Dose response of camptothecin induced DSBs in bulk and newly replicated DNA. SV40MRC5 cells were labelled as in Figure 1 except that during the 50 min exposure to [^{3}H]thymidine cells were also incubated with various concentrations of camptothecin (0–2.5 μM). Cells were immediately embedded in agarose and assayed for DNA DSBs by AFIGE. The error bars represent $\pm$ S.E.M. for at least four plugs from two or more independent experiments.

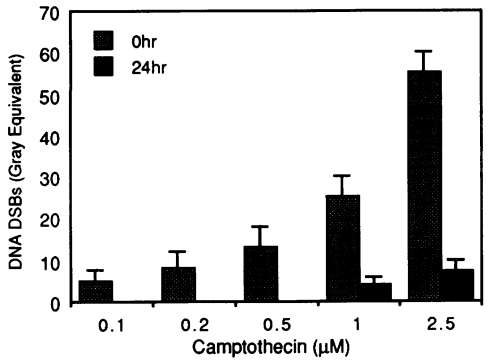

Figure 4. Quantitation of camptothecin induced DSBs in newly replicated DNA. SV40MRC5 cells were labelled and treated with camptothecin as in Figure 3. Cells were embedded in agarose immediately or after a 24 hr chase period in unlabelled medium without drugs. DNA DSBs in DNA nascent at the time of drug exposure were assayed by AFIGE. Results are expressed as Gray equivalent DSBs (see text for details). No DNA DSBs were detectable 24 hr after treatment with less than 1 μM camptothecin (AFIGE limit of detection = 2 Gray). The error bars represent ± S.E.M. for at least four plugs from two or more independent experiments.

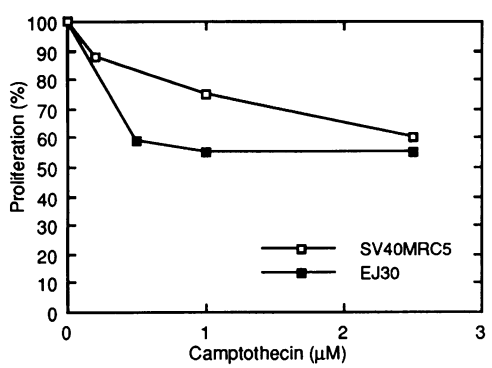

Figure 5. Inhibition of cell proliferation by camptothecin. Camptothecin cytotoxicity, measured by inhibition of cell proliferation, was determined for SV40MRC5 and EJ30 over a range of drug concentrations (0–2.5 μM).

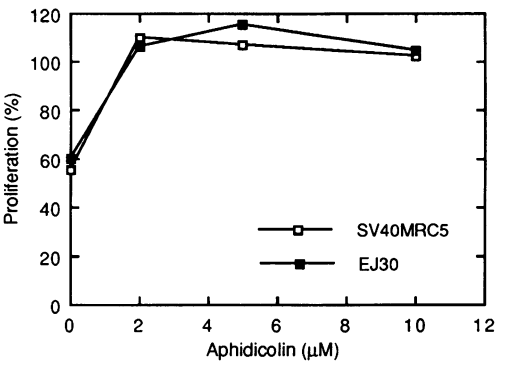

Figure 6. The effect of aphidicolin on inhibition of cell proliferation by camptothecin. Cells were incubated for 1 hr in the presence of camptothecin (1 μM—EJ30, 2.5 μM—SV40MRC5) together with various concentrations of aphidicolin (0–10 μM). Inhibition of cell proliferation was measured 4–6 days later. Results are expressed relative to cells treated with aphidicolin alone.

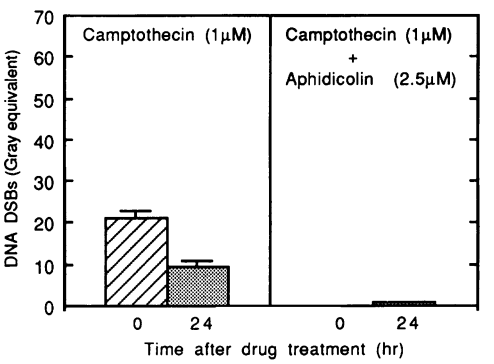

Figure 7. The effect of aphidicolin on camptothecin induced DSBs in newly replicated DNA. EJ30 cells were prelabelled with [^{3}H]thymidine and then incubated for 50 min in the presence of camptothecin (1 μM) and aphidicolin (0 or 2.5 μM). Cells were embedded in agarose immediately, or after a 24 hr chase period in unlabelled medium without drugs. DSBs were assayed by AFIGE and the results converted to Gray equivalent DSBs (see text) using a standard curve (Figure 1) constructed for EJ30 (data not shown). The error bars represent ± S.E.M. for four independent plugs.

Figure 2. Immediately after drug exposure, when replication-associated DNA is released poorly, a [^{3}H] FAR of 2.0 ± 0.1% is observed whereas 24 hr following the camptothecin-induced damage, when pulse-labelled DNA is released to a 5.6 fold greater extent for a given number of DSBs, the FAR is 1.08 ± 0.34%. The preferential induction of DSBs in replicating DNA is apparent over a range of camptothecin concentrations, and the increase in [^{3}H] FAR is approximately linear with dose up to 2.5 μM, the highest drug concentration tested (Figure 3).

Quantitation of camptothecin-induced double strand breaks in replicating DNA

Camptothecin-induced double strand DNA breaks in replicating DNA,measured as [^{3}H] FAR (%) in the AFIGE assay, can be expressed as Gray equivalent double strand breaks (i.e. the amount of gamma-irradiation measured in Gy which gives an equivalent FAR value). This is essential when comparing different cell types, where the relative response of replicating and bulk DNA in the AFIGE assay can vary, and also for measuring induced DNA damage at different times after various treatments (17). Immediately after drug treatment FAR in replicating DNA can bei expressed in Gray equivalent double strand breaks by using the lower curve in Figure 1a. After a 24 hr chase the [^{3}H] FAR can be expressed in Gray equivalents by using the lower curve in Figure 1b. Figure 4 shows such a transformation and it can be seen that at concentrations of camptothecin which are toxic to SV40MRC5 (1 μM and 2.5 μM, Figure 5) long-lived double strand breaks are detectable (for example 7.2 Gy equivalent at 24 hr for 2.5 μM camptothecin) in DNA nascent at the time of drug exposure, although a significant amount of repair seems to take place during this period. Transforming the data from Gy equivalent double strand DNA breaks to DNA DSB/cell is not yet straightforward. It has been estimated that 1 Gy of ionising radiation causes about 0.25–1.0 DSB/1.25×10^8 bp (18, 19). This corresponds to about 20–80 DSB/cell in SV40MRC5 assuming about 10^{10} bp/cell. Camptothecin specifically interacts with topoisomerase I and induces DNA DSBs in newly synthesised DNA which will probably lead to a clustering of these lesions at replication forks.

This makes difficult a direct numerical calculation of DSBs/cell by comparison to random DSBs induced by gamma irradiation.

The induction of double strand DNA breaks by camptothecin requires ongoing DNA synthesis

A model has been proposed whereby camptothecin cytotoxicity involves a collision of the DNA replication machinery with an immobilised topoisomerase I cleavable complex (1, 11). It has been reported that aphidicolin, a potent inhibitor of DNA polymerases and of DNA replication, can almost totally protect cells against camptothecin cytotoxicity, presumably by stalling/stopping the replication machinery and so preventing its collision with the topoisomerase I cleavable complex (7). We observed that co-incubation with aphidicolin can protect both SV40MRC5 and EJ30 against camptothecin-induced cell killing (Figure 6).

In order to test if aphidicolin could also protect against camptothecin-induced DSBs in newly replicated DNA of EJ30, a line more sensitive to the cytotoxic effects of camptothecin than SV40MRC5 (Figure 5) an experiment was devised whereby cells were pulse-labelled for 5 min with [^{3}H]thymidine. Following this pulse, cells were treated with aphidicolin (0 or 2.5 μM) and then 5 min later with camptothecin (1 μM). After a further 50 min incubation, drugs were washed out and samples prepared for AFIGE assay immediately or after a further 24 hr chase period. Camptothecin (1 μM) induces a similar number of replication-associated DSBs in EJ30 (21 $\pm$ 2.5 Gy equivalent) and SV40MRC5 (26 $\pm$6 Gy equivalent: compare Figures 7 and 4), although EJ30, the more sensitive line, has higher levels of drug-induced long-lived DSBs measured at 24 hr (EJ30 = 10 $\pm$ 1.0 Gy equivalent; SV40MRC5 = 3.8 $\pm$ 1.5 Gy equivalent). The presence of aphidicolin during camptothecin exposure abolishes the induction of DSBs in [^{3}H] pulse-labelled DNA. The camptothecin-induced topoisomerase I lesions associated with replicating DNA are rapidly and fully reversible, since when camptothecin and aphidicolin are removed no DSBs associated with the [^{3}H] pulse are observed, even after 24 hr. These results indicate that ongoing DNA synthesis during camptothecin exposure is required to generate DSBs at or near to replication forks.

DISCUSSION

In the present work we have examined whether the specific cytotoxic effect of camptothecin on S-phase mammalian cells can be explained by models developed from studies of the effects of the drug on SV40 replication in vitro and in vivo. Topoisomerase I appears to be the sole cytotoxic target of camptothecin, and the most attractive model proposes that interaction between a moving replication fork and a camptothecin-induced topoisomerase I cleavable-complex results in irreversible fork arrest and conversion of a reversible cleavable-complex into an irreversible enzyme-linked DNA strand break. A single strand break in parental strand DNA, exposed when the replication complex collides with a cleavable-complex, would be expressed as a DSB if the replication machinery halts at or near to this site; a result which has been confirmed by study of SV40 systems (12, 13, 14). We have shown that camptothecin induces DSBs in the replicating DNA of mammalian cells. This induction of DSBs is highly localised, with only very low levels of DSBs measured in bulk DNA at early times after drug treatment. Camptothecin-induced cytotoxicity requires ongoing DNA synthesis, which is also required for the generation of DSBs in replicating DNA. There is much evidence that DSBs are likely to be major cytotoxic lesions to the cell (20, 21, 22). The induction of DSBs in replicating DNA, with a requirement for ongoing synthesis, helps to explain the S phase specificity of camptothecin toxicity. We show that camptothecin-induced DSBs in replicating DNA disappear with time, although long-lived DSBs are still apparent 24 hr after induction at cytotoxic doses of drug. The cytotoxic nature of camptothecin and the presence of long-lived DSBs in cells indicates that these lesions are particularly difficult for cells to deal with. A given dose of camptothecin may be differentially cytotoxic to different cell types. In the present study we show that camptothecin induces similar levels of DSBs in the replicating DNA of two cell lines (SV40MRC5 and EJ30), but it is the level of long-lived DSBs which correlates better with drug sensitivity. Cell specific differences in the ability to process camptothecin-induced DSBs may account for differential drug sensitivity.

A caveat to the AFIGE measurement of DSBs in replicating DNA relates to the actual nature of the lesion in the nucleus. Sarkosyl treatment of samples prior to analysis means that the AFIGE assay might be expected to measure both frank DSBs, and cleavable complexes on single stranded DNA (gapped or nicked), or at stalled replication forks. Although the true nature of the DSBs measured by AFIGE in replicating DNA immediately after camptothecin treatment is difficult to ascertain at the present time, it is clear that S-phase camptothecin exposure is a potent inducer of sister chromatid exchanges and chromosomal (chromatid-type) aberrations, including chromatid breaks and gaps (23, H.Strutt, unpublished). Following exposure to camptothecin, the presence of these aberrations at the next mitosis indicates that the long-lived DSBs measured by AFIGE are likely to represent, at least in part, frank DSBs induced directly in S-phase, or produced through processing of the cleavable-complex/replication-complex lesion in the S or G2 phase of the cell cycle. We are at present attempting to determine the numerical relationship between camptothecin induced DSBs measured by AFIGE, and camptothecin induced chromatid breaks measured with a microscope.

Yeast RAD52 mutant cells which are deficient in recombination and double strand DNA break repair, are very sensitive to camptothecin (24, 25), indicating that the cytotoxic damage caused by the drug can be repaired in at least some systems, and that the processing of the damage includes some aspect of recombination and/or DNA double strand break repair.

In conclusion, we propose that unrepaired or long-lived DSBs induced in DNA replicating at the time of camptothecin exposure are responsible for the toxicity of the drug, and that the sensitivity of various cell types is attributable to differences in the level of DSBs induced and the ability of the cell to repair such damage.

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REFERENCES

1. Liu,L.F. (1989) *Annu. Rev. Biochem.* **58**, 351–375.
2. Nelson,E.M., Tewey,K.M. and Liu,L.F. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1361–1365.
3. Hsiang,Y.-H., Hertzberg,R., Hecht,S. and Liu,L.F. (1985) *J. Biol. Chem.* **260**, 14873–14878.

4. Horowitz,M.S. and Horowitz,S.B. (1971) *Biochem. Biophys. Res. Commun.* **45**, 723–727.
5. Hsiang,Y.-H., and Liu,L.F. (1988) *Cancer Res.* **48**, 1722–1726.
6. Covey,J.M., Jaxel,C., Kohn,K.W. and Pommier,Y. (1989) *Cancer Res.* **49**, 5016–5022.
7. Hsiang,Y.-H., Lihou,M.G. and Liu,L.F. (1989) *Cancer Res.* **49**, 5077–5082.
8. Kessler,D., Bosmann,M.B. and Lohr,K. (1972) *Biochim. Biophys. Acta* **269**, 210–216.
9. Li,L.H., Fraser,T.J., Olin,E.J. and Bhuyan,B.K. (1972) *Cancer Res.* **32**, 2643–2650.
10. Heck,M.M.S., Hittelman,W.N. and Earnshaw,W.C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1086–1090.
11. D'Arpa,P., Beardmore,C. and Liu,L.F. (1990) *Cancer Res.* **50**, 6919–6924.
12. Snapka,R.M. (1986) *Mol. Cell. Biol.* **6**, 4221–4227.
13. Avemann,K., Knippers,R., Koller,T. and Sogo,J.M. (1988) *Mol. Cell. Biol.* **8**, 3026–3034.
14. Shin,C.-G. and Snapka,R.M. (1990) *Biochem. Biophys. Res. Commun.* **168**, 135–140.
15. Denko,N., Giaccia,A., Peters,B. and Stamato,T.D. (1989) *Anal. Biochem.* **178**, 172–176.
16. Stamato,T.D. and Denko,N. (1990) *Radiation Res.* **121**, 196–205.
17. Shibuya,M.L., Buiddenbaum,W.E., Don,A.L., Utsumi,H., Suciu,D., Kosaka,T. and Elkind,M.M. (1991). *Cancer Res.* **51**, 573–580.
18. Ager,D.D. and Dewey,W.C. (1990) *Int. J. Radiat. Biol.* **58**, 249–259.
19. Cedervall,B., Erixon,K. and Lewensohn,R. (1990) *Journal of Cellular Biochem.* Supplement **14A**, UCLA Symposia on Molecular and Cellular Biology.
20. Frankenberg,D., Frankenberg-Schwager,M., Blocher,D. and Harbich,R. (1981) *Radiation Res.* **88**, 524–532.
21. Resnick,M.A. and Martin,P. (1976) *Mol. Gen. Genetics* **143**, 119–126.
22. Jeggo,P.A. and Kemp,L.M. (1983) *Mutat. Res.* **112**, 313–327.
23. Degrassi,F., De Silva,R., Tanzarella,C. and Palitti,F. (1989) *Mutat. Res.* **211**, 125–130.
24. Eng,W.K., Faucette,L., Johnson,R.K. and Sternglanz,R. (1988) *Mol. Pharm.* **34**, 755–760.
25. Nitiss,J. and Wang,J.C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7501–7505.