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Preclinical report

Topoisomerase I inhibitor (camptothecin)-induced apoptosis in human gastric cancer cells and the role of wild-type p53 in the enhancement of its cytotoxicity

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Camptothecin (CPT), a human topoisomerase I inhibitor, blocks DNA replication in human cancer cells. It represents a promising new class of chemotherapeutic agents with broad anti-tumor activity. However, its effect on gastric cancer cells remains unknown. We examined cell growth, apoptosis and cell cycle phase distribution in gastric cancer cells by exposing these cells to CPT for up to 72 h. Cell viability was determined by the Trypan blue exclusion assay. Cell cycle phase distribution and apoptosis were measured using flow cytometry, fluorescence microscopy and DNA ladder assay. Exposure of exponentially growing gastric AGS cancer cells to CPT induced time-dependent apoptosis and growth inhibition. Serum starvation-synchronized AGS cells (about 60% cells in G_0/G_1 phase) showed similar cellular responses. Analysis of cell cycle phase distribution of AGS cells treated with CPT for up to 72 h showed no obvious differences compared to untreated control cells. Although the induction of apoptosis was noticed in gastric cancer cell lines both with and without p53, cells lacking p53 showed less apoptosis compared to those cell lines possessing p53. Our data show that CPT is capable of inducing gastric cancer cell growth inhibition and apoptosis. Wild-type p53 may enhance the cytotoxicity of CPT against gastric carcinoma.

Key words: Apoptosis, camptothecin, cell cycle, gastric cancer, topoisomerase I.

Introduction

Gastric cancer is the second most common tumor type globally and the fourth in Europe. Although the overall incidence has been decreasing over the past few decades, the incidence of adenocarcinomas of the proximal stomach and esophagogastric junction is rising. Gastric cancer presenting at an early stage can be cured surgically; however, 80% of cases are too advanced at presentation. Moreover, penetration of the serosa by the tumor predicts a risk of recurrence of 80–85%.[1] There is considerable interest in the use of perioperative chemotherapy in gastric cancer where the aim is to reduce tumor bulk, increase resection rates, treat micrometastases and thereby improve survival. As yet there is no one established standard chemotherapy regimen in advanced gastric cancer and the benefit of combination chemotherapy over fluorouracil given alone remains controversial.[2]

Topoisomerases are ubiquitous enzymes that solve topological problems generated by key nuclear processes such as DNA replication, transcription, recombination, repair, chromatin assembly and chromosome segregation.[3] There are two types of topoisomerases. Drugs that target the related enzyme topoisomerase (Topo) II have been in use for some time, including anthracyclines (e.g. daunomycin) and podophyllotoxins (etoposide), but camptothecin (CPT) and its analogs, the first class of anti-cancer agents to target Topo I, have only recently been subjected to clinical trial in human cancer.[1,3] Anti-Topo-I drugs have the advantage that Topo I levels remain high in all phases of the cell cycle, whereas Topo II varies with the cell cycle and many slow-growing tumors have low levels of Topo II. CPTs have the additional advantage of being very poor substrates for the multidrug resistance 'pump' that ejects many compounds from cells. Moreover, CPTs are not detoxified by a glutathione-dependent mechanism, as are many of the other anti-cancer drugs.[3]

CPTs represent promising new class of chemotherapeutic agents with a novel mechanism of action. It

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has been shown that they have activity against several gastrointestinal tumors, including a gastric adenocarcinoma xenograft.[4] Some clinical trials have also shown promising results in advanced gastric cancer.[1,2]

It has been shown that a variety of cancer chemotherapeutic agents, including CPTs, exert their effects by inducing apoptotic cell death.[5,6] Indeed, the ability of tumor cells to respond to damage and eventually activate the apoptotic program might determine the ultimate success of cancer therapy.[7] Genes involved in neoplastic transformation, such as p53, the most frequently altered gene in human cancers, are also involved in the regulation of apoptosis.[8,9] Wild-type p53 protein has been shown to be a mediator of different types of apoptosis, including that induced by several chemotherapeutic agents such as adriamycin, etoposide and 5-fluorouracil.[10] In addition, mutations or deletions in the p53 gene have been associated, *in vivo* and *in vitro*, with resistance to apoptosis induced by the same chemotherapeutic agents.[7] CPTs have been shown to induce both p53-dependent or -independent apoptosis in many cancer cells.[6,11,12] A recent study suggests that wild-type p53 may enhance CPT cytotoxicity against some human cells.[11] However, whether CPT induces apoptosis in gastric cancer cells and whether cell p53 gene status influences the effect of CPT on gastric cancer cells are still unknown.

Therefore, this study examined the effect of CPT on gastric cancer cell growth, cell cycle phase distribution and apoptosis. The relationship between the effect of CPT on gastric cancer cells and cell p53 gene status was also assessed.

Materials and methods

Cell lines and culture conditions

Gastric cancer cell lines AGS, MKN 45, Kato3 and HSC39 were grown in culture medium, consisting of RPMI 1640 medium (Gibco, Paisley, UK), 10% fetal calf serum (FBS; Gibco), penicillin (100 IU/ml; Gibco), streptomycin (100 μg/ml; Gibco) and L-glutamine (2 mM; Gibco). The cells were maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

Treatment

To examine the effect of CPT on *in vitro* growth of AGS cells, exponentially growing and serum-starved AGS cells were, respectively, treated with culture medium alone or fresh medium plus 1 μM CPT (Sigma, Poole, UK) for up to 72 h. Cell viability was assessed by the Trypan blue exclusion method. Cells were harvested for the assessment of cell growth and cell cycle phase distribution. Both detached and adherent cells were collected for examination of apoptosis.

Flow cytometric analysis

Cell cycle phase distribution was analyzed using flow cytometry according to a previously published method.[13] Briefly, a pool of detached and adherent cells was washed in phosphate-buffered saline (PBS) and then the cells fixed in 70% ice-cold (4°C) ethanol overnight. The cell pellets were re-suspended in 1 ml solution containing 200 μg/ml ribonuclease (Sigma) and 50 μg/ml propidium iodide (Sigma) for 60 min at 37°C. Cell cycle analysis was performed on a FACScan flow cytometry (Becton Dickinson, Oxford, UK) equipped with a FACStation and CellQuest software (Becton Dickinson Immunocytometry Systems, Oxford, UK).

Debris was eliminated from the analysis using a forward angle light scatter threshold. Cell doublets and clumps were gated out of the analysis using a dot-plot display of area and width. In total, 10 000 cells were analyzed for each sample and the apoptotic cells were considered to constitute the sub-G_1 cell population. The induction of apoptosis was confirmed by fluorescence microscopy with acridine orange (Sigma) staining and DNA ladder assay as described previously.[14,15] All experiments were repeated at least 3 times.

Results

Induction of apoptosis and growth inhibition in gastric AGS cells by CPT

Exponentially growing AGS cells were treated for 0, 6, 12, 24, 48 and 72 h with 1 μM of CPT, the dose known to affect cell growth and induce apoptosis in other cancer cells.[6] Figure 1 shows that CPT induces apoptosis in AGS cells to a significantly greater degree than cells treated with medium containing no serum or untreated control cells.

To confirm the apoptotic nature of the observed cell death, morphological and molecular parameters were evaluated. The CPT-treated population (Figure 2A) shows the characteristic morphological features of apoptosis. Chromatin condensation at the margins of the nuclear membrane as well as chromatin granules resulting from DNA fragmentation are visible. The pathognomonic feature of apoptosis is the cleavage of DNA in the internucleosomal regions of chromatin. Analysis of DNA fragmentation in AGS cells treated with CPT for 48 h showed DNA ladder formation (Figure 2B, lane 2), which was absent in untreated cells (Figure 2B, lane 1).

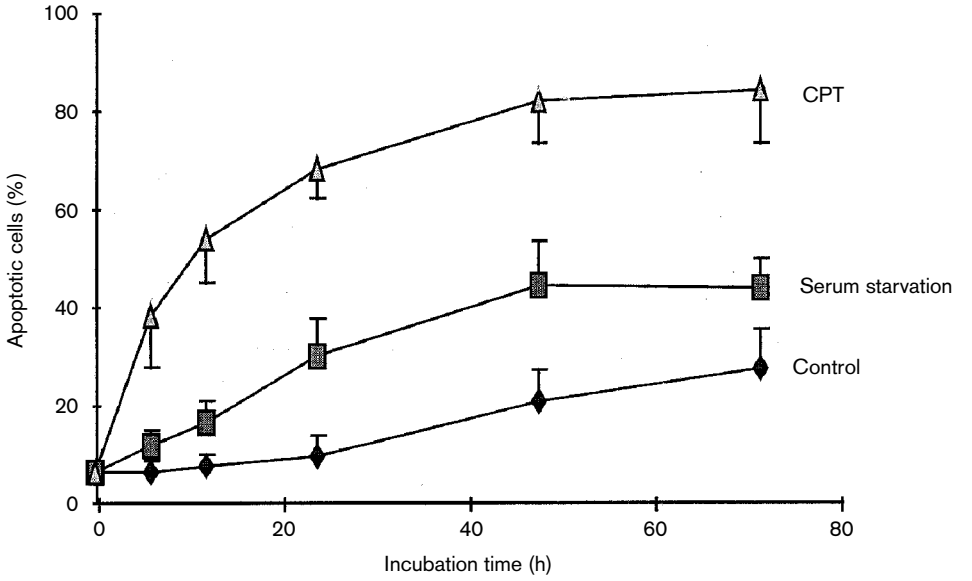


Figure 1. Induction of apoptosis in exponentially growing AGS cells after CPT treatment. Exponentially growing AGS cells were incubated in medium with or without CPT or medium without serum for up to 72 h. Both CPT treatment and serum starvation caused a significant induction of apoptosis, but the induction in CPT-treated cells was significantly higher compared to serum starved and untreated AGS cells. The results (mean ± SD) represent the average of three or more experiments.

To examine whether cell cycle phase affects the induction of apoptosis by CPT, AGS cells were serum starved for 72 h, which led to about 60% cells arrested in G_0/G_1 phases. Refeeding these cells with medium alone induced cell cycle progression, but medium containing CPT caused an enhanced induction of apoptosis and inhibition of cell cycle progression (Figure 3).

Effect of CPT on cell cycle progression

Induction of apoptosis is frequently associated with cell cycle modifications of surviving cells.[16] Analysis of cell cycle phase distributions of AGS cells treated with CPT revealed that although there was a marked induction of apoptosis in cells exposed to CPT, there was no significant difference in cell cycle phase distribution between cells exposed to CPT and untreated controls. However, cell proliferation index (PI%) progressively decreased as apoptosis increased (Figure 4). These findings indicate that CPT-induced gastric cell growth inhibition is mainly due to cell death by apoptosis.

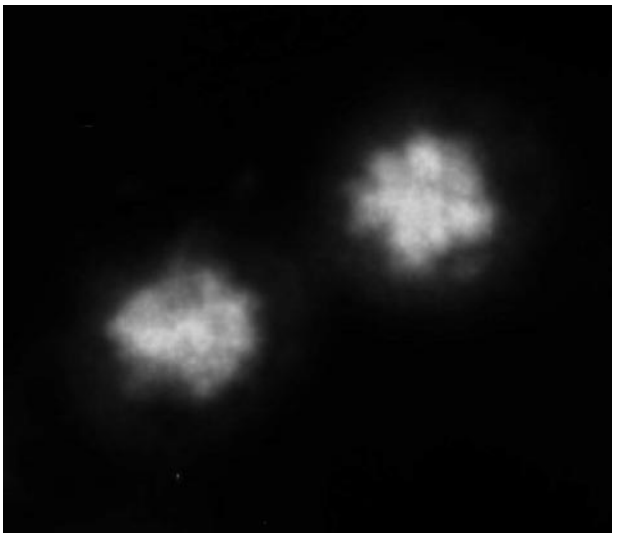
Role of p53 in CPT-induced gastric cancer apoptosis

To test the importance of p53 protein in the induction of gastric cancer cell apoptosis by CPT, MKN45 (with wild-type p53 gene), HSC39 (with mutated p53 gene) and Kato3 (with complete p53 gene deletion) cells[17,18] were further assessed for their responses following CPT exposure. The DNA contents of these cells were analyzed by flow cytometry. Figure 5 shows the effect of CPT on apoptosis in each of the cell lines after 48 h treatment. All the cell lines showed enhanced induction of apoptosis following CPT exposure and the effects were time dependent (Figure 6). However, Kato3 cells, which lack p53, had significantly reduced apoptosis compared to the other cell lines, all of which express p53.[17–19] These data indicate that p53 is not essential for the induction of CPT-induced apoptosis, but its presence enhances the cytotoxicity of CPT towards gastric cancer cells, as has been shown in non-gastric cancer cells.[11] Although the p53 gene in HSC39 cells is mutated, the cells still showed significant induction of apoptosis in response to CPT treatment. This finding suggests that the p53 gene mutation in this cell line may not affect the expression of normal p53 protein with respect to the induction of apoptosis or it may be re-activated by CPT as suggested recently.[20] Consistent with our findings in AGS cells, CPT also demonstrated a progressive inhibition in cell proliferation in MKN45, HSC39 and Kato3 cells, which was independent of p53 status (Table 1).

Discussion

The ability to activate the apoptotic program is a mechanism for anti-cancer drugs to destroy tumor cells

(A)



(B)

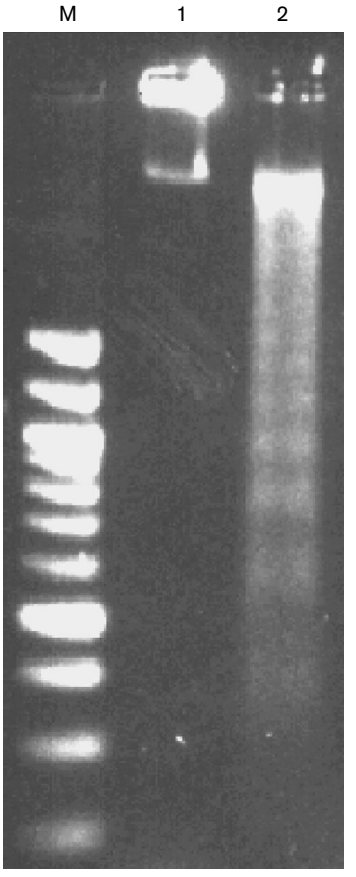


Figure 2. AGS cells were treated with CPT for 48 h. Fluorescence microscopy shows apoptotic cells (A) and DNA fragmentation by DNA ladder assay (B). Fluorescence microscopy with acridine orange staining shows chromatin condensation and chromatin granules (A). Ethidium bromide-stained agarose gel of electrophoresed DNA from AGS cells untreated (lane 2) or CPT treated (lane 3). Lane 1 contains a marker.

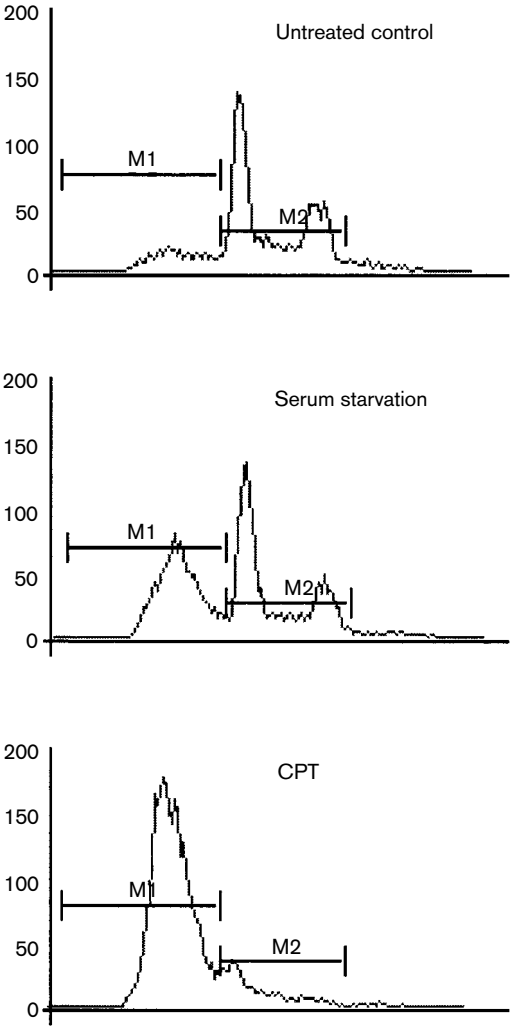


Figure 3. Induction of apoptosis in serum-starved AGS cells after CPT treatment. AGS cells were synchronized by serum starvation for 72 h and then were refed with medium in the presence or absence of CPT, or medium without serum for a further 48 h. Refeeding with medium alone induced cell cycle progression, but CPT induced a marked enhancement of apoptosis (sub-G_0/G_1 peak) compared to cells continuing on serum starvation. The results (mean $\pm$ SD) represent the average of three or more experiments.

and failure to induce apoptosis is an important cause of poor response in cancer treatment.[21] We have shown that CPT inhibited gastric cancer cell lines and induced apoptosis in several gastric cancer cell lines. This is consistent with the findings in other cell lines and suggests that apoptosis is a ubiquitous phenomenon following CPT exposure.[6,11,12] The sensitivity of cells to some anti-cancer agents is dependent on their position in the cell cycle.[22] Typically, asynchronous cultures consisting of cells in various phases of the cell

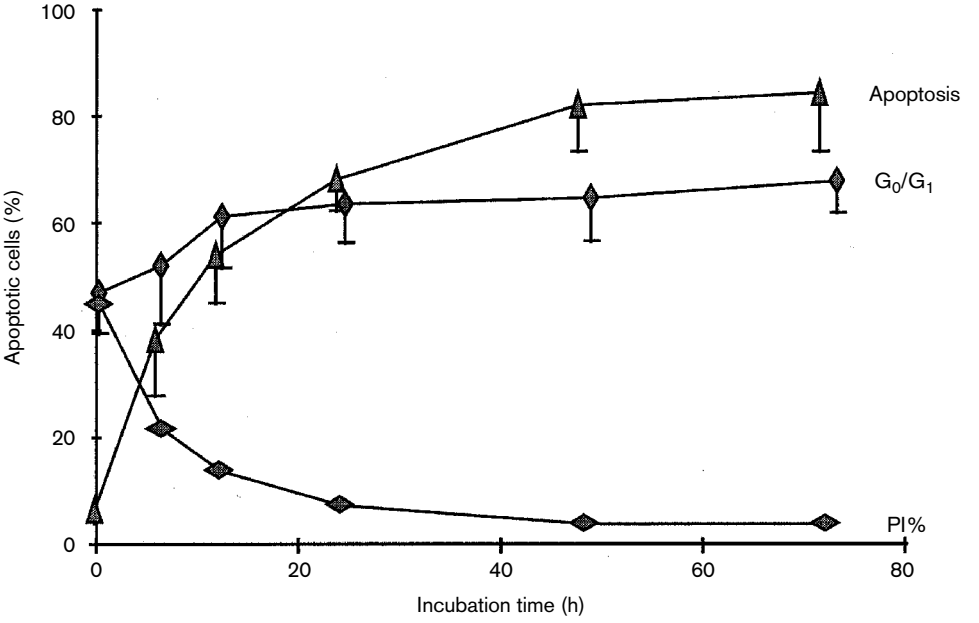


Figure 4. The relationship between apoptosis, cell cycle arrest and cell proliferation following CPT exposure. Exponentially growing AGS cells were incubated in the presence of CPT for up to 72 h. As apoptosis increased, there was a progressive decrease in AGS cell proliferation index (PI%). However, there was no significant change in cell cycle phase distribution following CPT exposure. The results (mean ± SD) represent the average of three or more experiments.

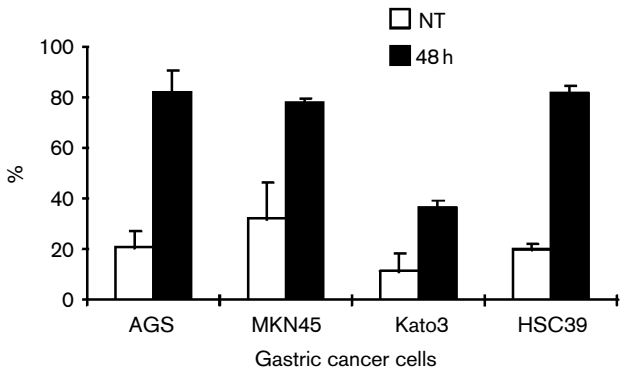


Figure 5. Induction of apoptosis in gastric cancer cell lines with or without functional p53. Exponentially growing gastric cancer cells AGS, MKN45, Kato3 and HSC39 were incubated in the presence or absence of CPT for 48 h. Apoptosis was quantified using flow cytometry. All cell lines showed an enhanced induction of apoptosis following CPT exposure. However, Kato3 cells, which lack p53, had less apoptosis when compared to the cell lines which contain the p53 gene. The results (mean ± SD) represent the average of three or more experiments.

cycle are used for studies of anti-cancer agent sensitivity. When such cultures are treated with anti-cancer agents, cells in different phases of the cell cycle may respond differently.[23] To assess whether CPT-induced gastric cancer cell growth inhibition is cell cycle phase specific, both exponentially growing and serum starvation synchronized AGS cells, which contain about 60% G_0/G_1 cells, were studied. We found no differences in apoptosis and growth inhibition between exponentially growing and serum starvation-synchronized cells, indicating that CPT anti-gastric cancer activity is not affected by cell cycle. This may be due to the fact that Topo I is not a cell cycle-dependent enzyme, remaining at a high level in all phases of the cell cycle,[3,24] and it suggests that CPT may be clinically effective in slow-growing gastric tumor cells.

Although the induction of apoptosis on gastric cancer cells following CPT exposure is not cell cycle phase specific, the Topo I inhibitor may cause surviving cell cycle arrest, which varies according to the cell type.[25–28] Del Bino and colleagues compared the effect of CPT on mouse lymphocytic L1210 cells and human promyelocytic HL-60 cells.[28] They found that exposure of mouse lymphocytic L1210 cells to CPT caused a reduction in the rate of cell progression through S and G_2 phases of the cell cycle. Some cells also enter higher DNA ploidy and progress through the cycle at that ploidy. CPT exerted similar effects on human lymphocytic MOLT-4 leukemia cells. In contrast, treatment of human promyelocytic HL-60 cells with CPT resulted in the immediate (occurring as early as 2 h after treatment) death of S and G_2/M phase cells;

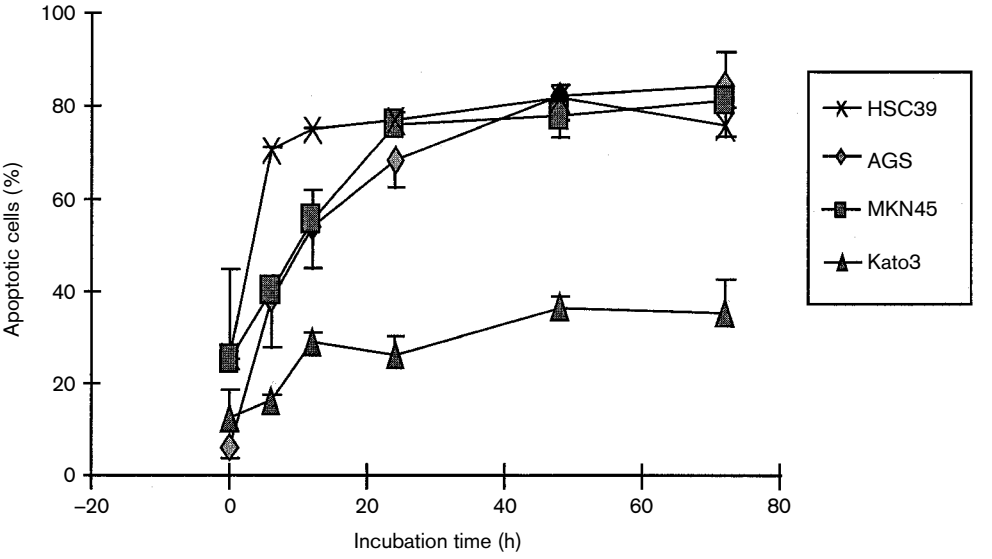


Figure 6. Time-dependent induction of apoptosis in gastric cancer cell lines with or without functional p53. Exponentially growing gastric cancer cells AGS, MKN45, Kato3 and HSC39 were incubated in the presence or absence of CPT. Apoptosis was quantified using flow cytometry. All cell lines showed a time-dependent induction of apoptosis following CPT exposure. However, Kato3 cells, which lack p53, showed an obviously lower induction of apoptosis when compared to the cell lines which contain the p53 gene. The results (mean ± SD) represent the average of three or more experiments.

Table 1. Effect of CPT on proliferation of gastric cancer cell lines with or without p53 gene

	Treatment	6 h	12 h	24 h	48 h	72 h	*p* values
MKN45	UT	28.5	28.0	24.0	23.2	21.8	0.02
	CPT	22.6	16.1	9.1	7.2	5.3	
AGS	UT	46.1	43.6	41.2	31.4	26.2	0.01
	CPT	23.4	15.7	9.4	6.1	5.7	
HSC39	UT	37.3	33.9	36.2	34.3	31.0	0.001
	CPT	11.7	8.4	7.8	8.2	11.8	
Kato3	UT	49.1	44.9	49.7	46.6	45.2	0.04
	CPT	50.1	35.5	36.0	33.5	34.6	

Exponentially growing gastric cancer cells AGS, MKN45, Kato3 and HSC39 were incubated in the presence or absence of CPT for up to 72 h. Cell proliferation was assessed as proliferation index (PI%), which was calculated as the percentage of cells in S and G_2/M phases. Cell cycle phase distribution was analyzed using flow cytometry. All the cell lines showed significant inhibition of cell proliferation when compared to untreated control cells and these changes were independent of cell p53 status. The results (mean) represent the average of three or more experiments.
UT, untreated control. The *p* values were calculated using single-factor one-way ANOVA.

the dead cells exhibited decreased DNA stainability with intercalating dyes, suggestive of DNA degradation. These data indicate that there may be tissue specificity in the response of cells to CPT. We analyzed the effect of CPT on gastric cancer cell cycle phase distribution. Like the effects on human promyelocytic HL-60 cells, we found that the gastric cancer cells rapidly underwent apoptosis but there was no obvious cell cycle arrest following CPT exposure. As apoptosis increased, there was a progressive decrease in cell proliferation index (PI%). These findings indicate that gastric cancer cells are highly susceptible to CPT-induced apoptosis, which is likely to be the major anti-cancer effect of CPT.

Many chemotherapeutic agents exert their cytotoxic activity by inducing a p53-mediated apoptotic cell death.[29,30] The role of p53 in the cytotoxicity of CPT against various cancer cells was investigated, and both p53-dependent and -independent cellular responses have been reported.[25,31–34] Arah *et al.* have recently examined the effect of CPT on human prostate tumor cells.[11] They infected human prostate PC3 cancer cells, which are null for the p53 gene, with an adenovirus-expressing human wild-type p53 gene

(Adwtp53). Infection induced the synthesis of wild-type p53, resulting in growth arrest of PC3 cells. In the presence of CPT, significant increases in p53 protein were detected in Adwtp53-infected PC3 cells. While Adwtp53 and CPT, as single agents, caused apoptosis, combinations of CPT and Adwtp53 enhanced the induction of apoptosis in PC3 cells. We have shown that CPT induces apoptosis in gastric cancer cells both with or without p53, suggesting that p53 is not essential for the induction of gastric cancer cell apoptosis during CPT treatment. However, cells lacking p53 (Kato3 cells) showed significantly lower induction rates of apoptosis compared to those cells with p53. These findings indicate that the presence of p53 protein may enhance the cytotoxicity of CPT against gastric cancer cells and introduction of the p53 gene with Topo I inhibitors may offer a clinical advantage for the treatment of gastric cancer containing null for the p53 gene.

In conclusion, our data have shown that CPT is able to induce growth inhibition and apoptosis in several gastric cancer cell lines. The cellular responses following CPT exposure are independent of cell cycle phase distribution. Although p53 did not play an essential role in the induction of apoptosis, the presence of this protein may enhance the cytotoxicity of CPT against gastric cancer cells.

References

1. Ellis P, Cunningham D. Current issues in cancer: management of carcinomas of the upper gastrointestinal tract. *Br Med J* 1994; **308**: 834–8.
2. Ajani JA. Current status of therapy for advanced gastric carcinoma. *Oncology* 1998; **12**: 99–102.
3. Rowe PM. Camptothecins: new enthusiasm for an old drug. *Lancet* 1996; **347**: 892.
4. Dancey J, Eisenhauer EA. Current perspectives on camptothecins in cancer treatment. *Br J Cancer* 1996; **74**: 327–38.
5. Strasser A. Dr Josef Steiner Cancer Research Prize Lecture: the role of physiological cell death in neoplastic transformation and in anti-cancer therapy. *Int J Cancer* 1999; **81**: 505–11.
6. Adjei PN, Kaufmann SH, Leung W, Mao F, Gores GJ. Selective induction of apoptosis in Hep 3b cells by topoisomerase I inhibitors: evidence for a protease-dependent pathway that does not activate cysteine protease p32. *J Clin Invest* 1996; **98**: 2588–96.
7. Fisher DE. Apoptosis in cancer therapy: crossing the threshold. *Cell* 1994; **78**: 539–42.
8. Brown JM, Wouters BG. Apoptosis, p53, and tumor cell sensitivity to anticancer agents. *Cancer Res* 1999; **59**: 1391–9.
9. Steele RJ, Thompson AM, Hall PA, Lane DP. The p53 tumour suppressor gene. *Br J Surg* 1998; **85**: 1460–7.
10. Lowe SW, Ruley HE, Jacks T, Housman DE. P53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 1993; **74**: 957–67.
11. Arah IN, Song K, Seth P, Cowan KH., Sinha BK. Role of wild-type p53 in the enhancement of camptothecin cytotoxicity against human prostate tumor cells. *Anticancer Res* 1998; **18**: 1845–9.
12. Li G, Tang L, Zhou X, Tron V, Ho V. Chemotherapy-induced apoptosis in melanoma cells is p53 dependent. *Melanoma Res* 1998; **8**: 17–23.
13. Ormerod MG. Analysis of DNA: general methods. In: M. G. Ormeron, eds. *Flow cytometry: a practical approach*. New York: Oxford University Press 1994: 119–36.
14. Ning ZQ, Murphy JJ. Calcium ionophore-induced apoptosis of human B cells is preceded by the induced expression of early response genes. *Eur J Immunol* 1993; **23**: 3369–72.
15. Gong J, Traganos F, Darzynkiewicz Z. A selective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. *Anal Biochem* 1994; **218**: 314–9.
16. Brady HJ, Gil-Gomez G. The cell cycle and apoptosis. *Results Probl Cell Different* 1999; **23**: 127–44.
17. Yamamoto M, Maehara Y, Sakaguchi Y, Kusumoto T, Ichiyoshi Y, Sugimachi K. Transforming growth factor-beta 1 induces apoptosis in gastric cancer cells through a p53-independent pathway. *Cancer* 1996; **77**: 1628–33.
18. Akama Y, Yasui W, Kuniyasu H, *et al*. Genetic status and expression of the cyclin-dependent kinase inhibitors in human gastric carcinoma cell lines. *Jpn J Cancer Res* 1996; **87**: 824–30.
19. Shirin H, Sordillo EM, Oh SH, *et al*. Helicobacter pylori inhibits the G_1 to S transition in AGS gastric epithelial cells. *Cancer Res* 1999; **59**: 2277–81.
20. Selivanova G, Kawasaki T, Ryabchenko L, Wiman KG. Reactivation of mutant p53: a new strategy for cancer therapy. *Semin Cancer Biol* 1998; **8**: 369–78.
21. Bergman PJ, Harris D. Radioresistance, chemoresistance, and apoptosis resistance. The past, present, and future. *Vet Clin North Am Small Anim Pract* 1997; **27:** 47–57.
22. Hall EJ, ed. *Radiobiology for the radiologist*. Philadelphia, PA: Lippincott 1994.
23. Okada SJ. Cells. In: Altman KI, Gerber GB, Okada SJ, eds. *Cells*. New York: Academic Press 1970: 191-239.
24. Sinha BK. Topoisomerase inhibitors. A review of their therapeutic potential in cancer. *Drugs* 1995; **49**: 11–9.
25. McDonald AC, Brown R. Induction of p53-dependent and p53-independent cellular responses by topoisomerase 1 inhibitors. *Br J Cancer* 1998; **78**: 745–51.
26. Bassi L, Palitti F, Mosesso P, Natarajan AT. Distribution of camptothecin-induced break points in Chinese hamster cells treated in late S and G_2 phases of the cell cycle. *Mutagenesis* 1998; **13**: 257–61.
27. Fertala J, Nieborowska-Skorska M, Calabretta B, Skorski T. The diverse effect of topoisomerase I specific inhibitor (camptothecin) on normal and BCR/ABL-dependent hematopoietic cells proliferation: therapeutic implications. *Folia Histochem Cytobiol* 1995; **33**: 187–92.
28. Del Bino G, Skierski JS, Darzynkiewicz Z. Diverse effects of camptothecin, an inhibitor of topoisomerase I, on the cell cycle of lymphocytic (L1210, MOLT-4) and myelogenous (HL-60, KG1) leukemic cells. *Cancer Res* 1990; **50**: 5746–50.

29. Peller S. Clinical implications of p53: effect on prognosis, tumor progression and chemotherapy response. *Semin Cancer Biol* 1998; **8**: 379–87.
30. Komarova EA, Gudkov AV. Could p53 be a target for therapeutic suppression? *Semin Cancer Biol* 1998; **8**: 389–400.
31. Nieves-Neira W, Pommier Y. Apoptotic response to camptothecin and 7-hydroxystaurosporine (UCN-01) in the 8 human breast cancer cell lines of the NCI Anticancer Drug Screen: multifactorial relationships with topoisomerase I, protein kinase C, Bcl-2, p53, MDM-2 and caspase pathways. *Int J Cancer* 1999; **82**: 396–404.
32. Zheng M, Wang H, Zhang H, Ou Q, Shen B, Yu B. The influence of the p53 gene on the *in vitro* chemosensitivity of colorectal cancer cells. *J Cancer Res Clin Oncol* 1999; **125**: 357–60.
33. Giuliano M, Lauricella M, Vassallo E, Carabillo M, Vento R, Tesoriere G. Induction of apoptosis in human retinoblastoma cells by topoisomerase inhibitors. *Invest Ophthalmol Vis Sci* 1998; **39**: 1300–11.
34. Shao RG, Cao CX, Shimizu T, O'Connor PM, Kohn KW, Pommier Y. Abrogation of an S-phase checkpoint and potentiation of camptothecin cytotoxicity by 7-hydroxystaurosporine (UCN-01) in human cancer cell lines, possibly influenced by p53 function. *Cancer Res* 1997; **57**: 4029–35.

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