Cancer Chemother Pharmacol (2000) 45: 362–368

ORIGINAL ARTICLE

Xuejun Xie · Keisuke Sasai · Keiko Shibuya
Seiji Tachiiri · Keij Nihei · Takeo Ohnishi
Masahiro Hiraoka

P53 status plays no role in radiosensitizing effects of SN-38, a camptothecin derivative

Received: 19 May 1999 / Accepted: 19 November 1999

Abstract *Purpose*: Topoisomerase inhibitors including camptothecin are being studied as potential radiosensitizers. CPT-11 is a derivative of camptothecin and is clinically available. In this study, we investigated the effects of SN-38 (an active metabolite of CPT-11) on four nonirradiated and irradiated murine fibroblast cell lines with different p53 statuses to clarify the role of p53 in the radiosensitizing activity of SN-38. *Materials and methods*: Four fibroblast cell lines, MT158, MT158/neo, MT158/wtp53 and MT158/mp53 with the same genetic background but with different p53 statuses, were used. Exponentially growing cells were treated with SN-38 (200 n*M*) and incubated with the drug for 30 min. Cells were then irradiated (0 to 12 Gy) and further incubated with the drug for 2 h. The cell survival rate was determined using a conventional clonogenic assay. The effects of the treatments on the cell cycle were analyzed with a flow cytometric assay. Apoptosis after these treatments was also detected by an annexin V assay. *Results*: There were no significant differences in sensitivity to radiation or SN-38 treatment among these cell lines. The combined treatment of irradiation and SN-38 showed supraadditive effects in all four cell lines independent of their p53 status. Transient arrest in G_2 with a decreased percentage of cells in both the S and G_1 phases was observed 8 h after treatment with either SN-38 alone, radiation or their combination, regardless of the p53 status. No significant differences in frequency of apoptosis were observed between treatment and control groups in two cell lines with or without wild-type p53. *Conclusion*: The combination of irradiation and SN-38 treatment showed supraadditive effects in all four cell lines tested, and the p53 status did not play a role in the combination effect.

Key words SN-38 · CPT-11 · Camptothecin · Radiation · p53

X. Xie (✉) · K. Sasai · K. Shibuya · S. Tachiiri
K. Nihei · M. Hiraoka
Department of Therapeutic Radiology and Oncology,
Graduate School of Medicine, Kyoto University,
Kyoto 606-8507, Japan
Tel.: +81-75-7513418; Fax: +81-75-7513418

T. Ohnishi
Department of Biology, Nara Medical University,
840 Shijo-cho, Kashihara, Nara 634, Japan

X. Xie
Department of Radiation Medicine,
Fourth Military Medical University,
Xi'an, Shaanxi, China

Introduction

Modulators of the DNA-unwinding enzyme, topoisomerase I, inhibit DNA repair and have been reported to increase the lethal effects of X-rays which create breaks in DNA [1, 2, 7, 15, 31, 33, 34, 36, 39]. Boothman et al. first reported that camptothecin, a specific inhibitor of topoisomerase I, significantly radiosensitizes human laryngeal epidermoid carcinoma (Hep-2) cells [1]. CPT-11 is a derivative of camptothecin and is clinically available as a novel anticancer agent. Topoisomerase inhibitors including camptothecin are being studied as potential radiosensitizers. The combined effects of SN-38 (an active metabolite of CPT-11) and irradiation for human and murine cell lines have been previously investigated, and supraadditive effects have been shown in some cell lines, but not in others [36]. No explanation for these differences has been proposed. Several mechanisms have been proposed as being involved in the radiosensitizing activity of camptothecin: its effect on the cell cycle [7, 28, 39] and the capacity to repair DNA damage [1, 2, 31, 33] after irradiation, and induction of apoptosis [43]. Falk and Smith have suggested that modification of topoisomerase I activity itself may change the cellular sensitivity to ionizing radiation [7].

The effect of the p53 protein is also a possible explanation for the mechanism. P53 is a tumor suppressor gene which is the most frequently mutated gene detected in all human cancers [44]. Functional p53 protein is

required to either arrest cell progression through the G_1/S interphase of the cell cycle and allow the repair of damaged DNA [14, 16, 22] or alternately to promote apoptosis when DNA damage is extreme [3, 25, 26]. Thus, the expression of wild-type p53 is associated with cellular sensitivity to various types of genotoxic stresses including radiation [17, 26] and cytotoxic agents [10, 25] as well as other growth arrest signals such as hypoxia [11, 12]. Conversely, mutations of p53 are associated with resistance to many of these agents [8, 18]. Alterations in p53 are responsible for the failure of most cancers to respond to radiotherapy and chemotherapy [19, 30].

In the study reported here we investigated the effects of SN-38 on four nonirradiated and irradiated murine fibroblast cell lines with the same genetic background but with different p53 statuses, to clarify the role of p53 in the radiosensitizing action of SN-38.

Materials and methods

Cells and cell culture

Four fibroblast cell lines, MT158, MT158/neo, MT158/wtp53 and MT158/mp53 were established as previously described [27]. MT158 fibroblasts were derived from a p53 knockout mouse. MT158/wtp53 and MT158/mp53 fibroblasts were different clones made by stable transfection of pCMVNc9 with wild-type p53 or pLTRp53cG with mutant-type p53 (val^{135}), respectively. The plasmid, pSV2neo containing the neo-resistance marker was also used in the transfection. MT158/neo cells contained vector alone. One of each kind of transfected clone was used in this study. All cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and kanamycin (40 μg/ml).

Drug preparation

SN-38 (7-ethyl-10-hydroxycamptothecin, MW 410.43) was kindly provided by Daiichi Pharmaceutical Co. The compound was prepared as a stock solution containing 2 m*M* in dimethylsulfoxide (DMSO), and aliquots were stored at −20 °C. Further dilutions with DMSO were made immediately before use to adjust the DMSO concentration in the growth medium to 0.2% for all experiments as previously described [36].

Irradiation

Irradiation was performed with an X-ray source (250 kV, 16 mA) with a 0.5-mm Cu filter at a dose rate of 1.9 Gy/min. The radiation dose was varied by changing the exposure time. Exponentially growing cells were treated with SN-38 (200 n*M*) and incubated with the drug for 30 min. Cells were then treated with radiation (0 to 12 Gy), and further incubated with the drug for 2 h, as previously described [36].

Cell survival curves

The cell survival rate was determined using a conventional clonogenic assay. Briefly, cells were trypsinized and plated onto dishes after treatment. The numbers of inoculated cells were adjusted such that about 100–200 colonies per dish could be expected. At 10–14 days after treatment, the cells were fixed with 70% ethanol for 10 min then stained with Giemsa solution for 4 h. Colonies of more than 50 cells were scored as survivors. The enhancement ratio was determined from the radiation doses required to reduce the surviving fraction to 0.1, when supraadditive effects of SN-38 and irradiation were observed.

Cell cycle analysis

Cell cycle distribution was analyzed by determining the DNA content. Cells were collected, fixed by dropwise addition of ice-cold 70% ethanol, and stored at 0 °C (> 18 h) until use. After fixation, cells were incubated for 60 min at 37 °C with RNase A (150 μg/ml) and propidium iodide (PI) (5 μg/ml). Before flow cytometric analysis, samples were filtered through 35-μm nylon mesh. Stained cells were analyzed with a FACS Calibur Cytometer (Becton Dickinson, San Jose, Calif.). The cell cycle distributions were assessed using Modfit software (Becton Dickinson).

Detection of apoptotic cells

Apoptosis was detected by an annexin V assay using an ApoAlert Annexin V-FITC Apoptosis Kit (Clontech Laboratories, Palo Alto, Calif.). Briefly, both the detached cells in the medium and the cells that remained attached to the dishes were collected and gently washed once with serum-containing medium, then cells were resuspended in 200 μl of 1 × binding buffer. Annexin V-FITC (5 μl, final concentration 0.5 μg/ml) and PI (5 μl) were added followed by incubation at room temperature for 5–15 min in the dark. The cells were examined by a FACS Calibur Cytometer using a single laser emitting excitation light at 488 nm and emission acquired at FL1. Data were analyzed using Cell Quest software (Becton Dickinson).

Western blot analysis

Exponentially growing cells were irradiated with 0 or 6 Gy (X-rays), and samples were collected 2 h later. Briefly, cells were washed with phosphate-buffered saline and lysed in 20 μl SDS lysis buffer (25 m*M* Tris-HCl, pH 6.5, 1% SDS, 0.24 M β-mercaptoethanol, 0.1% bromophenol blue, 5% glycerol). Following sonication and boiling, aliquots (routinely 50%) were subjected to 10% SDS-PAGE. After transfer to a nylon membrane, proteins were detected by anti-p53 monoclonal antibody (PAb240, Santa Cruz Biotechnology, Calif.) and HRP-conjugated antimouse Ig antibody (Amersham, Little Chalfont, UK) using Super Signal CL-HRP Substrate System (Pierce, Rockford, Ill.).

Statistical analysis

Student's *t*-test was used to compare the data in the clonogenic survival assay, cell cycle assay and the detection of apoptotic cells. *P*-values less than 0.05 were considered significant.

Results

p53 status

Western blot analysis of p53 expression in control and irradiated MT158/wtp53, MT158 and MT158/mp53 cells is shown in Fig. 1. High levels of p53 were observed in control and irradiated MT158/wtp53 and MT158/mp53 cells, indicating that the wild- or mutant-type p53 gene was successfully transfected into these cells and efficiently translated into immunoreactive p53 protein. However, the MT158 cell line did not show any detectable p53 expression either before or after irradiation, consistent with its p53 knockout status.

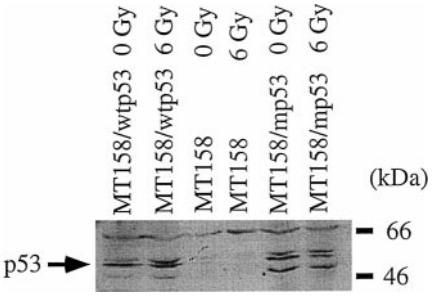

Fig. 1 Western blot analysis of p53 protein expression in control and irradiated (6 Gy) MT158/wtp53, MT158 and MT158/mp53 cells

Cell survival

The plating efficiencies for untreated cells were 0.51 ± 0.024 (SE), 0.39 ± 0.069, 0.49 ± 0.024 and 0.54 ± 0.038 for MT158, MT158/neo, MT158/wtp53 and MT158/mp53 fibroblasts, respectively. There were no significant differences in the clonogenic survival curves after irradiation among the four fibroblast cell lines (Fig. 2). This finding suggests that loss of p53 function is not a sole determinant of radiosensitivity in these cell lines.

SN-38 alone showed cytotoxicity against all cell lines after 2.5 h incubation. Survival rates of the four cell lines when treated with SN-38 at the concentration of 200 n*M* were 0.63 ± 0.048 (SE), 0.63 ± 0.070, 0.61 ± 0.047 and 0.71 ± 0.036 for MT158, MT158/neo, MT158/wtp53, and MT158/mp53 fibroblasts, respectively. There were no significant p53-dependent changes in sensitivity to treatment with SN-38 alone.

Figure 3 shows the combined effect of 200 n*M* SN-38 and irradiation on MT158 and MT158/wtp53 cells. When administered in combination with radiation, SN-38 sensitized the response of the cells to irradiation independent of their p53 status ($P < 0.05$, except for MT158/wtp53 cells at a dose of 12 Gy dose, which we cannot explain). Experiments utilizing MT158/neo and MT158/mp53 cells demonstrated similar results (not shown). The enhancement ratios of the four cell lines were 1.2, 1.3, 1.3, 1.3 for MT158, MT158/neo, MT158/wtp53, and MT158/mp53 fibroblasts, respectively.

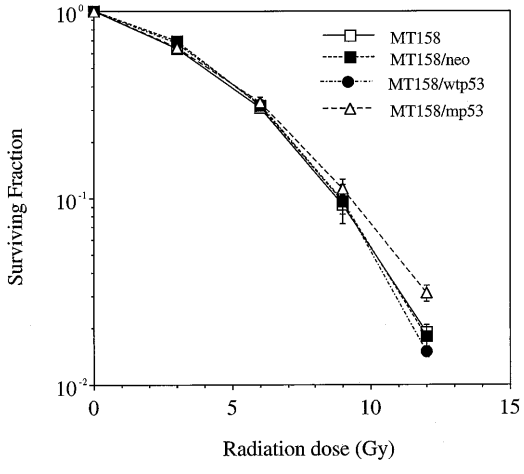

Fig. 2 Radiation dose-survival curves for MT158, MT158/neo, MT158/wtp53 and MT158/mp53 cells. Points represent the means from more than 18 samples in six independent experiments; *error bars* indicate 1 × SE

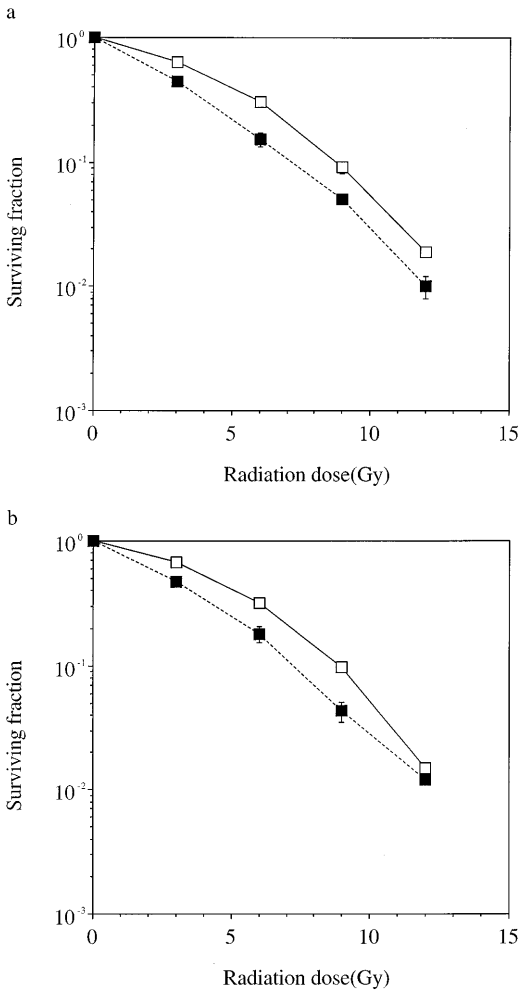

Fig. 3 Radiation dose-survival curves for MT158 (**a**) and MT158/wtp53 (**b**) cells with (*solid line*) or without (*dotted line*) treatment with 200 n*M* SN-38. Points represent the means from more than 18 samples in six independent experiments; *error bars* indicate 1 × SE

Effects of SN-38, radiation and their combination on the cell cycle

Figures 4a and 5a demonstrate that after 2.5 h exposure to SN-38 (200 n*M*) both MT158 and MT158/wtp53 cells displayed transient arrest in G_2 with a decreased percentage of cells in both the S and G_1 phases. The arrest peaked 8 h after treatment ($P < 0.05$) and was no longer apparent 24 h after treatment. There was no significant delay in G_1 observed in either cell line. Figures 4b and 5b show transient arrest in G_2 after irradiation with 9 Gy in the same manner, and there was no

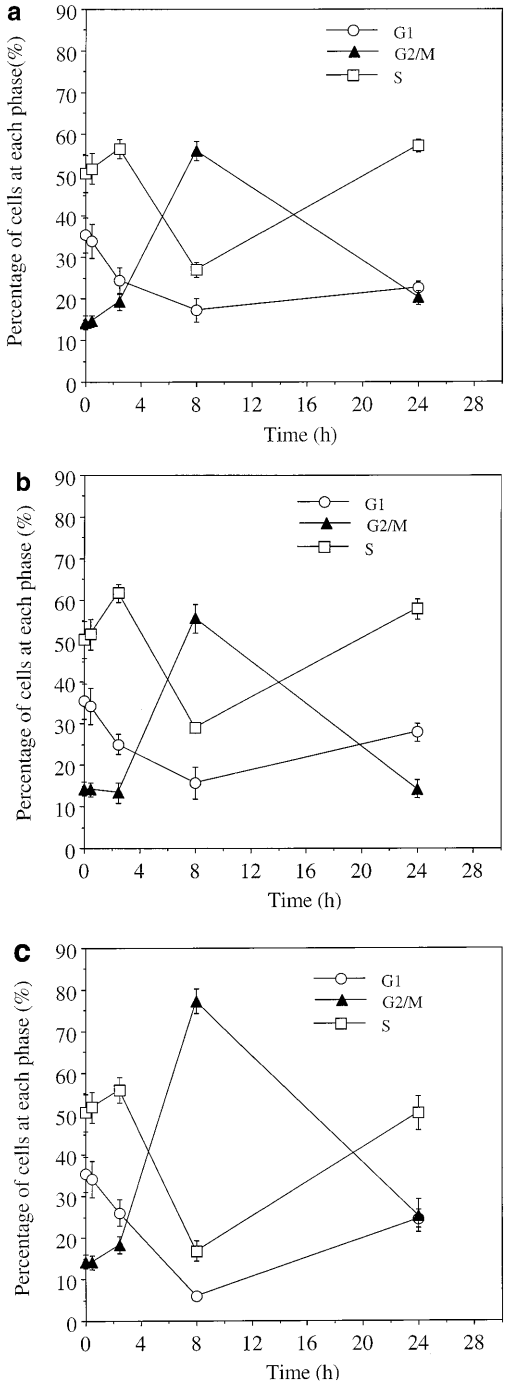

Fig. 4 Changes in cell phase distribution of MT158/wtp53 cells after irradiation (**a**), exposure to SN-38 (**b**), and treatment with a combination of the two (**c**). Points represent the means from three independent experiments; *error bars* indicate 1 × SE

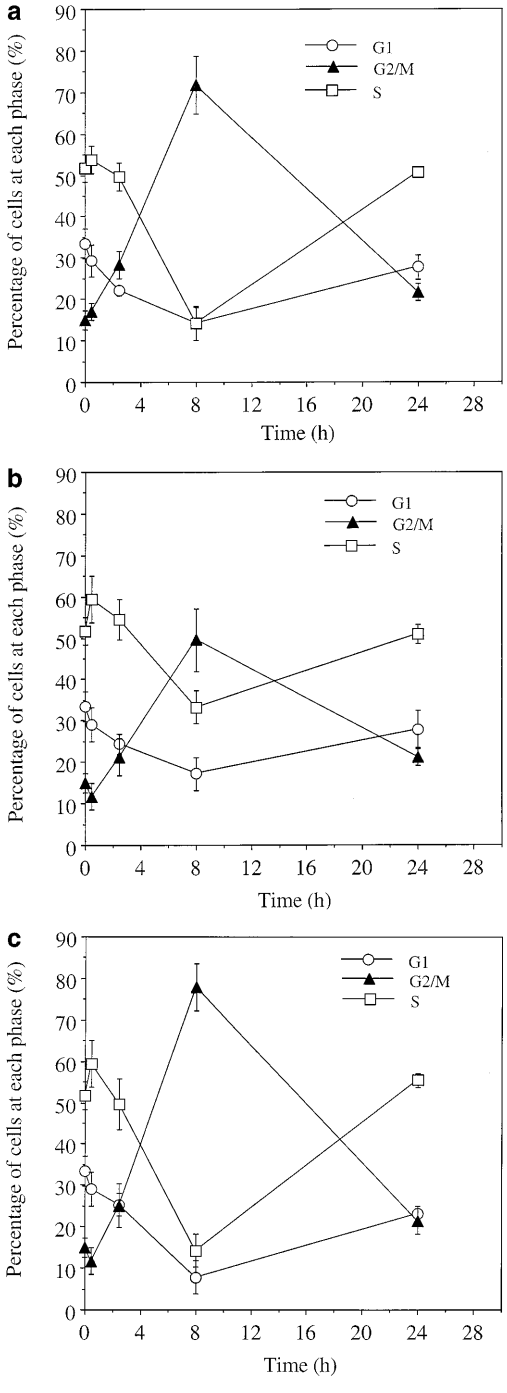

Fig. 5 Changes in cell phase distribution of MT158 cells after irradiation (**a**), exposure to SN-38 (**b**), and treatment with a combination of the two (**c**). Points represent the means of three independent experiments; *error bars* indicate 1 × SE

evidence of G_1 arrest detected in either cell line. When both cell lines were irradiated after 0.5 h continuous exposure to SN-38, and then further incubated with the drug for 2 h, significantly greater G_2 arrest was seen in both cell lines ($P < 0.05$) compared with that after treatment by either SN-38 or X-irradiation alone (Figs. 4c, 5c).

Effects of SN-38 and irradiation on apoptosis

Figure 6 shows the frequency of apoptosis in MT158 and MT158/wtp53 cells 48 h after treatment. Only slight differences in the frequency of apoptosis between different treatments and the control were observed in both cell lines.

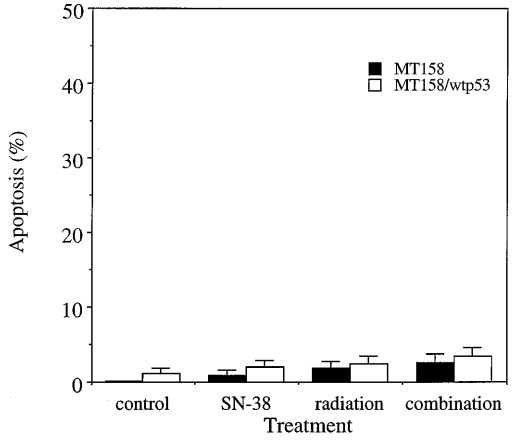

Fig. 6 The frequencies of apoptotic MT158 cells (*solid*) and MT158/wtp53 cells (*open*) 48 h after treatment with 200 n*M* SN-38 alone, irradiation at 9 Gy alone, and a combination of the two. Points represent the means of three independent experiments; *error bars* indicate 1 × SE

Discussion

Mammalian topoisomerases I and II are enzymes associated with DNA replication, transcription and possibly repair [33]. Camptothecin, a specific inhibitor of topoisomerase I, causes cell toxicity by stabilizing a ternary complex between the enzyme and double-stranded DNA. This leads to replication of fork-arrest, double DNA strand breaks and, possibly, illegitimate recombination of vital genes [35]. Camptothecin also causes single-strand breaks in DNA but the breaks are rapidly repaired after drug removal, while the cytotoxic action of camptothecin is sustained [43]. It has also been reported that exposure to camptothecin derivatives induces apoptosis [43]. Camptothecin affects the cell cycle. For example, it induces a dose-dependent delay in the S phase followed by dose-dependent trapping in the G_2/M phase [7].

Camptothecin and its derivative topotecan have been reported to enhance the cytotoxicity of ionizing irradiation in vitro and in vivo. CPT-11, a new semisynthetic derivative of camptothecin, is active in a number of tumor types in the clinic. CPT-11 is converted into the active metabolite SN-38 by carboxylesterase [15]. Treating cells with SN-38 for 3 h produces extensive degradation of DNA and apoptosis of the cells [43]. A combined effect of CPT-11/SN-38 and irradiation on human tumor cells has been reported. However, these reported data were limited to only one cell line. Omura et al. have reported that subtoxic concentrations of SN-38 can potentiate the cytotoxic effect of radiation only in cells grown as spheroids, but not in proliferating monolayer cells [34]. They showed that SN-38 completely inhibits potentially lethal damage repair (PLDR) after irradiation of cells in spheroids, suggesting that the mechanism of the radiosensitization by SN-38 is due to PLDR inhibition. Furthermore, they suggested that the SN-38 not only inhibits PLDR but also fixes potentially lethal damage in the spheroids [34]. Tamura et al. have also reported combined effects of CPT-11 and irradiation on transplanted human lung cancer tumors in athymic mice [39]. They concluded that SN-38 can sensitize the radiosensitivity of cells by modulation of the cell cycle.

The effects of cytotoxic agents including irradiation alone and in combination sometimes depend on the p53 status. Thymocytes in which the p53 alleles have been disrupted [3, 26] and hematopoietic cells expressing a mutant p53 allele [18] appear to be relatively radioresistant. The mechanism of radioresistance in these thymocytes appears to be the loss of a signal that induces apoptosis following irradiation [3, 26]. The expression of transdominant negative-mutant p53 ala^{143} in p53 wild-type A2780 cells induces resistance to radiation, cisplatin, doxorubicin, and cytarabine, but does not affect the susceptibility to Taxol or camptothecin [41]. Expression of mutant p53 in p53 wild-type RKO colon carcinoma cells abrogates the induced G_1 arrest, but except for enhanced sensitivity to cisplatin [9], does not modulate the sensitivity to irradiation or camptothecin [38].

Comparisons of the clonogenic survival curves of the four fibroblast cell lines did not reveal any significant differences in radiosensitivity or sensitivity to SN-38 in this study. This demonstrated that loss of p53 function is not the sole determinant of radiosensitivity or chemosensitivity to DNA-damaging agents at least in these murine fibroblast cell lines. These results are not necessarily contradictory. Several lines of evidence indicate that modulation of drug sensitivity and radiosensitivity by p53 may be both drug- and cell type-specific. Moreover, irradiation does not induce apoptosis in all cell types. For example, lymphoid cells are particularly sensitive to irradiation and many other agents [37].

The most plausible explanation for these differences is that cell type-specific differences exist in the response to radiation and DNA-damaging drug. Some cell types may undergo radiation-induced cell cycle arrest (e.g. fibroblasts), while an apoptotic pathway is induced by irradiation in other cell types. In this study, the low frequencies of apoptosis in both MT158/wtp53 and MT158 cell lines after treatment with SN-38 alone, radiation or both suggest that these cells normally do not induce p53 to undergo apoptosis after irradiation and SN-38 exposure. In contrast, the cells utilize the cell cycle arrest pathway and arrest at the G_2/M stage.

Cultured cells respond to ionizing radiation exposure by slowing or arresting their progression through the cell cycle. Reversible arrest at the G_2/M checkpoint (G_2 block) is a common finding in all cell types [40]. Although a prolonged G_1 arrest has been described in irradiated human diploid fibroblasts [23], such an effect has not been observed in tumor cells or transformed rodent cell lines. Lengthy G_2 phase delays clearly occur after irradiation in cells without p53, demonstrating that p53 is not necessary for the G_2 phase delay. Although

the loss of p53 function correlates perfectly with the absence of G_1 arrest, the retention of normal p53 function does not ensure that radiation-induced G_1 arrest will occur [20]. The lack of a pronounced G_1 arrest in the cell lines that express wild-type p53 found in the present study is consistent with the results of studies with human tumor cell lines from a variety of sources with both wild-type and mutant p53 status [20, 32]. Similarly, no radiation-induced G_1 arrest was observed in two closely related human lymphoblast cells with different p53 status isolated from the same donor [24]. Therefore, the disturbance in wild-type p53 function did not change the cell cycle responses or the sensitivity to irradiation and SN-38 of the cells in this study. This scenario is compatible with the observations that overexpression of wild-type p53 protein in a murine hematopoietic cell line utilizing a temperature-sensitive p53 gene product results in apoptosis [42], while only growth arrest has been noted in other cell types (such as fibroblasts) expressing this gene product in the wild-type conformation [29].

The radiosensitizing effect of topoisomerase I inhibitor in terms of the cell cycle has been investigated in several studies [4–6, 13, 16, 21]. Tamura et al. have reported that brief exposure to a low dose of SN-38 induces an initial cell cycle block in the G_2/M phase [39]. Li et al. have also reported the induction of a similar initial cell cycle change by exposure to a lower concentration of CPT-11 [21]. These findings suggest that a low dose of CPT-11 can alter cell cycle progression and increase the radiosensitivity of cells. Consistent with the results of these studies, our results also suggest that a 2.5-h exposure to a low dose of SN-38 increased the number of cells in the radiosensitive phase. This result might be useful to clarify the optimal timing of SN-38 treatment combined with irradiation. However, in our experiments, no significant changes in the cell cycle stage distribution were observed when the cells were irradiated after a 0.5-h exposure to SN-38. These findings suggest that mechanisms other than apoptosis, the cell cycle and p53 status also play a role in the radiosensitizing effect of SN-38.

In summary, the combination of irradiation and treatment with SN-38 showed supraadditive effects in all four cell lines tested here, and the status of p53 did not play a role in the combination effect.

Acknowledgement This work was supported in part by a Grant-in-Aid from the Japanese Ministry of Education, Science and Culture (09470199).

References

1. Boothman DA, Trask DK, Pardee AB (1989) Inhibition of potentially lethal DNA damage repair in human tumor cells by β-Lapachone, an activator of topoisomerase I. Cancer Res 49: 605
2. Boothman DA, Wang M, Schea RA, Burrows HL, Strickfaden S, Owens JK (1992) Posttreatment exposure to camptothecin enhances the lethal effects of x-rays on radioresistant human malignant melanoma cells. Int J Radiat Oncol Biol Phys 24: 939
3. Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML, Wyllie AH (1993) Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature 362: 849
4. D'Arpa P, Beardmore C, Liu LF (1990) Involvement of nucleic acid synthesis in cell killing mechanisms of topoisomerase poisons. Cancer Res 50: 6919
5. Del Bino G, Bruno S, Yi PN, Darzynkiewicz Z (1992) Apoptotic cell death triggered by camptothecin or teniposide. The cell cycle specificity and effects of ionizing radiation. Cell Prolif 25: 537
6. Drewinko B, Freireich E, Gottlieb J (1974) Lethal activity of camptothecin sodium on human lymphoma cells. Cancer Res 34: 747
7. Falk SJ, Smith PJ (1992) DNA damaging and cell cycle effects of the topoisomerase I poison camptothecin in irradiated human cells. Int J Radiat Biol 61: 749
8. Fan S, El-Deiry WS, Bae I, Freeman J, Jondle D, Bhatia K, Fornace AJJ, Magrath I, Kohn KW, O'Connor PM (1994) p53 gene mutations are associated with decreased sensitivity of human lymphoma cells to DNA damaging agents. Cancer Res 54: 5824
9. Fan S, Smith ML, Rivet DJ, Duba D, Zhan Q, Kohn KW, Fornace AJ, O'Connor PM (1995) Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. Cancer Res 55: 1649
10. Fujiwara T, Grimm EA, Mukhopadhyay T, Zhang W-W, Owen-Schaub LB, Roth JA (1994) Induction of chemosensitivity in human lung cancer cells in vivo by adenovirus-mediated transfer of the wild-type p53 gene. Cancer Res 54: 2287
11. Graeber TG, Peterson JF, Tsai M, Monica K, Fornace AJJ, Giaccia AJ (1994) Hypoxia induces accumulation of p53 protein, but activation of a G1-phase checkpoint by low-oxygen conditions is independent of p53 status. Mol Cell Biol 14: 6264
12. Graeber TG, Osmanian C, Jacks T, Housman DE, Koch CJ, Lowe SW, Giaccia AJ (1996) Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumors Nature 379: 88
13. Horwitz SB, Horwitz MS (1973) Effects of camptothecin on the breakage and repair of DNA during the cell cycle. Cancer Res 33: 2834
14. Huang T-S, Kuo M-L, Shew J-Y, Chou Y-W, Yang WK (1996) Distinct p53-mediated G1/S checkpoint responses in two NIH3T3 subclone cells following treatment with DNA-damaging agents. Oncogene 13: 625
15. Jansen WJ, Zwart B, Hulscher ST, Giaccone G, Pinedo HM, Boven E (1997) CPT-11 in human colon-cancer cell line and xenografts: characterization of cellular sensitivity determinants. Int J Cancer 70: 335
16. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW (1991) Participation of p53 protein in the cellular response to DNA damage. Cancer Res 51: 6304
17. Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB (1992) Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 89: 7491
18. Lee JM, Bernstein A (1993) p53 mutations increase resistance to ionizing radiation. Proc Natl Acad Sci U S A 90: 5742
19. Lee JM, Bernstein A (1995) Apoptosis, cancer and the p53 tumour suppressor gene. Cancer Metastasis Rev 14: 149
20. Li C-Y, Nagasawa H, William K, Dahlberg, Little JB (1995) Diminished capacity for p53 in mediating a radiation-induced G1 arrest in established human tumor cell lines. Oncogenetics 11: 1885
21. Li LH, Fraser TJ, Olin EJ, Bhuyan BK (1972) Action of camptothecin on mammalian cells in culture. Cancer Res 32: 2643
22. Lin D, Shield S, MT, Ullrich SJ, Appella E, Mercer WE (1992) Growth arrest induced by wild-type p53 protein blocks cells prior to or near the restriction point in late G1 phase. Proc Natl Acad Sci USA 89: 9210
23. Little JB (1968) Delayed initiation of DNA synthesis in irradiated human diploid cells. Nature 218: 1064
24. Little JB, Nagasawa H, Keng PC, Yu YJ, Li C-Y (1995) Absence of radiation-induced G1 arrest in two closely related

human lymphoblast cell lines that differ in p53 status. J Biol Chem 270: 11033
25. Lowe SW, Ruley HE, Jacks T, Housman DE (1993) p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 74: 957
26. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T (1993) p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature 362: 847
27. Matsumoto H, Takahashi A, Wang X, Ohnishi K, Ohnishi T (1997) Transfection of p53-knockout mouse fibroblasts with wild-type p53 increases the thermosensitivity and stimulates apoptosis induced by heat stress. Int J Radiat Oncol Biol Phys 39: 197
28. Mattern MR, Hofmann GA, McCabe FL, Johnson RK (1991) Synergistic cell killing by ionizing radiation and topoisomerase I inhibitor topotecan (SK&F 104864). Cancer Res 51: 5813
29. Michalovitz D, Halevy O, Oren M (1990) Conditional inhibition of transformation and of cell proliferation by a temperature-sensitive mutant of p53. Cell 62: 671
30. Müller H, Eppenberger U (1996) The dual role of mutant p53 protein in chemosensitivity of human cancers. Anticancer Res 16: 3845
31. Musk SRR, Steel GG (1990) The inhibition of cellular recovery in human tumor cells by inhibitors of topoisomerase. Br J Cancer 62: 364
32. Nagasawa H, Li CY, Maki CG, Imrich AC, Little JB (1995) Relationship between radiation-induced G1 phase arrest and p53 function in human tumor cells. Cancer Res 55: 1842
33. Ng CE, Bussey AM, Raaphorst GP (1994) Inhibition of potentially lethal and sublethal damage repair by camptothecin and etoposide in human melanoma cell lines. Int J Radiat Biol 66: 49
34. Omura M, Torigoe S, Kubota N (1997) SN-38, a metabolite of the camptothecin derivative CPT-11, potentiates the cytotoxic effect of radiation in human colon adenocarcinoma cells grown as spheroids. Radiother Oncol 43: 197
35. Rivory LP (1996) Irinotecan (CPT-11): a brief overview. Clin Exp Pharmacol Physiol 23: 1000
36. Sasai K, Guo GZ, Shibuya K, Oya N, Shibata T, Nagata Y, Hiraoka M (1998) Effects of SN-38 (an active metabolite of CPT-11) on responses of human and rodent cells to irradiation. Int J Radiat Oncol Biol Phys 42: 785
37. Sellins KS, Cohen JJ (1987) Gene induction by γ-irradiation leads to DNA fragmentation in lymphocytes. J Immunol 139: 3199
38. Sliche N, Myer WJ, Nelson WG, Slebos RJ, Kastan MB (1993) Loss of a p53-associated G1 checkpoint does not decrease cell survival following DNA damage. Cancer Res 53: 4164
39. Tamura K, Takada M, Kawase I, Tada T, Kudoh S, Okishio K, Fukuoka M, Yamaoka N, Fujiwara Y, Yamakido M (1997) Enhancement of tumor radio-response by irinotecan in human lung tumor xenograft. Jpn J Cancer Res 88: 218
40. Terasima T, Tolmach LJ (1963) Variations in several responses of Hela cells to X-irradiation during the division cycle. Biophys J 3: 11
41. Vasey PA, Jones NA, Jenkins S, Dive C, Brown R (1996) Cisplatin, camptothecin, and taxol sensitivities of cells with p53-associated multidrug resistance. Mol Pharmacol 50: 1536
42. Yonisch-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M (1991) Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature 352: 345
43. Yoshida A, Ueda T, Wano Y, Nakamura T (1993) DNA damage and cell killing by camptothecin and its derivative in human leukemia HL-60 cells. Jpn J Cancer Res 84: 566
44. Zambetti GP, Levine AJ (1993) A comparison of the biological activities of wild-type and mutant p53. FASEB J 7: 855