

Methylxanthine sensitization of human colon cancer cells to ^{186}Re -labeled monoclonal antibody

メタデータ	言語: eng 出版者: 公開日: 2017-10-03 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	http://hdl.handle.net/2297/2785

Methylxanthine Sensitization of Human Colon Cancer Cells to ^{186}Re -Labeled Monoclonal Antibody

Seigo Kinuya, Kunihiro Yokoyama, Miho Kudo, Yoshihito Kasahara, Katsutoshi Kobayashi, Shoji Motoishi, Katsuyuki Onoma, Hisashi Bunko, Takatoshi Michigishi, and Norihisa Tonami

Departments of Nuclear Medicine and Pediatrics, Kanazawa University School of Medicine, Kanazawa; Radioisotope Laboratory, Department of Research Reactor, Japan Atomic Energy Research Institute, Tokaimura; and Medical Informatics, Kanazawa University Hospital, Kanazawa, Japan

Tumor cells lacking the functional p53 suppressor gene may arrest at the G2 phase of the cell cycle after exposure to ionizing radiation, resulting in increased radioresistance. Methylxanthines (MTXs), such as pentoxifylline (PTX) or caffeine (CAF), can inhibit the G2-phase checkpoint arrest of damaged cells and thus radiosensitize them. However, the effect of MTX in cells irradiated with low-dose-rate β -emission is not well understood.

Methods: A clonogenic assay was performed with LS180 human colon cancer cells lacking the functional p53 suppressor gene. Cells were irradiated with increasing concentrations of ^{186}Re -mercaptoacetyltriglycine (^{186}Re -MAG3)-labeled A7 monoclonal antibody against colorectal cancer (0–925 kBq/mL) at 37°C in 5% CO_2 for 24 h in the presence or absence of PTX (0–2 mmol/L) or CAF (0–5 mmol/L). The enhancement ratio (ER) with MTX was calculated as a ratio of 50% cell-killing concentration of ^{186}Re -MAG3-A7 in control cells to that in cells treated with PTX or CAF. The cell cycle distribution was analyzed with a flow cytometer. **Results:** The concentration of 50% cell kill was 474 kBq/mL ^{186}Re -MAG3-A7. Both PTX and CAF dose dependently enhanced the cytotoxicity of ^{186}Re -MAG3-A7: ERs of 0.5 mmol/L PTX, 2 mmol/L PTX, 1 mmol/L CAF, and 5 mmol/L CAF were 1.50, 2.18, 1.54, and 2.63, respectively. Flow cytometry showed that the percentage nonirradiated cells in the G2/M phase of the cell cycle was $11.3\% \pm 1.66\%$. On the other hand, cells exposed to ^{186}Re -MAG3-A7 accumulated in the G2/M phase of the cell cycle ($40.2\% \pm 1.46\%$), which was inhibited by the presence of 1 mmol/L PTX ($19.8\% \pm 8.12\%$) or 2 mmol/L CAF ($26.9\% \pm 6.21\%$). **Conclusion:** Cellular modulation of the cell cycle with PTX and CAF radiosensitized LS180 colon cancer cells exposed to ^{186}Re radiation.

Key Words: radiosensitization; methylxanthine; ^{186}Re ; β -irradiation; cell cycle

J Nucl Med 2001; 42:596–600

Internal radiation therapy (IRT) using tumor-specific radiopharmaceuticals, such as radiolabeled monoclonal antibodies (mAbs), ^{131}I -metaiodobenzylguanidine, and ^{90}Y -anti-somatostatin receptor ligands, has been examined for the management of patients with malignant tumors. The response rate of patients with refractory malignant lymphoma to radioimmunotherapy is high (1,2). However, results in other types of tumors are less favorable, and therefore the sensitization of tumor cells to radiation with radionuclides should be beneficial in enhancing the efficacy of IRT.

Cells with DNA damage induced by high-dose-rate radiation are known to arrest at the G1 or G2 phase of the cell cycle and repair the damage, resulting in augmentation of their radioresistance (3). G1-phase arrest is mediated by the p53 suppressor gene function (4,5), and the G2-phase arrest is also affected by the p53 status to some extent (6,7). However, G2-phase arrest can occur in the absence of functional p53, as observed in many types of cancer cells (8). Substantial evidence indicates that inhibition of the G2 arrest with methylxanthines (MTXs), such as pentoxifylline (PTX) and caffeine (CAF), increases p53-independent apoptosis after radiation (9–15). Inhibition of the G2 checkpoint arrest also increases the death of cells with defective apoptotic pathways through the induction of giant cells, which would result from multiple rounds of DNA synthesis in the absence of mitosis (9,10).

Low-dose-rate radiation may also induce the checkpoint arrest of irradiated cells (16,17). However, most knowledge about the radiosensitization effect of MTX is concerned with high-dose-rate external-beam radiation, and little is known about the effect of low-dose-rate radiation with radionuclides emitting β -particles. Anderson et al. (16) showed the radiosensitization effect of CAF in Chinese hamster ovary cells irradiated with ^{131}I , and Macklis et al. (17) showed it in EL-4 lymphoma cells exposed to ^{90}Y . However, Macklis et al. indicated that Raji lymphoma cells were not sensitized by CAF and that the radiosensitization effect of CAF was not related to the cell cycle. Therefore,

Received Aug. 22, 2000; revision accepted Dec. 11, 2000.

For correspondence or reprints contact: Seigo Kinuya, MD, Department of Nuclear Medicine, Kanazawa University School of Medicine, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8640, Japan.

the cell cycle effect in cells exposed to low-dose-rate radiation seems specific to the cell line. It is generally accepted that the resistance of colorectal carcinomas to ionizing radiation is associated with p53 mutation, and the inhibition of G2 arrest with CAF reportedly increased the death of colorectal cancer cells exposed to high-dose-rate γ -radiation (10). The reported response rate of colon carcinomas to radioimmunotherapy was not high (18); therefore, the effect of MTX on colon cancer cells exposed to β -radiation is worth investigating.

^{186}Re has abundant intermediate-energy β -emission (71% of 1.07 MeV and 21% of 0.94 MeV). Its γ -emission of 137 keV (9%) is suitable for external detection with gamma cameras and produces a less nonspecific radiation dose than does the high-energy γ -emission of ^{131}I (364 keV), which is the most widely used nuclide in IRT. In this study, we examined the effect of PTX and CAF on the survival of human colon cancer cells lacking functional p53 protein after exposure to a ^{186}Re -labeled anti-colorectal cancer mAb in vitro.

MATERIALS AND METHODS

Cell Culture and Radiolabeling

LS180 human colon cancer cells lacking functional p53 protein (American Type Culture Collection, Rockville, MD) were maintained as a monolayer in a medium (Dulbecco's modified Eagle medium; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) with 10% fetal calf serum at 37°C in a 5% CO_2 atmosphere, harvested with 0.1% trypsin and 0.02% ethylenediaminetetraacetic acid, and inoculated into six-well culture plates. ^{186}Re -perrhenate ($^{186}\text{ReO}_4^-$) was produced by the $^{185}\text{Re}(n,\gamma)$ reaction (Japan Atomic Energy Research Institute, Tokaimura, Japan), chelated with *S*-benzoylmercaptoacetyltriglycine (*S*-benzoyl-MAG3) (a gift from Dr. Yasushi Arano, Kyoto University), and conjugated to A7 (an IgG1 anti-colorectal carcinoma antibody) (19). Briefly, the mixture of $^{186}\text{ReO}_4^-$, SnCl_2 , and *S*-benzoyl-MAG3 at the mol/L ratios of 2.3:1 for *S*-benzoyl-MAG3:Re and 8.0:1 for Sn^{2+} :Re was heated under a N_2 stream, resulting in ^{186}Re -MAG3, which was conjugated to A7 after esterification with 2,3,5,6-tetrafluorophenol (TFP) (Nacalai Tesque, Kyoto, Japan). ^{186}Re -MAG3-A7 was purified with a PD-10 column (Pharmacia LKB Biotechnology, Uppsala, Sweden), and its immunoreactivity was assessed using LS180 cells with an antigen excess.

Clonogenic Assay

LS180 cells were incubated in six-well culture plates with 0–925 kBq/mL ^{186}Re -A7 in 2 mL medium for 24 h. PTX (Sigma, St. Louis, MO) and CAF (Sigma) were added simultaneously to the wells at concentrations of 0–5 mmol/L and 0–2 mmol/L, respectively. The cells were washed with phosphate-buffered saline (PBS) twice and maintained in fresh medium for 10 d. Cell survival was determined by counting colonies fixed with 80% methanol and stained with crystal violet. Colonies containing ≥ 50 cells were considered as survivors. The surviving fraction was corrected by plating efficiency at each concentration of PTX and CAF. A triplicate assay was performed in all experiments. The data were fit to an exponential function to obtain a radioactivity concentration, producing a surviving fraction of 0.5 (i.e., 50% cell kill). An enhancement ratio (ER) at each concentration of PTX or

CAF was calculated as the ratio of 50% killing concentration in control cells to that in cells treated with PTX or CAF.

Cell Cycle Analysis

The distribution of cells in the phases of the cell cycle was analyzed with a flow cytometer (Cytoron Absolute; Ortho Diagnostic Systems, Raritan, NJ). Briefly, control cells and cells irradiated with 463 kBq/mL ^{186}Re -MAG3-A7 in the presence or absence of 1 mmol/L PTX or 2 mmol/L CAF were suspended at 2×10^6 cells/250 μL PBS, fixed with 250 μL 100% ethanol, and stored at 4°C. The cells were washed and resuspended with 250 μL ribonuclease A (500 U/mL; Sigma). They were then stained with 250 μL propidium iodide (50 $\mu\text{g}/\text{mL}$; Sigma). The DNA content was analyzed with a flow cytometer, and the cellular distribution in the cell cycle was determined. The results were analyzed by an unpaired *t* test. In the analyses, the level of significance was set at 5%.

RESULTS

The incorporation of ^{186}Re into *S*-benzoyl-MAG3 was 98%. The radiochemical yield of ^{186}Re -MAG3-TFP production was 89%, and 50% of ^{186}Re -MAG3-TFP was conjugated to A7 mAb. The specific activity of purified ^{186}Re -MAG3-A7 was 90 MBq/mg, and its immunoreactivity was 63%.

Figure 1 and Table 1 summarize the effect of PTX and CAF on the survival of LS180 colon cancer cells irradiated with ^{186}Re -MAG3-A7. The data of surviving curves were well fit to an exponential function, showing $R^2 > 0.96$ for all assay conditions. The concentration of 50% cell kill was

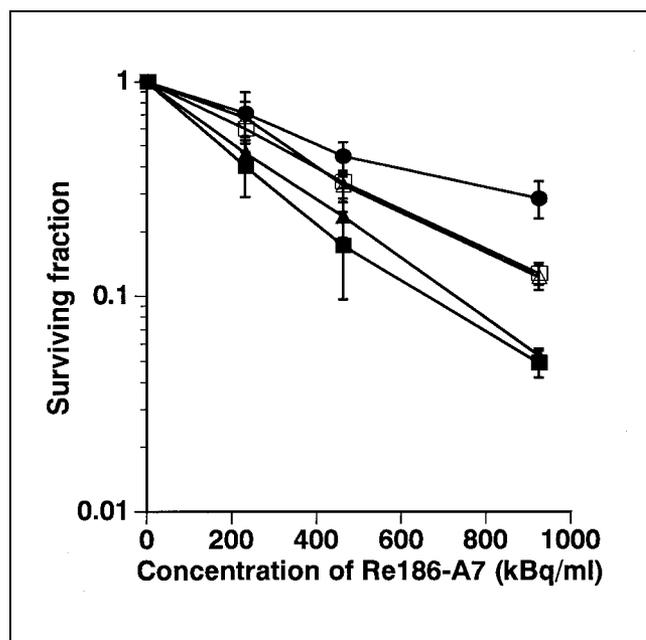


FIGURE 1. Surviving fraction of LS180 colon cancer cells exposed to ^{186}Re -MAG3-A7 anti-colorectal mAb in presence of PTX or CAF. ●, Control cells; △, PTX (0.5 mmol/L); ▲, PTX (2 mmol/L); □, CAF (1 mmol/L); ■, CAF (5 mmol/L). Values are mean \pm SD of triplicate assays.

TABLE 1
Radiosensitization Effect of MTXs

Treatment	50% kill dose* (kBq/mL)	ER†
Control group‡	474	—
PTX, 0.5 mmol/L	316	1.50
PTX, 2 mmol/L	217	2.18
CAF, 1 mmol/L	307	1.54
CAF, 5 mmol/L	180	2.64

*Concentration of radiocompound producing survival fraction of 0.5.

†Expressed as 50% kill dose of control/50% kill dose with PTX or CAF.

‡Irradiated without PTX or CAF.

474 kBq/mL, and both PTX and CAF dose dependently enhanced the cytotoxicity of ^{186}Re -MAG3-A7.

Flow cytometry revealed that the cells exposed to ^{186}Re -MAG3-A7 accumulated in the G2/M phase of the cell cycle; 40.2% of the irradiated cells were found in the G2/M phase compared with 11.3% of the control cells ($P < 0.0001$) (Fig. 2). The presence of PTX or CAF inhibited the G2/M-phase accumulation of irradiated cells ($P < 0.01$).

DISCUSSION

Once DNA damage is induced by ionizing radiation or cytotoxic drugs, some of the damaged cells die through the apoptotic pathway. Others survive by repairing the damage during transient arrest at cell cycle checkpoints in the G1 or G2 phase. G1-phase arrest is mediated by the p53 suppressor gene function (4,5), and many tumor cells fail to arrest at the G1 phase because of lack of functional p53. However, G2-phase arrest can occur in the absence of functional p53, as observed in many types of cells (8), and a correlation between G2-phase arrest and radioresistance has been shown (20,21). The inhibition of the G2 arrest with MTXs, such as PTX and CAF, increases p53-independent apoptosis after radiation (9–15), and the mechanism underlying the inhibitory effect of CAF on the G2-phase arrest was recently shown to be the inhibition of the Chk1 inhibitory function on the phosphatase Cdc25c (22).

Although the positive effect of MTX in cells exposed to high-dose-rate external-beam radiation has been well documented, only a few reports on low-dose-rate β -irradiation are available, and the effect of cell cycle modulation on β -irradiation is not fully understood. In this study, we presented evidence of the radiosensitizing effect of PTX and CAF in colon cancer cells exposed to ^{186}Re radiation and found a possible correlation between radiosensitization

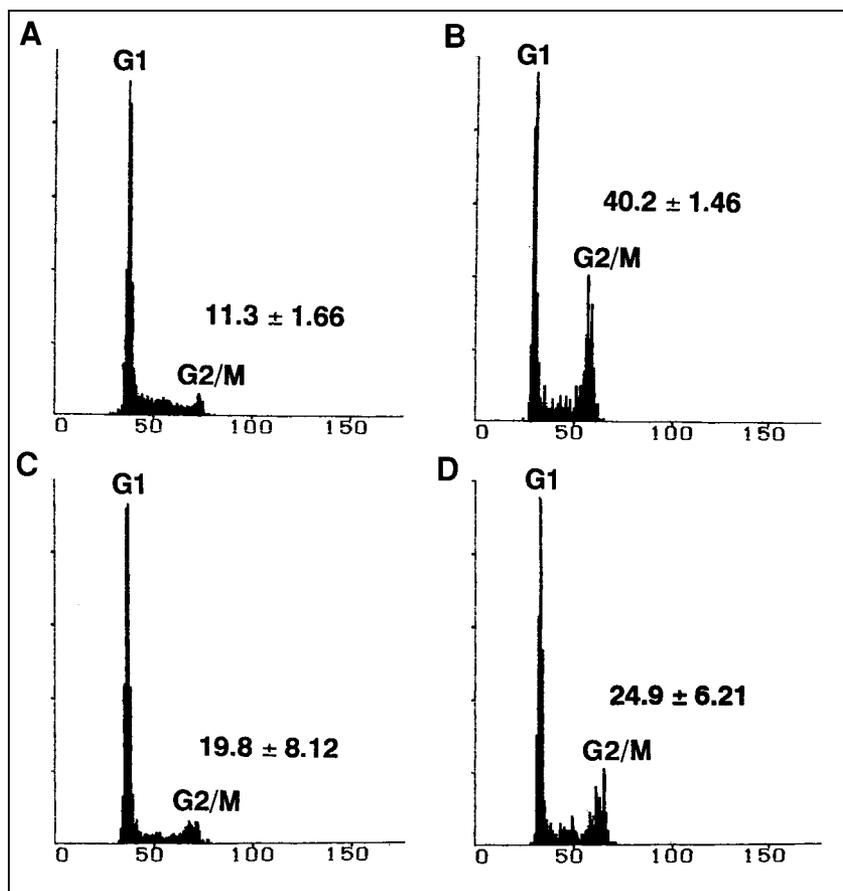


FIGURE 2. Representative histograms of flow cytometry of LS180 colon cancer cells. (A) Nonirradiated cells. (B) Cells irradiated with ^{186}Re -MAG3-A7 (463 kBq/mL for 24 h). (C) Cells irradiated in presence of PTX (1 mmol/L). (D) Cells irradiated in presence of CAF (2 mmol/L). Mean \pm SD ($n = 3$) of percentage cells in G2/M phase of cell cycle is indicated.

and inhibition of the G2-phase arrest of irradiated cells. Anderson et al. (16) similarly reported a radiosensitization effect of CAF in Chinese hamster ovary cells irradiated with ¹³¹I.

In contrast to these findings, the effectiveness of low-dose-rate irradiation is usually explained by the G2-phase accumulation of cells, because cells are radiosensitive in the G2 phase (23). Ning and Knox (24) recently reported the higher survival rate of CAF-treated HL60 leukemia cells exposed to low-dose-rate γ -radiation with a ¹³⁷Cs source. They concluded that the CAF-induced inhibition of the G2 arrest decreased the proportion of cells in the radiosensitive G2 phase, resulting in a higher survival rate of CAF-treated cells. On the other hand, the findings of Macklis et al. (17) are inconsistent with this hypothesis. They found that EL-4 lymphoma cells, showing less G2 accumulation than Raji lymphoma cells, were much more sensitive to low-dose-rate β -radiation of ⁹⁰Y than Raji cells, whereas these two cell lines showed equal sensitivity to high-dose-rate γ -radiation. Dewese et al. (25) indicated that cell cycle modulation was not a dominant determinant in five prostate cancer cell lines exposed to low-dose-rate γ -radiation. These inconsistent reports suggest that the effect of cell cycle modulation on the cytotoxicity of low-dose-rate radiation is likely specific to the cell line (26).

Radiation-induced apoptosis plays an important role in the responses of cells to irradiation, and MTX increases the p53-independent apoptosis of cells after irradiation (9,10). The expression level of Bcl-2, an anti-apoptotic oncogene, may therefore correlate with the induction of apoptosis by MTX (22). Other genes, such as p21, bax, RAF1, and p34, may also be involved (27–31). Investigation with many cell lines will be required to achieve a better understanding of the effect of MTX in cells exposed to low-dose-rate radiation with radiopharmaceuticals.

A limitation for the in vivo use of MTX is the difficulty in achieving the concentration required for radiosensitization through cell cycle modulation when administered systemically (16). To obtain a sufficiently high concentration, Tsuchiya et al. (32,33) intra-arterially infused CAF in patients with bone and soft-tissue sarcomas at a dose of 1.5 g/m² for 3 consecutive days in combination with chemotherapeutic agents. They found a much better response rate with this protocol than with conventional chemotherapy, suggesting the possible applicability of MTX to enhance the IRT effect. In addition, for the treatment of patients with intraperitoneal dissemination, we may administer these agents intraperitoneally, and it may be possible to obtain the lesional concentrations required for the radiosensitization effect of these agents in vivo. However, because IRT is a systemic therapy and patients with early systemic metastatic diseases would be the most suitable candidates for IRT (34), systemic administration of MTX would be needed. Therefore, the development of novel MTX derivatives of higher potential or other types of agents acting as cell cycle modulators would be a priority.

To enhance the efficacy of IRT, improved targeting of radiopharmaceuticals to tumors would be beneficial (35). It has been shown that PTX may improve tumor perfusion and oxygenation in vivo (36,37). Honess et al. (36) showed a 50%–70% increase of tumor perfusion by the administration of PTX in a nontoxic dose range in an animal model, and Song et al. (37) also showed an enhanced antitumor effect of x-irradiation with increased tumor oxygenation in a model administered with a similar nontoxic dose of PTX. These reports indicate that this kind of agent would play a significant role in enhancing IRT efficacy by increasing tumor accumulation of radiopharmaceuticals and improving tumor oxygenation status.

CONCLUSION

PTX and CAF inhibited the G2-phase arrest of LS180 human colon cancer cells exposed to ¹⁸⁶Re radiation and decreased their survival. These results encourage further investigation of the cell cycle checkpoint modulation of tumor cells for the enhancement of IRT.

ACKNOWLEDGMENTS

The authors thank Dr. Yasushi Arano (currently at Chiba University) for providing *S*-benzoyl-MAG3, Toshio Takahashi and Dr. Toshiharu Yamaguchi (Kyoto Prefectural University of Medicine) for A7 mAb, and John S. Gelblum for assistance in preparing the manuscript. This study was performed as a part of the Working Group on Radioactive Rhenium supported by the Consultative Committee of Research on Radioisotopes and the Subcommittee for Production and Radiolabeling in the Japan Atomic Energy Research Institute. This study was supported in part by grants-in-aid from the Ministry of Education, Science, Sports and Culture in Japan and from the Sagawa Cancer Research Foundation.

REFERENCES

1. Kaminski MS, Zasadny KR, Francis IR, et al. Iodine-131-anti-B1 radioimmunotherapy for B-cell lymphoma. *J Clin Oncol*. 1996;14:1974–1981.
2. Press OW, Eary JF, Appelbaum FR, et al. Phase II trial of ¹³¹I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. *Lancet*. 1995;346:336–340.
3. Hartwell LH, Kastan MB. Cell cycle control and cancer. *Science*. 1994;266:1821–1828.
4. O'Connor PM, Jackman J, Jondle D, Bhatia K, Magrath I, Kohn KW. Role of the p53 tumor suppressor gene in cell cycle arrest and radiosensitivity of Burkitt's lymphoma cell lines. *Cancer Res*. 1993;53:4776–4780.
5. McIlwrath AJ, Vasey PA, Ross GM, Brown R. Cell cycle arrests and radiosensitivity of human tumor cell lines: dependence on wild-type p53 for radiosensitivity. *Cancer Res*. 1994;54:3718–3722.
6. Stewart N, Hicks GG, Paraskevas F, Mowat M. Evidence for a second cell cycle block at G2/M by p53. *Oncogene*. 1995;10:109–115.
7. Guillof C, Rosselli F, Krishnaraju K, Moustacchi E, Hoffman B, Liebermann DA. p53 involvement in control of G2 exit of the cell cycle: role in DNA damage-induced apoptosis. *Oncogene*. 1995;10:2263–2270.
8. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res*. 1991;51:6304–6311.
9. Bracey TS, Miller JC, Preece A, Paraskeva C. γ -Radiation-induced apoptosis in human colorectal adenoma and carcinoma cell lines can occur in the absence of wild type p53. *Oncogene*. 1995;10:2391–2396.

10. Bracey TS, Williams AC, Paraskeva C. Inhibition of radiation-induced G2 delay potentiates cell death by apoptosis and/or the inhibition of giant cells in colorectal tumor cells with disrupted p53 functions. *Clin Cancer Res.* 1997;3:1371-1381.
11. Rowley R. Reduction of radiation-induced G2 arrest by caffeine. *Radiat Res.* 1992;129:224-227.
12. Russell KJ, Wiens LW, Demers GW, Galloway DA, Plon SE, Groudine M. Abrogation of the G2 checkpoint results in differential radiosensitization of G1 checkpoint-deficient and G1 checkpoint-competent cells. *Cancer Res.* 1995;55:1639-1642.
13. Powell SN, DeFrank JS, Connell P, et al. Differential sensitivity of p53(-) and p53(+) cells to caffeine-induced radiosensitization and override of G2 delay. *Cancer Res.* 1995;55:1643-1648.
14. Russell KJ, Wiens LW, Demers GW, et al. Preferential radiosensitization of G1 checkpoint-deficiency cells by methylxanthines. *Int J Radiat Oncol Biol Phys.* 1996;36:1099-1106.
15. Li Y-X, Weber-Johnson K, Sun L-Q, Paschoud N, Mirimanoff R-O, Coucke PA. Effect of pentoxifylline on radiation-induced G2-phase delay and radiosensitivity of human colon and cervical cancer cells. *Radiat Res.* 1998;149:338-342.
16. Anderson LL, Lau CC, Gracely EJ, Berkowitz RS, Kassis AI. Enhancement of ¹³¹I-mediated cytotoxicity by caffeine. *Gynecol Oncol.* 1997;65:253-257.
17. Macklis RM, Beresford BA, Humm JL. Radiobiologic studies of low-dose-rate ⁹⁰Y-lymphoma therapy. *Cancer.* 1994;73:966-973.
18. Divgi CR, Scott AM, Dantis L, et al. Phase I radioimmunotherapy trial with iodine-131-CC49 in metastatic colon cancer. *J Nucl Med.* 1995;36:586-592.
19. Kinuya S, Yokoyama K, Tega H, et al. Rhenium-186-mercaptoacetyltriglycine labeled monoclonal antibody for radioimmunotherapy: in vitro assessment, in vivo kinetics and dosimetry in tumor-bearing nude mice. *Jpn J Cancer Res.* 1998;89:870-878.
20. Nagasawa H, Keng P, Harley R, Dahlberg W, Little JB. Relationship between γ -ray-induced G(2)/M delay and cellular radiosensitivity. *Int J Radiat Biol.* 1994;66:373-379.
21. Su L, Little JB. Prolonged cell cycle delay in radioresistant human cell lines transfected with activated ras oncogene and/or simian virus 40 T-antigen. *Radiat Res.* 1993;133:73-79.
22. Kumagai A, Guo Z, Emami KH, Wang SX, Dunphy WG. The Xenopus chk1 protein kinase mediates a caffeine-sensitive pathway of checkpoint control in cell-free extracts. *J Cell Biol.* 1998;142:1559-1569.
23. Murtha AD. Radiobiology of low-dose-rate radiation relevant to radioimmunotherapy. *Cancer Biother Radiopharm.* 2000;15:7-14.
24. Ning S, Knox SJ. G2/M-phase arrest and death by apoptosis of HL60 cells irradiated with exponentially decreasing low-dose-rate gamma radiation. *Radiat Res.* 1999;151:659-669.
25. Deweese TL, Shipman JM, Dillehay LE, Nelson WG. Sensitivity of human prostatic carcinoma cell lines to low dose rate radiation exposure. *J Urol.* 1998;159:591-598.
26. Musk SRR. Reduction of radiation-induced cell cycle blocks does not necessarily lead to increased cell killing. *Radiat Res.* 1991;125:262-266.
27. Wouters BG, Denko NC, Giaccia AJ, Brown JM. A p53 and apoptotic independent role for p21^{waf1} in tumour response to radiation therapy. *Oncogene.* 1999;11:6540-6545.
28. Ofir R, Zhang LC, Kyne AP, Houtzager V, O'Connor L, Adams JM. Gamma-radiation-induced growth arrest and apoptosis in p53-null lymphoma cells is accompanied by modest transcriptional changes in many genes. *DNA Cell Biol.* 2000;19:29-37.
29. Bukholm IK, Nesland JM. Protein expression of p53, p21 (WAF1/CIP1), bcl-2, Bax, cyclin D1 and pRb in human colon carcinomas. *Virchows Arch.* 2000;436:224-228.
30. Warenus HM, Jones MD, Thompson CCM. Exit from G₂ phase after 2 Gy gamma irradiation is faster in radiosensitive human cells with high expression of the *RAF1* proto-oncogene. *Radiat Res.* 1996;146:485-493.
31. Yao S-L, Akhtar AJ, McKenna KA, et al. Selective radiosensitization of p53-deficient cells by caffeine-mediated activation of p34^{cdc2} kinase. *Nat Med.* 1996;2:1140-1143.
32. Tsuchiya H, Tomita K, Mori Y, et al. Caffeine-assisted chemotherapy and minimized tumor excision for nonmetastatic osteosarcoma. *Anticancer Res.* 1998;18:657-666.
33. Tsuchiya H, Tomita K, Yamamoto N, Mori Y, Asada N. Caffeine-potentiated chemotherapy and conservative surgery for high-grade soft-tissue sarcoma. *Anticancer Res.* 1998;18:3652-3656.
34. Juweid M, Sharkey RM, Behr T, et al. Radioimmunotherapy of patients with small-volume tumors using iodine-131 labeled anti-CEA monoclonal antibody NP-4 F(ab')₂. *J Nucl Med.* 1996;37:1504-1510.
35. Kinuya S, Yokoyama K, Kawashima A, et al. Pharmacologic intervention with angiotensin II and kinase inhibitor enhanced efficacy of RIT in human colon cancer xenografts. *J Nucl Med.* 2000;41:1244-1249.
36. Honess DJ, Dennis JF, Bleehen NM. Pentoxifylline: its pharmacokinetics and ability to improve tumour perfusion and radiosensitivity in mice. *Radiation Oncol.* 1993;28:208-218.
37. Song CW, Hasegawa T, Kwon HC, Lyons JC, Levitt SH. Increase in tumor oxygenation and radiosensitivity caused by pentoxifylline. *Radiat Res.* 1992;130:205-210.