been suggested that flavopiridol is an attractive agent for the treatment of esophageal cancer.[8] However, the potential value of flavopiridol as a radio-sensitizer for esophageal squamous cell carcinoma has not been clarified. Flavopiridol induces the highly radio-sensitive G_2/M phase of the cell cycle and inhibits several molecular factors that determine the sensitivity of tumor cells to radiation, such as EGFR and Bcl-2, so it seems to be a potential radiation sensitizer.

Several Phase I and Phase II trials have shown some evidence of the anticancer activity of this agent. However, flavopiridol has been reported to show dose-limiting toxicities of severe diarrhea and vascular thrombotic events,[13,14] including myocardial infarction, transient neurological ischemic attacks, deep vein thrombosis and pulmonary embolism.[15] Thus, flavopiridol is thought to be too toxic for clinical use as a single agent.

In the present study, we investigated the in vitro effect of flavopiridol alone and the in vitro radiosensitizing effect of low-dose flavopiridol on esophageal squamous cell carcinoma cell lines.

MATERIALS AND METHODS

Cell lines

The TE8, TE9 and KE4 cell lines, were derived from squamous cell carcinoma of the esophagus. TE8 and TE9 were obtained from the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer at Tohoku University,[16] while KE4 was kindly provided by Professor Kyogo Ito (Department of Immunology, Kurume University School of Medicine, Fukuoka, Japan). These cell lines were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and penicillin, and incubated at 37°C in an atmosphere of 5% CO_2.

Materials

Flavopiridol was obtained from Aventis Pharmaceuticals Inc. (Bridgewater, NJ). A 10-mg/mL stock solution was prepared in 0.05 mol/L acetic acid and stored at 4 C. This solution was further diluted in medium and used at final concentrations ranging from 0.01 nmol/L to 400 nmol/L.

In vitro proliferation assay

Growth inhibition was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. Cells were cultured in RPMI 1640 medium at 1×10^4 cells/well in 96-well plates for 24 or 48 h at 37 C with flavopiridol (0.01–400 nmol/L) or medium alone as the control. The plates were centrifuged at 1200 × *g* for 5 min and then washed with 150 μL of PBS and centrifuged again. After removing the supernatant, MTT (20 μL of a 0.5-mg/mL solution in PBS) was added to each well. After 4 h of incubation at 37°C, the cells were lysed with 150 μL of DMSO and the absorbance of each well at 560 and 655 nm was measured by a microplate reader (Bio-Rad, Richmond, CA). The mean absorbance of five wells was used as an indicator of relative cell growth.

Morphological examination

The apoptotic effect of flavopiridol was determined morphologically by fluorescence microscopy after labeling with Hoechst 33342 (Sigma Chemical Co., St. Louis, MO). After 48 h of exposure to flavopiridol (0.05 nmol/L or 300 nmol/L), cells were collected by trypsinization, washed in PBS and centrifuged twice at 2000 × *g* for 10 min each time. The pellet was resuspended in 50 μL of PBS and 10 μL of Hoechst 33342 was added. A 10-μL aliquot of the suspension was placed on a microscope slide, covered with a 22-mm^2 coverslip and examined under UV spectrum. Apoptosis was characterized morphologically by detection of condensed chromatin and fragmented nuclei.

Western blotting

Whole cell lysates (150 μg) obtained after 48 h of exposure to flavopiridol (0.05 nmol/L and 300 nmol/L) and control cell lysates were prepared for Western blot analysis. Proteins were resolved by SDS-PAGE and transferred to Immobilon-P membranes (Millipore, Danvers, MA). Nonspecific binding was blocked by incubation with Tris-buffered saline (TBS) and 5% non-fat dried milk. The membranes were probed with mouse monoclonal antibodies specific for Rb, cyclin D1 and Bcl-2 (PharMingen, San Diego, CA). A secondary horseradish peroxidase-conjugated antibody (Cappel) was applied for 1 h, followed by incubation with enhanced chemiluminescence reagents (Amersham, Buckinghamshire, United Kingdom). The level of expression of each target protein was quantified using a densitometric scanning system (Fuji Film, Tokyo, Japan).

Flow cytometric analysis

Cells (2.5×10^5 cells) were incubated with or without flavopiridol (0.05 and 300 nmol/L) for 48 h. After removal of the supernatant and dead cells, the viable cells were collected, washed with cold PBS, treated with 250 μL of 0.2% Triton X and 250 μL of 1% RNase A, and stained with 50 μg/mL propidium iodide (PI). Then the cell cycle distribution (DNA content) was determined by flow cytometry

with a FACScan (Becton Dickinson, San Jose, CA), with the Cell Quest (Becton Dickinson) and ModFit LT programs (Verity Software House, Inc., Topsham, ME) being used for analysis.

Colony-forming assay

The combined effect of radiation and flavopiridol was determined by a colony-forming assay. Cells (2.5×10^5) were incubated with or without 0.05 nmol/L flavopiridol for 48 h and then were harvested by trypsinization and centrifugation. Cells were irradiated at a dose rate of 1.4 Gy/min to a total dose of 2–10 Gy using an X-ray unit (Hitachi: 140 kV, 4 mA, 0.1 mm Al filter). Then the cells were plated (1×10^2 cells) in 60 mm petri dishes and colony formation was assessed after incubation for 12 days. Colonies were fixed with 96% methanol, stained with 2% Giemsa solution and counted to assess clonogenic survival. The Student's *t*-test was used for the comparison of the cell survival data.

RESULTS

Flavopiridol inhibits the proliferation of esophageal cancer cells

To determine whether or not flavopiridol could inhibit the cell growth of esophageal cancer cells, TE8, TE9 and KE4 cells were incubated with or without 0.01–400 nmol/L flavopiridol for 24 or 48 h and cell growth was evaluated by MTT assay. Growth inhibition was observed in all of the cells exposed to each concentration of flavopiridol during the first 24 h of incubation (Fig. 1A) and it was shown that flavopiridol caused dose-dependent and time-dependent inhibition of cell growth. The IC_{50} values at 48 h were approximately 110 nmol/L (KE4), 250 nmol/L (TE8) and 200 nmol/L (TE9) (Fig. 1B). In contrast, there was no obvious further growth inhibition of all three lines at the concentrations of 0.01–10 nmol/L from 24 h to 48 h (Fig. 1).

These results showed that flavopiridol could potently inhibit the growth of esophageal squamous cell carcinoma cells in vitro. Based upon these findings, we selected a concentration of 300 nmol/L that caused significant growth inhibition and a concentration 0.05 nmol/L that caused no obvious inhibition for subsequent experiments.

Flavopiridol induces apoptosis of esophageal cancer cells

To determine whether inhibition of the growth of esophageal cancer cells (TE8, TE9 and KE4) by flavopiridol was attributable to apoptosis, the cells were incubated with or without 0.05 or 300 nmol/L flavopiridol for 48 h, and were subjected to fluorescence microscopy after staining with Hoechst 33342. At a concentration of 300 nmol/L, high levels of Hoechst 33342 fluorescence were observed in all three lines and there was an increase of cells with nuclear fragmentation and chromatin condensation. In contrast, the cells incubated at 0.05 nmol/L showed low levels of Hoechst 33342 fluorescence similar to control cells (Fig. 2).

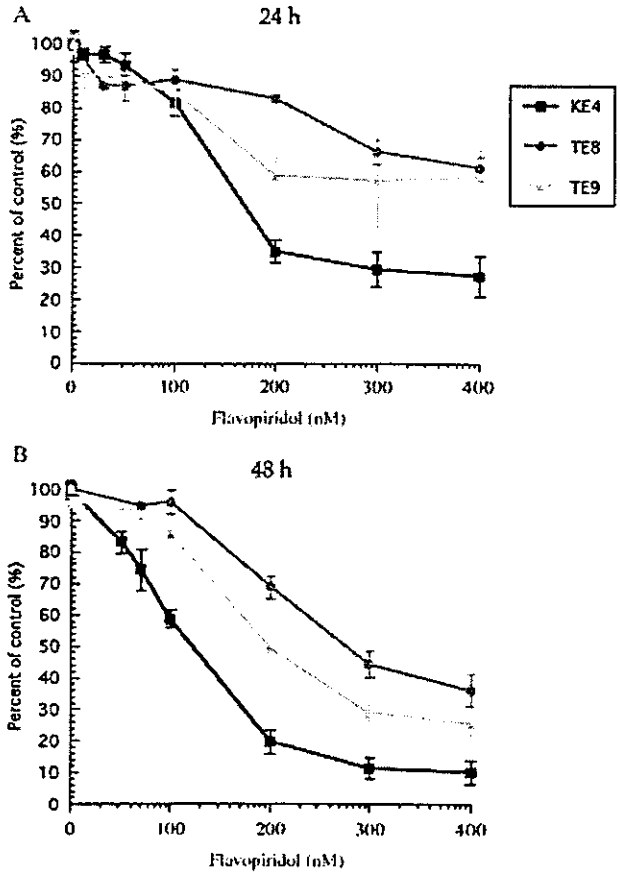

Fig. 1 Flavopiridol treatment inhibits the growth of esophageal cancer cells relative to control. Cells were incubated with or without 0.01–400 nmol/L flavopiridol for 24 or 48 h. Growth inhibition in the presence and absence of flavopiridol was assessed at the designated times by the MTT assay. Results are representative for one of three independent experiments: (A) after 24 h treatment; (B) after 48 h treatment. (Bars, ± SD.)

Expression of cyclin D1 and Rb decreases after exposure to a low dose of flavopiridol

To investigate whether the cell cycle and apoptotic factors were affected by flavopiridol, cyclin D1, Rb and Bcl-2 were detected by Western blotting in cells incubated with or without 0.05 or 300 nmol/L flavopiridol for 48 h. At a concentration of 300 nmol/L, Rb and cyclin D1 protein levels were decreased below control levels in TE8, TE9 and KE4 cells. In addition, Bcl-2 protein was decreased in KE4 cells. At a concentration of 0.05 nmol/L, Rb, cyclin D1 and Bcl-2 protein levels were slightly decreased to below control levels in KE4 cells, while, Rb and

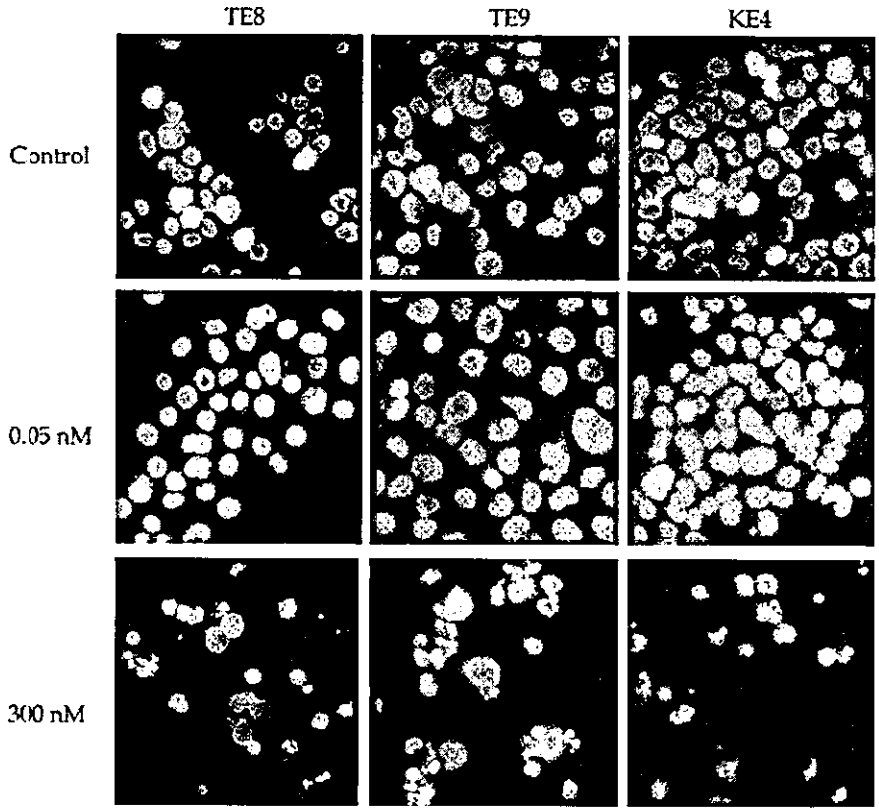

Fig. 2 Fluorescent microscopy of cells incubated with or without flavopiridol. Cells were stained with Hoechst 33342 as described under 'Materials and methods'.

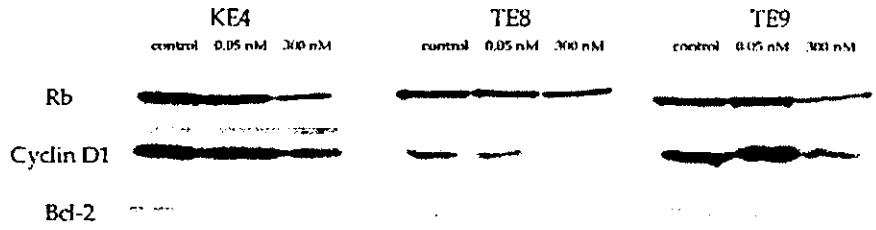

Fig. 3 Western blot analysis of cyclin D1, Rb and Bcl-2 protein expression by KE4, TE8 and TE9 cells incubated for 48 h with or without flavopiridol.

cyclin D1 were slightly decreased in TE8 cells. The levels of Rb, cyclin D1 and Bcl-2 protein were all unchanged in TE9 cells after treatment with 0.05 nmol/L flavopiridol (Fig. 3).

Flavopiridol induces cell cycle arrest at G_2/M and G_1 in esophageal cancer cells

The effect of flavopiridol on the cell cycle distribution of TE8, TE9 and KE4 cells is shown in Table 1. Consistent with the previously demonstrated inhibition of cell growth, exposure to 300 nmol/L flavopiridol caused G_1 arrest, manifested by a decrease of G_2/M and S cells in the cultured TE8, TE9 and KE4 cell lines. In contrast, exposure to 0.05 nmol/L flavopiridol caused G_2/M arrest in TE8, TE9 and KE4 cells. These results indicated that 0.05 nmol/L flavopiridol, which had no obvious effect on growth or apoptosis, was able to cause cell cycle arrest at the highly radio-sensitive G_2/M phase.

Flavopiridol increases the effect of radiation on esophageal cancer cells

To determine whether or not flavopiridol had a radio-sensitizer effect on esophageal cancer cells, cells were incubated with a concentration of 0.05 nmol/L for 48 h before irradiation. Then, cells were incubated for 12 days after irradiation and viability was determined by a clonogenic assay. It was found that 0.05 nmol/L flavopiridol showed a synergistic effect on radiation in TE8, TE9 and KE4 cells (Fig. 4).

DISCUSSION

This study obtained two novel findings. First, experimental evidence showed that combined treatment with low-dose flavopiridol enhanced radiation killing of esophageal cancer cell lines. Second, it was suggested that cell cycle progression may be arrested in G_2/M phase after exposure of esophageal cancer cells to a low dose of flavopiridol (0.05 nmol/L), while cell cycle arrest in G_1 phase occurs after exposure to a high dose of flavopiridol (300 nmol/L). In other words, our results raised the possibility that the cell cycle of tumor cells could be controllable by the flavopiridol treatment schedule.

Table 1 Effect of flavopiridol on the cell cycle

	Cell cycle distribution		
Cell line	% G_0/G_1	% S	% G_2/M
TE8			
Control	57.7	22.5	19.8
0.05 nmol/L	50.8	20.3	28.9
300 nmol/L	82.7	18.2	15.1
TE9			
Control	57.4	17.9	24.7
0.05 nmol/L	51.0	18.0	31.0
300 nmol/L	73.8	8.0	18.2
KE4			
Control	44.5	24.9	30.6
0.05 nmol/L	40.0	25.2	34.8
300 nmol/L	80.6	4.4	15.0

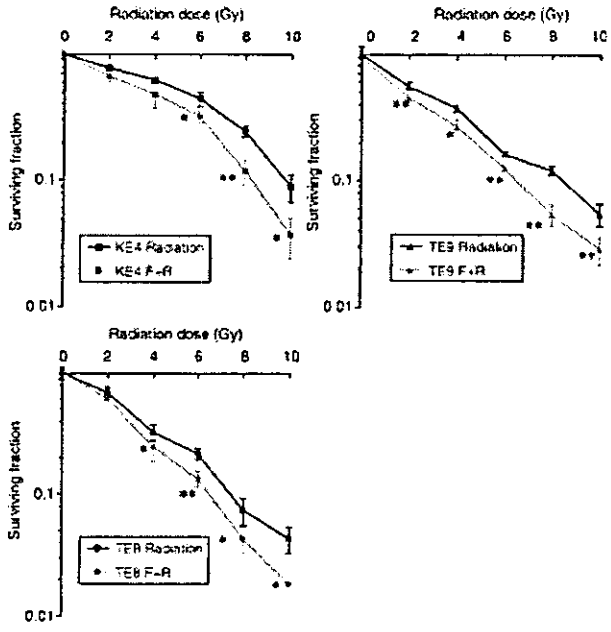

Fig. 4 TE8, TE9 and KE4 cells were irradiated (2–10 Gy) and viability was investigated with or without flavopiridol treatment. Exposure to flavopiridol for 48 h resulted in significant radiosensitization compared with untreated control cells. Bars, ± SD. (Significantly different to control (Student's *t*-test): $*P < 0.05$, $**P < 0.01$.)

It is unclear why flavopiridol arrests some cells in G_1 phase and other cells in G_2/M phase or both, yet the inhibition of cdks may play a key role in cell cycle arrest. Flavopiridol was reported to demonstrate potent inhibition of cell cycle progression at concentrations below 300 nmol/L,[17] while 200–300 nmol/L is the median steady-state concentration range that was achieved in clinical trials using the recommended dose of flavopiridol.[14] It has been shown to mediate either G_1 phase or G_2/M phase arrest with a progressive decrease of the S phase fraction in several cancer cell lines, including those derived from prostate, esophageal, lung and breast cancer.[6,8,18,19] In the present study, we demonstrated that flavopiridol mediated G_1 phase arrest of esophageal squamous cell carcinoma cell lines when used at a concentration of 300 nmol/L. In contrast, G_2/M phase arrest was found in other esophageal cancer cell lines.[8] Consistent with the initial inhibition of cell growth, flavopiridol-treated cells show an increase of the cell populations in sub G_1 phase,[19,20] and it has been reported that such cells are actually dying of apoptosis.[21] In the present study, there was no obvious increase of the sub G_1 population at a concentration of 300 nmol/L in all three cell lines, because only surviving cells were analyzed and floating cells which may have undergone apoptosis due to flavopiridol treatment were discarded before analysis. In fact, total cells (adherent plus floating cells) showed an increase of sub G_1 populations after flavopiridol exposure (data not shown).

The effect of flavopiridol on cdks has been investigated and the IC_{50} of cdks is 20–400 nmol/L.[6,22,23] Inhibition of these kinases is related to the intracellular concentration of flavopiridol, therefore the intracellular concentration (determined by the dose and treatment schedule), may determine whether G_1 phase or G_2/M phase arrest occurs. In this study, 48 h of exposure to flavopiridol could achieve dual cell cycle arrest (both G_1 and G_2/M phases) by changing the drug concentration. The value of flavopiridol as a radio-sensitizer would be greater if the cell cycle could be modulated as required.

Because a low dose of flavopiridol caused arrest at G_2/M which is the most radio-sensitive phase, we investigated whether flavopiridol could enhance the effect of radiation. The combination of flavopiridol and radiation was not reported to demonstrate significant activity against bladder cancer,[18] however, cells were irradiated before exposure to flavopiridol. Assuming that flavopiridol pretreatment before irradiation could mediate G_2/M arrest, this combination therapy would increase the effect of radiation. Recently, Raju *et al.* showed that treatment of ovarian carcinoma cells with 50–300 nmol/L flavopiridol augments their radio-sensitivity by accumulating cells in G_2/M phases of the cell cycle.[24] In

the present study, a low dose of flavopiridol treatment enhanced the radio-sensitivity of esophageal cancer cells. Interestingly, 0.05 nmol/L flavopiridol did not exhibit any inhibitory activity itself, yet synergistically enhanced the radio-sensitivity of the esophageal cancer cell lines. Since 0.05 nmol/L flavopiridol did not markedly increase the percentage of cells in G_2/M phase, the occurrence of G_2/M arrest does not seem to be a sufficient explanation for enhanced radio-sensitivity. Progression to G_2/M phase is thought to be regulated by cdk1/cyclin B and cdk1/cyclin A, and the IC_{50} of cdk1/cyclin B is approximately 30–400 nmol/L.[25] Because inhibitory activity of flavopiridol on cdks could not be expected at such low concentrations, another mechanism could modulate the effects of flavopiridol–radiation interaction.

In the present study, flavopiridol decreased cyclin D1, Bcl-2 and Rb protein expression by esophageal squamous cell carcinoma cells. Bcl-2 expression renders tumor cells resistant to apoptosis and is correlated with radio-resistance and recurrence in patients with squamous cell carcinoma of the head and neck.[26,27] Flavopiridol was reported to induce a decrease of Bcl-2 expression in leukemia cell lines.[7] In contrast, no obvious decrease of Bcl-2 was detected in esophageal cancer after flavopiridol treatment.[8] In the present study, 0.05 nmol/L flavopiridol only decreased Bcl-2 expression in KE4 cells, so a decrease of Bcl-2 expression cannot explain why flavopiridol enhanced the effect of radiation. There was no correlation between the cytotoxic action of flavopiridol and the decrease of Bcl-2 expression in several cell lines.[5,8,28] Although the mechanism of flavopiridol-induced antiproliferative activity in relation to Bcl-2 expression has not been fully elucidated, it is intriguing that the IC_{50} for KE4 cells was far lower than those of the other cell lines. Likewise, the relationship between radio-sensitivity and a decrease of cyclin D1 and Rb expression due to 0.05 nmol/L flavopiridol is unclear. The mechanism of action for flavopiridol plus radiation remains as a matter to be investigated further.

Decreases of cyclin D1, Rb and p107 expression may be an essential part of the mechanism by which flavopiridol induces cytotoxity in esophageal cancer, although the cytotoxity of flavopiridol is independent of the expression of pRb, p16 and p53.[8] In this study, consistent with the initial inhibition of cell growth, exposure to flavopiridol at 300 nmol/L decreased cyclin D1 and Rb expression in all three lines.

Cyclin D1 has been reported to show over-expression in several cancers and this is associated with a poor prognosis.[29,30] Over-expression of cyclin D1 has also been reported in esophageal cancer.[31,32] Amplification of cyclin D1 has been demonstrated to be a useful marker for predicting the outcome and distant metastasis in patients with squamous cell carcinoma of the esophagus.[33] It is suggested that a decrease of cyclin D1 over-expression may be useful in the treatment of esophageal cancer and premalignant esophageal lesions,[25] so flavopiridol may be helpful in the clinical management of esophageal cancer. However, treatment with flavopiridol sometimes causes side-effects such as severe diarrhea, vascular thrombosis and neutropenia.[13–15,34–36] For clinical purposes, a lower concentration of flavopiridol should be used, so combination with radiation may be more efficacious for the treatment of esophageal cancer. Flavopiridol is a strong radio-sensitizer and may achieve extended therapeutic use in the future. It will be effectively achieved at a low concentration of flavopiridol, which causes few side-effects. Moreover, flavopiridol shows cytotoxicity for esophageal cancer cell lines irrespective of the histology and tumor suppressor gene status,[8] indicating that the combination of flavopiridol and radiation may be useful for all esophageal cancer patients. In this study, only one treatment protocol was tested, so further investigations are required to explore the most effective treatment schedule to maximize the effect of flavopiridol combined with radiation.

In conclusion, these results suggest that combined treatment with low-dose flavopiridol can enhance radiation killing of tumor cells and this therapy may be promising for treatment of esophageal cancer.

Ackowledgements

We would like to thank Professor Keisuke Sasai (Department of Radiology, Niigata University School of Medicine, Niigata, Japan) for his helpful advice and discussion. We also thank Naomi Sato and Tamami Sakanishi for their excellent technical assistance. This work was supported in part by Grants-in-Aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

References

1 Daly J M, Karnell L H, Menck H R. National Cancer Data Base Report on esophageal carcinoma. Cancer 1996; 78: 1820–8.
2 Blot W J, McLaughlin J K. The changing epidemiology of esophageal cancer. Semin Oncol 1999; 26: 2–8.
3 Brown L M, Devesa S S. Epidemiologic trends in esophageal and gastric cancer in the United States. Surg Oncol Clin N Am 2002; 11: 235–56.
4 Daly J M, Fry W A, Little A G, *et al.* Esophageal cancer: results of an American College of Surgeons patient care evaluation study. J Am Coll Surg 2000; 190: 548–59.
5 Sedlacek H H. Mechanisms of action of flavopiridol. Crit Rev Oncol Hematol 2001; 38: 139–70.
6 Carlson B A, Dubay M M, Sausville E A, Brizuela L, Worland P J. Flavopiridol induces G1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. Cancer Res 1996; 56: 2973–8.

7 Konig A, Schwarz G K, Mohammad R M, Al-Katib A, Gabrilove J L. The novel cyclin-dependent kinase inhibitor flavopiridol down-regulates Bcl-2 and induces growth arrest and apoptosis in chronic B-cell leukemia lines. Blood 1997; 90: 4307–12.

8 Schrump D S, Matthews W, Chen G A, Mixon A, Altorki N K. Flavopiridol mediates cell cycle arrest and apoptosis in esophageal cancer cells. Clin Cancer Res 1998; 4: 2885–90.

9 Carlson B, Lahusen T, Singh S, *et al.* Downregulation of cyclin D1 by transcriptional repression in MCF-7 human breast carcinoma cells induced by flavopiridol. Cancer Res 1999; 59: 4634–41.

10 Zhai S, Senderowicz A M, Sausville E A, Figg W D. Flavopiridol, a novel cyclin-dependent kinase inhibitor, in clinical development. Ann Pharmacother 2002; 36: 905–11.

11 Sedlacek H H, Czech J, Naik R, *et al.* Flavopiridol (L86–8275, NSC-649890), a new kinase inhibitor for tumor therapy. Int J Oncol 1996; 9: 1143–68.

12 Melillo G, Sausville E A, Cloud K, Lahusen T, Varresio L, Senderowicz A M. Flavopiridol, a protein kinase inhibitor, down-regulates hypoxic induction of vascular endothelial growth factor expression in human monocytes. Cancer Res 1999; 59: 5433–7.

13 Innocenti F, Stadlar W M, Iyer L, Ramirez J, Vokes E E, Ratain M J. Flavopiridol metabolism in cancer patients is associated with the occurrence of diarrhea. Clin Cancer Res, 2000; 6: 3400–5.

14 Senderowicz A M, Headlee D, Stinson S F, *et al.* Phase I trial of continuous infusion flavopiridol, a novel cyclin-dependent kinase inhibitor, in patients with refractory neoplasms. J Clin Oncol 1998; 16: 2986–99.

15 Schwartz G K, Ilson D, Saltz L, *et al.* Phase II study of the cyclin-dependent kinase inhibitor flavopiridol administered to patients with advanced gastric carcinoma. J Clin Oncol 2001; 19: 1985–92.

16 Nishimura T, Hashimoto Y, Katayama M, Mori S, Kuroki T. Molecular and cellular features of esophageal cancer cells. J Cancer Res Clin Oncol 1993; 119: 441–9.

17 Zhai S, Senderowicz A M, Sausville E A, Figg W D. Flavopiridol, a novel cyclin dependent kinase inhibitor, in clinical development. Ann Phamacother 2000; 36: 905–11.

18 Chien M, Asturnian M, Liebowitz D, Rinker-Schaeffer C, Stadler W M. In vitro evaluation of flavopiridol, a novel cell cycle inhibitor, in bladder cancer. Cancer Chemother Pharmacol 1999; 44: 81–7.

19 Shapiro G I, Koestner D A, Matranga C B, Rollins B J. Flavopiridol induces cell cycle arrest and p53-independent apoptosis in non-small cell lung cancer cell lines. Clin Cancer Res 1999; 5: 2925–38.

20 Patel V, Senderowicz A M, Pinto D Jr, *et al.* Flavopiridol, a novel cyclin-dependent kinase inhibitor, suppresses the growth of head and neck squamous cell carcinomas by inducing apoptosis. J Clin Invest 1998; 102: 1674–81.

21 Nicoletti I, Migliorati G, Pagliacci M C, Grignani F, Riccardi C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J Immunol Meth, 1991; 139: 271–9.

22 Zhang J, Sanchez R J, Wang S, *et al.* Susbtrate specificity of cdc2 kinase from human HeLa cells as determined with synthetic peptides and molecular modeling. Arch Biochem Biophys 1994; 315: 415–24.

23 Losiewicz M D, Carlson B A, Kaur G, Sausville E A, Worland P J. Potent inhibition of cdc2 kinase activity by the flavonoid L86–8275. Biochem Biophys Res Commun 1994; 201: 589–95.

24 Raju U, Nakata E, Mason K A, Ang K K, Milas L. Flavopiridol a cyclin-dependent kinase inhibitor, enhances radiosensitivety of ovarian carcinoma cells. Cancer Res 2003; 63: 3263–7.

25 Schrump D S, Nguyen D M. Strategies for molecular intervention in esophageal cancers and their precursor lesions. Dis Esophagus 1999; 12: 181–5.

26 Condon L T, Ashman J N K, Ell S R, Stafford N D, Greenman J, Cawkwell L. Overexpression of Bcl-2 in squamous cell carcinoma of the larynx: a marker of radioresistance. Int J Cancer 2002; 100: 472–5.

27 Gallo O, Boddi V, Carlzolari A, Simonetti L, Trovati M, Bianchi S. Bcl-2 protein expression correlates with recurrence and survival in early stage head and neck cancer treated by radiotherapy. Clin Cancer Res 1996; 2: 261–7.

28 Byrd J C, Shinn C, Waselenko J K, *et al.* Flavopiridol induces apoptosis in chronic lymphocytic leukemia cells via activation of caspase-3 without evidence of Bcl-2 modulation or dependence on functional p53. Blood 1998; 92: 3804–16.

29 Gansauge S, Gansauge F, Ramadani M, Stobbe H, Rau B, Harada N. Overexpresson of cyclin D1 in human pancreatic carcinoma is associated with poor prognosis. Cancer Res 1997; 57: 1634–7.

30 Fredersdorf S, Burns J, Milne A M, Packham G, Fallis L, Gillett C E. High level expression of p27 (kip 1) and cyclin D1 in some human breast cancer cells: inverse correlation between the expression of p27 (kip 1) and degree of malignancy in human breast and colorectal cancers. Proc Natl Acad Sci USA 1997; 94: 6380–5.

31 Kanda Y, Nishiyama Y, Shimada Y, *et al.* Analysis of gene amplification and overexpression in human esophageal-carcinoma cell lines. Int J Cancer 1994; 58: 291–7.

32 Adelaide J, Monges G, Derderian C, Seitz J-F, Birnbaum D. Esophageal cancer and amplification of the human cyclin D gene CCND1/PRAD1. Br J Cancer 1995; 71: 64–8.

33 Shinozaki H, Ozawa S, Ando N, *et al.* Cyclin D1 amplification as a new predictive classification for squamous cell carcinoma of the esophagus, adding gene information. Clin Cancer Res 1996; 2: 1155–61.

34 Tan A R, Headlee D, Messmann R, *et al.* Phase I clinical and pharmacokinetic study of flavopiridol administered as a daily 1-hour infusion in patients with advanced neoplasms. J Clin Oncol 2002; 20: 4074–82.

35 Stadler W N, Vogelzang N J, Amato R, *et al.* Flavopiridol, a novel cyclin-dependent kinase inhibitor, in metastasic renal cancer: a University of Chicago Phase II Consortium study. J Clin Oncol 2000; 18: 371–5.

36 Shapiro G I, Supko J G, Patterson A, *et al.* A phase II trial of the cyclin-dependent kinase inhibitor flavopiridol in patients with previously untreated stage IV non-small cell lung cancer. Clin Cancer Res 2001; 7: 1590–9.

放射線治療の品質管理・品質保証

石倉　聡*

放射線治療において品質管理・品質保証（QC・QA）は欠かせないものである。しかしながらここ数年続けて「過剰照射事故」が報道されているように，医療現場ではQC・QAを行う体制が必ずしも整っていない現状がある。放射線治療の技術進歩はめざましく，三次元放射線治療（3D-CRT），強度変調放射線治療（IMRT），粒子線治療などの先端医療の普及，治療成績の向上のためにも，早急なQC・QA体制の確立が必要である。

Ⅰ．品質管理・品質保証の必要性

身体侵襲が少なく形態・機能温存を図れること，社会の高齢化とquality of lifeの視点などにより放射線治療を受ける患者数は増加の一途を辿っている。日本放射線腫瘍学会が行った構造調査結果によると1990年から1999年の10年間で放射線治療件数は約40%の増加がみられ，特にここ数年間の増加傾向は顕著である。

放射線治療の実施過程は複雑である。治療に先立つ計画の段階においては，放射線を照射する部位（標的体積という），方法，線量の決定およびモニターユニット値という放射線照射量の算出など多くの過程が存在する。また標的体積の決定一つを取ってみても，病巣進展範囲の認識や手術におけるリンパ節隔清に相当する予防照射領域の設定には治療計画者によりばらつきが生じうるところである。そのため放射線治療の実施にあたっては，その一連の過程に対する品質管理（quality control：QC）および品質保証（quality assurance：QA）の概念が必要となる[1]。もちろん誤って使用すれば死亡にもつながる障害を引き起こす可能性があり，放射線の照射装置そのものの精度管理も欠かせないものである。しかしながら，ここ数年たてつづけに「過剰照射事故」が報道されているように，医療現場ではこれらの精度管理を行う体制が整っていない状況が少なからず存在しており，その体制の早急な確立が必要である。

また，一般診療，臨床試験を問わず，異なる施設間での治療内容の差，施設間較差を解消する観点においても品質管理，品質保証活動は重要な役割を担っている。臨床試験における一つの悪い例として米国Southwest Oncology Group（SWOG）で過去に行われたホジキン病に対する臨床試験をあげる。この臨床試験では登録された症例のうち，36%の症例で放射線治療の

* *Satoshi Ishikura*　国立がんセンター東病院放射線部

プロトコール規定の逸脱が認められた。その結果プロトコールの規定を遵守していた症例では10%であった再発率が逸脱例では44%にも及んだことが報告がされている[2]。その他にもプロトコール規定の逸脱により治療成績が低下する複数の報告がある[3]。臨床試験が一般診療に適用可能な科学的結果を出すためには，異なる施設間において治療内容を比較することが可能でかつその較差が最小化されている必要があり，放射線治療における技術面を含めた治療の標準化は欠かせないものである。もちろん患者の安全を確保する，すなわち毒性の増強や効果の低減を防止する観点からも必須といえる。

Ⅱ．国外における品質管理・品質保証活動

米国においては放射線治療のQC・QAプログラムが確立されている。歴史的には1969年にNational Cancer Institute (NCI) の補助金を受けRadiological Physics Center (RPC) が活動を始めた[4]。その役割は多施設共同臨床試験に参加している施設の間で技術的に大きな乖離がないこと，適切なQCシステムにより施設間で比較可能な放射線治療が行われていることを第三者的に保証することである。RPCでは主として物理的な精度管理，すなわち施設間の線量のばらつきを解消するため，郵送可能な線量計を用いたoff-site auditによるスクリーニングや施設訪問による線量測定，施設のQC・QAプログラムの確認といったon-site auditを全米に約1,800存在する放射線治療施設のうち，NCIスポンサーの臨床試験に参加する全施設を含め，約1,350の施設を対象に実施している。このような活動は米国に限ったものではなく，International Atomic Energy Agency (IAEA)[5, 6]では主として発展途上国の115カ国，約1,200施設を対象として，European Society for Therapeutic Radiology and Oncology (ESTRO)[7]ではEU諸国の約450施設を対象として同様のQC・QAプログラムを実施している。これらにより全世界の約60%の施設がauditを受けていることになる。これらのプログラムに参加している施設においては，投与される放射線線量の誤差が5%以内であることが保証されている。放射線治療においては各過程にそれぞれ誤差が存在しその積み重ねがあるため，「5%以内の誤差」が精度管理を十分に行っていると判断される基準とされている。米国においてはRPCによるauditを受けていることが臨床試験に参加するための必須条件ともなっている。

物理的QC・QAプログラムとは別に，いわゆる照射野の設定方法など治療内容の臨床的QC・QAプログラムは主として臨床試験を通して実施されてきた。ここで臨床試験における放射線治療のQC・QA活動の草分け的存在であるQuality Assurance Review Center (QARC) による活動の歴史を紹介する[8]。QARCは多施設共同研究グループであるAcute Leukemia Group B (ALGB) の放射線治療委員会により1972年に設立された組織である。当時，臨床試験実施計画における放射線治療の項目は臨床腫瘍医にとって，同時に放射線腫瘍医にとってもいわばブラックボックスであった。臨床試験実施計画書には放射線治療の詳細については記載されておらず，実際に患者がどのような放射線治療を受けたかについてほとんど知られることはなかった。また実際に行われた治療内容を評価しようにも利用できる放射線治療の情報は20%にも至らなかった。そのため，ALGBの放射線治療委員会は，放射線治療の研究プログラムの策定のみならず，臨床試験に参加しているすべての施設研究者が確立されたガイドラインに従って均一な放射線治療が行えるように放射線治療手順を明確に規定することから着手した。同時に治療の適切さを評価するため，放射線治療に関わる資料を系統的かつ適切な時期に収集するシステムを確立した。これにより評価できる放射線治療の情報は2年間で30%未満から70%以上に上昇し5年間では90%以上となったが，これらの情報が集積されることにより多施設共同研究においては治療の均一性が達成されていないことも同時に明らかとなった。この結果を受けて放射線治療委員会では「プロトコール実施における問題点と落とし穴」と題した教育プログラムを定期的に実施し，それによりその後のプロトコール規定の遵守率は3年間で40%から70%へと改善，その後も堅調に上昇が認められた。

このような放射線治療の質の改善がきっかけとなり他の多施設共同研究グループにおいても同様のQC・

QAプログラムが実施されるようになり，同様の改善が示された[9,10]。その一方で，それぞれの多施設共同研究グループから，QC・QAプログラムを標準化し，同一の組織，均一な手順で実施する要求が高まり，1980年にQARCが正式に設立された。またその活動内容からQARCは多施設研究グループと独立してNCIから資金援助を受けている。欧州においてもEuropean Organisation for Research and Treatment of Cancer (EORTC)[11]で同様のプログラムが実施されており，放射線治療のQC・QAを行うことはglobal standardとして認識されている。2002年には米国内に5つあった放射線治療のQA組織：Image-Guided Therapy Center (ITC), Resource Center for Emerging Technology (RCET), RPC, Radiation Therapy Oncology Group (RTOG), QARCを統括する組織としてAdvanced Technology Consortium (ATC)が設立され，QC・QA手順の標準化，効率化がはかられている[12~15]。また同時に米国内のみならずNational Cancer Institute Canada (NCIC), EORTC, 日本臨床腫瘍研究グループ(Japan Clinical Oncology Group；JCOG)との間でも標準化のための共同プロジェクトが開始されている。

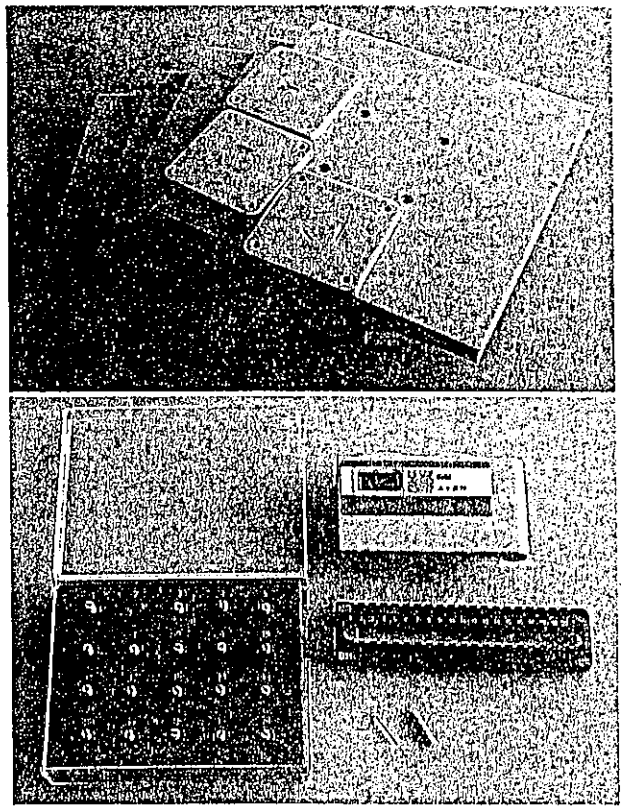

図1　ガラス線量計を用いた郵送調査
郵送調査で使用している固体ファントム（上段）とガラス線量計セット（下段）。
下段右上は大きさを比較するためにおかれたフィルムバッジケース（7×4 cm）。

Ⅲ．国内の状況

わが国においては，「線量計の校正」活動により各施設の線量計の精度は管理されてきたが，実際の治療装置等の線量管理を行なう物理的QC・QAおよび臨床的な照射野の設定等に関する臨床的QC・QAは，最近まで全国規模で体系的なQC・QA活動のシステム構築はなされてこなかった。日本放射線腫瘍学会(JASTRO)からQC・QAに関するガイドラインが出されてはいるものの，実際に各施設でどのようなQC・QA活動がなされているかの実態は明らかとはいえない。そのためわが国の放射線治療の質あるいは臨床試験の質は未だにブラックボックスで国際的に信頼性を得ることが出来ていないとともに，前述のようにここ数年間連続して過剰照射事故が判明するといった深刻な状況にある。「最近事故が増えた」のではなく，今まで知られていなかった事故の存在が「最近明らかになった」のであり，報告されていない事故も少なからず存在すると考えられる。これらの事故を防止することにもつながる物理的QC・QAについては厚生労働科学研究費補助金による研究班が米国RPCで実施されている手法に準じ，ガラス素子線量計の郵送によるoff-site audit（図1）および施設訪問によるon-site auditを2002年より開始した。研究班を基盤とした活動であり，自ずとマンパワーに限りがあり対象施設数は限られたが，その中でも各施設間で放射線照射線量のばらつきが許容範囲を超えて存在することが判明している。これには放射線治療を行なう上で物理的QC・QAに責任をもつ物理士の不足，急速な技術発展に伴い，すでに体制の充実した欧米とは対照的に，日本ではマンパワーおよび施設的条件が十分に整っていない中で技術導入が進められていることが背景にある。この研究班におけるaudit活動により照射線量の

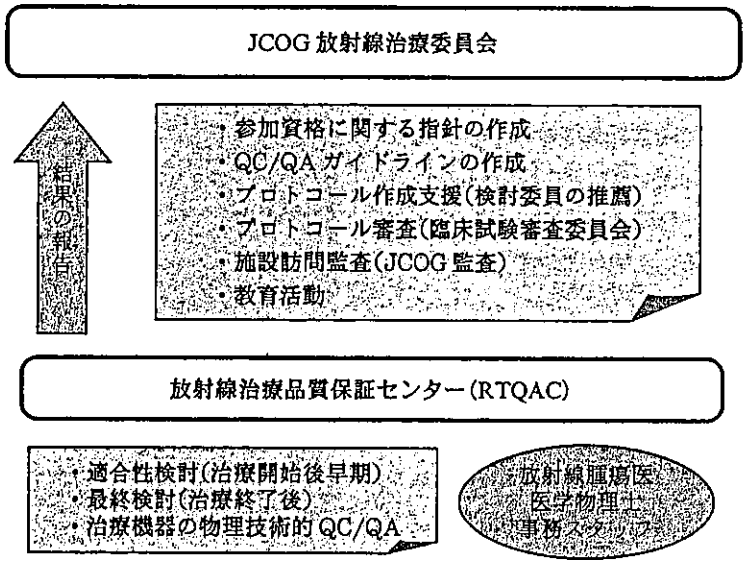

図2　JCOG 放射線治療委員会と JCOG 放射線治療品質保証センター
放射線治療委員会はプロトコール作成支援やプロトコール審査に関与し，放射線治療品質保証センターでは症例毎の検討とフィードバックを行っている。

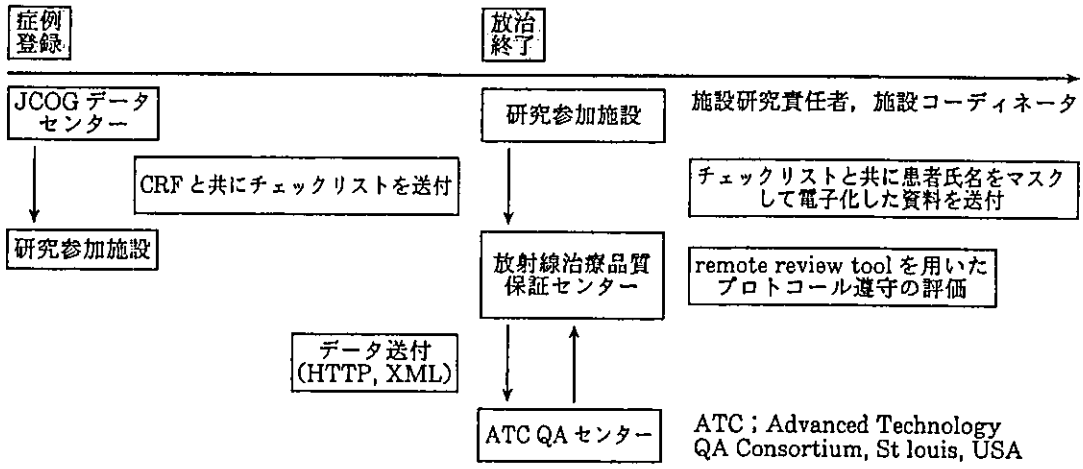

図3　放射線治療品質保証センターにおける品質保証の例(JCOG 放射線治療グループ，定位放射線治療の臨床試験)
研究参加施設から電子化して提出された資料を用いてプロトコール遵守の評価が行われる。米国 ATC と共同で，インターネットを用いた迅速かつ効率的な評価方法も開発されている。

ばらつきが解消されることも実証されており，全国規模で物理的 QC・QA 活動を早急に展開することが事故防止の観点からも必要である。

一方，臨床的 QC・QA に関して JCOG が 1999 年に放射線治療委員会を立ち上げた。2001 年には一つのランダム化比較試験において放射線治療のプロトコール規定の遵守に関する評価を行ったが遵守率はわずか 40%にとどまっており，わが国においても積極的に QC・QA プログラムを導入することが必要であることが認識された[16]。2002 年には放射線治療委員会の下に JCOG 放射線治療品質保証センターを立ち上げ，最近の臨床試験ではプロトコール作成の段階から放射線

治療規定の明確化をはかり，臨床試験への登録開始後には，登録症例における放射線治療の開始後早期の段階で治療内容の評価を行い各施設へのフィードバックを行うQC・QAプログラムを導入している[17]。これによりQARCの経験同様，今後短期間のうちにプロトコール規定の遵守率が改善することが期待されたが，実際にQC・QAプログラムを導入した臨床試験でプロトコール規定の遵守率が飛躍的に向上している。また，先述のように米国ATCを中心に進められているQC・QAプログラムの標準化にむけて，JCOGはEORTC，NCICとともに共同プロジェクトに参加している（図2，3）。

Ⅳ．今後の展望

近年のinformation technology（IT）技術の進歩により，放射線治療も従来の二次元的なものから三次元放射線治療（3D-CRT），強度変調放射線治療（IMRT），粒子線治療などへ急速な高度化が進んでいる。これらの先進的技術を行うために必要とされる物理的，臨床的QC・QAの内容も数倍に増加しかつ複雑となっている。これらのQC・QAを行うためには専門の知識を持ち，かつ主としてQC・QAを主たる業務とする「医学物理士」が安全管理，責任の明確化のために必須とされる。残念ながら現在まで施設で医学物理士を職制として認めている施設はごく一部に限られており，先進的な技術の発展，普及および治療成績の向上のためにも早急な職制の確立と人材の育成が求められている。またJCOG以外の国内臨床試験グループにおいてもQC・QAプログラムを導入することが求められている。これらを強力かつ効率的に推進するため，現在，特定非営利活動法人（NPO）放射線治療支援センターの設立準備が進められており，今後の発展に期待したい。

文　献

1) 加藤洋一：ISO9000による品質保証の基本的な考え方．臨床評価 28：33-50, 2000
2) Fabian CJ et al：Low-dose involved field radiation after chemotherapy in advanced Hodgkin disease. A Southwest Oncology Group Randomized Study. Ann Intern Med 120：903-912, 1994
3) White JE et al：The influence of radiation therapy quality control on survival, response and sites of relapse in oat cell carcinoma of the lung：preliminary report of a Southwest Oncology Group study. Cancer 50：1084-1090, 1982
4) http://rpc.mdanderson.org/rpc/
5) http://www.iaea.or.at/
6) Izewska J et al：The IAEA/WHO TLD postal dose quality audits for radiotherapy：a perspective of dosimetry practices at hospitals in developing countries. Radiother Oncol 69：91-97, 2003
7) http://www.estro.be/estro/Frames/QA.html
8) http://www.qarc.org/
9) Wallner PE et al：Impact of initial quality control review on study outcome in lung and head/neck cancer studies-review of the Radiation Therapy Oncology Group experience. Int J Radiat Oncol Biol Phys 17：893-900, 1989
10) Schaake-Koning C et al：The need for immediate monitoring of treatment parameters and uniform assessment of patient data in clinical trials. A quality control study of the EORTC Radiotherapy and Lung Cancer Cooperative Groups. Eur J Cancer 27：615-619, 1991
11) http://www.eortc.be/
12) http://itc.wustl.edu/
13) http://rcet.health.ufl.edu/Main_40.asp
14) http://www.rtog.org/
15) http://atc.wustl.edu/
16) Ishikura S et al：Initial experience of quality assurance in radiotherapy within the Japan Clinical Oncology Group (JCOG). Radiother Oncol 64：S224, 2002
17) http://www.jcog.jp/SHIRYOU/29_policy.pdf

Diseases of the Esophagus (2004) 17, 247–250
© 2004 ISDE

DISEASES OF THE ESOPHAGUS

Original article

Phase I study of the combination of nedaplatin, adriamycin and 5-fluorouracil for treatment of advanced esophageal cancer

M. Hirao, K. Fujitani, T. Tsujinaka

Department of Surgery, Osaka National Hospital, Osaka, Japan

SUMMARY. **This trial was conducted to determine the maximum-tolerated dose, principal toxicity, and recommended dose (RD) for the phase II study of the combination of nedaplatin (NED), adriamycin (ADM), and 5-fluorouracil (5-FU) in patients with advanced esophageal cancer. Patients with previously untreated esophageal cancer were eligible if they had performance status 0–1, were 75 years or younger and had adequate organ function. The dose of NED, the key anticancer platinum complex drug, was increased from 60 to 70, and 80 mg/m^2 on day 1. ADM and 5-FU were administered at fixed doses (30 mg/m^2 on day 1, and 700 mg/m^2 on days 1–5). The dose-limiting toxicities of NED were neutropenia and severe diarrhea, and its maximum-tolerated dose and RD were 70 mg/m^2 and 60 mg/m^2, respectively. There were four responders among the six patients administered the RD. The present study thus revealed combination chemotherapy with NED, ADM, and 5-FU to be active and well-tolerated and to warrant phase II study.**

KEY WORDS: **cancer, chemotherapy, esophagus, nedaplatin, phase I.**

INTRODUCTION

Esophageal cancer has a poor prognosis. In recent years, several strategies including presurgical chemotherapy, either alone or with radiotherapy, and chemoradiotherapy without surgery have been used in attempts to improve the prognosis.

Cisplatin (CDDP)-based combinations have been reported to yield high response rates and,[1] among the various combinations tested, that with 5-fluorouracil (5-FU) has proved to be a most effective, safe and standard regimen.[2] However, because CDDP itself has substantial toxicity, including renal and gastrointestinal toxicity, newer platinum analogs have been developed. (Glyconate-O,O) diammineplatinum (II) (Nedaplatin, hereafter NED), a second-generation platinum complex developed in Japan, is designed to reduce the adverse effects of CDDP and to further improve its antitumor effect.[3] It was reported that the response rate for NED as a single agent was 42.9% in a phase II study, with little toxicity observed.[4] It has been shown in vitro that NED can exert synergic effects with 5-FU.[5] Moreover, it was reported that the combination of NED and 5-FU yielded a 54% response rate among patients who had previously been treated with CDDP.[6]

Combination chemotherapy with 5-FU, adriamycin (ADM), and CDDP (FAP) has been reported to be useful in the treatment of advanced gastric cancer, esophageal cancer and other tumors.[7,8]

Based on these findings, we planned a phase I and II study of the combination of NED, ADM, and 5-FU for treatment of previously untreated advanced esophageal cancer. The main purpose of the present study was to determine the maximum-tolerated dose (MTD), principal toxicity, and recommended dose (RD) for the phase II study.

PATIENTS AND METHODS

Eligibility criteria

Between January 2003 and June 2003, 12 patients with advanced esophageal cancer cared for in Osaka National Hospital were enrolled for the study. Disease staging was performed according to the guidelines for the clinical and pathologic studies on carcinoma of the esophagus of the Japan Society for Esophageal Diseases.[9] Patients with histologically proven stage III or IVa or b disease who had not

Address correspondence to: Dr Motohiro Hirao, MD, Department of Surgery, Osaka National Hospital, 2-1-14 Hoenzaka, Chuouku, Osaka, 540–0006, Japan.
Tel/Fax: + 81 6 6946 5660, Email: hiraom@onh.go.jp

previously received chemotherapy, radiotherapy or surgical treatment were eligible for this study. Other eligibility criteria were: age between 20 and 75 years; Eastern Cooperative Oncology Group (ECOG) performance status of 1 or 0; a measurable lesion; life expectancy of 3 months or longer; adequate bone marrow function (white blood cell [WBC] count from 3000 to 12 000/μL, neutrophil count of 1500/μL or more, hemoglobin level of 8.0 g/dL, and platelet count level of 100 000/μL or more), acceptable renal function (serum creatinine levels less than 1.5 mg/dL and creatinine clearance rate of 50 mL/min or more) and hepatic function (total serum bilirubin level less than 1.5 mg/dL, AST and ALT levels less than or equal to two and one-half times the upper limits of the normal ranges), and normal ECG. Patients were excluded if they had active infection, severe heart disease, pregnancy, active synchronous carcinoma, interstitial pneumonia or pulmonary fibrosis, peripheral neuropathy, pleural effusion or ascites, symptomatic brain metastasis, syndrome of inappropriate secretion of ADH (SIADH), or severe drug allergy. Written informed consent was obtained from all patients.

Treatment protocol

ADM (30 mg/m^2) was diluted with 100 mL of normal saline and administered as an intravenous drip infusion over 60 min on day 1. 5-FU (700 mg/m^2) was diluted with saline and administered as an intravenous drip continuously from day 1–5. NED was diluted with 500 mL of normal saline before injection and given as an intravenous drip infusion over 60 min on day 1. This chemotherapy regimen was repeated every 4 weeks and given for at least 1 cycle for phase I and for more than 2 cycles for phase II study.

MTD and dose-limiting toxicity

Three dose levels of NED were chosen for investigation: step 1, 60 mg/m^2; step 2, 70 mg/m^2; and step 3, 80 mg/m^2. The dose of NED was increased on the basis of toxicity during the first cycle of chemotherapy. No dose escalation was permitted for individual patients. Decision on MTD was made on the basis of dose-limiting toxicity (DLT) events that occurred during the first cycle of chemotherapy. DLT was defined as follows: (1) a WBC count less than 1000/μL or neutrophil count less than 500/μL; (2) grade 3 febrile neutropenia; (3) a platelet count less than 25 000/μL; (4) any grade 3 non-hematologic toxicity that met NCI-CTC, except for alopecia, fatigue and nausea/vomiting. At least three patients were enrolled at each dose step. If DLT was observed in one patient, three additional patients were accrued. If DLT was observed in two or more of the initial patients or three or more of the six patients, patient accrual was discontinued and the dose level was considered the MTD. Once the MTD was determined, the previous dose level was chosen as the recommend dose (RD).

Toxicity and evaluation of response

Toxicities were assessed and graded according to NCI-CTC (National Cancer Institute Common Toxicity Criteria Version 2.0, 1999). A total of 21 courses were given. The median number of courses given per patient was two (range, 1–3). The World Health Organization criteria were used to define the following: complete response (CR), the disappearance of all known disease for at least 4 weeks; partial response (PR), 50% or more decrease in total tumor load of the lesions estimated by two observations no less than 4 weeks apart; no change (NC), no significant change for at least 4 weeks, which includes stable disease, estimated decrease of less than 50%, and lesions with estimated increase of less than 25%; and progressive disease (PD), appearance of any new lesions not previously identified or estimated increase of existing lesions by 25% or more.

RESULTS

Patient characteristics

Twelve patients were enrolled from January 2003. All patients could be assessed for toxicity (Tables 1 and 2). The study population included 11 men and one woman, with a mean age of 65 years (range, 46–74). Seven patients had performance status '0', and five patients '1'. The histologic diagnosis of 12 patients was squamous cell carcinoma of the esophagus. Four patients had stage III disease, three patients stage IVa disease, and five patients stage IVb disease. Moreover, four patients underwent esophagectomy after the combination chemotherapy.

Table 1 Dose-escalation scheme and dose limiting toxicity (DLT) in the first cycle of chemotherapy

Dose level	Nedaplatin (mg/m^2)	No. of patients	Total no. of courses	Patients with DLT
1	60	6	11	1
2	70	6	9	2

Table 2 Patient characteristics

Total no. of patients (level 1/level 2)	12 (6/6)
Sex, M/F	11/1
Age, years (range)	65 (46–74)
Stage, III/IVa/IVb	4/3/5
Performance status, 0/1	7/5

Table 3 Hematologic and nonhematologic toxicities by dose level in the first cycle

	Dose level	No. of patients	Neutropenia	Thrombocytopenia	Anemia	Nausea/ vomiting	Diarrhea	Elev. of creatinine	Elevation of transaminase
Grades of toxicity			1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
No. of patients	1	6	1 1 2 1	0 0 1 0	3 2 1 0	2 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
with each grade	2	6	2 1 1 1	0 0 0 0	4 1 0 0	3 0 0 0	1 0 1 0	1 0 0 0	0 0 0 0

DLT, MTD and RD as judged from toxicity

A total of 20 courses were given (Table 1). The median number of courses given per patient was two (range, 1–3). Administration of NED was started at 60 mg/m^2, and increased to 70 mg/m^2, which was determined to be the MTD. The RD of NED was 60 mg/m^2. DLTs were myelosuppression, especially neutropenia and grade 3 diarrhea during the first course (Table 1). Leukocytes and neutrophils reached a nadir in 9–22 days (median, 17 days) after the first administrations of NED, and 3 or 4 days (median, 3 days) were required for recovery from the nadir to 3000/mm^3 leukocytes. Grade 4 neutropenia occurred in two patients, and G-CSF was administered for 4 days to one of them. Platelet count reached a nadir in 7–20 days (median, 15 days) after the first administration, and 0–14 days (median, 7 days) were required for recovery from the nadir to 100 000/mm^3 (Table 3).

Nausea was the most frequent non-hematologic toxicity, and was grade 1 and transient. Moreover, only one patient, at the second step, had grade 3 non-hematologic toxicity diarrhea. Neither hepatic, cardiac or renal functions were impaired in any of the patients (Table 3).

Antitumor effects by dose of the first step of NED (60 mg/m^2) were follows: four patients achieved PR and two patients were NC, among six patients who were assessed for RD at the first step.

DISCUSSION

Patients with advanced esophageal cancer rarely benefit from chemotherapy. Several types of combination therapy have been employed, but the reported objective response rates have been only 15% with CDDP and bleomycin,[10] 42% with CDDP and mitomycin,[11] 29% with CDDP, bleomycin and vindesine,[12] and 35–60% with CDDP and 5-FU.[2,13,14] Cure is not possible and the prognosis of esophageal carcinoma remains unsatisfactory.

We designed the present study to determine the MTD, principal toxicity and RD of combination chemotherapy with NED, ADM and 5-FU (NAF) for advanced esophageal carcinomas.

The CDDP and 5-FU combination (FP) has been considered the standard regimen for patients with esophageal cancer, and investigators have reported response rates of 60% for resectable or localized tumors,[13] and 36% for recurrent, metastatic, or bulky unresectable carcinoma.[14] The most frequent toxicity of FP was gastrointestinal, and included nausea and vomiting.

Furthermore, combination chemotherapy with 5-FU, ADM and CDDP (FAP) has been reported to be useful in the treatment of advanced gastric cancer, esophageal cancer and other carcinomas.[7,8] Gisselbrecht *et al.* reported that the FAP regimen was administered to 21 patients with advanced esophageal cancer,[7] seven of them had an objective response (CR: 2, PR: 5), with no severe myelosuppression or nephrotoxicity observed.

NED, a novel second-generation platinum compound, has shown superior antitumor activity and less renal and gastrointestinal toxicity than CDDP in some preclinical and clinical studies.[3,4] With NED and 5-FU combination chemotherapy, a response rate of greater than 60% in assessable patients was achieved with a duration of 7 months (range 3–37) for advanced esophageal cancer.[15] Moreover, it was reported that a combination of NED and 5-FU yielded a 54% response rate among those who had previously been treated with CDDP.[6] In a phase II study of the combination of NED and 5-FU for metastatic squamous cell carcinoma of the esophagus, the overall response rate was 40% and the median survival time was 8.9 months. This phase II study showed that grade 4 neutropenia and thrombocytopenia occurred in 2–7%, and grade 3 diarrhea and nausea occurred in 2% and 12%, respectively. This combination therapy was previously found to be safe and active.[16] Our study showed that the combination of NED, ADM, and 5-FU was also generally well-tolerated and attractive.

A phase II study of combination chemotherapy with NED, ADM, and 5-FU for advanced esophageal cancer should be planned at the recommended dose.

References

1 Leichman L, Berry B T. Experience with cisplatin in treatment regimens for esophageal cancer. Semin Oncol 1991; 18: 64–72.
2 Ajani A J. Contributions of chemotherapy in the treatment of carcinoma of the esophagus: results and commentary. Semin Oncol 1994; 21: 474–82.
3 Totani T, Aono K, Komura M. Synthesis of (Glycolato-o,o') diammine platinum (II) and its related complexes. Chemistry Letters 1986; 4: 429–32.

4 Taguchi T, Wakui A, Nabeya K *et al.* A phase II clinical study of cis-diammine glycolato platinum, 254-S, for gastrointestinal cancers. Jpn J Cancer Chemother 1992; 19: 483–8.
5 Furukawa S, Yoshida T, Tukuda M *et al.* Experimental study on combination chemotherapy with platinum compounds and 5-fluorouracil. Jpn J Cancer Chemother 1989; 16: 419–22.
6 Fuwa N, Kodaira T, Kamata M *et al.* Phase I study of combination chemotherapy with 5-FU and nedaplatin. Am J Clin Oncol (CCT) 2002; 25: 565–9.
7 Gisselbrecht C, Calvo F, Mignot L *et al.* Fluorouracil, adriamycin, and cisplatin (FAP): combination chemotherapy of advanced esophageal carcinoma. Cancer 1983; 52: 974–7.
8 Wagener D J T, Yap S H, Wobbes T *et al.* Phase II trial of 5-fluorouracil, adriamycin and cisplatin (FAP) in advanced gastric cancer. Cancer Chemother Pharmacol 1985; 15: 86–7.
9 Japanese society for esophageal diseases. Guide Lines for the Clinical and Pathologic Studies on Carcinoma of the Esophagus, 9th edn. Tokyo: the Society, 1999.
10 Coonley C J, Ba ns M, Hilaris B, Chapman R, Kelsen D P. Cisplatin and b eomycin in the treatment of esophageal carcinoma, a final report. Cancer 1984; 54: 2351–5.
11 Engstrom P F, Lavin P T, Klaassen D J. Phase II evaluation of mitomycin and cisplatin in advanced esophageal carcinoma. Cancer Treat Rep 1983; 67: 713–15.
12 Dinwoodie W R, Bartolucci A A, Lyman G H, Velez-Garcia E, Martelo O J, Sarma P R. Phase II evaluation of cisplatin, bleomycin, and vindesine in advanced squamous cell carcinoma of the esophagus: a southeastern cancer Study Group trial. Cancer Treat Rep 1986; 70: 267–70.
13 Kies M S, Rosen S T, Tsang T K *et al.* Cisplatin and 5-fluorouracil in the primary management of squamous esophageal cancer. Cancer 1987; 60: 2156–60.
14 Iizuka T, Kakegawa T, Ide H *et al.* Phase II evaluation of cisplatin and 5-fluorouracil in advanced squamous cell carcinoma of the esophagus: a Japanese esophageal oncology group trial. Jpn J Clin Oncol 1992; 22: 172–6.
15 Yoshioka T, Gamoh M, Shineha R *et al.* A new combination chemotherapy with cis-diammine-glycolatoplatinum (nedaplatin) and 5-fluorouracil for advanced esophageal cancers. Intern Med 1999; 38: 844–8.
16 Muro K, Ando T, Nishimaki A *et al.* A phase II study of nadaplatin and 5-fluorouracil in metastatic squamous cell carcinoma of the esophagus: The Japanese clinical oncology group (JCOG) trial (JCOG 9905). Proc ASCO 2003; 22: 1112.

● 原 著

根治切除不能進行食道癌に対するNedaplatin/Adriamycin/5-FU (NAF) 併用療法のPhase I Study

平尾 素宏 藤谷 和正 辻仲 利政*

〔*Jpn J Cancer Chemother* 32(1): 53-56, *January*, 2005〕

Phase I Study of the Combination of Nedaplatin (NED), Adriamycin (ADM), and 5-Fluorouracil (5-FU) (NAF) for Treatment of Unresectable Advanced Esophageal Cancer: Motohiro Hirao, Kazumasa Fujitani, Toshimasa Tsujinaka (*Dept. of Surgery, Osaka National Hospital*)

Summary

Background: Esophageal cancer has a poor prognosis. Several strategies including chemotherapy (CDDP+5-FU), either alone or with radiotherapy, have been used to improve the prognosis. However, since CDDP itself has substantial toxicities, including renal and gastrointestinal toxicities, newer platinum analogues, such as nedaplatin (NED) have been developed, and it is of interest to test this new platinum analogue in a combination chemotherapy. Methods: We conducted a phase I-II study using a combination of NED (3 levels, 60-80 mg/m² on day 1), ADM (30 mg/m² on day 1), and 5-FU (700 mg/m² on day 1-5) for treatment of previously untreated advanced esophageal squamous cell carcinoma. Cycles were repeated every 28 days. The objectives were to determine dose-limiting toxicity (DLT), maximum-tolerated dose (MTD), recommended dose (RD) for a phase II study, and to determine antitumor effects. Results: Phase I: 12 patients (pts) (male/female=11/1) were evaluable. The median age was 65 (range 46-74), PS 0/1=7/5. At level 1, 1 pt developed DLT (grade 4, neutropenia). At level 2, 2 pts developed DLT (grade 4 neutropenia and grade 3 diarrhea). Level 2 (70 mg/m²) was determined as the MTD, and a level 1 dose (60 mg/m²) was recommended. Phase II: 7 pts (male/female=4/3) are at RD of level 1 at the present time. Median age 62 (range 46-75). The median number of cycles on phase II study at RD was 2 (range 1-3). 4 PRs were obtained. The response rate was 57.1%. Median survival time (MST) was not reached at the time. Conclusions: This combination therapy appears to be highly effective and generally well tolerated for advanced esophageal cancer. Key words: Nedaplatin, Esophageal cancer, Phase I-II (*Received May 1, 2004/ Accepted Jul. 7, 2004*)

要旨 今回われわれは，NED/ADM/5-FU併用療法（NAF療法）に着目し，予後不良な切除不能食道癌に対するfirst-lineとして，この併用化学療法の安全性および有効性の検討を計画した。key drugであるNEDを増量していき，step 2: 70 mg/m²で2例のdose limiting toxicity (DLT)（grade 4血液毒性とgrade 3非血液毒性）が出現したためstep 2を最大耐用量，step 1の60 mg/m²をNEDの推奨投与量とした。grade 1の悪心・嘔吐が最も多い非血液毒性であり，grade 1の腎毒性はstep 2で1例経験したが，すべて保存的に軽快した。また，step 1でのgrade 4好中球減少のDLT症例のみG-CSFを使用した。以上の第I相試験で明らかとなったNED 60 mg/m²，ADM 30 mg/m² day 1，5-FU 700 mg/m²/日 day 1〜5の持続点滴静注を推奨投与とした現在までの第II相での奏効率は57.1%と良好な結果が得られており，安全かつ有効なregimenとして十分期待できる。

はじめに

今回われわれは，予後不良な進行食道癌患者を対象としてnedaplatin (NED)，adriamycin (ADM)，および5-fluorouracil (5-FU) とのNAF併用療法 (NAF療法) の安全性および有効性の検討を目的にこの研究を行った[1,2]。臨床第I相として[2]，first-line NAF療法の安全性を検討し，NEDをkey drugとした本療法の推奨用量，用法の結果を報告する。また，現在進行中の第II相として，第I相で決定された推奨用量，用法における有効性

連絡先: 〒540-0006 大阪市中央区法円坂2-1-14 * 独立行政法人国立病院機構大阪医療センター・外科
平尾 素宏

0385-0684/05/¥500/論文/JCLS

および安全性を評価し，本併用療法のfeasibilityを明らかにする。当研究は当センターIRBにて承認され，患者には十分なインフォームド・コンセントを得てから行っている。以下の記載は，食道癌取扱い規約第9版に準じる。

I. 対　象

2003年1月から2004年3月までの期間，当院消化器科および外科を受診した前治療歴のない根治切除不能進行食道扁平上皮癌。根治切除不能進行食道癌とは，T4もしくはT4疑い，N4，M1，または腹部または頸部の2領域以上に及ぶ明らかなリンパ節転移が認められる症例。評価測定可能病変を有し，年齢は20歳以上75歳以下，performance status (PS): 0～1 (ECOG分類)，十分な心，骨髄，腎，肝機能を有し，重複癌のない患者を対象に施行。なお，選択基準や除外基準の詳細の記述は省略する[2]。

食道癌に対する補助化学療法としてのFAP (5-FU/ADM/CDDP) 併用療法の投与量はそれぞれ50 mg/body，100 mg/body，1,000 mg/body (day 1～5または7持続) と報告されている[3]。今回，NEDの投与量はNED単剤での至適投与量が100 mg/m²であることを考慮し，60 mg/m²を第一投与量とした。ADM，5-FUの投与量はそれぞれ30 mg/m² (day 1)，700 mg/m² (day 1～5持続) とし1日量はほぼ同量としたが，5-FUは5日間の持続投与とし，総投与量として減量した。この理由としてNEDはcisplatin (CDDP) より骨髄障害が強いため，5-FUの総投与量を減らすと同時に，患者のQOLを考えたためである。

II. 方　法

ADM 30 mg/m² day 1，5-FU 700 mg/m²/日 day 1～5の持続点滴静注で4週ごとに投与し，key drugであるNEDの投与量は以下に従い増量する。step 1: 60 mg/m²，step 2: 70 mg/m²，step 3: 80 mg/m²。step 1より試験を開始し，各stepのコースでのdose limiting toxicity (DLT) の発現頻度を評価し，以下に示した基準で投与量stepを移行する。1）同一投与量stepの3例にDLTが認められない場合は，次のstepに移行。2）3例中1例にDLTが認められた場合は3例追加し6例とする（①追加後の3例にDLTが認められない場合には，次のstepに移行する。②追加後の3例中1例にDLTが認められた場合は，その投与量stepを最大耐用量 (MTD) とする)。MTDが判明したのち，MTDより1段階低い投与量stepをRD（推奨投与量）とする。またDLT基準は以下のとおりである。①grade 4の白血球減少または好中球減少が出現，②38°C以上の発熱を伴うgrade 3の好中球減少が出現，③25,000/mm³未満の血小板減少，④悪心・嘔吐，食欲不振，疲労および脱毛を除く，grade 3以上の非血液毒性が出現した場合。中止基準を満たさない限り2コース以上行い，前コースでの発現副作用により減量基準も設けた[2]（詳細省略）。

Table 1　Patient characteristics on phase I study

Total number of patients (step 1/step 2)	12 (6/6)
Sex (M/F)	11/1
Age (years)	65 (46-74)
Stage (III/IV a/IV b)	4/3/5
Performance status (0/1)	7/5

第II相として，第I相で決定された推奨用量，用法における有効性を評価するためSimonのminimax designに従い，期待奏効率を50%，閾値率を30%として（$\alpha=0.05$，$\beta=0.20$），目標必要症例数を40例に設定した。評価病変に対する臨床的治療効果（腫瘍縮小効果）および奏効度の表現はWHO criteriaに従った。

III. 結　果

第I相は男性11例，女性1例の計12例（平均年齢65歳，46～74歳）が登録（Table 1）。進行度とPSもTable 1を参照されたい。各step 6例ずつでstep 2まで増量。step 1で1例のDLT (grade 4の好中球減少)，step 2で2例のDLT（grade 4の好中球減少とgrade 3の下痢）出現のためstep 2をMTD，step 1の60 mg/m²をNEDの推奨投与量とした（Table 2）。好中球減少のnadirは投与第1日目から9～22日（中央値17日）で，回復に3～4日を要した。grade 1の悪心・嘔吐が最も多い非血液毒性であり，grade 1の腎毒性はstep 2で1例経験したが，すべて保存的に軽快した。また，step 1でのgrade 4好中球減少のDLT症例のみG-CSFを使用した。また，進行中の第II相では，NEDの推奨用量をstep 1の60 mg/m²とし，現在のところ7例（男性4例，女性3例）（平均年齢62歳，46～74歳）を登録。施行コース数中央値は2（1～3）で，PR 4例，NC 3例であり，現在までの奏効率は57.1%であった（Table 3）。生存期間も追跡中である。

IV. 考　察

近年，手術手技の向上，術後管理の進歩，早期癌症例発見の増加などの理由で食道癌全体の治療成績は以前より改善したが，高度進行食道癌や再発食道癌は依然とし

Table 2 Hematologic and nonhematologic toxicities by dose level in the first cycle on phase I

Dose step	No. of patients	Neutropenia				Thrombocytopenia				Anemia				Nausea/vomiting				Diarrhea				Elevation of creatinine				Elevation of transaminase			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	6	1	1	2	1	0	0	1	0	3	2	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	6	2	1	1	1	0	0	0	0	4	1	0	0	3	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0

Table 3 Phase II study on going

Pt #	Gender	Age	TNM	Stage	No. of courses	Response	Evaluable sites
1	F	70	T4	IVa	2	PR	Main tumor
2	M	54	H1	IVb	2	PR	Liver
3	M	46	M1 (brain)	IVb	3	NC	Brain
4	M	74	T4	IVa	2	NC	Main tumor
5	F	68	T4, M1 (lung)	IVb	2	PR	Main tumor, Lung
6	M	60	H1	IVb	2	NC	Liver
7	F	61	T4	IVa	1	PR	Main tumor

て予後不良である。1980年代以降の高度進行食道癌や再発食道癌に対する化学療法成績をみると，単剤での奏効率は2～4剤の併用療法よりも低いことは判明している。当初，胃癌に対する効果からADMと5-FUとの併用が期待されたが，1984年のCDDP登場以来，それをkey drugとした多剤併用療法が中心となり効果をあげてきた[4]。1987年以後食道癌の標準併用化学療法となった5-FU/CDDP（FP）は，1992年Japanese Esophageal Oncology Group（JEOG）で行った進行食道癌を対象にしたFP療法の第II相臨床試験にてもその効果が認められ，わが国においても現在標準治療となっている[5]。同時にADMとCDDPの併用効果についても報告されるようになり，ADM，5-FUにCDDPを加えた3剤併用のいわゆるFAP療法の比較的良好な成績が報告されている[3]。しかし，key drugであるCDDPの腎毒性や消化器毒性などの副作用が強いため，QOLの改善が望まれている。

NEDはわが国で開発されたプラチナ誘導体であり，単剤で行われた第II相臨床試験でCDDP同様幅広い抗腫瘍スペクトルを有していることが示され，特に扁平上皮癌（頭頸部癌[6]，食道癌[7]，子宮頸癌[8]）に対する有効性が高いと評価された。また，CDDPに比較し，腎毒性が少なく，消化器障害の程度も低く，患者のQOL向上が期待できる。一方，NEDのDLTは骨髄抑制（白血球減少，血小板減少）であり，MTDは120 mg/m²であった。NED単剤での第II相臨床試験における食道癌に対する奏効率が51.7%（15例/29例）得られている。さらに，NED/5-FU併用療法が基礎的検討からも併用効果が証明されて以来[9]，NED/5-FU併用療法の良好な報告がされている[10]。食道癌と同様にFP療法が標準療法とされる頭頸部癌における報告[11]では，NED+5-FUはCDDP+5-FUと比較して奏効率はほぼ同等で血小板減少の頻度は高いが，腎毒性，悪心・嘔吐の消化器毒性が低いと報告されている。

また，ADMを加えたFAP/N療法の清水らの報告では，高度進行食道癌（高度リンパ節転移およびT4）に対し，抗腫瘍効果50%（FP療法5.9%）で，手術切除率92%（FP療法47%），根治率73%（FP療法25%）と，かなり良好な成績が得られ，毒性ではgrade 2～3の白血球減少と口内炎を認めたが腎毒性や消化器毒性はなく，いずれも保存的に対処可能であったとされている[12]。

taxane系を加えたnew regimenもでているなか[13]，食道癌の標準的治療として現実にはFP療法が行われており，欧米では40～60%の奏効率が報告されている[14,15]が，JEOGにおけるstudyでは奏効率33%（CR 1例+PR 11例/36例）であり，予想を下回る結果であった。以上より，今回われわれは，NED/ADM/5-FU併用療法（NAF療法）に着目し，切除不能進行食道癌に対するfirst-lineとして，この併用化学療法の安全性および有効性の検討を計画した。

NED 60 mg/m²，ADM 30 mg/m² day 1，5-FU 700 mg/m²/日 day 1～5の持続点滴静注を推奨投与法とした，現在までの第II相試験での奏効率は57.1%であり良好な結果が得られた。grade 4の血液毒性がみられたが，保存的に十分対応可能であり，CDDPにみられるような消化器，腎毒性はほとんどなかった。奏効度，安全性およびQOLの面からも期待できるregimenと思われる。治療後予後または生存期間はまだ解析中であるが，今後

症例を登録追加し，さらなる検討を進めたい。

文　献

1) Hirao M, Tsujinaka T and Fujitani K: Phase I-II study of the combination of nedaplatin (NED), adriamycin (ADM), and 5-fluorouracil (5-FU) for treatment of previously untreated advanced esophageal cancer. *Proc ASCO* **23**: 349, 2004.
2) Hirao M, Fujitani K and Tsujinaka T: Phase I study of the combination of nedaplatin, adriamycin, and 5-fluorouracil for treatment of previously untreated advanced esophageal cancer. *Dis Esophagus* **17**(3): 247-250, 2004.
3) 甲　利幸，安田卓司，古河　洋・他：食道癌の化学療法．消化器科　**25**(2): 148-153, 1997.
4) 栗原　稔：食道癌化学療法の現況．消化器癌　**2**(1): 57-62, 1992.
5) Iizuka T, Kakegawa T, Ide H, *et al*: Phase II evaluation of cisplatin and 5-fluorouracil in advanced squamous cell carcinoma of the esophagus: A Japanese Esophageal Oncology Group Trial. *Jpn J Clin Oncol* **22**(3): 172-176, 1992.
6) 犬山征夫，三宅浩郷，堀内正敏・他：頭頸部癌に対する新白金錯体254-Sの後期第II相臨床試験．癌と化学療法　**19**(6): 871-877, 1992.
7) 田口鐵男，涌井　昭，鍋谷欣市・他：254-Sの消化器癌に対する第II相臨床試験．癌と化学療法　**19**(4): 483-488, 1992.
8) 加藤　俊，西村治夫，薬師寺道明・他：婦人科癌に対する254-Sの臨床第II相試験．癌と化学療法　**19**(5): 695-703, 1992.
9) Takeda Y, Kasai H, Uchida N, *et al*: Enhanced antitumor efficacy of nedaplatin with 5-fluorouracil against human squamous carcinoma xenografts. *Anticancer Res* **19**: 4059-4064, 1999.
10) 石橋　悟，標葉隆三郎，宮崎修吉・他：高度進行・再発食道癌に対するNedaplatin/5-FU併用療法の効果—Cisplatin/5-FU併用療法と比較して．日消外会誌　**34**(8): 1269-1276, 2001.
11) Fujii M, Ohno Y, Tokumaru Y, *et al*: Comparison of nedaplatin plus 5-FU versus CDDP plus 5-FU for head and neck squamous cell carcinoma. *Proc ASCO* **18**: 405 a, 1999.
12) 清水孝王，西巻　正，小杉伸一・他：高度進行食道癌に対する術前FAP/N(5-FU+ADM+CDDP/Nedaplatin)療法の効果．日癌治療会誌　**36**(2): 761, 2001.
13) Ajani JA: Docetaxel for gastric and esophageal carcinomas. *Oncology* **16**(6): 89-96, 2002.
14) Kies MS, Rosen ST, Tsang T, *et al*: Cisplatin and 5-FU in the primary management of squamous esophageal cancer. *Cancer* **60**: 2156-2160, 1987.
15) Bleiberg H, Jacob JH, Bedenne L, *et al*: Randomized phase II trial of 5-FU and CDDP versus CDDP alone in advanced oesophageal cancer. *Proc ASCO* **10**: 447, 1991.

別刷

癌と化学療法

VOL.31(2004)

癌と化学療法社

Printed in Japan © 禁無断転載・複写複製