SPANDIDOS PUBLICATIONS

Investigation of optimal schedule of concurrent radiotherapy with S-1 for oral squamous cell carcinoma

KOJI HARADA, TARANNUM FERDOUS and HIDEO YOSHIDA

Department of Therapeutic Regulation for Oral Tumors, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8504, Japan

Received May 23, 2007; Accepted July 2, 2007

Abstract. In the present study, we examined the appropriate schedule of S-1 medication in the combination with radiation by investigating the safety, the clinical efficacy, and antitumor effects on tumors in nude mice. In the patients with oral squamous cell carcinoma (OSCC), S-1 was given orally according to a 4-week application followed by 2-week rest regimen (4-week regimen), or a 2-week application followed by a 1-week rest regimen (2-week regimen). Radiation was given (2 Gy/day; 5 days/week) for a total of 60 Gy. In nude mouse models, human oral cancer cell lines were used as subcutaneous xenografts in nude mice. The mice were treated by S-1 (10 mg/kg) and radiation (1 Gy) with a 4-week regimen or a 2-week regimen. Apoptotic cells were detected by TUNEL method. In the patients with OSCC, the response rate with the 4-week regimen was 100% and the response rate with the 2-week regimen was 92.3%. However, a high frequency of adverse effect was found in the 4-week regimen when compared to the 2-week regimen. Grade 3 toxicity of leukopenia, neutropenia and stomatitis were seen in 3 cases, grade 3 toxicity of anorexia and nausea were seen in 2 cases, and grade 3 toxicity of decrease of hemoglobin level, heartburn/dyspepsia and increase of bilirubin level were seen in a case of the 4-week regimen. On the other hand, grade 3 toxicity of stomatitis, anorexia, nausea, heartburn/dyspepsia and increase of bilirubin level were seen in a case of the 2-week regimen. In nude mouse models, the 2-week regimen was more effective than the 4-week regimen. In addition, significant increase in the percentage of apoptotic cells was observed in the tumors treated with the 4-week regimen when compared with the tumors treated with the 2-week regimen. No loss of body weight was observed in mice treated with the 2-week regimen during the experimental period. These results suggested that the 2-week regimen might reduce adverse effects, and enhance therapeutic effects compared to the 4-week regimen. Briefly, this 2-week regimen may be a useful concurrent chemo-radiotherapy improving the quality of life (QOL) of patients with OSCC.

Correspondence to: Dr Koji Harada, Department of Therapeutic Regulation for Oral Tumors, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8504, Japan
E-mail: harako@dent.tokushima-u.ac.jp

Key words: oral squamous cell carcinoma, S-1, chemo-radiotherapy, two-week regimen

Introduction

Preservation of oral function and sensuousness as well as improvement of survival rate is required in oral squamous cell carcinoma (OSCC) treatment. Though chemotherapy is very useful for preservation of oral function and sensuousness, single-agent chemotherapy only has a limited role in the treatment of advanced or recurrent OSCC patients. Thus, chemo-radiotherapy is a major approach in treatment of patients with OSCC.

We continued to treat advanced OSCC patients by radiotherapy in combination with oral fluoropyrimidine anticancer agent, UFT (combined drug of 1 M FT and 4 M uracil) (1). However, this treatment strategy was not effective in OSCC patients of stage IV. For further improvement of therapeutic effects, we started the combined therapy of S-1 and radiation.

S-1 is a novel oral fluoropyrimidine anticancer agent designed to enhance anticancer activity and reduce gastrointestinal toxicity through the combination of the following components: an oral fluoropyrimidine agent, tegafur (FT) which is a masked form of 5-fluorouracil (5-FU); a DPD inhibitor (CDHP: 5-chloro-2, 4-dihydroxypyridine) which is an inhibitor of 5-FU degradation and an OPRT (orotate phosphoribosyltransferase) inhibitor (Oxo: potassium oxonate) which is an inhibitor of 5-FU phosphorylation, which is localized in the gastrointestinal tract. Briefly, S-1 has cytotoxic mechanisms similar to those of 5-FU, but has been shown to have less toxic side-effects than 5-FU in clinical trials (2). Also, the response rate of S-1 single-agent chemotherapy was 46.2% (12/26) in late phase II study of advanced head and neck cancer (3). Thus, S-1 can exert remarkable antitumor effect through the maintenance of 5-FU level in the blood. Moreover, the concurrent radiotherapy with S-1 may produce enhanced therapeutic efficacy because 5-FU is thought to have radiosensitizing effects (4). We have reported that S-1 may have radiosensitization effects, and that the combination of S-1 and radiotherapy may be effective on human oral cancer cells in experimental models (5-7). However, the appropriate schedule of S-1 medication in combination with radiation

has not yet been determined, and a 2-week regimen, a 4-week regimen or other regimens are selected.

In the present study, we examined the appropriate schedule of S-1 medication in combination with radiation by investigating the safety and clinical efficacy, and tried to clarify the differences between the 4-week and the 2-week regimen by using nude mouse xenograft models.

Patients and methods

Patients. The subjects of the study were 27 patients with oral squamous cell carcinoma who received concurrent radiotherapy with S-1 between October 2002 and August 2004 at Tokushima University Hospital Faculty of Medicine and Dentistry. The characteristics of the patients are summarized in Table I.

Informed consent was obtained from each patient prior to the start of combined radiotherapy with S-1. The 14 patients were treated with the 4-week regimen of S-1 between October 2002 and September 2003. Briefly, they received the drug for 4-week periods followed by 2-week drug-free intervals (4-week regimen group; 4W/2W). The remaining 13 patients were treated with the 2-week regimen between October 2003 and August 2004. In short, these patients received the drug for 2-week periods followed by a drug-free interval for 1 week (2-week regimen group; 2W/1W).

Patients who satisfied all of the following requirements were included in the study: histologically confirmed OSCC; at least one measurable lesion; no history of prior antitumor treatment except resection; Eastern Cooperative Oncology Group (ECOG) performance status of 0-2; age of 20-80 years; adequate organ function, defined as a white blood cell count of 3500-12000 mm^3, a neutrophil count of >2000 mm^3, a platelet count of >100000 mm^3, a hemoglobin level of >9.0 g/dl, aspartate aminotransferase and alanine aminotransferase level of within 2 times the upper limit of facilities; a serum total bilirubin level of within 2 times the upper limit of facilities, a normal serum creatinine level of within the upper limit of facilities, an estimated life expectancy of at least 3 months. The exclusion criteria were: active concomitant malignancy, unable to take oral medication, a history of drug hypersensitivity, watery diarrhoea, severe complications (such as infection, interstitial pneumonia, lung fibrosis, heart disease, renal disease, liver disease and severe diabetes with administration of insulin), marked pleural effusion or ascites, pregnancy or lactation, men wishing to protect their ability to produce healthy sperm, and the judgement of exclusion by primary doctor. If all conditions were suitable, these patients were entered in the study. All patients provided written informed consent prior to enrollment in the study and the protocol was approved by the institutional ethics committee of our university hospital.

Methods of treatment. S-1 was administered orally at a dose of 65 mg/m^2 daily. The initial doses were determined according to the body surface area. One cycle of treatment in the 4-week regimen consisted of 4 weeks of drug application followed by a 2-week drug-free period. Each course of treatment in the 2-week regimen consisted of 2 weeks of drug application followed by a 1-week drug-free period. Concurrently, radiation was given (2 Gy/day; 5 days/week) for a total of 60 Gy (Fig. 1).

Table I. Patient characteristics.

Characteristics	4W-2W	2W-1W	P-value
Gender			
Male	7	8	NS (χ^2)
Female	7	5	
Age			
(Mean ± SD)	65.4±10.8	64.4±12.4	NS (t)
PS			
0	10	9	NS (χ^2)
1	3	2	
2	1	2	
Primary region			
Tongue	3	4	NS (χ^2)
Upper gingival	2	3	
Lower gingival	9	4	
Oral floor	0	1	
Palate	0	1	
Stage			
II	5	5	NS (χ^2)
III	3	4	
VIA	6	3	
VIB	0	1	

NS, not significant; χ^2, Chi-square test; t, Student's t-test. Stage grouping were classified according to the 1997 International Union Against Cancer criteria.

When a grade 3 or greater hematologic or nonheamatologic toxicity occurred, the temporary interruption of the S-1 administration and radiation was allowed until the toxicity subsided to grade 1 or less [the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0]. If the daily dose of S-1 was considered to be intolerable, the retreatment dose was reduced by 20 mg/day (minimum dose, 60 mg/day). Patients maintained a daily journal to record their intake of S-1 and any signs or symptoms that they experienced.

Assessment of response. The response after treatment was assessed according to the Japan Society for Cancer Therapy Criteria (8), which is similar to the World Health Organization Criteria. Briefly, a complete response (CR) means disappearance of all lesions and no occurrence of new lesions by therapy for 4 weeks or more. A partial response (PR) means reduction of ≥50% of lesions and no occurrence of new lesions by therapy for 4 weeks or more. No change (NC) was defined as a reduction of <50% or a <25% increase in the sum of the products of two perpendicular diameters of all lesions for a minimum of 4 weeks. A progressive disease (PD) was defined as an increase of ≥25% in the sum of the products of two perpendicular diameters of all lesions, the appearance

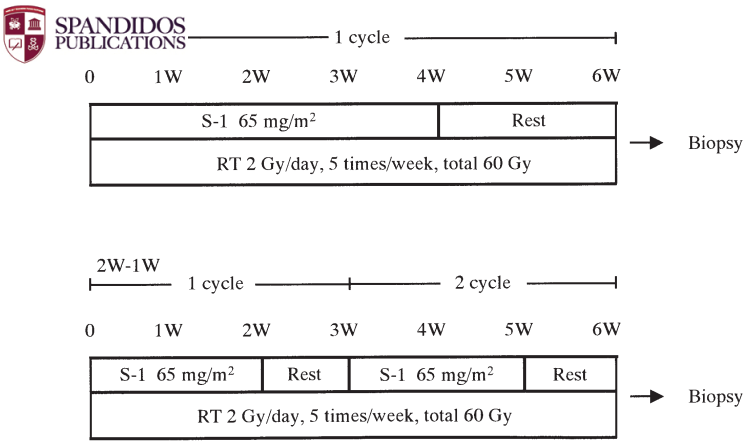

Figure 1. Schedule of concurrent radiotherapy with S-1 for OSCC patients. The patients of 4-week regimen group (4W/2W) received the S-1 for 4-week periods followed by 2-week S-1-free intervals. The patients of 2-week regimen group (2W/1W) received the drug for 2-week periods followed by a drug-free interval for 1 week. Radiotherapy (RT) was combined (2 Gy/day; 5 days/week) for a total of 60 Gy.

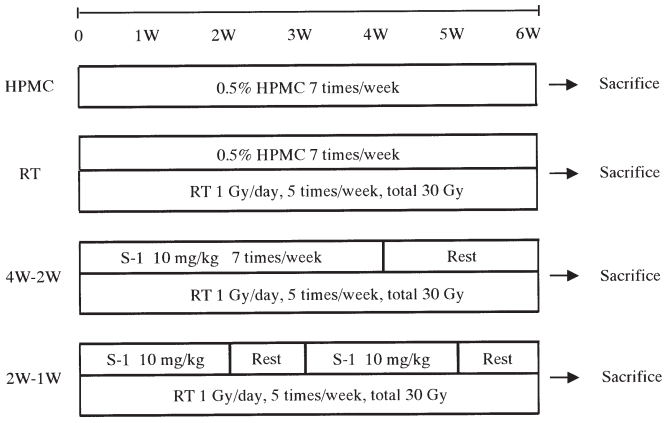

Figure 2. Schedule of concurrent radiotherapy with S-1 for nude mice. The mice of HPMC group were administered orally with 0.5% HPMC for 6 weeks (7 times/week). The mice of RT group were irradiated with 1 Gy per day for 6 weeks (5 times/week), and then administered orally with 0.5% HPMC for 6 weeks (7 times/week). The mice of 4-week regimen group (4W/2W) received the S-1 for 4-week periods followed by 2-week S-1-free intervals, and then irradiated with 1 Gy per day for 6 weeks. The mice of 2-week regimen group (2W/1W) received the drug for 2-week periods followed by a drug-free interval for 1 week, and were then irradiated with 1 Gy per day for 6 weeks.

of any new lesions, or the deterioration in the clinical status that was consistent with disease progression. Also, assessments of responses were performed by direct measurement.

Toxicity evaluation. Compliance was calculated for all treatment courses using the ratio of the total dose actually administered to the scheduled dose. Physical examinations, complete blood cell counts, biochemistry tests, and urinalyses were performed at least weekly. Adverse effects were evaluated according to NCI-CTC version 2.0. Objective responses and adverse effects were confirmed by an external review committee.

Cell lines and cell culture. B88 cells were isolated from an OSCC patient in our laboratory (9). HSC2 cells were obtained from American Type Culture Collection (Manassas, VA, USA). B88 and HSC2 cells were cultured in DMEM supplemented with medium supplemented with 10% fetal calf serum, 100 μg/ml streptomycin and 100 units/ml penicillin in a humidified atmosphere containing 5% CO_2/95% air.

Nude mice and tumor inoculations. Female athymic nude mice with CAnN.Cg-Foxnlnu/CrlCrlj genetic background (Clea Japan, Inc. Tokyo, Japan) were purchased at 4 weeks of age and kept under sterile conditions in a pathogen-free environment. The mice were provided with sterile water and food *ad libitum*, and all manipulations were carried out aseptically inside a laminar flow hood. Cells were used as a xenograft model in the nude mice. Briefly, tumor cells ($1x10^6$) were suspended in 0.1 ml of serum-free medium and injected into the subcutaneous tissue of 5-week-old nude mice using a 27-gauge needle. Tumors were allowed to grow for 10 days before treatment. Then, the mice were divided into 4 groups, each of 5 mice with almost equal mean tumor volume (~100-150 mm^3 in volume).

Reagents and treatment protocol. S-1 was obtained from Taiho Pharmaceutical Co., Ltd., (Tokyo, Japan). The drug was suspended in autoclaved 0.5% sodium hydroxypropylmethylcellulose (HPMC, Daiichi Seiyakukogyo, Kyoto, Japan) in sterile condition, at 1.0 mg/ml, and subsequently homogenized by stirring. The suspension was given to mice by a gastric tube in a volume of 0.1 ml/10 g body weight for 4 weeks (7 times/week) according to the each regimen. Also, tumors on the flanks of mice were irradiated with 1 Gy per day for 6 weeks (5 times/week) using an X-ray irradiator (MBR-1505R2, 150 kV, 5 mA, filter: 1.0 mm aluminum, Hitachi Medico). Control mice were administered orally with an equal volume of 0.5% HPMC for 6 weeks (7 times/week) (Fig. 2). The tumors were measured twice per week, and the relative tumor volumes were calculated. At 42 days, mice were sacrificed by cervical dislocation, and the tumors were dissected out, fixed in neutral-buffered formalin, and embedded in paraffin for further study.

TUNEL [terminal deoxynucleotidyl transferase (Tdt)-mediated nick end-labeling] assay. To detect apoptotic cells, the ApopTag Plus peroxidase *in situ* apoptosis detection kit (Intergen Company, Purchase, NY) was used. Paraffin sections of tumor (5 μm) were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. Tissue sections were incubated in 20 μg/ml proteinase K (Dako Corporation, Carpinteria, CA) for 15 min. After sections were rinsed in distilled water, endogenous peroxidase was blocked by incubating the slides in 3% hydrogen peroxide in PBS (0.05 M phosphate buffer containing 0.145 M sodium chloride, pH 7.4) for 5 min. After being washed with PBS, the sections were incubated with equilibration buffer and then TdT enzyme in a humidified chamber at 37˚C for 60 min. They were subsequently put into prewarmed working strength stop wash buffer for 10 min. After being rinsed in PBS, the sections were incubated with antidigoxigenin-peroxidase conjugate for 30 min. Peroxidase activity in each section was demonstrated

Table II. Response results.

Group	n	CR	PR	NC	PD	Response rate (%)
4W-2W	14	4	10	0	0	100.0
2W-1W	13	9	3	1	0	92.3

Complete response (CR) means the disappearance of all lesions and no occurrence of new lesion by therapy for 4 weeks or more. Partial response (PR) means the reduction of ≥50% lesions and no occurrence of new lesion by therapy for 4 weeks or more. No change (NC) was defined as a reduction of <50% or a <25% increase in the sum of the products of two perpendicular diameters of all lesions for a minimum of 4 weeks. Progressive disease (PD) was defined as an increase of ≥25% in the sum of the products of two perpendicular diameters of all lesions, the appearance of any new lesion, or the deterioration in the clinical status that was consistent with disease progression.

Table III. 4W-2W treatment-related adverse events (n=14): worst grade reported during treatment period.

Toxicity	Grade[a]					Grade 3-4 (%)
	0	1	2	3	4	
Hematologic						
Leukopenia	5	3	3	3	0	21.4
Neutropenia	6	2	3	3	0	21.4
Anemia	9	1	3	1	0	7.14
Thrombocytopenia	12	2	0	0	0	0
Nonhematologic						
Stomatitis	0	5	6	3	0	21.4
Anorexia	5	3	4	2	0	14.3
Nausea	8	3	1	2	0	14.3
Vomiting	12	1	1	0	0	0
Heartburn/dyspepsia	11	1	1	1	0	7.14
Diarrhoea	11	1	2	0	0	0
Rash	10	2	1	1	0	7.14
Pigmentation changes	11	2	1	0	0	0
Fatigue	8	3	2	1	0	7.14
ALT, AST	12	2	0	0	0	0
Total bilirubin	11	1	1	1	0	7.14

[a]NCI Common Toxicity Criteria, version 2.0. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

by the application of diaminobenzidine (peroxidase substrate kit; Vector Laboratories). Hematoxylin was used for a counterstaining. At least 1000 cells were counted under a microscope in several random fields of each section. The number of apoptotic cells was divided by the total number of cells, and the result was expressed as percentage of apoptotic cells.

Table IV. 2W-1W treatment-related adverse events (n=13): worst grade reported during treatment period.

Toxicity	Grade[a]					Grade 3-4 (%)
	0	1	2	3	4	
Hematologic						
Leukopenia	8	3	2	0	0	0
Neutropenia	8	3	2	0	0	0
Anemia	9	2	2	0	0	0
Thrombocytopenia	12	1	0	0	0	0
Nonhematologic						
Stomatitis	0	6	6	1	0	7.69
Anorexia	5	2	5	1	0	7.69
Nausea	9	1	2	1	0	7.69
Vomiting	13	0	0	0	0	0
Heartburn/dyspepsia	12	0	0	1	0	7.69
Diarrhoea	13	0	0	0	0	0
Rash	13	0	0	0	0	0
Pigmentation changes	11	2	0	0	0	0
Fatigue	11	1	1	0	0	0
ALT, AST	13	0	0	0	0	0
Total bilirubin	12	0	0	1	0	7.69

[a]NCI Common Toxicity Criteria, version 2.0. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Statistical analysis. All statistical significance was set at $p<0.05$. Statistical analyses were run using the StatView software (version 5.0J, SAS Institute Inc. Cary, NC, USA).

Results

Patient characteristics. The characteristics of the patients in each group are shown in Table I. There was no significant difference between the 4W/2W and the 2W/1W in terms of the gender, median age, performance status, primary region and disease stage.

Efficacy. Of the 14 patients, 4 patients achieved a CR and 10 patients showed a PR, giving an overall response rate of 100% in the 4W/2W. Nine patients achieved a CR and 3 showed a PR, giving an overall response rate of 92.3% in the 2W/1W (Table II).

Toxicity. All patients were assessed for toxicities that are listed in Tables III and IV. Both treatments were generally well tolerated throughout the study. Grade 3 leukopenia, neutropenia and stomatitis occurred in 3 patients each (21.4%), anorexia and nausea in 2 patients each (14.3%), and grade 3 anemia, heartburn/dyspepsia, rash, fatigue and high level of total bilirubin in one patient each (7.14%) in the 4W/2W. Grade 3 stomatatis, anorexia, nausea, heartburn/dyspepsia and high level of total bilirubin occurred in one patient each (7.69%) in the 4W/2W. Apart from the above adverse

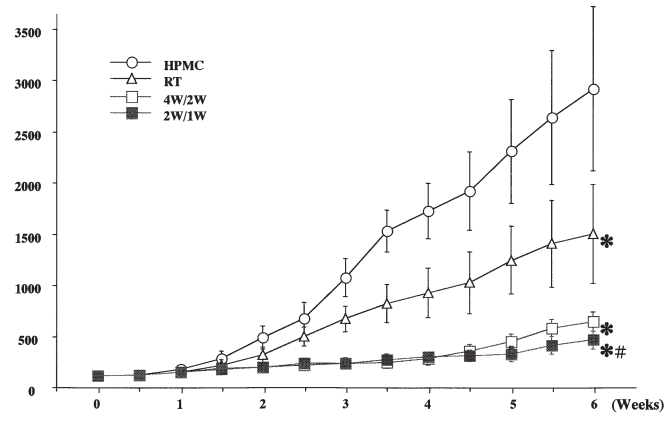

Figure 3. Effect of RT alone, the 4-week regimen, and the 2-week regimen on B88 tumor growth in nude mice. Growth inhibition effects of RT alone, the 4-week regimen, and the 2-week regimen were statistically significant when compared with that of HPMC (*P<0.01, by Mann-Whitney U test). The 2-week regimen was more effective than the 2-week regimen (#P<0.05, by Mann-Whitney U test). Bars, SD.

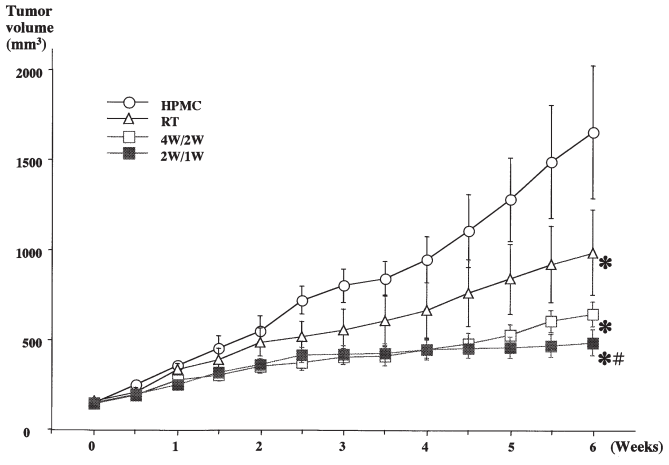

Figure 4. Effect of RT alone, the 4-week regimen, and the 2-week regimen on HSC2 tumor growth in nude mice. Growth inhibition effects of RT alone, the 4-week regimen, and the 2-week regimen were statistically significant when compared with that of HPMC (*P<0.01, by Mann-Whitney U test). The 2-week regimen was more effective than the 2-week regimen (#P<0.05, by Mann-Whitney U test). Bars, SD.

effects, hematologic and gastrointestinal toxicities were common, most of the toxicities were mild and transient. In addition, no grade 4 toxicities were observed in either group. No signs of cumulative toxicity were noted.

Compliance with the dose schedule. According to original schedule shown in Fig. 1, one course of chemotherapy with S-1 was received in the 4-week regimen, and two courses of chemotherapy with S-1 were received in the 2-week regimen. However, dose reduction of S-1 was required in 6 patients of the 4W/2W because of grade 3 adverse effects or medical judgment by the primary doctor. Dose reduction of S-1 was required in 2 patients of the 2W/1W because of grade 3 adverse effects or medical judgment by the primary doctor.

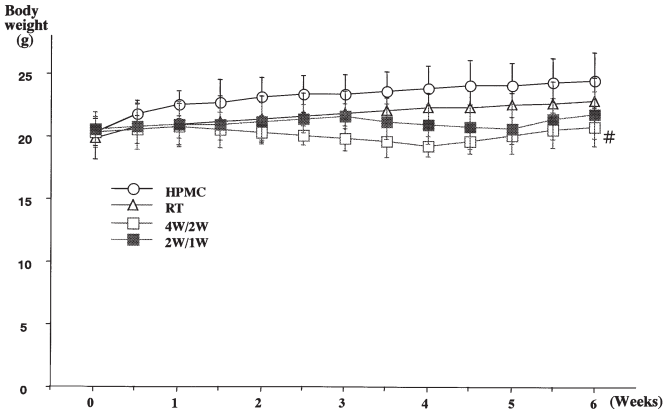

Figure 5. Change of body weight on nude mice bearing B88 tumors. During the experimental period, no loss of body weight was observed in mice treated with the 2-week regimen. However, loss of body weight was seen in mice treated with the 4-week regimen when compared with that of HPMC (#P<0.05, by Mann-Whitney U test). Bars, SD.

Moreover, interruption of radiation was required in 6 patients of the 4W/2W because of grade 3 stomatitis, and in 2 patients of the 2W/1W because of grade 3 stomatitis. The period of radiation interruption was one week each in the 8 patients.

Except for 4 patients, in whom treatment was abandoned because of toxicities (grade 3 stomatitis, anorexia, nausea and diarrhoea), the remaining 14 patients were treated as inpatients. The overall compliance rate was 77.8% (14/18) in the 4W/2W. Except for two patients, in whom treatment was abandoned because of toxicities (grade 3 stomatitis and nausea), the remaining 13 patients were treated as inpatients. The overall compliance rate was 86.7% (13/15) in the 2W/1W.

Relative dose intensity was 95.5±5.9 in the 4W/2W, and 98.9±2.8 in the 2W/1W. However, there was no significant difference between the 4W/2W and the 2W/1W.

Antitumor effects of 4-week regimen and 2-week regimen on nude mouse tumor. Mice were treated with 1 Gy/day radiation alone and in combination with 10 mg/kg S-1, and growth inhibitory effects were observed during the treatment period. Growth inhibition was seen in radiation alone or combined therapy, and these inhibitions were statistically significant when compared with that of control. Also, combined therapy was more effective than radiation alone. Moreover, the 2-week regimen was more effective than the 4-week regimen in the case of combined therapy. Furthermore, combined therapy preferentially suppressed the growth of B88 tumors when compared with that of HSC2 tumors (Fig. 4).

Effects of 4-week regimen and 2-week regimen on body weight of nude mouse. No loss of body weight was observed in mice treated with the 2-week regimen during the experimental period. However, the body weight of mice treated with the 4-week regimen was decreased when compared with that of control (Figs. 5 and 6).

Analysis of apoptosis after 4-week regimen and 2-week regimen on nude mouse tumor. To analyze the degree of

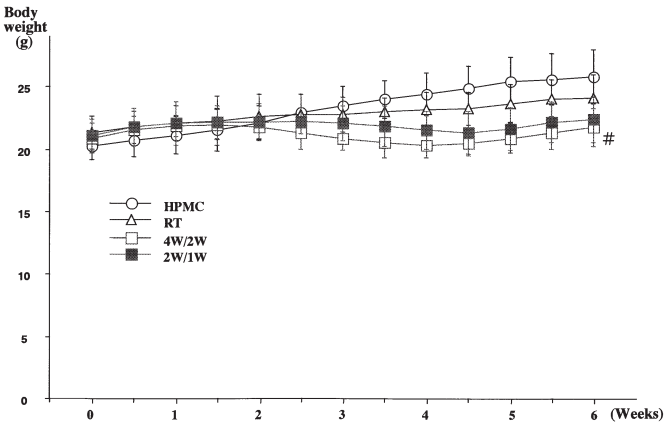

Figure 6. Change of body weight on nude mice bearing HSC2 tumors. During the experimental period, no loss of body weight was observed in mice treated with the 2-week regimen. However, loss of body weight was seen in mice treated with the 4-week regimen when compared with that of HPMC ($^{\#}P<0.05$, by Mann-Whitney U test). Bars, SD.

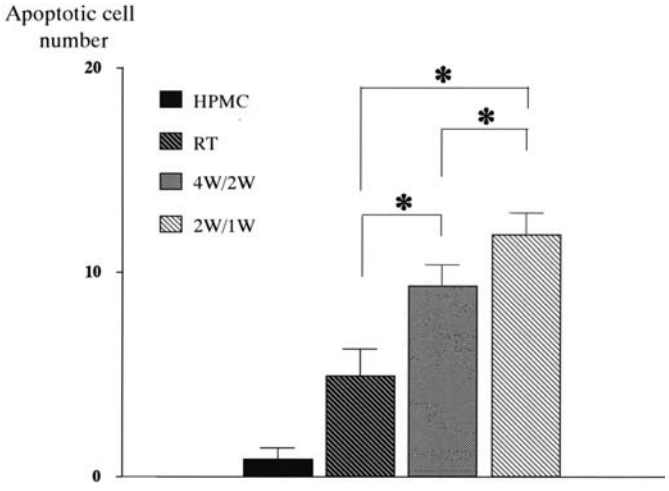

Figure 8. Analysis of induction of apoptosis in tumors treated with each regimen. The 2-week regimen markedly induced apoptosis compared to the 4-week regimen ($^{*}P<0.01$, by Mann-Whitney U test). Bars, SD.

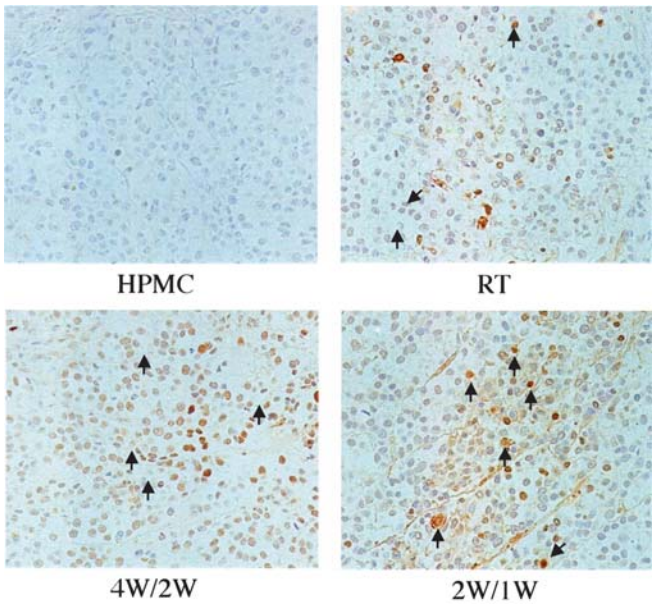

Figure 7. TUNEL assay. The largest number of apoptotic cells was seen in the tumors treated with a 2-week regimen. Arrows indicate apoptotic cells.

apoptosis, tumors were removed from mice after treatment, and the number of apoptotic cells was quantified by the TUNEL assay (Fig. 7). The degree of apoptosis in the tumors treated with either radiation alone or combined therapy was significantly higher than that of control. The largest number of apoptotic cells was seen in the tumors treated with the 2-week regimen. The extent of apoptosis in these groups were: Control group, 0.73±0.61; Radiation group, 4.88±1.22; 4-week regimen group, 9.39±0.98; 2-week regimen group, 11.9±1.01 (Fig. 8).

Discussion

The antitumor effect of S-1 has been demonstrated in a variety of solid tumors: the response rates for advanced gastric cancer (10), colorectal cancer (11), non-small cell lung cancer (12), and head and neck cancer (3) in the late phase II studies conducted in Japan were 44-49, 35, 22, and 29%, respectively. The efficacy of S-1 for the treatment of gastrointestinal cancer has also been reported in European patients: the response rates for advanced gastric cancer (13) and colorectal cancer (14) were 32 and 24%, respectively. However, few previous studies have described the efficacy and safety of S-1 for the treatment of oral cancer (15).

The incidence of adverse events following S-1 therapy is thought to be high (83.2%) in pre-marketing clinical trials (10,16). In the post-marketing survey, conducted on 3294 patients, evaluation of the S-1 related hematological toxicity revealed that the median time for the WBC, RBC, and platelet counts to reach their lowest levels was 22 days (17). In the same study, the median time to the onset of nonhematological adverse effects, such as diarrhea, skin symptoms, and stomatitis was 14-15 days. Briefly, they showed the therapeutic utility of a regimen of 2-week application followed by 1-week rest because the 2-week regimen can affect the medication during the onset of adverse effects. Originally, S-1 has been taken with the regimen of 4-week application followed by 2-week rest. In the present study, we tried to compare the safety and clinical efficacy between the conventional 4-week regimen and the new 2-week regimen in chemo-radiotherapy retrospectively.

The response rate with the 4-week regimen was 100%, and the response rate with the 2-week regimen was 92.3% (4/14). Both regimens showed good therapeutic effects. However, the CR rate with the 4-week regimen was 28.6%, and the CR rate with the 2-week regimen was 69.2% (9/13). In short, the 2-week regimen may have a therapeutic effect on patients with OSCC. The improvement of CR rate leads to preservation of oral function and sensuousness. A high frequency of adverse effect was found in the 4-week regimen when compared with the 2-week regimen. These results suggest that the 2-week regimen is connected to reduced adverse effects without decreasing therapeutic effects, compared with the 4-week regimen. Therefore, we studied the differences of these regimens for antitumor effects by using nude mouse xenograft models. Our investigation

that the 2-week regimen was more effective than k regimen on nude mouse tumors by the significant induction of apoptosis (Figs. 3, 5 and 6). Moreover, the antitumor effects were decreased during 2-week rest period of the 4-week regimen, compared with the 2-week regimen. However, the 2-week rest may be long in the combined therapy of S-1 and radiation. No loss of body weight was seen in mice treated with the 2-week regimen though loss of body weight was observed in mice treated with the 4-week regimen (Fig. 4). Briefly, the 2-week regimen may be able to reduce adverse effects, and enhance therapeutic effects when compared with the 4-week regimen in nude mouse xenograft models.

The above results suggest that the 2-week regimen may be a useful concurrent chemo-radiotherapy for patients with OSCC. Recently, we reported the results of phase I study of concurrent radiotherapy with S-1 for OSCC (18). In that clinical study, we selected the 2-week regimen. In addition, we are now conducting a phase II study of concurrent radiotherapy with S-1 for OSCC, and are expecting the results of a phase II clinical study.

References

1. Sato M, Harada K, Yoshida H, Yura Y, Azuma M, Iga H, Bando T, Kawamata H and Takegawa Y: Therapy for oral squamous cell carcinoma by tegafur and streptococcal agent OK-432 in combination with radiotherapy: association of the therapeutic effect with differentiation and apoptosis in the cancer cells. Apoptosis 2: 227-238, 1997.
2. Shirasaka T, Shimamato Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K and Fukushima M: Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs 7: 548-557, 1996.
3. Inuyama Y and Kida A: Late phase II study of S-1 in patients with advanced head and neck cancer. (In Japanese) Gan To Kagaku Ryoho 28: 1381-1390, 2001.
4. John E: Pharmacologic requirements for obtaining sensitization of human tumor cells *in vitro* to combined 5-fluorouracil or ftrafur and X-ray. Int J Radiation Oncol Biol Phys 8: 1923-1933, 1982.
5. Harada K, Kawaguchi S, Supriatno, Kawashima Y, Yoshida H and Sato M: S-1, an oral fluoropyrimidine anti-cancer agent, enhanced radiosensitivity in a human oral cancer cell line *in vivo* and *in vitro*: involvement possibility of inhibition of survival signal, Akt/PKB. Cancer Lett 226: 161-168, 2005.
6. Harada K, Kawaguchi S, Supriatno, Onoue T, Yoshida H and Sato M: Enhancement of apoptosis in salivary gland cancer cells by the combination of oral fluoropyrimidine anticancer agent (S-1) and radiation. Int J Oncol 25: 905-911, 2004.
7. Harada K, Kawaguchi S, Supriatno, Onoue T, Yoshida H and Sato M: Combined effects of the oral fluoropyrimidine anticancer agent, S-1 and radiation on human oral cancer cells. Oral Oncol 40: 713-719, 2004.
8. Japan Society for Cancer Therapy: Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. J Jpn Soc Cancer Ther 28: 101-130, 1993.
9. Supriatno, Harada K, Hoque MO, Bando T, Yoshida H and Sato M: Overexpression of $p27^{Kip1}$ induces growth arrest and apoptosis in an oral cancer cell line. Oral Oncol 38: 730-736, 2002.
10. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y and Taguchi T: Late Phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients. Eur J Cancer 34: 1715-1720, 1998.
11. Ohtsu A, Baba H, Sakata Y, Mitachi Y, Horikoshi N, Sugimachi K and Taguchi T: Phase II study of S-1, a novel oral fluoropyrimidine derivative, in patients with metastatic colorectal carcinoma. Br J Cancer 83: 141-145, 2000.
12. Kawahara M, Furuse K, Segawa Y, Yoshimori K, Matsui K, Kudoh S, Hasegawa K and Niitani H: S-1 Cooperative Study Group (Lung Cancer Working Group), Phase II study of S-1, a novel oral fluorouracil, in advanced non-small cell lung cancer. Br J Cancer 85: 939-943, 2001.
13. Chollet P, Schoffski P, Weigang-Kohler K, Schellens JH, Cure H, Pavlidis N, Grunwald V, De Boer R, Wanders J and Fumoleau P: EORTC Early Clinical Studies Group. Phase II trial with S-1 in chemotherapy-naive patients with gastric cancer. A trial performed by the EORTC Early Clinical Studies Group (ECSG). Eur J Cancer 39: 1264-1670, 2003.
14. Van den Brande J, Schoffski P, Schellens JH, Roth AD, Duffaud F, Weigang-Kohler K, Reinke F, Wanders J, de Boer RF, Vermorken JB and Fumoleau P: EORTC Early Clinical Studies Group early phase II trial of S-1 in patients with advanced or metastatic colorectal cancer. Br J Cancer 88: 648-653, 2003.
15. Tsukuda M, Kida A, Fujii M, Kono N, Yoshihara T, Hasegawa Y and Sugita M: Chemotherapy Study Group of Head and Neck Cancer. Br J Cancer 93: 884-889, 2005.
16. Sugimachi K, Maehara Y, Horikoshi N, Shimada Y, Sakata Y, Mitachi Y and Taguchi T: An early phase II study of oral S-1, a newly developed 5-fluorouracil derivative for advanced and recurrent gastrointestinal cancers. The S-1 Gastrointestinal Cancer Study Group. Oncology 57: 202-210, 1999.
17. Nagashima F, Ohtsu A, Yoshida S and Ito K: Japanese nationwide post-marketing survey of S-1 in patients with advanced gastric cancer. Gastric Cancer 8: 6-11, 2005.
18. Sato M and Harada K: Phase I study of concurrent radiotherapy with S-1 for oral squamous cell carcinoma. (In Japanese) Gan To Kagaku Ryoho 33 (Suppl 1): 179-183, 2006.