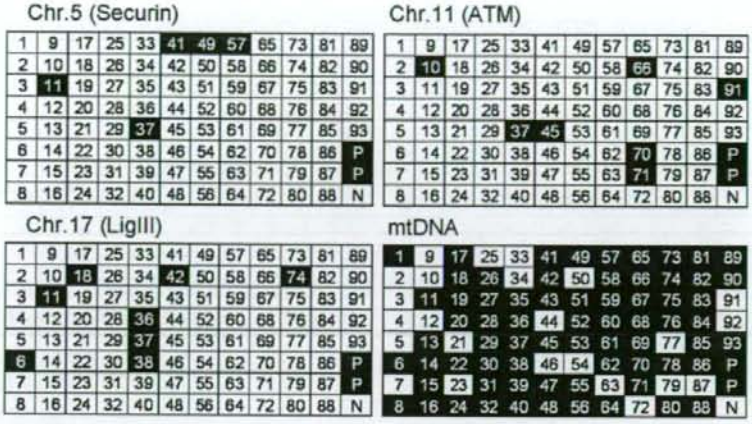

Fig. 3. Screening of target locus amplicons. Representatives of the first screening are shown. Gels were placed in a 96-well plate and serially numbered according to their positions. A black square with white lettering indicates a positive gel, and a white square with black lettering indicates a negative gel. Chr., chromosome; P, positive control gel; N, negative control gel; LigIII, DNA ligase III; mtDNA, mitochondial DNA.

ing gels contained only part of the region and were excluded from further analysis.

Multilocus genotyping of the whole ATM locus amplicons

The five gels that contained the entire *ATM* locus amplicon were subjected to visible genotyping array analysis for the presence of the seven SNPs within the seven loci used in the second screening. All seven SNP genotypes of the two positive control gels were identical to those of the genomic DNA extracted from an aliquot of blood from the same individual, demonstrating the accuracy of this method. Notably, three heterozygous SNPs were present in the positive control gels (Fig. 4, diplotype). None of the SNPs showed heterozygosity in the other three selected gels (66, 71, and 91), confirming the successful isolation of a single homologous chromosome-derived DNA molecule within these gels. Two gels (66 and 91) showed identical haplotypes (Fig. 4, upper haplotype), and the third gel (71) showed the complementary haplotype (Fig. 4, lower haplotype). These observations further demonstrate the effectiveness of this method. The method was also used to analyze 10 EBV-transformed human lymphoblastoid cell lines. The average number of gels showing positivity for chromosome 11 in the first screening was 19 (Table 1), 38% of which contained amplicons of the entire *ATM* locus. The genotyping results are summarized in Table 2. All of the experimentally determined haplotype patterns were also estimated by statistical analysis of the SNP data for 100 healthy volunteers, confirming the reliability of this method.

Discussion

Successful haplotyping depends on (i) the quality of the template chromosomal preparations, (ii) the separation of homologous chromosome-derived DNA molecules, and (iii) the efficiency of igMDA.

The quality of the template chromosomes, in particular the preservation of intact DNA, was ensured by placing the donor cells within the agarose solution, which contained 0.1 N NaOH, and maintaining the solution at 60 °C. Judging from the proportion of the gels that contained the entire 240-kb region (Table 1), DNA segments of at least a few hundred kilobases were retained within the agarose gels under these conditions. In the current study, amplicons up to 800 kb in length were obtained (data not shown), and these are more than sufficient for general haplotype analysis. Because the terminal regions of linear template DNA amplicons tend to be underrepresented, it might be necessary to include a preamplification ligation step to ensure full coverage [39].

The separation of homologous chromosomes is critical for reliable haplotyping. Because it is nearly impossible to isolate homologous chromosomes based on their physical properties, separation was achieved by the limiting dilution method. For this purpose, the desired number of cells within an individual agarose gel block was calculated to be 0.05 on average. At this cell concentration, the number of single-stranded homologous chromosomes within each gel is 0.2 (0.05 cells × 2 homologous chromosomes × 2 single-stranded chromosomes). Assuming a Gaussian distribution of chromosomes in the aliquots of agarose gel, colocalization of two homologous chromosomes within a single gel would occur infrequently. To ensure Gaussian distribution, thorough mixing of the stock DNA-containing agarose solution (to ensure uniformity and prevent aggregation of chromosomes) is important. The heated alkaline environment used in the current study was generally effective in preventing aggregation, although some aggregation might be observed in gel 37. Extensive colocalization of chromosomes (homologous and nonhomologous) in agarose gels was observed when neutral pH conditions were used (data not shown).

Efficient igMDA is required for successful haplotyping. The efficiency of igMDA increases as the amount of template DNA is reduced (Supplementary Fig. 1b), most likely due to the saturation effect described previously [18]. This property is both fortuitous and essential for molecular haplotyping because dilution of the chromosomes to a single copy number is required. MDA in the agarose gel reached a plateau after 4–6 h (Supplementary Fig. 2), as was the case in solution. However, because a longer incubation period would be necessary for the limited amount of template DNA, we decided to use an incubation period of 16 h. It is also important to provide constant shaking during incubation to ensure balanced amplification of long DNA molecules (Supplementary Fig. 2).

Experimental determination of both haplotypes of an individual eliminates potential false assignments related to the amplification of contaminating DNA molecules that might compete with the reduced amount of template DNA used in the current study. In all cases, the experimentally determined haplotypes were compared

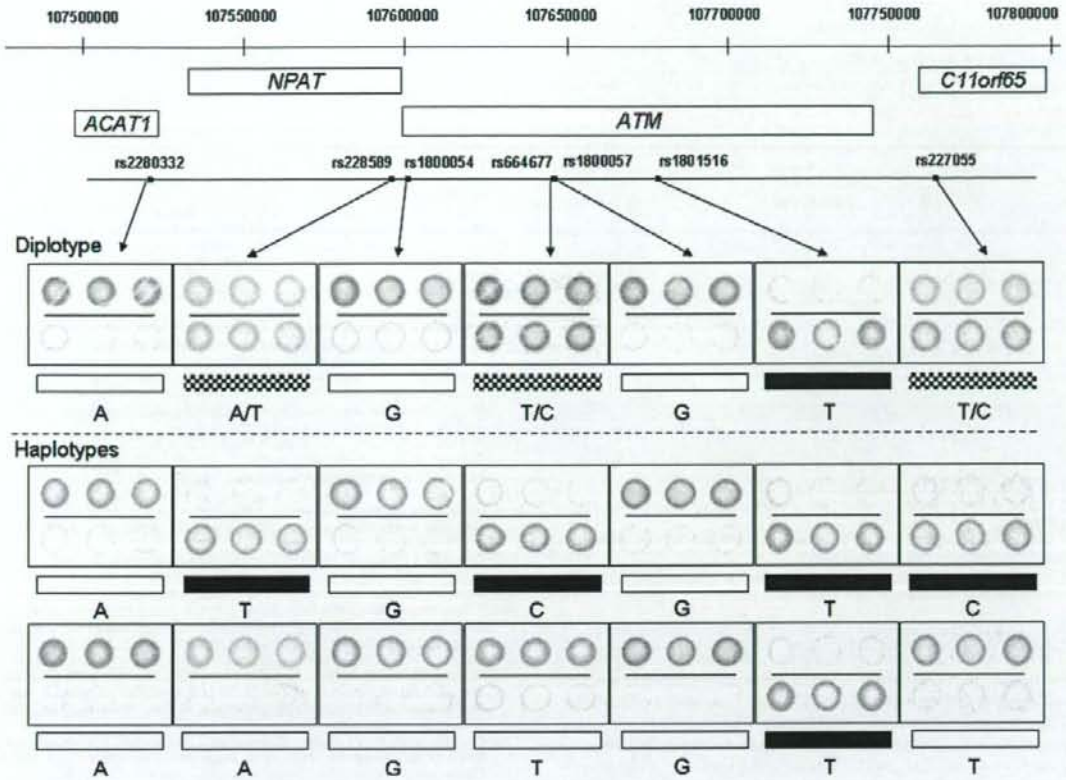

Fig. 4. Visible haplotype determination of the human *ATM* region. Seven SNPs, which span 240 kb of the human *ATM* region on chromosome 11, were determined for the amplified DNA. Allele-specific oligonucleotide primers were spotted in triplicate (allele 1 above and allele 2 below). Shown are spot images of the original diplotype and derived haplotypes taken with a digital camera. The white bars under the spot images indicate homozygosity for allele 1. The black bars indicate homozygosity for allele 2. The hatched bars indicate heterozygous alleles.

Table 1
Summary of *ATM* locus screening of EBV-transformed human lymphoblastoid cell lines

Patient ID	Concentration (cells/gel)	Number of gels	Screening 1[a]	Screening 2[b]	% Intact[c]
1	0.05	93	7	3	42.9
2	0.05	93	24	16	66.7
3	0.05	93	13	5	38.5
4	0.05	93	11	6	54.5
5	0.05	93	18	6	33.3
6	0.05	93	22	5	22.7
7	0.10	93	30	5	16.7
8	0.10	93	23	8	34.8
9	0.10	93	17	6	35.3
10	0.10	93	22	8	36.4
Average			18.7	6.8	38.2
SD			6.9	3.6	14.3

[a] Number of gels positive for the chromosome 11 (rs664677 SNP) amplicon.
[b] Number of gels positive for amplicons of the entire *ATM* locus.
[c] Proportion of positives in screen 2 that were also positive in screen 1.

with the original genotyping data to confirm correct assignment (Table 2).

To determine the two haplotypes of each individual, gels were screened repeatedly until the amplicons for both homologous chromosomes were obtained. For this, it was necessary to have at least 5 gels that were positive for the entire locus because the odds of obtaining only one homologous chromosome in 5 gels are 1 in 32 (2^5). Furthermore, considering that the average rate of obtaining intact DNA is 38%, at least 13 positive gels must be obtained in the first screening (Table 1). Thus, the starting number of gels containing 0.05 cells per gel required to obtain 13 positives is calculated to be more than 65 as follows:

$$13 \text{ chromosomes}/(0.05 \text{ cell} \times 2 \text{ alleles} \times 2 \text{ strands/gel}) = 13 \text{ chromosomes}/(0.2 \text{ chromosome/ gel}) = 65 \text{ gels}.$$

Because the actual concentration of cells fluctuates markedly at this dilution level, the preparation of multiple tubes of stock cell suspensions is recommended and the volume of the suspension should be adjusted in subsequent repeat experiments.

The proportion of DNA samples that contain a complete locus should vary according to the size of the locus because it is much easier to obtain intact amplicons of a shorter target locus than of a longer target locus. The *ATM* locus is relatively large, and the number of gels that contain a complete target locus of 50–100 kb, which is the normal range of linkage disequilibrium across the human genome [7], is expected to be higher. A higher proportion of intact template DNA would also lead to a reduction in the number of repeat experiments.

Table 2
Summary of *ATM* haplotype determinations

Estimation by EM algorithm ($n = 100$)[a]		
Haplotype ID	SNP alleles[b]	Frequency
1	C-T-G-C-G-G-C	0.419
2	A-A-G-T-G-G-T	0.257
3	C-A-G-T-G-G-T	0.132
4	A-T-G-C-G-G-C	0.095
5	A-A-G-C-G-G-T	0.060
6	C-A-G-C-G-G-T	0.015
7	C-A-G-C-G-G-C	0.009
8	A-A-G-C-G-G-C	0.006
9	C-T-G-T-G-G-T	0.005
Experimental determination[c]		
Cell line	Diplotype	
A	1–2	
B	1–2	
C	1–2	
D	1–3	
E	1–3	
F	1–5	
G	1–5	
H	2–9	
I	3–4	
J	3–5	

[a] Estimated based on the SNP genotypes of 100 healthy volunteers using the EM algorithm.
[b] SNPs are ordered from 5′ to 3′ as follows: rs2280332–rs228589–rs1800054–rs664677–rs1800057–rs1801516–rs227055.
[c] Experimentally determined for EBV-transformed B lymphoblastoid cell lines using the method developed in the current study.

In conclusion, the newly developed igMDA technique described herein, used in combination with the previously established visible multiple SNP typing array [32–34], allows convenient experimental haplotype determination with ordinary laboratory instruments. Currently, this method can be used to determine effectively the haplotypes of loci that contain multiple markers, and it allows precise mapping of genes for low numbers of samples such as for individual patients. For high-throughput, population-based haplotype analysis, it will be necessary to develop an automated gel handling system.

Acknowledgment

The authors acknowledge Ms. Masayo Terada for assistance with preparation of the manuscript.

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Radiotherapy and Oncology 87 (2008) 326–330
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Phase III randomised trial

Efficacy of novel hypoxic cell sensitiser doranidazole in the treatment of locally advanced pancreatic cancer: Long-term results of a placebo-controlled randomised study☆

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Abstract

Novel hypoxic cell radiosensitiser doranidazole was tested for unresectable pancreatic cancer administered at intraoperative radiotherapy. Short-term survival was not different. However, difference has been observed concerning 3-year survival (doranidazole group vs. placebo; 23% vs. 0%, $p = 0.0192$). This sensitiser might be effective in improving long-term survival for pancreatic cancer.
 Radiotherapy and Oncology 87 (2008) 326–330.

Keywords: Pancreatic cancer; Radiotherapy; IORT; Hypoxic cell radiosensitiser; Doranidazole

The development of hypoxic cell radiosensitisers has continued for more than 30 years [3,6,11,17–19]. Despite all these efforts, such agents still see relatively little use, and the main reasons are twofold. First, many agents do not successfully enhance radiotherapeutic effects. Secondly, the drug itself is often toxic to certain normal tissues, due to the high value of the partition coefficient (lipid–water partition). To decrease the value of the partition coefficient, great efforts have been put in on developing new compounds for more than a decade in Japan, finally resulting in the new compound doranidazole (PR-350; Pola Chemical Industries, Kanagawa, Japan), with three hydroxyls in the nitroimidazole side chain.

Following promising results from preclinical studies [10,21], this compound was used for patients with pancreatic cancer in a phase I study to determine its safety and efficacy [23]. Pancreatic cancer is known to be radioresistant and reportedly contains hypoxic tumour cells [4]. Pancreatic cancer also represents a good target for intraoperative radiation therapy (IORT), which provides a high single dose to the tumour, but for which hypoxic cells might pose more problems because the proportion of hypoxic cells dominates in the surviving fraction. Theoretically, the addition of hypoxic cell sensitiser should allow more hypoxic radioresistant cells to be killed and thus facilitate local tumour control. The phase I study was successfully closed with no severe adverse effects, no identification of any maximum tolerable dose, and promising survival data concerning locally advanced cases (median survival time, around 12 months).

Given these promising phase I study data, a multicentre double-blind phase III randomised study was conducted to compare outcomes for IORT with or without doranidazole followed by postoperative external beam radiotherapy. The preliminary results have been already published [24]. No differences were noted in 1-year overall survival rate between groups, but longer term results tended to be better for the doranidazole group. The data have been re-analysed to include at least 2 more years of follow-up and the long-term results are reported herein.

Materials and methods

Doranidazole (PR-350)

Radiosensitiser doranidazole was synthesised at Pola Chemical Industries. Doranidazole is a 2-nitroimidazole nucleoside analogue with a $CH_2OCH(CH_2OH)CH(OH)CH_2OH$ side chain at the N1 position.

☆ Presented at 13th ECCO, held October 30th to November 3rd, 2005, Paris.

0167-8140/$ - see front matter doi:10.1016/j.radonc.2008.02.007

Study design

Study design was reported in the previous report. The study protocols were approved by the ethical committees of the involved institutions.

Endpoints:

(1) Primary endpoints were overall 1-year survival, median survival time, and safety of the drug for 14 days following IORT.
(2) Secondary endpoints were effective response rate, tumour marker values, and the volume of analgesic drugs.

Eligibility criteria and exclusion criteria were also mentioned in the previous report, and are thus mentioned only briefly.

Eligibility criteria:

(1) patients must be 20- to 75-years-old;
(2) patients must have a performance status of 0–2, projecting a survival period >3 months;
(3) tumours must be unresectable due to invasion to the arterial system or peripancreatic nerve plexus;
(4) maximal diameters of tumours must be less than that required for radiotherapy; and
(5) liver metastasis, other organ metastasis and peritoneal seeding must be absent.

Exclusion criteria:

(1) previous radio- or chemotherapy;
(2) idiosyncratic reactions to drugs, including contrast media;
(3) presence of serious cardiovascular, pulmonary, renal, or hepatic disease;
(4) concomitant active neoplasia; or
(5) any condition believed by the physician-in-charge to preclude participation in the trial.

Following written informed consent to participate in the trial was obtained from the patient who met the above criteria, patients were registered as potential candidates at the trial central office. This was a prospective, randomized, closed-label, controlled study of IOR with or without radiosensitiser doranidazole. Patients were randomized by adaptive randomization method and notified by FAX. Final eligibility was determined based on operative findings by laparotomy.

After laparotomy, a biopsy specimen from each participant was analysed to confirm diagnosis. Infusions of doranidazole or placebo were strictly controlled to obtain a suitable concentration for radiotherapy; 2000 mg/m^2 of doranidazole or placebo was infused systemically for ~25 min before administration of IORT. Ten to 40 min after the completion of doranidazole or placebo administration, the patients were carried to the radiotherapy room and received 25 Gy of IORT at the maximum dose point covering gross tumour volume (the primary tumour and enlarged lymph nodes). The energy of the electron beam was selected so that all gross tumour volume was covered by 90% isodose line. Two weeks following surgery, all patients received EBRT. The total planned dose of 40 Gy at the isocentre was delivered in 20 fractions in 4 weeks using 10–14 MV photons. The radiation fields included the clinical target volume (gross tumour volume and the celiac and superior mesenteric artery) with 1–3 cm margins. CT-based multiple-port radiotherapy techniques were employed in order to lower the dose to the spinal cord.

No additional therapy, including chemotherapy and immunotherapy, was allowed for 6 months after IORT treatment to evaluate the efficacy of this compound unless there appeared locoregional recurrence and/or distant metastases.

Response criteria

Tumour response was graded as complete response (CR; 100% regression of the tumour and no new lesion), partial response (PR; more than 50% and no new lesion), minor response (MR; more than 25% and not greater than 50% and no new lesion), no change (NC; less than 25% regression and less than 25% progression and no new lesion), progressive disease (PD; not less than 25% progression or new lesion) and not evaluable (NE; either preoperative or postoperative CT was not clear enough to evaluate) evaluated by serial CT scans. Response of MR or better was considered effective. Effective response rate was defined as CR + PR + MR cases divided by overall cases.

Statistical methods

Kaplan–Meyer methods were used for the calculation of survival curves, and a generalised Wilcoxon test was used to assess the statistical significance of survival curves. Fischer's test was used to assess the statistical significance of a survival point. The χ^2 test was used to test significance for bivariate tables.

Results

Patient characteristics

Since the previous report, 1 case in the control group was found to be unsuitable for analysis, as the tumour was a mucin-producing carcinoma, representing a different disease entity from ordinary-type pancreatic cancer. All other cases were confirmed to be ordinary-type pancreatic cancer.

Between July 1999 and March 2002, a total of 81 patients were registered to participate in this trial. Of these, 34 patients were ineligible due to intraoperative findings of peritoneal seeding, liver metastasis or extensive tumours. Ultimately, 47 patients were enrolled in the trial and administered either doranidazole or placebo, and 46 cases were analysed (Fig. 1). Informed consent was obtained from all patients who participated in this trial.

Patient characteristics were as follows. In the doranidazole group (n = 22), male to female ratio was 15:7, and median age was 61.1 years (range 45–74 years). In the control group (n = 24), male to female ratio was 19:5, and median age was 61.3 years (range 50–74 years). No significant differences in background characteristics were noted between

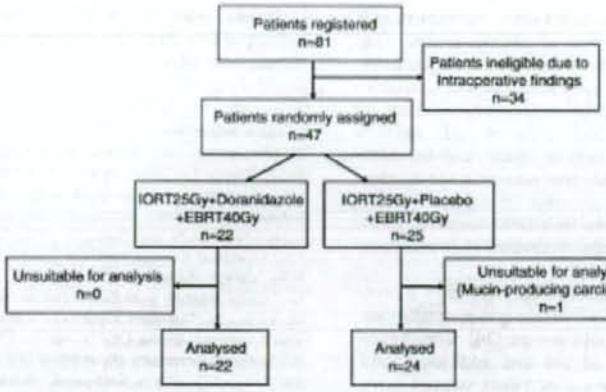

Fig. 1. CONSORT diagram.

groups. Median follow-up period for alive cases was 3.5 years.

Toxicity

As reported previously, the toxicity of doranidazole was not severe and the agent was thus considered safe. Also, there were no severe adverse effects regarding radiation therapy. All patients could receive IORT and EBRT as planned.

Efficacy

As reported previously, the efficacy of IORT with doranidazole for the treatment of pancreatic cancer was evaluated using computed tomography. The committee for evaluating efficacy reported that 9 of 19 patients (47%) in the doranidazole group showed higher effective response rate, compared with 4 of 22 patients (18%) in the control group ($p = 0.043$) (Table 1).

Survival

By the final follow-up in March 2005, all patients from the control group had died of the disease. Survival curves for both trial groups are shown in Fig. 2. Median survival period for the doranidazole group was 318.5 days, compared to 285.5 days for the control group. The 1-year survival rate was 36% ± 10% for the doranidazole and 29% ± 9% for the control group. Although the doranidazole group did not show significantly better survival than the control group, 5 of the 22 doranidazole patients (23%) remained alive >3 years after the trial ended (3-year survival rate: 23% ± 9%), compared with 0 of the 24 patients (3-year survival rate: 0%), in the control group. A significant difference in the 3-year survival rate was thus identified ($p = 0.0192$). As for the 3-year survival rates of the patients in both groups who did not develop distant metastases within 6 months, there was also a significant difference (39% ± 14% in the doranidazole group and 0% in the control group ($p = 0.0169$)).

Table 1
Response of the tumour

	Doranidazole group	Control group
CR	0	0
PR	1	2
MR	8	2
NC	9	15
PD	1	2
NE	0	1
Effective response	47%	18%

Discussion

Pancreatic cancer is known to be exceedingly refractory, and despite considerable effort, 5-year survival results have remained at about 4% for the last 30 years [20]. The standard treatment for locally advanced pancreatic cancer is surgical resection. However, such tumours are often unre-

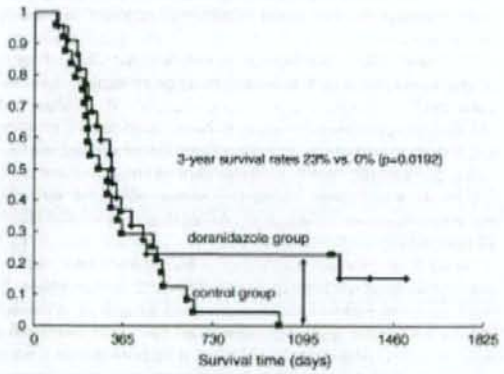

Fig. 2. Survival curves for both groups.

sectable and radiotherapy combined with chemotherapy is then used. With the development of promising new drugs such as gemcitabine [1], prognosis has been slowly improving, and median survival time has reached almost 12 months [5,12,13]. Radiotherapy comprises external beam radiation therapy (EBRT), IORT or a combination of both. IORT, if used by sparing surrounding normal tissue, can be safely combined with EBRT. Despite the technical difficulties, IORT has been used to increase radiation doses to the tumour and reportedly improves long-term survival results [14,15]. Efforts have been made to combine chemotherapy, IORT and EBRT [16].

We reported previously that although long-term survival tended to be better in the doranidazole group, median survival time was the same in both groups [24]. The present study involved a reanalysis of old and additional data, revealing a significant difference in 3-year survival rates. All five patients in the doranidazole group who had been alive at around 2 years or longer on initial analysis had achieved local control, developed no distant metastases and survived a relatively long period of time with local control.

As is often the case with trials involving pancreatic cancer, differences in survival were complicated by the early development of distant metastases even if the trial arm offered theoretically promising intensification of radio- and/or chemotherapy [2,7–9,22]. Obtaining definitive evidence is thus considered difficult in the treatment of pancreatic cancer.

Since this trial was performed in a multicentre double-blinded fashion, any achievement of a significant difference might well be considered attributable to the contribution of this radiosensitiser to the enhancement of IORT dose and thus enhancement of total radiation dose, in turn facilitating local tumour control and eventually improving long-term survival. Although various reports have suggested that escalating total irradiation dose to improve survival has no meaning, our data are among the first few to show that dose escalation can enhance local tumour effects, and thus enhance long-term survival.

The difference in 3-year survival rates was greater for cases in which distant metastases had not developed within 6 months (doranidazole group, 39%; control group, 0%). Survival rates might well be masked by early metastases.

This trial used radiotherapy alone, without chemotherapeutic agents such as 5-fluorouracil or gemcitabine, to evaluate the true efficacy of doranidazole. If a standard chemotherapy regimen was added, short-term survival might be improved due to the prevention of distant metastases. Data might then be comparable to those in curatively resected cases. Given these promising results, a well-designed clinical trial is necessary to optimise the combination of treatments.

Regarding adverse effects, as in the phase I trial, no severe adverse effects have been observed in this phase III trial. Doranidazole is considered quite a safe drug, although only intravenous administration can be performed. This point is quite essential, as numerous radiosensitisers have been abandoned due to inherent toxicities. Future studies need to confirm these promising findings.

Finally, since this drug is a hypoxic cell radiosensitiser, testing of this novel compound may be warranted for other tumours for which hypoxia poses a problem.

Acknowledgements

The authors are grateful to the PR-350 study group: Kumamoto University (Touru Beppu, Ryuiti Nishimura), Kyushu University (Kazuhiro Mizumoto), Kyusyu Medical Center (Michiyasu Nonaka, Satoru Uehara), Kurume University (Masao Hara, Etsuyo Ogo), Yamaguchi University (Kotaro Yamamoto, Yuichi Karino), Kochi Medical College (Michiya Kobayashi, Yasuhiro Ogawa), Shikoku Cancer Center (Minoru Tanada, Masaaki Kataoka), Ehime University (Humiki Kushihata, Takasi Fujii), Kobe University (Yasuyuki Suzuki, Toshinori Soejima), Osaka University (Masato Sakan, Eiichi Tanaka), Osaka City University (Yuji Nishino, Haruyuki Fukuda), Kyoto University (Ryuichirou Doi, Natuo Ooya), Fukui Medical University (Kanji Katayama, Masanori Yoshida), Fukui Red Cross Hospital (Yuki Hirose, Masato Noguchi), Aichi Cancer Center (Kenzo Yasui, Nobukazu Fuwa), Yokohama City University (Yasuhiko Miura, Ichiro Ogino), Toranomon Hospital (Goro Watanabe, Atushi Okazaki), Social Insurance Central General Hospital (Yasutugu Bandai, Kozou Makita), Chiba Cancer Center (Hiroshi Yamamoto, Kazuo Hatano), Saitama Cancer Center (Hirihiko Sakamoto, Mizuyoshi Sakura), Tsukuba University (Takeshi Todoroki, Kiyoshi Ohara), Tochigi Cancer Center (Shoichi Hishinuma, Iwao Tsukishima), Yamagata University (Kouichirou Ozawa, Yasushi Hamamoto) and Hirosaki University (Ken-ichi Hakamada, Masahiko Aoki).

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Received 22 June 2007; received in revised form 9 February 2008; accepted 9 February 2008; Available online 14 March 2008

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●特 集● 放射線治療の進歩—多分割照射の基礎と臨床—

頭頸部癌に対する過分割照射法の実際

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〔*Jpn J Cancer Chemother* 35(11): 1827-1832, *November*, 2008〕

Hyperfractionated Radiation Therapy for Head and Neck Cancers: Katsuyuki Karasawa[*1], Nahoko Hanyu[*1], Ta-Chen Chang[*1], Gencho Kuga[*1], Takuya Kaminuma[*1], Toshio Mitsuhashi[*2] and Hisao Miyashita[*2] ([*1]*Division of Radiation Oncology*, [*2]*Otorhinolaryngology, Head and Neck Tumor Surgery, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital*)

Summary

Among various altered fractionation regimens, hyperfractionated radiotherapy (HFRT) has been considered effective to raise survival rate (SR) as well as local control rate (LCR) in head and neck cancers. We reviewed our results of HFRT (117 cases) treated between 1995 and 2004 and compared with those of conventionally fractionated radiotherapy (CFRT; 80 cases) treated during the same period. By disease site, naso-/oro-/hypopharynx/larynx were 5/23/44/45 vs. 10/6/10/54. There were more advanced-stage cases in HFRT group (stage Ⅰ/Ⅱ/Ⅲ/Ⅳ=19/36/23/35 vs. 42/16/8/14). Median RT dose were 72 Gy vs. 66 Gy. In 71 cases, chemotherapy was added (HF/CF=54/17). In stage Ⅲ and Ⅳ cases, there was a borderline significant difference in LCR (at 5 years; 44.3% for HFRT group vs. 24.5% for CFRT group; p=0.0502), and a tendency in SR (at 5 years; 50.7% for HFRT group vs. 16.7% for CFRT group; p=0.1210). By disease site, LCR of HFRT group was higher in hypopharynx (p=0.0005) and oropharynx (p=0.0003), and SR of HFRT group was higher in hypopharynx (p=0.0023). Acute toxicity was heavy but in most cases it was tolerable and there were no severe late toxicities. From our data, it was suggested that HFRT might be effective in certain kinds of head and neck cancers. **Key words**: Head and neck cancer, Radiotherapy, Hyperfractionated radiotherapy, Hypopharyngeal cancer, Oropharyngeal cancer, Chemoradiotherapy, **Corresponding author**: Katsuyuki Karasawa, Division of Radiation Oncology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, Japan

要旨 頭頸部癌に対する1日複数回照射法のなかで1回1.1～1.3 Gyを1日2回照射する過分割照射法は，急性期の有害事象は増すものの局所制御率を改善し，一部疾患においては生存率も改善するとされている。今回当施設において行われている過分割照射法による頭頸部癌の方法と治療成績をreviewした。適応は喉頭癌もしくは咽頭癌で遠隔転移を有さず，過分割照射に耐え得る症例で，Shrinking field techniqueを用いて，GTVに対して1回1.2 Gy，1日2回，総線量72 Gy程度まで(60～80 Gy)腫瘍の反応に応じて投与する。1995～2004年までの10年間に過分割照射を施行された117例(HF群)と，同時期に単純分割照射を施行された80例(CF群)とを比較した。症例の偏りがあり全体としては局所制御率，生存率ともに有意差はなかったが，Ⅲ期，Ⅳ期の症例については5年局所制御率が(44.3% vs 24.5%; p=0.0502)，また5年生存率も(50.7% vs 16.7%; p=0.1210)と有意に良好な傾向を認めた。疾患別には下咽頭癌，中咽頭癌において，局所制御率が向上しており，また下咽頭癌においては生存率でも有意に向上していた。高度な晩期有害事象は認められなかった。過分割照射は特に局所進行例で局所制御に優れていた。今後は化学療法との併用治療の上で，どのように併用していくかが課題である。

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0385-0684/08/¥500/論文/JCLS

I．背　景

2007年の第31回日本頭頸部癌学会のシンポジウムで，初めて頭頸部癌における多分割照射法がテーマとして取り上げられた。

欧米において1日1回2 Gy，週間線量10 Gy，総線量60～70 Gyの照射法の成績では早期癌も局所進行癌も満足な成績をあげられていなかった。そこで1960年代より，癌細胞および正常細胞の放射線生物学的特徴から，分割回数を1日複数回に増やす一方で，1回線量を低下させることにより，治療可能比の向上をめざすことが始められた。これまで数多くの臨床試験において，局所制御率の改善は認められ，有用性が示唆されてきた[1-3]。2006年のBourhisらの1日複数回照射法のランダム化比較試験を集めたメタアナリシスにより，そのうちの過分割照射法が有意に生存率まで改善していると報告された[4]。

一方，わが国においては健康保険上の制限もあり，一部の施設を除いてあまり積極的に1日複数回照射法は行われてこなかったが，海外からの有望な報告および1996年の健康保険改訂により，複数回の照射が2回まで算定することが可能になったことにも起因して，本治療法が一般に行われるようになった。そのなかでも多くの施設は1回1.2 Gy程度の線量を使用した過分割照射法が行われてきている。しかし，多くの施設ではまとまった報告がなされてこなかった現状がある。

本シンポジウムでは多分割照射法の生物学的な原理，メタアナリシスの動向および臨床成績の報告がなされたが，本稿では頭頸部癌に対する1日複数回照射法のうち最も一般的な過分割照射法の実際の方法と，当院におけるこれまでの症例の成績を供覧し，考察する。

II．過分割照射の実際

1．適　応

喉頭癌（声門癌T1N0でnon bulkyなものを除く），中咽頭癌，下咽頭癌，上咽頭癌（T1/2N0例を除く）で遠隔転移を有さず，また過分割照射に耐えられる全身状態を有するcaseを対象とする。

2．照射方法

GTV（肉眼的腫瘍体積）は原発巣＋腫大したリンパ節，CTV（臨床的標的体積）は声門癌のT1，T2N0および1期の中咽頭癌，下咽頭癌を除き，原則として原発巣＋所属リンパ節を含む領域に設定する。原則として照射時には頭頸部固定具を使用する。CTVに5 mm程度の位置のずれ（セットアップエラー）を見込んで，PTV（計画標的体積）を設定する。

原発巣＋所属リンパ節を含む領域（鎖骨上リンパ節を含む）に左右対向二門＋前方一門照射で1回線量1.2 Gyにて40 Gy程度（39.6～40.8 Gy）まで投与（図1）する。その時点で照射範囲から脊髄を遮蔽し（図2），以後後頸部は電子線（9MeV）照射に変更する。その照射技法で照射を行い，予防照射領域は50～60 Gy程度にて終了する。視神経，脳幹部も50 Gyを超えないように注意する（50.4 Gyにて終了）。それ以降60 Gyからは原発巣および腫大リンパ節に照射範囲を限局させ，同部位には腫瘍の縮小に応じて72 Gy程度（66 Gyから最大81.6 Gy）投与する。放射線源としては4MVもしくは6MVのX線を使用する。

放射線生物学的に1日2回の照射の間には6時間の間隔を空ければよいとされているため，照射の時刻は典型的には勤務時間帯内の9時と15時というようなタイミングで行われる。月曜から金曜までの5日間，週10回照射を施行する。

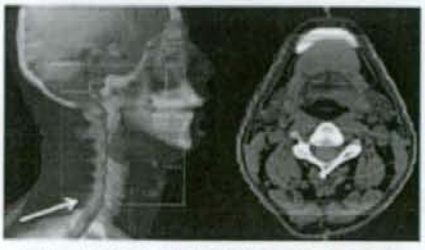

図1　最初の照射野と線量分布の例（中咽頭癌）
後頸部および咽頭周囲のリンパ節も含んで設定する。矢印部鎖骨上リンパ節の照射野とのオーバーラップを防ぐため，10 mmほど脊髄を遮蔽する。

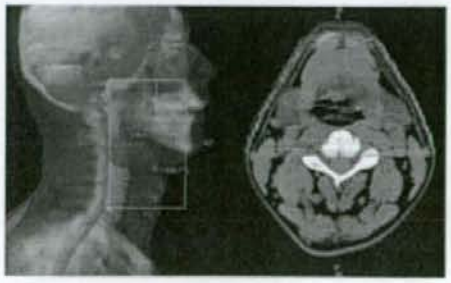

図2　40 Gyにて脊髄を遮蔽した後の照射野と線量分布
後頸部のリンパ節には脊髄への距離を考慮に入れ，9 MeVの電子線で照射を行う。

3．照射中の全身管理

治療の成否には照射範囲の設定と，照射中の全身管理が最も重要である。主たる急性有害事象である咽頭，口腔粘膜炎の軽減目的に粘膜保護剤もしくは粘膜保護作用のある含嗽剤を使用する。また治療中の栄養管理が重要であるため，鼻腔栄養，TPN，胃管からの栄養など，粘膜炎によって影響を受けない栄養補給法も検討する。さらに患者が高齢の場合には，早めに照射範囲を小さくさせることとともに，誤嚥性肺炎の併発に特に注意する。

表1 患者背景

		HF	CF
症例数	197	117	80
性別			
男性	177	104	73
女性	20	13	7
年齢	34～89	40～88	34～89
中央値	67	65	68
原発部位			
上咽頭	15	5	10
中咽頭	29	23	6
下咽頭	54	44	10
喉頭	99	45	54
Stage			
I	60	18（%）	42（%）
II	61	46（%）	15（%）
III	30	19（%）	11（%）
IV	46	34（%）	12（%）
総線量（Gy）		57.6～81.6	56～76
中央値		72	66
全治療期間（日）		35～65	40～64
中央値		44	46
化学療法の有無			
あり	71	54	17
なし	126	63	63

II．当院における治療成績

1995年1月～2004年12月に根治的な放射線治療を施行した頭頸部扁平上皮癌のうち上咽頭，中咽頭，下咽頭，喉頭癌を対象とした。対象患者は，197例で過分割照射群（HF群）117例，通常分割群（CF群）が80例であった。患者背景を表1，2に示す。後方視的な検討であるため，表2に示すように疾患・病期により治療法の選択に偏りが生じており，CF群では早期喉頭癌が多く，HF群では局所進行癌が多かった。放射線治療はHF群では1回線量は1.2 Gyを使用し，6時間以上の間隔を空けて1日2回の照射を行った。総線量は57.6～81.6 Gyで，基本的には治療終了時にCR-PRになるように治療に対する腫瘍の反応に応じて総線量を決定した。CF群では1回線量は1.8～2.0 Gyを使用した。総線量は56～76 Gyで，HF群と同様に腫瘍の反応により総線量を決定した。総線量はHF群で高く，治療期間は両群に差はなかった。

化学療法が71例で施行され，HF群で化学療法が併用された症例は54例であった。使用されたレジメンは，

表2 患者背景（2）

Stage	HF群				CF群			
	I	II	III	IV	I	II	III	IV
上咽頭	0	1 (1)	1 (1)	3 (2)	1	3 (2)	2 (2)	4 (2)
中咽頭	3 (1)	6 (2)	4 (2)	10 (7)	0	0	3 (2)	3 (2)
下咽頭	6 (2)	11 (5)	9 (6)	18 (13)	2 (2)	0	4 (3)	4 (2)
喉頭	9	28 (8)	5 (3)	3 (1)	39	12	2	1
Total	18 (3)	46 (16)	19(12)	34 (23)	42 (2)	15 (2)	11 (7)	12 (6)

＊（ ）内は化学療法施行症例数

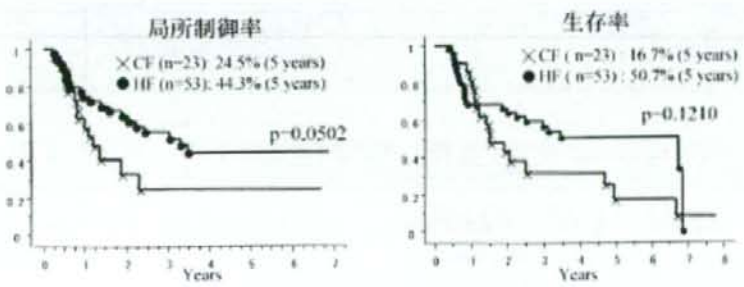

図3 stage III，IV症例の局所制御率と生存率
II期でやや差があるが，有意差は認められなかった。

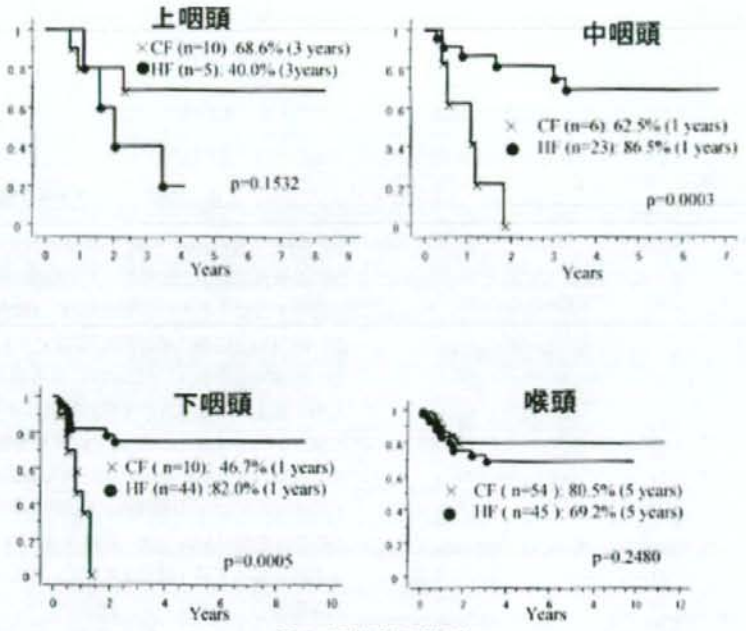

図 4　部位別局所制御率

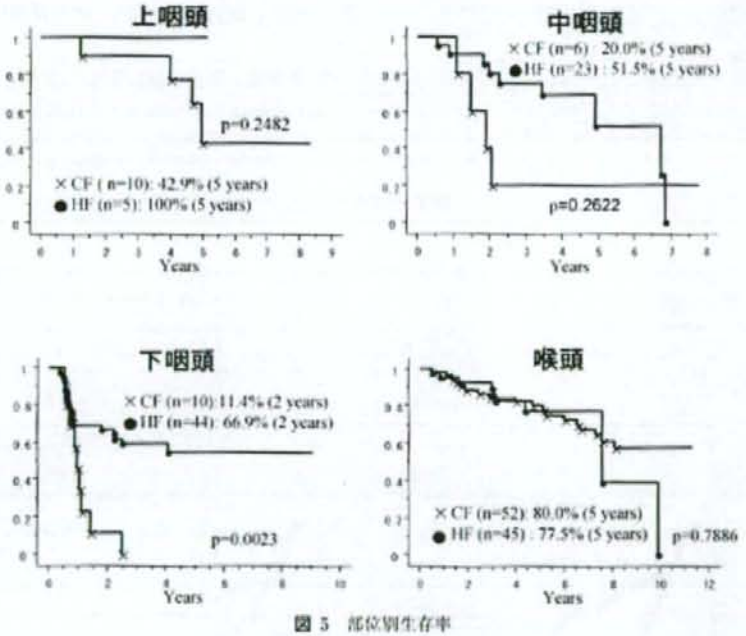

図 5　部位別生存率

導入化学療法・同時併用法としてはプラチナ系を含むものが多く，接治療としてはS-1, UFTの使用が多かった。

全症例の経過観察期間は，0.36～11.3年（中央値3.42年）であった。

5年局所制御率はHF群で67.4%，CF群で66.3%（p=0.6633），5年生存率はHF群で65.3%，CF群で63.0%（p=0.9757）といずれも有意差は認められなかった。早期癌，局所進行期癌の局所制御率・生存率を図3

に示す。統計的な有意差は認められなかったが，進行期癌の特に局所制御率でHF群が良好である傾向があった。部位別の局所制御率を図4，全生存率を図5に示す。下咽頭癌，中咽頭癌では，CF群と比較してHF群で局所制御率が有意に良好で，下咽頭癌では全生存率も有意に良好であった。

局所進行癌の化学療法の併用の有無による局所制御率と生存率は，4年の局所制御率が，CF群で化学療法あり25%，なしが23%（p=0.9433），HF群で化学療法あり44%，なしが43%（p=0.2315）であった。また5年の生存率は，CF群で化学療法あり34%，なしが10%（p=0.5699），HF群で化学療法あり56%，なしが41%（p=0.1215）であった。化学療法の併用による局所制御・生存率の有意な改善は認められなかった。

照射による粘膜炎のため，1週間以上の照射休止を必要とした患者は，HF群で6例（3%），CF群では1例（1%）であり，粘膜炎により麻薬が必要となった患者は，HF群で19例（9.6%），CF群で1例（1%）とHF群で急性期の粘膜炎が強い結果となった。しかし，grade 3以上の晩期の有害事象は認められなかった。また，化学療法を併用することによる急性期および晩期の有害事象の増加は認められなかった。

Ⅳ. 考　察

頭頸部癌治療において放射線治療の役割は非常に大きく，放射線治療が成功した場合には，嚥下・発声・美容面などで患者にもたらされる利益は非常に大きい。この領域における最近の手術技術の進歩，再建技術の進歩，放射線治療計画の技術の進歩に伴い，早期頭頸部癌の治療成績は比較的良好となったと考えられる。しかし局所進行癌では，いまだにその局所制御率・生存率・機能温存率において治療成績は満足がいくものとはいい難く，ここ最近では放射線生物学の概念に基づき，多分割照射法，なかでも過分割照射法において，局所制御率および一部においては生存率までも向上が認められている。

一方，Pignonらのメタアナリシス[5]により，化学療法を放射線療法に併用することにより生存率の向上が認められたことなどから，最近の傾向としては化学放射線療法が局所進行頭頸部癌の標準治療になりつつある。

またBrizelらは過分割照射法に化学療法を併用することで局所制御率をさらに改善させ，化学療法併用の有用性を示唆した[6]。Brizelによれば，過分割照射はすでに通常分割法の化学放射線療法が非常に有害事象が大きく，そこで用いる放射線治療を過分割照射法に代えることによるメリットはそう大きくはないといっている[7]。

しかしながら，化学療法の併用によってもいまだ十分良好な局所制御率，および生存率が得られているとは依然としていえず，さらなる治療成績の改善をめざす価値はあると考えられる。

Bourhisらのメタアナリシス[4]において，過分割照射法は比較的若年層には局所制御率の向上だけでなく生存率の向上も得られ，有用であるとされているが，高齢者には有害事象のためそのメリットが失われる，とされている。

高齢者に対しては，1回線量を通常の1.2 Gyから1.15 Gyもしくは1.1 Gyに低下させたり，N0例に対しては予防的な照射野を広範にとらないようにするという方策が考えられる。

一方で局所制御率を向上させることが必要な腫瘍は，一般的に切除不能で大きい腫瘍が多いため，広い照射野の設定が必要で，また最近は高齢の患者も多いことから，その照射野の設定法には一層の工夫が必要になってくる。

NCCNの診療ガイドライン[8]においては，頭頸部癌に対する多分割照射法は，化学放射線療法の普及もあってⅠ～Ⅱ期の声門癌や早期の中咽頭癌など限られた腫瘍において推奨されているにすぎないが，もう一つのガイドラインであるCancerNetにおいては，まだ研究中の治療として，過分割照射法が数多くの部位に対して，その治療法の選択肢としてあげられている[9]。

また化学療法の有用性を示したBrizelの報告においても試験群の放射線治療は過分割照射法が採用されており[6]，過分割照射法の有用性は局所進行咽頭喉頭癌において，さらに検討されるべき治療法であると考えられる。

もちろんその際には腫瘍の反応から患者の全身管理に至るまで，詳細な患者の観察が必要である。そのために耳鼻科と放射線科の間の密接な連携が極めて重要であることはいうまでもない。

また今後局所進行癌においては，いかに最適な化学療法の併用法を検討することと，さらに最近開発が著しい分子標的薬などとの併用方法についても検討が必要である[10]。

結　論

過分割照射法について，その実際と当院における治療成績を検討した。局所進行頭頸部癌の標準治療は化学放射線療法に移行しつつあるが，局所の制御は患者の社会生活にも重要な影響を与えるものであるため，いまだ十分とはいえない成績向上のためには，過分割照射法は有用な方法の一つである。部位的には下咽頭癌，中咽頭癌が有望である。

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●特集2●

放射線治療：切らずに治す早期の癌（1）

肛門癌

東京都立駒込病院 放射線科

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はじめに

肛門の扁平上皮癌は放射線感受性が高く，また初診時には病変が骨盤内に留まっている確率が高い。そのため根治的な放射線療法の適応になりやすく，また化学療法に対しても感受性が高いことから，化学放射線療法を行うとかなりの確率で肛門を温存でき，病変を治癒させることができることが知られてきた。一部の例外を除いて，肛門癌の根治手術は腹会陰式直腸切断術（APR）であるため，人工肛門造設が不可避である。患者は術後その管理を行わなければならず，QOLの点では非常に問題があった。欧米では，肛門癌は早期から局所進行期まで根治的化学放射線療法が適応となり，手術は化学放射線療法の後に再燃したとき，もしくは化学放射線療法の後，病変が消失しなかったときに，救済的に用いるようになっている。その意味では，人工肛門を避けるために治癒させなければならない癌といっても過言ではないであろう。

一方わが国では，本疾患がまれな疾患であることと放射線治療のスタッフが相対的に欠如していたことから，手術が先行して行われることが多かったが，放射線腫瘍学，腫瘍内科学の部門の発展により，次第に化学放射線療法が行われ始めている。本稿では肛門癌の化学放射線療法の歴史とその方法，および実際の症例について検討する。

肛門癌の放射線治療の歴史

歴史的にはWayne State大学のNigroらが，Mitomycin Cと5-FUの化学療法に放射線治療を同時併用させて効率に完全消失を得たという報告に始まる[1]。彼らが用いた線量はわずかに30Gyで，結果的には28例中24例が腫瘍の完全消失を得ている。当初，化学療法は術前の治療として考えられていたが，あまりにも高率な完全寛解率から，化学放射線療法単独で治療が行われるようになった。

Nigroらの報告のあとさまざまな報告がなされ，MMCと5-FUを併用した化学放射線療法により制御率および生存率ともに5年で約6割から7割と，手術成績に優るとも劣らない成績が次々と挙げられた。以来，少なくとも米国では，手術に代わる治療として化学放射線療法が標準治療とされて久しい。

併用化学療法の種類については，扁平上皮癌に有効とされるMMCが歴史的に用いられてきたが，MMCの有害事象の強さから，かわりのレジメンも試みられてきた。まずRTOG8704では，MMC＋5-FUのレジメンはMMCのない5-FU単独レジメンと比較された。5-FU単独レジメンは，4年時点での人工肛門造設率が9%対22%（p＝0.002），無病生存率が73%対51%（p＝0.0003）など，有害事象は減らせるものの有意に治癒率で劣った成績であったため，MMCの併用が薦められた[2]。

次の試験（RTOG9811）においてはCDDP＋5-FUとMMCと5-FUの比較が行われたが，中間解析において，肛門温存率，無再発生存率で少なくともCDDP＋5-FU群の優位性は示すことができず，現在においては標準治療はMMCと5-FUを同時併用する化学放射線療法であるとされている[3]。

わが国においては，前述のように肛門温存療法に対する理解が十分得られていなかったため，まとまった治療成績を出している報告はほとんどなかった[4]。そこで現状を調査する目的で，2006年に文部科学省山田班を中心に全国主要施設での肛門癌に対する（化学）放射線療法症例の後方視的調査を行った。その結果によれば，根治的に治療が行われた61例の5年生存率は77%で，5年肛門温存生存率は69%であった。有害事象はGrade 3が43%に，Grade 4が3%にそれぞれ認められた[5]。この成績はあくまでも後方視的であるが，わが国においても，欧米と同様（化学）放射線療法により優れた生存率および肛門温存率が得られることが示唆され，現在前向き試験が計画されている。

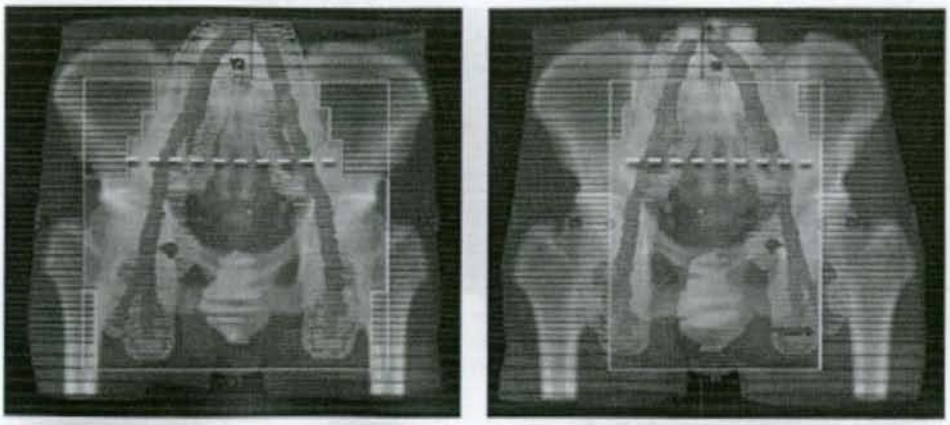

図1 肛門癌の照射野
a：前後方向の照射野
b：後前方向の照射野
上縁は終板を含み、下縁は肛門縁を十分に含む。外側縁はそ径リンパ節を十分含むように設定する。ただし上縁については、照射野が大きくなることを考慮し、腫大したリンパ節がなければ、30Gy程度で仙腸関節下縁（点線）まで縮小することが推奨される。

図1a 図1b

IMAGE PREVIEW 参照

標準的治療方法

肛門癌の標準治療は、いまだわが国では確立されていない。欧米では化学放射線療法が標準治療とされている。現在最も標準と考えられている治療方法は、Radiation Therapy Oncology Group（RTOG）の肛門癌の第3相試験であるRTOG98-11[7]の標準治療アームであるMMC＋5-FU＋放射線治療45Gy/25frという方法である。

放射線治療は6MV以上のエネルギーのX線を用いて行う。治療体位は背臥位にて行い、前後対向二門もしくは前後左右四門照射にて行う。1回線量は化学療法との併用のため1.8Gyが望ましい。最初の30.6Gy/17frまでは、原発巣および骨盤内リンパ節、そしてそ径リンパ節を標的体積とし、照射体積は上縁が1/5-S、下縁が肛門から2.5cm下方、そして側縁は前後方向からの照射では外そ径リンパ節を含む範囲であるのに対し、後前方向からの照射では外そ径リンパ節を含まずに座骨結節から2cm外側に設定する。側方からの照射野の照射範囲は骨盤とそ径リンパ節を含む範囲に設定する。そして30.6Gy以降は照射野上縁を仙腸関節の下縁にまで下げて、14.4Gy/8fr追加する（図1）。後方からの照射野にそ径リンパ節が含まれないため、線量が不足する分は電子線で追加する。総線量45Gyを5週間にて投与した後、T2例で残存が認められる例、N＋例、T3～4例に関しては、最初の腫瘍の位置から2～2.5cmのマージンを設定して、10～14Gy/5～7fr追加する。照射法および治療体位については、背臥位にてX線照射を行うか、砕石位にて肛門部を直接電子線で照射するかはケースバイケースとする。

化学療法は放射線治療開始の週と第5週に2コース同時併用する。MMCは第1日と第29日に10mg/m²をボーラス投与する。5-FUは第1日から第4日と第29日から第32日に1日あたり1,000mg/m²を持続投与する。なお、5-FUの投与量に関しては、あくまでもアメリカ人での量であるので、日本では800mg/m²程度の量で投与する方が安全である。

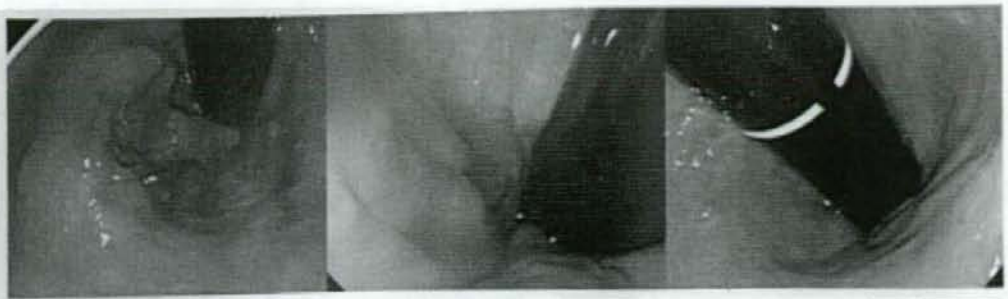

図2a | 図2b | 図2c

図2　内視鏡所見
a：治療前。肛門管から下部直腸にかけて長径3cm程度の2型の腫瘍を認める。
b：治療後1ヵ月。腫瘍は消失し、クレーターの部分は瘢痕化している。同部の粘膜は易出血性であった。
c：治療後6ヵ月。腫瘍部は正常な粘膜に置き換わっていて、わずかに瘢痕を認めるのみである。

IMAGE PREVIEW 参照

治療奏効例

以下に化学放射線療法でCRが得られた症例を提示する。

症例：82歳、女性
主訴：肛門部の痛み、出血
病理：扁平上皮癌
病期：cT2N0M0、Ⅱ期
治療方針：化学放射線療法（RT＋5FU＋MMC）
現病歴：半年前より、排便時の出血、痛みを自覚して、近医で痔と診断され、外用薬を処方されていた。しかし改善しないため、1ヵ月前に前医を受診し、CF施行された。この際肛門管9時方向に3cm大の2型の腫瘍を指摘され、生検では扁平上皮癌と診断された。放射線治療などの適応と判断され、当院消化器内科に紹介された。
既往歴：32年前子宮頸癌、14年前S状結腸癌（いずれも放射線治療歴なし）。
検査所見：
CT：肛門部の壁肥厚があり、周囲脂肪組織の濃度上昇を認める。明らかなリンパ節転移を認めない。
CF：肛門管からRb右壁中心に2型の腫瘍を認める送気して進展性が悪く、mp程度の病変が疑われる（**図2a**）。
治療：病理、年齢その他の点を考慮し、化学放射線療法を選択され、放射線科紹介受診となった。RTOG-9811のプロトコルにのっとり治療を施行した。

1）放射線治療
背臥位、前後対向2門、10MV、1.8Gy/fr ―― TD36.0Gy/20fr（day1～29）
照射野縮小、前後対向2門、10MV、1.8Gy/fr ―― TD9.0Gy/2fr（day30～36）
合計：TD45Gy/25fr/36days

2）化学療法
5FU、450mg/body ―― day1～5、29～33（96hCI）
MMC、80mg/body ―― day1、one shot
82歳の高齢および体格（145cm/35kg）より、5FUの投与量は450mg/bodyとした。

3）治療効果
効果判定：CR（触診＋CT＋CF上）（**図2b**）
有害事象：血液－白血球G3
下痢G3、放射線皮膚炎G3
いずれも保存的に観察可能であった。治療終了1ヵ月後のCFでは、腫瘍は消失し、クレーターの部分は

増悪化している。同部の粘膜は易出血性であった。6ヵ月後のCFでは有害事象の影響もなくなり、正常な粘膜に置き換わっている（図2c）。腫瘍の再発は認められない。治療後経過2年7ヵ月後、無病生存中である。

治療による有害事象および経過観察上の注意点

総線量は60Gy程度で極端に多くはないものの、化学療法の同時併用からさまざまな有害事象が生じる。急性期に頻発するものとしては、皮膚粘膜炎、血球減少症、下痢、悪心嘔吐等の消化管症状などである。遅発性の有害事象としては、皮膚粘膜の萎縮、消化管の瘻孔形成、そしてリンパ節への照射による下肢の浮腫、肛門部への過線量による肛門の壊死などである。

そ径リンパ節への再発は好発するため、同部の触診および超音波検査は重要である。局所制御を観察するために直腸診は必須である。また定期的に大腸内視鏡および腹骨盤部CTを行う。

肛門癌の場合反応が緩徐であるため、CRとならない症例でも数ヵ月してCRとなることがある。米国では、6週間ごとに経過を観察し、腫瘍が大きくなってこなければ経過観察を続ける方針をとっている施設もある[7]。

また、本治療法は局所を制御すると同時にQOLを保持することが重要であるため、原発巣がCRになるまで時間がかかることから、規定の治療の終了時にCRとなっていないために、総線量を60Gyを超えて投与していく際には有害事象に対する注意が必要である。

まとめ

肛門癌、特に扁平上皮癌は、早期癌および局所進行癌ともに、化学放射線療法による良好な治療成績が報告され、欧米では化学放射線療法が標準治療とみなされて久しい。またわが国の調査においても、その高い生存率並びに肛門温存率が確かめられた。患者にとって、人工肛門を造設することはQOLを非常に大きく低下させるため、少なくとも肛門扁平上皮癌に関しては、早期でも骨盤内に限局した局所進行期でも、まず化学放射線療法で治療が施行されることが望まれる。

肛門癌が比較的まれな疾患であるため、今後わが国での化学放射線療法のエビデンスの確立のためには、多施設共同した臨床試験が行なわれなければならない。またその際には外科医の協力が必須である。

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Int. J. Radiation Oncology Biol. Phys., Vol. 72, No. 4, pp. 1168–1173, 2008

0360-3016/08/$–see front matter

ELSEVIER

doi:10.1016/j.ijrobp.2008.02.054

CLINICAL INVESTIGATION **Brain**

INCIDENCE OF BRAIN ATROPHY AND DECLINE IN MINI-MENTAL STATE EXAMINATION SCORE AFTER WHOLE-BRAIN RADIOTHERAPY IN PATIENTS WITH BRAIN METASTASES: A PROSPECTIVE STUDY

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Purpose: To determine the incidence of brain atrophy and dementia after whole-brain radiotherapy (WBRT) in patients with brain metastases not undergoing surgery.
Methods and Materials: Eligible patients underwent WBRT to 40 Gy in 20 fractions with or without a 10-Gy boost. Brain magnetic resonance imaging or computed tomography and Mini-Mental State Examination (MMSE) were performed before and soon after radiotherapy, every 3 months for 18 months, and every 6 months thereafter. Brain atrophy was evaluated by change in cerebrospinal fluid–cranial ratio (CCR), and the atrophy index was defined as postradiation CCR divided by preradiation CCR.
Results: Of 101 patients (median age, 62 years) entering the study, 92 completed WBRT, and 45, 25, and 10 patients were assessable at 6, 12, and 18 months, respectively. Mean atrophy index was 1.24 ± 0.39 (SD) at 6 months and 1.32 ± 0.40 at 12 months, and 18% and 28% of the patients had an increase in the atrophy index by 30% or greater, respectively. No apparent decrease in mean MMSE score was observed after WBRT. Individually, MMSE scores decreased by four or more points in 11% at 6 months, 12% at 12 months, and 0% at 18 months. However, about half the decrease in MMSE scores was associated with a decrease in performance status caused by systemic disease progression.
Conclusions: Brain atrophy developed in up to 30% of patients, but it was not necessarily accompanied by MMSE score decrease. Dementia after WBRT unaccompanied by tumor recurrence was infrequent. © 2008 Elsevier Inc.

Whole-brain radiation, Brain metastasis, Brain atrophy, Dementia, Mini-Mental State Examination.

INTRODUCTION

Before the establishment of stereotactic radiosurgery (SRS), whole-brain radiotherapy (WBRT) was the golden standard of treatment for patients with brain metastases (1). Currently, patients with single or oligometastases frequently are treated with SRS, whereas those with four or more metastases are considered to be indicated for WBRT; after SRS alone, the expected probability of tumor recurrence in the unirradiated areas is very high (2, 3). Nevertheless, many patients with four or more metastases are treated by means of SRS alone without undergoing WBRT, especially in Japan (4, 5). One of the major reasons for avoiding WBRT is the fear that WBRT may cause dementia, as well as brain atrophy. However, there are no data clearly indicating the incidence of such late adverse effects of cranial irradiation, and there are only retrospective studies suggesting the occurrence of these complications (6–11). Many patients reported previously were treated with surgery and radiation (9, 10); therefore, it is unclear whether these complications are attributable solely to radiation therapy.

Brain atrophy and dementia may be related not only to surgery, but also to tumor status and chemotherapy (12, 13). To

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Presented at the 48th Annual Meeting of the American Society for Therapeutic Radiology and Oncology (ASTRO), Philadelphia, PA, November 5–9, 2006.

Conflict of interest: none.

Acknowledgments—We thank Drs. Chikao Sugie, Kana Kobayashi, Natsuo Tomita, Chisa Hashizume, and Souichiro Mori for their contribution in collecting data. Author affiliations are Department of Radiotherapy, Aizawa Hospital, Matsumoto, Japan (K.O.), Department of Radiology, Gifu University Hospital, Gifu, Japan (S.H.), Institute of Biomedical Research and Innovation, Kobe, Japan (M.K.), Department of Radiology, Nagoya University Hospital, Nagoya, Japan (S.I., Y.I.), and Faculty of Radiological Technology, Fujita Health University School of Health Sciences, Toyoake, Japan (M.K.).

Received Jan 21, 2008, and in revised form Feb 16, 2008. Accepted for publication Feb 21, 2008.