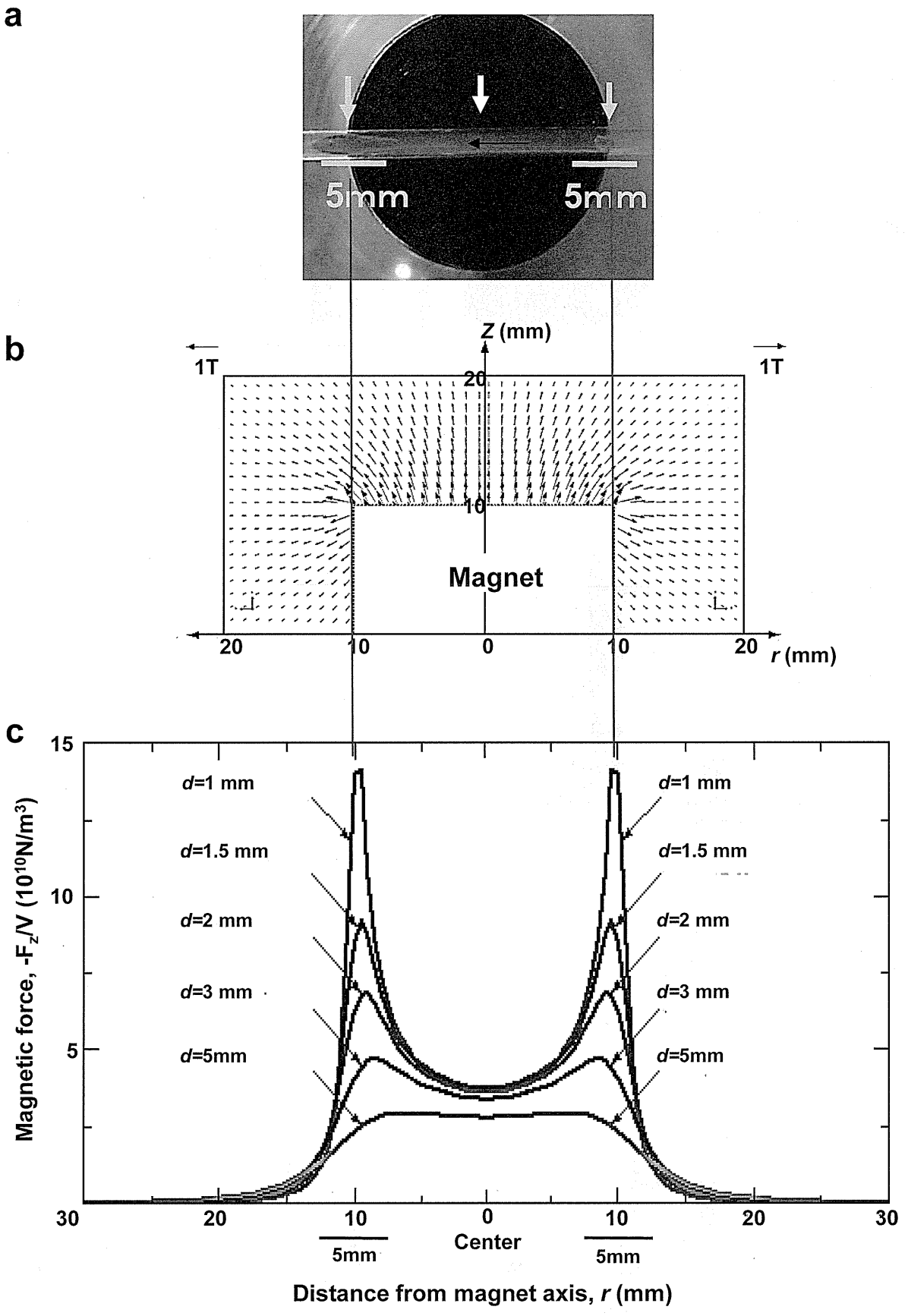
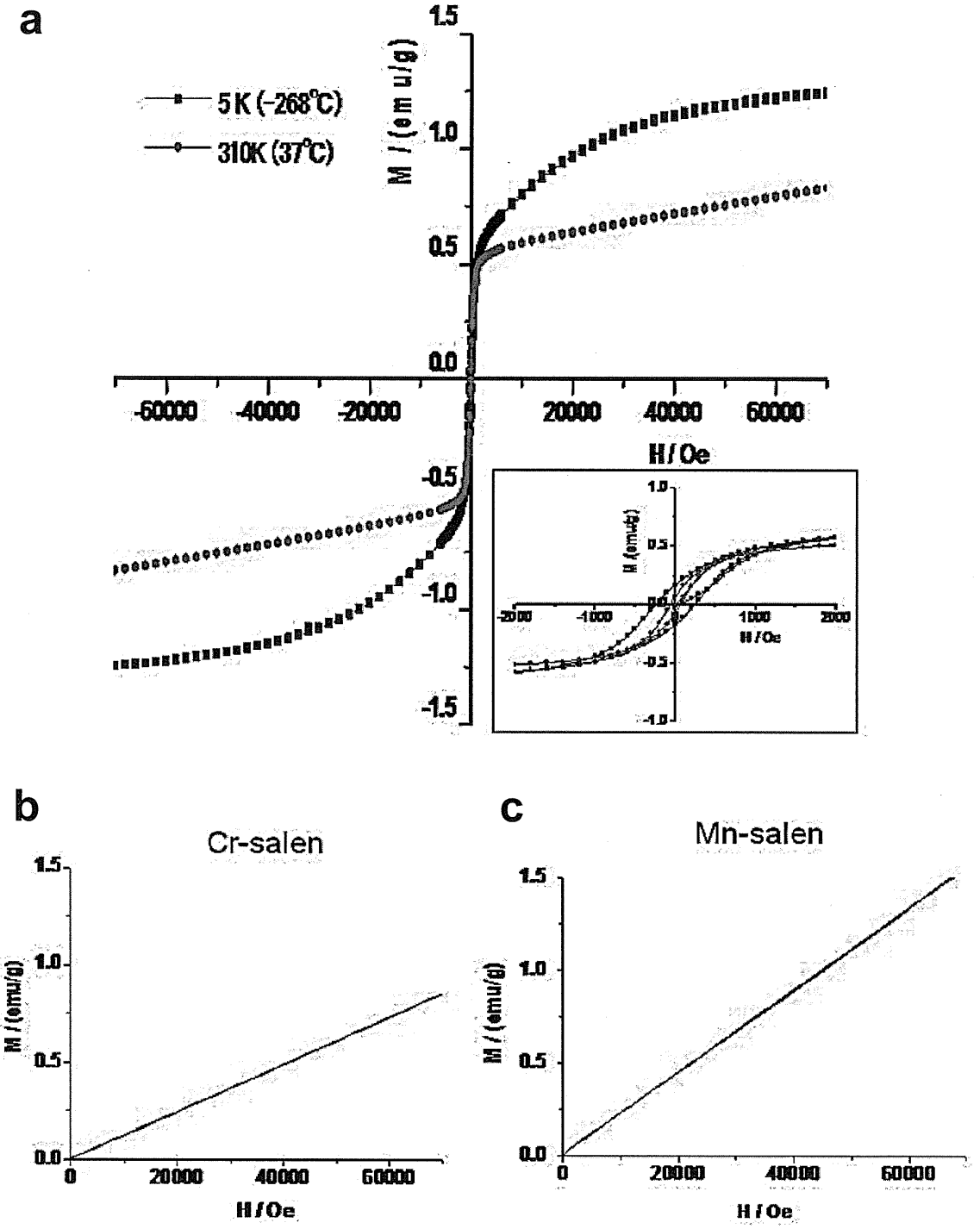
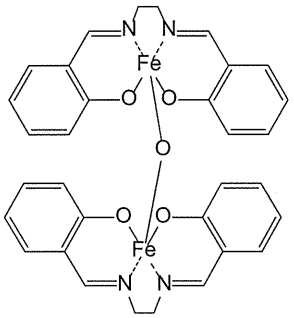
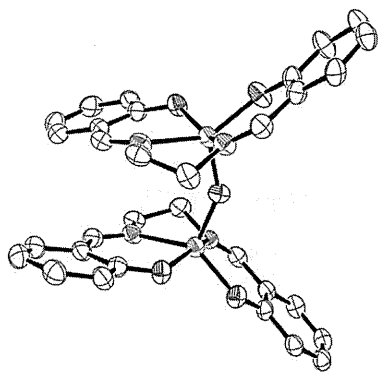

a
5mm
5mm
b
Z (mm)
1T
1T
20
10
Magnet
20
10
0
10
20
r (mm)
c
15
10
5
30
20
10
0
10
20
30
Magnetic force, -F_z/V (10^10 N/m^3)
d=1 mm
d=1.5 mm
d=2 mm
d=3 mm
d=5mm
d=1 mm
d=1.5 mm
d=2 mm
d=3 mm
d=5mm
5mm
Center
5mm
Distance from magnet axis, r (mm)


a
5K (-268°C)
310K (37°C)
M / (emu/g)
H / Oe
b
Cr-salen
M / (emu/g)
H / Oe
c
Mn-salen
M / (emu/g)
H / Oe

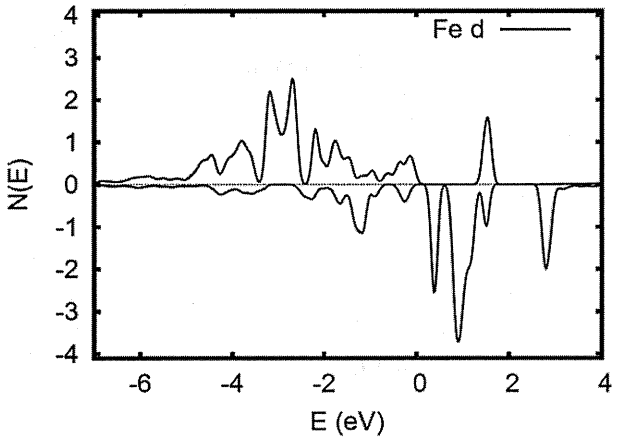
a



b

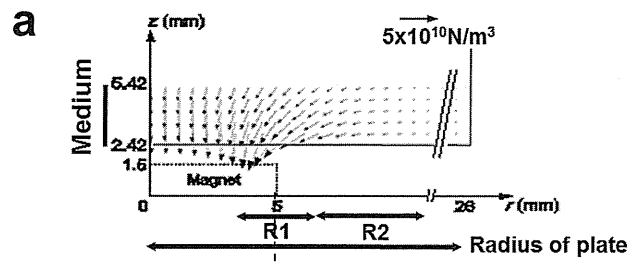


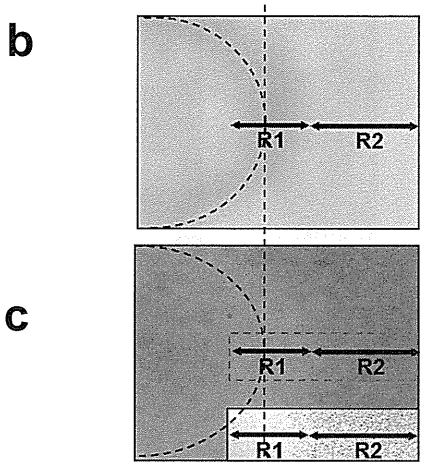
c

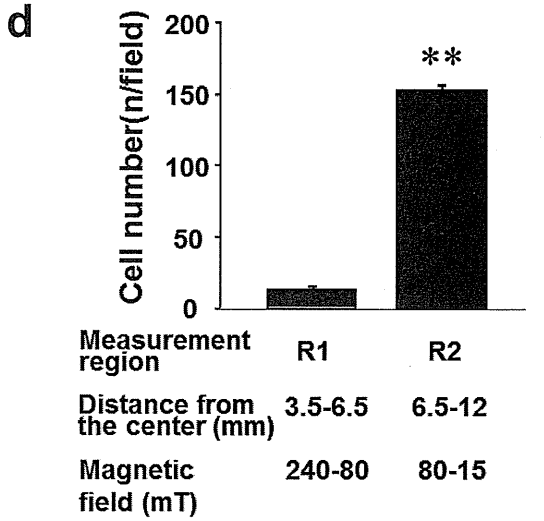


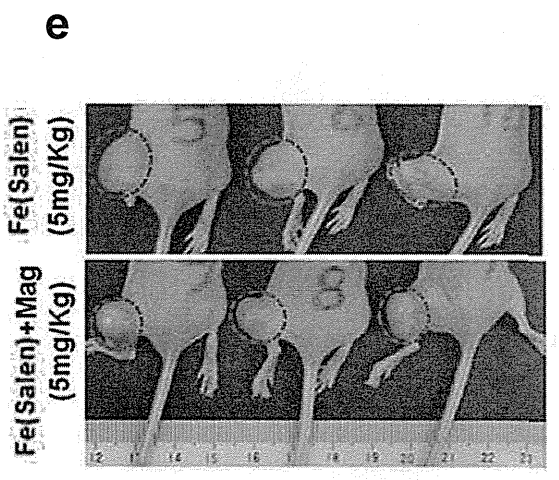
d

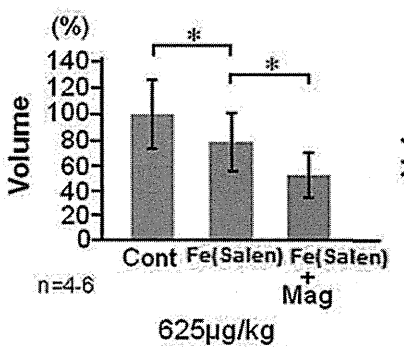
Crystal Data	
Compound	$Fe_2C_{32}H_{28}O_5N_4$
Crystal system	Triclinic
Space group	*P*1-
Cell constants with esd's in parentheses	
a, *b*, *c*	10.748(10)Å, 10.76(2) Å, 13.768(10) Å
α, *β*, γ	66.49(4) °, 81.10(2) °, 73.12(5) °
V	1411.4
Z	2
μ	0.543 mm^{-1}
Data Collection	
Wavelength of X-rays	0.6888 Å
Temperature	100 K
Crystal dimensions	5 × 5 × 40 μm
Beam size at crystal position	1 (horizontal) × 1.5 (vertical) μm
Rotation angle/image	5 deg
Exposure time/image	1 sec
Total no. of images	38
Resolution range	15-0. 87 Å
Refinement Statistics	
Reflections with $I > 4\sigma(I)$	3,236
No. of parameters	429
Weight	$w=1/[\sigma^2(F_o^2)+(0.1000P)^2]$ where $P=(F_o^2+2F_c^2)/3$
Shift/esd	less than 2.089
R_{index}	$R = 0.0800$, $wR2 = 0.1818$
S	1.335
$\Delta\rho_{max}$ and $\Delta\rho_{min}$	0.625 e/$Å_3$ and -0.670 e/$Å_3$

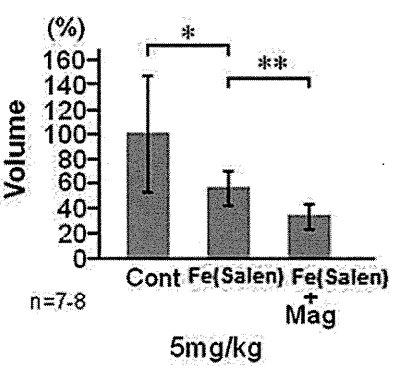

a
z (mm)
5x10^10N/m^3
Medium
5.42
2.42
1.6
Magnet
0
5
26
r (mm)
R1
R2
Radius of plate

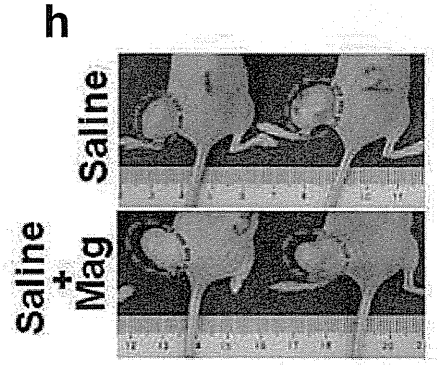

b
R1
R2
c
R1
R2
R1
R2

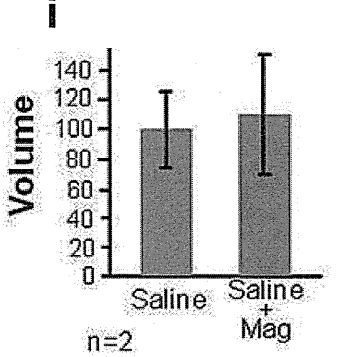

d
Cell number(n/field)
200
150
100
50
0
**
Measurement region
R1
R2
Distance from the center (mm)
3.5-6.5
6.5-12
Magnetic field (mT)
240-80
80-15

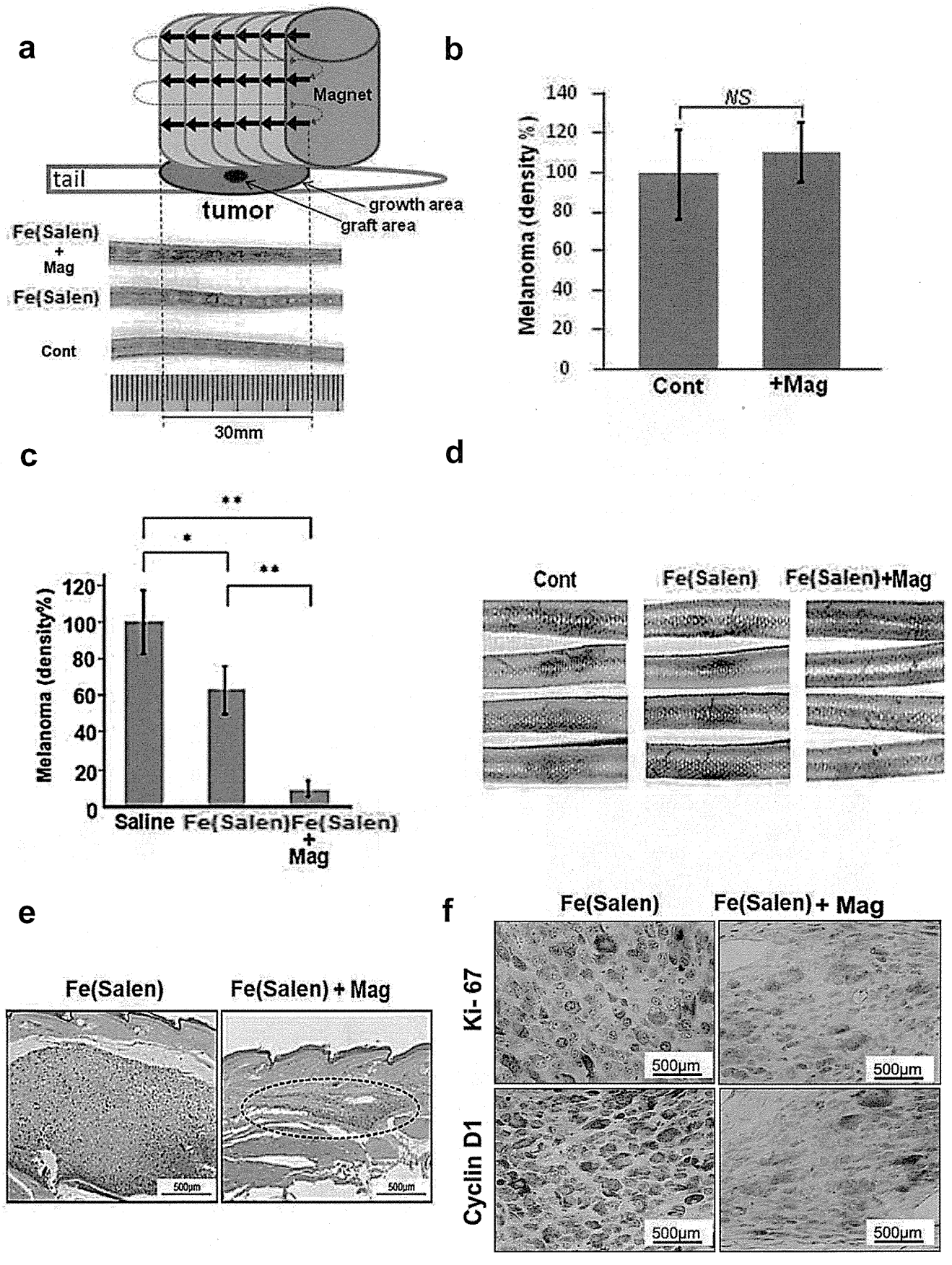

e
Fe(Salen) (5mg/Kg)
Fe(Salen)+Mag (5mg/Kg)


f
(%)
Volume
140
120
100
80
60
40
20
0
*
*
Cont
Fe(Salen)
Fe(Salen) + Mag
n=4-6
625μg/kg

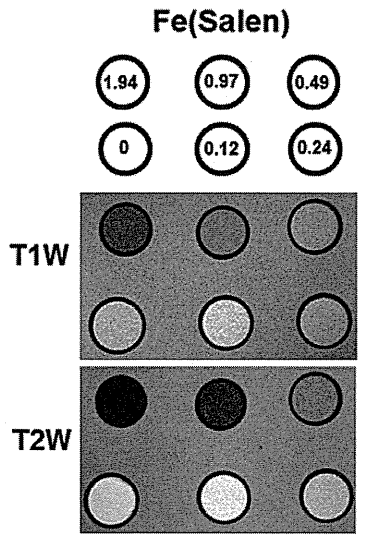

g
(%)
Volume
160
140
120
100
80
60
40
20
0
*
**
Cont
Fe(Salen)
Fe(Salen) + Mag
n=7-8
5mg/kg


h
Saline
Saline + Mag

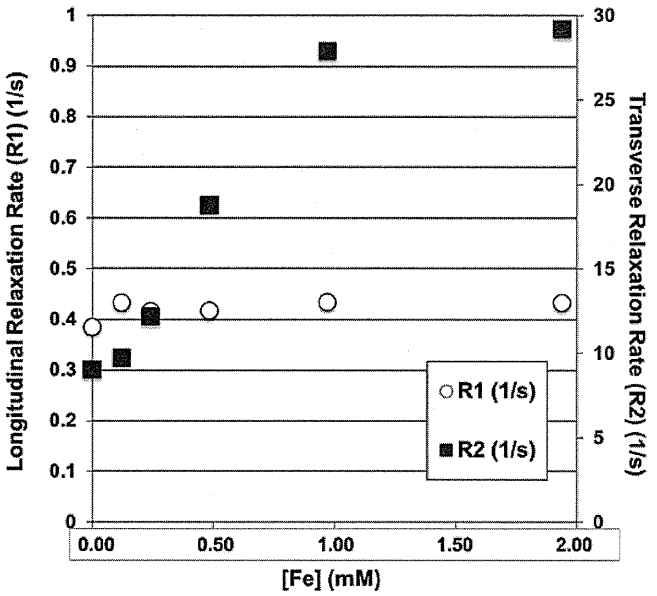

i
Volume
140
120
100
80
60
40
20
0
Saline
Saline + Mag
n=2


a
Magnet
tail
tumor
growth area
graft area
Fe(Salen) + Mag
Fe(Salen)
Cont
30mm
b
NS
Melanoma (density %)
140
120
100
80
60
40
20
0
Cont
+Mag
c
**
*
**
Melanoma (density%)
120
100
80
60
40
20
0
Saline
Fe(Salen)
Fe(Salen) + Mag
d
Cont
Fe(Salen)
Fe(Salen)+Mag
e
Fe(Salen)
Fe(Salen) + Mag
500μm
500μm
f
Fe(Salen)
Fe(Salen) + Mag
Ki- 67
Cyclin D1
500μm
500μm
500μm
500μm

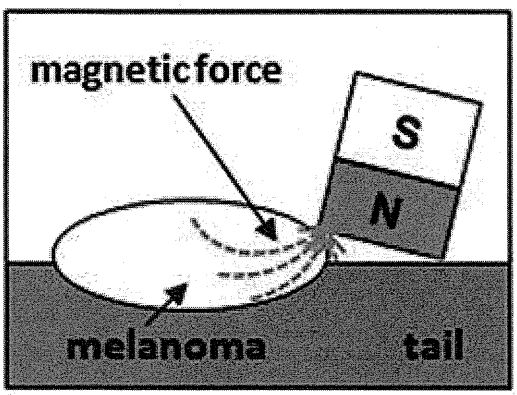
a


Fe(Salen)
1.94
0.97
0.49
0
0.12
0.24
T1W
T2W

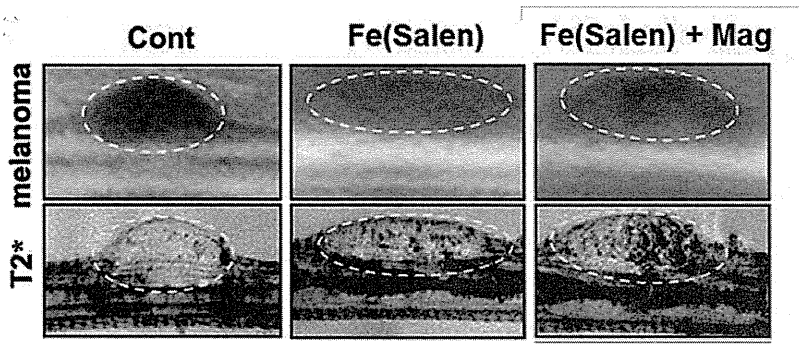
b


Longitudinal Relaxation Rate (R1) (1/s)
Transverse Relaxation Rate (R2) (1/s)
R1 (1/s)
R2 (1/s)
[Fe] (mM)
0.00
0.50
1.00
1.50
2.00

c


magnetic force
S
N
melanoma
tail

d


Cont
Fe(Salen)
Fe(Salen) + Mag
melanoma
T2*

Radiotherapy and Oncology 111 (2014) 306–310

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Intra-arterial radiochemotherapy

Retrograde superselective intra-arterial chemotherapy and daily concurrent radiotherapy for stage III and IV oral cancer: Analysis of therapeutic results in 112 cases

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Survival rate

ABSTRACT

Purpose: To evaluate the therapeutic results and rate of organ preservation in patients with stage III or IV oral cancer treated with retrograde superselective intra-arterial chemotherapy and daily concurrent radiotherapy.

Materials and methods: One hundred and twelve patients with stage III and IV oral squamous cell carcinoma underwent intra-arterial chemoradiotherapy. Catheterization from the superficial temporal and occipital arteries was performed. Treatment consisted of superselective intra-arterial chemotherapy (docetaxel, total 60 mg/m^2, cisplatin, total 150 mg/m^2) and daily concurrent radiotherapy (total of 60 Gy) for 6 weeks.

Results: The median follow-up for all patients was 46.2 months (range, 10–76 months). After intra-arterial chemoradiotherapy, primary site complete response was achieved in 98 (87.5%) of 112 cases. Five-year survival and local control rates were 71.3% and 79.3%, respectively. Grade 3 or 4 toxicities included mucositis in 92.0%, neutropenia in 30.4%, dermatitis in 28.6%, anemia in 26.8%, and thrombocytopenia in 7.1% of patients. Grade 3 toxicities included dysphagia in 72.3%, nausea/vomiting in 21.4%, fever in 8.0%, and renal failure in 0.9% of patients.

Conclusion: Retrograde superselective intra-arterial chemotherapy and daily concurrent radiotherapy for stage III and IV oral cancer provided good overall survival and local control.

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For patients with locally advanced head and neck cancer, including the oral cavity, surgery with or without radiotherapy is widely accepted as the standard treatment and is thought to be the most effective curative therapy. However, extended surgery markedly causes loss of oral function, including swallowing and speech, and affects the patient's social life, reducing the quality of life (QOL). To preserve function while maintaining or improving locoregional control and survival rates, concurrent chemoradiotherapy (CRT) represents one of the standard treatment modalities for definitive treatment of locoregionally advanced squamous cell carcinoma of the head and neck, particularly in resectable advanced cases [1]. However, treatment results remain unsatisfactory. Superselective intra-arterial chemotherapy for head and neck cancer has the advantage of delivering a high concentration of the chemotherapeutic agents to the tumor bed. It can be classified into the following two types: selective arterial infusion through the femoral artery by Seldinger method [2]; and retrograde selective infusion via the superficial temporal artery (STA) and/or occipital artery (OA) [3–5]. Retrograde superselective intra-arterial chemotherapy with radiotherapy for advanced head and neck cancers has been developed over the last 20 years [3,4], and can be used to provide daily concurrent CRT for patients with advanced head and neck cancer. Treatment results from arterial injection therapy combined with radiotherapy for locally advanced oral cavity cancer have been reported to be similar to those of surgery, suggesting the usefulness of this treatment modality [6]. This method can be used for patients with T3, 4 head and neck cancer, and it may allow organ preservation, even in cases of locally advanced head and neck cancer [7]. The purpose of the present study was to evaluate therapeutic results and rate of organ preservation in 112 patients with stage III and IV (M0) oral cancer treated with retrograde superselective intra-arterial chemotherapy and daily concurrent radiotherapy.

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0167-8140/

Materials and methods

Patients

Between August 2006 and July 2011, 118 patients with stage III and IV squamous cell carcinoma of the oral cavity and no evidence of distant metastasis when initially evaluated underwent retrograde superselective intra-arterial chemotherapy and daily concurrent radiotherapy. Six of these patients were found to be ineligible for the study: 2 due to a catheter infection, 1 due to pneumonia, 1 due to edema of neck and pharynx, 1 due to liver dysfunction, and 1 due to withdrawal of consent during treatment. Thus, 112 patients (78 male and 34 female; median age, 59 years; range, 28–87 years) were eligible for evaluation (Table 1). The primary lesion and cervical lymph nodes were assessed by positron emission tomography–computed tomography (PET–CT), magnetic resonance imaging (MRI) and ultrasound examination before treatment. Staging was performed according to the 2002 UICC staging system [8]. Patients who had received previous chemotherapy, radiotherapy, or surgery were excluded. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status (http://ecog.dfci.harvard.edu/general/perf_stat.html) of 0 or 1, a white blood cell count of at least 3500 cells/mm^3, a platelet count of at least 100,000/mm^3, and a hemoglobin level of at least 9 g/dL. Patients with cerebral infarction, or severe dysfunction of the liver, kidney, heart, or lung were ineligible. The primary tumor sites included the tongue ($n = 60$), upper gingiva ($n = 16$), lower gingiva ($n = 14$), floor of mouth ($n = 7$), buccal mucosa ($n = 6$), hard palate ($n = 4$), and other lesion ($n = 5$). Forty patients had stage III disease, and the remaining 72 had stage IV disease. The local institutional research board approved this study, and informed consent was obtained from each participant.

Retrograde superselective intra-arterial infusion procedure

Before treatment, 3-dimensional computed tomography angiography (3D-CTA) of the carotid artery was performed to identify the main tumor-feeding arteries and determine the morphology of the tumor-feeding artery originating from the external carotid artery. Catheterization from the STA was performed according to the method described by Tohnai et al. [3] and Fuwa et al. [4] (HFT method) [7]. A hook-shaped catheter (Medikit Corp., Tokyo, Japan) was superselectively inserted into the target artery and fixed to the periauricular skin. Catheterization from OA was performed according to the method of Iwai et al. [5]. When the tumor had 2 or more feeding arteries, catheters were inserted into the 2 arteries via STA and OA or bilaterally. After catheterization, flow check digital subtraction angiography (DSA) and angio-CT were performed in all cases. Angio-CT can help to detect tumors by confirming enhancement of the feeding area and enabling the catheter to be placed at the appropriate position. Furthermore, weekly confirmation of the feeding artery by injection of a small amount of indigo carmine is important. When catheterization using a hook-shaped catheter was not stable, the guidewire exchange method was used to replace it with a P–U catheter (Toray Medical Co., Ltd., Tokyo, Japan) [4].

Table 1
Patients and disease characteristic ($n = 112$).

Characteristics	No. of patients (%)
Gender	
Male	78 (70)
Female	34 (30)
Age, year	
Range	28–87
Median	59
Primary tumor site	
Tongue	60 (54)
Upper gingiva	16 (14)
Lower gingiva	14 (13)
Floor of mouth	7 (6)
Buccal mucosa	6 (5)
Hard palate	4 (4)
Other	5 (4)
T classification	
T2	18 (16)
T3	43 (38)
T4a	47 (42)
T4b	4 (4)
Stage classification	
III	40 (36)
IVA	61 (54)
IVB	11 (10)
Total	112 (100)

UICC staging system, Sobbin et al.

Radiotherapy

Radiotherapy was planned for all patients after appropriate immobilization using a thermoplastic mask and 3-dimensional CT-based techniques. Conventional radiotherapy was performed at 4 or 6 MV and 2 Gy/fraction/day. The irradiation field was changed according to lymph node status. In cases of N0 disease, the field contained the primary site and levels I to III of the neck on the ipsilateral side. The dose was delivered to 40 Gy/20 fractions. The portal was then reduced to only the primary site to spare the spinal cord. The total dose delivered to the primary tumor was 60 Gy/30 fractions. In cases of N1–N2a, b disease, the field contained the primary site and the levels I–V of the neck on the ipsilateral side. The dose was delivered to 40 Gy/20 fractions. The portal was then reduced to the primary site and lymph node metastases. The total dose delivered to the primary tumor was 60 Gy/30 fractions, and that to the metastatic lymph node sites was 50 Gy/25 fractions. In cases of N2c disease, the field contained the primary site and the levels I–V of the neck on bilateral sides. The dose was delivered to 40 Gy/20 fractions. The portal was then reduced to the primary site and lymph node metastases. The dose to the spinal cord ranged from 40 to 45 Gy. The total dose delivered to the primary tumor was 60 Gy/30 fractions, and, if at all possible, the total dose delivered to the metastatic lymph node sites was to 50 Gy/25 fractions.

Superselective intra-arterial chemotherapy

The anticancer agent was injected in a bolus for 1 h through the intra-arterial catheter when radiotherapy was performed. The total dose of docetaxel (DOC) was 60 mg/m^2 (10 mg/m^2/week), and that of cisplatin (CDDP) was 150 mg/m^2 (5 mg/m^2/day) (Fig 1). Sodium thiosulfate (STS) (1 g/m^2) was administered intravenously to provide effective cisplatin neutralization after the anticancer agent was given as soon as possible. All patients were given a 5-HT3 receptor antagonist before administration of the anticancer agent.

Follow-up after the treatment

All patients were evaluated 4 weeks after completion of treatment by PET–CT, MRI and ultrasound examination. The purpose of this combined CRT using retrograde superselective intra-arterial infusion was to improve the local control rate and achieve good QOL without surgery. If residual primary tumor was present after this treatment, a salvage operation was performed 6–8 weeks after completion of intra-arterial CRT. If residual metastatic lymph nodes were present after treatment, radical neck dissection was performed.

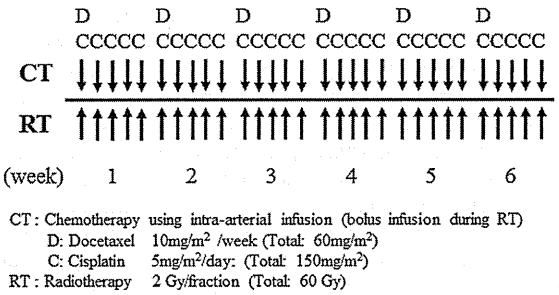

Fig. 1. Treatment schedule for chemoradiotherapy using retrograde superselective intra-arterial infusion. The total dose of docetaxel (DOC) was 60 mg/m^2 (10 mg/m^2/week × 6), and that of cisplatin (CDDP) was 150 mg/m^2 (5 mg/m^2/day × 30). When catheters were inserted into the 2 arteries or bilaterally, a half dose of anticancer agents was injected into the two lines, respectively. External irradiation was performed 5 times a week at 2 Gy per fraction, to a total of 60 Gy, for 6 weeks.

Toxicity assessment

Toxicities encountered during therapy were evaluated according to the National Cancer Institute – Common Terminology Criteria for Adverse Events v3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). The evaluation categories were blood cell counts, nausea/vomiting, oral mucositis, dermatitis, dysphagia, renal function and fever.

Statistical analysis

Overall survival (OS) and local control (LC) rates were estimated using the Kaplan–Meier method. Cases of residual or recurrent primary lesion after treatment were considered to be local failures unless salvage operation was successful. The differences between stage III and stage IV OS and LC rates were assessed by the log-rank test.

Results

Treatment results

For all patients, the median follow-up was 46.2 months (range, 10–76 months). After intra-arterial chemoradiotherapy, primary site complete response was achieved in 98 (87.5%) of 112 cases, and residual tumor was seen in 14 (12.5%) cases. Eight patients (7.1%) was detected local recurrence during follow-up. Thirty patients (26.8%) died: 21 of pulmonary metastasis, 5 of progression of the primary lesion, 3 of the cervical lymph node, and 1 of non-cancer-related causes.

The Kaplan–Meier method was used to estimate the 1-year, 3-year, and 5-year OS rates, which were 85.7%, 74.6%, and 71.3%, respectively (Fig. 2a). At 5 years, OS rates of stage III and stage IV oral cancer patients were 83.1% and 64.5%, respectively. Five-year OS rate of stage III oral cancer patients was significantly higher than that of stage IV oral cancer patients ($P = 0.033$) (Fig. 2a). The 1-year, 3-year, and 5-year LC rates were 82.0%, 79.3%, and 79.3%, respectively (Fig. 2b). At 5 years, LC rates of stage III and stage IV oral cancer patients were 85.1% and 75.4%, respectively. No significant difference was observed between the LC rates of stage III and stage IV oral cancer patients ($P = 0.120$) (Fig. 2b).

Toxicities

Table 2 shows the acute toxicities experienced during therapy. Grade 3 or 4 toxicities included mucositis in 103 cases (92.0%), neutropenia in 34 cases (30.4%), dermatitis in 32 cases (28.6%), anemia in 30 cases (26.8%), and thrombocytopenia in 8 case

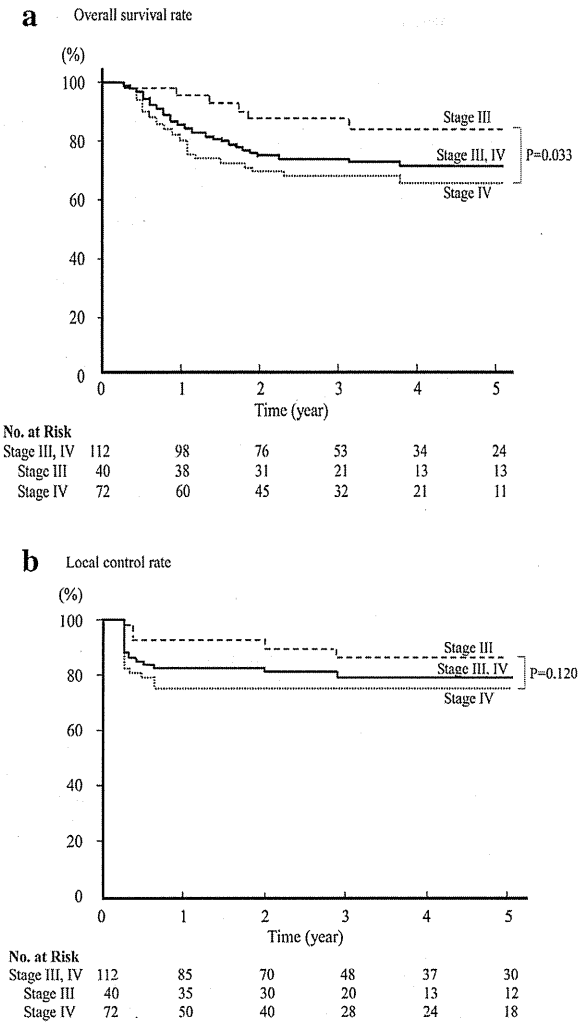

Fig. 2. Overall survival rate (a) and local control rate (b) using the Kaplan–Meier method. (a) Three and 5-year OS rates were 74.6%, and 71.3%, respectively. At 5 years, the OS rate of stage III oral cancer patients was 83.1% (95% confidence interval, 80.8–85.3%), and the OS rate of stage IV oral cancer patients was 64.5% (95% confidence interval, 61.0–67.9%), 5-year OS rate of stage III oral cancer patients was significantly higher than that of stage IV oral cancer patients ($P = 0.033$). (b) Three and 5-year LC rates were 79.3% and 79.3%, respectively. At 5 years, the LC rate of stage III oral cancer patients was 85.1% (95% confidence interval, 83.3–86.1%), and, the LC rate of stage IV oral cancer patients was 76.1% (95% confidence interval, 73.6–77.3%); no significant difference was observed between the LC rates of stage III and stage IV oral cancer patients ($P = 0.120$).

Table 2
Toxicity ($n = 112$).

Toxicity	No. of patients by toxicity grade			
	I	II	III	IV
Neutropenia	15	22	31	3
Anemia	36	39	26	4
Thrombocytopenia	56	5	6	2
Nausea/Vomiting	23	31	24	
Mucositis		9	54	49
Dermatitis	13	61	28	4
Dysphagia	1	22	81	
Renal failure	27	3	1	
Fever	37	24	9	

National Cancer Institute – Common Terminology Criteria for Adverse Events v3.0.

(7.1%). Grade 3 dysphagia occurred in 81 cases (72.3%) with severe mucositis. Grade 3 toxicities included nausea/vomiting in 24 cases

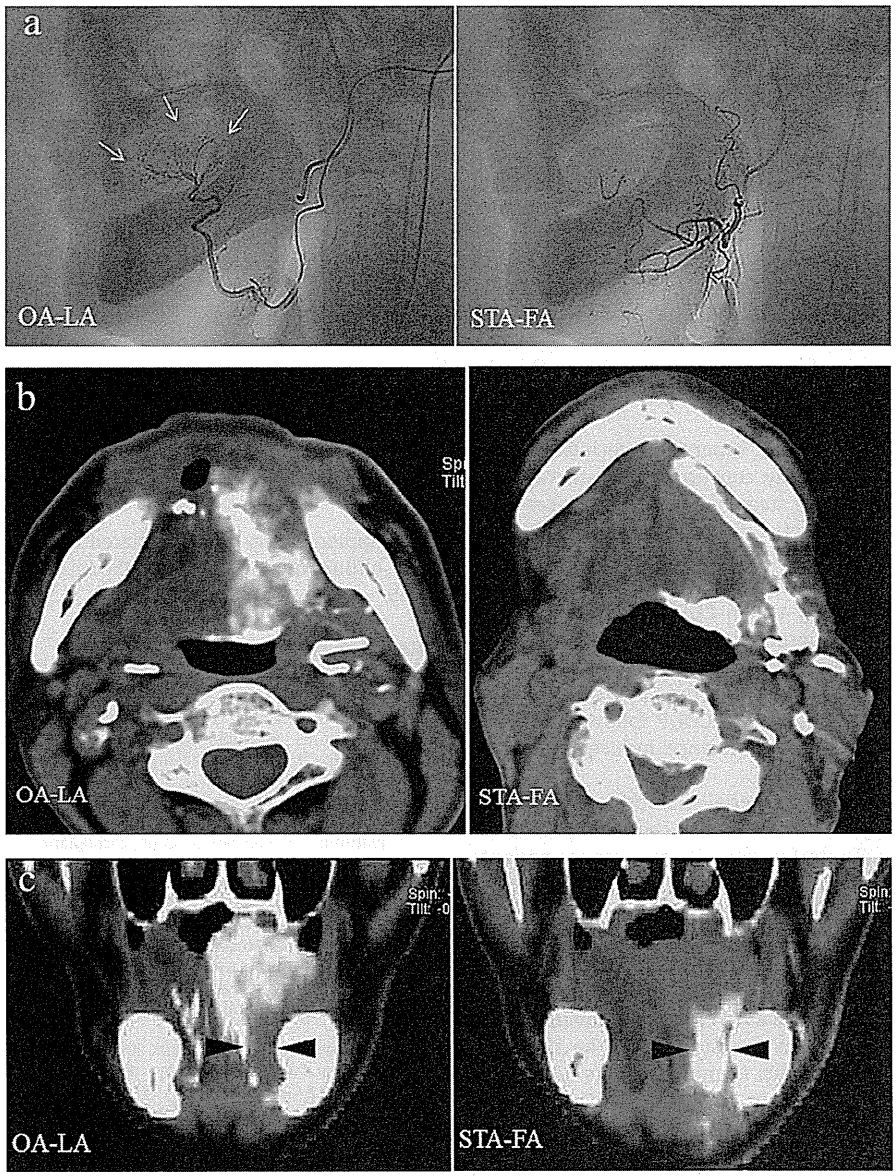

Fig. 3. Squamous cell carcinoma of the tongue (T4aN1M0). (a) Digital subtraction angiograms (DSA) of retrograde superselective intra-arterial infusion. Two catheters were superselectively inserted into the left lingual artery (LA) via the occipital artery (OA–LA) and left facial artery (FA) via the superficial temporal artery (STA–FA). Tumor stain is seen with the use of contrast medium on flow check DSA (OA–LA: arrowhead). (b, c) Axial and coronal views of angio-CT. The left side of the tongue tumor extends to the floor of the mouth and extrinsic muscles of the tongue. Angio-CT images showed that tumor staining of left tongue from left LA and left mouth floor from left FA can be seen with the use of contrast medium. The perfusion area from the left LA was not visible to the floor of the mouth and inside the mandible (c: OA–LA arrow), the perfusion area of floor of the mouth and inside the mandible was seen from the left FA (c: STA–FA arrow).

(21.4%), fever in 9 cases (8.0%), and renal failure occurred in 1 case (0.9%). Renal toxicity was significantly low: this finding is thought to be due to the low dose of CDDP and the STS-based neutralization of CDDP. No patients died as a result of treatment toxicity.

Discussion

Intra-arterial administration of anticancer agents might result in increased levels of anticancer agents being delivered to tumors and more potent antitumor effects compared to intravenous administration [3]. Present strategies at our institution for patients with locally advanced oral cancer include avoiding extended surgery and preserving primary organ function using retrograde superselective intra-arterial chemotherapy and daily concurrent radiotherapy. This method has been performed since 1996 for organ preservation and improvement of treatment results in patients with advanced head and neck cancer [7]. For patients with N2 and N3 cervical lymph node metastases, retrograde superselective intra-arterial CRT combined with hyperthermia was utilized [9].

In cases of locally advanced tongue carcinoma (T3 and T4), the primary tumor extends to the floor of the mouth, lower gingiva and/or extrinsic muscle of the tongue. In such cases, the catheter must be placed in both the lingual and facial arteries. After catheterization, it is necessary to check the flow to the tumor on DSA (Fig 3a) and angio-CT (Fig 3b and c). Determining whether arterial infusion of an anticancer drug actually permeates the entire tumor is important for successful arterial infusion therapy. Angio-CT images were obtained in 5-mm-thick continuous sections in two

planes (axial and coronal) depending on the extent of the tumor. Angio-CT images showed that the perfusion area from the left lingual artery was not visible to the floor of the mouth and the inside of the mandible with use of contrast medium (Fig 3b and c: OA-LA). On the other hand, the perfused area on the floor of the mouth and inside the mandible was seen from the left facial artery (Fig 3b and c: STA-FA). Therefore, the position of the catheter must be checked to ascertain of the catheter was put in the proper position after catheterization. However, flow check angio-CT has sometimes difficulty to confirm the tumor feeding area due to the artifacts of crowned teeth. Nakamura et al. used MRI to ascertain the area reached by infusion of an arterial anticancer drug instead of angio-CT [10]. The MRI flow check method in the present report allows acquisition of images from various directions, thereby delineating the perfusion area by arterial infusion more accurately than conventional methods.

In the present trial of retrograde superselective intra-arterial DOC/CDDP chemotherapy and daily concurrent radiotherapy, OS and LC rates among patients with stage III and IV oral cancer were excellent. DOC enhances the effect of radiotherapy by causing cell synchronization at the most radiosensitive phase of the cell cycle (G2/M). CDDP enhances radiosensitivity through inhibition of DNA repair. The mechanisms by which CDDP and DOC serve as either a cytotoxic agent or radiosensitizer are distinct from each other [11]. DOC treatment followed by CDDP demonstrates a synergistic effect on cell survival inhibition, with increased intracellular platinum accumulation compared to CDDP followed by DOC, and DOC improves the multidrug resistance induced by single treatment with CDDP [12].

Retrograde superselective intra-arterial chemotherapy and daily concurrent radiotherapy for advanced oral cancer improved OS and LC rates. Five-year OS rate for stage III oral cancer patients was significantly higher than that for stage IV oral cancers ($P = 0.033$), 21 of 30 patients died due to the pulmonary metastasis; 18 of stage IV patients, 3 of stage III patients. On the other hand, 5-year LC rates for stage III and stage IV oral cancer patients were not significantly different ($P = 0.120$), indicating that this method provided good local control even for locally advanced oral cancer. These results suggest that the disease can be managed without a primary surgical approach in most patients with stage III and IV oral cancer.

In the present study, grade 3 or 4 toxicities included mucositis in 103 cases (92.0%) and grade 3 dysphagia with severe mucositis during treatment in 81 cases (72.3%). Intra-arterial chemotherapy can deliver a high dose of anticancer agents to head and neck lesions, and mucositis is a significant toxicity that can affect swallowing function. Thus, dysphagia is likely to improve with time in most cases within 3 months: in the present study only one patient was dependent on tube feeding over 1 year. Newman et al. [13] investigated swallowing and speech function after treatment for head and neck cancer with intra-arterial versus intravenous CRT, and found no statistically significant differences between the intra-arterial CRT group and the systemic CRT group in relation to swallowing function. Speech function was comparable; however, it was significantly worse in the intra-arterial CRT group than in the systemic CRT group. The present method was not associated with any major complications during follow-up, and no patients died as a result of treatment toxicity. These findings indicate that retrograde superselective intra-arterial CRT is safe and suitable for advanced oral cancer.

Multiple trials, particularly those using high-dose CDDP (RADPLAT), have reported a high response rate intra-arterial CRT [14]. On the other hand, a previous multicenter randomized phase 3 trial covering 239 patients with advanced unresectable head and neck cancer in the Netherlands concluded that CDDP-based intra-arterial CRT was not superior to intravenous CRT in relation to locoregional control and survival [15]. Robbins had questioned if this randomized trial was related to the technique used to deliver the intra-arterial infusions [16]. To prove the effectiveness and feasibility of retrograde intra-arterial CRT, further randomized trials comparing retrograde intra-arterial and intravenous chemotherapy combined with concurrent radiation therapy are necessary.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.radonc.2014.03.005.

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ORIGINAL PAPER

Hyperthermia generated with ferucarbotran (Resovist®) in an alternating magnetic field enhances cisplatin-induced apoptosis of cultured human oral cancer cells

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Abstract Hyperthermia is a promising anti-cancer treatment in which the tissue temperature is increased to 42–45 °C, and which is often used in combination with chemotherapy or radiation therapy. Our aim in the present work was to examine the feasibility of combination therapy for oral cancer with cisplatin and hyperthermia generated with ferucarbotran (Resovist®; superparamagnetic iron oxide) in an alternating magnetic field (AMF). First, we established that administration of ferucarbotran at the approved dosage for magnetic resonance imaging provides an iron concentration sufficient to increase the temperature to 42.5 °C upon exposure to AMF. Then, we examined the effect of cisplatin combined with ferucarbotran/AMF-induced hyperthermia on cultured human oral cancer cells (HSC-3 and OSC-19). Cisplatin alone induced apoptosis of cancer cells in a dose-dependent manner, as is well known. However, the combination of cisplatin with ferucarbotran/AMF was significantly more effective than cisplatin alone. This result suggests that it might be possible to reduce the clinically effective dosage of cisplatin by administering it in combination with ferucarbotran/AMF-induced hyperthermia, thereby potentially reducing the incidence of serious cisplatin-related side effects. Further work seems justified to evaluate simultaneous thermo-chemotherapy as a new approach to anticancer therapy.

Keywords Ferucarbotran · Hyperthermia · Oral cancer · Anti-cancer effect · Cisplatin · Resovist®

Abbreviations

AMF	Alternating magnetic field
MNPs	Magnetic nanoparticles
MRI	Magnetic resonance imaging
SPIO	Superparamagnetic iron oxide

Introduction

Cancer cells are more vulnerable to increased temperature than normal cells [1]. Thus, hyperthermia is viewed as a promising approach in cancer therapy [2]. Many techniques have been reported to increase the temperature of cancer tissues, such as whole-body hyperthermia [3], radiofrequency hyperthermia [4], microwave-induced hyperthermia [5], and implantable needles [6]. However, with all

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these modalities, it remains difficult to increase the temperature of only the cancer tissues in a controlled manner without damaging surrounding normal tissues.

More than 40,000 people are diagnosed with oral cancer, including cancers of the mouth, tongue, tonsils, and throat, every year in the US alone. Oral cancer can cause functional damage and disfigurement, and, in its advanced stages, it invades surrounding organs, causing disorders of speech, swallowing, and even chewing. Surgery may have serious adverse effects, so chemotherapy or radiation therapy is often favored in oral cancer patients, not with standing potentially serious systemic side effects. Hyperthermia is often preferred, e.g., for metastatic N3 cervical lymph nodes, because it has fewer adverse side effects. However, it is difficult to induce hyperthermia in a metastatic node-specific manner. Nevertheless, selective hyperthermia has been studied as a possible approach to obtain tumor-specific cytotoxicity, e.g., by ferromagnetic embolization [7]. More recently, magnetic nanoparticles (MNPs) have been investigated for this purpose, because MNPs generate heat when they are exposed to an alternating magnetic field (AMF) as a result of hysteresis and relaxational losses [8].

Ferucarbotran (Resovist®) is an organ-specific contrast agent used in magnetic resonance imaging (MRI) of local tumors, and the permissible dose in humans has been established by at least two studies [9, 10]. Because ferucarbotran consists of superparamagnetic iron oxide (SPIO) coated with carboxydextran, it generates heat when it is exposed to an AMF [11, 12], and it has been reported to induce selective hyperthermia when used in arterial embolization [11]. However, it has not been established whether ferucarbotran is suitable for inducing hyperthermia in cancer treatment.

Cisplatin (*cis*-diaminedichloroplatinum II; CDDP) is widely used in chemotherapy in many types of cancer, including oral cancers [13]. However, it has serious side effects, including acute kidney damage and/or renal failure [14–16]. Recent studies have demonstrated that hyperthermia stimulates cellular uptake of cisplatin [17, 18] and consequently enhances the cytotoxicity of cisplatin in cancer cells, both in vitro and in vivo [19–21]. Thus, combined treatment with cisplatin plus hyperthermia may allow the effective dose of cisplatin to be decreased sufficiently to minimize serious side effects.

Accordingly, in order to examine the feasibility of using combination therapy with cisplatin and ferucarbotran/AMF-induced hyperthermia in the therapy of oral cancer, in this study we examined the effect of the combined treatment on oral cancer cells in culture. Our results confirmed that ferucarbotran/AMF-induced hyperthermia significantly enhances the effect of cisplatin. Because both cisplatin and ferucarbotran have already been approved for clinical use, early introduction of this technique, at least for oral cancers, should be feasible.

Materials and methods

Reagent, drug and cell lines

Ferucarbotran (Resovist®) was purchased from FUJIFILM Pharma (Tokyo, Japan) [11]. Cisplatin was purchased from Wako Pure Chemical Industries (Osaka, Japan). Human oral squamous cell carcinoma cell lines OSC-19 and HSC-3 were purchased from the Japan Health Sciences Foundation, Health Science Research Resources Bank (Osaka, Japan). In all cases, cells from early passage cultures were stored and used for the experiments. OSC-19 and HSC-3 were cultured in Dulbecco's modified Eagle's medium (DMEM), 1 % penicillin–streptomycin, and 1 % L-glutamine.

Thermography

Thermal images were taken using a thermograph (infrared thermal imaging camera InfReC R300SR; Nippon Avionics, Tokyo, Japan). Temperature was also measured using a thermograph.

Alternating magnetic field (AMF) generator

An AMF was generated by a vertical coil with an inner diameter of 6.5 cm, driven by a transistor inverter (HOT SHOT; Ameritherm, New York, USA) operated at a

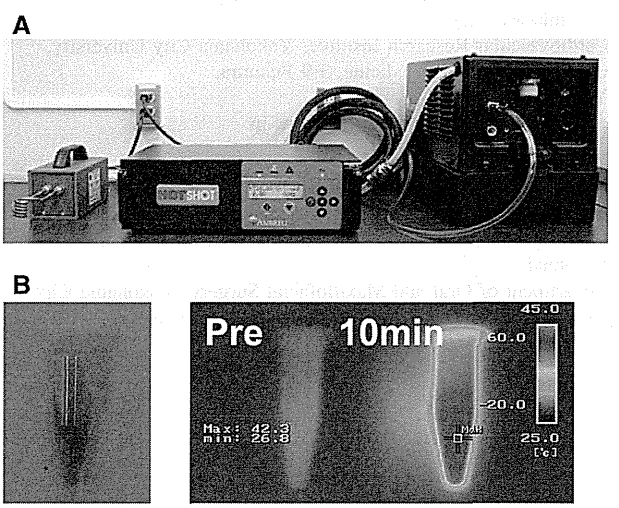

Fig. 1 Heat generation by ferucarbotran in an alternating magnetic field (AMF). **a** The alternating magnetic field (AMF) generator, **b** a photograph of ferucarbotran in medium (*left*), and thermal images of ferucarbotran in medium before (*middle*), and 10 min after AMF (308 kHz, EC 270 A) (*right*)

frequency of 308 kHz and electric current (EC) 250 A [12, 22–26]. Temperature was measured using a hand-held thermometer, HA-200 (Anritsu Meter, Tokyo, Japan).

Apoptosis assay

HSC-3 cells and OSC-19 (6×10^4 cells/well) were seeded on 6-cm dishes and incubated for 24 h. Cisplatin was then added to a concentration of 0 μM (control), 7.5 or 15 μM. When hyperthermia was to be applied, 10 mM ferucarbotran was added and AMF was performed with a HOT SHOT under the conditions described above [22, 25, 26]. Incubation was continued for 12 h at 37 °C, in an atmosphere of 5 % CO_2 in air. Cells were washed twice with cold PBS and suspended in 1× binding buffer at a concentration of 1×10^6 cells/ml. Next, a 100-μl aliquot of the solution, containing 1×10^5 cells, was transferred to a 5-ml culture tube. Then, 5 μl of allophycocyanin (APC) Annexin V and 5 μl of 7-aminoactinomycin D (AAD) (BD Biosciences, CA, USA) [27] were added to the tube. Incubation was continued for 15 min at room temperature (25 °C) in the dark. Finally, 400 μl of 1× binding buffer were added to each tube. Cells were examined by flow cytometry (BD FACSCanto II; BD Biosciences).

Cell cycle analysis

Cell cycle analysis was performed using The Cycletest™ Plus DNA Reagent Kit (BD Biosciences) according to the manufacturer's protocol [28]. Briefly, HCS-3 and OSC-19 cells treated with 0 μM (control), 7.5 or 15 μM cisplatin, with or without hyperthermia (10 mM ferucarbotan/AMF), were washed in PBS and fixed in 90 % ethanol. Fixed cells were washed twice in PBS and stained with 50 μM propidium iodide containing 5 μg/ml DNase-free RNase for 1 h, then analyzed by flow cytometry using a FACScan (BD FACSCanto II).

Statistical analysis

Data were analyzed using BD FACSDiva software (BD Biosciences). Data are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by the Tukey post hoc test using GraphPad Prism software (GraphPad Software, CA, USA). The criterion of statistical significance was set at $p < 0.05$.

Results

Heat generation by ferucarbotran in an alternating magnetic field (AMF)

Heat production is determined by the magnetic properties of ferucarbotran, its concentration, and the strength of the AMF [12]. Therefore, we examined the heating effect of AMF on medium containing ferucarbotran by thermography (Fig. 1b). As shown in Fig. 2, the temperature increased time-dependently, and the extent of the increase was dependent on the concentration of ferucarbotran (Fig. 2a) and the magnitude of the EC used to generate AMF (Fig. 2b). The results showed that AMF produced at

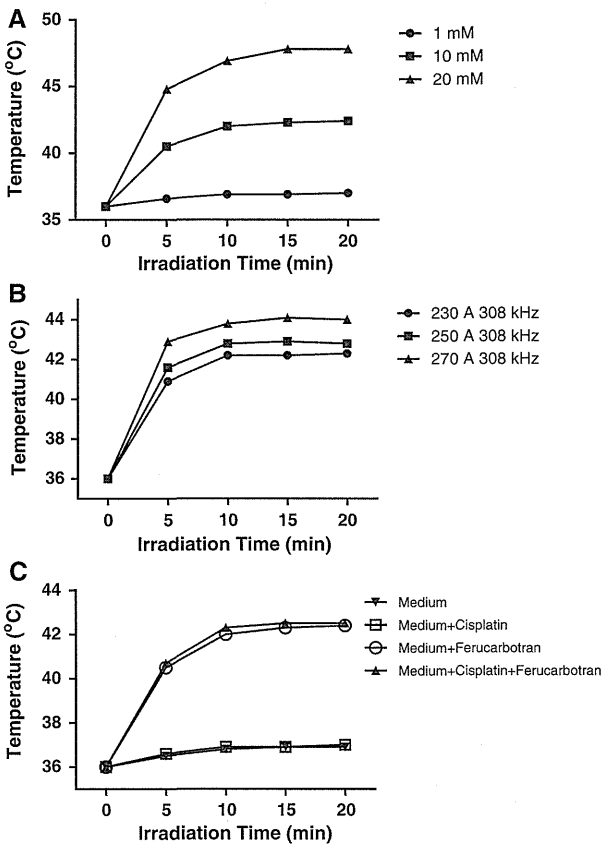

Fig. 2 Dependence of heat generation on ferucarbotran concentration and alternating magnetic field (AMF) strength. **a** Temperature–time curves at different concentrations of ferucarbotran (1, 10, or 20 mM equivalent of iron) on AMF at 308 kHz and EC 230 A. **b** Temperature–time curves in the presence of 10 mM ferucarbotran on AMF at different levels of electric current (230–270 A) at 308 kHz. **c** Effect of cisplatin (30 μM) on ferucarbotran (1, 10, or 20 mM equivalent of iron)/AMF (308 kHz, EC 230 A)-induced increase of temperature; medium only, medium + cisplatin, medium + ferucarbotran, and medium + cisplatin + ferucarbotran

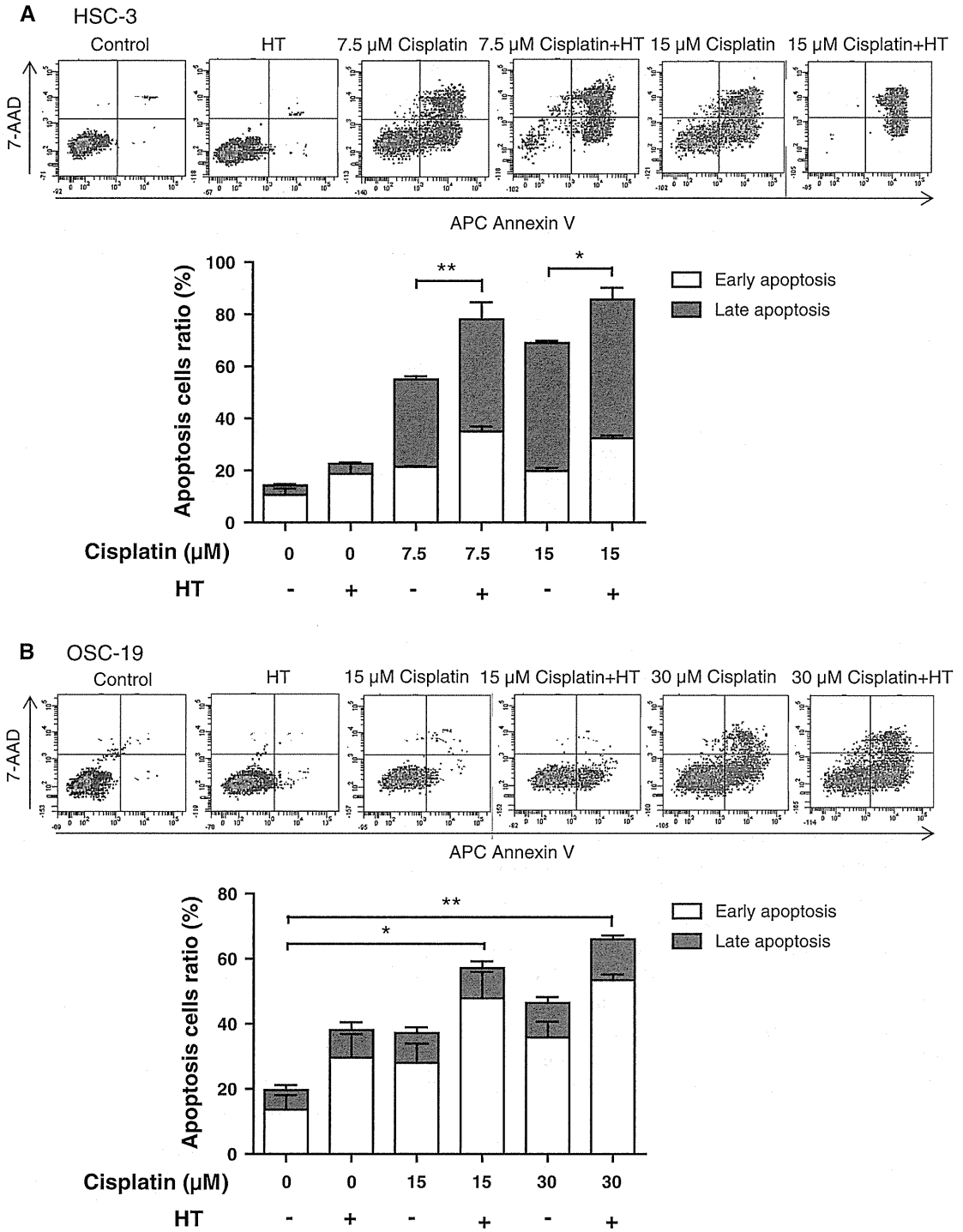

Fig. 3 Ferucarbotran/AMF-induced hyperthermia enhances the pro-apoptotic effect of cisplatin in human oral cancer cells. Annexin-V/PI staining of human oral cancer cells at 12-h intervals after treatment with 0, 7.5, or 15 μM cisplatin with or without hyperthermia (HT) in HSC-3 cells, and 0, 15, 30 μM cisplatin with or without HT in OSC-19 cells. **a** Representative analysis of apoptosis of HCS-3 cells and OSC-19 cells exposed to cisplatin and ferucarbotran with or without AMF. Annexin-V/PI method with FACS scan dot plot analysis was used to divide the treated and control cells into four groups: (1) living cells (*lower left quadrant*); (2) necrotic cells (*upper left quadrant*); (3) early apoptotic cells (*lower right quadrant*); and (4) late apoptotic cells (*upper right quadrant*). **b** Representative analysis of apoptosis of OSC-19 cells exposed to cisplatin with or without AMF. $*p < 0.05$, $**p < 0.01$; $n = 4$

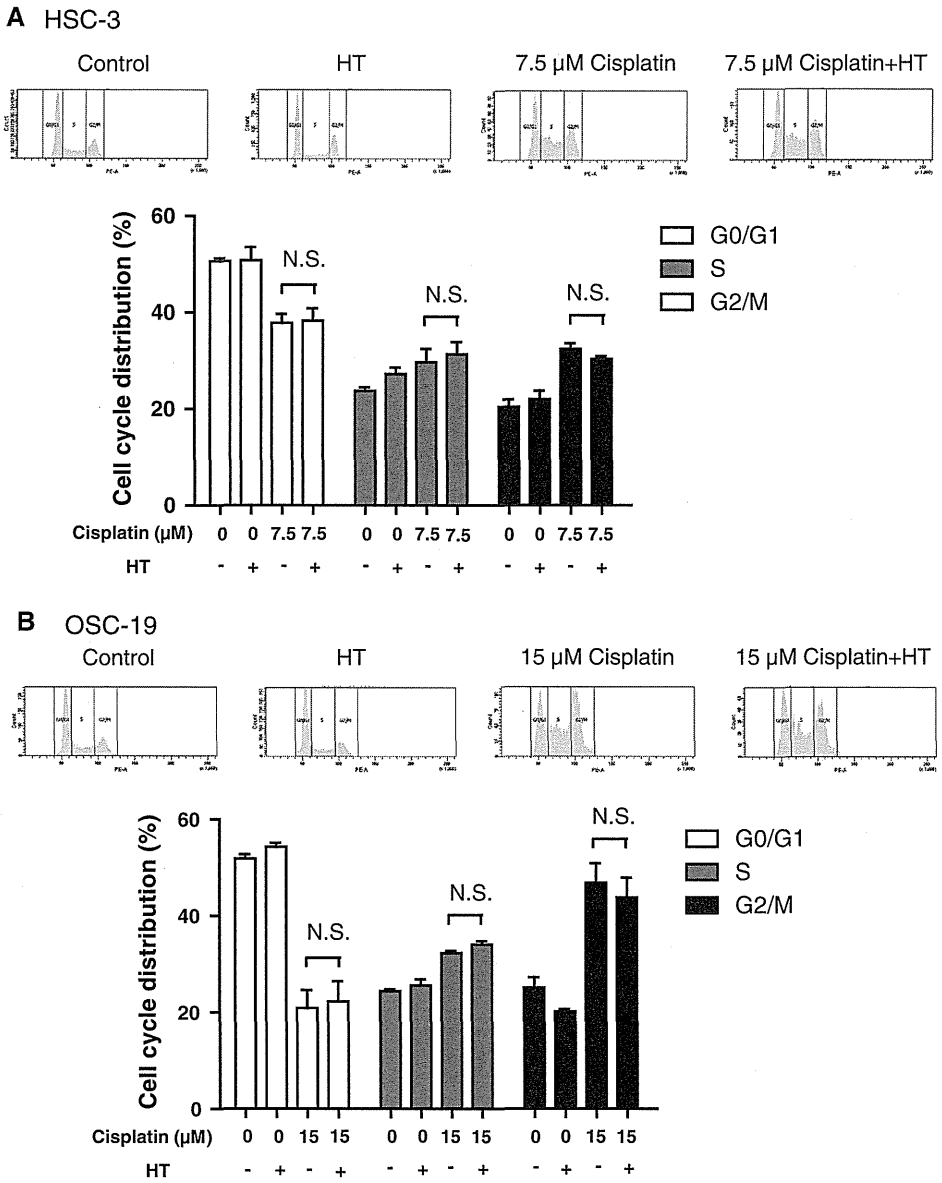

Fig. 4 Combination of cisplatin and ferucarbotran/AMF-induced hyperthermia causes G2/M arrest. Cell cycle analysis of HSC-3 and OSC-19 cells at 48-h intervals after treatment with 0, 7.5, or 15 μM of cisplatin with or without hyperthermia (HT). **a** Representative cell cycle analysis of HSC-3 cells in response to cisplatin treatment with or without AMF (*upper panel*) and of OSC-19 cells in response to cisplatin treatment with or without AMF (*lower panel*). *NS* not significant; $n = 4$

generator settings of 308 kHz and EC 250 A in the presence of 10 mM (equivalent of iron) ferucarbotran was sufficient to generate a temperature of 42.5 °C, and we adopted these conditions for the subsequent assays. We confirmed that cisplatin did not alter the heating effect under these conditions (Fig. 2c).

Ferucarbotran-enhanced cisplatin-mediated apoptosis

It has been reported that cisplatin induces apoptosis in cancer cells [29]. We thus examined whether ferucarbotran/AMF-induced hyperthermia further increased cisplatin-induced apoptosis in oral cancer cells. FACS analysis demonstrated that cisplatin increased both early and late apoptosis in a dose-dependent manner in HSC-3 cells (Fig. 3a) and OSC-19 cells (Fig. 3b). Ferucarbotran/AMF-induced hyperthermia for an hour significantly increased the apoptotic effect of cisplatin.

Cisplatin-induced G2/M arrest of human oral cancer cells was unaffected by hyperthermia

To examine whether hyperthermia modifies the mechanism of anti-cancer action of cisplatin, flow-cytometric cell-cycle analysis of treated cells was performed. Cisplatin induced potent G2/M arrest in both HSC-3 cells (Fig. 4a) and OSC-19 cells (Fig. 4b). We found that ferucarbotran/AMF-induced hyperthermia did not alter the effect of

cisplatin on the cell cycle. Thus, hyperthermia per se had no effect on the anti-cancer mechanism of cisplatin.

Discussion

Ferucarbotran is an organ-specific superparamagnetic contrast agent used in MRI, and its safety and maximum dosage (10 mM; 0.016 mL/kg, which contains 8 μmol (0.45 mg) Fe/kg equivalent of iron [30]) have been well established [9, 10]. Since hyperthermia has already been shown to enhance the anti-cancer effect of cisplatin [31] in the treatment of oral cancer, we anticipated that combination therapy with cisplatin and ferucarbotran/AMF-induced hyperthermia might be suitable for oral cancer treatment, making it possible to reduce the necessary dose of cisplatin and consequently reduce the risk of serious side effects.

Hyperthermia to induce apoptosis of cancer cells is best performed at about 42 °C, because temperatures above 44 °C have been reported to cause necrosis and damage to surrounding normal tissues [32]. Therefore, we first confirmed that the above concentration of ferucarbotran was sufficient to maintain a temperature of 42.5 °C under appropriate AMF conditions, and this level of hyperthermia could induce apoptosis of oral cancer cells, as evaluated by FACS analysis. It should be noted that it would still be necessary to optimize AMF conditions for clinical treatment. Similarly, it would be desirable to deliver cisplatin and ferucarbotran to oral cancer tissue in a selective manner. This may be achieved by the use of superselective intra-arterial infusion with a catheter, as we previously reported in oral cancer patients [33].

We previously reported that ROS production was higher in cancer cells than in normal cells, and was further increased when the temperature was increased [34]. Cisplatin also increases ROS production, and this is most likely the mechanism responsible for its anti-cancer effect [34, 35]. We confirmed that the combination of cisplatin and ferucarbotran/AMF-induced hyperthermia further enhanced ROS production (data not shown). This is important, because cisplatin may cause ototoxicity [36], so it is desirable to minimize the necessary cisplatin dose, as far as is consistent with therapeutic effectiveness, in the clinical context.

It is well known that cisplatin causes accumulation of cells in S phase and blocks the G0/G1 phases in xenografted human head and neck carcinoma cells [37], leading to apoptosis. [38, 39]. Our data showed that ferucarbotran/AMF-induced hyperthermia enhanced the anti-cancer effect of cisplatin without altering its characteristic effect on the cell cycle. Accordingly, ferucarbotran/AMF-induced hyperthermia did not appear to modify the mechanism of action of cisplatin in human oral cancer cells. Because both cisplatin and ferucarbotran are already in clinical use, we believe the combination of cisplatin with ferucarbotran/AMF-induced hyperthermia has the potential for early clinical application. It should at least be possible to reduce the clinically effective dosage of cisplatin by administering it in combination with ferucarbotran/AMF, thereby reducing the risk of serious cisplatin-related side effects. Further investigation seems warranted to confirm the safety and effectiveness of this combined treatment for oral cancers in humans.

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Conflict of interest The authors declare no potential conflicts of interest.

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口腔腫瘍　26巻2号　31～36頁　2014

口腔癌患者の上部消化管領域における同時性重複癌のスクリーニング

―上部消化管内視鏡検査と^{18}F-FDG-PET/CTの比較―

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要旨：われわれは口腔癌患者の上部消化管領域における同時性重複癌のスクリーニングとしての上部消化管内視鏡検査（GIF）とFDG-PET/CTについて比較したので報告する。

2006年9月から2009年8月までの3年間に口腔癌患者133例が治療前にGIFとFDG-PET/CTを受けた。GIFで5人の患者（3.8%）に食道や胃の同時性重複癌が発見され，それらは全例早期癌であった。しかし，FDG-PET/CTでは1人のみしか同時性重複癌（食道癌）が発見できず，その感度と特異度はそれぞれ20%と100%であった。本研究は口腔癌患者の上部消化管領域における治療前スクリーニングとしてのGIFの有用性と必要性を示した。

キーワード：上部消化管内視鏡検査（GIF），FDG-PET/CT，スクリーニング，重複癌，口腔癌

緒　言

口腔癌患者における重複癌の存在は治療の優先順位や治療方針に影響を与えるため[1,2]，治療前のスクリーニングは極めて重要である。口腔癌を含む頭頸部癌では上部消化管領域に重複癌が発生することが多いとされ，上部消化管内視鏡検査（Upper gastrointestinal fiberscopy：GIF）は食道癌や胃癌の検出に有用であると報告されている[1-17]。

一方，近年の画像診断技術の進歩にともない，原発巣やリンパ節転移の診断だけでなく重複癌や遠隔転移の診断にも有用とされる^{18}F-FDG-PET（FDG-PET）が口腔癌にも広く行われるようになってきた[2,18-22]。しかし，PETは空間分解能が低く解剖学的情報が乏しいため，CT画像をPET画像に重ね合わせた^{18}F-FDG-PET/CT（FDG-PET/CT）がその後に導入されてきた[23-25]。そのため，口腔癌患者における治療前の上部消化管領域の同時性重複癌のスクリーニングとしてGIFとFDG-PET/CTが行われることが多くなってきたものの，これまで上部消化管領域における同時性重複癌の検出に関して両者の比較は十分に行われていない。今回われわれは口腔癌患者の上部消化管領域における重複癌の治療前スクリーニングとして行ったGIFとFDG-PET/CTの同時性重複癌の検出について比較検討したので報告する。

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対象および方法

2006年9月から2009年8月までの3年間に横浜市立大学附属病院歯科・口腔外科・矯正歯科を受診した口腔癌患者290例中，上部消化管領域の重複癌の治療前スクリーニングとしてGIFとFDG-PET/CTが行えた口腔癌患者133例（男性84例，女性49例，平均年齢64歳（28～86歳））を対象とした。本検討では上顎洞癌や唾液腺癌などは除外し，UICCで定義される口腔癌のみを対象とした。対象の内訳は舌癌62例（46.6%），下顎歯肉癌30例（22.6%），上顎歯肉癌14例（10.5%），口底癌14例（10.5%），頬粘膜癌7例（5.3%），硬口蓋癌6例（4.5%）であった。各部位別の年齢の中央値は舌癌：65歳，下顎歯肉癌：63歳，上顎歯肉癌：68歳，口底癌：65歳，頬粘膜癌：66歳，硬口蓋癌：62歳であった。

重複癌の定義はWarren and Gates[26]に従い，①各腫瘍は一定の悪性像を呈する，②各腫瘍は互いに離れた部位を占める，③一方の腫瘍が他の腫瘍の転移でないものとした。GIFではルゴール染色を行った後に不染部位がある場合や粘膜の異常所見を認めた場合には生検を施行した。口腔癌患者の上部消化管領域における重複癌の治療前スクリーニングとして行ったGIFとFDG-PET/CTの同時性重複癌の検出について比較検討した。また，上部消化管領域の異時性重複癌（第2癌までの診断期間：1年以上）についても検討した。

表1　重複癌症例の内訳

症例	性別	初診時年齢	原発部位	重複部位	重複癌の病理診断（GIFによる生検）	PET/CTによる上部消化管の集積
1	男	74	硬口蓋（T2）	食道（T1）	扁平上皮癌	+
2	男	59	口底（T4a）	食道（T1）	扁平上皮癌	−
3	男	57	舌（T3）	食道（T1）	扁平上皮癌	−
4	男	64	舌（T2）	食道（T1）	扁平上皮癌	−
5	女	69	舌（T2）	胃（T1）	腺癌	−

（　）：原発部位および重複癌のT分類

結　果

口腔癌患者に対する治療前スクリーニングとして施行したGIFにて同時性重複癌を認めたのは133例中5例（3.8%）であり，すべて上部消化管の早期癌（T1）であった。その内訳は食道癌4例（扁平上皮癌），胃癌1例（腺癌）であり，食道癌と胃癌の同時性重複癌は認めなかった（表1）。性別での重複癌発症率は男性で4.8%（4/84例），女性で2%（1/49例）であった。口腔癌の原発部位は舌3例（T2N0M0：2例，T3N0M0：1例），口底1例（T4aN2bM0），硬口蓋1例（T2N0M0）ですべて扁平上皮癌であり，部位別発症率はそれぞれ硬口蓋癌：16.7%（1/6），口底癌：7.1%（1/14），舌癌：4.8%（3/62）であった。同時性重複癌を認めた口腔癌患者5例はすべて食道や胃の早期癌であったため，口腔癌の治療を優先して行った。これら5例のうち，FDG-PET/CTにて上部消化管領域にFDGの異常集積を認めたのは硬口蓋癌の1例（0.8%）のみであり，重複癌である食道癌のSUVmaxは5.5であった（図1）。口腔癌患者に対する上部消化管領域の同時性重複癌のスクリーニングにおいて，FDG-PET/CTの感度：20%（1/5），特異度：100%（128/128），偽陰性率：80%（4/5），偽陽性率：0%（0/128），陽性反応的中度：100%（1/1），陰性反応的中度：97%（128/132）であった（表2）。

本検討では経過観察中に上部消化管領域の異時性重複癌を認めた症例はなかった。

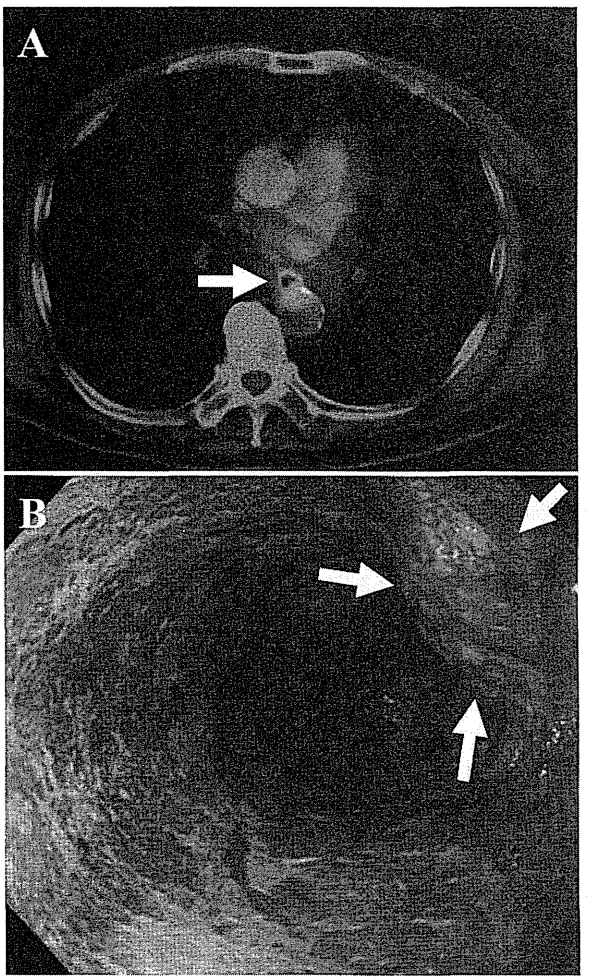

図1　症例1のPET/CTおよび内視鏡画像
A：PET/CTでは食道にSUVmax＝5.5のFDGの集積（矢印）を認めた。B：GIFでも食道に腫瘤性病変（矢印）を認めた。

表2　FDG-PET/CTによる食道癌・胃癌の検出

	食道癌・胃癌（+）	食道癌・胃癌（−）
FDG-PET/CT（+）	1	0
FDG-PET/CT（−）	4	128

考　察

頭頸部癌患者は他臓器の癌よりも重複癌の頻度が高いとされ[4,5]，上部消化管領域に重複癌が生じることが比較的多い[10,13]。われわれが渉猟しえた限り，頭頸部癌患者の上部消化管領域に生じる重複癌の頻度は2.8～10.8%[1,3-5,8-10,15,17]と報告されているが，一般的な癌検診と比べ10倍以上の頻度で重複癌が検出されている[2]。上部消化管領域の同時性重複癌（第2癌までの診断期間：1年未満）のみでなく，異時性重複癌（第2癌までの診断期間：1年以上）も含めて検討している報告も多いため，加療後経過観察中のGIF