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ORIGINAL ARTICLE

Advantage of FMISO-PET over FDG-PET for predicting histological response to preoperative chemotherapy in patients with oral squamous cell carcinoma

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Abstract
Purpose Hypoxia, a prognostic factor in many types of cancer, can be detected by ^{18}F-fluoromisonidazole (FMISO) positron emission tomography (PET). It is unclear whether hypoxia reflects the response to chemotherapy in patients with oral squamous cell carcinoma (OSCC). The correlations of FMISO-PET and FDG-PET with histological response to preoperative chemotherapy were therefore assessed in patients with OSCC.
Methods This study enrolled 22 patients with OSCC undergoing preoperative chemotherapy. The T-stages were T2 in 6 patients, T3 in 3, and T4a in 13, and the N-stages were N0 in 14 patients, N1 in 3, and N2 in 5. Each patient was evaluated by both FMISO-PET and FDG-PET before surgery, and the maximum standardized uptake value (SUV_{max}) of FDG- and FMISO-PET and tumor-muscle ratio (TMR) of FMISO-PET were measured. The threshold for the hypoxic volume based on TMR was set at 1.25. The histological response to preoperative chemotherapy was evaluated using operative materials.
Results FMISO-PET and FDG-PET detected uptake by primary OSCCs in 15 (68 %) and 21 (95 %) patients, respectively, and median SUV_{max}s of FMISO- and FDG-PET in the primary site were 2.0 (range, 1.3–3.5) and 16.0 (range, 1.0–32.2), respectively. The median of FMISO TMR was 1.5 (range, 0.99–2.96). There were five cases whose FMISO TMR was less than 1.25. Histological evaluation showed good response to preoperative chemotherapy in 7 patients (32 %) and poor response in 15 (68 %). Good response was significantly more prevalent in patients with negative than positive FMISO uptake ($P<0.001$) and without the hypoxic area evaluated by FMISO-PET TMR ($P=0.04$), whereas FDG uptake was not significantly correlated with response to chemotherapy response. Multivariate logistic regression analysis showed that FMISO uptake was an independent significant predictor of response to preoperative chemotherapy ($P=0.03$, odds ratio=0.06, 95 % confidence interval=0.004–0.759).
Conclusions An advantage of FMISO-PET over FDG-PET for predicting histological response to preoperative chemotherapy in patients with OSCC was observed.

Keywords Hypoxia · FMISO-PET · FDG-PET · Preoperative chemotherapy · HIF-1α · Oral squamous cell carcinoma

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Introduction

Hypoxia is rare in normal tissues, but is common in cancers and is a prognostic factor for many types of cancer [1, 2]. Clinically, patients with tumors having low oxygenation

levels have a poor prognosis, with strong evidence showing that this is due to the effects of hypoxia on therapy resistance and malignant progression [2]. In particular, hypoxia is a negative factor in the treatment of head and neck cancers, reducing the chance of cure [1]. Hypoxia also contributes to resistance to chemotherapeutic agents [2, 3]. Although the significance of preoperative chemotherapy in patients with oral squamous cell carcinoma (OSCC) is not clear, conventional roles of preoperative chemotherapy, such as organ preservation and reducing distant metastases, are generally accepted [4]. Non-responders to chemotherapy not only suffer from side effects, but also lose precious time to take advantage of other possible treatment [5]. Thus, accurate prediction of responses to chemotherapy may allow treatment to be tailored to individual patients, improving outcomes and avoiding unnecessary treatments [5]. Few studies to date, however, have assessed the correlation between hypoxia and response to preoperative chemotherapy in patients with OSCC, because monitoring of the response during the course of chemotherapy is difficult [6].

^{18}F-fluoro-2-deoxyglucose positron emission tomography (FDG-PET) is frequently used in tumor diagnosis and in evaluating of treatment outcomes. In patients with head and neck cancer, FDG-PET has been reported clinically useful in evaluating the therapeutic effects of neoadjuvant chemotherapy (NAC) and relapse [7–11]. The extent of FDG uptake by the tumor may indirectly reflect the tumor microenvironment, including areas of hypoxia [7, 12, 13]. In vitro studies have suggested that accumulation of FDG in cancer cells is associated with regional hypoxia [12–15]. Little is known, however, about the relationships between FDG uptake and tumor hypoxia in the clinical setting, because the exact mechanism by which FDG accumulates in malignant tumors is not fully understood [7, 13].

Multiple radiotracers have been developed for hypoxia imaging [1]. ^{18}F-misonidazole (FMISO)-PET is a promising noninvasive method of measuring hypoxia [16–19]. This method is sensitive to the presence of hypoxia in viable cells and can cover the entire region of interest [20, 21]. A recent study in our institutions demonstrated high reproducibility of tumor hypoxia evaluated by FMISO-PET for head and neck cancer [22]. Hypoxia achieves many effects by activating the transcription factor, hypoxia-inducible factor-1 (HIF-1) [23, 24], a key player in the transcriptional response to hypoxia [25–27]. Elevated HIF-1α has been closely correlated with chemo-resistance of tumor cells, and HIF-1α has been shown to inhibit the induction of apoptosis in tumor cells [28, 29]. We recently reported that FMISO but not FDG uptake correlated with the immunohistochemical expression of HIF-1α in patients with OSCC [23].

Identifying reliable predictors of chemotherapy outcome in patients with OSCC is of clinical interest [30]. This study was designed to elucidate the correlations between uptake of FMISO-PET and of FDG-PET and histological response to preoperative chemotherapy in patients with OSCC. The findings of this study may contribute to the development of improved strategies for treatment of OSCC.

Material and methods

Patients

The study enrolled 22 consecutive patients (14 men, 8 women; median age 65 years; range, 42–86 years) with untreated primary OSCC who received preoperative chemotherapy followed by radical surgery between October 2009 and March 2013 in our department (Table 1). All 22 patients were evaluated by FMISO- PET and FDG-PET before surgery. None received palliative treatment. The primary tumor sites were the tongue (n=5), upper gingiva (n=7), lower gingiva (n=6), buccal mucosa (n=2), and oral floor (n=2). Six tumors (27 %) were classified as T2, 3 (14 %) as T3, and 13 (59 %) as T4a. The N-classifications were N0 in 14 patients (64 %), N1 in 3 (13 %), and N2 in 5 (23 %) [31].

Intraoperative resected materials were stained with hematoxylin-eosin and evaluated histopathologically by a specialist in oral pathology (MS) blinded to the specimen origin. The degree of histological differentiation was determined in accordance with the 1997 WHO criteria. Of the 22 tumors, 9 (41 %) were classified as grade 1, 6 (27 %) as grade 2, 3 (14 %) as grade 3, and 4 (18 %) as unclear [32]. The histological mode of cancer invasion was classified according to the Yamamoto and Kohama (YK) classification system [33], with YK-1 tumors having well-defined borders and YK-4 tumors having diffuse growth or invasion. Of the 22 tumors, 6 (27 %) were classified as YK-2, 10 (45 %) as YK-3, 1 (5 %) as YK-4, and 5 as unclear.

This study was approved by the Institutional Ethics Committee (2009) and was performed in accordance with the guideline of the Helsinki II Declaration. All patients provided written informed consent.

Preoperative chemotherapy

All of the patients received preoperative chemotherapy with oral anticancer agent. Two received oral tegafur-uracil (UFT; Taiho Pharmaceutical Co., Ltd, Tokyo, Japan), 18 received oral tegafur-gimeracil-oteracil-potassium (S-1; Taiho Pharmaceutical Co., Ltd, Tokyo, Japan), and 2 received both agents. In the latter two cases, S-1 was changed to UFT because of side effects. The median duration of chemotherapy was 14 days (range, 6–31 days). None of the patients received preoperative radiation therapy.

Table 1 Patient characteristics

Case no.	Age/gender (years)	T-/N- classification	Primary site	FMISO/FDG uptake	FMISO SUV_{max}/TMR	FDG SUV_{max}	HIF-1α expression	Chemotherapy response (grade)
1	65/M	4a/1	Upper gingiva	−/+	1.27/1.03	30.05	−	1
2	62/M	4a/0	Upper gingiva	+/+	2.20/1.64	12.20	+	1
3	62/M	3/2	Tongue	+/+	1.98/1.79	17.90	+	1
4	79/F	2/0	Lower gingiva	−/+	1.23/0.99	5.90	−	2
5	72/M	4a/0	Upper gingiva	−/+	1.37/1.33	2.99	−	1
6	56/F	4a/0	Upper gingiva	−/+	1.59/1.20	9.40	−	2
7	73/F	2/2	Lower gingiva	−/+	1.14/1.13	4.00	−	2
8	57/M	4a/0	Oral floor	+/+	2.21/1.89	32.20	−	0
9	59/M	4a/2	Oral floor	+/+	2.38/1.88	29.10	+	0
10	83/F	2/0	Lower gingiva	+/+	1.93/1.72	7.70	+	1
11	59/F	4a/1	Tongue	+/+	2.40/1.70	21.80	+	1
12	67/M	4a/0	Lower gingiva	+/+	1.64/1.37	16.60	−	1
13	64/M	4a/0	Lower gingiva	+/+	2.14/1.95	13.10	−	1
14	70/M	2/0	Tongue	+/+	1.87/1.41	25.50	+	1
15	59/F	3/0	Tongue	+/+	1.53/1.59	16.5	−	2
16	79/M	4a/2	Tongue	+/+	2.73/2.14	23.40	+	0
17	42/M	2/2	Tongue	−/−	1.83/1.10	1.00	−	3
18	68/M	4a/0	Lower gingiva	+/+	3.46/2.96	12.00	Not done	2
19	86/F	2/0	Upper gingiva	+/+	2.48/1.48	19.70	Not done	0
20	69/F	3/0	Buccal mucosa	+/+	3.36/2.33	25.58	Not done	0
21	78/M	4a/0	Upper gingiva	−/+	1.70/1.35	11.60	Not done	2
22	63/F	4a/1	Buccal mucosa	+/+	1.98/1.48	15.40	Not done	1

Histological evaluation of preoperative chemotherapy

The histological effects of preoperative chemotherapy were evaluated using operative resected materials according to the General Rules for Clinical Studies on Head and Neck Cancer published by the Japan Society for Head and Neck Cancer (2002) [34], with grades 0–3 indicating no histological response, slight response (>1/3 cancer cell viable), moderate response (<1/3 cancer cells viable), and excellent response (no viable cancer cells). In this study, grades 2 and 3 were defined as good response to preoperative chemotherapy.

Immunohistochemical assay for HIF-1α

The immunohistochemical detection of HIF-1α was conducted using operation materials with formalin-fixed paraffin-embedded tissue sections, as described [23]. The sections were incubated with a primary mouse monoclonal antibody to HIF-1α (sc-13515, 1:100 dilution, Santa Cruz Biotechnology: Santa Cruz, CA) overnight at 4 °C. The epitope of this antibody is mapped within amino acids 329–530 of HIF-1α of human origin, and the antibody has no cross-reactivity to HIF-2α or HIF-3α. Negative controls in which the primary antibody was replaced with normal rat IgG were run with each specimen. HIF-1α positivity was evaluated by counting positive cells among 500–1,000 tumor cells at a magnification of ×200 in three different areas. We set the cutoff value of HIF-1α-positive cells at 5 % of the positively stained cells [35]. This work was performed by two of the authors (MS and JS) who were blind to the identities of the patients from whom the specimens had been obtained.

FMISO- and FDG-PET imaging

All of the patients underwent FMISO-PET and FDG-PET before surgery, after providing written informed consent. None of the patients had insulin-dependent diabetes. PET imaging was performed before chemotherapy in 9 patients (41 %) and after starting chemotherapy in 13 (59 %). Of the latter 13 patients, 7 underwent PET examinations after finishing chemotherapy (median, 20 days; range, 19–40 days, mean; 25.3±20.3 days), and 6 underwent PET examinations during chemotherapy, a median of 6 days (range, 2–14 days), and mean 6.0±5.0 days after starting chemotherapy.

For FMISO-PET, 10-min static PET images were acquired in the 3D mode using a PET/CT scanner (True Point Biograph 64 with true V option Siemens Japan, Tokyo, Japan) 4 h after the injection of 400 MBq of FMISO, because the cap of FMISO in our instruction is 400 MBq [22, 23]. The energy window of the PET/CT scanner was 425–650 keV, its

transaxial field of view (FOV) was 216 mm, and its reconstruction matrix was 168×168 [16, 17]. For FDG-PET, a 3-min static scan was obtained 1 h after the injection of 4.5 MBq/kg FDG. Images were reconstructed using the iterative TrueX reconstruction method, which included partial volume correction. The spatial resolution was 6.7 mm after reconstruction [22]. The detailed methods of FMISO-PET and high reproducibility of this method for evaluating hypoxia in head and neck tumors in our institution have been described [22, 23]. FMISO-PET images were analyzed quantitatively, including assessment of the maximal standardized uptake values (SUV_{max}) and the tumor-to-muscle ratio (TMR). The SUV_{max} was calculated as the activity concentration divided by injected dose/body weight [22]. For calculation of the TMR, a region of interest was placed over the primary lesion and posterior cervical muscle. The TMR was then defined as the tumor uptake divided by the uptake of the posterior cervical muscle [22]. The SUV_{max} and TMR were determined qualitatively evaluated and determination by researchers blinded to CT results. For the semiquantitative evaluation of FMISO and FDG uptake by the primary tumor, the highest uptake level of one voxel in the tumor was estimated using the SUV_{max}. In this study, the threshold for hypoxic volume based on TMR was set at 1.25 as described previously [22].

PET images were also visually evaluated by specialists in nuclear medicine (SO, TS, and NT), blinded to the clinical information. Since each patients underwent FMISO- and FDG-PET on different days, the nuclear medicine specialists evaluated each image independently on different days. When necessary, they referred to enhanced CT images to confirm the tumor region. The average and median of the interval between FMISO- and FDG- PET were 3.0±3.8 days and 1 day (range, 1–16 days), respectively.

Serum C-reactive protein concentrations were measured just before FDG-PET examination.

Statistical analysis

The Spearman correlation coefficient was used to compare the relationship between the SUV_{max}s of FMISO- and FDG-PET. Chi-square test and univariate logistic regression analysis were used to compare histological response to preoperative chemotherapy and PET uptake or other factors, including patient age, T- and N-classifications, clinical stage, degree of histological differentiation, histological mode of invasion, duration of chemotherapy, chemotherapy regimen, and expression of HIF-1α. The factors assessed included T-classification (T1+2 vs. T3+4), N-classification (N0 vs. N1+2), clinical stage (stage I+II vs. III+IV), degree of histological differentiation (grade 1 vs. grade 2+3), and mode of invasion (YK-1+2 vs. YK-3+4). Moreover, Mann-Whitney U-tests were performed to compare uptake of FMISO or FDG SUV_{max}, and serum CRP concentrations. All statistical analyses were performed using Stat View J-5.0 statistical software (Abacus Concepts, Berkeley, CA). In all analyses, $P<0.05$ was taken to indicate statistical significance.

Results

FMISO- and FDG-PET

FMISO- and FDG-PET detected uptake by primary OSCCs in 15 (68 %) and 21 (95 %) of the 22 patients, respectively (Table 1). Only one patient (no. 17) showed no FDG uptake. The median SUV_{max}s of FMISO- and FDG-PET at the primary site were 2.0 (range, 1.3–3.5) and 16.0 (range, 1.0–32.2), respectively (Table 1 and Fig. 1). The median of FMISO TMR was 1.5 (range, 0.99–2.96). There were five cases whose FMISO TMR was less than 1.25 (Table 1 and Fig. 2).

There was a weak positive correlation between FMISO and FDG SUV_{max} ($P=0.03$, $r=0.39$) (Fig. 1). Serum CRP concentration was not significantly correlated with either FMISO uptake ($P=0.13$) or FDG SUV_{max} ($P=0.07$). The median CRP concentrations in patients positive and negative for FMISO uptake were 0.21 mg/dl (range, 0.02–7.25 mg/dl) and 0.02 mg/dl(range, 0.02–0.21 mg/dl), respectively. The median CRP concentrations of patients with high and low FDG SUV_{max} were 0.21 mg/dl (range, 0.02–7.25 mg/dl) and 0.02 mg/dl (range, 0.02–0.66 mg/dl), respectively.

Immunohistochemical staining for HIF-1α and PET image

We were able to immunohistochemically analyze HIF-1α expression in 17 consecutive patients (nos. 1–17); of these, 7

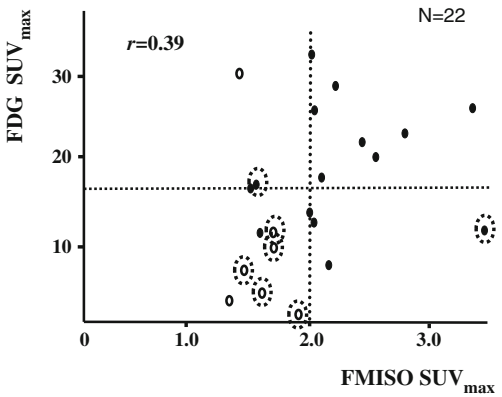

Fig. 1 Relationships between SUV_{max} of FMISO- and FDG-PET and responses to preoperative chemotherapy. SUV_{max} of FMISO- and FDG-PET showed a weak positive correlation by Spearman correlation analysis ($P=0.03$, $r=0.39$). The median SUV_{max}s of FMISO- and FDG-PET were 2.0 and 16.0, respectively. White and black circles indicate patients without ($n=7$) and with ($n=15$) FMISO uptake evaluated by visual examination, respectively. Dotted circles indicate patients showing good histological response to preoperative chemotherapy ($n=7$)

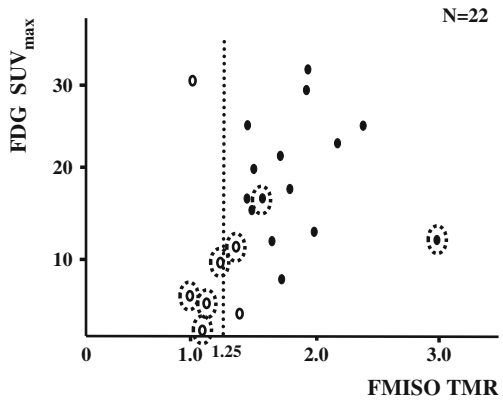

Fig. 2 Relationships between TMR of FMISO or SUV_{max} of FDG-PET and responses to preoperative chemotherapy. There were five cases whose TMR of FMISO was less than 1.25. White and black circles indicate patients without (n=7) and with (n=15) FMISO uptake evaluated by visual examination, respectively. Dotted circles indicate patients showing good histological response to preoperative chemotherapy (n=7)

(41 %) were positive for HIF-1α expression (Table 1). HIF-1α was detected in the cytoplasm and nucleus of cancer cells (Figs. 3 and 4) [23]. The prevalence of HIF-1α-positivity was significantly higher in patients with than without FMISO uptake at primary sites (7/11 vs. 0/6: P<0.025) (Table 2). The median SUV_{max} of FMISO-PET was significantly higher in HIF-1α-positive than HIF-1α-negative patients [2.2 (range, 1.6–2.7) vs. 1.6 (range, 1.3–2.0), P=0.005]. The median TMR of FMISO-PET was higher in HIF-1α-positive than HIF-1α-negative patients [1.7 (range, 1.4–2.1) vs. 1.3 (range, 1.0–2.0), P=0.05] (Fig. 5). In contrast, the median SUV_{max} of FDG-PET was not significantly correlated with HIF-1α positivity [21.8 (range 7.7–29.1) vs. 7.7 (range, 3.0–32.2): P=0.27] (data not shown).

FMISO- and FDG-PETs and histological response to preoperative chemotherapy

Histological evaluation of preoperative chemotherapy showed good response in seven patients (32 %) including 6 patients with grade 2 and 1 with grade 3, and poor response in 15 patients (68 %), including 5 with grade 0 and 10 with grade 1 (Table 1). Good response was significantly more likely in patients negative than positive for FMISO uptake (2/15 vs. 5/7: P<0.001) (Fig. 1 and Table 3), but was not correlated with FDG uptake (6/21 vs. 1/1: P >0.05) (Table 4). Moreover, good response was significantly more likely in patients with low FMISO TMR (<1.25) than high TMR >1.25) (4/5 vs. 3/17: P<0.01) (Fig. 2 and Table 5). Moreover, the prevalence of HIF-1α positivity was significantly lower in patients with good than poor histological response to chemotherapy (0/5 vs. 7/12: P<0.05) (Tables 6).

Univariate logistic regression analysis showed a significant correlation between FMISO uptake or FMISO TMR and response to preoperative chemotherapy [P=0.03 and 0.04, odds ratios (ORs)=0.08 and 0.07, 95 % confidence interval (CI)=0.01–0.74 and 0.01–0.95] (Table 7). However, significant correlations were not observed between FMISO SUV_{max} (P=0.06, OR=0.10, 95 % CI=0.001–1.013) or FDG SUV_{max}

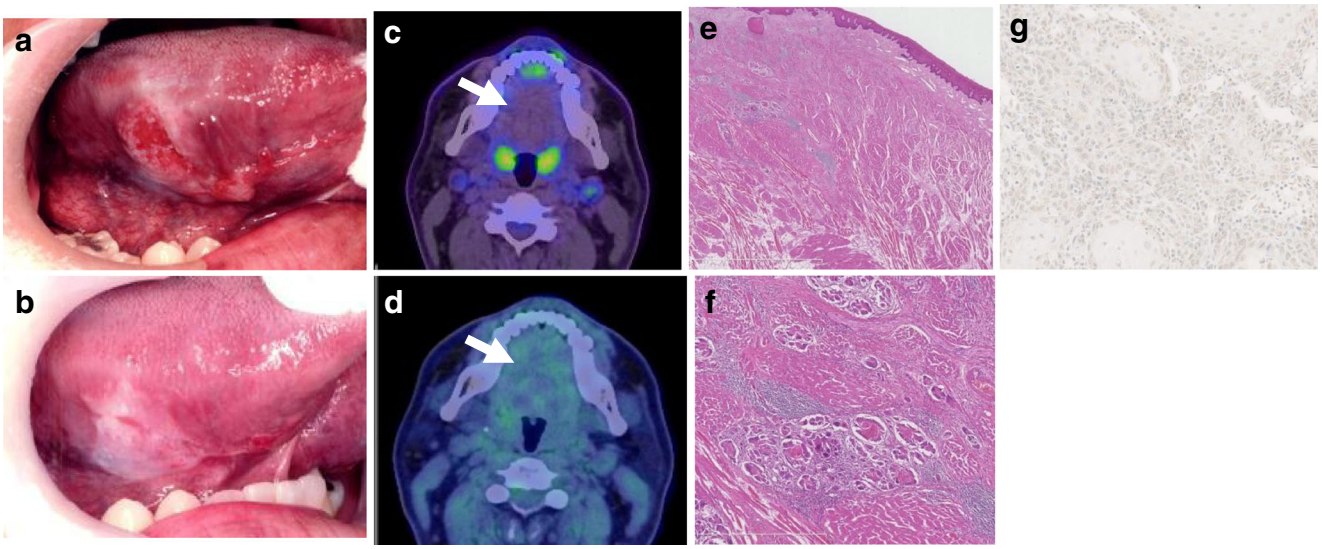

Fig. 3 Clinical findings, FMISO- and FDG-PET images, and histological findings and immunohistochemical staining of HIF-1α of resected material in patient no. 17. **a** Photograph showing an SCC (T2N2b) on the right side of the tongue at the first visit. **b** Photograph showing that tumor size decreased after preoperative chemotherapy (S-1 for 14 days). **c** FDG-PET results, showing no definitive FDG uptake by the primary lesion (SUV_{max}, 1.00). The white arrow indicates the primary site. **d** FMISO-PET results, showing no definitive FMISO uptake by the primary lesion (SUV_{max}, 1.83). The white arrows indicate the primary site. (**e** and **f**) Histological findings of the resected material at low (**e**) and high (**f**) magnifications. Most cancer cells were denatured and necrotic after preoperative chemotherapy. The histological response to preoperative chemotherapy was excellent (grade 3). **g** A weak HIF-1α expression was observed in cancer cells by immunohistochemical analysis

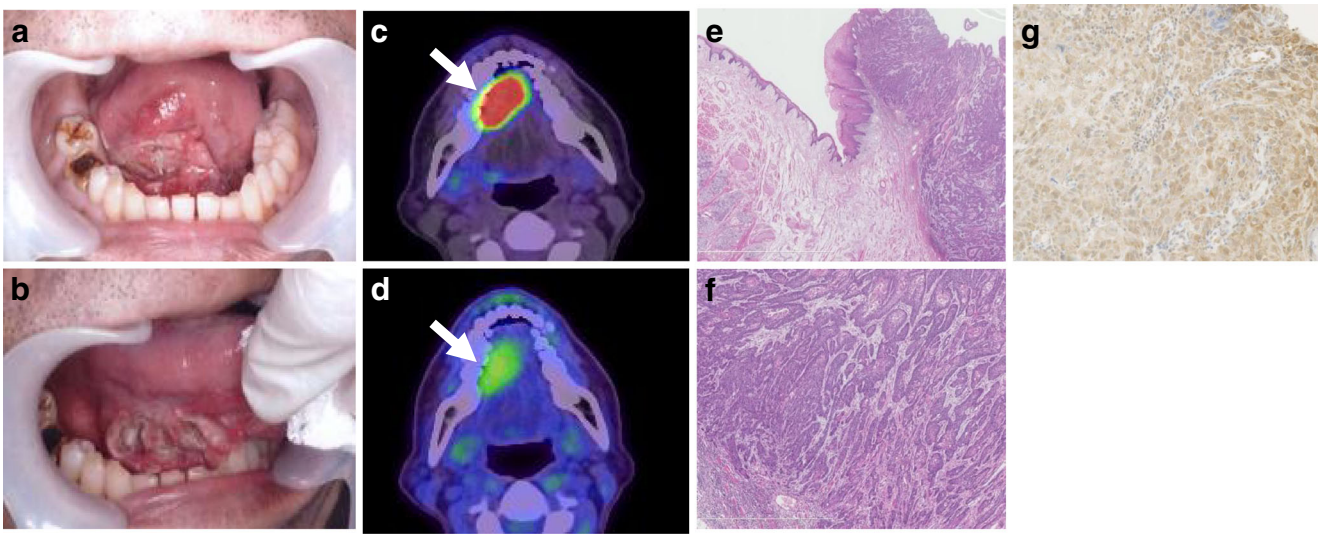

Fig. 4 Clinical findings, FMISO- and FDG-PET images, and histological findings and immunohistochemical staining of HIF-1α of resected material in patient no. 16. Photograph showing an SCC (T4aN0) on the oral floor at the first visit. **b** Photograph showing that the tumor did not decrease in size after preoperative chemotherapy (S-1 for 14 days). **c** FDG-PET results, showing definitive FDG uptake by the primary lesion (SUV_{max}, 32.2), indicated by the white arrow. **d** FMISO-PET results, showing definitive FMISO uptake by the primary tumor site (SUV_{max}, 2.21), indicated by the white arrows. **e** and **f** Histological findings of the resected material at low (**e**) and high (**f**) magnifications: Most cancer cells were viable and not denatured after preoperative chemotherapy. The histological response to preoperative chemotherapy was poor (grade 0). **g** HIF-1α was clearly detected in the nucleus and cytoplasm of cancer cells

(*P*=0.06, OR=0.10, 95 % CI=0.01–0.74) and response to preoperative chemotherapy (Table 7). Moreover, response to preoperative chemotherapy was not significantly correlated with patient age (*P*=0.41), T classification (*P*=0.52), N classification (*P*=0.32), clinical stage (*P*=0.86), degree of histological differentiation (*P*=0.60), histological mode of invasion (*P*=0.41), or duration of chemotherapy (*P*=0.95) (Table 7).

Multiple logistic regression analysis, including factors with relative low *P*-values (<0.60) on univariate logistic regression analyses, showed that FMISO uptake was an independent predictor of response to preoperative chemotherapy (*P*=0.03, OR=0.06, 95 % CI=0.004–0.759) (Table 8), whereas FDG SUV_{max} was not (*P*=0.08, OR=0.12, 95 % CI=0.010–1.339) (Table 9). We excluded the factor of the histological mode of invasion (*P*=0.41) because of some deficit data from multivariate analysis.

Chemotherapy regimen

The 22 patients were divided into three different chemotherapy regimens, including the only oral tegafur-uracil (UFT) group (2 cases), only oral tegafur-gimeracil-oteracil-potassium (S-1) group (18 cases), and both agents group (2 cases). So, we divided the patients into two groups, those who were (*n*=4) and were not (*n*=18) treated preoperatively with oral tegafur-uracil (UFT). Histological response to preoperative chemotherapy did not differ significantly in those two groups (*P*>0.05) (Table 10).

Table 2 Relationship between FMISO uptake and expression of HIF-1α

	HIF-1α (+)	HIF-1α (−)	Total
FMISO uptake (+)	7 cases	4 cases	11cases
FMISO uptake (−)	0 case	6 cases	6 cases
Total	7 cases	10 cases	17 cases

7/11 vs. 0/6 chi-square=6.49, *P*<0.025

Order of PET examinations and chemotherapy

There were no significant differences between FMISO-PET (*P*=0.20) and FDG-PET (*P*=0.52) SUV_{max} or FMISO-PET

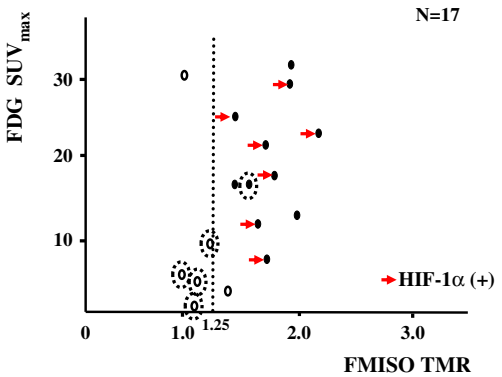

Fig. 5 Relationships between the TMR of FMISO-PET or SUV_{max} of FDG-PET and response to chemotherapy. There are five cases whose TMR of FMISO was less than 1.25. *White and black circles* indicate patients having tumors without (*n*=7) and with (*n*=15) FMISO uptake evaluated by visual examination, respectively. *Dotted circles* indicate the seven patients who showed good histological response to preoperative chemotherapy. *Red arrows* indicate the seven patients who showed positive staining for HIF-α

Table 3 Relationship between FMISO uptake and response to preoperative chemotherapy

	Good response	Poor response	Total
FMISO uptake (+)	2 cases	13 cases	15 cases
FMISO uptake (−)	5 cases	2 cases	7 cases
Total	7 cases	15 cases	22 cases

2/15 vs. 5/7 chi-square=7.43, $P<0.001$

Table 5 Relationship between response to preoperative chemotherapy and FMISO TMR

	Good response	Poor response	Total
FMISO TMR<1.25	4 cases	1 case	5 cases
FMISO TMR>1.25	3 case	14 case	17 case
Total	7 cases	15 cases	22 cases

4/5 vs. 3/17 chi-square=6.92, $P<0.01$

TMR ($P=0.80$) and the orders of PET examinations and chemotherapy (data not shown). Moreover, histological response to chemotherapy did not differ in the patient groups divided by the orders of PET examinations and chemotherapy ($P=0.58$, OR=0.57, 95 % CI=0.079–4.129) (Table 7). We could observed that there was a significant relationship between the FMISO TMR and response to chemotherapy ($P<0.025$) even in the 13 patients who scanned PET after initiating chemotherapy (data not shown).

Discussion

We observed a significant correlation between FMISO uptake or FMISO-PET TMR and histological response to preoperative chemotherapy in patients with OSCC. However, FDG uptake was not significantly correlated with response to chemotherapy response. Moreover, FMISO uptake was an independent significant predictor of histological response to preoperative chemotherapy. Previous studies have shown association between tumor hypoxia and resistance to chemotherapeutic agents [2, 36, 37]. Hypoxia is characteristic of solid tumors due to their less ordered vasculature and necrosis [3, 38, 39]. As hypoxic cells are often at a distance from blood vessels, drug concentrations are often insufficient for effective killing of these cells, because of diffusion limitations and drug uptake by intervening well-oxygenated cells. In addition, prolonged hypoxia can lead to cell cycle inhibition and a decrease in the growth fraction. As most current chemotherapeutic agents are more effective in killing proliferating cells, hypoxia can lead to resistance to theses agents [2, 40, 41]. Moreover, hypoxia can also induce major changes in gene expression, thereby enhancing the metastatic ability and increasing the malignancy of tumor cells [1]. Intratumoral hypoxia is one of the most important mechanisms promoting tumor aggressiveness, metastasis, and poor prognosis [25].

Recent findings indicate that elevated expression of HIF-1α is closely correlated with the chemoresistance of tumor cells [28]. One of the genes increased in expression in response to hypoxia and though to contribute to drug resistance is the multidrug resistance (MDR) gene, which encodes P-glycoprotein (P-gp) [28, 36, 42]. In response to hypoxia, MDR gene expression, with subsequent functional P-gp expression, is markedly upregulated in a manner dependent on HIF-1 [28, 42]. Miyawaki et al. [7] demonstrated that higher expression of HIF-1α was associated with a poor histological response to NAC in 37 patients with OSCC. We observed a similar result, in that the prevalence of HIF-1α positivity was significantly lower in patients with good than with poor histological response to preoperative chemotherapy ($P<0.05$).

Several studies, however, have reported that the susceptibility of tumor cells to chemotheraupeutic drugs is not correlated with the level of HIF-1α expression [28, 43]. Because the mechanisms involved in chemotheraupeutic resistance are more complex, many factors other than HIF-1α are involved in the chemoresistance of cancer cells [28]. Among the proteins implicated in response to chemotherapy are apoptosis regulators, including p53 [44]; the cell cycle regulators p16, p21, p27, cyclin D1, and BCL2; the growth regulators EGFR and P-ATK; and hypoxia response proteins such as HIF-1α [45]. This study focused on the relationship between hypoxia and chemotherapy response in patients with OSCC.

Miyagaki et al. [5] demonstrated that the expression of PETK1, also known as cyclin-dependent kinase 14, in not only resected cancer tissues but also in biopsy samples obtained before the treatment was a predictor of the response to chemotherapy in patients with oesophageal SCC. Therefore,

Table 4 Relationship between FDG uptake and response to preoperative chemotherapy

	Good response	Poor response	Total
FDG uptake (+)	6 cases	15 cases	21 cases
FDG uptake (−)	1 case	0 case	1 case
Total	7 cases	15 cases	22 cases

6/21 vs. 1/1 chi-square=2.24, $P>0.05$

Table 6 Relationship between response to preoperative chemotherapy and expression of HIF-1α

	HIF-1α (+)	HIF-1α (−)	Total
Good response	0 case	5 cases	5 cases
Poor response	7 cases	5 cases	12 cases
Total	7 cases	10 cases	17 cases

0/5 vs. 7/12 chi-square=4.96, $P<0.05$

Table 7 Univariate logistic regression analysis of factors associated with histological response to preoperative chemotherapy

Factors	Chi-square	P value	Odds ratio (95 % CI)
FMISO uptake	5.00	0.03	0.08 (0.01–0.74)
FMISO SUV_{max}	3.54	0.06	0.10 (0.01–1.10)
FMISO TMR	4.01	0.04	0.07 (0.01–0.95)
FDG SUV_{max}	3.54	0.06	0.10 (0.001–1.10)
Age	0.67	0.41	0.44 (0.06–3.16)
T classification	0.41	0.52	0.50 (0.06–4.15)
N classification	0.98	0.32	0.30 (0.03–3.25)
Clinical stage	0.03	0.86	1.25 (0.10–15.11)
Histological grading	0.52	0.60	4.20 (0.33–53.13)
Mode of invasion	0.83	0.41	2.86 (0.24–33.916)
Duration of chemotherapy	0.01	0.95	1.09 (0.09–13.78)
Orders of PET and chemotherapy	0.31	0.58	0.57 (0.08–4.13)

CI confidence interval

we have tried to perform an immunohistochemical analysis for HIF-1α of the biopsy samples. However, we were unable to confirm the usefulness of most of theses samples because of the inadequate quality and quantity of our biopsy specimens.

Other studies in patients with head and neck cancer reported that FMISO-PET results could not predict response to NAC [4, 46]. Yamane et al. [4] applied 1 cycle of NAC (S-1 plus nedaplatin) in 13 patients with advanced head and neck SCC. The median FMISO-PET SUV_{max} of the primary tumor was lower for the nine responders than for the four non-responders, but the difference was not statistically significant [2.2 (range, 0.7–3.2) vs. 2.3 (range, 1.5–3.6), P=0.94]. In that study, therapeutic response was based on the response evaluation criteria in solid tumors (RESIST), which evaluate the clinical size reduction of cancer. The authors commented that, although the effects of chemotherapy would be better evaluated by assessing pathological changes, their inclusion only of patients who underwent surgical tumor resection limited their pathological results [4].

In contrast to FMISO-PET, FDG-PET is widely available in hospitals and clinics worldwide. Recent studies indicated that tumor hypoxia and high-level FDG uptake have been associated with poor outcomes in patients with head and neck cancer [3, 12, 47]. Although the high FDG uptake by malignant tumors is due to increased glucose metabolism, the exact mechanism by which FDG accumulates in malignant tumors is not fully understood [7]. The avid uptake of glucose and FDG by malignant tumors is likely due to increased membrane glucose transporter and glycolytic enzyme activities in tumor cells [48, 49]. The uptake of glucose and other hexoses by human cells can take place via three transport mechanisms: passive diffusion, Na^+-dependent glucose transporter, and facilitative glucose transporters (Glut) [48]. Among 13 subtypes of the latter, Glut-1, Glut-3, and Glut-4 have a relatively high affinity for glucose [48]. Hypoxia leads to an increase in the rate of glycolysis, which in turn increases the FDG uptake [7]. Glut-1 and −3 largely mediate basal glucose transport in cancer cells, facilitating the maintenance of glycolytic energy metabolism when the substrate is in limited supply: e.g., in moderate to poorly perfused regions [48]. The extent of FDG uptake by a tumor may indirectly reflect its level of hypoxia, because tumor hyperglycolysis is driven by the expression of HIF1-α [12, 13, 50, 51].

A study of 24 patients with head and neck SCC and metastatic lymph nodes who underwent FDG-PET, FMISO-PET, and PO_2-polarography within 1 week found that FMISO uptake (r=0.80, P<0.001), but not FDG uptake was correlated with the results of PO_2-polarography [52], whereas other results have indicated that, although FDG uptake may indicate the presence of hypoxia, it should not be considered a surrogate marker for hypoxia [53]. Our results suggest that FMISO-PET, but not FDG-PET, can identify hypoxic tumor [34].

In contrast, Miyawaki et al. demonstrated that the preoperative FDG SUV_{max} was significantly lower in patients with higher histological response to NAC [7]. The SUV_{max} of

Table 8 Multivariate logistic regression analysis associated with histological response to preoperative chemotherapy

Factors	Chi-square	P value	Odds ratio (95 % CI)
FMISO uptake	4.71	0.03	0.06 (0.004–0.76)
T classification	0.11	0.74	0.66 (0.05–8.05)
N classification	1.31	0.25	0.17 (0.01–3.53)

CI confidence interval

Table 9 Multivariate logistic regression analysis associated with histological response to preoperative chemotherapy

Factors	Chi-square	P value	Odds ratio (95 % CI)
FDG SUV_{max}	2.98	0.08	0.12 (0.01–1.34)
T classification	0.17	0.68	0.61 (0.06–6.44)
N classification	0.34	0.56	0.46 (0.03–6.45)

CI confidence interval

Table 10 Relationship between chemotherapy regimen and response to preoperative chemotherapy

	UFT or UFT+S-1	Only S-1	Total
Good response	2 cases	5 cases	7 cases
Poor response	2 cases	13 cases	15 cases
Total	4 cases	18 cases	22 cases

UFT: oral tegafur-uracil, S-1: oral tegafur-gimeracil-oteracil-potassium
2/7 vs. 2/15 chi-square=0.75, P>0.05

responders and non-responders to chemotherapy was 9.1±3.8 vs. 13.7±4.3, respectively. In contrast, we observed no significant correlation between FDG SUV_{max} and response to chemotherapy, a discrepancy that may be due to differences in treatment methods, evaluation of treatment outcomes, and patient characteristics. Preoperative treatment of our patients consisted only of oral anticancer agents, whereas, in the study by Miyawaki et al., patients were treated with cisplatin or carboplatin plus 5-fluorouracil in combination with radiation therapy (30Gy) [7]. We evaluated response to chemotherapy using the criteria of the Japan Society for Head and Neck Cancer (2002) [34], whereas the Miyawaki et al. [7] evaluated response by other criteria by Shimosato et al. [54]. Tumor sizes in the two studies differed, with 20 of their 37 patients (54 %) having T2-sized tumors [7], whereas 13 of our 22 patients (59 %) were T4a-sized.

Although high tumor uptake of FDG is largely due to increased glucose metabolism, FDG metabolism also reflects a nonspecific inflammatory response and scarring around a necrotic tumor [6, 46]. It is difficult to evaluate the degree of inflammation or other active processes that also affect SUV_{max} [50]. Although we attempted to evaluate inflammatory conditions by measuring the serum CRP concentration at the time of FDG PET, we could not evaluate localized inflammation in those patients.

S-1, an oral anticancer agent containing tegafur and two modulators of 5-fluorouracil (5-FU) metabolism, is frequently used to treat patients with OSCC. This drug was designed to enhance the efficacy of tegafur, a prodrug of 5-FU [16]. Although we usually treat OSCC patients preoperatively with S-1, this agent should not be used in selected patients, including elderly patients and those with low performance status. Thus, our 22 patients received three different oral chemotherapy regimens. However, these three subgroups did not differ in their histological responses to preoperative chemotherapy.

The major limitation of this study was the small size of the patient population. Further studies in larger numbers of patient are required to address these issues. To our knowledge, however, no previous clinical studies have assessed the correlation between FMISO-PET and histological response to preoperative chemotherapy in patients with OSCC.

In the evaluation of FMISO uptake, our study evaluated FMISO-PET images using SUV, TMR, and visual evaluation. There was a possibility that visually evaluated FMISO uptake would be "negative" despite a relatively high SUV because the background SUV differed in each image. Our findings suggest that the SUV_{max} of the tumor and visual evaluation of uptake are not always identical. In the present study, the hypoxic area was also defined as the area with TMR of ≥1.25. This threshold is now a standard in our institution because the upper limit of the 95 % confidence interval for normal muscle uptake without carcinoma is 1.25 [22].

Another study limitation is the orders of PET examination and chemotherapy in the present study. Although we could observe no significant differences between PET SUVs or FMISO-PET TMR and the orders of PET examinations and chemotherapy response, we could not deny the possible bias for evaluation of PET images, because reduction of FMISO uptake after NAC for head and neck SCC was reported [4]. We are sure that it would have been ideal to perform all PET examinations before initiation of treatment, including preoperative chemotherapy. However, this was difficult because some patients required more immediate treatment. Moreover, we were not able to evaluate the relationships between the hypoxia and other factors that might affect treatment efficacy in patients with OSCC. We are sure that several factors are considered independent prognostic factors in patients with head and neck cancer including the expression of human papillomavirus (HPV) [55].

Multivariate logistic regression analyses included with relatively low P-value ($P<0.60$) on univariate analyses, including FMISO uptake, FDG SUV, T-classification, and N-classification. Our multivariate analyses could not include three important factors, FMISO uptake, FMISO TMR, and FDG SUV, in one statistical model, because these three factors had a positive correlation. We therefore had to construct two models to compare the contributions of FMISO-PET and FDG-PET.

Conclusion

We demonstrated a significant relationship between FMISO uptake or FMISO TMR and histological response to preoperative chemotherapy in patients with OSCC. We could demonstrate the advantage of FMISO-PET over FDG-PET for predicting histological response to preoperative chemotherapy in patients with OSCC. In the future, FMISO-PET might be used in the decision-making process regarding treatment strategies in these patients.

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Conflict of interest None of the authors of this manuscript has any financial relationship with any organization, or any conflict of interest, regarding this study.

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