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USPIO-enhanced MRI of highly invasive and highly metastasizing transplanted human squamous cell carcinoma: an experimental study

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Objective: The aim of this study was to investigate the signal intensity characteristics of highly invasive and highly metastasizing transplanted human squamous cell carcinoma using ultra-small super-paramagnetic iron oxide (USPIO)-enhanced MRI and to correlate them with USPIO distribution to tumour components revealed by histological examination.

Methods: 13 nude mice with transplanted human squamous cell carcinoma in the oral cavity were imaged before and 24 hours after intravenous administration of USPIO. The difference in signal intensity between pre-contrast and post-contrast MR images was visually evaluated. For quantitative analysis, signal intensity within a region of interest was measured. Histological findings were correlated with MR findings. The approximate USPIO concentration was evaluated using USPIO phantoms.

Results: Seven tumours had an area showing signal intensity increase on post-contrast T_1 weighted images. Histopathologically, six of those tumours contained a small amount of iron particles in the stroma. The USPIO concentration was presumed low. Two tumours had an area showing signal intensity decrease on post-contrast T_1 and T_2 weighted images. The areas had a large amount of iron particles in the stroma and the USPIO concentration was presumed high. There was a minimal amount of iron particles in tumour parenchymal cells.

Conclusions: The amount of USPIO accumulation into tumour stroma was considered to affect MR signal intensity. A small amount increases T_1 weighted signal intensity, whereas a large amount decreases T_1 and T_2 weighted intensity. The USPIO accumulation into the tumour parenchyma was not thought to affect MR signal intensity.

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Introduction

Squamous cell carcinoma accounts for more than 90% of head and neck cancers, and head and neck squamous cell tumours recur in more than 40% of patients.^{1,2} Detecting tumour recurrence in the head and neck using clinical modalities including imaging, CT and MRI is difficult

because some types of treatment cause deformations in surrounding tissues.²

Ultra-small super-paramagnetic iron oxide (USPIO) has a long plasma half-life and comprises extremely small particles, so it is useful as a tissue-specific MR contrast agent. USPIO can cross capillary walls and widely distribute into tissues including lymph nodes and bone marrow.³ Some studies have shown that USPIO penetrates the capillary endothelium of tumours and becomes internalized in tumour cells.^{4,5} Thus, USPIO might provide valuable information for differentiating

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recurrent tumours from interstitial inflammation caused by radiotherapy and chemotherapy. USPIO-enhanced MRI can differentiate metastatic from normal lymph nodes in head and neck squamous cell carcinoma.⁶ However, few studies have described the signal intensity characteristics of primary tumours. Varallyay *et al*⁷ evaluated USPIO-enhanced MR images of intracranial squamous cell carcinoma in two patients and reported that they both showed iron uptake and signal intensity changes. However, they did not perform a quantitative analysis, owing to minimal intracranial spread. The biological and pathological characteristics of squamous cell carcinoma widely vary and the degree of malignancy depends on the type. Thus, identifying the characteristics of USPIO-enhanced MR images of highly invasive and highly metastatic squamous cell carcinoma is important.

The purpose of this study was to investigate the signal intensity characteristics of highly invasive and highly metastasizing transplanted human squamous cell carcinoma using USPIO-enhanced MRI and to correlate them with USPIO distribution in tumour components such as parenchyma, stroma and necrosis revealed by histological examination.

Materials and methods

Mice

The study protocol was approved by our institutional animal care and use committee. 18 female CAnN.Cg-Foxn1^{nu}/CrJCrJ (BALB/c-nu/nu) nude mice, 6–13 weeks old, (Charles River Japan, Yokohama, Japan) were used.

Cell line and cell culture

OSC-19 cells derived from a human tongue squamous cell carcinoma were used (the cells were provided by Professor E Yamamoto, Department of Oral and Maxillofacial Surgery, Graduate School of Medical Science, Kanazawa University).⁸ Tumours of OSC-19 cells transplanted into the backs of nude mice showed growth without active invasion into the surrounding tissue, similar to benign tumours. In contrast, when transplanted into the oral cavities of nude mice, the tumours showed invasive growth.⁹ Another biological characteristic is that regional neck lymph node metastases are detected in more than 80% of cases. The histological characteristics are diffuse invasion into the surrounding tissue with cord-like microtumour nests, well-differentiated squamous cell carcinomas, and fibroblastic reaction and mononuclear cell infiltrates around the tumour nest.

OSC-19 cells were maintained in RPMI 1640 medium (Sigma-Aldrich Corp., St Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum at 37°C in a humidified atmosphere of 5% CO₂ in the air. The mice were anaesthetized with ether and tumour cells were injected into the submucosa of the oral cavity

(tongue, floor of oral cavity or buccal mucosa) via an intraoral approach at 1.0×10^6 viable cells/0.025 ml in phosphate buffered saline per inoculum using a tuberculin syringe with a 26-gauge disposable needle. They were imaged using MRI approximately 3–4 weeks later when the size of the tumour was approximately 3 mm–8 mm in diameter.

Contrast agent

As a contrast agent, the USPIO Combidx® (AMAG Pharmaceuticals, Inc., Cambridge, MA) was used. It was provided as lyophilized powder consisting of USPIO particles covered with low-molecular-weight dextran with a total particle diameter in solution of between 17 nm and 21 nm. Combidx was dissolved in 20 ml of physiological saline and the concentration of USPIO was 10.52 mg Fe ml⁻¹ as a result. The mice were randomly divided into 3 groups of 6 and each group was intravenously administered with USPIO at a dose of 2.6 mg, 8.5 mg or 17.0 mg per kilogram of body weight (mg Fe kg⁻¹). The doses of USPIO were determined with reference to a previous study in which 2.6 mg Fe kg⁻¹ was the recommended ferumoxtran (Combidx) dose for detecting nodal metastases in humans and another in which 17.0 mg Fe kg⁻¹ of USPIO was used in experimental tumour models.^{10,11} We also used 8.5 mg Fe kg⁻¹ as an intermediate dose. The USPIO solution was administered intravenously through the tail vein (0.03–0.07 ml). Five mice were excluded from the study: two died after tumour cell injection before imaging, two died after anaesthesia when they were imaged and the remaining one was injected with an incorrect dose of USPIO. Thus, our study population ultimately consisted of 13 mice: 3 with 2.6 mg Fe kg⁻¹ injection (tumours A–C), 4 with 8.5 mg Fe kg⁻¹ injection (tumours D–G) and 6 with 17.0 mg Fe kg⁻¹ injection (tumours H–M).

MR imaging

The mice were imaged twice, before and 24 hours after intravenous administration of USPIO. After obtaining pre-contrast MR images, each mouse was administered USPIO with the above described dose. All images were obtained with a 1.5 T superconducting magnet system (Magnetom Vision; Siemens, Erlangen, Germany) by using a 4 cm diameter surface coil. Before imaging, the mice were anaesthetized by intraperitoneal injection of approximately 35 mg kg⁻¹ pentobarbital. Then the mouse was fixed in a plastic device and placed prone in the coil. The MR images were obtained using the following two sequences: *T*₁ weighted spin-echo (repetition time/echo time, 500 ms/20 ms) and *T*₂ weighted fast spin-echo (3000 ms/96 ms, echo train length of 7). All images were acquired at a section thickness of 2 mm without an intersection gap; matrix, 160 × 256; field of view (FOV), 31 × 50 mm. At imaging, 2 extension tubes with 3.3 mm internal diameter were placed in the FOV as reference phantoms: 1 tube was filled with deionized water, which was used as a phantom for *T*₁ weighted

imaging, and the other was filled with 120 mg of manganese(II) chloride tetrahydrate per litre of deionized water, which was used for T_2 weighted imaging.

Image analysis

The difference in signal intensity between the pre-contrast and post-contrast MR images was assessed. 2 observers (AT and SN with 14 years of experience in diagnosis of head and neck MR imaging) evaluated qualitative changes in signal intensity (increase, decrease, no change) by consensus. In quantitative analysis for signal intensity, an operator-defined circular region of interest (ROI), which was as large as possible (approximately 2 mm–5 mm in diameter) was set on both the tumour and the phantom by a single observer (AT). The area in which the signal intensity showed increase, decrease or no change after contrast agent administration was visually identified. Specific small ROIs were then set on one of these areas in each tumour visualized in post-contrast images and on the corresponding areas in pre-contrast images, and then we measured the mean signal intensity of these ROIs. The relative intensity (RI) was determined as follows: $RI = \text{signal intensity of tumour} / \text{signal intensity of reference phantom}$.

Statistical analysis

The paired *t*-test was used to detect significant differences in the RI of the tumour between pre-contrast and post-contrast images in the same group of mice. We detected differences in RI for a large ROI covering large areas of tumours at different concentrations of USPIO using the one-way analysis of variance (ANOVA). $p < 0.05$ was considered statistically significant. ANOVA was performed with the statistical software SPSS version 16.0 or 18.0 (SPSS Inc., Chicago, IL).

Histological analysis

After the mice were sacrificed by intraperitoneal injection of an overdose of pentobarbital, the tumours were removed, stored in phosphate buffered 10% formalin and embedded in paraffin. Histological sections 4 μm thick were prepared in the same direction as the coronal section of MR images and stained with haematoxylin–eosin and diaminobenzidine-enhanced Perls' Prussian

blue staining and weak haematoxylin counterstaining. The MR findings were compared with the histological findings.

Phantom study

To investigate the relationship between RI and USPIO concentration, contrast agent phantoms were prepared by adding the USPIO into the following five types of reference standard phantom (Table 1): type 1, 0.125 mg ammonium nickel(II) sulfate hexahydrate per litre of deionized water showing intermediate signal intensity similar to the tumour parenchyma and stroma on T_1 weighted images; type 2, deionized water showing low signal intensity similar to the necrotic area on T_1 weighted images; type 3, 120 mg of manganese(II) chloride tetrahydrate per litre of deionized water showing low signal intensity similar to a low intensity component of tumours on T_2 weighted images; type 4, 60 mg of manganese(II) chloride tetrahydrate per litre of deionized water showing intermediate signal intensity similar to a intermediate intensity component of tumours on T_2 weighted images; type 5, deionized water showing high signal intensity similar to a high intensity component of tumours on T_2 weighted images. The concentrations of USPIO were 0 mg, 1 mg, 2 mg, 4 mg, 6 mg, 12 mg, 24 mg, 48 mg, 100 mg and 150 mg Fe ml^{-1} for the contrast agent phantom for T_1 weighted imaging (type 1 and type 2), and 0 mg, 1 mg, 2 mg, 4 mg, 6 mg, 12 mg, 24 mg, 48 mg and 100 mg Fe ml^{-1} for T_2 weighted imaging (type 3, type 4 and type 5). MR images of the phantoms were obtained three times with the same parameters in the mouse study. The RI of the concentration phantom and reference standard phantom was calculated as follows: $RI = \text{signal intensity of concentration phantom} / \text{reference standard phantom} / \text{signal intensity of reference phantom}$.

Results

On pre-contrast T_1 weighted images, all tumours showed homogeneous intermediate signal intensity except one tumour containing a large low signal intensity area. On pre-contrast T_2 weighted images, all tumours showed heterogeneous low-to-high signal intensity.

Table 1 Reference standard phantoms

Type	MRI	SI	RI	Area	Solution (concentration)
1	T_1 weighted image	Intermediate	1.466	Tumour parenchyma and stroma	$(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ (0.125 mg l^{-1})
2	T_1 weighted image	Low	1.000	Necrotic area	Deionized water
3	T_2 weighted image	Low	1.000	Low signal intensity area	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (120 mg l^{-1})
4	T_2 weighted image	Intermediate	3.919	Intermediate signal intensity area	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (60 mg l^{-1})
5	T_2 weighted image	High	12.78	High signal intensity area	Deionized water

RI, relative intensity; SI, signal intensity

Figure 1 (a) Pre-contrast T_1 weighted image shows a tumour with homogeneous low signal intensity (arrow). (b) Post-contrast T_1 weighted image shows areas with increased signal intensity (arrow). (c) Pre-contrast T_2 weighted image shows a tumour with heterogeneous low-to-high signal intensity (arrow). (d) Post-contrast T_2 weighted image does not show any signal intensity change visually (arrow). (e) Histological section demonstrates a small amount of iron particles in stroma around a large vascular space (diaminobenzidine-enhanced Perls' Prussian blue staining and weak haematoxylin counterstaining; original magnification $\times 40$)

Visual analysis

1. T_1 weighted images: In the 2.6 mg Fe kg^{-1} group, one of the three tumours (tumour C) had a large low signal intensity area on the pre-contrast image. The area showed an increase of signal intensity, which was similar to the remaining area, on the post-contrast image. In the 8.5 mg Fe kg^{-1} group, 1 of the 4 tumours (tumour E) had an area showing increased signal intensity. In the 17.0 mg Fe kg^{-1} group, 3 of the 6 tumours (tumours I, K and M) had an area showing increased signal intensity (Figure 1) and 2 (tumours H and J) had an area showing both increase and decrease of signal intensity (Figure 2). The remaining six tumours did not show any signal intensity change.

2. T_2 weighted images: For all tumours in the 2.6 mg and 8.5 mg groups, the signal intensity change could not be detected by visual evaluation on T_2 weighted images. In the 17.0 mg Fe kg^{-1} group, 2 of the 6 tumours (tumours H and J) had areas showing signal intensity decrease on T_2 weighted images (Figure 2). These two tumours had areas showing both increase and decrease of signal intensity on T_1 weighted images. The remaining four tumours did not show any signal intensity change.

Quantitative analysis

Overall RI for a large ROI covering a large area of tumours did not show a significant change on either

T_1 or T_2 weighted images with each dose group ($p > 0.05$) (Table 2). The RI for a large ROI covering large areas of tumours in both T_1 and T_2 weighted post-contrast images did not significantly differ among the three USPIO concentrations ($p > 0.05$). Table 3 lists RI for a specific small ROI set on the area showing either increase or decrease of signal intensity by visual evaluation on T_1 weighted images. Table 4 summarises statistics for RI of the area showing signal intensity increase. The RI showed a significant increase on post-contrast images ($p < 0.05$). Because of the small number of areas (two areas) showing signal intensity decrease on T_1 weighted images, the statistical analysis could not be performed. Table 5 shows RI for a specific small ROI set on the area showing signal intensity decrease on T_2 weighted images. Statistical analysis concerning T_2 weighted images could not be performed because of the small number of tumours that showed signal intensity changes (two tumours).

Histological analysis

In six of the seven tumours containing a signal intensity increased area, a small amount of iron particles was found in the stroma. The remaining tumour (tumour C) contained a large empty area which was considered a liquefactive necrosis. In two tumours with a decreased signal intensity area on both T_1 and T_2 weighted images, a large amount of iron particles was found in the stroma. Mononuclear cell infiltration was

Figure 2 (a) Pre-contrast T_1 weighted image shows a tumour with homogeneous low signal intensity (arrow). (b) Post-contrast T_1 weighted image shows areas with increased signal intensity and with decreased signal intensity (arrow). (c) Pre-contrast T_2 weighted image shows a tumour with heterogeneous low-to-high signal intensity (arrow). (d) Post-contrast T_2 weighted image shows areas with decreased signal intensity (arrow). (e) Histological section demonstrates a large amount of iron particles in stroma (diaminobenzidine-enhanced Perls' Prussian blue staining and weak haematoxylin counterstaining; original magnification $\times 100$)

also found in these areas. These seven tumours contained a minimal amount of iron particles in tumour parenchymal cells. No signal intensity change was detected in 6 of the 13 tumours. Only a minimal amount of scattered iron particles was found in these tumours.

Phantom study

Table 1 shows the RI of the reference standard phantoms. As the USPIO concentration increased, both reference standard phantoms type 1 and type 2 showed RI increase until reaching a peak at the dose of 12 mg Fgl⁻¹, after which RI decreased (Figure 3). With increasing USPIO concentration, reference standard phantom type 3 and type 4 showed RI decrease and reference standard phantom type 5 showed RI increase until reaching a peak at the dose of 4 mg Fgl⁻¹, after which RI decreased (Figure 4).

USPIO concentration in tumours

1. Areas with signal intensity increase on T_1 weighted images: For six of the seven tumours (excluding tumour C) showing signal intensity increase, the pre-contrast RI was 1.265–1.652. The USPIO concentration of the areas was presumed to be approximately 1 mg–2 mg Fgl⁻¹ or 60 mg Fgl⁻¹ using the reference standard phantom type 1, which showed a similar RI value (Figure 3). To determine the USPIO concentration, the RI of the same region on T_2 weighted images was used.

It was difficult to select the appropriate reference standard phantom for T_2 weighted imaging because the pre-contrast RI showed 3.757–7.884. If the USPIO concentration in the same region was supposed to be approximately 60 mg Fgl⁻¹, RI was presumed to be close to 0 on T_2 weighted images (Figure 4). Therefore, the USPIO concentration in the region was thought to be approximately 1 mg–2 mg Fgl⁻¹. The USPIO concentration of the necrotic area (tumour C) was estimated as approximately 1 mg–2 mg Fgl⁻¹ or 60 mg Fgl⁻¹ using the reference standard phantom type 2. The USPIO concentration of this area was presumed to be approximately 1 mg–2 mg Fgl⁻¹ using the reference standard phantom type 5.

2. Areas with signal intensity decrease on T_1 weighted imaging: The USPIO concentration of the area showing signal intensity decrease on T_1 weighted images was estimated as approximately 80 mg Fgl⁻¹ using reference standard phantom type 1. In contrast, using RI in the same region on T_2 weighted images it was estimated as more than approximately 10 mg Fgl⁻¹.

3. Areas with signal intensity decrease on T_2 weighted imaging: Concerning the area showing signal intensity decrease on T_2 weighted images, pre-contrast RI of tumours H and J did not have equivalent values to the reference standard phantom type 3 and type 4 and type 3, respectively. The USPIO concentrations of tumours H and J were presumed to be approximately 12 mg Fgl⁻¹ using the reference standard phantom type 5 and

Table 2 Overall relative intensity for large region of interest covering a large area of tumours

USPIO dose (mg Fe kg ⁻¹)	Tumour	<i>T</i> ₁ weighted image		<i>T</i> ₂ weighted image	
		Pre-contrast RI	Post-contrast RI	Pre-contrast RI	Post-contrast RI
2.6	A	1.755	2.019	3.597	6.268
	B	1.075	1.480	4.244	5.761
	C	2.008	2.030	8.587	7.239
	Mean ± SE	1.613 ± 0.279	1.843 ± 0.182	5.476 ± 1.567	6.423 ± 0.434
8.5	D	2.240	1.592	9.374	8.396
	E	1.479	1.498	7.526	7.404
	F	1.288	1.148	5.328	4.404
	G	1.290	1.401	4.471	4.199
	Mean ± SE	1.574 ± 0.226	1.410 ± 0.956	6.675 ± 1.106	6.101 ± 1.509
	H	1.577	1.812	6.303	5.172
17.0	I	1.510	1.467	5.020	4.846
	J	1.577	1.363	3.057	1.695
	K	1.208	1.408	5.738	5.088
	L	1.138	1.566	3.789	5.946
	M	1.682	1.927	3.740	5.188
	Mean ± SE	1.449 ± 0.090	1.591 ± 0.973	4.608 ± 0.521	4.656 ± 0.611

RI, relative intensity; SE, Standard error; USPIO, ultra-small super-paramagnetic iron oxide

approximately 10 mg Fe l⁻¹ type 4 and 24 mg Fe l⁻¹ type 5, respectively. On the other hand, using RI in the same region on *T*₁ weighted images, the USPIO concentration was presumed to be more than approximately 65 mg Fe l⁻¹.

Discussion

7 of the 13 tumours contained an area showing signal intensity increase on post-contrast *T*₁ weighted images by visual analysis. The post-contrast RI of the specific small ROI on these areas showed a significant increase compared with the pre-contrast RI (*p* < 0.05). This suggested that the signal intensity change caused by USPIO could be detected on MR images, and it was confirmed by the histological findings that iron particles were found in these areas. The increased signal intensity indicated a *T*₁ shortening effect of the USPIO. A low concentration of USPIO on *T*₁ weighted sequences was reported to induce positive MR signal

enhancement;¹² therefore, a small amount of iron particles was sufficient to cause a *T*₁ shortening effect. The two tumours showing signal intensity change on *T*₁ weighted images also showed signal intensity change on *T*₂ weighted images: both of these tumours had areas of signal intensity decrease. Histologically these tumours had areas containing a large amount of USPIO and thus a signal intensity change could be detected on both *T*₁ and *T*₂ weighted images. At high concentrations of USPIO, the signal intensity decreases on *T*₁ weighted images.¹² Larger amounts of USPIO particles were found histologically in the areas showing signal intensity decrease on *T*₁ weighted images than in areas of increase. On the other hand, overall RI did not show any significant change on either *T*₁ or *T*₂ weighted images. This was probably owing to the following two reasons: the sizes of these areas showing signal intensity change were too small to show overall RI change by quantitative analysis, and some tumours contained the areas showing signal intensity increase as well as decrease.

Table 3 Relative intensity for a specific small region of interest set on the area showing either increase or decrease on post-contrast *T*₁ weighted image and the corresponding area on pre-contrast *T*₁ weighted image

USPIO dose (mg Fe kg ⁻¹)	Tumour	SI change	<i>T</i> ₁ weighted image		<i>T</i> ₂ weighted image	
			Pre-contrast RI	Post-contrast RI	Pre-contrast RI	Post-contrast RI
2.6 (<i>n</i> = 1)	C	Increase	1.516	2.084	15.98	14.87
		No change	2.013	2.054	8.624	7.098
8.5 (<i>n</i> = 1)	E	Increase	1.477	2.041	7.884	11.57
		No change	1.493	1.516	6.484	6.878
17.0 (<i>n</i> = 5)	H	Increase	1.565	2.281	6.562	6.301
		Decrease	1.610	1.075	6.332	2.537
		No change	1.562	1.749	6.283	5.310
	I	Increase	1.465	1.856	5.723	5.825
		No change	1.520	1.383	3.941	4.862
		Decrease	1.638	1.826	3.757	1.907
	J	Increase	1.620	0.714	3.059	0.822
		Decrease	1.265	1.696	6.330	5.274
	K	Increase	1.131	1.333	5.536	5.063
		No change	1.652	2.273	3.922	5.583
	M	No change	1.670	1.691	3.333	4.191

n, number; RI, relative intensity; SI, signal intensity; USPIO, ultra-small super-paramagnetic iron oxide

Table 4 Statistics for specific small region of interest of the area showing signal intensity increase on T_1 -weighted image

SI change	T_1 weighted image		p-Value	T_2 weighted image		p-Value
	Pre-contrast RI	Post-contrast RI		Pre-contrast RI	Post-contrast RI	
	(mean $\pm$ SE)	(mean $\pm$ SE)		(mean $\pm$ SE)	(mean $\pm$ SE)	
Increase ($n = 7$)	1.511 $\pm$ 0.495	2.008 $\pm$ 0.852	<0.05	7.165 $\pm$ 1.570	7.332 $\pm$ 1.654	0.826
No change ($n = 6$)	1.565 $\pm$ 0.117	1.621 $\pm$ 0.109	0.322	5.702 $\pm$ 0.781	5.557 $\pm$ 0.475	0.758

n , number; RI, relative intensity; SE, Standard error; SI, signal intensity

The number of tumours showing signal intensity change was seven on T_1 weighted images but only two on T_2 weighted images. Five of the seven tumours showed a signal intensity increase on T_1 weighted images but not on T_2 weighted images. The USPIO concentrations of these areas were presumed low. Figure 4 shows that the RI on T_2 weighted images was low using the concentration phantom type 3, intermediate using the concentration phantom type 4 and high using the concentration phantom type 5 at low USPIO concentration. The signal intensity change could thus not be detected by visual analysis at low USPIO concentrations on T_2 weighted images despite being detected on T_1 weighted images. It has been reported that the T_1 effect prevails over the T_2 effect at low USPIO concentration.¹³ Our result was similar to the previous study. T_1 weighted sequences thus seem to be more effective than T_2 weighted sequences for detecting a small amount of USPIO in tumours.

The RI for large ROIs covering large areas of tumours in both T_1 and T_2 weighted post-contrast images did not significantly differ among the three USPIO concentrations ($p > 0.05$). However, areas of decreased signal intensity on T_2 weighted images appeared only from tumours in the high dose (17.0 mg Fe kg⁻¹ of USPIO) group. This finding suggests a relationship between the USPIO concentration and RI on post-contrast images. Zimmer *et al*⁴ found tumoral USPIO uptake in a glioma model, particularly when a higher dose was applied. Our findings were similar to these. The number of tumours in which signal intensity changed increased as the dose of USPIO increased; the signal intensity of tumours with the highest dose decreased on T_2 weighted images, whereas that of the others did not. Histologically, tumours had areas containing a large amount of USPIO, whereas the latter had areas with a small amount of USPIO. However, further studies will be

required to determine the influence of the internal heterogeneity of each tumour on the RI.

Gradient-echo sequence imaging is more sensitive to the magnetic susceptibility effects of USPIO than spin-echo sequence imaging. However, Hudgins *et al*¹⁰ found that signal intensity decreased on both T_2 weighted fast spin-echo and on T_2^* weighted gradient-echo images after administering USPIO for nodal evaluation. We could not obtain T_2^* weighted gradient-echo images with high spatial resolution using our MR system. However, we consider that the findings might be similar to those on T_2 weighted fast spin-echo images. Small amounts of USPIO can be visualized more sensitively on T_1 weighted spin-echo than T_2^* weighted gradient-echo images.¹³ Thus, changes in signal intensity might not have been detectable in the five tumours in which signal intensity changed only on T_1 weighted spin-echo images. However, further studies are required to confirm this notion.

The presumed USPIO concentration in the area showing signal intensity decrease was higher than that which increased. However, using reference standard phantoms for T_1 weighted imaging, the value differed from those using reference standard phantoms for T_2 weighted imaging. This may have been because the tumour showed homogeneous signal intensity on pre-contrast T_1 weighted images, but heterogeneous low-to-high intensity on pre-contrast T_2 weighted images. Thus, the heterogeneity of tumour signal intensity on pre-contrast T_2 weighted images prevented selection of the appropriate standard reference phantom for estimation of USPIO concentration and made it difficult to accurately estimate the USPIO concentration. The contrast agent exists homogeneously in the concentration phantoms but heterogeneously in tumour tissues. This discrepancy may also affect the estimation of USPIO concentrations in tumours.

Table 5 Relative intensity for a specific small region of interest set on the area showing signal intensity decrease on post-contrast T_2 weighted image and the corresponding area on pre-contrast T_2 weighted image

USPIO dose (mg Fe kg ⁻¹)	Tumour	SI change	T_2 weighted image		T_1 weighted image	
			Pre-contrast RI	Post-contrast RI	Pre-contrast RI	Post-contrast RI
17.0 ($n = 2$)	H	Decrease	6.254	4.221	1.556	1.562
		No change	6.730	6.063	1.620	2.196
	J	Decrease	3.089	1.453	1.625	1.212

n , number; RI, relative intensity; SI, signal intensity; USPIO, ultra-small super-paramagnetic iron oxide

Figure 3 Graph shows relative intensity of reference standard phantom and concentration phantoms type 1 and type 2 on T_1 weighted images. The data are represented as the mean and standard deviation of three measurements

Histological observation revealed iron particles mainly in tumour stroma, while those in tumour parenchymal cells were limited and much smaller. This finding suggested that the amount of USPIO in tumour parenchymal cells was not sufficient to induce signal intensity changes. Moore *et al*⁵ demonstrated that iron particles were identified in stroma but preferentially located in tumour parenchymal cells in a gliosarcoma rodent model. Differences of the iron uptake into the tumour parenchymal cells may be related to the tumour type. One tumour containing a large area showed a signal intensity increase on post-contrast T_1 weighted images. This corresponded histologically to an empty area and was probably a liquefactive necrosis. The liquefactive necrotic area might have contained some USPIO that was lost during the preparation of histological sections.

Our study had limitations. This study was performed in a small number of animals. This was mainly because some mice died before they were imaged. One reason may be that the tumour implanted into the oral cavity prevented feeding. The spatial resolution of the MR images was not sufficient to differentiate tumour parenchyma from stroma, and therefore it was difficult to compare the MRI findings with histological findings completely. Our study was unable to establish the influence of tumour-associated macrophages because

Figure 4 Graph shows relative intensity of reference standard phantom and concentration phantom type 3, type 4 and type 5 on T_2 weighted images. The data are represented as the mean and standard deviation of three measurements

we used nude mice. To investigate this influence, further studies using other animals and immunostaining will be needed.

In conclusion, for oral-transplanted human squamous cell carcinoma, the signal intensity of some tumours was significantly increased after USPIO administration on T_1 weighted images. The histological observation showed that this was mainly caused by the USPIO accumulation in the tumour stroma. Some tumours showed decreased signal intensity on T_1 and T_2 weighted images and large USPIO accumulation in the stroma was found histologically. The USPIO accumulation in the tumour parenchyma was relatively small, and thus could not affect the signal intensity change. One tumour contained a large necrotic area; this showed increased signal intensity on T_1 weighted images, which might have been caused by the accumulation of USPIO.

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