

ORIGINAL ARTICLE

Comparison of [18F]-FMISO, [18F]-FAZA and [18F]-HX4 for PET imaging of hypoxia – a simulation study

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ABSTRACT

Background. To investigate the effect of hypoxia tracer properties on positron emission tomography (PET) image quality for three tracers [18F]-fluoromisonidazole (FMISO), [18F]-fluoroazomycinaraboside (FAZA) and [18F]-flortanidazole (HX4), using mathematical simulations based on microscopic tumor tissue sections.

Material and methods. Oxygen distribution and tracer binding was mathematically simulated on immunohistochemically stained cross-sections of tumor xenografts. Tracer diffusion properties were determined based on available literature. Blood activity and clearance over a four-hour period post-injection (p.i.) were derived from clinical dynamic PET scans of patients suffering from head and neck or bronchial cancer. Simulations were performed both for average patient blood activities and for individual patients, and image contrast between normoxic and hypoxic tissue areas was determined over this four-hour period p.i.

Results. On average, HX4 showed a six-fold higher clearance than FMISO and an almost three-fold higher clearance than FAZA based on the clinical PET data. The absolute variation in clearance was significantly higher for HX4 than for FMISO (standard deviations of $5.75 \cdot 10^{-5}$ s⁻¹ vs. $1.55 \cdot 10^{-5}$ s⁻¹). The absolute tracer activity in these scans at four hours p.i. was highest for FMISO and lowest for HX4. Simulated contrast at four hours p.i. was highest for HX4 (2.39), while FMISO and FAZA were comparable (1.67 and 1.75, respectively). Variations in contrast of 7–11% were observed for each tracer depending on the vascularization patterns of the chosen tissue. Higher variations in clearance for HX4 resulted in an increased inter-patient variance in simulated contrast at four hours p.i.

Conclusions. In line with recent experimental and clinical data, the results suggest that HX4 is a promising new tracer that provides high image contrast four hours p.i., though inter-patient variance can be very high. Nevertheless, the widely used tracer FMISO provides a robust and reproducible signal four hours p.i., but with a lower contrast. The simulations revealed tracer clearance to be the key factor in determining image contrast.

Tumor cell hypoxia causes radiation resistance compared to normoxic cells resulting in a negative impact on outcome of anticancer treatments, including radiotherapy [1]. Assessment of presence, quantity and localization of hypoxia prior to and during treatment has been a subject of ongoing research [2]. These studies do not only investigate its value as a prognostic factor, but also as a predictive factor for allowing for patient stratification and treatment

adjustments that could potentially overcome hypoxia-induced radioresistance. These modifications include treatment approaches, such as increasing the oxygen supply to tumor cells, e.g. through hyperbaric oxygen or carbogen breathing, or administering hypoxic cell radiosensitizers, such as nimorazole [3]. Another frequently suggested option is escalating delivered radiation dose specifically to hypoxic sub-volumes inside the tumor, a process referred to as

dose painting. The establishment of intensity-modulated radiotherapy and other high-precision radiotherapy techniques in routine clinical practice has made such an approach technically feasible [2].

However, there is no clinically validated gold standard to non-invasively identify tumor hypoxia at present [3]. Frequently, dedicated tracers for positron emission tomography (PET) that accumulate specifically in hypoxic tissue volumes are used for non-invasive hypoxia imaging. In contrast to most invasive methods, hypoxia PET imaging provides a spatially resolved quantitative image of tissue oxygenation [4].

Many of the hypoxia-related PET tracers are [^{18}F]-labeled nitroimidazole compounds, including [^{18}F]-fluoromisonidazole (FMISO), which has been used in many clinical and pre-clinical studies [5,6]. Due to the low signal-to-background ratios of FMISO PET, alternative hypoxia tracers have been proposed. One of them, [^{18}F]-fluoroazomycinaraboside (FAZA), is a focus of current research due to its higher clearance and has found its way into clinical trials [7,8]. The tracer [^{18}F]-flortanidazole, commonly referred to as HX4, is also a nitroimidazole compound. Similar to FAZA, it is more water-soluble than FMISO [9], and a first study in 12 head and neck squamous cell carcinoma (HNSCC) patients has shown better tumor-to-muscle ratio in HNSCC tumors compared to FMISO [10].

Attempts to quantify and compare the imaging properties for these tracers are ongoing, and several of them have been compared in preclinical and clinical studies [10–12]. However, the results of these studies are non-conclusive, and further investigations are warranted before deciding for the optimal tracer and to facilitate comparative analyses from studies using different tracers.

In addition to pre-clinical and clinical studies, mathematical simulations have been used as a more theoretical approach to investigate the imaging properties of hypoxia PET tracers under a variety of conditions [13]. For simulating oxygen and tracer distributions in the tumor microenvironment, several approaches have been used. These included methods using Green's function, finite difference and finite element methods [13,14], or the heat kernel [15], carried out in two-dimensional (2D) [13,14] or 3D [15,16].

The hypothesis of this study is that the imaging characteristics of hypoxia PET tracers vary according to their respective physical and chemical properties and can therefore be studied based on mathematical simulations. Major goal was to investigate the imaging properties of the tracers FMISO, FAZA, and HX4 by mathematically simulating their diffusion-retention properties on the basis of 2D models of

tumor vasculature. Using this method, the interdependence between clearance, diffusion and image contrast was investigated.

Material and methods

Dedicated hypoxia PET tracers are bound to cells depending on the local oxygen concentration. Therefore, to simulate hypoxia PET signals, a two-step process is used: 1) Simulation of the diffusive oxygen supply from blood vessels that is in equilibrium with cellular oxygen consumption (steady state); and 2) modeling of tracer diffusion and retention depending on its specific diffusion and clearance properties as well as the previously simulated oxygen distributions. In this work, a mathematical model has been used which has been described in detail before [13]. A brief description of the model is given here.

Simulation of steady-state oxygen distribution

Oxygen distribution is described as the steady-state of oxygen diffusion and consumption, which can be modeled by a reaction diffusion equation [13], where oxygen consumption was modeled by a Michaelis-Menten relationship:

$$\frac{\partial P}{\partial t} = D_{O_2} \cdot \nabla^2 P - \frac{M_0 \cdot P}{P + P_0}$$

where P is the local oxygen partial pressure, P_0 is the Michaelis-Menten coefficient of oxygen consumption, D_{O_2} is the diffusion coefficient and M_0 is maximum oxygen consumption rate. This diffusion problem can be solved by using a Gaussian fundamental solution [15]:

$$G_{O_2}(x, y, t) = k_{O_2} \cdot t^{-1} \cdot e^{-\frac{(x^2 + y^2)}{4 \cdot D_{O_2} \cdot t}}$$

where D_{O_2} is the diffusion coefficient and k_{O_2} is a normalization factor chosen in a way that

$$\iint_{0,0}^{\infty,\infty} G_{O_2}(x, y, t) dx dy \equiv 1$$

Using the fundamental solution of the diffusion equation assumes that the initial distribution at $t = 0$ can be described as a δ -function. As each pixel on the vessel map was regarded as a separate oxygen source of a size of approximately $2.5 \times 2.5 \mu\text{m}^2$ and thus much smaller than the distance of hypoxic regions from the vessels, this was judged to be a valid approximation [15]. Therefore, if the initial pO_2 distribution is known, pure diffusion at any point in time can be determined by convolving the initial oxygen distribution with the diffusion kernel.

However, since the oxygen consumption is a function of oxygen partial pressure, the model was run repeatedly with time steps of 10 ms, as proposed in a previous study [15]. Oxygenation was calculated iteratively for each pixel, taking into account the local oxygen consumption. Within blood vessels, the initial value of $P = 40$ mmHg was kept constant. The simulation was repeated until a steady state was reached, i.e. a change of less than 0.1% between two iterations was used as a convergence criterion.

Our simulations were carried out in 2D, therefore, the following equations are specific for 2D, but the model can easily be extended to perform 3D simulations [15].

Simulation of hypoxia tracer distribution and uptake

Similar to the oxygen distribution, the distribution and uptake of hypoxia PET tracers was simulated by a numerical solution of the respective reaction-diffusion equation [13], where C_f is the concentration of free tracer and $K(P)$ accounts for tracer binding depending on oxygen levels and cell viability:

$$\frac{\partial C_f}{\partial t} = D_T \nabla^2 C_f - K(P) C_f$$

Nitroimidazoles are transported through tumor tissue solely by diffusion. Therefore, the fundamental solution of the diffusion equation was also applied here to create a convolution kernel analogous to the oxygenation model, with time steps of 1 second:

$$G_T(x, y, t) = k_T \cdot t^{-1} \cdot e^{-\frac{(x^2 + y^2)}{4 \cdot D_T \cdot t}}$$

The method gave 2D results comparable to the well established but computationally more expensive finite element method that was used in [13].

Blood activity curves

The blood activity curves that were used as input functions for the simulations were derived from clinical dynamic PET images (FMISO: $n = 10$, FAZA: $n = 3$, HX4: $n = 5$).

FMISO and FAZA scans were acquired in a GE Advance scanner (GE Medical Systems, Milwaukee, WI, USA). Images were acquired in dynamic acquisition mode with a slice thickness of 4.25 mm, and a pixel spacing of 4.3 mm. Reconstruction was performed using OSEM 2D with four iterations and eight subsets. A scatter correction (convolution subtraction) was applied. The FMISO group included 10 patients aged 57–71 years, including seven men and three women, while the FAZA group consisted of three men aged 58–66 years, both suffering from HNSCC.

For HX4, five non-small cell lung cancer (NSCLC) patients were imaged in a Philips Gemini TF 64 PET/CT scanner (Philips Healthcare, Best, The Netherlands). The imaging parameters were set to an axial field of view of 18 cm, slice thickness of 4 mm, in plane pixel spacing of 4 mm, and a spatial resolution of approximately 5 mm FWHM. CT-based attenuation correction and scatter correction (SS-SIMUL) were performed. Image reconstruction was performed using 3D ordered-subset iterative time-of-flight reconstruction technique (BLOB-OS-TF) using three iterations and 33 subsets. The field-of-view for computed tomography (CT) and PET imaging was positioned on the primary tumor. The five patients imaged with HX4 were aged between 59 and 71 years.

From each scan, the tracer concentration in the blood was determined at 20–45 time points over a period of four hours post-injection (p.i.). Blood activity for a given time point was determined by averaging over the activity of six voxels in the heart or a major blood vessel for each patient. This was followed by fitting to the following function to the data:

$$A(t) = A_1 \cdot e^{-\tau_1 \cdot t} + A_2 \cdot e^{-\tau_2 \cdot t}$$

For each parameter, the average was taken over all patients of the same group to derive an average input function (AIP) for all three tracers individually. For the calculations of the standard deviation, the 95% confidence interval of each fit was taken into account.

Parameters

The oxygen and hypoxia PET tracer model parameters used for these simulations were derived from previous publications and are listed in Table I.

Tracer diffusion coefficients were calculated as average of the two formulas presented by Pruijn et al. [17], which estimate the diffusion coefficient of tirapazamine analogs through multicellular layers based on their partition coefficient and molecular structure.

Partition coefficients were taken from literature, except for FAZA, for which published values varied by several orders of magnitude [11,18]. Therefore, we used the simulation softwares XLOGP3, ALOGPS 2.1, Molinspiration and LogKow and took the average of the simulated values as suggested in [19].

The parameter $P_{1/2}$, the pO_2 at which the FMISO binding rate is half-maximal, was taken from [13] and [20]. For FAZA and HX4, the same parameter was used as their uptake follows the same binding mechanism [3,7].

Table I. Simulation parameters used for calculation of the steady state oxygen and tracer distribution. Bold font indicates the value chosen for this simulation out of a range of values.

Symbol	Meaning	Value
P_{ic}	Intraerythrocyte pO_2 in tumors	40 mmHg [13,14]
D_{O_2}	O_2 diffusion coefficient	$2 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$ [13]
M_0	Maximum O_2 consumption rate	15 mmHg/s [13,14]
P_0	Michaelis-Menten coefficient of oxygen consumption	2.0–2.5 mmHg [13,14]
		FMISO FAZA HX4
M	Molecular weight	189.14 247.18 269.22
D_T	Tracer diffusion coefficient [$\cdot 10^{-11} \text{ m}^2/\text{s}$]	7.90 2.99 3.55 [17]
P	Partition coefficient	0.41 0.30 0.20 [9,21]
P_1	pO_2 inhibiting binding by 50%	0.8–1.5 mmHg [7,13,20]

Simulation details

All simulation steps were implemented in MATLAB R2009b.

Simulations were carried out on vessel maps derived from tumor xenografts which were immunohistochemically stained for CD31, a marker of endothelial tissue. Tissue sections were obtained as described before [4]. Based on this, simulations of oxygen distributions and tracer diffusion-retention over a period of four hours p.i. were performed for six different vessel maps.

Vessel maps were divided into $1 \times 1 \text{ mm}^2$ subsections. For each of those, the median pO_2 (mp O_2) and tracer activity was determined. Sections with an mp O_2 of 35 ± 0.2 mmHg and 2.5 ± 0.2 mmHg were chosen to represent normoxic and hypoxic tissue areas, respectively. Their mean tracer activities at different time points p.i. were then used to generate normoxic and hypoxic time activity curves (TACs).

Image contrast ($K(t)$) was defined as the ratio between the mean signal in hypoxic tissue (S_{hyp}) and normoxic tissue (S_{norm}).

$$K(t) = \frac{S_{hyp}(t)}{S_{norm}(t)}$$

TACs and contrast results were compared using a Wilcoxon rank-sum test for median and a Levene test for variance using MATLAB R2009b. p-Values equal or lower than 0.05 were considered significant.

Results

Blood activities

For all three investigated hypoxia PET tracers, the average fit parameters for the input functions are listed in Table II. HX4 has the highest clearance rate

Table II. Average fit parameters for blood activity of the three tracers and their standard deviation.

	A_1 (kBq/ml)	τ_1 ($\cdot 10^{-3} \text{ s}^{-1}$)	A_2 (kBq/ml)	τ_2 ($\cdot 10^{-5} \text{ s}^{-1}$) (clearance rate)
FMISO	18.11 ± 7.08	2.0 ± 0.71	10.93 ± 2.49	1.90 ± 1.55
FAZA	13.1 ± 4.5	1.3 ± 0.6	13.1 ± 3.70	4.27 ± 1.51
HX4	33.55 ± 12.81	3.1 ± 0.94	12.31 ± 3.27	11.78 ± 5.75

($\tau_{2,HX4} = 11.8 \cdot 10^{-5} \text{ s}^{-1}$), followed by FAZA ($\tau_{2,FAZA} = 4.3 \cdot 10^{-5} \text{ s}^{-1}$) and FMISO ($\tau_{2,FMISO} = 1.9 \cdot 10^{-5} \text{ s}^{-1}$). Clearance is significantly different for all tracers. τ_1 , the tracer wash-in, was significantly higher for HX4 than for FMISO ($p = 0.037$). HX4 also showed the highest inter-patient variation in clearance, which was significantly higher than for FMISO ($p = 0.047$). All other parameters were not significantly different.

The resulting AIPs with the respective 95% confidence intervals are presented in Figure 1.

Hypoxia contrast for AIPs

Figure 2 shows the simulated distributions for FMISO, FAZA and HX4 at two and four hours p.i. for the same xenograft tumor section.

Tracer retention in viable hypoxic tissue areas is already distinguishable at two hours p.i. for all tracers. The distribution patterns for FMISO and FAZA appear almost identical. In contrast, for HX4, the background activity is lower due to the fast tracer clearance. Consequently, less HX4 is retained than FMISO and FAZA, because less unbound HX4 was available during the uptake period. At four hours p.i.,

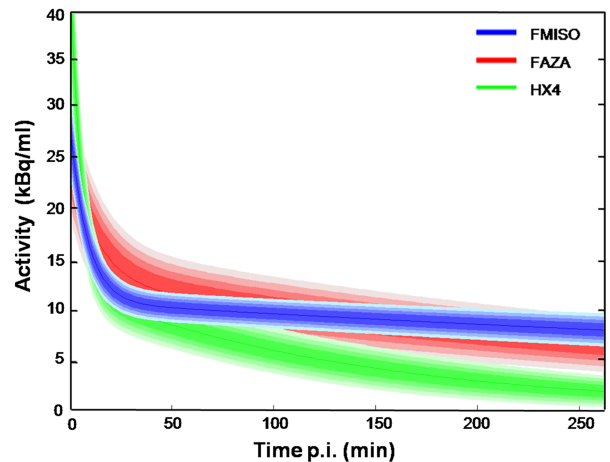


Figure 1. Average input function (AIP) for FMISO, FAZA and HX4 over an interval of 4 h post-injection (p.i.). At 4 h p.i., FMISO shows the highest blood activity, followed by FAZA and HX4, reflecting their respective clearance rates (Parameter τ_2). Shaded areas indicate the respective 95% confidence intervals.

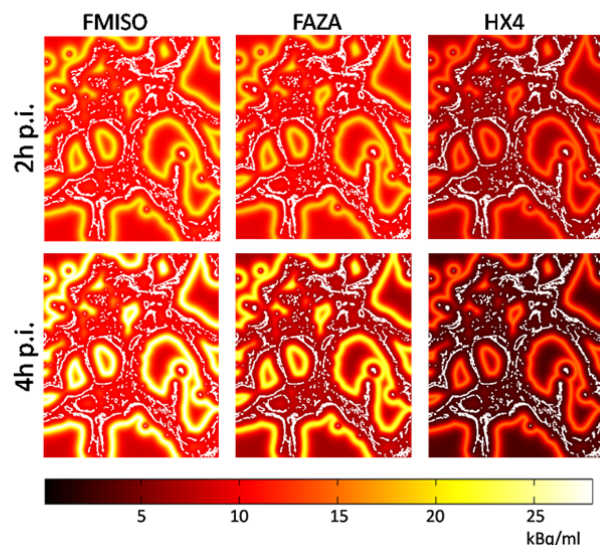


Figure 2. Representative simulated activity distributions using average input functions for FMISO, FAZA and HX4 on the same tissue section 2 and 4 h post-injection. Vessels are highlighted in white.

the tracer wash-out caused a decrease in background signal, which is more pronounced for faster clearing tracers. Meanwhile, tracer binding in hypoxic cells continued. This leads to a net increase of signal in viable hypoxic areas for FMISO, due to the larger supply of free tracer in the blood, and a constant signal for the faster-clearing tracer HX4.

The simulated contrast $K(t)$ of the image signal between hypoxic and normoxic tissue over time is shown in Figure 3. For the first hour p.i., there is little difference in contrast which may be due to a low overall effect of the difference in diffusivity of

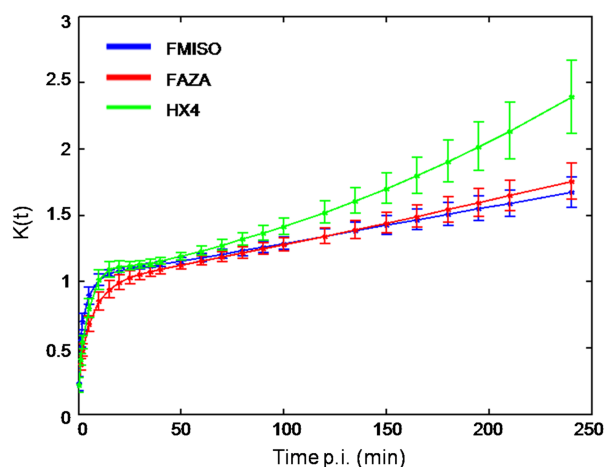


Figure 3. Simulated hypoxia (2.5 ± 0.2 mmHg) to normoxia (35 ± 0.2 mmHg) image contrast for the three tracers over an interval of 4 h post-injection. Error bars reflect variations due to different vascularization patterns in tissue sections with identical median oxygen partial pressure.

the tracer. For all tracers, contrast continues to increase over time. A faster increase can be observed for tracers with a higher clearance rate, due to a faster elimination of free tracer and a resulting decrease of background signal. In comparison to FMISO, HX4 and FAZA show an image contrast four hours p.i. that is 43% and 3% higher, respectively. Variations in the vascularization patterns of tissue sections with the same median pO_2 can affect overall tracer retention in that section. This leads to a standard deviation for contrast at four hours p.i., which is smallest for FMISO (6.9%) and largest for HX4 (11.4%).

Patient-specific input functions

Furthermore, the influence of variations between patient-specific input functions on the resulting contrast four hours p.i. was investigated. Each input function results in a different simulated contrast. Contrast distributions for each tracer are shown in Figure 4.

HX4 shows the highest median contrast (2.08, range 1.87–2.73) over all patients, followed by FAZA (1.64, range 1.60–1.70) and FMISO (1.58, range 1.54–1.64), reflecting the clearance behavior of each tracer. The differences for the median contrasts tested significant for FMISO and HX4 ($p = 0.001$), as well as FAZA and HX4 ($p = 0.036$), but not for FMISO and FAZA ($p = 0.161$).

Of all tracers, HX4 showed the largest absolute contrast range, which was significantly larger than for FMISO ($p = 0.025$). This reflects a larger inter-patient variation of clearance observed for HX4 which may be due to differences in renal and hepatic clearance between patients. Other significant range differences were not observed.

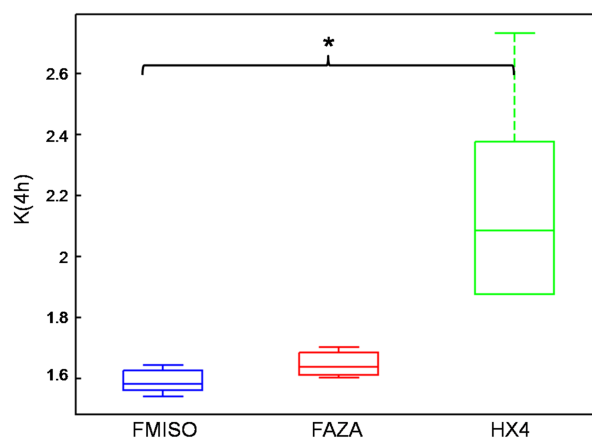


Figure 4. Boxplots representing the distributions of contrast 4 h post-injection. Contrast was simulated individually for each patient. The asterisk indicates significant differences in variation.

Discussion

In this study, we compared three nitroimidazole-based hypoxia PET tracers, which show similar binding properties in hypoxic cells but differ in their diffusion and clearance characteristics. We could show that both parameters have an impact on final image contrast.

Based on blood input functions from clinical dynamic PET data, HX4 shows the highest clearance, followed by FAZA and FMISO. This corresponds well to previous clinical and preclinical studies, in which it was found that HX4 has a higher clearance than FMISO, which ultimately leads to higher image contrast [9,10,21]. Chen et al. [10] reported that image contrast for HX4 1.5 hours p.i. is the same as for FMISO two hours p.i.. This finding was corroborated by our simulations based on average patient input functions as shown in Figure 3.

Tracer uptake in hypoxic cell culture is the same for FMISO and FAZA, suggesting equal nitroreductase affinity for both tracers, which was one of the working assumptions for the simulations. Comparisons between FMISO and FAZA based on patient data are currently not available. However, overall activity is lower for FAZA than for FMISO in pre-clinical studies, indicating that mechanisms other than enzyme activity, such as tracer clearance and diffusion, are responsible for this observation [11,12,22]. The well established hypoxia tracer FAZA has been argued to show better imaging properties than FMISO due to its higher hydrophilicity and increased clearance, although results from earlier pre-clinical studies directly comparing the two tracers regarding better contrast behavior remain non-conclusive [11,18].

Recently, two preclinical studies have been published that directly compared the imaging properties of the three tracers in the same tumor. Carlin et al. [12] showed a clear superiority of FMISO to HX4 and FAZA in murine models 90 minutes p.i. both in terms of contrast and SUV. Another study by Peeters et al. [22] conducted in rats three hours p.i. found HX4 to be the superior tracer with respect to image contrast, followed by FAZA and FMISO, which is in good agreement with our simulation study. HX4 also showed a faster elimination from the blood as well as lower overall activities and larger ranges compared to FMISO, and the TACs shown in the publication are similar to the simulated activities we observed.

Additionally, the study by Carlin et al. [12] showed good correlation between pimonidazole and all three hypoxia PET tracers, indicating that they accumulate in roughly the same areas and at similar levels of hypoxia, which agrees well with our assumption that the parameter P_1 , which defines the pO_2

inhibiting tracer binding by 50%, is roughly the same for all three tracers.

In the simulations presented here, contrast four hours p.i. between FMISO and FAZA showed differences of only a few percent despite the fact that FAZA has a significantly higher clearance. When actual patient input functions were used, no significant difference in contrast four hours p.i. was observed. The lower diffusion coefficient of FAZA leads to a decrease in tracer binding that largely negates the positive influence of its higher clearance rate.

The simulations showed that a higher clearance rate leads to lower absolute activities for both FAZA and HX4. Moreover, HX4 showed both the highest clearance and the largest contrast, but also the lowest absolute activity, both in normoxic and hypoxic tissue areas. This has a negative impact on the signal-to-noise ratio (SNR), particularly since hypoxia PET inherently suffers from a low SNR. One option to improve SNR could be to increase the injected activity, however, this would lead to a higher radiation dose to the patient. Another option could be to prolong acquisition time [21].

Diffusion coefficients were calculated using a previously published model. Our value for FMISO was reasonably close to the experimental value for the related compound misonidazole ($D = 5.5 \cdot 10^{-11} \text{ m}^2/\text{s}$) [13]. Consequently, it was assumed that the approach is valid for FAZA and HX4 as well. However, diffusion can vary significantly between different tumor types and even throughout a tumor volume [17]. Nonetheless, the simulation model is insensitive to changes in diffusion coefficient, with activity changes of less than 5% at two hours p.i. for variations of 50% in the diffusion coefficient. Therefore, any errors caused by the modeling of partition coefficients and, based on this, the diffusion coefficient, should have a negligible impact on the simulation results.

Regarding the use of parameters derived from literature, there is a large range of values particularly for the parameters used for the simulation of oxygen distribution. Most of this variation is likely to be caused by variation between cell lines. We found that while changes in these parameters sometimes have a visible impact on tracer retention and image contrast, they affect all tracers equally. This ultimately leads to no change in the ranking of tracers regarding image contrast and image contrast variation.

Even though the simulations shown here were performed in 2D only, the method can easily be extended to 3D, and has originally been developed for this purpose [15]. Previous publications have shown that oxygen distributions do not strongly depend on whether simulations have been carried out in 2D or 3D [23]. This suggests that 2D

simulations are an adequate and computationally far less expensive approximation.

Two sources of signal uncertainties were investigated: variations of vascular patterns in tissue areas with approximately the same median pO_2 , and variations of blood activity curves between patients. Changes in the vascular pattern introduced absolute signal changes of 5–8%, which can lead to contrast variations of 7–11%. HX4 showed the highest susceptibility to these contrast changes, but the overall differences between tracers were too small to justify a preference for one tracer over the others.

More relevant for clinical application is the inter-patient variability of clearance. FMISO is eliminated mostly through the entero-hepatic pathway, while HX4 and FAZA are also cleared via renal excretion [24]. HX4 clearance and, to a lesser extent, FAZA clearance is therefore also dependent on patient kidney function, and may hence affect the suitability for patients treated with (concurrent) nephrotoxic systemic agents. This adds variability to the total rate at which the tracer is cleared from the system, resulting in larger absolute ranges of clearance rates. Consequently, the final contrast four hours p.i. between hypoxic and normoxic tissue areas varies much more for HX4 than for FMISO. This can be of concern for clinical imaging since the large differences between patients make it difficult to find a reliable threshold value to define tumor hypoxia.

Acquisition modes and patient collectives varied between institutions. This might affect the PET scans used for the determination of blood input functions. Only blood activity was determined from the scans, which should be mostly unaffected by patient gender or the type of tumor. Test runs showed that changes in relative signal which may result from different acquisition modes have no impact on the simulated contrast. Nonetheless, an impact on the results cannot be completely ruled out. Also, the number of patients in this study is rather small, particularly for FAZA and HX4. Therefore, a preclinical or even clinical validation of the simulation results by conducting PET imaging of all three tracers in the same test subject would be required to fully validate the results of this simulation study.

Conclusion

According to the simulations in this study, HX4 showed the highest clearance and image contrast and the lowest background signal, followed by FAZA and FMISO, but also the largest variance between patients, both in clearance and contrast. Differences in vascularization patterns are an additional source of signal variance, causing an uncertainty of 7–11%. HX4 is a promising tracer providing PET images

with a higher contrast than FAZA and FMISO, whereas FMISO is a very reproducible hypoxia tracer, however with a lower image contrast. The results agree well with previously published preclinical data, showing that tracer clearance is the key factor in determining image contrast.

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