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[^{18}F]Fluoro-azomycin-2´-deoxy-β-D-ribofuranoside — A new imaging agent for tumor hypoxia in comparison with [^{18}F]FAZA☆,☆☆

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ABSTRACT

Introduction: Radiolabeled 2-nitroimidazoles (azomycins) are a prominent class of biomarkers for PET imaging of hypoxia. [^{18}F]Fluoro-azomycin-α-arabinoside ([^{18}F]FAZA) – already in clinical use – may be seen as α-configuration nucleoside, but enters cells only *via* diffusion and is not transported by cellular nucleoside transporters. To enhance image contrast in comparison to [^{18}F]FAZA our objective was to ^{18}F-radiolabel an azomycin-2´-deoxyriboside with β-configuration ([^{18}F]FAZDR, [^{18}F]-β-**8**) to mimic nucleosides more closely and comparatively evaluate it *versus* [^{18}F]FAZA.

Methods: Precursor and cold standards for [^{18}F]FAZDR were synthesized from methyl 2-deoxy-D-ribofuranosides α- and β-**1** in 6 steps yielding precursors α- and β-**5**. β-**5** was radiolabeled in a GE TRACERlab FX$_{F-N}$ synthesizer in DMSO and deprotected with NH_4OH to give [^{18}F]FAZDR ([^{18}F]-β-**8**). [^{18}F]FAZA or [^{18}F]FAZDR was injected in BALB/c mice bearing CT26 colon carcinoma xenografts, PET scans (10 min) were performed after 1, 2 and 3 h post injection (p.i.). On a subset of mice injected with [^{18}F]FAZDR, we analyzed biodistribution.

Results: [^{18}F]FAZDR was obtained in non-corrected yields of 10.9 ± 2.4% (9.1 ± 2.2 GBq, n = 4) 60 min EOB, with radiochemical purity >98% and specific activity >50 GBq/μmol. Small animal PET imaging showed a decrease in uptake over time for both [^{18}F]FAZDR (1 h p.i.: 0.56 ± 0.22% ID/cc, 3 h: 0.17 ± 0.08% ID/cc, n = 9) and [^{18}F]FAZA (1 h: 1.95 ± 0.59% ID/cc, 3 h: 0.87 ± 0.55% ID/cc), whereas T/M ratios were significantly higher for [^{18}F]FAZDR at 1 h (2.76) compared to [^{18}F]FAZA (1.69, $P < 0.001$), 3 h p.i. ratios showed no significant difference. Moreover, [^{18}F]FAZDR showed an inverse correlation between tracer uptake in carcinomas and oxygen breathing, while muscle tissue uptake was not affected by switching from air to oxygen.

Conclusions: First PET imaging results with [^{18}F]FAZDR showed advantages over [^{18}F]FAZA regarding higher tumor contrast at earlier time points p.i. Availability of precursor and cold fluoro standard together with high output radiosynthesis will allow for a more detailed quantitative evaluation of [^{18}F]FAZDR, especially with regard to mechanistic studies whether active transport processes are involved, compared to passive diffusion as observed for [^{18}F]FAZA.

1. Introduction

Tumor hypoxia represents one of the key hallmarks of malignant cancers and is associated with tumor aggressiveness, metastasis, aberrant angiogenesis, increased resistance to radiotherapy and a negative predictive value [1,2]. Thus, identifying and specifically targeting hypoxic areas in solid tumors is of utmost interest for patient stratification, management and targeted therapy [3,4]. Tumor hypoxia has a pivotal role in driving disease severity in various cancer types, including head and neck cancer, melanoma, breast cancer or glioma [5–8].

In the past decades, tumor hypoxia imaging has gained momentum — allowing non-invasive *in vivo* quantification of hypoxic tumor areas [9]. For this purpose, hypoxia-specific tracers for positron emission tomography (PET) were developed and under investigation in the past [10,11]. Yet, different hypoxia PET tracer classes have evolved thereof. [^{64}Cu]ATSM was introduced by M. J. Welch [12] and has been largely evaluated *in vivo* and *in vitro*. Similarly, 2-nitroimidazole-based compounds have been investigated over the last decades, with [^{18}F]FMISO being the most prominent example [13–15]. Besides [^{18}F]FMISO, more 2-nitroimidazole derivatives have been developed, ^{18}F-labeled 1-(5´-deoxy-5´-fluoro-α-D-arabinofuranosyl)-2-nitroimidazole

☆ Research paper.
☆☆ Prof. Dr. Anton Rieker, Institute of Organic Chemistry, University of Tübingen, Tübingen, Germany, on the occasion of his 85th birthday.

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([^{18}F]FAZA) [16–18] or ^{68}Ga-labeled 2-nitroimidazole derivatives [19] may stand as examples.

Recently, we further contributed to the *in vivo* evaluation of [^{18}F]FAZA, confirming its hypoxia specificity [20,21]. However, according to current knowledge, the cellular uptake mechanism of [^{18}F]FAZA results from passive diffusion with the consequence of relatively low signal-to-noise ratios and thus low image contrast at early time points after tracer injection — which is unfavorable for the application in clinical routine. Reasons for this might be the sugar moiety in [^{18}F]FAZA – arabinose – and the α-configuration at C-1′, resulting in an uptake dominated by passive diffusion over the cell membrane. To possibly make use of the nucleoside transporter system, in recent years attempts were made to use other sugars and to change to β-configuration at C-1′. Emami et al. [22] described synthesis, transport and hypoxia selective binding of 1-(5′-deoxy-5′-fluoro-β-D-ribofuranosyl)-2-nitroimidazole *in vitro*. Later, from the same group, Kumar et al. [23] reported on synthesis, physicochemical properties and preliminary *in vitro* assessment of the radiosensitization properties of 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-2-nitroimidazole and 1-(3′-deoxy-3′-fluoro-β-D-lyxofuranosyl)-2-nitroimidazole finding similar properties as with FAZA. So far, no ^{18}F-radiolabeling and evaluation of these new sugar-azomycin derivatives as radiotracers have been reported.

Thus, we aimed at the design of a novel 2-nitroimidazole-based PET tracer – mimicking endogenous nucleosides – hypothetically achieving enhanced early image contrast, while maintaining specificity by linking the 2-nitroimidazole ring to 2-deoxy-D-ribofuranose, to finally obtain [^{18}F]fluoro-azomycin-2′-deoxyriboside. Organic syntheses of precursors and standards had to be developed, labeling was to be established, followed by first small animal PET investigations as a proof of concept.

2. Materials and methods

2.1. General

Acetonitrile for azeotropic drying before ^{18}F-radiolabeling was from Merck (DNA synthesis grade, Darmstadt, Germany). DMSO (dried over molecular sieves) as solvent for labeling was used from Fluka (Germany). Kryptofix 2.2.2. was purchased from Merck.

All other chemicals and solvents (either Fluka or Merck) were of the highest purity available and used as received. Deuterated solvents were ordered from Eurisotop GmbH (Saarbrücken, Germany).

^{1}H and ^{13}C (*J* modulated, not *J* modulated spectra were recorded of 2-nitroimidazole derivatives) and ^{19}F-NMR spectra were obtained from compounds dissolved in $CDCl_3$, DMSO-d_6, and acetone-d_6 at 300 K using a Bruker Avance DRX 400 at 400.13 MHz and 100.61 MHz and a Bruker AV III 700 at 658.9 MHz, respectively. Chemical shifts were referenced to residual $CHCl_3$ (δ_H = 7.24) and $CDCl_3$ (δ_C = 77.00), residual $CHD_2S(O)CD_3$ (δ_H = 2.50) and $CD_3S(O)CD_3$ (δ_C = 39.50), and residual $CHD_2C(O)CD_3$ (δ_H = 2.05) and $CD_3C(O)CD_3$ (δ_C = 30.50). IR spectra of liquid samples were measured on a PerkinElmer 1600 FT-IR spectrometer as films on a silicon disc [24]. ATR spectra of solid samples were measured on a Bruker Vertex 70 instrument. Optical rotations were measured at 20 °C on a PerkinElmer 351 polarimeter in a 1 dm cell. TLC was carried out on 0.25 mm thick Merck plates, silica gel 60 F_{254}. Flash (column) chromatography was performed with Merck silica gel 60 (230–400 mesh). Spots were visualized by UV and/or dipping the plate into a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (23.0 g) and of $Ce(SO_4)_2\cdot 4H_2O$ (1.0 g) in 10% aqueous H_2SO_4 (500 mL), followed by heating with a heat gun. Melting points were determined on a Reichert Thermovar instrument and were uncorrected.

2.2. Synthesis of precursors

Tetrabutylammonium salt of 2-nitroimidazole[25]: NaOH (0.62 g) was added to a cooled (20 °C) and stirred solution of tetrabutylammonium hydrogen sulfate or bromide (1 mmol) in water (2.0 mL). After 5 min 2-nitroimidazole (0.113 g, 1 mmol) and $CHCl_3$ (10 mL) were added and stirring was continued for 5 min. The organic layer was separated and the aqueous one was extracted with $CHCl_3$ (2 × 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated at reduced pressure. The residue was dissolved in toluene/CH_2Cl_2 (1/1, 15 mL) and concentrated again at reduced pressure to leave a brownish oil, which crystallized.

Methyl 3-*O*-acetyl-5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-ribofuranoside and methyl 3-*O*-acetyl-5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-ribofuranoside (α- and β-2): *tert*-Butyldiphenylchlorosilane (TBDPSCl, 1.98 mL, 7.77 mmol, 1.05 equiv) was added to a stirred and cooled solution (−20 °C) of the mixture of anomeric methyl 2-deoxy-D-ribofuranosides (1.095 g, 7.39 mmol, prepared according to a literature procedure [26] except that HCl in MeOH was replaced by TMSCl) in dry pyridine (11 mL) under argon. After allowing the mixture to warm up to room temperature in the bath within 18 h (TLC: hexanes/EtOAc = 2/1), Ac_2O (5.0 mL) was added and stirring was continued for 30 min at 50 °C. Volatile components were removed at reduced pressure and the residue was taken up in water (10 mL) and 1 M HCl (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (10 mL) and water (10 mL), dried (Na_2SO_4) and concentrated at reduced pressure. The crude product was purified by flash chromatography (hexanes/EtOAc = 4/1, α: R_f = 0.47; β: R_f = 0.39) to give a mixture of protected methyl glycosides α- and β-**2** (2.003 g, 63%; α/β ~ 1/1) as an oil; the analytical samples of both oily anomers were obtained by flash chromatography (hexanes/EtOAc = 10/1) of the mixture.

Methyl 2-deoxy-β-D-ribofuranoside β-2: 29%, $[\alpha]_D^{20}$ = −35.64 (*c* = 1.65, acetone). IR (Si): ν = 2931, 2858, 1741, 1241, 1113 cm^{-1}; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 7.71–7.64 (m, 4H), 7.45–7.35 (m, 6H), 5.35 (ddd, *J* = 6.9, 4.3, 3.0 Hz, 1H), 5.11 (ddd, *J* = 5.6, 3.0 Hz, 1H), 4.13 (ddd, *J* = 6.7, 5.6, 3.0 Hz, 1H), 3.73 (AB system, *J* = 10.6, 6.7, 5.6 Hz, 2H), 3.29 (s, 3H), 2.32 (ddd, *J* = 14.1, 6.9, 3.0 Hz, 1H), 2.09 (ddd, *J* = 14.1, 5.6, 4.3 Hz, 1H), 2.03 (s, 3H), 1.06 (s, 9H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ = 170.29, 135.59 (2C), 135.57 (2C), 133.35, 133.29, 129.67 (2C), 127.65 (4C), 105.47, 84.02, 75.34, 64.72, 55.23, 39.02, 26.72 (3C), 21.00, 19.16. Anal. calcd for $C_{24}H_{32}O_5Si$: C 67.26, H 7.53. Found: C 67.29, H 7.30.

Methyl 2-deoxy-α-D-ribofuranoside α-2: 34%, $[\alpha]_D^{20}$ = +80.45 (*c* = 0.88, acetone). IR (Si): ν = 2931, 2858, 1739, 1472, 1428, 1241, 1209, 1113, 1071, 1023 cm^{-1}; ^{1}H NMR (400. 13 MHz, $CDCl_3$): δ = 7.71–7.65 (m, 4H), 7.45–7.35 (m, 6H), 5.27 (ddd, *J* = 8.1, 2.9, 1.5 Hz, 1H), 5.12 (d, *J* = 5.3 Hz, 1H), 4.16 (ddd, *J* = 3.5, 3.3. 2.9 Hz, ≈q, 1H), 3.83 (AB system, *J* = 11.1, 3.5, 3.3 Hz, 2H), 3.39 (s, 3H), 2.43 (ddd, *J* = 14.4, 8.1, 5.3 Hz, 1H), 2.06 (s, 3H), 2.01 (br d, *J* = 14.4 Hz, 1H), 1.05 (s, 9H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ = 170.88, 135.60 (2C), 135.53 (2C), 133.22, 133.18, 129.67, 129.62, 127.66 (2C), 127.62 (2C), 105.26, 83.86, 74.58, 64.14, 54.99, 39.40, 26.74 (3C), 21.11, 19.19. Anal. calcd for $C_{24}H_{32}O_5Si$: C 67.26, H 7.53. Found: C 67.13, H 7.33.

1-(3′-*O*-Acetyl-5′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-α-D-ribofuranosyl)-2-nitroimidazole and 1-(3′-*O*-acetyl-5′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-β-D-ribofuranosyl)-2-nitroimidazole (α- and β-3): A cold (−20 °C) solution of HCl in dry Et_2O (6 mL, 8 M) was added in one portion to a solution of the mixture of methyl glycosides α- and β-**2** (1.029 g, 2.4 mmol) in dry CH_2Cl_2 (6 mL), cooled and stirred at −15 °C under argon. After 45 min (TLC: hexanes/EtOAc = 4/1, showed 4 spots, assigned to β-OMe with R_f = 0.61, α-OMe with R_f = 0.53, β-Cl with R_f = 0.42 and α-Cl with R_f = 0.21), when the bath temperature had risen to −5 °C, volatile components were removed at reduced pressure (10 mm). The residue was twice dissolved in dry CH_2Cl_2 (5 mL) and concentrated again (10 mm), then dried for 5 min at 0.8 mbar (10 °C) to leave the chlorides as an unstable colorless oil (decomposes when left at ambient; by ^{1}H NMR: β-OMe:α-OMe:β-Cl:α-Cl = 0.51:0.26:0.14:1.0 M ratio).

One half of the crude chloride was dissolved in dry CH_3CN (8 mL), the other one in dry 1,2-$C_2H_4Cl_2$ (8 mL) and cooled under argon to −30 °C.