Biodistribution and Pharmacokinetics of the Radiosensitizer 3-Aminobenzamide: Assessment with Fluorine-19 NMR Imaging

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Introduction

Intact DNA repair systems are a prerequisite for all cells to correct spontaneous or induced DNA damage and to maintain the integrity of the genetic information. Inhibition of these repair systems leads to a marked increase in DNA strand breakage, cytogenetic damage, and cell killing. The aromatic amide 3-aminobenzamide (3-ABA) is a potent agent, which inhibits the repair of radiation-induced DNA strand breaks [1] and, thus, enhances the antitumor activity of radiotherapy ('radiosensitization'). The effective use of radiosensitizers, however, requires the development of drug targeting systems and the investigation of imaging techniques to noninvasively monitor the biodistribution of the agent.

Purpose

To apply a ^{19}F-labeling technique in combination with a chemical-shift selective ^{19}F NMR imaging sequence to map the biodistribution and pharmacokinetics of 3-ABA in tumor-bearing animals.

Methods

3-ABA was labeled with ^{19}F by trifluoroethylization. To this end, 3-nitrobenzoic acid chloride reacted with trifluoroethylamine in dioxane to the crystalline *N*-alkylamide. Afterwards, the nitro group was reduced to the amino group under Raney-Nickel catalysis (70 bar hydrogen pressure). Finally, 3-amino-*N*-2,2,2-trifluoroethylbenzamide (3-ABA-TFE) was obtained with a yield of 86 %.

The biodistribution and kinetics of 3-ABA-TFE were mapped in nine Copenhagen rats bearing *s. c.* transplanted Dunning prostate adenocarcinoma (R3327-AT1) at both hind legs. The animals received 3-ABA-TFE (400 mg/kg-bw *i. p.*) dissolved in rat serum albumin and 20 % DMSO. Each rat was examined with ^{19}F and ^{1}H NMR 1.5 h, 26 h, 52 h, and 190 h after 3-ABA-TFE administration.

NMR imaging was performed at a 1.5-Tesla whole-body system (MAGNETOM 63/84 SP®, Siemens AG) equipped with a frequency selective duplexer and preamplifier for ^{19}F NMR spectroscopy. A double-tuned linear-polarized animal resonator was used for RF transmission and detection at ^{19}F and ^{1}H frequencies. For ^{19}F NMR [2], a FLASH pulse sequence (TR = 100 ms, TE = 2.7 ms, TH = 15 mm, MS = 16×16, FOV = 160 mm, NEX = 2000) was employed with an acquisition time of 53 min for a 3-ABA-TFE image.

Results

All rats tolerated the high 3-ABA-TFE dose well and could be examined in inhalation narcosis (0.4 vol.-% halothane) during the whole experimental trial. High resolution ^{19}F NMR spectra of 3-ABA-TFE showed both *in vitro* and *in vivo* a sharp single resonance line ($FWHM$ = 1.4 ppm) from the trifluoroethyl group, resonating at $\delta = -2.4$ ppm with respect to trifluoroacetic acid. No contamination, 3-ABA-TFE by-products, or ^{19}F-containing metabolites could be detected even 190 h after administration. With an acquisition time of 53 min, metabolic ^{19}F images were measured with an excellent signal-to-noise ratio. Figure 1 shows serial NMR images of a Copenhagen rat with Dunning prostate carcinoma at both hind legs. A high 3-ABA-TFE uptake was observed in the peritoneum and the liver while a small heterogeneous 3-ABA-TFE signal distribution could be detected in the tumor. Quantitative image analysis with a ROI technique and normalization of the ^{19}F signals to 3-ABA-TFE standards revealed a liver-to-tumor ratio of 8.5 to 11.9. The maximum 3-ABA-TFE uptake in the liver and the tumor was found about 52 h after *i. p.* 3-ABA-TFE administration.

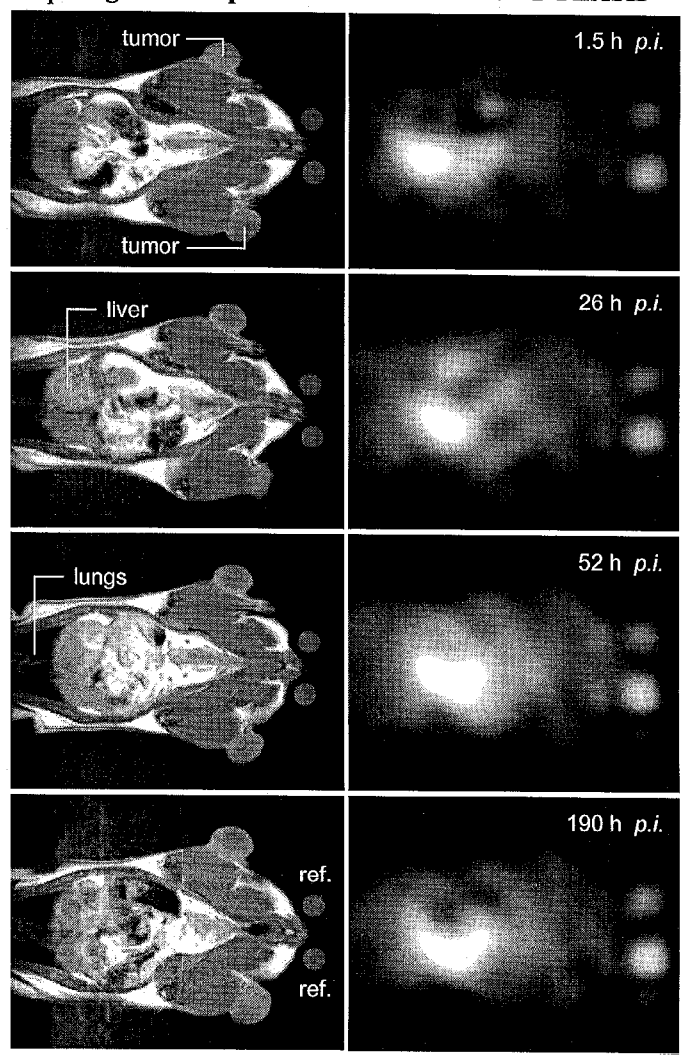

Figure 1. Serial coronal NMR images of a Copenhagen rat bearing Dunning prostate carcinoma together with two ^{19}F reference vials. Different time points after *i. p.* administration of 3-ABA-TFE.

Discussion

Metabolic ^{19}F NMR imaging provides an elegant means to measure the biodistribution and pharmacokinetics of ^{19}F-labeled drugs such as 3-ABA-TFE. For the *in vivo* quantification of drug concentrations with ^{19}F NMR, however, the exact knowledge of the T_1 and T_2* relaxation times in the various tissue compartments is mandatory. In order to obtain a strong ^{19}F NMR signal, we chose a trifluoroethyl group (three chemically identical fluorine-19 atoms) for conjugation. Owing to its size, however, the labeling group may alter the physicochemical properties of the low molecular weight radiosensitizer. To ameliorate this problem and to improve the uptake of the lipophilic 3-ABA conjugate in the tumor, we are presently investigating drug targeting systems which allow us to link 3-ABA and 3-ABA-TFE to potent macromolecular carriers.

References

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