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Antilipolytic drug boosts glucose metabolism in prostate cancer ☆

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ABSTRACT

Introduction: The antilipolytic drug Acipimox reduces free fatty acid (FFA) levels in the blood stream. We examined the effect of reduced FFAs on glucose metabolism in androgen-dependent (CWR22R*v*1) and androgen-independent (PC3) prostate cancer (PCa) xenografts.
Methods: Subcutaneous tumors were produced in nude mice by injection of PC3 and CWR22R*v*1 PCa cells. The mice were divided into two groups (Acipimox vs. controls). Acipimox (50 mg/kg) was administered by oral gavage 1 h before injection of tracers. 1 h after i.v. co-injection of 8.2 MBq (222 ± 6.0 μCi) ^{18}F-FDG and ~0.0037 MBq (0.1 μCi) ^{14}C-acetate, ^{18}F-FDG imaging was performed using a small-animal PET scanner. Counting rates in reconstructed images were converted to activity concentrations. Quantification was obtained by region-of-interest analysis using dedicated software. The mice were euthanized, and blood samples and organs were harvested. ^{18}F radioactivity was measured in a calibrated γ-counter using a dynamic counting window and decay correction. ^{14}C radioactivity was determined by liquid scintillation counting using external standard quench corrections. Counts were converted into activity, and percentage of the injected dose per gram (%ID/g) tissue was calculated.
Results: FDG biodistribution data in mice with PC3 xenografts demonstrated doubled average %ID/g tumor tissue after administration of Acipimox compared to controls (7.21 ± 1.93 vs. 3.59 ± 1.35, P = 0.02). Tumor-to-organ ratios were generally higher in mice treated with Acipimox. This was supported by PET imaging data, both semi-quantitatively (mean tumor FDG uptake) and visually (tumor-to-background ratios). In mice with CWR22R*v*1 xenografts there was no effect of Acipimox on FDG uptake, either in biodistribution or PET imaging. ^{14}C-acetate uptake was unaffected in PC3 and CWR22R*v*1 xenografts.
Conclusions: In mice with PC3 PCa xenografts, acute administration of Acipimox increases tumor uptake of ^{18}F-FDG with general improvements in tumor-to-background ratios. Data indicate that administration of Acipimox prior to ^{18}F-FDG PET scans has potential to improve sensitivity and specificity in patients with castration-resistant advanced PCa.

1. Introduction

1.1. Tumor energy metabolism

In general, glucose appears to be the most important energy substrate for tumors. Under normoxic conditions glucose is preferentially metabolized by the tricarboxylic acid (TCA) cycle and electron transport chain (oxidative phosphorylation), while under hypoxic conditions the glycolytic pathway is used. Despite the efficiency of the TCA cycle and electron transport chain to produce adenosine 5'-triphosphate (ATP) from glucose, some tumor cells continue to use glycolysis for ATP production in the presence of oxygen, also known as the Warburg effect [1]. Additionally, Herbert Crabtree observed an inhibition of oxygen consumption by adding glucose to microorganisms that have a high rate of aerobic glycolysis, which also occurs in most malignancies (the Crabtree effect) [2]. Summarized, both the Warburg and Crabtree effects suggest that an altered metabolic control and/or enzymes play a role in tumor glucose metabolism. However, tumors can also use other energy substrates, including fatty acids, which are the most reduced energy substrate available to cells. They provide the most ATP when metabolized, although their metabolism is oxygen dependent. There are two sources of fatty acids for tumors; free fatty acids and endogenous lipid esters from both tumor and normal tissues.

1.2. Interaction between tumor glucose and fatty acid metabolism

The interaction between glucose and free fatty acids (FFAs) metabolism was first demonstrated in a perfused rat heart [3].

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Thereafter the glucose–FFA cycle has been shown to be operational in the human heart [4,5], skeletal muscles [6], as well as at whole body level [7]. Although several enzymes are involved in glycolysis, only a few key enzymes regulate the glucose flux. Phosphofructokinase is an enzyme that controls partially the selection of fuels. The activity of phosphofructokinase is inhibited by excess of citrate and ATP produced by metabolism of FFA and lactate. This leads to accumulation of glucose 6-phosphate, which restrains further uptake and phosphorylation of glucose by allosteric inhibition of hexokinase [8]. Conversely, when citrate and ATP levels are decreased, the inhibitory effects are reduced and the activity of phosphofructokinase increases, leading to increased glucose transport and phosphorylation. While the function of the glucose–FFA cycle has been well established in normal tissues and healthy subjects, the picture is different in cancer patients who show tumor-associated changes in host metabolism.

1.3. Effects of the antilipolytic drug Acipimox

Acipimox is a long-acting ($t_{½plasma}$ ~2 h) nicotinic acid derivative, which through its antilipolytic actions is used to inhibit the release of non-esterified fatty acids from adipose tissue and reduce total serum triglyceride and cholesterol levels. Administration of Acipimox has no effect on whole body glucose utilization after an overnight fast [9]. This does not preclude redistribution of glucose uptake between tissues or a change in the fate of glucose. In keeping with the latter alternative, Acipimox enhances glucose and decreases lipid oxidation. Acipimox also decreases glucose utilization for gluconeogenesis, which might in part explain why total glucose utilization remains unchanged although glucose oxidation increases [9].

1.4. Nuclear medicine imaging of glucose metabolism in prostate cancer

Prostate cancer is the most common malignancy among men in the United States, accounting for approximately one third of all cancer diagnoses. It is estimated that 240,890 men will be diagnosed with and 33,720 men will die of cancer of the prostate in 2011 [10]. Prostate cancers vary widely in their rate of growth, aggressiveness, and tendency to metastasize. The biology of this disease evolves from a small, slow-growing, androgen-dependent 'indolent' carcinoma toward a more and more aggressive, androgen-independent tumor during the course of progression [11,12]. Among the currently available nuclear medicine imaging modalities positron emission tomography (PET) offers several advantages compared to single-photon emission computed tomography (SPECT), especially in terms of spatial resolution and acquisition time (whole-body imaging in three dimensions in a relatively short amount of time). This has resulted in a clear tendency of development and application of PET agents in a number of clinical settings (diagnosis, staging, treatment evaluation). PET imaging with the glucose analogue 18-fluorine fluoro-2-deoxy-D-glucose (^{18} F-FDG) takes advantage of the increase in glycolytic flux in cancer. Unfortunately, a fraction of prostate cancers possesses a relatively slow metabolic rate and expresses fewer GLUT-1 binding sites (a hexose transporter), leading to lower FDG uptake compared with other cancers.

The results of prostate cancer PET imaging with ^{18} F-FDG are in general disappointing [13–17]. Despite its poor reputation from earlier reports, ^{18} F-FDG is in fact not an unsuitable tracer for the investigation of prostate carcinoma, but at present needs to be used in carefully selected groups of patients [18–22]. The effect of Acipimox on fatty acid and glucose metabolism in malignant prostate cells has not been investigated — we hypothesize that Acipimox through its antilipolytic effect may increase FDG tumor uptake in prostate cancer xenografts. Consequently, the diagnostic properties of ^{18} F-FDG PET regarding different clinical settings in patients with prostate cancer may be improved.

2. Methods

2.1. Generation of tumor xenografts

All animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of Memorial Sloan-Kettering Cancer Center and followed National Institutes of Health guidelines for animal welfare. Tumor cell lines were obtained from the American Type Culture Collection and cultured under conditions provided by the supplier. The cell lines included PC3 (derived from androgen independent prostate cancer bone metastasis) and CWR22Rv1 (derived from a more androgen dependent prostate cancer primary tumor). Subcutaneous tumors were produced on the right shoulder of nude mice (20–25 g; Taconic) by subcutaneous injection of tumor cells (PC3: 5×10^6 cells, n = 10; CWR22Rv1: 10×10^6 cells, n = 10) in 200 μL consisting of 100 μL of cell culture medium and 100 μL of Matrigel (BD Biosciences) under 2% isoflurane anesthesia.

2.2. Small-animal PET

The mice were divided into two groups (Acipimox (n = 5 for each xenograft) vs. control (n = 5 for each xenograft)). Acipimox (50 mg/kg) was administered to the mice using the oral gavage technique 1 h before injection of the tracers [23]. Imaging was performed by use of a dedicated high-resolution small-animal PET scanner (microPET R4 Rodent; Concorde Microsystems Inc., Knoxville, TN, USA). The mice were maintained under 2% isoflurane anesthesia in oxygen at 2 L/min during the entire scanning period. Imaging was performed 1 h after co-injection of 8.2 MBq (222 μCi ± 6.0 μCi) ^{18} F-FDG and ~0.0037 MBq (0.1 μCi) ^{14}C-1-acetate (^{14}C-acetate) via the tail vein. An energy window of 350–700 keV and a coincidence timing window of 6 ns were used. The image data were corrected for non-uniformity of the scanner response, dead-time count losses, and physical decay to the time of injection. No correction was applied for attenuation, scatter, or partial-volume averaging. The measured reconstructed spatial resolution of the R4 microPET scanner is approximately 2.0 mm in full width at half maximum at the center of the field of view. The counting rates in the reconstructed images were converted to activity concentrations (percentage injected dose [%ID] per gram of tissue) by use of a system calibration factor derived from the imaging of a mouse-sized water equivalent phantom containing ^{18} F. Quantification was obtained by region-of-interest analysis using ASIPro VM software (v. 6.3.3.0, Concorde Microsystems Inc., Knoxville, TN, USA).

2.3. In vivo biodistribution studies

Immediately after PET imaging the mice were euthanized and then blood samples and organs were harvested. ^{18} F radioactivity was measured in a calibrated γ-counter (2480 Wizard^2 Automatic Gamma Counter; PerkinElmer, Inc.) using a dynamic counting window (peak 511 keV; threshold 20%) and decay correction. For measuring ^{14}C activity, all samples were solubilized (Soluene-350; Packard Instrument Co., Inc.) after the ^{18} F radioactivity counting. The samples were then stored at 4 °C for 2 days to allow for ^{18} F decay. A scintillant agent (Insta-Fluor; Packard Instrument Co., Inc.) was added to solubilized samples, and ^{14}C radioactivity was determined by liquid scintillation counting (Tri-Carb Liquid Scintillation Analyzer 1600TR; Packard Instrument Co., Inc.) using external standard quench corrections. The counts were converted into activity, and %ID/g was calculated by dividing by decay-corrected injected activity and the weight of the organ.