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Increased choline uptake in macrophages and prostate cancer cells does not allow for differentiation between benign and malignant prostate pathologies

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ABSTRACT

Introduction: Inflammatory cells may contribute to the choline uptake in different prostate pathologies. The aim of this study was (i) to assess if inflammatory cells incorporate choline and (ii) to potentially detect differences compared to FDG uptake. Therefore we investigated the uptake of [^{3}H]choline and [^{18}F]FDG in human prostate carcinoma cells and human inflammatory cells.

Methods: Macrophages were cultured from isolated mononuclear cells, gained by density gradient centrifugation of human buffy coats. T-lymphocytes, B-lymphocytes and granulocytes were enriched by density gradient centrifugation before cell sorting by means of flow cytometry was performed. [^{3}H]choline and [^{18}F]FDG uptake of isolated inflammatory cells as well as of LNCaP and PC-3 human prostate carcinoma cells was assessed simultaneously in dual tracer uptake experiments.

Results: Macrophages showed highest [^{3}H]choline and [^{18}F]FDG uptake compared to the tracer uptake rates of leukocytes. [^{3}H]choline uptake of macrophages was in the same range as in prostate cancer cells. Lipopolysaccharide stimulation of macrophages resulted in an increase of [^{18}F]FDG uptake in macrophages, but not in an increased [^{3}H]choline uptake.

Conclusions: The high [^{3}H]choline uptake in macrophages may be a source of false-positive PET results in diagnosis of prostate cancer by choline-PET/CT. As already known from FDG-PET, discrimination between tumor and inflammation in prostate cancer patients is not possible *via* choline-PET.

Advances in knowledge and implications for patient care: The application of choline-PET for reliable primary prostate cancer detection and delineation has to be queried.

1. Introduction

For prostate cancer imaging, radioactively labeled choline derivatives are used for diagnosis staging and especially for re-staging [1,2]. Increased choline uptake in benign prostate lesions is probably one of the reasons for the limited specificity of choline PET/CT for diagnosing prostate cancer [3,4]. Particularly the benign prostate hyperplasia (BPH) as well as inflammatory changes seem to be associated with an increased choline uptake [5,6]. In staging and re-staging of prostate cancer the diagnosis of lymph node metastases with high sensitivity and especially specificity is also of crucial interest. For lymph node staging in prostate cancer false positive findings due to choline uptake in lymph nodes with inflammatory changes have also been described [7,8].

Benign prostate lesions such as chronic prostatitis [9] and benign prostate hyperplasia [10,11] as well as prostate carcinoma [12] reveal an inflammatory component involving leukocyte infiltration. It has been postulated that choline is also accumulated in non-tumor lesions [13–15], the inflammatory component of benign and malignant prostate lesions being the reason of increased choline uptake in these lesions.

The aim of this study was to test the hypothesis that different types of inflammatory cells might mediate an increased choline uptake. Therefore *in-vitro* studies were performed to examine the [^{3}H]choline and [^{18}F]FDG uptake of the different inflammatory cells (macrophages, granulocytes, B-lymphocytes, T-lymphocytes) as well as of LNCaP and PC-3 prostate carcinoma cells. The [^{18}F]FDG uptake was also measured to assess if there was a difference between the [^{18}F]FDG - and [^{3}H]choline uptake – with and without lipopolysaccharide stimulation – in these different cells.

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2. Materials and methods

2.1. Culture of prostate carcinoma cell lines

The human prostate cancer cells LNCaP and PC-3 used for the uptake studies were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids and 1% penicillin/streptomycin. Cells were kept in an incubator at 37 °C in a humidified 5% CO_2 atmosphere. For tracer uptake experiments 1×10^6 cells were seeded per well of 24-well plates. After confluent growth (for PC-3 cells after 3–4 days and for LNCaP cells after 7 days) the uptake experiments were performed.

2.2. Preparation of human inflammatory cells

All human inflammatory cells such as macrophages, granulocytes, T- and B-Lymphocytes were isolated from buffy coats supplied by the blood bank of the Bavarian Red Cross in Wiesentheid, Germany. For preparation and culture of macrophages, mononuclear cells (monocytes and lymphocytes) were isolated from buffy coats by density gradient centrifugation using Histopaque®-1077 (Sigma, Steinheim, Germany). Mononuclear cells (2×10^6 per well) were seeded in 24-well plates and incubated in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C and 5% CO_2. Two hours after seeding, the growth medium was removed and adherent growing monocytes were washed twice with PBS to remove the non-adherent lymphocytes. To receive macrophages from the adherent monocytes, cells were cultured for 14 days before uptake experiments were performed. Growth medium was changed every 2 or 3 days. Macrophages were stimulated for 48 h in growth medium containing lipopolysaccharide (LPS) in different concentrations (0.1 μg/ml, 1 μg/ml and 10 μg/ml).

Isolation of granulocytes, B- and T-lymphocytes was done using fluorescence activated cell sorting (FACS) to separate the cells according to the expression of the cellular surface molecules CD19 for B-Lymphocytes, CD3 for T-Lymphocytes and CD15 for granulocytes. The lymphocytes were isolated from the mononuclear cells obtained by density gradient centrifugation of the Buffy Coat with Histopaque®-1077. Before sorting of granulocytes, these cells were enriched by means of two density gradient centrifugations with Histopaque®-1077 and Histopaque®-1119. After incubation of the enriched cells with fluorochrome-labeled antibodies anti-CD3-APC (Beckman Coulter, Marseille, IM2467), anti-CD19-PE (Beckman Coulter, Marseille, A07769) or anti-CD15-Pacific Blue (Beckman Coulter, Marseille, A74775) cell sorting was performed in a high speed cell sorter (MoFLo™ LEGACY1). To exclude dead cells, cells were stained with propidium iodide (PI) shortly before the sorting was started.

2.3. Radiopharmaceuticals

[^{18}F]FDG was synthesized at the Department of Nuclear Medicine of the Technische Universiät München. [^{3}H]choline (TRK 593) was purchased from GE Healthcare (Freiburg, Germany).

2.4. Combined [^{18}F]FDG and [^{3}H]choline uptake experiments

Before uptake experiments were initiated, growth medium was removed by washing the cells three times with uptake buffer (25 mM Tris/HCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 $MgSO_4$, 5 mM glucose, 140 mM NaCl, pH 7.4). For the adherent growing cells (macrophages, LNCaP and PC-3 cells) the uptake experiments were performed in 24 well plates without detaching the cells from growth plate. Pre-incubation in 100 μl uptake buffer was done for 10 min at 37 °C. For uptake experiments of suspension cells such as lymphocytes and granulocytes, 5×10^5 cells in 100 μl of uptake buffer were transferred to Eppendorf vials. Pre-incubation was done as described above. Uptake experiments were started by adding 100 μl uptake buffer supplemented with [^{3}H]choline (1,85 kBq) and [^{18}F]FDG (370 kBq). Uptake was allowed for 10 min at 37 °C and was stopped by addition of 1 ml of ice-cold phosphate-buffered saline (PBS) (Biochrom, Berlin, Germany). Subsequently, cells were washed three times with ice-cold PBS (1 ml each). Washing was combined with centrifugation in case of non-adherent cells. Prior to determination of [^{18}F]FDG uptake, cells were lysed in 0.5 ml of 2% sodium dodecyl sulfate (SDS) and transferred to new Eppendorf vials. The ^{18}F-activity was measured in a gamma counter (Wallac, Finland). After 48 h, *i.e.* after complete decay of the ^{18}F-activity, ^{3}H-activity was quantified by liquid scintillation counting using a beta-counter (Wallac, Finland). Uptake rates were expressed in % of the applied standard ^{18}F- and ^{3}H-activities. Protein content of the samples was determined according to the method of Bradford (Bio-Rad, Munich, Germany). Uptake rates of each sample were expressed as %/mg protein.

2.5. Statistics

The uptake experiments were repeated three times with four samples for each cell line. Including 12 uptake values per cell line, mean values and 95% confidence intervals were calculated. Statistical significance concerning the uptake rates of the cell lines was evaluated by means of Student's t test. $p < 0.05$ was considered to be statistically significant. To correct type I error due to multiple testing, a Bonferroni correction for p-values was performed.

For stimulation of macrophages the Z-scores of each sample were calculated according to the equation shown below. Stimulation experiments with LPS were performed three times at three different days and with 4 different LPS concentrations. By calculating the Z-scores for each sample, the effect of different basal rates of every experiment is minimized by revealing the uptake values to a mean value. Statistical testing for differences between the Z-scores was done as mentioned above. Linear correlation between the different LPS concentrations used for stimulation and the Z-scores was performed by non-parametric correlation testing according to Spearman using the following equation:

$$Z\text{—}Scores = \frac{U - \overline{U}}{\sigma}$$

U- sample uptake value (from any of the four LPS concentrations assayed); $\bar{U}$- mean uptake including all 4 samples of a chosen LPS stimulation experiment; σ- standard deviation of the mean uptake rate from all 4 samples of a chosen LPS stimulation experiment.

3. Results

3.1. Cellular [^{18}F]FDG uptake rates

The PC3- and LNCaP prostate carcinoma cell lines showed significantly higher FDG uptake rates than macrophages, granulocytes, B- and T-lymphocytes ($p < 0.05$, respectively) (Fig. 1). Moreover, the androgen independent cell line PC-3 was characterized by a significant higher FDG uptake compared to the androgen dependent cell line LNCaP ($p < 0.05$). Among the inflammatory cells, macrophages most avidly incorporated FDG resulting in a significantly higher uptake than in B-lymphocytes and T-lymphocytes ($p < 0.05$, respectively). There was no significant difference between the uptake rates of the small leukocytes like granulocytes, B-lymphocytes and T-lymphocytes ($p = 1.000$, respectively).

3.2. Cellular [^{3}H]choline uptake rates

Incorporation of [^{3}H]choline in %/mg protein was distinctly higher than uptake of FDG in all cell lines analyzed. Uptake of [^{3}H]choline in PC-3 was significantly higher compared to LNCaP cells, macrophages and small leucocytes (granulocytes, B-lymphocytes and T-lymphocytes)