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Dynamic behaviour of selected PET tracers in embryonated chicken eggs

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ABSTRACT

Positron emission tomography/computer tomography (PET/CT) is an established method in preclinical research in small animal disease models and the clinical diagnosis of cancer. It combines functional information of the positron-emitting biomarker with the anatomical data obtained from the CT image. Thus, it allows for 4D in vivo investigation of biological processes. Recently, PET/CT was used to monitor bone growth of chicken embryos using ^{18}F-fluoride as a bone-seeking tracer. We are interested in investigating the adequacy of additional PET/CT tracers in chicken embryos as an in vivo model system. For this reason, we evaluated several positron emitting compounds typically used in clinical tests or if these were not commercially available, we synthesised them. We studied the properties of these ^{18}F- and ^{68}Ga-labelled tracers and of ^{64}Cu-chloride in catheterised eggs via small animal microPET/CT. 2-Deoxy-2-[^{18}F]fluoroglucose ([^{18}F]FDG) was primarily absorbed at the sites of bone growth. ^{64}Cu chloride and a ^{68}Ga-labelled amyloid-fibril-binding antibody accumulated in the liver, while the ^{68}Ga-albumin desferrioxamine conjugate signal in liver decreased over time. These results indicate that these biomarkers can potentially be used for the monitoring of biological processes in chicken eggs as an animal model.

Comportamiento dinámico de marcadores PET seleccionados en huevos de gallina embrionados

Palabras clave:
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RESUMEN

La tomografía por emisión de positrones/tomografía computarizada (PET/TC) es una técnica establecida en la investigación preclínica de enfermedades en modelos de animales pequeños y en el diagnóstico clínico de cáncer. Esta técnica combina la información funcional del biomarcador emisor de positrones con los datos anatómicos de la imagen TC, permitiendo de este modo la investigación in vivo de los procesos biológicos en 4 dimensiones. Recientemente, el crecimiento de los huesos de los embriones de pollo se ha podido monitorizar usando PET/TC con ^{18}F fluoruro como trazador óseo. Estamos interesados en la investigación de otros trazadores PET/TC en embriones de pollo como sistema de modelo in vivo. Para ello, evaluamos varios radiotrazadores usados habitualmente en pruebas clínicas o sintetizamos aquellos que no estaban disponibles comercialmente. Utilizando un microPET/TC de animales pequeños, evaluamos las características de ^{18}F, ^{68}Ga y ^{64}Cu cloruro en huevos con sondaje. La 2-Deoxy-2[^{18}F]fluorodeoxiglucosa (^{18}F FDG) estaba absorbida en los sitios de crecimiento de los huesos. El ^{64}Cu cloruro y un anticuerpo de unión a fibrillas de amiloide marcado con ^{68}Ga se acumularon en el hígado, mientras que el ^{68}Ga ligado a un conjugado de desferroxamina disminuyó con el tiempo en ese mismo órgano. Los resultados indican que estos biomarcadores pueden ser utilizados para la monitorización de procesos biológicos en huevos de pollo como modelo animal.

Introduction

In basic research, molecular imaging methods are a means of studying biological processes in living systems. PET/CT combines two modalities. In positron emission tomography (PET), the distribution of a tracer compound carrying a positron-emitting isotope can be followed because it interacts with potential target sites. Thereby, regions of interest (ROIs) deep in the body can also be observed and quantified because the 511 keV gamma ray photons can easily penetrate organic material. Computed tomography (CT) provides the anatomical information necessary to specify the point of tracer uptake. The resolution of microPET is in the range of 1.5 mm, and that of CT can reach 50 μm.[1]

The chick embryo is a well-established model organism in infection biology,[2–5] oncology,[2–8] and pharmacology.[9,10] Avian embryogenesis has been extensively studied by in vivo imaging methods.[11] However, only recently has microPET/CT been implemented to investigate metabolic processes in the chick embryo.[12] Innovative anaesthetic[13] and tracer-injection techniques have

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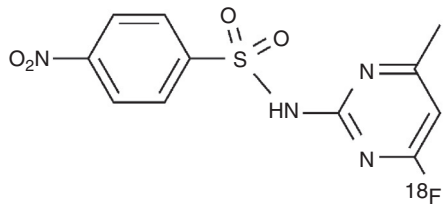

Fig. 1. Structure of the ^{18}F-sulphonamide (**1**).

resolved the problems encountered during the molecular imaging of chick embryos via microPET/CT.

Following the success of experiments in which [^{18}F]fluoride was injected,[12] we investigated biologically active isotopes and molecules in the in vivo chick embryo. These included 2-deoxy-2-[^{18}F]fluoroglucose ([^{18}F]FDG), a glucose analogue that accumulates in regions of enhanced metabolism, ^{68}Ga citrate, which can help diagnose bone infection,[14] a ^{68}Ga-labelled albumin desferrioxamine conjugate as a blood pool marker,[15] and a ^{68}Ga-labelled amyloid-fibril-binding antibody (^{68}Ga-B10).[16] We also took advantage of the chick embryo model to investigate the properties of a recently synthesised sulphonamide, N-(4-[^{18}F]fluoro-6-methylpyrimidin-2-yl)-4-nitrobenzenesulphonamide (Fig. 1), which was developed as an experimental precursor compound.[17]

Although these tracers were designed for application in mammals, we demonstrate that microPET/CT can be successfully utilised to investigate the general distribution and accumulation patterns in healthy chick embryos, thereby increasing the usefulness of this model organism.

Material and methods

^{18}F-labelled compounds

The [^{18}F]FDG was from Eckert & Ziegler, f-con Deutschland GmbH (Holzhausen, Germany).

N-(4-[^{18}F]fluoro-6-methylpyrimidin-2-yl)-4-nitrobenzenesulphonamide was synthesised as described.[17]

^{68}Ga-labelled compounds

Preparation of desferrioxamine conjugates

The ^{68}Ga-albumin desferrioxamine conjugate was synthesised as described[18] with some modifications. Before derivatisation of the albumin, 5 ml of 0.68% BSA (Carl Roth, Karlsruhe, Germany) in Ca^{2+}- and Mg^{2+}-free Dulbecco's PBS (PAA Laboratories GmbH, Pasching, Austria) were concentrated twofold using Microsep 3K Centrifugal Devices (Pall, Dreieich, Germany, MCP003C41). The albumin solution was then washed once with 2 ml of 86 mM EDTA and four times with PBS. The volume was then adjusted to 5 ml with PBS. To 0.5 ml of the solution were added 6 μl of desferrioxamine-p-SCN (0.07 mg in DMSO, 2.4 fold excess, Macrocyclics, Dallas/TX, USA). The pH was adjusted to 9.0 with 0.1 M sodium carbonate, and the reaction was carried out at 37 °C over 45 min. The conjugate was then purified by size exclusion chromatography (PD-25, GE Healthcare Europe GmbH) with 0.25 M sodium acetate (pH 5.5) as the eluent. The flow through and an additional 250 μl of eluate were discarded. The next 700 μl was collected and stored at 4 °C. Further purification was performed with 3 K centrifugal filters (Amicon Ultra-0.5 ml, Merck Millipore, Schwalbach, Germany). For this, 500 μl of the albumin desferrioxamine conjugate were concentrated fivefold and washed five times with 400 μl of 0.25 M ammonium acetate. After removal from the filter unit, the volume was adjusted to 0.5 ml.

The desferrioxamine conjugate of the amyloid fibril-binding antibody (B10)[16] was synthesised as described for the albumin. It was diluted in PBS to give a concentration of 5 mg/ml. After size exclusion chromatography, the conjugate was labelled without further purification.

Labelling of proteins

[^{68}Ga]Gallium chloride was eluted from a ^{68}Ge/^{68}Ga generator (iThemba LABS, Somerset West, South Africa). The eluate was concentrated as described,[19] mixed with 100 μl of 1.1 M sodium acetate, and the pH was adjusted stepwise to 4.7–5.0 with 2.0 M sodium carbonate. A volume of 200 μl of the albumin desferrioxamine conjugate or B10 desferrioxamine conjugate was added to the eluate, followed by incubation for 5 min.

As quality control, instant thin layer chromatography (ITLC) was performed on dark green tec-control strips (Elimpex-Medizintechnik GesmbH, Mödling, Austria) using 20 mM citric acid (pH 4.9) as the eluent. The labelled proteins were found at the origin, while free $^{68}Ga^{3+}$ ran with the solvent front. Additionally, radio HPLC was performed with a C-4 reverse phase column (Jupiter 5u, 300 A, 150 mm × 4.6 mm, Phenomenex, Aschaffenburg, Germany) and a gradient system which started from 80% buffer A with 20% buffer B coming to 5% buffer A with 95% buffer B over 20 min changing then to 5 min of initial conditions. Buffer A was 95% water/5% ACN/0.05% TFA (v/v/v) and buffer B was 75% ACN/20% 2-propanol/4.1% water/0.85% TFA (v/v/v/v). Free ^{68}Ga salt was detected at 2–3 min, and the labelled tracer was detected at approximately 10 min. The labelling efficiency was between 90 and 97%.

For ^{68}Ga citrate, 50 μl of 0.01 M citric acid was added to 150 μl of the $^{68}GaCl_3$ eluate, and the pH was adjusted to 6.0 with 2.0 M sodium carbonate. Quality control was performed using ITLC as described above. The pH of the tracer solution was adjusted to 6.2–6.5 prior to injection.

Ligand free ^{64}Cu

^{64}Cu chloride was produced as described by Thieme et al.[20]

Preparation of embryonated chicken eggs, catheter system and tracer injection

Gallus gallus domesticus eggs were prepared as it is fully described by Würbach et al.[12] Briefly, for dynamic experiments, 15–18 day old embryos were catheterised via the chorioallantoic membrane (CAM). For this the custom-made catheter of 22 cm in length with a 30 G needle was inserted through an opening of about 1 cm^2 into an blood vessel of sufficient size on the CAM and closed with a stopper. The integrity of the system was checked (leakage of blood), and the opening was closed with paraffin wax, also fastening the catheter.

Injection of tracers was made after the CT measurement. Approximately 5 s before tracer injection, the PET scan was started. Tracers (150 μl, diluted in PBS) were injected over 40–60 s.

Anaesthesia and assembly during measurement

The embryos were anaesthetised as described[13] during the full time of measurement. Briefly, the embryos were kept under red light and with an oxygen flow of 3 l/min (5% isoflurane) for 15 min in a commercially available anaesthesia induction chamber (inner dimensions: 180 mm × 260 mm × 140 mm, Rothacher Medical GmbH, Switzerland) anaesthesia was induced. For microPET/CT measurement the embryos were then placed in a 14 cm × 18 cm plastic bag and fixed with transparent tape. The reclosable bag