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PET Imaging of Multidrug Resistance in Tumors Using ¹⁸F-Fluoropaclitaxel

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Abstract

The failure of solid tumors to respond to chemotherapy is a complicated and clinically frustrating issue. The ability to predict which tumors will respond to treatment could reduce the human and monetary costs of cancer therapy by allowing pro-active selection of a chemotherapeutic to which the tumor does not express resistance. PET/CT imaging with a radiolabeled form of paclitaxel, F-18 fluoropaclitaxel (FPAC), may be able to predict the uptake of paclitaxel in solid tumors, and as a substrate of P-glycoprotein, it may also predict which tumors exhibit multidrug resistance (MDR), a phenotype in which tumors fail to respond to a wide variety of chemically unrelated chemotherapeutic agents. This article reviews the synthetic, preclinical and early human data obtained during the development phase of this promising new radio-pharmaceutical.

Keywords

Drug development; Molecular Imaging; F-18 fluoropaclitaxel; Multidrug resistance; Paclitaxel; PET; Pgp

Introduction

The failure of solid tumors to respond to chemotherapy is a complicated and clinically frustrating issue. Often a patient appears to respond initially; however the therapy fails to destroy all of the cancer cells, leading to incomplete response and/or rapid recurrence. Even more disconcerting is the fact that tumor cells exhibiting resistance to one drug are also likely to be resistant to other chemotherapeutic agents, a condition known as multi-drug resistance (MDR) [1].

Prior exposure to a given drug increases the probability that the tumor will not respond to that drug and possibly numerous other chemotherapeutics. While MDR can be an innate tumor characteristic, it can also be induced by prior exposure (selective pressure). Additionally, the primary tumor may respond to a given drug while metastatic foci exhibit

resistance. This means that intensive molecular analysis of a biopsy sample from the primary tumor may not be able to predict successful treatment of the metastases.

Drug delivery to tumors is dependent on numerous patient specific and tumor specific factors. Once administered, many drugs are metabolized to various degrees to active or inactive metabolites. When the active drug is in the bloodstream, delivery to the tumor and normal tissues is initially defined by tissue/tumor perfusion. Retention of drug within the tumor depends on the specific mechanism of the chemotherapeutic agent (i.e. binding to microtubules or incorporation into DNA) and characteristics of the tumor cells (i.e. intracellular transport and drug efflux mechanisms).

Many studies have focused on the ability of tumor cells to actively remove cytotoxic drugs from their intracellular compartments as this mechanism is likely a significant component of MDR. A family of proteins, known as ABC (ATP Binding Cassette) transporters has been shown to actively remove, i.e. pump out, a wide variety of chemotherapeutic agents *in vitro* [2,3]. Numerous *in vivo* rodent xenograft studies that compare tumors with and without over-expression of ABC transporters, including knockout models, support the role of these transporters in MDR. The best characterized ABC transporter involved with MDR is p-glycoprotein (Pgp). Pgp is a 170-kDa membrane protein encoded by the *mdr1* gene (located on chromosome 7 in humans). In normal human subjects, Pgp expression is observed in the liver, kidneys, intestines, blood-brain-barrier, blood -testicular-barrier, and hematopoietic stem cells [4,5]. Pgp appears to perform a protective function by actively removing potentially toxic substances against a concentration gradient. Over-expression of this gene in tumors has been shown to result in MDR *in vitro* and *in vivo*.

There is increasing interest in the use of adjunctive drugs such as anti-angiogenic agents, which are thought to normalize blood flow to tumors (thus potentially enhancing delivery of a cytotoxic agent), vascular disrupting agents (which may be able to “trap” the cytotoxic drug in the tumor) and efflux pump modulators (which block the removal of cytotoxic drugs from the intracellular space). While the efficacy of efflux pump modulators has been demonstrated *in vitro*, the clinical translation has been slow and disappointing [6]. While the first generation of modulators often showed normal tissue toxicity and interactions with cytochrome p450 (resulting in altered chemotherapeutic drug levels/ distribution) [7,8], studies using more recently developed “third-generation” modulators, exhibit fewer of these problems, and early clinical studies suggest increased drug accumulation in tumors [9]. However, clinical studies demonstrating improved tumor response in the presence of MDR modulators are lacking.

The goal of chemotherapy is to deliver an adequate amount of active drug to the tumor while minimizing normal tissue toxicity. Conventional methods of evaluating chemotherapeutic effectiveness involve monitoring for normal tissue toxicities and waiting for clinical or anatomical imaging to show signs of tumor regression (or lack of progression). Determination of MDR modulator effectiveness is therefore also performed in this fashion. Thus, the effectiveness of therapy is only evaluated after a significant time lapse. Because aggressive treatment of tumors is generally required, clinicians need a more rapid feedback on the effectiveness of a given therapy in order to avoid causing unnecessary toxicities (i.e. risks without benefit). We sought to develop an imaging method to determine the drug concentration of a particularly effective chemotherapeutic drug, paclitaxel, within the tumor. Paclitaxel, a derivative of a natural product, has proven effective in the treatment of numerous solid organ tumors. This method could be used both to evaluate the propensity of a given tumor to exclude paclitaxel and to evaluate the efficacy of MDR modulators in enhancing paclitaxel uptake.

New molecular imaging probes could address the issue of tumor MDR. As defined by the Society of Nuclear Medicine, molecular imaging is: “the visualization, characterization, and measurements of biological processes at the molecular and cellular levels” [10]. A powerful tool in the molecular imaging arsenal is positron emission tomography (PET). By radiolabeling “trace” amounts of a drug with an isotope which decays by positron emission, the biodistribution of that drug can be quantitatively evaluated *in vivo*. Positron emitting isotopes decay by ejecting a positron (a positively charged electron) from the nucleus. The positron can only traverse a short distance (up to a few millimeters depending on the emission energy and tissue density) before encountering an electron and “annihilating”, resulting in two 511 keV photons which are emitted in diametrically opposite directions. The PET scanner contains sensitive detectors which record the number and location of photon pairs, which can mathematically be reconstructed into images. Just prior to or following this “emission” scan, a transmission scan (either using x-rays (CT) or gamma photon emitting source) is obtained and this data is used to correct the emission image for soft tissue attenuation, resulting in an image that displays actual radioactivity (measured in counts /sec). If the specific activity of the radiotracer is known, drug concentration can be quantitatively determined throughout the image. As the functional images may contain little anatomic information, the PET scanner is often combined with a CT scanner resulting in functional-anatomic imaging.

Through this technology, PET/CT imaging may perform a “virtual biopsy” of the entire patient. While a standard diagnostic biopsy examines only a small portion of the tumor at a single time point, PET imaging can non-invasively provide information of molecular and cellular events throughout the entire body over time. It images not just the tumor cells, but provides a systemic view of how the tumor interacts with its microenvironment. It is non-destructive and minimally invasive, can evaluate the whole body, and can be performed at multiple time points (i.e. pre-therapy and post-therapy). By changing the drug to which the PET emitter is attached, varying molecular interactions can be observed. This virtual biopsy is intended to be complementary to the physical biopsy, which provides high resolution pathological and molecular diagnostic information. While the relative levels of Pgp expression have been correlated with MDR in cell killing and cytotoxin uptake studies, the presence of the pump does not necessarily represent its function or level of function [11]. The function of Pgp is also dependent on the affinity of the substrate and specific Pgp mutation present [12, 13]. The ability to non-invasively visualize drug accumulation in tumors would be beneficial for the design of patient specific cancer therapy.

F-18 Paclitaxel ([¹⁸F]FPAC)

One commonly used natural diterpenoid chemotherapeutic agent, paclitaxel (PAC, Fig. 1), is a substrate of Pgp [14]. The compound was originally isolated from the bark of the Pacific Yew tree (*Taxus brevifolia*) at great expense to the trees. Subsequently, the non-cytotoxic tricyclic baccatin-III and biosynthetic precursors were isolated from needles of the European yew species *Taxus baccata* and the search for semi-synthetic and more potent analogues led to the discovery of docetaxel (Fig. 1).

Paclitaxel binds to β -tubulin subunits to stabilize the formation of microtubules. This results in cell cycle arrest at the G2/M stage and ultimately apoptosis. This neutral β -tubulin binding drug does not require an electrostatic potential gradient for intracellular retention and is not a significant substrate for other MDR associated ABC transporters (i.e. MRP) [15]. For these reasons, the development of a PET tracer derivative of PAC is predicted to be useful in estimating intratumoral drug concentration and from this, PET imaging may be used to predict which tumors are likely to respond to the drug prior to treatment.

Basic scientists frequently use C-14 or H-3 labeled drugs to quantify biodistribution, but their long half-lives and method of decay limits their utility in patients. DTPA PAC labeled with In-111, a single photon emitting radionuclide, has been investigated in mice bearing paclitaxel-sensitive mammary tumors [16] with a maximum tumor to background ratio of 6.9 at 24 hours; however further xenograft testing using several drug sensitive and resistant cell lines showed no correlation between tumors which responded to paclitaxel therapy and those that did not. It was postulated that this unexpected finding may be due to the use of the chelating agent, DTPA [17, 18]. C-11 paclitaxel, a positron emitting tracer was synthesized by Ravert *et al.* in 2002 [19]. The short half life of C-11 (~20 minutes) would limit its clinical utility. Docetaxel was radiolabeled with carbon-11 in two different positions; the t-butylcarbamate [20] and the 2-oxybenzoate carboxyl group [21]. When compared with two radiohalogenated paclitaxel derivatives (I-124 and Br-76), F-18 fluoropaclitaxel ([¹⁸F]FPAC) showed the highest uptake and rapid clearance from tissues in rats [22], so further development was pursued.

The large number of functional groups and stereochemical centers of paclitaxel posed both an opportunity and a challenges for radiochemical syntheses. The large number of hydroxyl functional groups raised questions regarding the ability to selectively functionalize a specific position; fortunately the tricyclic ring structure and the stereochemical presentation of the functional groups provided opportunities to achieve selectivity of reaction. We chose to take advantage of the highest reactivity exhibited by the amino functionality in the phenylisoserine moiety of paclitaxel. The authentic non-radioactive FPAC had been prepared prior to our work and was shown to retain its biological cytotoxicity [23].

We observed that the previously reported synthesis of [¹⁸F]fluorobenzoic acid would allow synthesis of fluoropaclitaxel by coupling to a 3'-debenzoylated precursor. The debenzoylation exposes a primary amine that would be the most reactive site for acylation. A method had been reported in the patent literature for conversion of paclitaxel into this primary amine precursor [24] and this amine became commercially available *via* custom chemical synthesis. The authentic standard fluoropaclitaxel was easily prepared by reaction of the primary amine with 4-fluoro-benzoyl chloride. Because the amine was much more reactive than the free hydroxyl groups, this reaction proceeded specifically and efficiently.

Several precursors have been utilized for the preparation of various esters of [¹⁸F]fluorobenzoic acid. The optimal leaving group for aromatic nucleophilic substitution is a trimethylammonium functionality situated para to the electron withdrawing ester group [25]; typical esters are ethyl or pentamethylbenzyl [26]. The pentamethylbenzyl ester can be cleaved under acidic conditions; the ethyl ester was cleaved under basic conditions. The two different esters have not been directly compared for the radiochemical synthesis of [¹⁸F]FPAC, but synthetic procedures would be somewhat different.

We chose to use the pentamethylbenzyl ester because the cleavage could be accomplished with neat trifluoroacetic acid. The subsequent coupling step that we utilized required nearly anhydrous conditions that were easily accomplished by careful evaporation of the trifluoroacetic acid at sub-ambient temperatures. We explored two different routes to the radiochemical synthesis of [¹⁸F]FPA[22]. Our initial effort was to prepare [¹⁸F]benzoyl chloride, which would be a more reactive moiety for formation of the desired amide. However, the high volatility of the acid chloride and propensity to hydrolyze made the radiosynthesis unreliable. The volatile [¹⁸F]fluoro-benzoyl chloride was impossible to handle reliably using manual radiochemical methods. We changed to an alternative amide coupling reaction outlined in Scheme 1 that provided much higher reproducibility at the cost of a somewhat lower overall yield.

Incorporation of [^{18}F]fluoride into the pentamethylbenzyl trimethylammonium benzoate triflate could be accomplished in about 50% radiochemical yield in a 10 min reaction time. The initial reaction solution was diluted with ether and passed through a silica gel column to remove unreacted [^{18}F]fluoride. Evaporation of the eluate and addition of trifluoroacetic acid effected the cleavage of the pentamethylbenzyl ester to give [^{18}F]fluorobenzoic acid. The trifluoroacetic acid was evaporated carefully with a gentle stream of argon after cooling the reaction solution to 0-4 °C. This pre-cooling minimized product losses due to evaporation of the [^{18}F]fluorobenzoic acid. Coupling of this acid to the primary amine of paclitaxel was accomplished by treating a solution of the two with diethylcyanophosphonate and diisopropylethylamine. After a 5 min reaction time, [^{18}F]fluoropaclitaxel was purified by HPLC in an overall yield from [^{18}F]fluoride of $15 \pm 5\%$ (uncorrected) in a synthesis time of about 80 min [27]. The robustness of this manual synthetic procedure is supported by its successful transfer to another institution for use in biological studies [28].

Minor modifications to the procedure were required for successful automation with a modified GE automated synthesis device [29]. The automated method provides [^{18}F]FPAC in 21 % (corrected to EOB) radiochemical yield with specific activities of about 150 GBq/micromole. This automated procedure provides a radiochemical synthetic method with appropriate controls to allow utility of the product in human pilot studies.

Preclinical Studies

In Vitro—Preliminary cytotoxicity studies performed in drug sensitive (KB 3-1, non-Pgp expressing) and drug resistant (KB 8-5, Pgp expression induced) human epithelial cells [30] showed PAC and FPAC to have comparable cell killing abilities. *In vitro* radiotracer uptake studies were performed in these same cell lines using [^{18}F]FPAC, [$^{99\text{m}}\text{Tc}$] sestamibi and [$^{99\text{m}}\text{Tc}$] tetro-fosmin (other known MDR substrates), as well as [^{18}F]FDG (as a negative control). A similar differential uptake between the sensitive and resistant cell lines was seen in all of the radiolabeled MDR substrates, but not for [^{18}F]FDG (the uptake of which has not been associated with MDR) [31].

Rodent Studies—The biodistribution of [^{18}F]FPAC (i.v. 50-60% ethanol vehicle) was initially performed in male rats (Spague-Dawley) by Kiesewetter *et al.* [22] with and without treatment with unlabeled PAC (200 nmol, i.p.). Using differential uptake ratios [$\text{DUR} = (\% \text{ injected dose/gram} \times \text{body mass in grams})/100$], the organs with the highest activities (without unlabeled PAC) at 60 min post injection were liver (1.6 ± 0.25), kidney (1.6 ± 0.17), heart (1.3 ± 0.30) and lungs (1.0 ± 0.14). The retention of radioactivity in these organs increased significantly ($p = 0.001$) in the rats receiving unlabeled PAC. Metabolism of [^{18}F]FPAC was determined by extraction of liver and serum and analyzing the supernatant for parent; percent parent compound in serum and liver was 78% and 87%, respectively. The DURs of the parent compound in blood were 0.082 and 0.033 at 30 and 60 minutes respectively.

This same publication reported the biodistribution in wild type and *mdr 1a/1b* (comparable to human *mdr 1* gene) knockout mice using a co-injection of [^3H]PAC and [^{18}F]FPAC, with and without either cold PAC or an MDR modulator (XR9576, tariquidar) treatment [32]. XR9576, an anthranilic acid derivative, is a non-competitive Pgp modulator that binds to a Pgp binding site separate from that of PAC transport and its uptake appears to be due to passive diffusion (independent of Pgp expression) [33]. The organ distribution of [^{18}F]FPAC in the wild type mice was similar to the rats. The uptake of [^{18}F]FPAC and [^3H]PAC were similar with the exception of the kidneys, which again showed higher [^3H]PAC uptake. Comparing the [^{18}F]FPAC uptake in wild type and knockout mice, the

heart (79%), lungs (143%), femur (26%), muscle (38%) and brain (1400%) uptake increased significantly ($p>0.05$) in the knockout; while the uptake of [^3H]PAC increased in the blood (1.6%), heart (76%), liver (22%), muscle (59%), and brain (640%) of the knock-out. Wild-type mice pre-treated with PAC (20 nmol) showed significant increases in the heart, lungs, liver kidney and brain. Pre-treatment with XR9586 showed significant increases in the heart (95%), lungs (230%), femur (23%) and muscle (45%). These data suggest that overall; the biodistribution of [^{18}F]FPAC is similar to that of PAC and the uptake of [^{18}F]FPAC increases with administration of cold PAC or MDR modulator.

Biodistribution and microPET studies were performed by Gangloff *et al.* [28], in human tumor mouse xenografts. Athymic mice with and without MCF-7 (human breast cancer cell line) were co-injected with [^{18}F]FPAC and [^3H]PAC in 100% dimethyl sulfoxide (DMSO) *via* tail vein. Eight of these mice were pre-treated with cyclosporine (10 mg/kg), a known Pgp inhibitor [34]. A non-significant trend towards increased tumor uptake ($p=0.10$) was noted in mice receiving cyclosporine for both those receiving [^{18}F]FPAC and those receiving [^3H]PAC. The biodistribution of [^{18}F]FPAC DMSO was similar to [^3H]PAC DMSO, again with the exception of higher bladder uptake in the [^3H]PAC DMSO mice. The addition of 20 mg/kg of unlabelled PAC DMSO did not significantly change the tumor or normal organ uptake in either group.

Hsueh *et al.* [35] studied [^{18}F]FPAC uptake using microPET in human tumor mouse xenograft models that were treated with paclitaxel. Athymic mice bearing either MCF-7 or MCF-7/AdrR (doxorubicin-selected MCF-7 human breast tumor cell line is associated with over-expression of Pgp) tumors [36] were injected with [^{18}F]FPAC DMSO, *via* tail vein. These mice were subsequently treated with a single dose (40 mg/kg) of PAC DMSO, *i.p.* Preliminary experiments showed that the tumor to background ratio after a 30 minute uptake period did not change as a result of the paclitaxel treatment. The rate of change in tumor volume (measured weekly) was compared with [^{18}F]FPAC uptake (expressed as a measured standardized uptake value (mSUV [cps tumor/cps whole body])). Their results showed an inverse correlation between tumor [^{18}F]FPAC uptake and rate of tumor growth ($r=-0.55$, $p<0.02$; $n=19$). If the two outliers (MCF tumors showing [^{18}F]FPAC uptake which did not respond to therapy) are removed from the analysis the correlation strength increased to $r=0.83$, $p<0.001$; $n=17$) [35]. It is possible that the outliers were due to the presence of alternate resistance mechanisms in MCF-7/AdrR cells not associated with drug uptake (*i.e.* compromised DNA synthesis) [37].

Studies performed by injecting [^{18}F]FPAC in 50-60% ethanol vehicle, *via* tail vein, in adult female nude mice bearing both drug sensitive (KB 3-1, $n=8$) and drug resistant (KB 8-5, $n=9$) human epithelial cell xenografts showed a trend toward increased accumulation of [^{18}F]FPAC in the drug sensitive tumors; however statistical significance was not achieved ($p=0.06$) [31].

Non-Human Primates—To further evaluate the biodistribution of [^{18}F]FPAC, multiple PET imaging studies were performed on three non-tumor bearing, non-human primates to obtain human dosimetry estimates. [^{18}F]FPAC remained >99% protein bound. The mean parent fraction in blood plasma was >50% during the first two hours after injection, with 2 metabolites of approximately equal fraction and a small amount of a third metabolite, found only in the female monkey. The baseline biodistribution showed > 75% of the injected dose (ID) in the liver, gall bladder and intestines consistent with primary hepatic excretion. Human dosimetry estimates were performed using the dynamic image data and MIRDose 3.1. The organs receiving the highest doses include the gall bladder, 0.692 rads/mCi, liver, 0.523 rads/mCi, and the upper large intestine, 0.347 rads/mCi. The whole body effective dose is estimated at 0.083 rem/mCi [27].

To assess changes in [^{18}F]FPAC uptake in organs normally expressing Pgp, imaging was also performed both with and without the pre-administration of an MDR modulator (XR-9576, administered as an i.v. bolus, 1-hour prior to [^{18}F]FPAC). Using the slope of the Logan plot as an imaging parameter (estimate of the drug volume of distribution) [38], only the liver showed a significant increase following modulator administration ($p=0.02$). When simply using the area under the organ concentration/activity curves normalized to the ID and animal weight as the imaging parameter, the liver, lung and the kidney showed significant increases ($p=0.03$, 0.04 and 0.05 respectively), consistent with known Pgp expression in these organs [27]. Surprisingly, the brain uptake (which was negligible at baseline) did not change with Pgp modulation. As the log P value for paclitaxel has been reported to be 4.4 (lipophilic) [39], this finding suggests that Pgp may not be the primary method preventing FPAC's accumulation in the monkeys' brains. No measurement of the log P for FPAC has been reported.

Clinical Studies

First in human imaging was performed on three normal volunteers (2 male, 1 female) receiving a mean injected [^{18}F]FPAC dose of 4.3 mCi (range 2.7-5.9). Serial whole-body images were acquired after the administration of [^{18}F]FPAC to obtain time activity curves from all organs. The organs accumulating the highest activity include the gall bladder, intestines and liver [31]. In this same study, imaging was also performed in three female breast cancer patients with primary tumors > 1.5 cm (Fig. 2). Although, the mean tumor uptake in all 3 subjects was low (SUV 0.84), the tumor was visible against the low background of uninvolved breast tissue (SUV of 0.15). The average tumor to background ratio at one hour post injection was 6.0 [31].

These preliminary data show that [^{18}F]FPAC PET imaging is feasible, that the dosimetry is within the range of other clinically used tracers, and that uptake differences between normal breast tissue and breast tumors allow tumor visualization. Further analysis of this data is ongoing, and a pilot study characterizing [^{18}F]FPAC uptake in patients with extra-abdominal tumors historically sensitive to PAC and those historically resistant to PAC will begin shortly.

Conclusion

Drug resistance in solid tumors is a significant clinical problem. With the recent advancements in molecular imaging, implementation of non-invasive techniques to predict therapeutic response is becoming a reality.

An advantage of imaging a radiolabeled chemotherapy agent, such as [^{18}F]FPAC, is that regardless of the mechanism(s) of tumor uptake and/or removal, the concentration of the drug within the tumor can be estimated *a priori*. Such a tool can be used to further investigate the mechanisms of drug resistance and investigate the effectiveness of adjunctive and or combination therapies.

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Fig. (1).
Paclitaxel and docetaxel.

Fig. (2). 6.1 mCi of [¹⁸F]FPAC was administered i.v. (left antecubital fossa). These static wholebody images were acquired 80 minutes later. The maximum SUV of the 4.4 cm right primary breast tumor [a] was 1.3. The SUV of the involved 2.5 cm right axillary node [b] was 1.6. The majority of the tracer is found in the excretory pathway (liver and intestines), with lesser uptake seen in the bone marrow (BM) and heart. Uptake is also seen in the pituitary (open arrow) and salivary (solid arrow) glands

Scheme 1.
Radiochemical synthetic route to $[^{18}\text{F}]$ fluoropaclitaxel.