Clinical Pharmacokinetic and Metabolism of PET Radiotracers for Imaging P-glycoprotein in Chemoresistant Tumor of Colorectal Cancer

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Abstract: The pharmacological treatment of colorectal tumour leads to MultiDrug Resistance due to overexpression of several ABC transporters such as P-glycoprotein and some Multidrug associated Resistance Proteins (MRPs) that are able to efflux the chemotherapeutic agent out of the cell.

A strategy to reverse MDR is the co-administration of antineoplastic agent with a P-glycoprotein inhibitor. These inhibitors are an useful tool for investigating, by PET, the expression and the activity of P-gp and MRPs that are overexpressed in chemoresistant colorectal tumor cells. In this review will be focused the aspect on P-gp and MRPs ligands employed as PET radiotracers considering their pharmacokinetic pharmacodynamic profile and their selectivity towards ABC transporters involved in chemoresistant cell of colorectal tumour.

Keywords: Colorectal cancer, PET, P-glycoprotein, MultiDrug Resistance, 11-C radiotracers, Everted gut sac.

INTRODUCTION

MultiDrug resistance is the adaptive response of cancer cells to produce a resistant phenotype and it could be a complex process that involves several pathways. Several resistance mechanisms that affect the efficacy of numerous unrelated anticancer drugs are reported and the best characterized and most investigated mechanism involves the ABC transporters (ATP-ase Binding Cassette) [1]. It has been demonstrated that prolonged exposure of cancer cells to chemotherapeutic drugs reduced drug accumulation due to the efflux activity of pumps localized into the bilayer of tumor cell membrane [2].

The most involved efflux pump is a 170 kDa protein termed the permeability glycoprotein, or P-glycoprotein [3]. Several anticancer drugs, are known to be substrates of P-gp such as taxanes (e.g. paclitaxel), epipodophyllotoxins (e.g. etoposide), anthracyclines (e.g. doxorubicin) and vinca alkaloids (e.g. vinblastine). Expression of the protein has been observed in many cancer types and a number of endogenous tissue sites, mainly localized at sites of excretion or at barriers.

This crucial localization indicates a protective role for the body against chemical insult. Although the expression of P-gp has been confirmed in many cancer types, a substantive role in mediating resistance is most convincing in neuroblastoma, breast cancer and colon adenocarcinoma [4,5]. In these cancer types, the expression of P-gp has been associated with poor prognosis, reduced rates of remission and lower overall survival.

ABC Transporters Involved in MDR: P-gp BCRP and MRPs

Human ATP binding cassette (ABC) transporters belong to a family of 49 genes classified into seven subfamilies: ABC-A, ABC-B, ABC-C, ABC-D, ABC-E, ABC-F, ABC-G [6,7]. Some of these transporters are known in MDR, in particular ABC-B1, better known as Pglycoprotein (P-gp), ABC-G2 or Breast Cancer Resistance protein (BCRP) and ABC-C1-6 or Multidrug Resistance associated Proteins (MRP1-6). These transporters are characterized by a highly conserved structure, in detail P-gp is organized in two Membrane Spanning Domains (MSDs) each containing six transmembrane helices and two Nucleotide Binding domains (NBDs), responsible for ATP binding [8,9]. BCRP is a “half-transporter” because it is formed by only one MSD and one NBD although it dimerizes to be fully active [10]. MRPs differ from P-gp because they display three MSDs and the additional domain contains five transmembrane domains. Moreover, P-gp is localized in apical compartment of cell membrane in liver, kidney, placenta and the villus tip of enterocytes in the gut. This localization allows the efflux transport of xenobiotics and several anticancer drugs into the intestine lumen, bile, urine and blood [11]. In the gut, P-gp displays a strategic activity modulating access of drugs to the CYP3A4 enzyme, thereby regulating drug metabolism and absorption. BCRP is expressed on apical cell membrane of breast, ovary, small intestine, colon and liver; this pump effluxes the same substrates as P-gp and regulating drug absorption and disposition. MRP1 is localized in basolateral membrane of polarized epithelial cells, renal distal and collecting tubules, and liver. This protein protects cells through the efflux of substrates into the blood, it is involved in detoxification of endogenous metabolites such as glutathione conjugates, bilirubine glucuronides and diamionic bile salts [7].

Positron Emission Tomography for imaging P-gp and other ABC Transporters in Tumors

PET is a nuclear medicine imaging technique that employs radiotracers for visualizing in vivo the target involved in cancer diseases. PET analysis discloses high spatial resolution and it providing a quantitative measure of radioactivity in tissue [12]. In PET studies are employed radioisotopes such as carbon-11 (11C), nitrogen-13 (13N), oxygen-15 (15O), fluorine-18 (18F) or gallium-68 (68Ga) that decaying emit positron, with different half-lives: 20.4, 9.96, 2.04, 109.7 and 67.6 min, respectively. The radioisotopes are generated in a cyclotron and they are chemically introduce into the molecule of interest immediately prior to administration.

ABC RADIOTRACERS

The development of ABC transporter radioligands is a critical step in medicinal chemistry and radiochemistry fields because of the difficulty to obtain probes with useful selectivity towards the P-gp, BCRP and MRP1. Another critical parameter in drug penetration across the small intestine and other barriers is the metabolizing enzyme CYP3A4 activity that modifies the structure and the amount of radiotracer [13]. Finally, it is important to establish the interacting mechanism of radiotracer [14]. Indeed, P-gp substrate is able to detect the activity of ABC transporter whereas P-gp inhibitor permit to visualize the expression of pump [15].
In oncology field an important aspect is the early diagnosis of a chemoresistant tumors with a P-gp inhibitor as a PET probe to imaging the expression of pump in tumor cells. In the meantime is important to evaluate the brain uptake of radiotracer with respect to the uptake in peripheral tumor cell.

Several attempts have been carried out to develop useful PET radiotracers for early diagnosis in oncology field but to date the proposed radioligands failed because of pharmacodynamic and or pharmacokinetic limitations.

The calcium channel inhibitor verapamil is used as a reference P-gp inhibitor in experimental pharmacology but actually in PET experiments, verapamil is transported by Pgp. Therefore $^{11}$C-labelled verapamil has been developed as the first Pgp substrate radiotracer with low selectivity towards P-gp because it is also a substrate of MRP1 [16].

Since for quantitative PET studies an enantiomerically pure PET tracer is preferred over a racemic mixture, the synthesis of the individual enantiomers of $^{11}$C-verapamil has been developed [17]. P-gp expression estimation cannot be performed with Pgp substrate and in order to overcome this limitation several P-gp inhibitors belonging to claimed third-generation ligands have radiolabelled such as $^{11}$C-laniquidar [18], $^{11}$C-elacridar [19,20] and $^{11}$C-tariquidar (Fig. 1) [21].

The goal was to map Pgp expression levels with P-gp inhibitor radiotracers that should bind to Pgp without being transported giving higher baseline PET signals than substrates. Unfortunately, all mentioned Pgp inhibitors were found in vivo to be similar to radiolabelled substrates [22].

In Vitro and Ex Vivo Evaluation of a P-gp Radiotracer to Imaging Chemoresistant Tumor of Colorectal Cancer

Lack of suitable experimental models a major drawback in the study of the mechanisms responsible for intrinsic resistance in colorectal cancer cells.

Few studies have been reported to establish the mechanism and the pathways involved chemoresistant gastrointestinal malignancies [23] although the overexpression of ABC transporters seems to be the most considered hypothesis. Moreover, another critical aspect is the presence of a large panel of ABC transporters involved in MDR in each selected tumour cell of gastrointestinal tract [24].

In particular, in LoVo [25] and Caco-2 cells a number of proteins, including P-gp, MRP, LRP (liver resistance-related using biochemical and immunocytological techniques) have been detected and so the role of each transporter in MDR cannot established.

Caco-2 cells [26] have been largely employed in in vitro assays to define specific pharmacokinetic parameters such as the Apparent Permeability ($P_{app}$) In this assay was determined the basolateral-apical flux (BA) and apical-basolateral flux (AB). The first flux represents passive diffusion, while the second represents active P-gp-modulated transport. The BA/AB ratio identifies substrates (BA/AB from 18 to 20) and inhibitors (BA/AB < 2), while modulators display an intermediate BA/AB ratio (ranging from 2 to 18) [27].

Moreover, since P-gp and CYP3A4 are co-expressed in the intestine and liver they modulate together to the Absorption Distribution Metabolism Excretion Toxicity (ADMET) of drugs [28, 29]. Therefore, intestinal drug metabolism is dependent not only on the activity of CYP3A4 in the intestine, but also on the activity of P-gp. The importance to take into account transport and metabolism simultaneously, is demonstrated by several clinical studies in which, the concomitant administration of a CYP3A4 inhibitor and a P-gp inhibitor, has been considered a possible strategy to increase the bioavailability of drugs and to design useful PET radiotracers for gastrointestinal tumours [30].

Everted Gut Sac

Traditional in vitro methods separately analyze absorption and metabolism, but the results taken together did not permit to have predictive values to transfer in vivo model [31,32]. In fact, in some cases, in vivo results do not confirm in vitro experiments for the same compounds, i.e. elacridar which was in vitro classified as P-gp inhibitor resulted to be a substrate in in vivo PET experiments.

A strategy to better estimate the in vivo potentials of new radiotracer could be to evaluate it in the everted gut sac assay, an ex vivo model that, at first, gives simultaneous results on absorption and metabolism, secondly, the results obtained by this assay display an high prediction degree for in vivo experiments [33,34]. Since it is accepted that small intestine is the major site of drug absorption, everted gut sac assay is usually used to study drug transport across the small intestine epithelium and to assess the role of metabolism during drug absorption.

This isolated organ bearing both CYP450 and P-gp, permits to evaluate various aspects of drug absorption including P-gp activity.
and intestinal metabolism CYP450-mediated. The metabolism data obtained by the everted gut sac are predictive for the in vivo response because derived from the interaction of substrates with CYP450 enzymes inside enterocytes during the passage through the cells.

**Requirements of a P-gp Radiotracer for Imaging Gastrointestinal Chemoresistant Tumour**

Several attempts are reported to develop Pgp inhibitors as PET tracers because they are expected to bind to Pgp without being transported. This mechanism permit the visualization of Pgp expression levels matching the goal of PET tracers which are employed to label the distribution of biological targets such as P-gp that could be considered as a dynamic biomarker in chemoresistant tumours.[14]

Recently several radiotracers such as [11C]elacridar, [11C]tariquidar and [11C]MC18 [15] belonging to third-generation Pgp inhibitors have been in vivo evaluated (Fig. 1).

These in vivo experiments carried out both in rats and mice showed that, [11C]elacridar and [11C]tariquidar displayed very low brain uptake, and surprisingly, this uptake was several-fold increased in the presence of unlabeled inhibitor. These results display a discrepancy between in vitro and in vivo results. Indeed, these ligands defined P-gp inhibitors for the results obtained in in vitro assays, could be considered P-gp substrates for their results in in vivo microPET studies.

To date only our radiotracer namely [11C]MC18, showed different behavior because it had about more higher brain activity uptake in baseline scans than the other radiotracer.

Moreover, unpublished data, the co-administration of CsA did not affect the brain uptake.

Conversely, the in vivo data reported for [11C]elacridar [20] and [11C]tariquidar [21] suggested that these radiotracers are transported by Pgp and other ABC pump such as BCRP.

In conclusion, as data standing, it is possible to affirm that [11C]MC18 could be considered an useful radiotracer for imaging P-gp expression in gastrointestinal malignancies because it was a P-gp inhibitor both in vitro and in vivo. Moreover, all in vitro studies to assess the activity of our radiotracer have been carried out in Caco-2 cells to define Apparent Permeability (BA/AB = 1.6), the ATP-ase activation (it was unable to activate the enzyme) and the potency (EC50 = 1.6 μM) evaluating [3H]vinblastine transport inhibition in Caco-2 monolayer [35]. Finally these results were corroborates in everted gut sac method where our ligand was not transported and, in addition, was unable to interfere with the enzymatic activity [14].

**REFERENCES**


