Dynamics of multidrug resistance. Analyses with PET and single photon imaging
Hendrikse, Nelis Hendrik

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Chapter 9

Summary

Multidrug resistance (MDR) is characterized by the occurrence of cross-resistance of a broad range of structurally and functionally unrelated drugs [1]. Drugs which are involved in MDR belong to the group of natural drugs, such as the anthracyclines (doxorubicin, daunorubicin), vinca alkaloids (vincristine, vinblastine), epipodophyllotoxins (etoposide), and taxanes (paclitaxel). Several mechanisms are involved in MDR, such as decreased topoisomerase II enzyme levels or increased glutathione (GSH) and glutathione S-transferase levels [2]. One of the most well-known mechanism is the overexpression of P-glycoprotein (P-gp), encoded by the MDR1 gene. P-gp is a 170 kDa transmembrane drug efflux pump, belonging to the ATP-binding cassette (ABC) superfamily [3].

P-gp is extensively expressed in the human body, namely in the adrenal cortex, the intestinal mucosal cells, the biliary hepatocytes, the renal proximal tubule epithelium, the pancreatic ductulus, the pregnant uterus, the gastrointestinal epithelium, the blood-capillaries of the brain and the testes, and in CD34+ bone marrow stem cells [4, 5]. Furthermore, P-gp can be present on membranes in several tumor types [6]. The function of the drug efflux pump P-gp is most likely to protect these organs against endogenous and exogenous toxins.

In rodents P-gp expression is encoded by two genes, namely the mdr1a and the mdr1b gene. Considering the distribution of these genes in rodents, it is suggested that these two genes accomplish the same role as the MDR1 gene in humans.

Another drug efflux pump, which is involved in MDR is the 190 kDa multidrug resistance associated protein (MRP), encoded by the MRP1 gene. This was firstly described by Cole et al in a MDR small cell lung carcinoma cell line without P-gp expression in which the MRP gene was overexpressed [6]. MRP1 is expressed in nearly all organs and in several tumor types. Beside MRP1, other homologues of this gene are known. MRP2 (cMOAT), which is mainly expressed at the canalicular membrane of hepatocytes in vivo, is involved in the transport of anionic conjugates into bile. In addition, in vitro studies demonstrated that the functional transport of MRP2 is equal to MRP1. MRP3 most likely is mainly expressed in the liver. MRP1 gene expression is only low in a few tissues such as bladder, tonsil, lung and kidney while MRP5 is expressed in nearly all tissues of the human body [7]. Only from MRP1 and MRP2 the function is defined. Drugs can be transported by MRP1 as well as MRP2 after conjugation or co-transport with GSH. To modulate MRP mediated drug resistance, buthionine sulfoximine can be used, which results in intracellular GSH depletion.
Overexpression of the P-gp and MRP drug efflux pumps on tumor membranes, which result in MDR in human tumors. To circumvent this resistant phenotype, several modulators for P-gp, such as cyclosporin A are potentially available for clinical use. These modulators are also substrates for P-gp and are able to compete with cytostatic drugs for the drug efflux pump. The result is that the P-gp mediated transport of cytostatic drugs is inhibited, and the concentration of the cytostatic drug in the cell is increased. However, even large randomized studies with P-gp blockers in addition to chemotherapeutic drugs show disappointing results [8-11]. These may partly be due to the fact that it is difficult to have a proper patient selection. For proper patient selection, knowledge of functionality of P-gp and MRP might be helpful. In this thesis, several experiments where the functional transport of P-gp and MRP is studied by PET and planar imaging are described.

In chapter 1 a review is described giving an overview of measurement P-gp and MRP functionality with PET and SPECT. For SPECT studies several studies with Tc-complexes, such as $^{99m}$Tc-sestamibi and $^{99m}$Tc-disofenin and for PET, studies with $^{11}$C-radiolabeled P-gp substrates are discussed.

To study functional P-gp transport with PET, two $^{11}$C radiolabeled P-gp substrates have been developed. In chapter 2 the labeling of the chemotherapeutic agent daunorubicin and the calcium channel blocker verapamil with $^{11}$C is described. Both radiolabeled P-gp substrates were evaluated in vitro and in vivo. $[^{11}\text{C}]$daunorubicin was synthesized from $[^{11}\text{C}]$diazomethane with an aldehyde precursor, followed by mild alkaline hydrolysis. $[^{11}\text{C}]$verapamil was synthesized by a reaction of $^{11}$C-methyliodide and N-nor-methylverapamil. Labeling with $[^{11}\text{C}]$ has the advantage that the native compound is available to study pharmacokinetics with PET. With these radiolabeled P-gp substrates the cellular accumulation in an ovarian carcinoma cell line (A2780) and its P-gp overexpressing subline (A2780AD) were studied. The cellular accumulation of $[^{11}\text{C}]$daunorubicin and $[^{11}\text{C}]$verapamil were respectively 16 and 4-5 fold higher in A2780 than in A2780AD.

The chapters 3 and 4 describe visualization of P-gp function in vivo.

In chapter 3 imaging of the P-gp function and the effects of a modulator on this function in mdr1a gene disrupted mice (mdr1a(-/-) mice) and wild type mice mdr1a(+/+) mice) was evaluated. Ex vivo biodistribution studies revealed 9.5-fold higher $[^{11}\text{C}]$verapamil levels in the brain and 3.4-fold higher levels in the testes of mdr1a(-/-) mice than in mdr1a(+/+) mice. The $[^{11}\text{C}]$verapamil levels were dose-dependently increased by the P-gp blocker cyclosporin A in mdr1a(+/+) mice. No modulating effects of cyclosporin A were found in the mdr1a(-/-) mice. In vivo positron camera data showed lower $[^{11}\text{C}]$verapamil levels in the brain of mdr1a(+/+) mice than in mdr1a(-/-) mice. Time activity curves demonstrated that $[^{11}\text{C}]$verapamil accumulation in the brain of mdr1a(+/+) mice was increased by cyclosporin A to levels comparable with those in mdr1a(-/-) mice, indicating that reversal of P-gp mediated efflux can be monitored by PET.
Chapter 4 describes the results obtained with $^{[14]}$C-verapamil and $^{[14]}$C-daunorubicin in a 2-sided tumor bearing rat. Rats were inoculated with GLC$_4$ cells in one flank and with the MDR1-gene transfected P-gp overexpressing subline GLC$_4$/P-gp in the other flank. Biodistribution studies demonstrated higher accumulation of $^{[14]}$C-verapamil and $^{[14]}$C-daunorubicin in GLC$_4$ than in the GLC$_4$/P-gp tumors. The decreased accumulation of radioactivity in the GLC$_4$/P-gp tumors was completely modulated by cyclosporin A. In vivo positron camera data of $^{[14]}$C-verapamil kinetics and modulating effects with cyclosporin A showed that P-gp function and its reversal can be visualized non-invasively.

In chapter 5 a compartment model was developed and evaluated to study P-gp facilitated kinetics and its modulation in rat brain. The results were optimal described by a 2-compartment model, where the influx was defined by passive diffusion and the efflux was defined by a combination of passive diffusion and active non-competitive Michaelis-Menten kinetics. This model allows description of P-gp facilitated transport in terms of $\text{eV}_{\text{max}}$ (pmol.ml$^{-1}$.min$^{-1}$) and $k_{2D}$ (min$^{-1}$). Firstly, assuming a correlation between P-gp mediated transport and drug resistance, this might be of clinical interest in e.g. acquired drug resistance during tumor treatment. In that case, an increase in P-gp facilitated transport, expressed in terms of $\text{V}_{\text{max}}$, may give information about drug resistance during chemotherapeutic treatment. Secondly, comparison of P-gp mediated efflux in terms of $\text{V}_{\text{max}}$ at different doses of various modulators, may give insight in the efficiency of modulation.

Chapter 6 describes the drug distribution and the kinetics of a bolus injection of the amine drug $^{[14]}$C-verapamil in solid tumors and in several tissues in the human body. It was demonstrated that the $^{[14]}$C-verapamil uptake in the lung was approximately 40% of the injected dose, possibly due to binding of $^{[14]}$C-verapamil to serotonin transporters in the lung. The $^{[14]}$C-verapamil half-lives in the lungs were 46.2 min, in the heart 73.8 min, and in the tumor 23.7 min, which is indicative for a relatively fast verapamil efflux from tumor tissue. Therefore, this rapid tumor efflux of bolus verapamil should be taken into consideration in clinical studies where verapamil modulation is used in solid tumors patients.

Chapter 7 and 8 described several experiments with $\gamma$-emitting compounds to study the transport function of P-gp and MRP in vitro and in vivo.

Chapter 7 describes an in vitro study in which it is shown that $^{99m}$Tc-sestamibi is a substrate for both the P-gp and MRP, drug efflux pump. It was already known that $^{99m}$Tc-sestamibi was a substrate for P-gp. Along several lines it was shown that $^{99m}$Tc-sestamibi is also a substrate for MRP, Accumulation and efflux studies were performed in a small cell lung carcinoma cell line (GLC$_4$) and in its doxorubicin-resistant MRP, but not P-gp overexpressing sublines (GLC$_4$/ADR) as well as in the non-small cell carcinoma cell line (S1) and its MRP, -transfected subline (S1-MRP). In the GLC$_4$ cell lines and in the S1-MRP cell line the accumulation of $^{99m}$Tc-sestamibi was decreased with increasing MRP expression and after depletion of GSH
the cellular $^{99m}$Tc-sestamibi concentration was increased. Sensitivity testing showed cross-resistance of $^{99m}$Tc-sestamibi in GLC$_4$/ADR$_{150x}$ versus GLC$_4$ and a synergistic effect on vincristine cytotoxicity in GLC$_2$/ADR$_{150x}$.

In chapter 8 the substrate specificity of $^{99m}$Tc-sestamibi and $^{99m}$Tc-disofenin for P-gp and MRP was investigated. Furthermore, the possibility to discriminate in vivo between MRP- and P-gp facilitated transport was evaluated. $^{99m}$Tc-sestamibi is normally used in the clinic to study myocard perfusion and $^{99m}$Tc-disofenin for diagnostic imaging of hepatobiliary disorders. To study whether $^{99m}$Tc-disofenin is a substrate for P-gp and/or MRP, accumulation studies were performed in GLC$_4$, GLC$_4$/ADR$_{150x}$, and GLC$_4$/P-gp cells. $^{99m}$Tc-disofenin cellular accumulation was lower in GLC$_4$/ADR$_{150x}$ than in GLC$_4$. The accumulation of $^{99m}$Tc-disofenin in GLC$_4$/P-gp and GLC$_4$ was equal. Bile secretion of $^{99m}$Tc-sestamibi in MRP deficient GY/TR Wistar rats and in normal Wistar rats was equal, while $^{99m}$Tc-disofenin transport was lower in GY/TR than in Wistar rats. Transport activity of $^{99m}$Tc-sestamibi and $^{99m}$Tc-disofenin was decreased after GSH depletion. Gamma-camera experiments with $^{99m}$Tc-sestamibi and $^{99m}$Tc-disofenin in GY/TR Wistar rats and in normal Wistar rats visualized a delayed liver excretion of $^{99m}$Tc-disofenin in GY/TR rats compared to normal Wistar rats, while radionuclide activity of $^{99m}$Tc-sestamibi was equal in both types of rats. $^{99m}$Tc-disofenin was also studied in healthy volunteers and in a Dubin-Johnson patient. Due to a defect in the MRP, drug efflux pump, camera data visualized that in the Dubin-Johnson patient the hepatobiliary transport of $^{99m}$Tc-disofenin was delayed compared to healthy volunteers.

CONCLUSIONS AND FUTURE PERSPECTIVES

The occurrence of drug resistance is a major obstacle in the clinical treatment of cancer patients. Several mechanisms leading to drug resistance exist, and one of them is MDR. Various mechanisms are underlying MDR, namely 1) increased expression of drug efflux pumps (P-gp and MRP), 2) increased expression of LRP, 3) reduced expression of the topoisomerase II enzyme. Focussing on P-gp and MRP, it might be helpful to know the status of drug efflux pumps for a more successful treatment of clinical response to cancer. Much research has been initiated to get more insight in the contribution of P-gp and MRP in MDR and the possibility to inhibit transport activity of these pumps. One potential way to overcome MDR is to treat cancer patients with chemotherapeutic drugs in combination with relative non-toxic modulating agents. Until now, clinical randomized studies demonstrated limited to no effect in solid tumors after addition of modulators [8-11]. One of the reasons for this poor clinical outcome may be due to the patient selection. Therefore, to select a patient group who will benefit from MDR modulators combined with cytostatic agents, it is useful to study the functional transport of P-gp and MRP. As diagnostic tool, PET and planar...
Sutr,tvaRy scintigraphy with radiolabeled substrates are interesting techniques which might allow a non-invasive, quantitative study of the transport kinetics by drug efflux pumps. It can be concluded from the results of the studies described in this thesis, that visualization of the P-gp and MRP function in vivo may be a helpful tool in the design of modulator studies in the clinic. To define the exact role of these new functional diagnostic tests, further research including studies in various tumor types is necessary. It would be interesting to know the protein expression of drug efflux pumps in these tumors, to study whether there is a good correlation between P-gp and MRP expression and efflux. When a correlation between protein levels and drug efflux would exist, it might give more insight in the transport functionality of P-gp and MRP in vivo. It is of relevance to study whether efflux activity of P-gp and MRP can both be efficiently inhibited by modulators, such as biricodar [12].

When the best diagnostic tool for measuring P-gp functionality is developed, the most suitable radiolabeled tracer should be found for MDR studies in vivo. Until now, it is unclear which tracer is most useful to study P-gp and MRP functionality.

The development of a diagnostic tool to measure P-gp mediated kinetics in patients, might have a broad impact in further research e.g. the blood-brain barrier. The blood-brain barrier consists of an endothelial cell layer in the brain microvessels and maintain homeostasis. Due to the presence of tight junctions between endothelial cells in the brain, transport of hydrophylic drugs is limited. Normally, transport of drugs can be predicted by their lipophilicity. However, due to P-gp expression in the blood-brain barrier, the brain uptake is low for several clinical relevant and hydrophylic drugs. For instance, several CNS-active drugs (chlorpromazine, clomipramine, phenytoin) and HIV protease inhibitors (saquinavir, indinavir) interact with P-gp. Therefore P-gp overexpression might be an underlying mechanism for drug therapy resistance in certain psychiatric and AIDS patients. Furthermore, for P-gp modulation, co-administration with these drugs in combination with other P-gp substrates (dexamethasone, cyclosporin A) may improve drug delivery to the brain [13, 14]. However, sometimes after co-medication neurotoxicity is observed. This is described many times for transplantation patients, where central neurotoxicity was observed due to the treatment with dexamethasone and cyclosporin A [15, 16]. Besides P-gp, recently MRP has also been localized in the blood-brain barrier [17-20]. Therefore, novel insights in the pathophysiology and in the pharmacology of drugs would be gained if the P-gp and MRP function in the blood-brain barrier could be visualized and quantified with a non-invasive imaging technique such as PET. When the function of the drug efflux pumps in the blood-brain barrier can be measured quantitatively, a modulator can be administered in an effective dose due to inhibit the transport function of these drug efflux pumps. Then the concentration of clinical relevant drugs which work on the central nervous system can be increased in the brain. In this way, the measurement of the function of the drug efflux pumps in the blood-brain barrier can be involved to improve the therapeutic treatment in the
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central nervous system. Finally, from this thesis it can be concluded that insight in mechanisms of MDR in tumors may be helpful in the insight of other physiological and pathological processes in which P-gp and MRP are involved.

REFERENCES


