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Patel, Sharvil Pankaj (2007) Development of Immobilised Biopolymer Stationary Phases based on the Efflux Transporters. Doctoral thesis, University of Sunderland.

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## **ABSTRACT**

The body is continuously exposed to a variety of toxins and metabolic waste products but is able to rid itself of these by using various detoxification mechanisms such as enzymes and transmembrane transporters. A large number of the transporters which play an essential role in the detoxification mechanisms are found in the liver, kidney and intestines. The largest family of transporters is the Solute Carrier (SLC) Superfamily with 255 members in humans. While most SLC transporters are highly specialized, a number of these transporters are polyspecific, generalized transporters that play a major role in the elimination process. Accordingly, the aim of this research programme was to develop novel methodology to enable the study of the interaction of drugs and related compounds with these transporters, in a rapid and facile manner.

In the initial studies the application of affinity chromatography was carried out with the use of  $\alpha_1$ -acid glycoprotein (AGP) column to achieve enantioselective separation of the drug ketamine and its metabolites. The enantioselective separation of ketamine and norketamine from plasma samples was achieved and the assay conducted was sensitive and reproducible. Building upon the experience gained in this work on affinity chromatography and LC-MS, these useful tools for the determination of chiral drugs in biological fluids were also used in the study of drug-protein interactions. With the nicotinic acetylcholine receptors it was possible to explore the use of immobilized liquid chromatographic stationary phases containing drug transporters in on-line high throughput screening (HTS).

Thus having demonstrated that a stationary phase containing immobilized membranes can be used to identify substrate/inhibitors of an expressed receptor/transporter the next and principal phase of the programme was to adapt the methodology to other target biopolymers such as P-glycoprotein which over the last decade has been focused upon for its role in drug resistance in cancer treatments. In particular liquid chromatographic columns containing the drug transporters, P-glycoprotein (Pgp) and human organic cation transporter 1 (hOCT1) were prepared, evaluated and exploited.

Initial studies were conducted to confirm the functionality of a stably transfected cell line expressing Pgp (Pgp(+), LCC6/MDR1 cell line) through a comparison with the non-transfected cell line (Pgp(-), LCC6 cell line).

Initially membranes from the Pgp(+) and Pgp(-) cell lines were then immobilized on immobilized artificial membranes. However, although the resulting column could actively bind the Pgp substrates, strong non-specific interactions with the IAM backbone led to large retention times and peak tailing. A more successful approach was to immobilize on the surface of open tubular capillaries. Such columns were used to sort compounds with or without affinity for Pgp, by comparing the differential retention time on the Pgp (+) and Pgp (-) columns. In this way the non-specific interactions with the constituents of the cellular membrane were compensated for. The results from the sorting by differential chromatography were compared with the behavior of the same compounds in Caco-2 monolayers cultured in 96-well transwell plates, the standard method for the determination of substrates for Pgp. A group of 14 compounds

previously characterized as substrates or non-substrates of Pgp were studied using the chromatographic and Caco-2 methods. In the parallel chromatographic screen, the value of  $\Delta t$  varied from 0.11 min (nicardipine) to 21.53 min (domperidone). The ratio of the value of the apparent permeability coefficient ( $P_{app}$ ) in the basal-to-apical direction ( $P_{app_{B-A}}$ ) to  $P_{app}$  in the apical-to-basal direction ( $P_{app_{A-B}}$ ), ( $P_{app_{B-A}}/P_{app_{A-B}}$ ) among the same set of compounds ranged from 1.4 (nicardipine) to 45.0 (domperidone). In almost every case, when  $\Delta t$  exceeded 0.25 min, the value of  $P_{app_{B-A}}/P_{app_{A-B}}$  was greater than 2.0, suggesting that the compound was a Pgp substrate. Also there was a positive correlation between the two data sets ( $r^2 = 0.95$ ). It was therefore clear that the parallel screening of new pharmaceutical compounds on Pgp(+) and Pgp (-) open tubular columns could be interchanged with the conventional, widely used Caco-2 model and could be used to pre-screen and identify Pgp substrates and inhibitors, prior to moving onto a more expensive and time consuming functional assays to determine functional activity. Also the success of these experiments would help to generate affinity chromatography models for other transport proteins.

An illustration of this was a study on the human organic cation transporter, hOCT1 which plays an essential role in the detoxification mechanisms mediating the inactivation and excretion of toxins and metabolic waste products. This transport protein was immobilized on an IAM based stationary phase, with no significant non-specific interactions occurring in this cases, and frontal affinity chromatography was used to characterize the activity of the immobilized transporter. The data indicated that the hOCT1 bound known hOCT1 substrates and the calculated  $K_d$  value for the marker

ligand N-methyl-4-phenylpyridinium (MPP) was 6.06 pM, consistent with previously reported  $K_i$  values. The previously found enantioselectivity of hOCT1 was confirmed here by determining the ratio of the  $K_d$  values for the stereoisomers of propranolol (R/S = 2.98), atenolol (R/S = 2.11), psuedoephedrine (S/R = 1.53) and verapamil (S/R = 86.5). The observed differences in the  $K_d$  values of the stereoisomers was used to design a pharmacophore which identified the different interactions between the substrate/inhibitor and hOCT1 to help to explain the enantioselectivity of the hOCT1 transporter. The pharmacophore contained a hydrophobic region ( $\pi$ - $\pi$  interaction, or  $\pi$ -cation interactions), a hydrogen bond donor and an ion pair interaction region. It was then expanded to include other ligands and a general approach to the determination of whether a compound is a substrate/inhibitor of the hOCT1 was formulated. This general approach shows great promise for making a greater contribution in the future for the general understanding of drug protein interactions and in particular to the pre-Development screening of drug candidates.

## **ACKNOWLEDGEMENTS**

First and foremost, I would like to thank my supervisors and mentors, Dr Lough and Dr Wainer, for all their help and guidance during the course of the research project. I cannot fully express my gratitude to their exceptional support and encouragement to me for the research project right from the start. My sincere appreciation to the entire team at the National Institute of Health, Laboratory of Clinical Investigations, with special thanks to Dr Moaddel for his generous assistance in the research project. Finally I would like to thank my mom, dad, grandma and my sweet sister for their faith and support during the full research time. I would like to dedicate this work and effort of mine to my beloved and loving grandfather Shri Ramanbhai Patel, who is not with us anymore.