Cum Laude

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Evaluation of P-glycoprotein modulation by pharmaceutical excipients

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Abstract

Modulation of P-glycoprotein (P-gp) may play a significant role in the systemic delivery of poorly bioavailable drugs e.g. anti-neoplastic drugs. Many compounds from different chemical classes, including pharmaceutical excipients, have been proven to have inhibitory effects on P-gp mediated efflux transport, which leads to increased bioavailability. For the purpose of this study, different sub-classes of a group of excipients (i.e. disintegrants) were selected to evaluate the effect of *ex vivo* transport of a model compound across excised pig intestinal tissue. The model compound, namely Rhodamine 123 (R123), was selected on the basis that it is a known P-gp substrate. Results showed that certain of the selected disintegrants showed P-gp modulation but other disintegrants had no effect on R123 transport.

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Introduction

P-glycoprotein (P-gp) is a well described active efflux transporter, which forms part of the ATP-binding cassette (ABC) family. It can be found throughout the human body, most notably in the liver, the intestinal epithelia and the blood-brain barrier. P-gp is capable of actively transporting xenobiotica (e.g. drugs/drug-like compounds) out of the cell back to the apical side of the membrane, thus altering absorption, distribution and elimination of substrates (i.e. bioavailability). Therefore, any modulation (including inhibition or induction) of P-gp related efflux transport can directly influence the bioavailability of certain drugs (Varma *et al.*, 2003; Zhao *et al.*, 2016).

Pharmaceutical excipients are compounds employed in dosage form designs other than the active pharmaceutical ingredient(s). Initially regarded as pharmacologically inert, they do exert certain effects on drug release kinetics and play a major role during the design and manufacturing of different dosage forms. More recently, however, excipients have been found to alter pharmacokinetics of drugs such as P-gp related transport (Dave *et al.*, 2015; Zhao *et al.*, 2016).

Rhodamine 123 (R123) is a known P-gp substrate, which was used as a marker compound at a concentration of 5 µM in this study. We confirmed the selective P-gp transport of R123 by finding an increased secretory percentage transport compared to the transport in the absorptive direction. Any additive which causes an increase in R123 transport in the absorptive direction and a decrease in the secretory direction may be classified as a P-gp inhibitor and *vice versa* (Wang *et al.*, 2009). Therefore, any compound that is able to modulate P-gp related transport of a substrate can potentially affect bioavailability.

Materials and methods

Materials

Rhodamine 123, Krebs-Ringer bicarbonate (KRB) buffer and sodium alginate were purchased from Sigma-Aldrich (Johannesburg, South Africa). Croscarmellose sodium (Ac-di-sol®) was purchased from FMC Corporation (Cork, Ireland). Crospovidone (Kollidon® CL-M) was purchased from BASF (Ludwigshafen, Germany). Costar® 96-well plates were purchased from The Scientific Group (Randburg, South Africa) and pig intestine was collected from a local abattoir in Potchefstroom, South Africa.

Preparation of excised pig jejunum

The research project involving *ex vivo* transport studies across the excised pig intestinal tissue was approved by the Animal Ethics Committee of North-West University (NWU-00025-15-A5). A section of approximately 20 cm of pig proximal jejunum was

collected from the abattoir directly after slaughter of the pig. The excised tissue was rinsed with and submerged in ice-cold KRB and transported to the laboratory on ice. In the laboratory, the tissue segment was pulled over a glass tube and the serosa was removed by means of blunt dissection. Thereafter the intestinal section was cut along the mesenteric border and the resultant tissue sheet was cut into smaller sections to be mounted between two Sweetana-Grass diffusion half cells. Six complete Sweetana-Grass diffusion chambers were connected to a 37°C heating block and a carbogen (95% O_2:5% CO_2) source (Legen *et al.*, 2005; Pietzonka *et al.*, 2002).

Transport studies across excised pig jejunum

All transport studies were conducted in two directions, i.e. apical-to-basolateral (A-B) and basolateral-to-apical (B-A). Initially the chambers were incubated with pre-heated (37°C) KRB on both sides of the membrane and allowed to acclimatise for 15 min. Two sets of test solutions were prepared and pre-heated. Test solutions for A-B studies were prepared by mixing R123 with enough of each of the selected disintegrants to make up to the required concentration and incubated on the apical side. On the other hand, for B-A experiments, solutions of R123 alone was incubated on the basolateral side while suspensions of the disintegrants alone was incubated on the apical side. This was done to ensure that experimentation mimics biological conditions as close as possible because the disintegrant remains on the apical side of the membrane. KRB was aspirated from each donor side before test solutions were applied to the donor chamber. Test samples of 180 µl were collected from the acceptor chamber at 20 min intervals for a period of 120 min, amounting to six samples for each transport study (Legen *et al.*, 2005; Pietzonka *et al.*, 2002).

Fluorescent spectroscopy analysis method

All analyses for R123 content of the test samples were conducted using a validated fluorescent spectroscopic analysis method with the aid of a Spectramax Paradigm® multi-mode detection platform plate reader. Excitation wavelength was set at 480 nm and the emission wavelength at 520 nm (Kaprelyants & Kell, 1992; Wang *et al.*, 2009).

Statistical analysis of data

The apparent permeability coefficient (P_{app}) was calculated from the obtained percentage transport data for each bi-directional transport study, using equation 1 (Legen *et al.*, 2005).

$$P_{app} = \frac{dQ}{dt}\left(\frac{1}{A.60.C_0}\right) \qquad \text{(Eq. 1)}$$

Where P_{app} is the apparent permeability coefficient ($cm \cdot s^{-1}$), $\frac{dQ}{dt}$ is the permeability rate (amount permeated per minute), A is the diffusion area of the membrane (cm^2) and C_0 is the initial concentration of R123.

Statistical analyses were performed on all P_{app} values to determine if the addition of disintegrants had any effect on R123 transport. Analysis of variance (ANOVA) was performed on all data and compared to the control followed by Dunnett's t-test and the Kruskal-Wallis test for non-parametric data. P-values of less than 0.05 were considered to indicate statistically significant differences between data sets.

The efflux ratio (ER) was calculated using equation 2 to obtain a ratio which indicates the extent to which R123 undergoes efflux.

$$ER = \frac{P_{app}(B\text{-}A)}{P_{app}(A\text{-}B)} \qquad \text{(Eq. 2)}$$

Where P_{app} (B-A) is the permeability coefficient for the permeation in the basolateral-to-apical direction and P_{app} (A-B) the same variable in the apical-to-basolateral direction.

Results and discussion

Croscarmellose sodium (Ac-di-sol®)

Croscarmellose sodium (CCS), commercially distributed as Ac-di-sol®, is classified as a superdisintegrant and was tested at concentrations of 0.0005% (w/v), 0.001% (w/v), 0.005% (w/v) and 0.01% (w/v) during R123 transport studies.

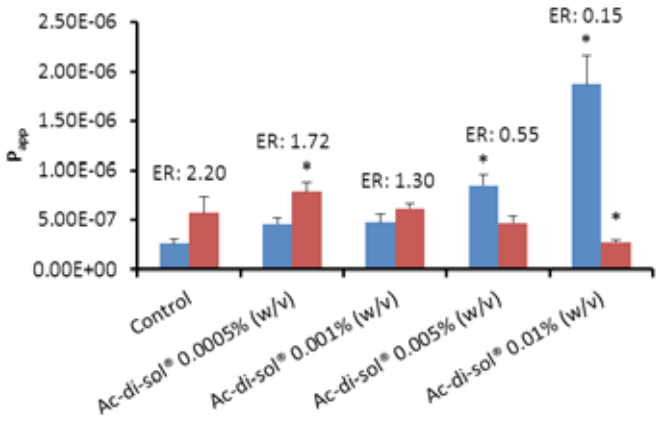

Figure 1: Average P_{app} with efflux ratio (ER) values for bi-directional transport of Rhodamine 123 in the presence of croscarmellose sodium (Ac-di-sol®) across excised pig jejunum (*statistically significant differences compared to the control, $p \leq 0.05$)

The calculated ER values take both directions of transport into consideration to indicate the main transport mechanism across a membrane. An ER value greater than 1 indicates active efflux as the main transport mechanism, while an ER value of less than 1 indicates that active uptake is the primary mechanism of transport (Bock *et al.*, 2003). Figure 1 shows that CCS had concentration dependent inhibitory effect on P-gp related efflux of R123 when compared to the control, and it was proven by a decrease in ER values.

Crospovidone (Kollidon® CL-M)

Crospovidone (CPD), commercially available as Kollidon® CL-M, is also classified as a superdisintegrant and was tested at

concentrations of 0.002% (w/v), 0.004% (w/v), 0.005% (w/v) and 0.01% (w/v) during this study.

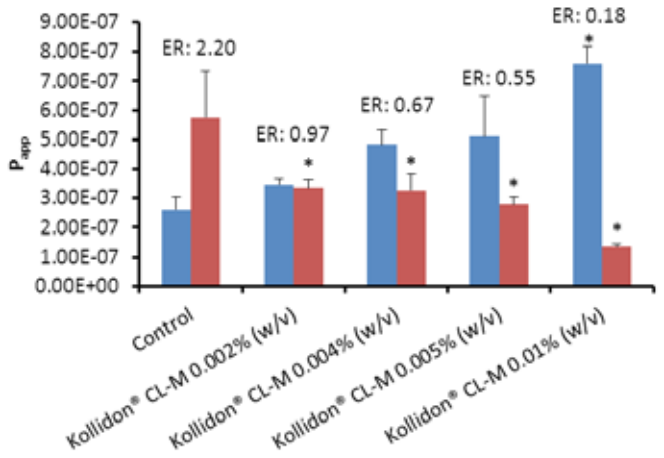

Figure 2: Average P_{app} with efflux ratio (ER) values for bi-directional transport of Rhodamine 123 in the presence of crospovidone (Kollidon® CL-M) across excised pig jejunum (*statistically significant differences compared to the control, $p \leq 0.05$)

Figure 2 shows that CPD inhibited P-gp mediated efflux transport of R123 to approximately the same extent as CCS for the two higher concentrations tested, i.e. 0.005% (w/v) and 0.01% (w/v). Furthermore, the calculated ER values of R123 for all the CPD concentrations reduced in a concentration dependent manner. This indicates significant inhibition of P-gp related efflux along with the increase in P_{app} values in the A-B direction and the decrease in P_{app} values in the B-A direction.

Sodium alginate

Sodium alginate (SAL) is a multi-functional excipient which is often used as a tablet disintegrant at concentrations of 2.5 – 10% (w/w) (Cable, 2005). In this study, concentrations of 0.0025% (w/v), 0.005% (w/v), 0.01% (w/v) and 0.025% (w/v) were tested.

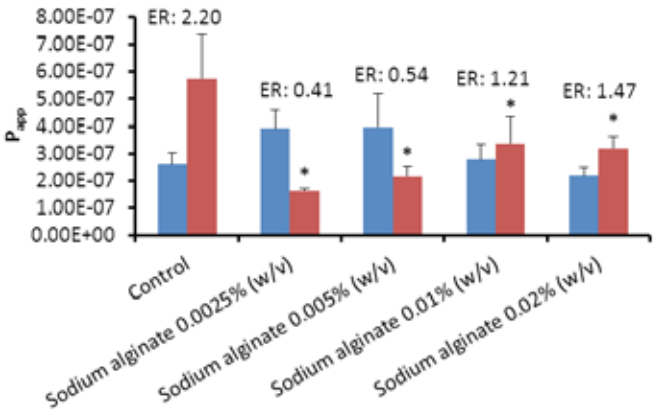

Figure 3: Average P_{app} with efflux ratio (ER) values for bi-directional transport of Rhodamine 123 in the presence of sodium alginate across excised pig jejunum (*statistically significant differences compared to the control, $p \leq 0.05$)

Transport of R123 in the presence of selected SAL concentrations presented with increasing ER values, albeit all lower than the control (Figure 3). This indicates a relative induction of R123 related efflux, but inhibition of efflux when compared to the control group. This is indicative that other transport mechanisms for R123 might have been affected by the presence of SAL in the test solutions. Trans-epithelial electrical resistance (TEER) was also measured at 20 min intervals during the transport studies (results not shown) and showed a concentration dependent increase in TEER. TEER measurements are often used to monitor potential changes in membrane integrity during paracellular transport studies. An increase in TEER points to more cohesive intercellular tight junctions and as a result, less paracellular transport. R123 has recently been proven to be transported via the paracellular route in addition to P-gp mediated efflux (Takizawa *et al.*, 2013). Therefore, R123 was probably not able to move via the paracellular route and could only be transported via P-gp mediated efflux, which resulted in an apparent increase in the calculated ER values as concentration increases.

Conclusion

P-gp mediated efflux transport of R123 was modulated by the selected disintegrants by either inhibition or possibly induction of the transporter protein. CCS and CPD both inhibited P-gp in a concentration dependent manner. SAL showed an apparent induction in efflux transport of R123. This phenomenon may be substantial due to an inhibition of paracellular transport via tightening of the tight junctions, rather than a direct effect on P-gp.

Two other excipients, namely microcrystalline cellulose and sodium starch glycolate was also tested (results not shown) but no P-gp related transport modulatory effects were found.

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