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 pharmaceutically 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-AS). 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% O₂:5% CO₂) 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).

\[
P_{\text{app}} = \frac{dQ}{dt} \left( \frac{1}{A \cdot 60 \cdot C_0} \right) \quad \text{(Eq. 1)}
\]

Where P_app is the apparent permeability coefficient (cm·s⁻¹), dQ/dt
is the permeability rate (amount permeated per minute), A
is the diffusion area of the membrane (cm²) and C₀ 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.

\[
ER = \frac{P_{\text{app}}(B-A)}{P_{\text{app}}(A-B)} \quad \text{(Eq. 2)}
\]

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.

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.

Crosovidone (Kollidon’ CL-M)

Crosovidone (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.

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.

**References**