Poster no.

531

Relationship between Particle and Biological Properties of Emulsion-templated, Freeze-dried LPV Nanoparticle Dispersions

Darren L. Smith[1], Phillip Martin[1], Tom McDonald[2], Marco Giardiello[2], Steve Rannard[2] and Andrew Owen[1]

[1]Department of Pharmacology and Therapeutics, University of Liverpool, UK; [2]Department of Chemistry, University of Liverpool

Correspondence to:
Dr. Darren Smith
HIV Pharmacology Group
70 Pembroke Place,
Block H (first floor),
Liverpool, UK, L69 3GF
e-mail: smithdx@liv.ac.uk
Tel: + 44 (0)151 794 5919
Fax: + 44 (0)151 794 5656

Introduction

- Nanomedicine has the potential to enhance bioavailability and delivery of low solubility compounds to sanctuary sites.
- Lopinavir (LPV) is a protease inhibitor (PI) with low bioavailability (<2%) that requires boosting with ritonavir (RTV).
- Conversely to current approaches this study uses a 'bottom up' process to manufacture an impact on the bioavailabilty of LPV by nanoformulation.
- Oil in water, freeze dried nanoformulation (Fig 1) allows hydrophobic drugs with low solubility potential to be dissolved in an oil with a polymer and surfactant.
- Nanoparticles are formed through freeze drying of this emulsion.
- On rehydration the nanoparticles do not dissolve, but disperse into water.
- Altering the excipients enables the modulation of particle characteristics.
- This study investigates the relationship between particle properties including; size (z-average), surface charge (zeta potential) and polydispersity on cellular accumulation (CAR) and transcellular permeability of LPV dispersions.

Aims

The aim of this study was to identify LPV nanoformulation properties which confer lower cytotoxicity, enhanced transcellular permeability and accumulation.

Methods

Cell culture

All cell lines were propagated in DMEM (HepG2 and Caco-2) or RPMI 1640 (THP-1 and CEM). A-THP-1 (monocyte derived macrophages; MDMs) were differentiated from THP-1 by activation using phorbol 12-myristate 13-acetate (PMA; 100nM) for 7 days.

Nanoformulations

74 LPV dispersions containing 0.1 µCi ^{3}H-LPV were generated. Table 1 details the particle properties including median value including; zeta potential (surface charge); z-average (size range) and polydispersity. Table 1 also includes the list of surfactants and polymers used in creating this bank of dispersions and the frequency of use across the 74 formulations.

Cellular Accumulation

Cells were propagated to a cell density of 5 million cells per assay, using the same cell passage for each cell line per experiment. The cells were washed (x2) in Hanks Balanced Salt Solution (HBSS) and incubated with each LPV dispersion (10 µM, formulated with 0.1 µCi $^{3}H^{+}$ LPV). After 1 hr an an extracellular sample was taken for scintillation counting. The cells were then washed in ice cold HBSS (x2) and the cells lysed using 100 µl water. The lysate was then taken for quantitation by scintillation counting as an intracellular measurement. The extracellular to intracellular comparison of the concentrations of LPV present were then used to then calculate a cellular accumulation ratio (CAR).

Caco-2 transpermeability assay

A 0.4 µM permeable transwell support (polycarbonate) (Corning, Ltd), was seeded with 35K Caco-2 cells and propagated on over a 21 day period to form a polarised monolayer with tight junctions mimicking the endothelial layer of the gut. The monolayer integrity of each well was determined by the level of transepithelial resistance. Only monolayers with values >1000 Ω were used for subsequent experiments. Apical>basolateral transport was measured over a 2hr time period. Radiolabelled LPV nanoformulation was added to the apical chamber at a concentration of 10 µM, containing ^{3}H-LPV as with the cellular accumulation.

Nanoparticle comparison of Cellular Accumulation and Caco-2 transpermeability

Data were log transformed and models to describe relationships between particle properties and biological characteristics were constructed using multiple linear regression.

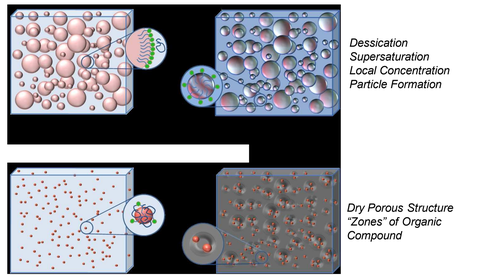

Fig 1: Method of formulation; *Zhang et. al. Nature Nanotechnology 3, 2008, 506 – 511*

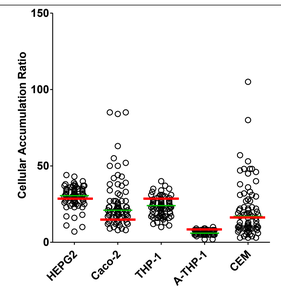

Fig 2: Cellular accumulation ratio (CAR) of LPV nanoformulations across 5 immortalised cell lines. The red line corresponds to the CAR value for the parental drug, the green line represents the median value for the accumulation dataset.

Excipients	Range / number of samples
Zeta Potential median	-3.7 (-56.7- 39.7) mV
Z average median	436 (184-3145) nm
Polydispersity Median	0.3 (0.1-0.9)
PVA	12
Pluronic F68	7
Pluronic F127	10
Hyamine	12
CTAB	12
PEG 1K	5
Kollicoat	11
PVP 30K	6
HPC	4
HPMC	10
Hydrolysed Gelatine	6
NaCMC	3
Na Alginate	3
Na Mystriate	1
Na Deoxycholate	4
Na Caprylate	5
Vit E-PEG succinate	6
Sisterna 11	2
Sisterna 16	1
Chremophor	8
Solution HS	6
Tween 20	6
Tween 80	9
Brij 58	8

Table 1: Nanoparticle properties including; the frequency of individual surfactants and polymers used in formulation; median value and low to high range of zeta-potential, z-average and polydispersity.

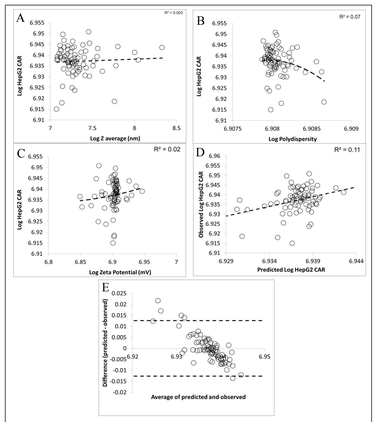

Fig 3: Linear regression analysis between Log CAR values and; Log z-average (A), Log polydispersity (B), Log zeta potential(C). Graph (D) linear regression to model predicted Log HepG2 CAR against observed Log HepG2 CAR. (E) Bland and Altman plot of predicted/observed CAR values.

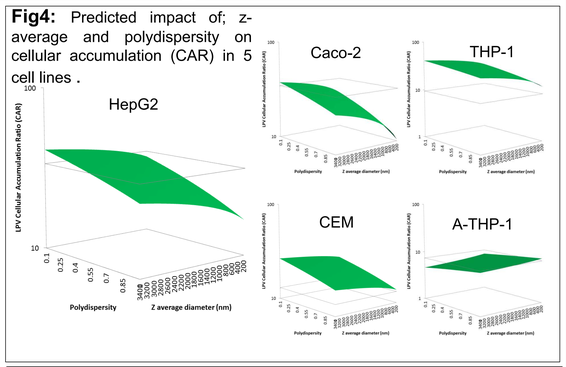

Fig4: Predicted impact of; z-average and polydispersity on cellular accumulation (CAR) in 5 cell lines .

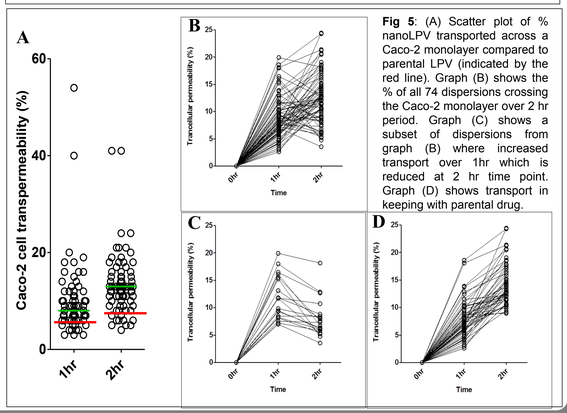

Fig 5: (A) Scatter plot of % nanoLPV transported across a Caco-2 monolayer compared to parental LPV (indicated by the red line). Graph (B) shows the % of all 74 dispersions crossing the Caco-2 monolayer over 2 hr period. Graph (C) shows a subset of dispersions from graph (B) where increased transport over 1hr which is reduced at 2 hr time point. Graph (D) shows transport in keeping with parental drug.

Results

- Previous work at the University of Liverpool, (data not shown), has identified that nanoformulation of LPV reduces the cellular toxicity when compared to aqueous solution of the drug.
- Fig 2 illustrates the Cellular accumulation of the 74 nanoLPV dispersions compared to the parental LPV. Certain dispersions exhibited a higher CAR than the aqueous LPV *e.g.* the median CAR value for nanoLPV in CEMs was 17.0 with a range of values from 2.8-105.1 compared to 13.1 for aqueous LPV.
- Accumulation assays for each cell line identified formulations with higher LPV accumulation.
- Accumulation of nanoLPV in Caco-2, HepG2 and CEM cells had median CAR values greater than the CAR value for the aqueous solution of the drug.
- Fig 3, uses the LogHepG2 CAR values as an example of how it is possible to correlate particle properties to cellular accumulation (CAR) and that modelling can be used to predict CAR in a certain cell lines using particle properties.
- A Bland and Altman plot in Fig 3E compares the same data set of average observed/predicted data to show that most points lie within 2 standard deviations.
- Data not shown identified that HepG2 (r =-0.38), Caco2 (r = -0.39), THP1 (r = -0.14) and CEM (r = -0.09) showed inverse correlation with polydispersity and z-average.
- Interestingly, in phagocytic ATHP1 cells a direct relationship was evident (r = 0.28).
- Fig 5, expands on these data, showing prediction of CAR on altering z-average and polydispersity where the single line on each chart represents the CAR of the aqueous drug solution.
- Transcellular permeability of LPV was higher for some nano LPV dispersions where the median value for A>B permeation across Caco2 monolayer was compared to 2.5 %/hr for aqueous LPV. No correlation with particle properties were seen.
- Interestingly, certain formulations (Fig 5.C) transversed the Caco-2 monoalyer at a higher rate within the first hour, but were decreased at the 2 hr time point. This may be due increased ability of the drug to enter/transverse the cell, or may be transported as a particle, degraded and effluxed back accross the membrane in its aqueous form.

Conclusions

- Nanoformulation has the ability to increase both LPV accumulation and transpermeability.
- Nanoformulation of LPV has a lesser impact on accumulation of LPV in MDM cell line ATHP-1 when compared to other cell lines used in this study.
- By increasing polydispersity and lowering particle size increases cellular accumulation in Caco-2, THP-1, CEM, HepG2.
- Conversely, lowering polydispersity, and increasing the particle size has the potential to increase cellular accumulation in MDM cell line ATHP-1.
- LPV dispersions surpass the aqueous solution of the drug in crossing the Caco-2 monolayer during transcellular permeability assays, although certain formulations efflux at rate that is lower at the 2 hr time point than at the 1 hr time point.
- By using a large number of surfactants and polymers, inferring changes in particle size, surface charge and polydispersity means that using experimental values from cellular accumulation/transpermeability/cytotox etc, models can be derived that aid design of nanoparticles with increased biological potential.

REFERENCES

- Zhang H, Wang D, Butler R, Campbell NL, Long J, Tan B, Duncalf DJ, Foster AJ, Hopkinson A, Taylor D, Angus D, Cooper AI and Rannard SP (2008) Formation and enhanced biocidal activity of water-dispersable organic nanoparticles. *Nat Nanotechnol* **3**:506-511.