Enhancing the bioavailability of darunavir by lipophilic prodrug approach without the use of pharmacokinetic boosters

Yunning Du, Giuseppe Mantovani, Pavel Gershkovich

School of pharmacy, University of Nottingham, Nottingham, United Kingdom

Background: Starting Antiretroviral Therapy is recommended for all individuals newly diagnosed as HIV positive. Darunavir is an HIV protease inhibitor currently intensively used in HIV treatment. Oral bioavailability of darunavir is limited by first-pass metabolic loss in the intestinal wall and liver. Therefore, the co-administration with hepatic enzyme inhibitor (such as ritonavir) is the main reason for adverse effects in clinical usage. The first-pass metabolism could be avoided through intestinal lymphatic pathway, which resulted in an improved bioavailability of drugs without the usage of hepatic enzyme inhibitors. However, most of the compound such as darunavir cannot be encapsulated into chylomicrons due to the low lipophilic property. Highly lipophilic prodrug approach has been proposed by our group as a strategy to achieve intestinal lymphatic transport of drugs. The aim of this project is to improve the oral bioavailability and prolong the elimination half-life of darunavir through highly lipophilic prodrug strategy.

Methods: HPLC-UV and LC-MS bioanalytical methods were developed to investigate the in-vitro and in-vivo drug metabolism and pharmacokinetics of darunavir. A combination of protein precipitation using cold acetonitrile and liquid-liquid extraction was utilised to extract darunavir from rat plasma and artificial chylomicrons emulsion. Samples were then evaporated and reconstituted in mobile phase and injected into an HPLC-UV or LC-MS system. Separation was achieved using ACE C18-PFP 150 mm × 4.6 mm, 3 µm HPLC column with elution using a mobile phase consisting of water, acetonitrile or menthol in appropriated proportion. Lipophilic prodrugs of darunavir with high intestinal lymphatic transport potential were designed using previously reported in silico model. Physicochemical parameters values were obtained using ACD Lab software.

Results: Extraction using diethyl ether demonstrated superior efficiency in liquid-liquid extraction compared to hexane, methyl tert-butyl ether (MTBE) and ethyl acetate. ACE C18-PFP 150 mm × 4.6 mm, 3 µm HPLC column at 55 °C with isocratic elution using a mobile phase consisting of acetonitrile-water (60:40, v/v) at 0.6 mL/min for 30 min provided the best separation efficiency in HPLC-UV system for quantification of darunavir from artificial chylomicrons emulsion, but the chromatography resulting from this condition showed interference between background plasma peaks and the peaks of darunavir when the concentration is lower than 20 ng/mL. Cannabidiol as internal standard showed appropriate elution time in this method. Darunavir was detected at 265 nm, and internal standard (cannabidiol, CBD) was detected at 220 nm. In-silico model showed that chylomicron association of darunavir is negligible 2.58% but for most of long-chain alkyl group prodrugs is up to 99%.

Conclusions: The HPLC-UV method for the determination of darunavir achieved quantification of darunavir from artificial chylomicrons emulsion, but additional optimization is needed using LC-MS methodology for quantification of the drug from plasma matrix. Intestinal lymphatic transport potential of darunavir is negligible without chemical modification. Highly lipophilic prodrugs of darunavir with high lymphatic transport potential have been successfully designed.