Characterization of in vivo activity of a novel Lecithin: cholesterol acyltransferase inhibitor

I. Zanotti[1], S. Simonelli[2], I. Eberini[2], C.Sensi[2], G. Lusardi[1], G. Franceschini[2], F. Bernini[1], L. Calabresi[2]

[1]Dept. of Pharmacy, University of Parma, Italy
[2]Dept. of Pharmacological and Biomolecular Sciences, University of Milan, Italy

High density lipoproteins (HDL) are thought to exert their antiatherosclerotic activity mainly by the promotion of the reverse cholesterol transport (RCT), the process by which excess cholesterol from peripheral tissues is transported to the liver, for the ultimate elimination in the feces. Recent epidemiological studies suggest that HDL capacity to promote the first step of RCT, cholesterol efflux from cells, is a better predictor of atheroprotection than HDL levels. This function is intimately related to HDL structure, that affects particle capacity to interact with lipid transporters expressed on cell plasma membrane. Lecithin:cholesterol acyltransferase (LCAT) is a crucial enzyme in HDL remodeling, where it promotes the conversion of small, lipid poor prebeta-HDL into the largest HDL2 and HDL3. Since pre-beta HDL promote cholesterol efflux specifically via ATP Binding Cassette transporter A1, currently considered the most atheroprotective mechanism, inhibition of LCAT can be proposed as a novel strategy for the development of antiatherosclerotic drugs. A potential inhibitor was designed, mimicking the intermediate of the cholesterol esterification reaction. The molecule (heptadecylcholesteryl-(R,S)-phosphonyl chloridrate) proved to irreversibly inhibit LCAT in vitro. In the present work we assessed the activity of the compound in vivo. In a preliminary experiment, aimed to select the most suitable procedure, the compound was administered to eight week old, male C57BL/6 mice, by either oral gavage (n=3) or intraperitoneal injection (n=3) at the dose of 150mg/kg. Whereas mice receiving the compound per os showed a highly variability in LCAT inhibition (from 0 to 100%), the intraperitoneal injection caused the complete inhibition of enzyme activity in all animals. In the next test, 5 eight week old, male C57BL/6 mice received 150mg/kg of the compound in a single intraperitoneal injection and plasma was collected at different time points. LCAT activity was time-dependently reduced, until a complete inhibition 45 hours after the administration: 4.5±2.1 nmol cholesteryl ester/ml/h, 1.8±1.6 nmol/ml/h, and 0±0 nmol/ml/h before the injection, and after 30 hours and 45 hours, respectively. Concomitantly, the free to total cholesterol ratio increased from 0.35 at baseline to 0.41 and 0.37 after 30 hours and 45 hours, respectively. In conclusion, the compound demonstrated to efficiently inhibit LCAT activity in mice, in absence of evident toxic effects. Whereas its impact on RCT still needs further investigations to support the identification of LCAT as a novel target, our molecule could represent a lead compound for the development of a new class of antiatherosclerotic drugs.