Screening of a marketed drug library identifies dantrolene as an activator of the antioxidative enzyme paraoxonase-1

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Objectives: Human paraoxonase 1 (PON1) is a hydrolytic enzyme, which is bound to high-density lipoprotein (HDL) in serum. This enzyme can hydrolyze a variety of substrates displaying activities of paraoxonase, arylesterase and lactonase. The hydrolysis of pro-atherogenic oxidized phospholipids represents the major function of PON1, thus contributing to the antioxidant and antiatherogenic properties of HDL. Reduced PON1 activity has been associated with an increased risk for cardiovascular disease.
Methods: In the present study, a library of commercially available drugs (956 compounds) was screened to identify small molecules that can increase the HDL-associated PON1 activity. Screening was performed by a kinetic absorbance assay in a 96-well plate using human HDL as enzyme source, and paraoxon and phenyl acetate as substrates to measure paraoxonase and arylesterase activities of PON1 respectively. In addition, we studied the effect of certain compounds on the activity of specific PON1 variants (polymorphisms 192Q/R, 55L/M and mutation M127R). Furthermore, mechanistic investigation of the effect of compounds on enzyme activity was performed using recombinant human wild-type PON1 and the PON1[L55M] variant associated with reduced activity in humans, as well as molecular docking of drugs onto the structure of PON1.
Results: Library screening led to the identification of seven compounds that increase the paraoxonase activity, four of which also increase the arylesterase activity of PON1. Analysis of the effect of these compounds on the activity of PON1 variants showed that dantrolene is an activator of all enzyme variants studied, inducing the stronger activation to PON1[L55M]. Further analysis showed that dantrolene, at a low μM concentration, has the capacity to activate dose-dependently both WT PON1 and PON1[L55M] by primarily increasing the V_{max} of the enzymatically catalyzed reactions. Molecular docking suggested that dantrolene binding to PON1 can result to direct stabilization of Ca(II) atoms that either participate in catalysis or stabilize the protein.
Conclusion: Our findings support that existing drugs, such as dantrolene, can increase HDL-associated PON1 activity and pave the way for new therapeutic approaches for atherosclerosis and cardiovascular disease.