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Kinetic Studies on Human Paraoxonase 1

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The human paraoxonase 1 (PON1), an HDL-associated esterase has been implicated in the prevention of atherosclerosis. Previously, we have described some qualitative aspects of PON1 namely the allelic frequencies of the $PON1_{192QR}$ and $PON1_{55LM}$ polymorphisms in a Malaysian population. The estimation of its quantitative characteristics may shed useful information for comparison purposes. In the present study, kinetic and inhibition studies on PON1 were conducted to assess three common parameters: the Michaelis constant (K_M) and maximal rate of metabolisme (V_{max}) of paraoxonase, and inhibition constant (K_i) of phenylacetate. PON1 activity was measured spectrophotometrically at 405 nm, using selected plasma samples in both basal (without added NaCl) and salt-stimulated assays (1 M NaCl), pH 8.5. Inhibition studies were performed using phenylacetate as an inhibitor of PON1 in basal assays, pH 8.0. Estimates of K_M and V_{max} were obtained from the Lineweaver-Burk plot. Estimates of K_i were obtained from the secondary plot of apparent K_M ($K_{M,app}$) versus inhibitor concentration. The parameter values were evaluated for the genotypes $PON1_{192QQ}$, $_{-QR}$ and $_{-RR}$.

In salt-stimulated assays, the V_{max} increased twofold from 121 to 253 U/L for the $PON1_{192QQ}$ samples and three- to fivefold from 294 to 1197 U/L for the $PON1_{192RR}$ samples compared with basal assays. Similarly, it increased fourfold from 194 to 742 U/L for $PON1_{192QR}$ samples. K_M was comparable with or without 1.0 M NaCl across the three genotypes. In $PON1_{192RR}$ samples, K_M was approximately 0.45 mM for basal assays and 0.58 mM for salt-stimulated assays, and for $PON1_{192QR}$ samples, K_M was 0.34 mM for basal and 0.31 mM for salt-stimulated assays. In $PON1_{192QQ}$ samples, K_M values were 0.58 mM for basal assays and 0.53 mM for salt-stimulated ones. The Lineweaver-Burk and Dixon plots revealed that phenylacetate was a predominantly competitive inhibitor exhibiting linear mixed type inhibition. The K_i were 0.25, 0.27 and 0.39 for $PON1_{192QQ}$, $_{-QR}$ and $_{-RR}$ respectively. We conclude that the three kinetic parameters of PON1 in the Malaysian population estimated in the present report were comparable with those reported by other studies on PON1 from Caucasian populations.