Human renal lipoxygenase

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While the exact mechanism(s) of drug-induced renal papillary necrosis remains unclear, an attractive hypothesis is that drugs undergo metabolic activation within the kidney, producing reactive intermediates. We have previously shown that metabolic activation of xenobiotics in human kidney occurs primarily via a lipoxygenase pathway (1). Although lipoxygenase is frequently cited as a cytosolic enzyme, we detected lipoxygenase activity in both the cytosolic fraction of the renal cortex and the microsomal fraction of the renal medulla/papilla. Three major lipoxygenases exist (5-, 12- & 15-LO) and the aim of this study was to partially purify and characterise the human renal lipoxygenase(s).

Using the method of Marnett et al (1984), human renal lipoxygenase was purified separately from medullary/papillary microsomes and cortical cytosol by ion-exchange chromatography. LO activity was measured by investigation of the arachidonic acid (AA)-dependent metabolism of the model compound tetramethylphenylenediamine (TMPD) (2). A 2-fold increase in AA-dependent activity was achieved in the purified medullary/papillary microsomal fraction compared with that of microsomes (25.6 ± 2.9 vs 11.0 ± 1.4 nmol/min/mg, n=3). This activity was further stimulated by addition of ferrous ions (500 μM) (146 ± 16 nmol/min/mg, p<0.01), whereas ferric ions had no effect.

Purification of the cytosolic cortical enzyme yielded a 3-fold increase in the AA-dependent activity (7.1 ± 2.5 vs 2.0 ± 0.5 nmol/min/mg, n=3). Again this activity was further stimulated by the addition of ferrous ions, yielding a 48-fold increase in the rate of cooxidation (90 ± 15 nmol/min/mg, P=0.0005). Therefore the purified cortical cytosolic fraction exhibited ferrous-dependent AA-mediated cooxidation of TMPD similar to the levels observed in the purified microsomal medulla/papilla fraction.

The use of selective inhibitors enabled further investigation of the purified enzymes. Nordihydroguaiaretic acid (10 μM, non-specific LO inhibitor), BWA4C (50 μM, specific 5-LO inhibitor) and BW755C (50 μM, dual prostaglandin synthetase/LO inhibitor) all caused significant inhibition of AA-mediated activity (p<0.01 compared with control) of both purified enzymes. In contrast, the inhibitors baicalein (1 mM, specific 12-LO inhibitor), 5,11,14-eicosatetraynoic acid (50 μM, specific 12/15-LO inhibitor) and indomethacin (100 μM, prostaglandin synthetase inhibitor) had no effect on the activity of either of the purified enzymes. These results are consistent with both purified enzymes being 5-lipoxygenases.

Although arachidonic acid has been used as the fatty acid of choice in these studies, lipoxygenases may utilise a wide range of fatty acid cosubstrates. The purified human renal microsomal LO was found to be equally active with arachidonic acid (AA) and α-linolenic acid (α-LNA) but inactive with linoleic acid (LA), γ-linolenic acid (γ-LNA) and dihomogammalinolenic acid (DGLA) both in the presence and absence of ferrous ions. 5-LOs react predominantly with C20 fatty acids and are almost inactive with C18 polyenoic acids (eg, LA, γ-LNA, DGLA) (3). Based on the observed substrate specificity and the results obtained using the selective inhibitors, it is probable that the purified human renal microsomal lipoxygenase is a 5-LO.

Although the purified renal cytosolic lipoxygenase was active with both AA and γ-LNA, the preferred cosubstrate was α-LNA while LA and DGLA were poor cosubstrates. 12-LOs utilise C18 and C20 fatty acids as active substrates (4). However due to the low levels of activity seen with the C18 polyenoic acids - LA, DGLA & γ-LNA - and considering the results obtained using the selective LO inhibitors, it is likely that the purified renal cytosolic enzyme is a 5-LO which has a slightly different substrate specificity to the purified microsomal enzyme.

Analysis of the primary lipoxygenase products (5-, 12- & 15-hydroperoxyeicosatetraenoic acids (HPETEs)) enabled further investigation of renal lipoxygenase activity. The lipoxygenase products were analysed by RP-HPLC using a procedure which separated the 5-, 12- and 15-HPETEs. HPLC analysis of the primary lipoxygenase products of the purified enzymes revealed formation of only 5-HPETE (0.278 ± 0.032 μg/mg, n=3, for the microsomal enzyme and 0.268 ± 0.058 μg/mg, n=3, for the cytosolic enzyme). No 12-HPETE nor 15-HPETE production was detected. These results support the conclusion that the purified enzymes are 5-LOs.

Increased formation of 5-LO products has been demonstrated in several models of renal disease (6, 7, 8). 5-LO products are predominantly pro-inflammatory. Therefore 5-LO inhibitors may provide protection from drug- or disease-induced papillary necrosis. These findings have obvious implications for the protection from, and potential reversal of, papillary necrosis. This work was funded by Glaxo Group Research.