Functional characterization of 7-ethoxyresorufin deethylase in naïve male rat liver microsomes

Emilija Makajii, Ghaznia Khan, Cristina S Trambitas, Alison C Holloway, Denis J Crankshaw

McMaster University, Hamilton, ON, Canada

The fluorogenic substrate 7-ethoxyresorufin (ERES) is biotransformed by cytochrome P450 (CYP) enzymes collectively referred to as 7-ethoxyresorufin deethylase (EROD). In animals exposed to aryl hydrocarbon receptor (Okey et al, 1994) agonists EROD activity is used as a biomarker for CYP1A1 (Anger et al, 2005). Conversely, EROD activity is often used as an index of exposure to aryl hydrocarbon receptor agonists (Behnisch et al, 2001). However, in naïve rats CYP1A1 does not contribute to EROD activity, which may be mediated by CYPs 2B1 and 2C6 (Burke et al, 1994).

The goal of the present study was to functionally characterise EROD activity in liver microsomes from naïve male rats with respect to development, enzyme kinetics and sensitivity to inhibitors.

EROD activity in rat liver microsomes and recombinant rat CYP1A1 was determined using fluorescence intensity assays as we have described (Anger et al 2005). All data are expressed as mean ± s.e.mean.

EROD activity was undetectable in liver microsomes at birth, peaked at 14 ± 3 pmol/min/mg at 21 days of age and declined steadily to the last time point we measured (120 days) where it was 1.5 ± 0.8 pmol/min/mg (n = 5). In all subsequent experiments livers from 65 day-old animals were used. EROD had complex kinetics compatible with a two site model with a Vmax1 of 8.3 ± 0.9 pmol/min/mg, Km1 of 11.8 ± 7.0 µM, Vmax2 of 3.2 ± 0.5 pmol/min/mg and Km2 of 0.1 ± 0.06 µM (n = 3). The EROD activity of recombinant rat CYP1A1 was fully inhibited by 3-methylcholanthrene (3MC) whereas that of naïve rat liver microsomes was unaffected by 3MC at concentrations up to 10 µM. We searched for inhibitors of the enzyme by screening a small (1120) compound library. The library was obtained from Prestwick Chemical and consisted of 90% marketed drugs and 10% bioactive alkaloids. Assay conditions were optimized for the screen and each compound was tested once at a concentration of 10 µM. Compounds that inhibited EROD to 24% of control or lower were considered to be active. We identified 13 active compounds of which we were able to examine 5 in detail with full concentration-response curves starting from pure substance. These experiments confirmed that 2 of the 5 compounds were true inhibitors of EROD. Acacetin and chrysin had pIC50 values of 5.6 ± 0.07 and 5.9 ± 0.2, respectively (n = 3), though chrysin only inhibited activity to 28 ± 1% of control. The screen also identified two compounds that activated EROD to more than 200% of control. Both compounds were tested further and dicumarol was confirmed as an activator (pEC50 = 5.5 ± 0.2, max = 230 ± 34% control, n = 3). The effect of dicumarol did not appear to be mediated through inhibition of NAD(P)H: quinone oxidoreductase 1.

Thus, we have confirmed that EROD activity in naïve rats is not mediated by CYP1A1. Changes in liver EROD activity with age suggest that it plays a role in development. EROD is likely comprised of two enzymes, both of which are sensitive to acacetin, and one of which is sensitive to chrysin.

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