In vitro Characterization of Bupropion Metabolism using Two Methodologies

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Background

- Bupropion is a commonly prescribed antidepressant with relatively poorly understood metabolism.
- While the role of CYP2B6, a known pharmacogene, in bupropion conversion to 6-OH-bupropion is well-described, other active metabolites (threohydrobupropion, erythrohydrobupropion) have also been observed *in vivo*.
- The relative contribution of the various enzyme pathways to bupropion clearance and antidepressant efficacy is not well understood.
- In this study, two *in vitro* hepatic systems were used to examine bupropion metabolism and identify the enzymes involved in bupropion breakdown and formation of its three active metabolites, including pathways outside of the P450 system which may identify additional sources of variability in metabolism.

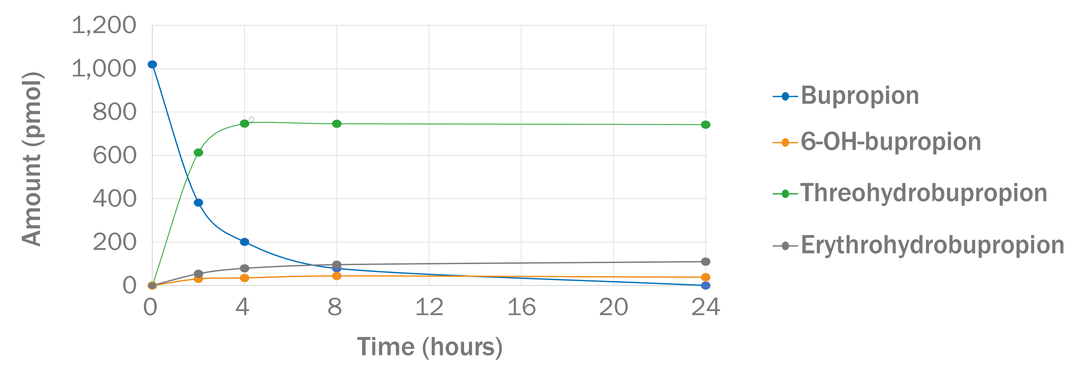
Methods

- Two methods were used to model bupropion hepatic metabolism *in vitro*:
  1. Cryopreserved, plated human hepatocytes (cPHHs), which recapitulate the *in vivo* quantity of enzymes and cofactors
  2. S9 liver fraction, a simpler system that permits the use of chemical inhibitors to identify the enzymes involved in the formation of specific metabolites
- Bupropion (5 µM) was incubated, in triplicate, with cPHHs (1.25 million cells/mL, 30 mixed gender donors) for 24 hours and with S9 fraction (1 mg protein/mL, 50 mixed gender donors) and an NADPH-regenerating system with or without UDPGA for 1 hour. Donors were assessed to be normal for various CYP and UGT isoform activities.
- S9 incubations included solvent controls or chemical inhibitors for CYP2B6 (30 µM phencyclidine), CYP2C9 (20 µM tienilic acid), CYP2C19 (10 µM esomeprazole), CYP2D6 (5 µM paroxetine), CYP3A (1 µM ketoconazole), HSD11B1 (1 µM glycyrrhetinic acid), AKR1C1/1C2/1C3 (20 µM flufenamic acid) and AKR1C1/1C4 isoforms (20 µM phenolphthalein), or CBR1 (100 µM quercetin).
- Bupropion, 6-OH-bupropion, threohydrobupropion, and erythrohydrobupropion concentrations were detected via liquid chromatography with tandem mass spectrometry and quantified using standard curves. When area ratios were detected but were below the lower level of quantification (LLOQ), the LLOQ was used in calculations. Zero was used when no peak was detected.
- Positive controls tested the general competency of both systems and each enzyme targeted in the S9 fraction. Negative controls measured substrate degradation and metabolite formation in the absence of cells or cofactors.

Results

- In cPHHs, 99.6% of the initial bupropion was depleted (below LLOQ) and 87.4% was recovered as 6-OH-bupropion, threohydrobupropion, and erythrohydrobupropion, indicating that these 3 metabolites are the major metabolites and that no major metabolite was missing in this evaluation (Figure 1).

Figure 1. Amount of Bupropion Depleted and Metabolites Formed in Hepatocytes after 24 Hours



- The most abundant metabolite was threohydrobupropion, accounting for 73.1% of the depleted bupropion, while erythrohydrobupropion and 6-OH-bupropion accounted for 10.8% and 3.8%, respectively.
- In the S9 fraction, 44.2% of the initial bupropion was depleted, with 82.6% accounted for by the metabolites (Figure 2). Like the cPHHs findings, all three active metabolites were formed in the S9 fraction, and 70.3% of the depleted bupropion was recovered as threohydrobupropion; however, unlike in cPHHs, erythrohydrobupropion constituted the smallest proportion.

Figure 2. Amount of Bupropion Depleted and Metabolites Formed in S9 Fraction after 1 Hour

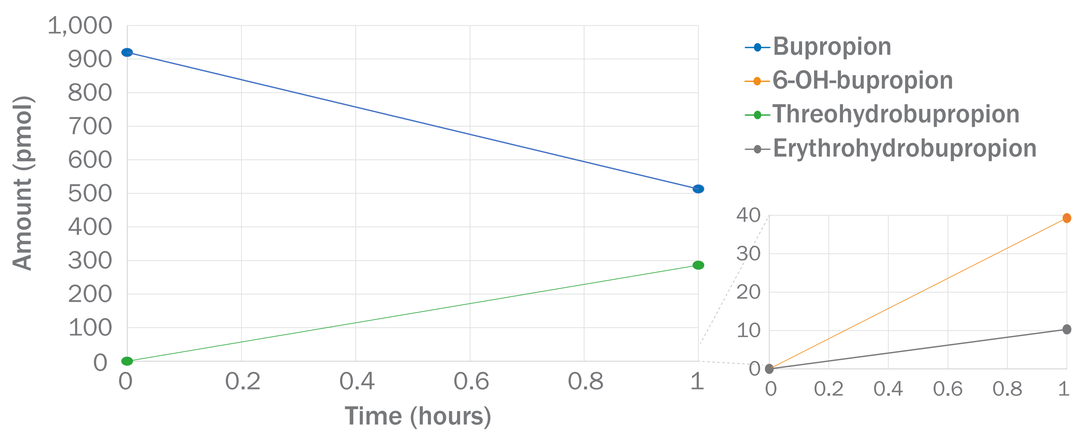


Table 1. Percent Inhibition of Bupropion Depletion and Metabolite Formation in the Presence of Enzyme Inhibitors

Enzyme Target for Inhibition	Bupropion	6-OH-Bupropion	Threohydro-Bupropion	Erythrohydro-Bupropion
CYP2B6	6.5	86.9	—	6.4
CYP2C9	—[b]	3.2[a]	—[a]	—[a]
CYP2C19	9.9	8.6	—	10.4
CYP2D6	—[a]	73.5[d]	4.2	2.3
CYP3A	—	32.6	—	—
HSD11B1	58.8	—	94.0	92.6[c]
AKR1C1, AKR1C2, AKR1C3	21.5	7.5	1.1	2.5
AKR1C1, AKR1C4	—	59.8[d]	8.2	3.7
CBR1	39.9	21.5	5.7	8.5

Mean percent inhibition of bupropion depletion or metabolite formation from triplicate observations, unless otherwise noted.

— no inhibition; [a]duplicate; [b]single; [c]below LLOQ; [d]possible nonspecific inhibition

- Chemical inhibition of bupropion metabolism in the S9 fraction implicated HSD11B1, CYP2B6, and CYP3A enzymes (Table 1).
- Specifically, glycyrrhetinic acid (HSD11B1 inhibitor) inhibited bupropion depletion 58.8%, and the formation of threohydrobupropion and erythrohydrobupropion 94.0% and 92.6%, respectively.
- Phencyclidine (CYP2B6 inhibitor) inhibited the formation of 6-OH-bupropion 86.9%; however, it did not inhibit bupropion depletion nor the formation of threohydrobupropion or erythrohydrobupropion.
- Similarly, ketoconazole (CYP3A inhibitor) inhibited 6-OH-bupropion formation 32.6% and did not impact bupropion or the other metabolites.
- The apparent inhibition by paroxetine (CYP2D6 inhibitor) may reflect non-specific inhibition of other enzymes, including CYP2B6.
- The apparent inhibition by the AKR1C1/1C4 inhibitor may reflect a lack of specificity of phenolphthalein.
- Inclusion of UDPGA did not meaningfully change the amount of bupropion depleted or metabolites formed except for threohydrobupropion, suggesting subsequent glucuronidation of this metabolite.

Conclusions

- The major *in vitro* bupropion metabolites were threohydrobupropion, 6-OH-bupropion, and erythrohydrobupropion, with the most abundant being threohydrobupropion.
- While CYP2B6 and CYP3A were involved in the formation of 6-OH-bupropion, HSD11B1 was found to be the main enzyme responsible for the formation of the major metabolite threohydrobupropion, and the depletion of bupropion.
- Understanding interindividual differences in HSD11B1 activity may be useful in predicting exposure to bupropion and its active metabolites.
- Future directions could also include evaluation of how antidepressant efficacy of bupropion is impacted by exposure to these metabolites and variation in HSD11B1, CYP2B6, and CYP3A activity.