T2306

Metabolic Kinetics of Bupropion in Human Liver and Intestinal Subcellular Fractions

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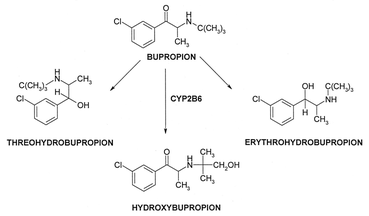
INTRODUCTION

Bupropion is a nonepinephine/dopamine reuptake inhibitor used for major depressive disorder and smoking cessation. A single dose bioequivalence (BE) study was performed for generic versions of bupropion and the name brand, Wellbutrin. Due to seizure issues associated with the 300 mg dose, BE studies with the 150 mg dose was extrapolated to the 300 mg dose. However, after complaints of efficacy and adverse effects, the FDA conducted a pilot study on one generic (Budeprion 300 XL) with Wellbutrin 300 XL. It was found that the AUC and Cmax of Budeprion XL is only 86% (90% CI 77-96%) and 75% (90% CI of 65-87%) of Wellbutrin XL respectively. Since then, some generics have been pulled from the market. Understanding issues associated with this problem is not straight forward since there could be many factors involved. However, we explored the metabolism aspect of this issue.



ABSTRACT

Purpose:

Bupropion is a clinically available drug product used for depression. Generics of Wellbutrin extended release (XL) were approved based on bioequivalence (BE) studies comparing the 150mg strength of the products to Wellbutrin XL 150mg. The results were extrapolated to establish bioequivalence of the 300mg product. However, BE studies for 300mg XL showed bioinequivalence for some generics. One possible factor may be the lack of understanding the metabolism of bupropion, particularly relating to liver and intestinal metabolism. Hydroxybupropion and threohydrobupropion/erythrohydrobupropion are the active primary metabolites of bupropion (25-50% of bupropion activity) by the enzymes CYP450 2B6 and Carbonyl Reductase respectively. The purpose of this study was to understand the metabolite formation in vitro using liver and intestinal microsome and S9 fractions.

Methods:

Liver and intestinal microsome and S9 stability experiments were conducted using concentrations of bupropion from 1-4000µM. NADPH (20mM) was used for the cofactor and to initiate the reaction. Sample was collected at time points from 0-90 minutes, and the reaction was terminated by spiking the sample in methanol containing the internal standard (venlafaxine) at 500nM. All samples were quantified by LC-MS/MS and the method was validated for specificity, matrix effect, and linearity.

Results:

In these studies, we found that hydroxybupropion was formed in the liver but was not detected in microsome and S9 fraction of intestine. The estimated V_{max} was 131±5.8 and 51±1.9pmol/min/mg and the K_m was 87.9±20.2 and 99.5±18.9µM for the liver microsome and S9 fractions, respectively. For the diastereomer, threohydrobupropion was the dominant metabolite in both the liver and intestines. For the liver microsome and S9 fractions, threohydrobupropion was formed at a V_{max} of 98.37±6.6 and 99±7.58pmol/min/mg and the K_m 186.3±53.48 and 265±77.79µM respectively. In the intestines, threohydrobupropion was formed at a lesser extent in both microsome and S9, the V_{max} was 5.5±0.3 and 25.87±2.8pmol/min/mg and K_m 149.9±28.86 and 573.4±88.9 µM respectively. Erythohydrobupropion was not detectable in either microsome or S9 fraction of intestine.

Conclusion:

The metabolism of bupropion *in vitro* shows that the liver and intestines have distinct metabolite profiles of bupropion. These differences in metabolism might provide evidence towards the understanding of bioinequivalence of bupropion products.

MATERIALS AND METHODS

Microsome and S9 stability assay: Both liver and intestinal microsome and S9 fraction were used at a final concentration of 1mg/mL. Compound was added to reactions ranging from 1 µM- 4 mM. For the master mix, microsome or S9, compound and PBS (100 mM photassium phosphate buffer + 3.3 mM MgCl2). For each reaction, 20 mM of freshly prepared NADPH was prepared. The master mix and NADPH were heated at 37 °C for 3 minutes, 20 µL of NADPH was then added to the master mix to initial the reaction. At time points 0, 30, 60, and 90 minutes, 50 µL of reaction was added to150 µL of methanol containing the internal standard (venlafaxine) at 500 nM.

Standard Preparation: The stock solutions of bupropion, hydroxybupropion, threohydrobupropion, and erythrohydrobupropion were prepared in methanol to generate a stock solution of 100 µg/mL. An aliquot of this solution was diluted in methanol to get a series of working standard solutions of 1, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, and 5000 ng/mL. Internal standard (IS) solution was prepared by diluting the stock solution of venlafaxine to yield a final concentration of 500 ng/mL and 150 µL of the IS was added with 50 µL of PBS solution.

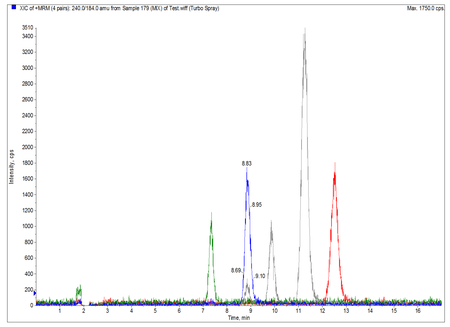
Sample Preparation: One hundred-fifty µL of IS solution (500 nM of venlafaxine) was added to 50 µL of sample from microsome or S9 reaction at each time point. All samples also had an additional 50 µL of MeOH. The mixture was then vortexed for 1 min, followed by centrifugation for 15 min at 14,000 rpm in an Eppendorf centrifuge. The supernatant was transfer to vials and 5 µl was injected for LC-MS/MS analysis.

LC-MS/MS analysis: The LC-MS/MS analysis was conducted using an Agilent 1200 HPLC system coupled to an API 3200 mass spectrometer (Applied Biosystems, MDS Sciex Toronto, Canada) equipped with an API electrospray ionization (ESI) source. Quantitative analysis was accomplished on a Supelco C18 (150 x 4.6 mm I.D., 5 µm). The mobile phases were 0.04% formic acid in purified water (A) and 0.04% formic acid in methanol (B). An isocratic gradient was held constant at 35% and the flow rate was set at 0.8 mL/min. The quantitation of bupropion, hydroxybupropion, threohydrobupropion, and erythrohydrobupropion in *in vitro* assay at different times and concentration were performed by multiple reaction monitoring (MRM) of the $[M-H]^-$ ion. For bupropion channel m/z 240-> 184, hydroxybupropion channel m/z 256-> 238, threo/erthrohydrobupropion channel m/z 242-> 168, and venlafaxine channel m/z 278-> 260.

Data Analysis: All data was modeled simulated in Prism5 using the Michaelis-Menten model.

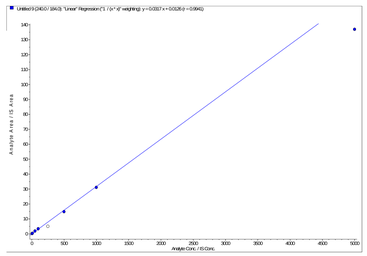
RESULTS

Figure 1: Separation of bupropion and metabolites

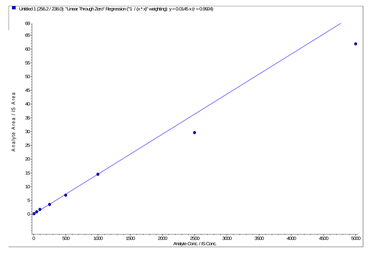


Analyte	Time (minutes)
Bupropion	8.8
Hydroxybupropion	7.3
Threohydrobupropion	9.85
Erythrohydrobupropion	11.2
Venlafaxine	12.5

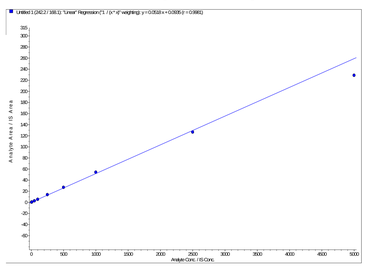
Figure 2: Standard curves for bupropion and metabolites



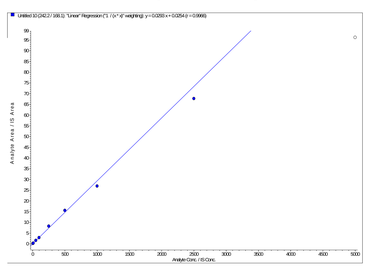
a) Calibration curve for bupropion quantification ($y = 0.0317x + 0.0126$, $r = 0.9941$). Linear range 5-5000 ng/mL



b) Calibration curve for hydroxybupropion quantification ($y = 0.0145X$, $r = 0.9924$). Linear range 10-5000 ng/mL

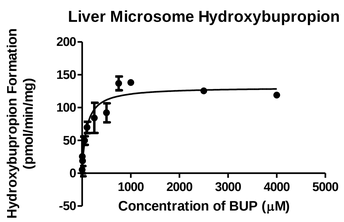


c) Calibration curve for threohydrobupropion quantification ($y = 0.0518x+0.0935$, $r = 0.9981$). Linear range 5-5000 ng/mL



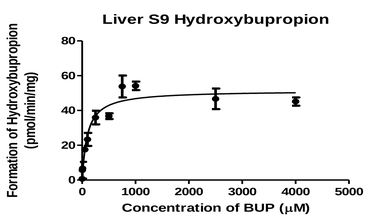
d) Calibration curve for erythrohydrobupropion quantification ($y = 0.0293X+0.0254$, $r = 0.9966$). Linear range 5-2500 ng/mL

Figure 3: Liver Microsome & S9 Quantification



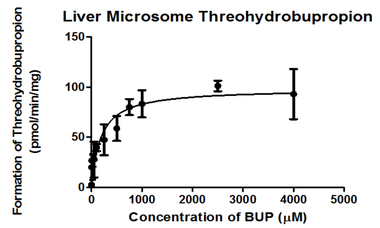
Vmax	131.2 ± 5.8 pmol/min/mg
Km	87.98 ± 20.2 µM

a) Hydroxybupropion formation in liver microsome

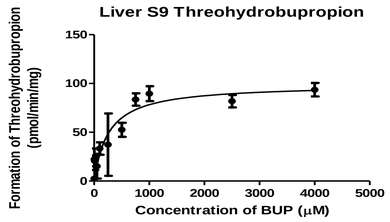


Vmax	51.45 ± 1.9 pmol/min/mg
Km	99.54 ± 18.91 µM

b) Hydroxybupropion formation in liver S9 fraction



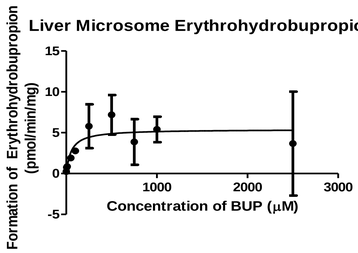
Vmax	98.37 ± 6.6 pmol/min/mg
Km	186.3 ± 53.48 µM



Vmax	99 ± 7.5 pmol/min/mg
Km	265.7 ± 77.79 µM

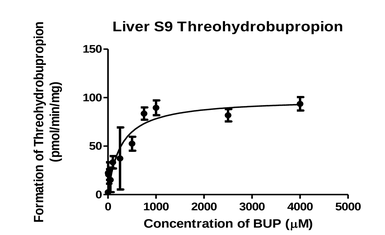
c) Threohydrobupropion formation in liver microsome

d) Threohydrobupropion formation in liver S9 fraction



Vmax	2.649 ± 0.3 pmol/min/mg
Km	41.45 ± 26.62 µM

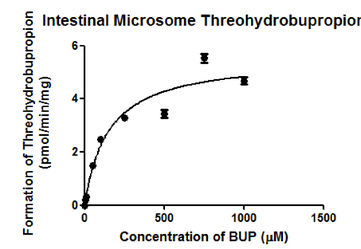
e) Erythrohydrobupropion formation in liver microsome



Vmax	4.23 ± 0.286 pmol/min/mg
Km	107 ± 32.14 µM

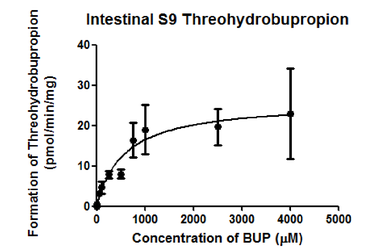
f) Erythrohydrobupropion formation in liver S9 fraction

Figure 4: Intestinal Microsome & S9 Quantification



Vmax	5.55 ± 0.3 pmol/min/mg
Km	149.9 ± 28.8 µM

a) Threohydrobupropion formation in intestine microsome



Vmax	25.87 ± 2.8 pmol/min/mg
Km	573.4 ± 188.9 µM

b) Threohydrobupropion formation in intestine S9 fraction

CONCLUSION/ FUTURE DIRECTIONS

In both microsome and S9 fractions of the liver, all three metabolites were formed. In the liver microsomes, hydroxybupropion was formed at a greater extent with the highest affinity of 87µM. This is likely due to the metabolism by CYP2B6 which are concentrated in microsomes. In the liver S9 fractions, formation of threohydrobupropion was almost 2 fold higher than hydroxybupropion, yet showed very low affinity. In both liver fractions erythrohydrobupropion formation was minor.

In the intestines, the only metabolite formed was threohydrobupropion. However, the formation of threohydrobupropion in the intestinal S9 fraction was 25% of the formation formed in the liver. Both hydroxybupropion and erythrohydrobupropion were not formed in the intestines.

In future studies, we will examine the carbonyl reductase pathway to understand which carbonyl reductase enzymes are important for this metabolism.

ACKNOWLEDGEMENTS OR REFERENCES

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