Inhibition of Presystemic Oxidative and Conjugative Metabolism of Buprenorphine Using GRAS Compounds or Dietary Constituents: In Vitro Proof of Concept

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Purpose
Suboxone™ (buprenorphine: naloxone (4:1)) is one of the widely used therapeutic formulations for treating opiate addiction. Buprenorphine (BUP) undergoes extensive oxidative metabolism mainly by CYPs 3A4, 2C8 and 2C9 to form norbuprenorphine (NBUP) and conjugative metabolism by glucuronidation (UGTs 1A1, 1A3 and 2B7) to form buprenorphine-3β-D-glucuronide (BUPG). This study evaluated the ability of several GRAS compounds and dietary substances (called inhibitors hereafter) to inhibit the metabolism of BUP. In addition, the contribution of gut wall and liver to the presystemic metabolism of BUP was also predicted.

Methods
BUP (5 or 8 μM) with and without the inhibitors (25 μM) was incubated with 0.2 or 0.3 mg/mL human intestinal microsomes or HIM (15 or 30 mins) and 0.1 mg/mL human liver microsomes or HLM (11 or 15 mins), depending on the metabolic pathway (oxidation or glucuronidation) being tested. Effect on 28 putative inhibitors on formation of BUPG and NBUP was monitored (individually) in the microsomes. Reverse phase HPLC coupled with UV absorbance and Acquity QDa mass detection was used for quantitation of BUP, BUPG and NBUP. Significant inhibition was tested by comparing the mean rate of formation of BUPG or NBUP in presence of inhibitors to the respective mean formation rate of BUPG or NBUP in control (no inhibitor) using one-way ANOVA with Dunnett’s post-hoc test (α = 0.05). Of the inhibitors that produce statistically significant inhibition, only the ones exhibiting more than 50% of UGT inhibition and/or more than 30% of CYP inhibition were reported as substantial inhibitors. Qgut model was used to determine Fg (intestinal availability) and hepatic well-stirred model to determine Fh (hepatic availability) of BUP. The absolute oral bioavailability (Foral) of BUP was also predicted using the Fg and Fh values determined above (Fraction absorbed or Fa assumed to be 1).

Results
In both HLM and HIM, more than 80% mass balance was attained. Metabolite formation was linear and saturable. Of the 28 inhibitors tested in HLM, chrysin, curcumin, ginger extract, hesperitin, magnolol, peppermint oil, quercetin and silybin substantially inhibited BUPG formation whereas chrysin, curcumin, ginger extract, 6-gingerol, peppermint oil, pterostilbene, resveratrol and silybin exhibited substantial inhibition of NBUP formation. In the HIM, curcumin, ginger extract, α-mangostin, peppermint oil, quercetin and silybin substantially inhibited BUPG formation while chrysin, ginger extract, α-mangostin, peppermint oil, pterostilbene and resveratrol exhibited substantial inhibition of NBUP formation. The oxidative intrinsic clearance (4.9 ml/min/kg NBUP formation) was 6 fold higher than glucuronidative intrinsic clearance (0.8 ml/min/kg BUPG formation) in HIM and about 4 fold higher in HLM (Clint,oxid = 157 ml/min/kg, Clint,gluc = 40.6 ml/min/kg). The Fg and Fh of BUP were determined to be 0.2 and 0.1 respectively. The Foral was predicted to be 2%, which is consistent with the reported in vivo Foral of BUP (< 15%; NIDA, 1993) Inhibition of 75% of intestinal extraction and 50% of hepatic extraction of BUP by the GRAS inhibitors or their combinations would result in a Foral of 44%, similar to the absolute bioavailability of the sublingual BUP (42 ± 9%).

Conclusion
The IVIVE results suggest oxidation to be the major metabolic pathway in gutwall and liver. This study demonstrates feasibility of using various GRAS/dietary compounds to substantially inhibit oxidative and conjugative presystemic metabolism of BUP and improve its Foral. Above results exhibit promising potential of developing an efficacious oral formulation of BUP and inhibitors (or their combination) to serve as an attractive alternative to sublingual BUP.