Naltrexone and its Active Metabolite 6β-Naltrexol Metabolic Analyses In Vitro

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Background
Naltrexone (NAL) is a prescription drug for the treatment of alcohol dependence. There are contradictory treatment responses while using NAL; some patients response to NAL, but some patients don't.

Aims & Objectives
NAL metabolism may affect the treatment response. This study compared the metabolism of NAL and its active metabolite 6β-naltrexol from incubating with liver microsome and isoenzymes.

Methods
A fixed amount of NAL and its active metabolite 6β-naltrexol were incubated with the fixed amount of recombinant human liver S9 fraction, liver recombinant metabolic enzymes of cyto-chrome P-450 iso-enzymes 1A2 (CYP1A2), CYP2B6, CYP2C19, CYP2D6, CYP3A4 in vitro for 24h. The NAL and 6β-naltrexol concentration reduction levels were calculated from the chromato-gram peak area of high performance liquid chromatography (HPLC), and compared to a standard NAL or 6β-naltrexol concentration without incubated with any enzyme.
A total of 50 μL of recombinant human liver S9 fraction, cytochrome P-450 iso-enzyme of CYP1A2, CYP2B6, CYP2C19, and CYP3A4, and CYP2D6 was mixed with 40 μL of 100 μg/mL NAL or 6β-naltrexol at room temperature for 20 min. To initiate the cytochrome enzymatic reaction, 10 μL of 1 mM NADPH was added to each well. The 100 μL of total volume in each well con-tained 100 pmol/mL of baculovirus-infected insect cell expressing each enzyme microsome, 40μg/mL NAL or 6β-naltrexol and 0.1 mM NADPH. Incubations were performed at 37℃ for 24h and stopped by adding 100 μL of 70% Acetonitrile. A 200 μL aliquot of mixture was mixed and filtered through 0.22 μm PVDF Millex-GV, and 20 μL of the aliquot was chromatographed through HPLC. The analytical column was a Luna C18 column (5 μm, 150 × 3 mm) with a C8 guard column (10 × 3 mm). The column oven was set at 30℃. The mobile phase was composed of 0.2% N,N,N,N-Tetramethylethylenediamine, 12% Acetonitrile, with a final pH of 6.0. The flow rate was 0.7 mL/min.

Results
NAL after incubated with recombinant human liver S9 fraction for 24 hours has created a concen-tration reduction for 35% in comparison with a standard NAL concentration which did not incubate with any metabolic enzymes. This suggested that liver metabolic enzymes have a high catalytic ac-tivity toward NAL. The rank order of these liver isozymes catalytic activity for NAL was CYP3A4> CYP2D6= CYP2C19= CYP2B6= CYP1A2. On the contrary, recombinant human liver S9 fraction did not show catalytic activity toward 6β-naltrexol after 24 hours of incubation. How-ever, CYP2C19 was the only isoenzyme showed catalytic activity toward 6β-naltrexol after 24 hours of incubation.

Discussion & Conclusion
1. This study has demonstrated that the liver metabolic enzymes have a strong impact for the NAL concentration. NAL can be metabolized by liver enzymes faster than its active metabolite 6β-naltrexol.
2. 6β-naltrexol may have a longer influence than its parent compound for the response of NAL due to its less susceptible for the liver metabolism.
3. According to the different catalytic activity of liver cytochrome P-450 isozymes, the CYP3A4 and CYP2C19 may significantly contribute to the NAL treatment responses.