Investigation of Phase I Metabolism for Piperine in Rats
T. Ren, C. Li, Q. Wang, Y. Zhang, Z. Zuo
Chinese University of Hong Kong

Purpose
As the major alkaloid from *Piper nigrum* Linn. (black pepper) and *Piper longum* Linn. (long pepper), piperine has been associated with miscellaneous pharmacologic effects, including antiepileptic effects, anti-tumor properties, anti-oxidative and anti-inflammatory activities. However, few metabolites of piperine have been reported and no study indicated the rate and extent of its metabolism. The current study is proposed to illustrate the phase I metabolism characteristic of piperine.

Methods
Around 35 mg/kg piperine in saline with 20% ethanol was given to rat for 2h. Then liver and bile were collected and treated by protein precipitation method. Potential metabolites of piperine after orally administration in SD rats were subsequently identified by LC/Q-TOF/MS method. In-vitro enzyme kinetics study was further carried out to explore the hepatic and intestinal metabolic rate and extent of piperine in liver and intestinal microsomes. Piperine ranging from 1 to 160 μM were incubated with 0.5 mg/ml rat liver or intestinal microsomes and NADPH regenerating system for 1.5 h under 37 degree Celsius. The metabolic profile was generated by plotting reaction rate versus concentration of piperine. The enzyme kinetic parameters including maximum velocity (Vmax), substrate concentration at half maximal velocity (Km) and intrinsic clearance (Clint) were further obtained by fitting data to the Michaelis-Menten equation.

Results
After oral administration of piperine, metabolite E,E-1-[5-(3-methoxy-4-hydroxy-phenyl)-1-oxo-2,4-pentadienyl]piperidine was found in both rat liver and bile, and another metabolite, 5-(3,4-methylenedioxy phenyl)-2E,4E- pentadienoic acid-N-(3-yl propionic acid)-amide was identified in bile. The in-vitro enzyme kinetic study showed that piperine had limited hepatic phase I metabolism with Vmax, Km and Clint to be 56.03±5.85 pmol/min/mg, 27.02±9.2 μM and 2.07 μl/min/mg, respectively. In addition, there is no reduction of piperine content after incubation with rat intestinal microsomes, indicating minimal intestinal phase I metabolism.

Conclusion
Our current in-vitro and in-vivo studies consistently indicate that there is no extensive phase I metabolism after consumption of piperine. [Financial support from the Macau Science and Technology Development Fund (Project Code No. 0203) and CUHK 7010298]