

- Title** : ENZYME KINETICS OF BUPIVACAINE, ETIDOCAINE, LIDOCAINE, AND 2,6-PIPECOLOXYLIDIDE (PPX) USING RAT HEPATOCYTES
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Introduction: Amide local anesthetics are extensively metabolized by liver enzymes. The rate and extent of metabolism is described by the intrinsic clearance term CL_{int} . Intrinsic clearance can be defined as the ratio of the enzyme kinetic terms maximum velocity (V_{max}) and the Michaelis-Menton constant (K_m). Propranolol has recently been reported to inhibit lidocaine uptake and metabolism, using rat hepatocytes.⁽¹⁾ Unfortunately, no data as to whether this inhibition was competitive, noncompetitive, or uncompetitive was reported. The present study was designed to test the feasibility of using primary rat hepatocyte cultures as a model with which to study local anesthetic metabolism. This paper reports a detailed evaluation of the use of rat hepatocytes for the determination of K_m , V_{max} , and intrinsic clearance of bupivacaine, etidocaine, lidocaine, and 2,6-pipecoloxylidide (PPX), a principal metabolite of bupivacaine.

Methods: Hepatocytes were isolated from Fisher 344 rats by modification of the collagenase perfusion method of Seglen (2). Briefly, the liver is perfused *in situ* with calcium free Hank's balanced salt solution (HBSS), pH 7.6, containing 10 mM HEPES buffer. The liver is then digested with collagenase (75 mg/ml) and calcium free HBSS for 20 to 30 minutes. The perfusing agent is oxygenated, adjusted to pH 7.5, and recirculated through the liver. Following digestion, the liver capsule is disrupted and the hepatocytes freed. Following a 30-minute incubation at 37°C, the cells are washed three times at room temperature with complete HBSS and then suspended in RPMI-1640 culture media at 1.1×10^6 cells/ml. Viabilities are then determined using a Trypan Blue exclusion test. Minimum acceptable viability for use was 75%. Each drug was studied over a range of five concentrations (0.069-0.431 mM). 9 ml of suspended hepatocytes were pipetted into each of the 5 flasks and incubated at 37°C for 10 minutes. 1.0 ml of the desired drug was then introduced, and 1.0-ml samples were withdrawn at 10, 20, and 30 minutes. Metabolism was terminated by placing the sample in a 60°C water bath for one minute. Samples were then centrifuged and the supernatant withdrawn for analysis of the drug by gas chromatography. Cytochrome P-450 determinations were performed on composite hepatocyte suspensions using a Beckman DU-8 spectrophotometer⁽³⁾. Initial velocities were estimated from the absolute differences between the 10- and 20- and the 20- and 30-minute time points at each concentration. Lineweaver-Burke double reciprocal plots of $1/V_0$ versus $1/S$ were constructed for the determination of K_m and V_{max} . Intrinsic clearances were then calculated. K_m and V_{max} were determined from a minimum of six independent hepatocyte harvests for each drug of interest. At least three drugs were run using each harvest for intra-animal comparisons. Statistical analysis was accomplished using a *t* test for independent means and Scheffe's analysis of variance. $P < .05$ were considered significant.

Table 1: K_m , V_{max} , and CL_{int} for bupivacaine, etidocaine, lidocaine, and PPX^a

	K_m (μM)	V_{max} (nmoles/h/ 10^6 cells)	CL_{int}
Bupivacaine	271 \pm 75	331 \pm 59 ^e	1.36 \pm 0.22
Etidocaine	169 \pm 25 ^b	523 \pm 16 ^d	3.63 \pm 0.54 ^f
Lidocaine	313 \pm 58	513 \pm 69	1.84 \pm 0.22
PPX	179 \pm 34 ^c	348 \pm 72	1.38 \pm 0.24

a. mean \pm sem; N=6; b. $p < .025$ compared to lidocaine; c. $p < .05$ compared to lidocaine; d. $p < .025$ compared to bupivacaine or PPX; e. $p < .05$ compared to lidocaine; f. $p < .005$ compared to bupivacaine, lidocaine, and PPX.

Results: All cellular suspensions were found to contain 0.27 ± 0.04 n moles of Cytochrome P450/ 10^6 cells. Preliminary studies demonstrated that metabolic integrity and viability were maintained for a minimum of four hours. No significant differences in metabolic activity could be detected for viabilities between 75 and 95% at a concentration of 10^6 cells/ml. Lidocaine has a significantly higher K_m than etidocaine and PPX. Lidocaine and etidocaine have significantly higher V_{max} than bupivacaine. Intrinsic clearance increases in the order bupivacaine < lidocaine < etidocaine.

Discussion: The methods described in this paper establish rat hepatocyte cultures as a useful model for the study of local anesthetic metabolism. The increase in intrinsic clearance follows that reported for man⁽⁴⁾. The data reported here suggest that the rat hepatocyte model is sensitive and reliable for detecting changes in V_{max} and K_m and thus in intrinsic clearance. This model is therefore ideally suited for investigations of drug interactions between adjuvant drugs and local anesthetics.

References:

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