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Short communications

Effects of clonidine on lidocaine metabolism in human or rat liver microsomes

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Although lidocaine is a commonly used local anesthetic, large doses of lidocaine may cause systemic toxicity. It is important to understand whether one drug inhibits metabolism of another drug (i.e., lidocaine) when they are administered simultaneously. Clonidine is a common medication used to treat patients with hypertension, and a useful sedative for reducing anesthetic requirements [1,2]. Although in one in vivo rodent study [3] clonidine was reported to increase concentrations of bupivacaine in plasma, another in vivo human study [4] suggested that clonidine is likely to decrease concentrations of lidocaine. Previously, we reported that oral clonidine premedication (dose of 4μg/kg) reduces lidocaine concentrations in plasma by 25%–50% in children with continuous thoracic epidural anesthesia [5]. Several reasons may account for these different results. First, the difference in these results may partially depend on differences between species. Second, clonidine may influence drug metabolism in a liver cytochrome P450 (CYP) system. In an in vivo study, the negative hemodynamic effects of clonidine, which causes a decrease in blood pressure and/or heart rate, are also likely to decrease hepatic blood flow, and may lead to diminished drug metabolism. In an in vitro study, the metabolic effects of the drug can be isolated from their systemic, especially hemodynamic, effects. For that reason, we performed an in vitro study to test whether clonidine influences lidocaine metabolism in human and rat liver microsomes.

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The study protocol was approved by the Animal Experimental Procedures Committee. Human microsomes were purchased from Gentest (pooled human liver microsomes, H161 Lot number 16, Protein 20mg/ml in 250mM sucrose, Woburn, MA, USA), and were stored at −80°C. After thawing, the human microsomes were stored on ice.

Male Sprague-Dawley rats (weighing 280–350g, 8–10 weeks of age) were obtained from Nihon Clea Co. (Tokyo, Japan). The rats were kept in an air-conditioned room (25 ± 1°C, 50%–60% humidity) with a 12-h light–dark cycle (8:00 a.m.–8:00 p.m.), and given free access to commercial rat chow (Oriental-MF, Tokyo, Japan) and water. The untreated rats were made to fast overnight and then were decapitated under ether anesthesia. The liver was immediately removed. The livers were homogenized in 4 vols (w/v) ice-cold 1.15% KCl, the homogenates were centrifuged at 10000*g* for 20min, and the supernatants were then ultracentrifuged at 105000*g* for 60min. The resulting microsomal pellets were resuspended in 50mM potassium phosphate buffer (pH 7.4) containing 0.1mM ethylenediaminetetraacetate, and stored at −80°C.

The incubation mixture contained 0.125mg liver microsomal protein, 0.1M potassium phosphate buffer (pH 7.4), 4mM nicotinamide adenine dinucleotide phosphates reduced (NADPH), 20μM lidocaine (equal to a plasma concentration of 4.7μg/ml in vivo), and clonidine (0, 1, 2.5, 5, 10, 25, 50, and 100nM; three samples each) in a total volume of 0.5ml. The incubations were initiated following a 5-min preincubation at 37°C by the addition of NADPH and carried out for 15min in a shaking water bath at 37°C. The reaction was terminated by adding 50μl 1N NaOH and 1.5ml ethyl acetate containing 0.5μg/ml trimethoprim (internal standard). Clonidine and trimethoprim were purchased from Sigma (St. Louis, MO, USA). After vortex mixing for 5min, the tubes were centrifuged at 1200*g* for 5min, and the aqueous phase was removed by aspiration. The

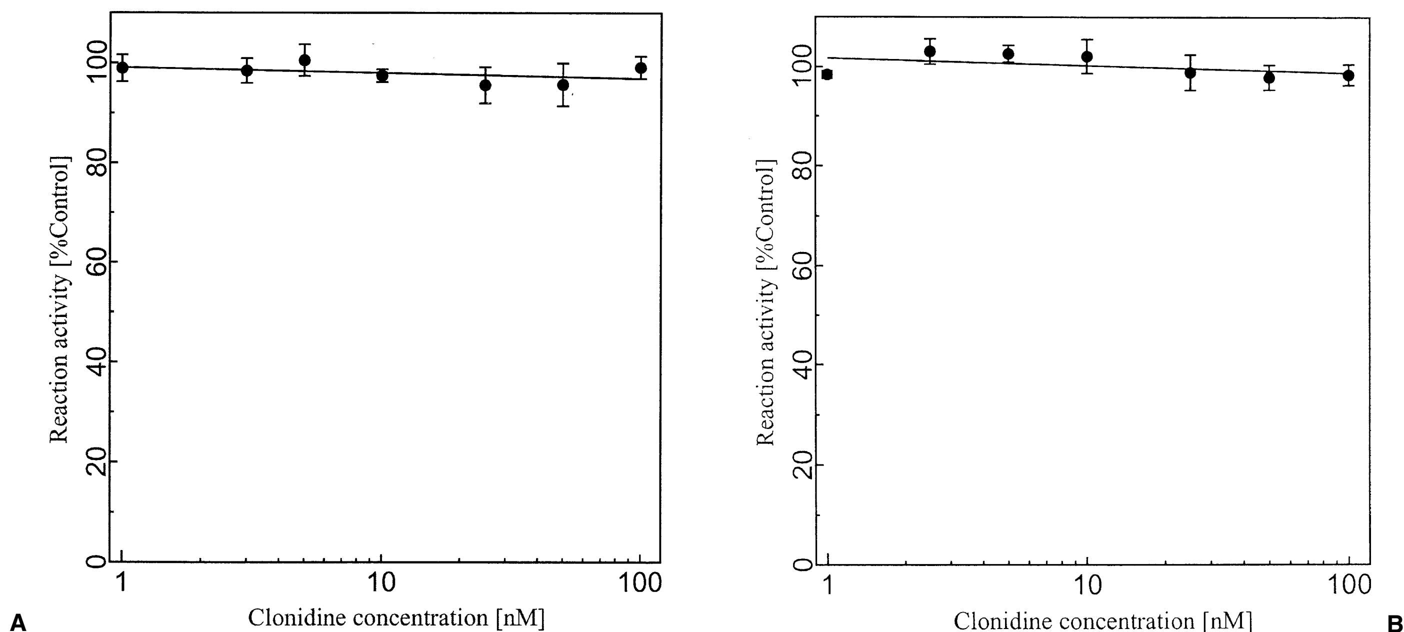

Fig. 1A,B. **A** Effect of clonidine concentration (1–100 nM) on 20 μM lidocaine metabolism [monoethylglycinexylidide (MEGX) formation] in in vitro human liver microsomes. Data points are means ± SD (n = 3). **B** Effect of clonidine concentration (1–100 nM) on 20 μM lidocaine metabolism (MEGX formation) in in vitro rat liver microsomes. Data points are means ± SD (n = 3)

organic phase was transferred to a clean conical tube and evaporated in a water bath at about 40°C under a gentle stream of nitrogen. The residue was dissolved in a 200 μl mobile phase, and 50 μl was injected into the high-pressure liquid chromatography (HPLC) apparatus as described below. The P450 contents of the microsomes were 0.556 and 0.553 nmol·mg^{-1} protein in the rats and humans, respectively.

We used the assay reported by Tanaka et al. [6] to determine the lidocaine and monoethylglycinexylidide (MEGX) concentrations. Lidocaine and MEGX were kindly provided by Astra Japan (Osaka, Japan). The HPLC equipment consisted of a pump (Model CCPS, Tosoh, Tokyo, Japan) and a variable-wavelength ultraviolet (UV) detector (Model UV-8000, Tosoh, Tokyo, Japan). Separation was achieved using a C_{18} reversed-phase column (150 mm × 4.6 mm I.D., particle size 5 μm, TSK-gel ODS80-TM; Tosoh, Tokyo, Japan). The mobile phase was composed of 0.05 M KH_2PO_4-acetonitril (86 : 14, v/v) (pH 4.0), and the flow rate was 0.8 ml/min. The absorbance of the eluate was monitored at 205 nm. All instruments were operated at ambient laboratory temperature (23°C). The retention times of the MEGX, internal standard, and lidocaine were about 8.4, 10, and 13 min, respectively. With this assay method, the extraction recoveries from plasma for lidocaine and MEGX were 96.6% and 91.2%, respectively, at 10 μg/ml. The maximum coefficient of variation for within-run or between-run precision was 3.3%, and the detection limits for lidocaine and MEGX were 10 ng/ml using 250 μl of the plasma sample.

Data were expressed as the means ± SD. Statistical comparisons were performed using repeated measurements analysis of variance (ANOVA) within each group, and significance was assessed using the Scheffe F-test. In all cases, a P value less than 0.05 was considered the minimum level of statistical significance.

The effects of clonidine on lidocaine metabolism (MEGX formation) in human and rat liver microsomes are shown in Fig. 1. Reaction activity (% control), was 99 ± 6% (P = 0.4) or 100 ± 4% (P = 0.4) for concentrations of clonidine between 1 and 100 nM (1, 2.5, 5, 10, 25, 50 and 100 nM; 3 samples each) in human or rats, respectively. The mean activity of lidocaine deethylation (MEGX formation) in the three liver microsomal samples was 0.897 nmol·mg^{-1} protein·min^{-1} in rat and 0.446 nmol·mg^{-1} protein·min^{-1} in human when the substrate concentration was 20 μM lidocaine.

No studies of in vitro drug interactions involving clonidine and lidocaine analyzed from the metabolic point of view have been published. The plasma clonidine concentrations have been reported as 1.5–4 ng·ml^{-1} in humans receiving therapeutic doses of clonidine [7,8]. According to the results of our in vitro

study, the plasma clonidine concentration in clinical usage is unlikely to inhibit lidocaine metabolism in liver microsomes.

Previous studies showed that lidocaine is metabolized by both CYP3A4 and CYP1A2 in humans [9,10] and by both CYP3A2 and CYP2B1 in rats [11]. There are no reports concerning which CYP subfamily metabolizes clonidine. The results of the current study indicate that clonidine is likely to be metabolized by different CYP subfamilies from those that metabolize lidocaine in both human and rat microsomes.

In this in vitro study, microsomal lidocaine deethylation was not altered by clonidine concentrations as high as 100 nM. However, some previous in vivo studies using clonidine found increased [12] or decreased [4,5] lidocaine concentrations. Several possible reasons may account for the increased concentrations of lidocaine. First, a previous study showed that clonidine at a larger dose of $100\,\mu g \cdot kg^{-1}$ ip increased the plasma concentration of lidocaine in mice [3]. A large dose of clonidine injected ip may diminish hepatic blood flow and the hepatic metabolism of lidocaine because of its vasoconstrictive effect. Second, since clonidine often decreases blood pressure and/or heart rate, this reduction in cardiac output may also cause decreased hepatic blood flow, which may then inhibit drug metabolism in the liver. Changes in hepatic blood flow may also account for the contradictory results obtained by previous studies. Third, both diazepam [13] and lidocaine [9,10] are partially metabolized by CYP3A4. If patients receive diazepam as a premedicant, lidocaine metabolism may be affected (inhibited), leading to an increase in lidocaine concentrations in such patients. Another possible mechanism by which clonidine may decrease concentrations of lidocaine is a slowing of lidocaine elimination from the injection site of the clonidine [14]. These differences could also account for the contradictory results.

In conclusion, we performed an in vitro study to test whether clonidine influences lidocaine metabolism, isolated from their systemic effects, in human or rat liver microsomes. Clonidine of clinical concentration is unlikely to influence the metabolism of lidocaine in vitro in either human or rat liver microsomes.

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