

Letter to the Editors

Lack of effect of subject posture on intravenous midazolam clearance: implications for hepatic cytochrome P450 3A phenotyping

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Intravenous (i.v.) midazolam is used to phenotype hepatic cytochrome P450 (CYP) 3A [1]. Midazolam clearance (CL) is believed to correlate with the intrinsic hepatic CL (CL_{int}) of midazolam and thus acts as a surrogate for hepatic CYP3A activity. Midazolam is a moderate extraction ratio drug [2]. Based on the well-stirred model of hepatic clearance [3], midazolam CL depends on CL_{int} and hepatic blood flow. Subject posture alters hepatic blood flow, is often not controlled during blood sample collection [1], and impacts pharmacokinetic studies [4]. The magnitude of posture-induced blood flow changes has been seen in a study of six subjects. Plasma midazolam CL decreased 48% in an ambulant (sitting/walking) posture compared with a supine posture [5] and suggests that changes in midazolam CL may be dependent on hepatic blood flow and not exclusively CYP3A activity. This study evaluated the effect of subject posture on midazolam CL to confirm previous findings. We quantified posture-induced hepatic blood flow changes with i.v. indocyanine green as a biomarker. Results are published elsewhere [6].

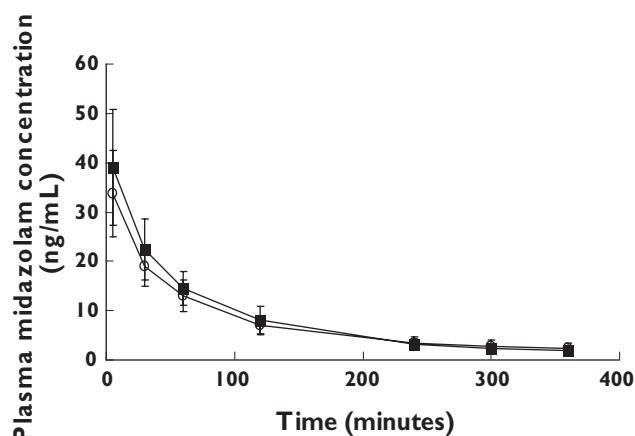
The Institutional Review Board of Bassett Healthcare (Cooperstown, NY, USA) approved the study. Written informed consent was obtained. Healthy adults 18–55 years old who were not on any medications were enrolled. Subjects were randomized to a 7-h supine or ambulant posture. A minimum of 7 days passed between study phases. Subjects maintained strict horizontal bed rest during supine posture. During ambulant posture, subjects were seated for the first 3 h and then allowed to stand, walk, or sit. Sessions began between 06.00 h and 07.00 h to minimize temporal variation [5]. Subjects fasted (except water) overnight and for the duration of each posture. To stabilize hepatic blood flow, subjects were in the assigned posture for 1 h prior to midazolam 0.025 mg kg^{-1} i.v. (midazolam hydrochloride, 1 mg ml^{-1} injection, North Chicago, IL, USA) administration. Eight blood samples were

obtained over 6 h. Samples were centrifuged and stored at -80°C . Plasma concentrations were analysed by liquid chromatography/mass spectrometry/mass spectrometry as described elsewhere [7]. The linear range of the assay was $0.50\text{--}100 \text{ ng ml}^{-1}$, with intra- and interday coefficients of variation of $\leq 7.9\%$ at 0.75, 7.5 and 75 ng ml^{-1} .

Midazolam pharmacokinetics was determined by non-compartmental analysis (WinNonlin v3.1; Pharsight, Cary, NC, USA). Sample size calculations were based on previously published data [5, 8–10]. Using an $\alpha = 0.05$ and $\beta = 0.20$, a sample size of 12 would detect a 25% difference in midazolam CL. Bioequivalence testing was used by calculating the least squares geometric mean ratios (LS-GMR; $CL_{ambulant}/CL_{supine}$) and 90% confidence intervals (CI). Bioequivalence was concluded if the 90% CIs were within the 80–125% interval.

Thirteen (seven female) subjects completed both postures. Plasma midazolam concentrations vs. time are shown in Figure 1. Bioequivalence was observed for the geometric mean midazolam CLs in the supine and ambulant phases (595.6 vs. $565.1 \text{ ml min}^{-1}$; LS-GMR 0.95, 90% CI 0.88, 1.02). Intersubject variation of midazolam CL was small, varying 1.7- and two-fold in the supine and ambulant postures, respectively. The volume of distribution (V_d) decreased 18% in the ambulant posture. The difference in V_d may be due to changes in tissue binding, but for CYP3A phenotyping in healthy adults this change is unlikely to be of clinical significance.

Studies have observed posture-induced changes in hepatic blood flow of 17–37% for several medications [11, 12]. In the current study, subject posture did not affect midazolam CL. In contrast to the study of Klotz *et al.* [5], the current study used a smaller midazolam i.v. dose (0.025 vs. 0.075 mg kg^{-1}), had a larger sample size (13 vs. 6), and had subjects remain fasting during the entire sampling period.

**Figure 1**

Mean \pm SD midazolam plasma concentrations vs. time in the ambulant (closed squares) and supine (open circles) postures ($n = 13$)

Food intake increases hepatic blood flow and lasts up to 190 min [13]. We speculate that the small sample size and food intake may have affected midazolam CL in the Klotz *et al.* study [5]. Neither study reported the exact amount of time during the ambulant posture that subjects were in a sitting, walking and standing posture, and potential differences between subjects and studies may have existed in the amount of time spent in a specific ambulant position.

Phenotyping studies using i.v. midazolam as a hepatic CYP3A probe do not need to control for subject posture during blood sample collection. These findings are not applicable when using oral midazolam to phenotype intestinal and hepatic CYP3A activity, as i.v. midazolam exclusively measures hepatic CYP3A.

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RECEIVED

1 October 2008

ACCEPTED

5 December 2008

PUBLISHED Early View

12 February 2009

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