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Acute kidney injury reduces the hepatic metabolism of midazolam in critically ill patients

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Abstract *Introduction:* Acute kidney injury (AKI) is a common and serious complication increasing morbidity and mortality from all causes of hospital admission. We have previously shown that AKI decreases midazolam metabolism, a substrate of the cytochrome P450 3A (CYP3A) enzymes and our primary aim was to determine if this effect is dependent on the severity of AKI. We also present preliminary data on the functional impact of different genotypes of CYP3A. *Methods:* Critically ill patients at risk of AKI and admitted to a general intensive care unit were categorised after initial resuscitation according to the RIFLE criteria for AKI. Midazolam (1mg) was administered and the serum concentration of midazolam measured at 4 h. Samples were taken for CYP3A genotyping. *Results:* Seventy-three patients were assigned to categories R, I and F of the RIFLE criteria or C (controls). Midazolam concentrations (ng mL^{-1}) increased significantly ($p = 0.002$) as the severity of AKI worsened [control 3.1 (1.4–5.9), risk 4.7 (1.3–10.3), injury 3.9 (2.0–11.1) and failure 6.8 (2.2–113.6)] and were predicted by the duration of AKI ($p = 0.000$) and γ-glutamyl transferase (p = 0.005) concentrations. Increasing BMI negatively predicted the midazolam concentration ($p = 0.001$). Preliminary data suggest this effect is diminished if the patient expresses functional CYP3A5. *Conclusion:* Increasing severity and duration of AKI are associated with decreased midazolam elimination. We propose that this is caused by impaired CYP3A activity secondary to AKI. The exact mechanism remains to be elucidated. This may have important implications for our drug treatment of critically ill patients.

Keywords Acute kidney injury · Pharmacogenetics · Cytochrome P450 · Midazolam · Hepatic metabolism · Critical illness

Introduction

Acute kidney injury (AKI) is a common and important problem for hospital patients and is associated with increased risk of death [1, 2]. Recent retrospective analysis of outcome from AKI in all hospital admissions revealed that an acute increase in creatinine (>26 μmol L^{-1}), even if recovered to normal and regardless of underlying cause for admission, was associated with poor outcome compared to patients with normal renal function or indeed patients admitted with chronic kidney disease (CKD) [3]. With the development of consensus criteria [4, 5] for the description of AKI, there is increasing recognition of cross-talk between kidneys and other organs including cardiorenal, neurorenal, respiratory-renal and heptaorenal interactions [6–9]. In a recent pilot study, we presented preliminary data

to suggest that AKI may impair the hepatic metabolism of midazolam, but we were unable to relate this to the severity of AKI [10]. A similar phenomenon has been described for CKD [11–14].

The pharmacokinetics and pharmacodynamics of drugs administered to critically ill patients are often unpredictable and complex, and efficacy and toxicity of drugs may be dose-critical. However, most dosing regimens are based on data extrapolated from studies in normal individuals or those experiencing chronic disease states or are based on empirical principles. The effects of acute organ failures are not fully understood, and the impact of AKI on hepatic drug metabolism remains to be characterized.

The cytochrome P450 3A (CYP3A) enzymes are the most abundantly expressed subfamily of cytochrome P450 enzymes. The human *CYP3A* gene locus comprises four functional genes, *CYP3A4, CYP3A5, CYP3A7* and *CYP3A43*, and two pseudogenes [15]. Only *CYP3A4* and *CYP3A5* are functionally relevant in adults and are responsible for metabolism of >50% of all drugs [16]. They differ in terms of substrate intrinsic clearance and regioselectivity [17]. The majority of activity of these enzymes occurs in the liver although they are also expressed in intestinal mucosa, lung and kidney. Intestinal CYP3A activity has a marked effect on the bioavailability of orally administered drugs, but following intravenous administration, the main determinant of drug metabolism is hepatic CYP3A enzyme content [18].

All adults express CYP3A4. Many single nucleotide polymorphisms (SNP) exist in the CYP3A4 gene, but all are present at low frequency (<1–2%) and do not relate to drug metabolism phenotype [19]. In contrast, individuals are either functional expressers or non-expressers of CYP3A5. The most frequent SNP is an A > G substitution within intron 3, known as *CYP3A5*3* (*6986 A > G*). The substitution leads to improperly spliced *CYP3A5 mRNA* and a non-functional protein truncated at amino acid 102 [17]. Individuals with at least one wild-type allele (*CYP3A5*1*) are expressers and *CYP3A5*3/*3* homozygotes are non-expressers of CYP3A5. When expressed, *CYP3A5* represents >50% of total *CYP3A* activity [17]. The frequency of the allele *CYP3A5*3* is 87% in Caucasians and 28% in African Americans [20] and accounts for differences in metabolism of CYP3A substrates observed among ethnic groups. Renal transplant recipients heterozygous or homozygous for *CYP3A5*1* have a twofold lower dose-normalized blood concentration of tacrolimus than patients homozygous for *CYP3A5*3* [21].

Measuring the the total systemic clearance of midazolam is an established in vivo method of probing CYP3A enzyme activity, and a single point determination of midazolam concentration 4 h after intravenous administration has been shown to be a safe and accurate representation of total midazolam exposure in healthy volunteers and critically ill patients [10, 15]. Our primary aim was to test the hypothesis that worsening AKI decreases the hepatic metabolism of midazolam by CYP3A enzymes. A secondary aim was to define the role of the *CYP3A5* genotype on inhibition of hepatic drug metabolism in AKI.

Methods

Critically ill patients were enrolled from the general critical care unit of a London university hospital over a 1 year period (May 2008–May 2009). The unit admits 1,400 patients per year, including for elective surgery and emergency admissions (medical and surgical).

Approval for the study was granted by the Research Ethics Committee. Written assent for the patient to participate was obtained from a next of kin, in accordance with advice received from the Research Ethics Committee in relation to the Mental Capacity Act of 2006 (UK).

Patients

All patients requiring >48 h admission to the general intensive care unit (GICU) were reviewed and were considered at immediate risk of AKI if they had either a urine output <0.5 mL kg^{-1} h^{-1} or a serum creatinine >110 μmol L^{-1} (Fig. 1). Patients then underwent a 24 h period of resuscitation by clinicians not involved in the study, before allocation to one of the RIFLE criteria: risk (R), injury (I) and failure (F) at the start of the 4 h study period [4]. Seventeen patients did not fulfil any of the RIFLE criteria after resuscitation and were designated controls.

Exclusion criteria were (1) acute or chronic liver disease, (2) immediate requirement for renal replacement therapy, (3) renal transplantation, (4) pregnancy, (5) prior use of benzodiazepines (during current hospital admission) or (6) administration of major inhibitors (amiodarone, macrolide antibiotics, imidazole compounds) or inducers (rifampicin, phenytoin, dexamethasone) of CYP3A enzymes.

Length of time of AKI was estimated based on the time since baseline serum creatinine and/or normal urine output (if the patient was in hospital prior to AKI), or as time since admission to hospital, if admitted as an emergency.

Data collection

Patient length and weight were recorded and body mass index (BMI) and body surface area (BSA) calculated [22].

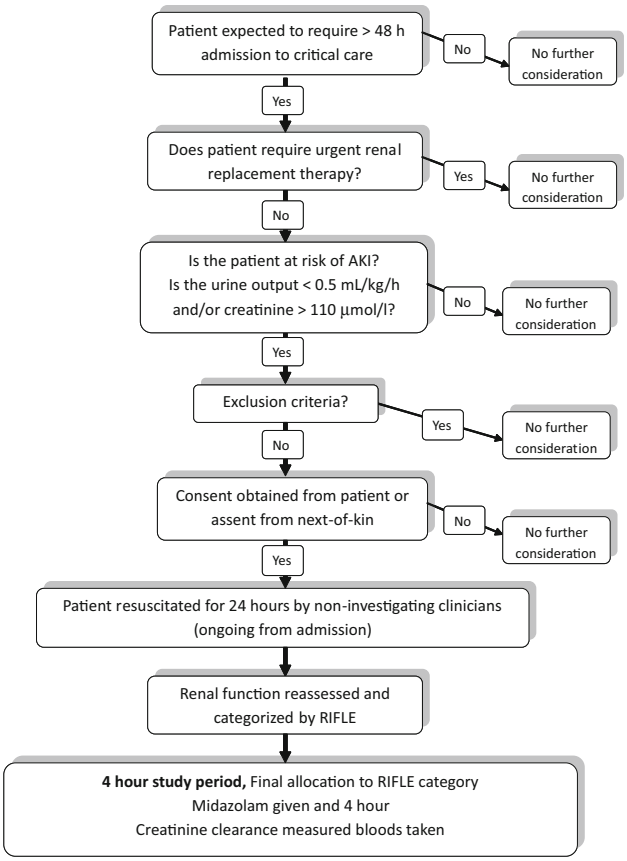

Fig. 1 Flow diagram describing patient recruitment

Baseline serum creatinine concentrations were established using the following methods: (1) from hospital admission blood tests if admitted prior to developing AKI, (2) from results recorded in the notes or by the patient's general practitioner (GP) within the previous year, (3) if a previous result was unavailable and if renal function recovered to within normal range following the ICU stay, this value was used as the baseline. Using these methods a baseline creatinine measurement was obtained for all patients.

4 h study period

All patients had blood tests during the 4 h study period for renal biochemistry (urea, creatinine), serum electrolytes, alkaline phosphatase (ALP), alanine transaminase (ALT), gamma-glutamyl transferase (γ-GT), albumin, bilirubin and haematology including the international normalised ratio (INR) and a full blood count. Arterial blood was analysed for pH, partial pressure of oxygen (PaO_2) and bicarbonate (HCO_3^-) concentration. An ethylenediaminetetraacetic acid (EDTA) sample was taken for genotyping of *CYP3A5*.

Intravenous midazolam (1 mg) was given at time zero (T0) and two blood samples collected 4 h later (T4) for analysis of midazolam concentration and for repeat serum creatinine, urea and albumin.

Urine was collected for 4 h and a sample sent for spot albumin and creatinine concentrations. The 4 h creatinine clearance (^{4}CrCl) (mL min^{-1}) was calculated as U $\times$ V/P [U urine creatinine concentration (mg mL^{-1}), V urine flow (mL min^{-1}) and P plasma creatinine concentration (mg mL^{-1})]. ^{4}CrCl was adjusted to the average BSA (1.73 m^2).

Midazolam assay

Total serum midazolam concentration was determined by high performance liquid chromatography with tandem mass spectrometry (HPLC/MS/MS) using a Sciex API 4000^{TM} (AME Bioscience, Norway). This is a standard method, based on one previously published [23] with in-house modifications such that deuterate midazolam is used as internal standard to give a much superior assay. Sample size was 100 µL, and the lower limit of quantitation (LLOQ) was set to 0.25 ng mL^{-1}. The limit of detection (LOD) was 0.05 ng mL^{-1}.

Urine creatinine was quantified by the Jaffe reaction and urine albumin by a turbidimetric assay.

Genotype at the CYP3A5*6986 A > G* (*CYP3A5*1/*3*) SNP was determined by real-time PCR with fluorescently labelled hybridisation probes and melt curve analysis on a Roche Lightcycler 1.0 [21].

Power for this study was calculated from previous data published by this group [10]. Assuming a difference in midazolam concentration of 1.5 ng mL^{-1} between groups and using a standard deviation of 2.1, 15 patients per RIFLE criterion were required to achieve a power of 80% with a significance level (α, two-sided) of 0.05 for the primary aim.

Statistical analysis

All statistical analysis was done using SPSS® (v16.0). Changes with RIFLE criteria for non-parametric data were tested for significance using the Kruskal-Wallis test, and a two-tailed P value <0.05 was considered significant. A backward-selected linear multiple regression was employed to look for predictors of midazolam concentration.

Funding

Funding was obtained from the St George's Research Fund.

Results

After excluding patients requiring urgent renal replacement therapy, 166 critically ill patients were considered at risk of AKI. Of these, 93 were excluded for the following reasons: (1) prescribed exclusion medications, (2) exposure to midazolam, or (3) permission was refused. In total 73 patients were studied, including 56 patients with AKI after initial resuscitation and 17 critically ill patients without AKI.

Patient characteristics

A summary of patient demographics is given in Table 1. Significant co-morbidities are listed and the Charlson co-mobidity score calculated. Patients who developed AKI were more likely to have an increased serum creatinine at baseline or a history of chronic renal impairment. There were no other significant differences between RIFLE categories.

Clinical variables on admission to the study are summarized in Table 2. The total Sequential Organ Failure Assessment (SOFA) score increased as renal function worsened but was entirely attributable to the deteriorating score for the renal component (Table 2).

Nineteen of 73 patients were genetically predicted to express functional CYP3A5 (heterozygous or homozygous for the *CYP3A5*1* allele). All black (genetically sub-Saharan African) patients expressed at least one *CYP3A5*1* allele compared to 18% of Caucasian patients. Ethnic groups were evenly distributed across the RIFLE criteria (Table 1).

Findings at T_4

The renal and hepatic indices at T_4 are shown (Table 3). There were no significant differences in pH, serum albumin, ALT, γ-GT, INR or bilirubin. ALP did increase significantly with worsening RIFLE criteria ($P = 0.034$).

Midazolam concentrations increased with the severity of AKI (Kruskal-Wallis, $P = 0.002$, Table 3). There were two major outliers who may have skewed the results in favour of the hypothesis with midazolam concentrations of 60.47 and 113.61 ng mL^{-1}. Without these results the statistical significance persists (Fig. 2, $P = 0.009$).

The raw midazolam data were skewed, and in order to determine the predictive value of variables, the data were normalized by $\log_{10}$ transformation. The relationships between the log-transformed midazolam concentrations

Table 1 Patient demographics and baseline measurements, including significant co-morbidities, renal indices and type of admission (elective or emergency, surgical or medical)

	All patients	Controls	RIFLE criteria			*P* value
			Risk (R)	Injury (I)	Failure (F)	
Number of patients	73	17	24	16	16	
Age (years)	73 (23–90)	73 (23–86)	71 (37–90)	72 (39–85)	73 (25–85)	0.944
Gender (M/F)	44/29	10/7	19/5	7/9	8/8	0.106
Ethnicity						
Black	6	1	2	1	2	
South Asian	4	1	2	1	0	
White	63	15	20	14	14	0.982
Height (cm)	171 (144–197)	173 (152–196)	175 (150–190)	173 (150–197)	165 (144–178)	0.129
Weight (kg)	75 (40–175)	72 (48–128)	82 (55–160)	71 (52–175)	65 (40–110)	0.275
Body surface area (m^2)	1.88 (1.26–3.09)	1.84 (1.48–2.50)	2.05 (1.51–2.67)	1.79 (1.55–3.09)	1.74 (1.26–2.33)	0.210
Body mass index (kg m^{-2})	25 (17–67)	26 (18–42)	26 (21–62)	26 (17–67)	25 (19–42)	0.989
Baseline creatinine (μmol L^{-1})	78 (38–298)	71 (38–298)	80 (43–198)	84 (49–130)	92 (49–220)	0.021
Baseline urea (mmol L^{-1})	6.4 (1.5–18.2)	6.3 (3.6–13.0)	6.4 (3.2–18.2)	7.0 (1.5–11.6)	7.6 (2.9–13.5)	0.498
Medical	26	5	6	7	8	
Emergency surgery	30	6	12	8	4	
Elective surgery	17	6	6	1	4	0.287
Charlson co-morbidity score	4 (0–11)	5 (0–11)	4 (0–10)	4 (1–10)	5 (0–7)	0.724
Hypertension	38	6	13	9	10	0.432
Diabetes mellitus	16	6	4	3	3	0.525
Chronic kidney impairment	6	0	2	0	4	0.033
Cancer	15	6	5	3	1	0.244
Ischaemic heart disease	14	3	3	4	4	0.681
Peripheral vascular disease	5	0	1	2	2	0.375
Connective tissue disorder	9	0	4	3	2	0.302
Chronic respiratory disease	9	1	5	2	1	0.525

Values are number or median (range). Differences tested by non-parametric tests: Kruskal-Wallis test (asymptomatic significance $P < 0.05$) and Fisher's exact test (exact two-sided significance, $P < 0.05$)

Table 2 SOFA scores with and without renal component according to RIFLE criteria, use of alfentanil and propofol between groups and variation in cardiac indices between groups, and expression of *CYP3A5* genotypes between groups

Variable	All patients	Controls (C)	RIFLE criteria			*P* value[a]
			Risk (R)	Injury (I)	Failure (F)	
Number of patients (n)	73	17	24	16	16	
Total SOFA scores	6 (1–15)	4 (1–9)	6 (1–12)	8 (2–13)	9 (4–15)	0.013
Renal score	1 (0–4)	0 (0–1)	1 (0–2)	2 (0–4)	3 (1–4)	0.000
SOFA without renal score	5 (0–12)	5 (1–11)	5 (1–12)	5 (2–12)	6 (0–11)	0.793
Patients administered						
Alfentanil (n)	30	8	10	5	7	0.834
Propofol (n)	32	8	10	7	7	0.987
Cardiac index (n)	36	6	15	8	7	
Cardiac index (L min^{-1} m^2)	3.2 (1.4–5.4)	3.75 (3.5–4.5)	3.2 (1.8–5.4)	3.0 (2.3–4.2)	3.1 (1.4–4.4)	0.314
CYP3A5 genotype (n)						
AA (**1/*1*)	4	0	1	1	2	
AG (**1/*3*)	15	4	3	5	3	
GG (**3/*3*)	54	13	20	10	11	0.571

Values are number or median (range). *CYP3A5 *1/*1* is the wildtype (active gene), *CYP3A5 *3/*3* homozygous for inactive gene

[a] Kruskal-Wallis test (asymptomatic significance, $P < 0.05$), Fisher's exact test (exact significance, two-sided, $P < 0.05$)

Table 3 Midazolam concentrations and renal and hepatic indices according to RIFLE criteria during the 4 h test period

Variable	All patients	Controls (C)	RIFLE criteria			*P* value
			Risk (R)	Injury (I)	Failure (F)	
Number of patients (*n*)	73	17	24	16	16	
Midazolam (ng mL^{-1})	4.8 (1.3–113.6)	3.1 (1.4–5.9)	4.7 (1.3–10.3)	3.9 (2.0–11.1)	6.8 (2.2–113.6)	0.002
Renal indices						
Time with AKI (h)	24 (0–168)	0	15 (6–72)	24 (8–168)	72 (24–120)	0.000
Serum urea (mmol L^{-1})	12.5 (2.7–58)	6.7 (2.2–18.0)	11.9 (4.9–34.9)	14.6 (6.4–57.9)	23.9 (10.3–39.1)	0.000
Serum creatinine (μmol L^{-1})	136 (39–606)	73 (40–312)	130 (43–314)	183 (39–207)	327 (160–606)	0.000
Corrected ^{4}CrCl (ml min^{-1} m^2)	39 (0–146)	90 (50–146)	46 (9–83)	23 (7–65)	8 (0–27)	0.000
Cystatin C (mg L^{-1})	1.52 (0.4–3.9)	0.8 (0.4–2.0)	1.2 (0.5–3.0)	1.9 (0.7–3.5)	2.8 (2.0–3.9)	0.000
Urine output (ml kg^{-1} h^{-1})	0.55 (0–3.82)	0.84 (0.5–3.8)	0.53 (0.26–3.67)	0.48 (0.1–1.53)	0.25 (0–2.12)	0.001
Hepatic indices						
Albumin (g L^{-1})	18 (10–32)	22 (10–30)	18 (10–32)	17 (10–27)	18 (11–28)	0.294
Alkaline phosphatase (IU L^{-1})	64 (23–464)	59 (38–104)	60 (23–220)	58 (32–257)	128 (29–464)	0.034
Alanine transaminase (IU L^{-1})	26 (5–623)	17 (5–48)	24 (7–518)	21 (10–197)	46 (9–323)	0.078
Gamma glutamyl transferase (U L^{-1})	34 (7–821)	29 (7–121)	28 (12–222)	54 (9–392)	45 (8–821)	0.284
Bilirubin (μmol L^{-1})	10 (3–63)	8 (4–38)	10 (5–63)	9 (3–30)	10 (4–28)	0.591
pH	7.37 (7.17–7.49)	7.4 (7.17–7.49)	7.38 (7.20–7.45)	7.4 (7.23–7.44)	7.34 (7.19–7.46)	0.585
International normalised ratio (INR)	1.2 (0.9–4.5)	1.1 (0.9–1.5)	1.2 (0.9–4.5)	1.2 (0.9–2.2)	1.4 (1.1–4.1)	0.170

Values are median (range). Kruskal-Wallis test (asymptomatic significance, $P < 0.05$)

and predicting variables were tested using Pearson correlations, and significant results were entered into a backward-selected stepwise linear regression analysis. These included length of time with AKI, serum urea, serum creatinine, serum albumin, BMI, ALP and γ-GT. The results are summarized in Table 4. Length of time with AKI was the strongest predictor of the midazolam concentration. BMI was a negative predictor of midazolam concentration.

Figure 3 illustrates the increase in midazolam concentration as the RIFLE criteria worsen from controls to failure with patients classified by genotype.

Discussion

Acute kidney injury may reduce the hepatic metabolism of midazolam in critically ill patients who do not have underlying liver disease. Midazolam concentrations increased with worsening RIFLE category, and the length of time with AKI was predictive of this effect. We have hypothesised that AKI decreases the hepatic metabolism of midazolam via inhibition (functional or expressive) of the CYP3A4 and 5 enzymes, and our data are supportive of this. However, there are limitations to our method and

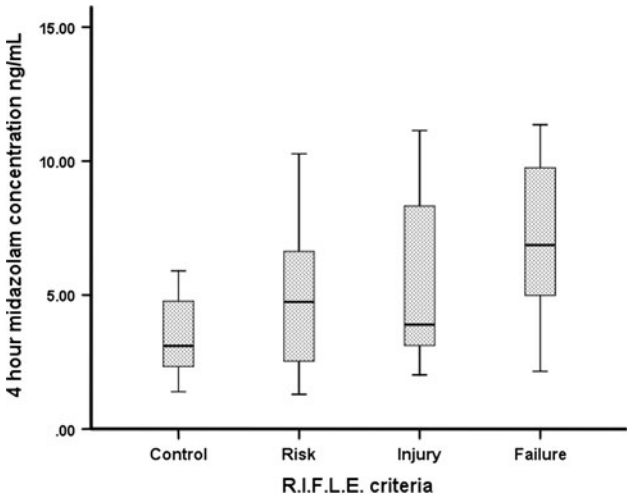

Fig. 2 Boxplot (median, interquartile range, minimum and maximum) of midazolam concentrations (ng mL^{-1}) according to the R.I.F.L.E criteria, excluding two outliers in the F group (Kruskal-Wallis, two-tailed significance, $P = 0.009$)

Table 4 Regression analysis of predictors of midazolam concentration

Independent variable	Dependent variable: $\log_{10}$ midazolam concentration			
	B	SE B	β	P value
Time with AKI	0.004	0.001	0.374	0.000
γ-GT	0.001	0.000	0.294	0.005
BMI	−0.012	0.004	–0.319	0.001

coincidental hepatic impairment cannot be excluded as a cause.

Two patients had extremely high midazolam concentrations (60.47 and 113.61 ng mL^{-1}). Both patients had severe renal dysfunction over a prolonged period, and an alternative explanation for the midazolam concentrations was not found. However, such results may skew the data and distort interpretation. Statistics were performed excluding these outliers, and the results and conclusions were not changed (data not presented).

Patients who had raised baseline serum creatinine concentrations were more likely to develop AKI. This is in keeping with previous observations that people with pre-existing CKD, however mild, are more at risk of developing AKI when critically ill [24].

Serum midazolam concentrations in vivo are determined by the dose administered, its volume of distribution (V_d) and its elimination. It has been shown previously that renal impairment significantly increases the half-life of both midazolam and its metabolites [25] although the authors were unable to explain the mechanism by which the midazolam half-life was affected. We measured total midazolam concentration, using the UK standard. This

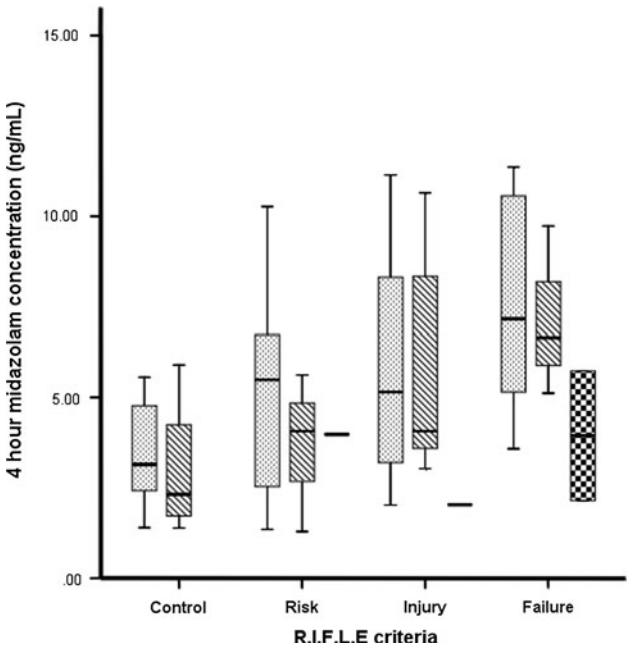

Fig. 3 Boxplot (median, interquartile range, minimum and maximum) of midazolam concentrations according to the RIFLE criteria and patient *CYP3A* genotype, excluding the two outliers in the F group. *CYP3A5* genotypes: *dotted squares CYP3A5*3/*3* (GG), *hatched squares CYP3A5*1/*3* (AG), *checked squares CYP3A5**1/*1 (AA). The *single horizontal bars* in the risk and injury categories represent single patients with *CYP3A5*1/*1* genotype

method is calibrated with deuterate midazolam thus allowing low concentrations of midazolam to be detected with accuracy, minimising the required dose for midazolam but limiting our ability to detect metabolites.

Midazolam is highly protein bound (94–96%) predominantly to albumin, with little redistribution within red blood cells [26] and has a V_d of 1.2–2.7 L kg^{-1}, independently determined by adipose tissue, pH and plasma albumin concentrations [27]. None of these parameters differed significantly between RIFLE groups. Although we did not measure V_d, critical illness, including AKI, would be expected to increase the V_d for midazolam (decreased serum albumin and decreased pH) [28] thus decreasing the expected serum total midazolam concentration at T4. This is in opposition to our hypothesis, but nevertheless, we observed an increase in T4 serum midazolam with worsening AKI despite potential changes in V_d. Indeed, it is possible this study underestimates the true magnitude of effect.

The BMI was significantly but negatively associated with serum midazolam concentrations in keeping with its effect on V_d. We considered varying the dose of midazolam according to an assessment of adiposity, but two considerations made this impractical. First, assessing the amount of adipose tissue is difficult and imprecise [29, 30], and secondly, the dose of midazolam, for ethical and safety reasons, must be small and unlikely to cause

clinical effect. This restricts dose variability according to the limits and accuracy of the method of detection.

In our study, serum urea concentration did not predict the midazolam concentration, but in patients with CKD, erythromycin (an alternative probe of CYP3A4 activity) elimination was decreased as urea increased [31]. The most likely reason for our study's failure to find a relationship with urea is the complex perturbations of urea observed in critically ill patients. Urea concentrations depend on co-existing pathology (e.g. gastrointestinal haemorrhage and liver disease), hydration status and nutritional status making it a crude marker of poor renal perfusion.

Multivariate analysis showed that the duration of AKI was an important predictor of midazolam concentration although this cannot be separated from the severity of AKI, and the relative importance of each remains uncertain. However, if time is an important consideration it may provide a clue to the underlying mechanism, perhaps implying a change in gene expression and production of the enzyme rather than direct inhibition of the enzyme activity. This would be in keeping with data from CKD studies [13].

Serum alkaline phosphatase concentrations were significantly increased as the RIFLE criteria worsened, and γ-GT concentrations were predictive of midazolam concentrations. It is possible that coincidental liver impairment is the cause of the observed altered midazolam metabolism. However, patients with known acute or chronic liver disease were excluded, and the other important data are not supportive of severe liver dysfunction (e.g. INR, lactate, ALT). These tests however do lack both specificity and sensitivity for assessing liver metabolic and synthetic function in critically ill patients [32]. Alternatively, AKI may impact more widely on hepatic enzyme systems, in addition to CYP450 enzymes, than we have previously supposed. This warrants further investigation.

One confounding factor we were unable to address fully was the potential effect of hepatic blood flow. An ideal drug probe of CYP3A has an extraction ratio <0.3 and thus is unaffected by hepatic blood flow. Midazolam has an extraction ratio of between 0.33 and 0.95 (mean 0.55) [26]. Measuring liver blood flow is challenging in critically ill patients, the available methods being either indirect and imprecise, or invasive and complex [33]. In healthy volunteers, the relationship between midazolam clearance and blood flow has been investigated using the 'MEG-X' method (the appearance of monoethylglycinexylidide, a metabolite of lignocaine). Under normal conditions the clearance of lignocaine and midazolam is correlated, but in the presence of a CYP3A4 inhibitor, this correlation is lost [34]. In 36 patients we measured cardiac output and found no difference between categories of AKI, but whether this can be related to liver blood flow is uncertain. In sepsis, however, hepatic blood flow has been shown to change little, even in patients with raised cardiac output [35]. Overall there is a lack of extensive studies of hepatic blood flow in critically ill patients.

Midazolam as a drug probe has two major advantages: first it is metabolized exclusively by CYP3A, and second, it has a good safety profile at a dose which provides detectable samples. Erythromycin is the most established alternative probe [36] but its use has two problems. First, it demands a steady state of background carbon dioxide (CO_2) production and elimination, and expired CO_2 is highly variable in critically ill patients, affected by both underlying pathology and medical interventions [37]. Second, erythromycin elimination is affected by P-glycoprotein (pgp) activity, and this has been shown to be decreased in chronic renal impairment [38] whereas midazolam is unaffected by pgp. Alfentanil is another potential alternative; however the doses required are not insignificant for critically ill patients [39].

Although underpowered to make firm conclusions, our data do provide preliminary evidence enabling us to hypothesize that patients who have a *CYP3A*1* allele, i.e. who express functional CYP3A5, metabolize midazolam faster than patients with the *CYP3A5*3/*3* genotype, partially protecting them from the impact of AKI on CYP function.

The importance of these changes in enzyme activity, either through the effects of AKI or as a consequence of genotype variations, remains unknown in critically ill patients. However, in renal transplant patients, the administration of fluconazole (a potent CYP3A inhibitor) decreases tacrolimus metabolism. This effect is diminished in patients who express the *CYP3A5*1* allele thus demanding less change to the dose of tacrolimus [40], and it is easy to envisage similarly important situations in the critically ill. The list of CYP3A substrates is large, and our evidence suggests that not only should we be considering patients' genotype but also their renal function when dosing drugs eliminated by the liver. Understanding more about the effects of a patient's illness on drug metabolism in combination with genotype may allow more efficient, individualized therapy.

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Conflict of interest None.

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