Enzymes of drug metabolism during delirium

SUSAN WHITE, B. L. CALVER, VICKY NEWSWAY, R. WADE, S. PATEL, A. BAYER, M. SINEAD O’MAHONY

Department of Geriatric Medicine, Cardiff University, Academic Centre, Llandough Hospital, Penarth CF64 2XX, UK

Address correspondence to: M. S. O’Mahony. Fax: (+44) 029 20 71 1267. Email: omahonyms@cf.ac.uk

Abstract

Background: delirium is common in ill medical patients. Several drugs and polypharmacy are recognised risk factors, yet little is known about drug metabolism in people with delirium.

Objective: the aim of this study was to investigate the activities of plasma esterases (drug metabolising enzymes) in delirium.

Design: this was a prospective study of delirium present at time of hospital admission (community acquired) or developing later (hospital acquired) in patients admitted as a medical emergency and aged 75 years or over.

Methods: following informed consent or assent cognitive screening was completed on all patients on admission and every 48 hours subsequently. Delirium was diagnosed by Confusion Assessment Method and DSM IV criteria. Blood samples were taken on admission and at onset of delirium if this was later. Four plasma esterase assays were performed spectrophotometrically: acetylcholinesterase, aspirin esterase, benzoylcholinesterase, butyrylcholinesterase.

Results: 283 patients (71% of eligible) were recruited, with mean age 82.4 years and 59% female. 27% had community acquired delirium, 10% developed hospital acquired delirium, 63% never developed delirium. On admission the mean activities of all four esterase assays were statistically significantly lower in delirious than non delirious patients. There were no significant differences on admission in any plasma esterase activity between patients with hospital and community acquired delirium. In-hospital mortality was associated with low plasma esterase activities on admission.

Conclusion: plasma esterase activities are suppressed during delirium. These data reinforce the need for extreme caution with drugs in this vulnerable population.

Keywords: delirium, frailty, drug metabolism, enzymes, elderly

Introduction

Delirium is common in hospitalised elderly patients with prevalence estimates varying from 14 to 56% [1]. The underlying biological mechanisms are not understood. Previous research has focused mainly on risk factors associated with delirium. Drugs including analgesics, hypnotics, sedatives and anticholinergics were found to be the precipitating cause in 11% of cases of delirium occurring in elderly patients admitted to a District General Hospital [2]. Drugs were found to be a contributory factor in 30% of cases of delirium and the definite aetiology in 14% of cases in an American study of delirium amongst acute medical admissions aged 70 years and over [3]. In a case controlled study amongst elderly hospitalised patients, Schor et al. found neuroleptic use and narcotic use were independently associated with the development of delirium [4]. In a prospective cohort study of the development of delirium amongst acute medical admissions aged 70 years and over, Inouye and Charpentier examined several medication-related variables as possible precipitating factors for delirium, including the use of major tranquillisers, narcotics, anti-emetics, psychoactive medications and polypharmacy [5]. Of these, the addition of more than three medications during acute hospitalisation was found to be the best predictor of the occurrence of delirium [5]. The pathophysiological mechanisms underlying such drug toxicity in delirium are not well understood. To date, no studies have investigated drug metabolism during delirium.

Indeed very little is known about drug metabolism during acute illness. In animal models of trauma, both oxidative and conjugative metabolism are reduced following acute vascular injury [6–8]. Human studies have found that the clearance of some drugs, meperidine and morphine, is reduced in patients following trauma [9, 10]. However, in vivo investigation has been difficult in humans and limited by the impractical nature of undertaking pharmacokinetic studies with frequent blood sampling in acutely ill patients.

Esterases are Phase I drug metabolising enzymes present in blood and their activities can be easily measured directly in vitro. These enzymes are synthesised in the liver.
and are found in blood, intestinal mucosa, central and peripheral nervous tissue as well as in the liver. Esterases are involved in the metabolism of a number of drugs including aspirin, heroin, cocaine, procaine, rivastigmine and anesthetic drugs [11–14]. The aspirin hydrolysing activity of serum is mainly due to butyrylcholinesterase, also known as pseudocholinesterase [15]. Esterases are also involved in the activation of several prodrugs to their active form, including carbimazole [16] and angiotensin converting enzyme inhibitors, such as enalapril, a prodrug which is hydrolysed by esterases to its active form enalaprilat, thus delaying its onset and duration of action [17]. Previous work investigating the activity of plasma esterases in man has shown that activity is reduced in frail institutionalised older people compared with independently living older subjects [18], but activity is well maintained in healthy ageing [19]. Reduced esterase activities have also been found in acutely ill older patients following hip fracture [20], and in older patients with pneumonia, the reduced enzyme activities correlating with poor prognostic score [21].

The aim of the current study was to investigate whether enzymes of drug metabolism are altered in delirium measuring acetylcholinesterase, aspirin esterase, benzoylcholinesterase and butyrylcholinesterase activities. C-reactive protein (CRP) and albumin were also measured as markers of the acute phase response.

Methods

Subjects aged 75 years and over were recruited from emergency medical admissions. All patients on alternate takes were approached over a 6 month period. Subjects were recruited within 24 hours of admission following informed consent or assent where appropriate. Cognitive assessments including Mini Mental State Examination (MMSE) [22]; clock drawing [23] and verbal fluency tests [24] were performed on all patients on admission and repeated every 48 hours for the first 10 days of admission. Assessments were done daily if the patient was delirious or thought to be developing delirium. Delirium was diagnosed using the Confusion Assessment Method [25] and DSM IV criteria. Delirious subjects were classified as having community acquired delirium if delirious on admission and having hospital acquired delirium if it developed during admission. Pre-admission cognitive status was also assessed. Previous diagnosis of dementia made by a geriatrician or psychogeriatrician was recorded as ‘definite’ dementia. Relatives and carers were interviewed using the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE). The informant was asked specifically about cognitive function prior to the current acute illness and a diagnosis of ‘probable’ dementia was based on the IQCODE score and collateral history [26]. This study was given ethical approval by Bro Taf Local Research Ethics Committee.

On admission venous blood was obtained from each subject. Further blood samples were obtained when possible at onset of any subsequent delirium (hospital acquired) and after convalescence one month following discharge from hospital. Samples were sent immediately for standard biochemical and haematological tests including albumin measurement. Blood was centrifuged at 400 g (1500 rpm) for 10 min at room temperature. Plasma was then separated and stored at –80°C, for subsequent determinations of enzyme activities and CRP levels. CRP concentration was measured by ELISA using an Olympus A600 and CRP reagent pack.

Esterase assays

Four different substrates were used to probe plasma esterase activity in vitro spectrophotometrically. The enzyme assays in this study have been named according to the substrate used. Plasma acetylcholinesterase and butyrylcholinesterase activities were assayed by measuring the production of thiocholine from the hydrolysis of the respective specific substrates acetylcholine iodide and S-butyrylthiocholine iodide. The reaction product, thiocholine, reacts with 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), producing the yellow anion 5-thio-2-nitrobenzoate. The acetylcholinesterase assay mixture comprised 10 µl plasma, 160 µl of stock solution of substrate, to give a final acetylcholine iodide concentration of 4 mM, 100 µl 10 mM DTNB, 2.86 ml 0.1 M phosphate buffer, pH 8.0. The butyrylcholinesterase assay mixture comprised 4 µl plasma, 400 µl stock solution of substrate, to give a final S-butyrylthiocholine iodide concentration of 10 mM, 100 µl of 10 mM DTNB and 2.626 ml 0.1 M phosphate buffer, pH 8.0. The production of 5-thio-2-nitrobenzoate was monitored spectrophotometrically at 412 nm over time [27]. Following an initial delay of 120 seconds, repeated absorbance readings were taken at 60 second intervals for 360 seconds. Over this time period 5-thio-2-nitrobenzoate production as measured by change in absorbance was linear. Both enzyme activities were expressed as µmol of DTNB transformed per ml of plasma per min. Ellman et al. found change in absorbance/minute divided by 13600 is equivalent to production of 1 mole of 5-thio-2-nitrobenzoate per litre per minute [27]. The following formula was therefore used to calculate acetylcholinesterase activity:

$$\text{activity (µmol/ml/min)} = \frac{\Delta A}{\text{min} \times 10^6} \times 313 \times 13600 \times 10^3$$

where 13600 is the extinction coefficient of the yellow anion and 313 is the correction factor for plasma dilution.

Butyrylcholinesterase activity (µmol/ml/min) was calculated as:

$$\Delta A/\text{min} \times 10^6 \times 782.5/13600 \times 10^3$$

where 782.5 is the correction factor for plasma dilution.

Plasma aspirin esterase activity was assayed by direct measurement of the concentration of the product salicylate from the hydrolysis of the substrate acetylsalicylic acid after 20 minute incubation [18]. The aspirin esterase activity assay was performed using a substrate concentration of 1.5 mM, plasma volume 200 µl, incubation time 20 minutes and 2.76 ml Tris buffer, pH 7.4. The concentration of salicylate was
measured by comparing the absorbance at 300 nm with a standard curve. Activity was expressed as nmol salicylate formed per ml plasma per min.

Plasma benzoylcholinesterase activity was determined spectrophotometrically by measuring the disappearance of the substrate benzoylcholine iodide from the incubation solution [28]. The benzoylcholinesterase assay mixture comprised 15 µl plasma, 20 µl substrate and 2.965 ml sodium/potassium 0.1 M phosphate buffer, pH 7.4. Using these assay conditions, Kalow and Lindsay found a change in absorbance of 0.165 was equivalent to a disappearance in substrate of 0.025 µmol [28]. The following formula was therefore used to calculate enzyme activity:

\[
\text{activity (nmol/ml/min)} = \frac{\Delta A}{\min} \times 0.025 \\
\times 200 \times 1000/0.165
\]

where 200 is the correction factor for plasma dilution and 1000 is the conversion from µmol to nmol.

The statistical package STATA was used for data analysis. Mean values are given, ±1 SE. The CRP concentration exhibited a skewed distribution which was normalised with a log transformation. The four plasma enzyme activities were normally distributed.

The chi-squared test was used to compare the proportions of delirious and non delirious patients that died whilst in hospital, and the proportions dying in upper and lower quartiles of esterase activity.

Independent sample t-tests were used to compare the mean plasma esterase activity, albumin and CRP concentrations between subjects with delirium and no delirium and to compare community acquired delirium with hospital acquired delirium. Paired t-tests were used to compare the mean plasma esterase activity at admission with the activity at the onset of delirium and after convalescence.

Results

435 patients aged 75 years and over were admitted via the Emergency Admissions Unit during the study period. Of these, 398 (91.5%) were eligible for inclusion in the study. 37 (8.5%) patients were excluded because they died (5), were moribund (3), transferred to another hospital (4) or discharged home (17) prior to assessment. Five patients were not acute admissions but elective transfers from another hospital and three patients spoke insufficient English to participate in the study.

283 patients (71% of eligible) agreed to take part. Their average age (mean ± SE) was 82.4 ± 0.3 years and 166 (59%) were female. On admission, 76 (27%) had delirium, 29 (10%) developed delirium during their hospital stay and 178 (63%) never developed delirium. There were no significant differences in gender between any of these groups. Patients with delirium were significantly older than those without (83.7 ± 0.6 versus 81.7 ± 0.4 years; P=0.006), but there was no significant difference in age between patients with hospital acquired and community delirium. 25% of delirium patients were known to have previously diagnosed dementia compared with 6% of non-delirium patients (P<0.001). 60% of delirium patients had probable dementia based on their ICODE scores [26], compared with 24% of non-delirium patients (P<0.001).

On admission, plasma esterase activities were lower in the delirious patients than in the non delirious patients (Table 1). Log CRP concentration was higher and albumin concentration was lower in delirium than in non delirium. There were no statistically significant differences at admission in any plasma esterase activity between hospital and community acquired delirium.

Upon onset of delirium another blood sample was taken from 7 of the 29 patients who developed delirium during the course of their hospital admission (22 patients declined venesection at this time). Though the data available are limited, the mean activities of acetylcholinesterase and butyrylcholinesterase were significantly lower at onset of delirium than the mean activities at admission (Table 2a). Delirious patients who survived and agreed to follow-up also had blood taken after convalescence one month post discharge. 32 were available for comparison with admission bloods. Plasma acetylcholinesterase and butyrylcholinesterase activities were significantly higher for these subjects at convalescence than at admission (Table 2b).

Of the 105 delirious subjects, 38 (37%) died in hospital compared with 11 (6%) of the 178 non delirious subjects.

Table 1. Mean (±SE) plasma esterase activities, CRP and albumin on admission to hospital in 283 acutely ill medical patients aged 75 years or over

<table>
<thead>
<tr>
<th>Activity (µmol of DTNB)</th>
<th>No Delirium (n=178)</th>
<th>Community acquired delirium (n=76)</th>
<th>Hospital acquired delirium (n=29)</th>
<th>Delirium (n=105)</th>
<th>P value (delirium versus no delirium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase (µmol of DTNB)</td>
<td>2.3 (0.1)</td>
<td>1.9 (0.1)</td>
<td>1.9 (0.1)</td>
<td>1.9 (0.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>transformed per ml of plasma per min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin esterase (nmol salicylate formed/ml plasma/min)</td>
<td>80.3 (2.2)</td>
<td>72.8 (3.3)</td>
<td>63.6 (4.9)</td>
<td>70.4 (2.8)</td>
<td>0.006</td>
</tr>
<tr>
<td>Benzoylcholinesterase (nmol of benzoylcholine iodide utilised/ml plasma/min)</td>
<td>841.0 (21.6)</td>
<td>689.1 (29.4)</td>
<td>691.5 (48.9)</td>
<td>689.8 (25.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Butyrylcholinesterase (µmol of DTNB)</td>
<td>5.1 (0.1)</td>
<td>4.2 (0.2)</td>
<td>4.2 (0.3)</td>
<td>4.2 (0.2)</td>
<td>0.0001</td>
</tr>
<tr>
<td>transformed per ml of plasma per min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log of CRP concentration (log of mg/l)</td>
<td>2.2 (0.2)</td>
<td>3.1 (0.2)</td>
<td>3.0 (0.4)</td>
<td>3.1 (0.2)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Albumin concentration (g/l)</td>
<td>34.8 (0.4)</td>
<td>31.5 (0.6)</td>
<td>32.1 (1.2)</td>
<td>31.7 (0.6)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
In addition, of the remaining patients, a higher percentage of delirious patients died within 1 month of discharge than non-delirious patients (11% versus 2%) \((P=0.007)\). There was a strong inverse relationship between plasma esterase activity on admission and in-hospital mortality. When the group is divided into quartiles according to plasma esterase activities on admission the lower quartiles (0–25% and 25–50%) have between a 4-fold and 10-fold higher in-hospital mortality rate than the higher quartiles (Figure 1). In-hospital mortality was 31.7% in the lowest quartile of plasma esterase activity on admission compared to 3.3% in the highest quartile (Figure 1).
quartiles of acetylcholinesterase and butyrylcholinesterase compared with 3.3% in the highest quartile (P<0.0001), 21.7% in quartile 1 of aspirin esterase compared with 6.7% in quartile 4 (P<0.0005) and 35.6% in quartile 1 of benzoylcholinesterase compared with 3.3% in quartile 4 (P<0.0001).

Discussion

In this study of 283 patients aged 75 years and over admitted as a medical emergency, 27% had delirium on admission and 10% developed delirium during their subsequent hospital stay. Compared with patients who never developed delirium, those with delirium (both community and hospital acquired) had significantly lower plasma esterase activities on admission to hospital. There was a further small but significant decline in acetylcholinesterase and butyrylcholinesterase activities at the time of onset of hospital acquired delirium. There was also a small but significant rise in these enzyme activities during convalescence. This indicates that the activities of these enzymes are not fixed, but change with the physiological environment.

To our knowledge this is the first study to investigate enzymes of drug metabolism during delirium. The fact that enzyme activities are already low on admission to hospital in both those with delirium and those who subsequently develop delirium indicates that low enzyme activities are a part of the vulnerability of this group. Low esterase activities do not occur due to age alone [19], but do occur in vulnerable older populations either at the time of acute illness [20, 21] or during chronic frailty [18]. The finding of decrements in enzyme activities in patients with delirium does not imply a direct cause effect relationship for delirium, but does emphasise the need for extreme caution with drugs and drug dosing in vulnerable older populations.

The cause of delirium, like other geriatric syndromes, is often multifactorial. Several studies of delirium amongst acute elderly admissions have identified multiple aetiological factors within individual patients [2, 3, 29]. Even though infection is often considered to be the commonest ‘cause’ of delirium, infection is equally common in medical emergencies with delirium and those without [2]. This clearly indicates that other factors must determine why some elderly patients with acute problems or infections get delirium and some do not. Inouye [5, 30] hypothesised that delirium occurs because of the interplay between predisposing factors (vulnerability) and precipitating factors, including the use of physician restraints, the addition of more than three medications, use of bladder catheters, malnutrition and iatrogenic events. Our study suggests that reduced activity of drug metabolising enzymes is one of the host factors that contribute to vulnerability to delirium. Reduced enzyme activities may be a biomarker of frailty in that they were also strongly associated with in-hospital mortality in our study.

The main limitation of our study is that we have measured the activities of only one family of Phase I drug metabolising enzymes, namely esterases. We have focused on esterases because they happen to be readily accessible and are measurable by means of a simple blood test. We cannot extrapolate from our study and generalise to all enzymes of drug metabolism. However, experiments in animals and human hepatocyte cultures indicate that activation of the acute inflammatory response downregulates the expression of major cytochrome P450 enzymes in liver [31, 32]. Interestingly, the addition of plasma from critically ill patients to isolated human hepatocyte cultures adversely affects the ability of hepatocytes to metabolise drugs [33]. Further studies are needed to investigate hepatic drug metabolism and P450 expression during delirium. The data reinforce the need for great care with prescription and dosing of drugs in this vulnerable population.

Key points

• Amongst emergency medical admissions aged 75 years and over, plasma esterase activities are significantly lower in delirious than non-delirious patients.
• Esterase activities are low on admission to hospital in patients who already have delirium as well as in patients who subsequently develop delirium.
• Low esterase activities on admission to hospital are associated with increased mortality.
• The findings of this study are consistent with the hypothesis that reduced ability to metabolise drugs is one of the host factors that contributes to vulnerability to delirium.

Acknowledgement

The project was funded by a BGS Start-Up Grant and the Glyn Penrhyn Jones Fellowship (administered by the Welsh BGS).

References


Received 23 April 2005; accepted in revised form 17 August 2005