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Changes in the concentrations of creatinine, cystatin C and NGAL in patients with acute paraquat self-poisoning

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ABSTRACT

An increase in creatinine >3 μmol/L/h has been suggested to predict death in patients with paraquat self-poisoning and the value of other plasma biomarkers of acute kidney injury has not been assessed. The aim of this study was to validate the predictive value of serial creatinine concentrations and to study the utility of cystatin C and neutrophil gelatinase-associated lipocalin (NGAL) as predictors of outcome in patients with acute paraquat poisoning. The rate of change of creatinine (dCr/d*t*) and cystatin C (dCyC/d*t*) concentrations were compared between survivors and deaths. Receiver-operating characteristic (ROC) curves were constructed to determine the best threshold for predicting death. Paraquat was detected in 20 patients and 7 of these died between 18 h and 20 days post-ingestion. The dCr/d*t* ROC curve had an area of 0.93 and the cut-off was >4.3 μmol/L/h (sensitivity 100%, specificity 85%, likelihood ratio 7). The dCyC/d*t* ROC curve had an area of 0.97 and the cutoff was >0.009 mg/L/h (sensitivity 100%, specificity 91%, likelihood ratio 11). NGAL did not separate survivors from deaths. Death due to acute paraquat poisoning is associated with changes in creatinine and cystatin concentrations. Further validation of these measurements is needed before they can be adopted in guiding intensive treatments.

1. Introduction

Paraquat (1,1′-dimethyl-4,4′-bipyridinium) dichloride is a non-selective contact herbicide widely used in many countries since the 1960s. It is an important cause of fatal self-poisoning in some countries, particularly in South-East Asia (Gunnell et al., 2007). The outcome of paraquat poisoning is variable but in large cohort studies typically between 40 and 60% of cases die, most within 24–72 h from multi-organ failure (Dawson et al., 2010; Gil et al., 2008; Senarathna et al., 2009). However, patients with smaller exposures may die over the following weeks from respiratory failure secondary to progressive pulmonary fibrosis. Better prognostic indicators to identify this group would be very useful as ongoing interventions are most likely to be beneficial for this group with delayed toxicity. Paraquat produces free radicals which induce cellular toxicity (Eddleston et al., 2003). Many treatments have been proposed and trialled, including extracorporeal elimination, immunosuppressants and antioxidants, but the mortality remains high even in centres using all these treatments (Gil et al., 2008) (and JL Lin, unpublished observation 2010).

A very strong predictor of death in large cohort studies is the volume of paraquat consumed (Wilks et al., 2008, 2011), but estimates of this are often unreliable in individual patients. The concentration of paraquat in blood or urine can be used as a surrogate for ingested dose to predict survival or death using a nomogram. These have a positive predictive value for death of 92–96% (Senarathna et al., 2009). Unfortunately paraquat assays are not widely available, particularly in the developing world, and the time of ingestion may be unknown, so alternative biomarkers are required which should ideally be able to be interpreted independent of the time of exposure.

A range of alternative clinical and biochemical investigations for prognosis following acute paraquat poisoning have been assessed, but inadequately validated (Eddleston et al., 2003). For example,

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acute kidney injury is a prominent manifestation of acute paraquat poisoning which has prompted research into renal biomarkers (Gil et al., 2009; Ragoucy-Sengler and Pileire, 1996). One small study ($n = 18$) suggested that an increase in creatinine of >3 μmol/L/h (dCr/dt) predicts death (Ragoucy-Sengler and Pileire, 1996). The rise in creatinine is probably due to progressive renal impairment and a direct reflection of organ toxicity (Pond et al., 1993). However, paraquat interferes with some creatinine assays that utilise the Jaffe (picric-acid) method (Aitken et al., 1994; Fairshter et al., 1986; Price et al., 1995; Webb and Davies, 1981). Therefore, the increase in creatinine may reflect both exposure and toxicity. The apparent creatinine concentration increases with increasing paraquat concentrations (Aitken et al., 1994; Fairshter et al., 1986; Price et al., 1995; Webb and Davies, 1981), although minimally with concentrations less than 10 mg/L, in contrast to concentrations greater than 100 mg/L where interference is marked (Fairshter et al., 1986; Webb and Davies, 1981). In clinical practice the majority of paraquat concentrations are less than 100 mg/L (Senarathna et al., 2009).

The predictive value of other plasma biomarkers of acute kidney injury, such as cystatin C or neutrophil gelatinase-associated lipocalin (NGAL), have not been assessed in acute paraquat poisoning. A single study noted that urinary NGAL correlated with changes in creatinine concentration in patients with acute kidney injury (Gil et al., 2009).

The objective of this study was to further explore the utility of serial creatinine concentrations for predicting death and to examine the utility of plasma cystatin C and NGAL as alternative predictive biomarkers.

2. Materials and methods

This study was approved by Human Research Ethics Committees in Australia, Sri Lanka and UK. We prospectively identified all patients with acute paraquat exposure presenting to Anuradhapura and Polonnaruwa Hospitals in Sri Lanka. These are regional referral hospitals that provide 24-h medical and nursing care to patients in dedicated medical wards. Patients were directly admitted to a medical ward or via transfer from a remote hospital where they were medically assessed.

Every patient presenting to these study hospitals with a history of an acute paraquat exposure was reviewed by on-site study doctors. Following an initial clinical assessment and resuscitation, the history of exposure (including co-ingestants) was obtained on presentation for each patient. All patients received supportive care, including intravenous fluids and ventilatory and haemodynamic support as required; oxygen supplementation is withheld in patients with paraquat poisoning unless treatment is palliative and the patient is hypoxic. Patients were followed by dedicated study doctors until discharge or death. Follow up visits to the patient's home were attempted approximately 6 months after discharge to confirm survival.

Written informed consent was provided by 26 patients between 23rd April 2005 and 3rd September 2006 for the collection of additional blood samples. Blood samples were obtained at least 4 h post-ingestion (well after the peak plasma concentration), immediately centrifuged and plasma was taken off and stored at −23 °C until analysis. Samples were shipped to the UK to quantify the concentration of paraquat, creatinine and cystatin C. Available duplicate samples were shipped to Australia to quantify the concentration of NGAL.

Paraquat and creatinine analyses were conducted by Syngenta CTL (Alderley Park, Macclesfield, Cheshire, UK) in October 2006. The paraquat concentration was measured using HPLC, LC–MS–MS, and LC fluorescence (Blake et al., 2002). The creatinine concentration was measured utilising the modified Jaffe (picric-acid) method according to product guidelines (Labmedics, UK).

The cystatin C concentration was measured by Chemical Pathology, St. Thomas' Hospital, London, UK in April 2007. This utilised a particle-enhanced nephelometric assay on a Dade Behring BNII nephlometer (Milton Keynes, UK) with antisera and calibrators supplied by Dade Behring (Bandaranayake et al., 2007).

NGAL concentration was measured by Therapeutics Research Centre, University of Queensland, Brisbane, Australia in October 2009. These assays were conducted using the Triage® NGAL Test, a point-of-care fluorescence immunoassay using the Triage Meter according to product guidelines.

3. Calculation

Median values and inter-quartile ranges were determined for each renal biomarker and compared non-parametrically. The rate of change of creatinine and cystatin C concentrations in serial samples were determined and compared between survivors and deaths. Receiver-operating characteristic (ROC) curves were constructed to determine the best threshold (as determined by Youden's index (Youden, 1950)) for the rate of change of creatinine (dCr/dt) and cystatin C (dCyC/dt) concentrations for predicting death, including likelihood ratios, sensitivities and specificities. Sensitivity is the proportion of all deaths that were predicted to die by the test (cut-off), specificity is the proportion of survivors predicted to survive by the test. All analyses were conducted using GraphPad Prism version 4.03 for Windows, GraphPad Software, San Diego, USA, www.graphpad.com and $P < 0.05$ was considered statistically significant. Prediction of outcome on the basis of the admission paraquat concentration was determined according to Senarathna et al. (2009).

4. Results

4.1. Clinical outcomes

Paraquat exposure was confirmed in 20 patients who were eligible for inclusion; the other 6 patients were excluded. 14 of the 16 patients who were discharged alive were followed up in the community and three of these patients subsequently died. Altogether, seven patients died at 18 h, 48 h, 65 h, 11 days, 12 days, 15 days and 20 days after exposure. On the basis of the admission paraquat concentration, all actual deaths were predicted to die according to the Proudfoot nomogram (Eddleston et al., 2003). A total of 86 blood samples from different time points were assayed, although in some cases the volume was too small for every test to be conducted.

4.2. Changes in biomarkers of acute kidney injury post-admission

Serial concentrations of creatinine and cystatin C for individuals are shown in Fig. 1a and b, respectively. In the case of creatinine and cystatin C, increasing concentrations during the first 24–48 h were observed which were suitable for further analyses. Because biochemical data from patients who died were unavailable beyond 75 h post-ingestion, all subsequent analyses in surviving patients were limited to data obtained within the same period.

The plasma concentration of NGAL was measured in 14 patients and serial changes are shown in Fig. 1c. No relationship was observed that could be used to separate survivors from the four deaths captured in this study (which occurred 48 h, 65 h, 11 days and 12 days post-ingestion). Of these deaths, NGAL was not elevated in one patient while in the other three patients the highest concentration was 331 ng/mL and most were less than 100 ng/mL. The NGAL plasma concentration in one survivor was as high as 608 ng/mL.

4.3. Temporal trends in biomarkers of acute kidney injury

Where multiple samples were available in a single patient, in many cases the rates of increase in creatinine and cystatin C concentrations were approximately linear for the deaths (Fig. 1a and b) and the overall rate of change (estimated by linear regression of all samples) was used to construct ROC curves. The dCr/dt and dCyC/dt in survivors were also estimated using linear regression for direct comparison to data from survivors.

Of the 13 survivors, only four were found to have a positive gradient for dCr/dt that was statistically different to zero (data not shown). The gradients were much higher for deaths [medians 9.0 μmol/L/h (IQR 5.3–14.8) for deaths and 0.3 μmol/L/h (IQR −0.3 to 3.3) for survivors; $P = 0.002$, Mann–Whitney test]. The ROC curve had an area of 0.93 (95% CI 0.83–1.04). The best dCr/dt cut-off was