

Circulating Paracetamol Metabolites Accurately Predict Hepatotoxicity And Represent New Clinical Toxicokinetic Biomarkers

Paracetamol (acetaminophen) is the commonest cause of drug-induced liver injury in the Western world. Paracetamol is predominantly metabolised by conjugation in the liver to form non-toxic sulphate and glucuronide conjugates. A fraction of paracetamol is oxidized by P450 enzymes to form the highly reactive potentially toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI). After NAPQI is formed it reacts with glutathione (GSH) to form mercapturic acid and cysteine conjugates. However, if NAPQI production exceeds the capacity of GSH for detoxification then it can bind covalently to cellular proteins and result in oxidative stress and hepatocyte death. The primary objective of this study was to explore the toxicokinetics of circulating paracetamol metabolites in patient samples with and without acute liver injury (ALI) from the recently published SNAP trial.[1] The secondary objective was to explore why significantly more SNAP trial patients treated with the antiemetic drug ondansetron developed ALI after paracetamol overdose.

Paracetamol, paracetamol-glucuronide, paracetamol-sulphate (Para-Sul), paracetamol-GSH (P450 mediated), paracetamol-mercapturate (P450 mediated) and paracetamol-cysteine (Para-CYS) (P450 mediated) were measured in plasma samples from 116 patients before ('pre-treatment'), after 12 hours, and at the end of acetylcysteine (NAC) treatment for a single acute paracetamol overdose. Metabolite analysis was performed by liquid chromatography tandem mass spectrometry.

Patients who developed ALI had longer paracetamol plasma half-lives and higher relative amounts of metabolites formed by P450 activity. There was no difference between ondansetron and placebo treated patients. At pre-treatment the fraction of metabolites formed by the P450 pathway was significantly higher in patients that subsequently developed ALI (ALI: 2.2% (1.0-3.6). No ALI: 1.0% (0.6-1.6) $P=0.0004$). Receiver operator curve (ROC) analysis demonstrated that the most accurate predictor of ALI was the ratio of Para-Cys/Para-Sul, with a ROC curve AUC of 0.91 (0.83-0.98). By contrast, the current standard markers, alanine transaminase (ALT) and paracetamol parent drug had ROC-AUC of only 0.67 (0.50-0.84) and 0.50 (0.33-0.67), respectively. Post hoc analysis of the SNAP trial by logistic regression modelling demonstrated that when pre-treatment Para-Cys/Para-Sul was added to the stratified randomisation process the incidence of ALI in the ondansetron treated patients was not different from placebo. There was no effect on the SNAP trial's primary outcome.

In conclusion, circulating paracetamol metabolites are toxicokinetic biomarkers that accurately stratify patients at first presentation to hospital. They are superior predictors of toxicity compared with measurement of paracetamol parent drug concentration and may refine clinical trial design by identifying patients at risk of hepatotoxicity, at trial entry, before current biomarkers are elevated. In the SNAP trial, by chance, more patients with a toxic metabolite profile at randomisation received ondansetron compared with placebo.

[1] Bateman DN et al. Reduction of adverse effects from intravenous acetylcysteine treatment for paracetamol poisoning: a randomised controlled trial. *Lancet* 2014; 383: 697-704.