Articles You Might Have Missed

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Received: 30 May 2017 / Revised: 2 June 2017 / Accepted: 5 June 2017 / Published online: 26 June 2017
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Keywords Acetylcholinesterase · Amanita phalloides · Fractionated plasma separation and absorption (FPSA) · Litchi · Oxcarbazepine

Shrivastava A, Kumar A, Thomas JD et al.
Association of Acute Toxic Encephalopathy with Litchi Consumption in an Outbreak in Muzaffarpur, India, 2014: A Case-Control Study. *Lancet Glob Health* 2017;5:e458–455.

Background In the region of Muzaffarpur in India, children have been suffering from unexplained neurologic illness since 1995 with recurring outbreaks coinciding with the litchi (lychee fruit) harvesting season.

Research Question What is the cause of this recurring, regional, seasonal, unexplained pediatric neurologic disease and what interventions can best prevent and treat this condition?

Methods This study had three main components. First, cases of this condition were identified using predetermined clinical criteria (<16 years of age admitted for new onset seizures or altered sensorium). Demographic and clinical data were collected and analyzed. Second, there was a nested case-control study of cases ($n = 104$) matched with similar patients admitted for non-neurologic conditions. Cases and controls participated in a complex epidemiological survey. Finally, there was

laboratory analysis of subjects' clinical specimens (blood and CSF) and locally harvested litchis were tested for the presence of toxins, heavy metals, pesticides, and infectious agents.

Results A total of 390 cases were identified over a period of 2 months in the summer of 2014. There were 122 (31%) deaths. Most children were previously well and had sudden onset of symptoms in the early morning. Most ($n = 204$; 62%) presented with hypoglycemia (blood glucose <70 mg/dL); median admission glucose was 47 mg/dL. Litchi consumption, odds ratio (OR) = 9.6 (95% CI 3.6–24), and absence of an evening meal prior to admission, OR = 2.2 (1.2–4.3), were associated with illness. The absence of an evening meal prior to admission increased the apparent effect of eating litchis on illness: OR 7.8 (3.3–18.8) without an evening meal, OR = 3.6 (1.1–11.1) with an evening meal. No pattern of significant metal or pesticide exposure was identified in clinical specimens or fruit samples. Metabolites of methylenecyclopropylglycine (MCPG) and hypoglycin A were found in 46 (66%) of 73 cases but none from controls ($n = 15$). A total of 72 (90%) of 80 cases had abnormal plasma acylcarnitine profiles, suggesting disruption of fatty acid metabolism. The concentration of hypoglycin A in tested litchi fruit ($n = 36$) ranged from 12.4 to 152 mcg/g and the concentration of MCPG ranged from 44.9 to 220 mcg/g. There were no differences in socioeconomic status between subjects and matched controls.

Conclusion Severe seasonal neurologic illness in children from Muzaffarpur, India, was apparently caused by hypoglycin A and MCPG found in litchi fruit. Not eating an evening meal appeared to worsen the effects of impaired fatty acid metabolism. To prevent illness in that region, litchi consumption should be minimized, and children presenting with suggestive symptoms should be administered glucose.

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Critique This was a very well-executed epidemiologic study that makes a compelling case for the toxins hypoglycin A and MCPG in litchi fruit as the cause of a seasonal neurologic disorder. Limitations included relying on reported litchi fruit ingestion (not confirmed or quantified) and variations in specimen collection and interviewing control subjects. Many, but not all possible variables (e.g., genetic predilection) were studied.

Implication for Toxicologists Toxicologists should be aware that hypoglycin A and MCPG can be found in litchi fruit and may cause hypoglycemia and severe neurologic illness in the correct clinical context. Additionally, the article demonstrates the multipronged approach needed to establish toxic etiology in unexplained epidemic illness.

Spiller HA, Strauch J, Essing-Spiller SJ, Burns G: Thirteen Years of Oxcarbazepine Exposures Reported to US Poison Centers: 2000 to 2012. Hum Exp Toxicol 2016;35(10):1055–1059.

Background Approved by the US Food and Drug Administration in 2000, oxcarbazepine (OXC) was developed to have better tolerability and less toxicity than carbamazepine (CBZ). As a prodrug, OXC is metabolized to the active compound 10-hydroxy-carbazepine by cystolic ketoreductase. Unlike CBZ, OXC does not have a toxic epoxide metabolite, does not induce CYP3A4, or interact with drugs metabolized by CYP3A4. The few reports available suggest that OXC is indeed less toxic than CBZ.

Research Question What adverse effects are associated with supratherapeutic doses and/or overdose of OXC?

Methods A retrospective analysis of single-substance OXC ingestions reported to the National Poison Data System (NPDS) between January 2000 and December 2012.

Results A total of 18,867 cases were reported to NPDS; 55% were female. The majority of patients were adults with 29% of exposures in children <6 years. The most frequently reported clinical effects were drowsiness (25.3%), vomiting (8.4%), dizziness/vertigo (6.5%), tachycardia (3.2%), and agitation (1.8%). Other effects, including hypotension (0.9%), coma (0.8%), and seizure (0.6%) were less common. A major effect was reported in 176 patients (0.9%); 31 of whom were children. Moderate effects were reported in 1728 patients (9%); 300 were children. Five adult deaths were reported. All fatalities and 68% of patients with major effects ($n = 176$) were intentional exposures (e.g., suicide; $n = 5142$). Healthcare utilization remained consistent

over the 13-year period, with 53% of adults and 38% of children being managed in a healthcare facility. The most common therapies were oxygen (1.9%), intubation (1%), and benzodiazepines (0.9%).

Conclusion Thirteen years of data from NPDS suggests that serious outcomes are rare following OXC exposures. Accidental pediatric exposures are likely to result in only mild symptoms and it may be appropriate for most of these cases to be observed at home via a poison control center (PCC). Among adult cases, including intentional overdoses, there were few neurologic and cardiovascular complications. Therapeutic interventions for major clinical effects were primarily supportive care with infrequent use of oxygen, intubation, sedation, or anticonvulsant medications.

Critique This was a retrospective study of OXC exposures voluntarily reported to PCCs and may have suffered from reporting bias. Ingestions of OXC were not verified through serum measurement of OXC or its metabolite. It is possible that reported exposures were not actual exposures, and that other true exposures (some with symptoms) were managed without PCC involvement. No data concerning (reported) ingested amounts were provided. Lastly, other unknown ingestions were not ruled out.

Implication for Toxicologists This study supports the improved safety profile of OXC compared to CBZ. Many patients with non-intentional OXC exposures may be managed appropriately at home.

Stankiewicz R, Lewandowski Z, Kotulski M, et al. Effectiveness of Fractionated Plasma Separation and Absorption as a Treatment for Amanita Phalloides Poisoning. Ann Transplant 2016;21:428–432.

Background Fractionated plasma separation and absorption with high-flux dialysis (FPSA) is an extracorporeal system for removing toxins in the setting of acute liver failure (ALF). For severe *Amanita phalloides* mushroom poisoning (overall mortality ~22%), liver transplantation is the only definitive treatment.

Research Question Can FPSA improve post transplantation survival in patients with ALF from *A. phalloides* poisoning?

Methods This was a retrospective chart review of 10 patients who received liver transplantation after poisoning with *A. phalloides*. The primary outcome was survival at 30 days post-transplantation. In order to receive

transplantation, all patients had to meet predetermined clinical criteria based on the King's College criteria. Six of 10 patients met additional criteria identifying more severe disease, which qualified them for FPSA prior to transplantation.

Results The FPSA group had higher mean Model for End-Stage Liver Disease (MELD) score (40 vs. 36.8), total bilirubin (9.83 vs. 4.56), INR (7.67 vs. 3.04), and ammonia (322.3 vs. 196.3), as well as more grade 3 or 4 encephalopathy (6/6 vs. 1/4) and hypoglycemia (6/6 vs. 2/4). One subject, in the FPSA group, had cerebral edema prior to transplant. The FPSA group also had a longer mean waiting time for transplantation: 3.5 days (range 2–8) vs. 1.25 days (range 0–2). In the six patients who received FPSA only, one (16%) survived to 30 days post-transplant, compared to 4/4 survival in the non-FPSA group.

Conclusion Despite lower survival rate after transplantation, FPSA prolonged presurgical waiting time, providing an opportunity for life-saving liver transplantation.

Critique The study was limited by the small number ($n = 10$) of subjects and the inability to prove *A. phalloides* as the cause of ALF. It was difficult to determine if the FPSA group benefited from treatment with FPSA since they were more severely ill at baseline and had a longer wait for transplant. The study design only included patients who received a liver transplant, so it is not clear if some patients would have died prior to transplantation without FPSA.

Implication for Toxicologists In the future, FPSA and other extracorporeal, non-biologic liver support (such as molecular absorbents recirculating system) may prove to be important tools in the treatment of toxin-induced ALF, including *A. phalloides*.

Yan L, Chen J, Xu B, et al.: A Liquid Chromatography Tandem Mass Spectrometric Method on in vitro Nerve Agents Poisoning Characterization and Reactivator Efficacy Evaluation by Determination of Specific Peptide Adducts in Acetylcholinesterase. J Chromatogr A 2016;1450:86–93.

Background Nerve agents (NAs) exert their toxic effect through the rapid inhibition of acetylcholinesterase (AChE) with subsequent excessive cholinergic nerve transmission. Although all chemical warfare agents are prohibited, NAs still pose a threat through potential use in terrorist attacks and ongoing war zones. Analysis of AChE activity is commonly performed by the colorimetric Ellman assay, but this method lacks

specificity and cannot determine the status (free, unaged, or aged) of AChE.

Research Question Can liquid chromatography tandem mass spectrometry (LC-MS/MS) be used to simultaneously measure the in vitro poisoning, aging, and reactivation status of AChE adducts after poisoning by sarin or VX?

Methods A protocol was established to identify specific peptides by LC-MS/MS and the method was validated for linear range, intra-day precision and accuracy, inter-day precision and accuracy, matrix effect and stability. AChE (from *Electrophorus electricus*; electric eel) was poisoned with sarin or VX at various concentrations for different lengths of time. The resulting free peptide, unaged peptide, and aged peptide adducts were measured by LC-MS/MS. For comparison, AChE activity was also measured using a modified Ellman assay. To evaluate the efficacy of AChE reactivators, typical reversal agents (HI-6, obidoxime and pralidoxime) were added after AChE was poisoned for 30 min and then incubated for various lengths of time before termination of the reaction. The authors determined that adding 5% formic acid was the best method to terminate poisoning without contaminating peptide adducts. Once an optimal reactivation time was determined for each reversal agent, a series of concentrations were used to calculate the half maximal effective concentration (EC_{50}) for each reactivator.

Results Measurement of AChE inhibition by LC-MS/MS was consistent with the Ellman assay, indicating that the new method was reliable. Interestingly, the LC-MS/MS assay determined that sarin-induced AChE inhibition was slightly lower than rates measured via the Ellman assay. Exposure to sarin or VX produced peptide adduct profiles consistent with reported kinetics. Nearly complete reactivation of inhibited AChE was achieved with 1 mM HI-6, obidoxime, and pralidoxime, with incubation times of 40, 60, and 80 min, respectively. When tested at different concentrations, HI-6 was the most efficacious in reactivating sarin-inhibited AChE, followed by obidoxime and pralidoxime. These results are supported by most (but not all) prior work with these agents. In contrast, all three reversal agents had a similar EC_{50} for VX inhibition.

Conclusion LC-MS/MS can be used to determine both AChE inhibition and the aging status of AChE poisoned by NAs, unlike the Ellman assay which can only determine inhibition. This method may be used to investigate new reactivators in development as antidotes and to better understand the toxicology of other NAs and organophosphate pesticides. The use of formic acid to immediately terminate ongoing NA-induced AChE poisoning was shown to be effective and should assist with future research for “precision capturing” of time-dependent NA effects.

Critique This study was performed entirely in vitro, so the LC-MS/MS method cannot be used to assess AChE inhibition in patients, unlike the Ellman assay which can be used to evaluate blood and tissue samples. The AChE used was from *E. electricus*, which may not adequately represent these effects on human AChE peptide adducts. Lastly, suboptimal grammar made several sections within the article difficult to understand.

Implication for Toxicologists LC-MS/MS is a novel tool available for researchers working to better understand the inhibition of AChE by organophosphates and (possibly) to develop

antidotes. However, this assay has no current clinical applications for bedside toxicologists.

Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflict of interest.

Sources of Funding None