Articles You May Have Missed

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Keywords Acute liver failure · Drug metabolism methods · Marijuana · Synthetic cannabinoid · Seizure · Lead · Firing range

Article Title Koch DG, Speiser JL, Durkalski V, et al.: The natural history of severe acute liver injury. *American Journal of Gastroenterology* 2017; 112 (9): 1389–1396

Background Acute liver failure (ALF) has been defined by the presence of hepatic encephalopathy. The natural course and clinical predictors of acute liver injury (ALI) without hepatic encephalopathy (HE) has not been well studied or determined.

Research Question What is the natural history of acetaminophen (APAP) and non-APAP ALI, and which patients are most at risk for a poor outcome?

Methods Prospective study of consecutively enrolled registry patients by the Acute Liver Failure Study Group, from 2008 to 2013, at 23 academic hospitals in the USA has been undertaken. For this study, an ALI subject was defined as having no HE, INR ≥ 2.0 , and ALT $\geq \times 10$ upper limit of normal; non-APAP cases required bilirubin ≥ 3.0 mg/dL. Peak INR > 2 needed to occur within 48 h of enrollment, and those with preexisting liver disease were excluded. Determination of APAP-induced ALI was based on ingestion history and/or elevated serum APAP, with ALT or AST ≥ 1000 IU/L and bilirubin < 10 mg/dL.

Analyses included univariate analyses, the Wilcoxon rank sum, and χ^2 tests. Random forest (RF), a newer modeling technique composed of decision trees used for its improved ability to manage missing data, was used to

estimate the probability that individual ALI patients would have a poor outcome. The authors defined poor outcome as progression to ALF, liver transplantation (LT), or death within 21 days of enrollment.

Results All 386 ALI subjects meeting criteria were enrolled. Most were women (61%) and Caucasian (72%), with a median age of 38 years (interquartile range 28–49). APAP accounted for 50% of cases, followed by 12% due to autoimmune hepatitis, 6% from non-APAP drug-induced liver injury, and 6% from hepatitis A. A total of 26% were due to “other” or indeterminate causes. Median AST was 2270 U/L (929–5219), ALT 2784 U/L (942–5713), bilirubin 5.1 mg/dL (2.6–15.6), and INR 2.4 (2.1–3.4). Patients developed symptoms (nausea/emesis, abdominal pain, joint pain, and edema) a mean of 5 days prior to enrollment and jaundice a mean of 4 days prior. Only 2% required renal replacement therapy for kidney injury.

APAP subjects were younger (35 vs. 44 years), with almost fourfold higher aminotransferase concentrations. APAP cases had lower bilirubin (3.2 vs. 15.7 mg/dL) and phosphate (2.2 vs. 3.3 mmol/L) and shorter durations of symptoms (3 vs. 11 days) and jaundice (1 vs. 8 days) prior to enrollment. A poor outcome occurred in 40% of non-APAP cases (mainly ALF) compared to 7.2% of APAP cases. Overall, 68% of patients received N-acetylcysteine (NAC) (90% of APAP vs. 30% of non-APAP cases). For non-APAP cases, NAC was not associated with outcome.

Only 23% developed ≥ 1 of the 3 primary outcomes; 72 (19%) progressed to ALF, 44 (11%) underwent LT, and 19 (5%) died. Of those who died (including four from APAP toxicity), 47% lacked preceding ALF. The etiologies of ALI requiring LT included autoimmune hepatitis (15/44; 34%), indeterminant (13/44; 30%) and APAP (3/44; 7%). Non-Caucasians had increased risk of poor outcome compared with Whites (31 vs. 20%), as did older subjects.

Using RF modeling, the variable most predictive of a poor outcome was etiology, followed by duration of jaundice from

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onset to enrollment, and serum concentrations of APAP, bilirubin and INR. The model's prediction accuracy was 81%, with area under the ROC of 0.84. Correct predictions were made for 83% of ALI patients who recovered, and 76% of patients with a poor outcome.

In a separate validation cohort of 163 ALI patients (enrolled 2013–2015), model accuracy was 75%, with correct predictions for 67% of ALI patients who resolved, and 88% of patients with a poor outcome.

Conclusion This analysis of a defined cohort of ALI subjects described the history and progression of the disease. A derived model showed variables most predictive for poor outcome: ALI etiology (non-APAP induced), jaundice duration, and blood concentrations of APAP, bilirubin, and INR.

Critique Assessment of HE and ALI etiology was entirely at the discretion of each site's primary investigator. These assessments were not audited by a second investigator, and inter-rater reliability was not examined. The authors correctly pointed out that their results were limited to this study cohort, and readers should not generalize the lack of NAC's efficacy for non-APAP ALI, given uniquely inclusion criteria for this study and previously shown benefit.

Implication for Toxicologists This study used a novel definition of ALI lacking HE, with RF modeling, to follow and predict the course and outcomes of these patients. More work (including prospective analysis) is required.

Article Title Bhushan A, Martucci NJ, Usta OB, Yarmush ML: New technologies in drug metabolism and screening: organ-to-organ interaction. *Expert Opinion on Drug Metabolism & Toxicology* 2016; 12 (5): 475–7

Background The development of new pharmaceutical therapies requires extensive evaluation of both potential toxicities and effectiveness in treating disease. Current methods of evaluation such as in vitro cell-based assays or animal models do not always predict a drug's effects or toxicities in vivo. However, new technologies continue to emerge which may increase confidence in predicting drug metabolism and toxicity in humans.

Research Question What new technologies exist (or are in development) that might help to better predict the toxicities and therapeutic effects of drugs in humans?

Methods The authors outlined three new or emerging technologies, discussing each of their potential uses and limitations, and then concluded with opinions.

Results Three areas of emerging technologies were discussed. The first was the *humanized mouse model*, in which a mouse gene is replaced by a portion of a human gene. These mice then produce progeny carrying the human gene and used for further study. Because the offspring carry only one (or a few) humanized proteins, specific enzymes and pathways can be studied. However, this model limits assessment of complex interactions within a particular organ or within an organ-organ model. The recent development of three chimeric liver humanized mouse models completely repopulating the mouse liver with human hepatocytes. These chimeric models involve complete humanized hepatic genes, proteins, and pathways and have already predicted human toxicity for two compounds (fialuridine and bosentan) that were not recognized previously by other preclinical studies.

A second area of interest involved *in vitro microphysiological cell constructs*. After a drug is metabolized by the liver, its metabolites may be toxic to other organs. Historically such cross-organ toxicity involved animal models. However, multi-organ in vitro microphysiological cell constructs offer an alternative, and by utilizing human cells, may in fact recognize more potential human toxicities. Use of multiple “organ-on-a-chip” platforms, connected by physiologically relevant fluid streams, allows for the assessment of interactions between multiple organs including liver, small intestine, kidney, skin, and neurologic tissue. These constructs can be limited by their high media to cell ratio which dilutes metabolites, reducing therapeutic or toxic effects. For this reason, newer low media to cell ratio devices have been developed. Several compounds already tested on in vitro microphysiological cell constructs include troglitazone and 2,5-hexadione.

Finally, several efforts have been made to derive human hepatocytes from induced pluripotent stem cells (*iPSC*), as human hepatocytes are considered the gold standard for liver metabolism and toxicological assessments. If taken from individual patients, *iPSCs* may allow for patient-specific testing and facilitate advances in personalized (precision) medicine. Additionally, several groups are using pluripotent stem cells to represent other organs, which may then allow for the investigation of organ-organ interactions.

Conclusion The authors concluded that while there are challenges with each of these new technologies, they show great promise towards predicting human metabolism and toxicity which could lead to more rapid introduction of new therapies.

Critique This article was an informative review of some recent advances which may aid in more rapidly bringing new therapies to market. While it addressed the potential benefits and pitfalls of each of these new advances, it did not discuss any particular study in depth or provide more specific evidence.

Implication for Toxicologists The understanding of drug metabolism and toxicity, including organ-organ interactions, is germane to our daily practice. Toxicologists should be aware of new areas of study that assist with identifying drug toxicities or interactions and advance precision medicine.

Article Title Malyshevskaya O, Aritake K, Kaushik MK, et al.: Natural (Δ^9 -THC) and synthetic (JWH-018) cannabinoids induce seizures by acting through the cannabinoid CB₁ receptor. *Scientific Reports* 2017; 7 (1): 10516

Background Access to marijuana is increasing as more US states and countries legalize medicinal and recreational use. Even as marijuana becomes more accessible, use of synthetic cannabinoids (SCs) has increased. Both SCs and Δ^9 -THC, the psychoactive ingredient in marijuana, mediate their effects via agonism at cannabinoid receptors (CB₁R and CB₂R). Previous studies of cannabinoid effects on seizure activity have been contradictory, showing both proconvulsive and antiepileptic effects, typically involving CB₁R. Controlled human studies on cannabinoid (and related compounds') pharmacokinetics and toxicokinetics are difficult to conduct, resulting in data being derived from case studies.

Research Question What are the epileptogenic effects of Δ^9 -THC and SCs on the CB₁R in a mouse model?

Methods Experiments were conducted using adult male C57BL/6J mice. Electroencephalography (EEG) electrodes were implanted in the epidural space of the right frontal and parietal cortices. EMG electrodes were implanted (under pentobarbital sedation) into bilateral neck muscles, 3 days prior to experimental studies. Animal movements and behavior were monitored with infrared sensors and camera.

Each animal served as a self-control by receiving a vehicle, via intraperitoneal (IP) injection, on the day prior to IP injection of a cannabinoid (Δ^9 -THC or SC, JWH-018). Measurement of serum JWH-018 and metabolites were performed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Power analysis was performed and estimated a requirement of six mice per group assuming a normal distribution, a 20% change in mean and 15% variation. In some cases, mice were excluded from analysis due to damage to (or loss of) the EEG electrode.

Results Mice that received IP Δ^9 -THC and JWH-018 exhibited decreased locomotor and electromyogram activity and had electroencephalography (EEG) evidence of seizure activity. Seizures persisted longer after administration of JWH-018 (344 ± 12 min) than Δ^9 -THC (256 ± 15.3 min; $p < 0.001$), with significantly more frequent EEG spikes after JWH-018 (25.1 ± 3.1 spikes/min; $p < 0.001$) compared to Δ^9 -THC

administration (12.3 ± 1.4 spikes/min). JWH-018 increased EEG spikes in a dose-dependent manner. Doses of 1.5, 2.5, and 5 mg/kg caused 18.7 ± 2.7 , 25.1 ± 3.1 , and 37.1 ± 7.3 spikes/min, respectively [$p < 0.01$ (1.5 vs. 5 mg/kg) and $p < 0.05$ (2.5 vs. 5 mg/kg), one-way ANOVA]. Pretreating mice with AM-251, a CB₁R-selective antagonist, via IP injection 30 min prior to administration of JWH-018 (2.5 mg/kg) resulted in normal EEG activity without seizure activity.

The investigators also obtained serial serum concentrations of JWH-018 and metabolites after administration. Peak serum concentrations (87.8 ng/ml) of JWH-018 were reached 15 min after IP injection and remained at 40% of peak concentration (35.7 ng/ml) at 3 h post-injection. The metabolite JWH-018 N-(4-hydroxypentyl) [JWH-018 N-(4-OH)] reached its peak of 9.1 ng/ml at 15 min and slowly decreased over 3 h. The metabolite JWH-018-N-pentanoic acid (JWH-018 N-COOH) reached peak concentration of 4.9 ng/ml at 3 h after JWH-018 administration.

Conclusion Analysis of EEG activity and locomotor behavior suggests that naturally occurring and SCs have epileptogenic properties. The authors note that while EEG activity showed seizure activity, video recordings showed locomotor depression, i.e., subclinical seizures. JWH-018 achieved much higher serum concentrations in study mice than have been recorded in human trials. While the metabolite JWH-018-N-COOH usually demonstrates the highest concentration in humans after JWH-018 consumption, in mice, the JWH-018N-(4-OH) metabolite exhibited the highest concentration and mimicked the time/concentration curve of JWH-018. Administration of a CB₁R antagonist, AM-251, inhibited induction of seizure activity by a SC.

Critique Interpretation of these results faces the inherent challenges of applying animal models to human toxicology. Mice received IP Δ^9 -THC and JWH-018, whereas human exposures are typically inhalational (Δ^9 -THC, JWH-018) or oral (Δ^9 -THC). The study used very high doses of Δ^9 -THC and JWH-018 and reached much higher plasma concentrations of JWH-018 than those observed in humans. Metabolism showed a longer time to peak serum JWH-018 concentration compared to human inhalational studies, which might have affected the kinetics of CB₁R agonism. The authors did not test whether AM-251 inhibits Δ^9 -THC-induced seizure activity. Lastly, the small sample size, and exclusion of several animals due to testing failures, limited confidence in results.

Implication for Toxicologists Human exposures to marijuana and SCs have been shown to cause seizures, likely mediated via CB₁R. It is possible that patients (both adults and children) exposed to high doses of marijuana and SCs may experience subclinical seizures. However, marijuana products contain other compounds including cannabidiol, which may possess

antiepileptic activity. The inhibition of SC-induced seizures by a CB₁R inhibitor suggests a potential clinical therapy for treatment of this condition.

Article Title Laidlaw MAS, Filippelli G, Mielke H, Gulson B, Ball AS: Lead exposure at firing ranges—a review. *Environmental Health* 2017; 16: 34

Background Lead is a toxic substance that can lead to acute and chronic health problems including but not limited to anemia, renal injury, and encephalopathy. Lead can be found in various settings, including firing ranges where exposure to lead dust can cause toxicity.

Research Question What does the literature report on blood lead levels (BLLs) and the adverse health effects in the population involved with firearms?

Methods Using various search engines, the terms “blood lead,” “lead poisoning,” “lead exposure,” “marksmen,” “firearms,” “shooting,” “guns,” “rifle,” and “firing ranges” were used to identify literature that reported on BLLs and the health effects associated with individuals involved with shooting firearms.

Results Thirty six articles, published between 1975 and 2016, were identified that reported on lead exposures at firing ranges. Most (21/36; 58%) studies were from the USA but 14 other countries were represented and included shooters, shooting range employees, and law enforcement/military personnel and men, women, and children. A total of 31 studies reported BLLs > 10 µg/dL; 18 had BLLs > 20 µg/dL, 17 had BLLs > 30 µg/dL, and 15 had BLLs > 40 µg/dL. All articles reported BLLs > 2 µg/dL among enrolled subjects. Several reports described an increase in BLLs among individuals involved in shooting training, followed by a decrease in BLLs after the course was completed. For example, one cited study

showed an increase in mean BLLs ($n = 21$) from 3.3 µg/dL before training to 18.4 µg/dL, 3 days after training ended. This, and other data, suggested a positive correlation between frequency of shooting at firing ranges and BLLs. Sources of lead exposure, including projectiles, primers, dust/particles, and inhaled fumes, were discussed. The paper also included a review of several organizational recommendations concerning BLLs.

Conclusion Shooting lead bullets and employment at firing ranges can result in elevated BLLs. The BLLs can be high enough to have health implications, particularly among exposure pregnant women and children. There is a need for revised engineered safety mechanism at firing ranges to mitigate the risk of lead exposure.

Critique This simple review of the literature described potential health concerns for elevated BLLs related to firing range exposures. It did not, however, describe the specific adverse health effects or provide data (or hypotheses) concerning long-term exposures and clinical effects. It would have been beneficial to determine if these elevated BLLs were clinically significant and describe the types of clinical findings in more detail. The paper would have benefited from a systematic review and, where possible, meta-analysis of pooled data.

Implication for Toxicologists Toxicologists and poison center staff should be aware of shooting ranges as a potential setting for lead exposure. An appreciation of the subsequent clinical, occupational, and legal implications of these exposures is also warranted.

Compliance with Ethical Standards

Conflicts of Interest None.

Sources of Funding None.