Newsletter for the TDM and Toxicology Division of AACC

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Therapeutics & Toxins News

Inside this issue:

"Immunosuppressive drug assays are used to monitor therapeutic drug concentrations in solid-organ transplant recipients."

The Time Has Come for Standardization of Tacrolimus Assays

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Background

My first appreciation of the need for standardization of clinical laboratory assays came back in 1987. The Rogosin Institute was one of several institutions around the world pioneering a life-saving therapy for homozygous and severe heterozygous familial hypercholesterolemia called LDL-apheresis (1-3, see 4 for a review). This cholesterol reduction therapy was goal-oriented to a specific time-averaged blood cholesterol concentration between treatments which required knowing the "true" cholesterol concentration. We required a standardized cholesterol assay that was accurate, precise, and traceable to the CDC-modified Abell-Kendall reference method for blood cholesterol (5). This would make our study comparable to other NIH-funded cholesterol studies.

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Early Days of Cholesterol Testing

We re-wrote the assay parameters and value assigned the calibrators of a commercially available cholesterol assay resulting in improved accuracy with an imprecision of about 1-1.5% CV. Consistent correct recovery of CDC control material was obtained that was traceable to the Abell-Kendall reference method. Patients anxiously awaited their weekly pre- and post- cholesterol levels, and so did we as researchers, as a marker of successful cholesterol reduction. Clinically, our patients improved and felt better within months and many returned to work and other normal activities. The National Heart, Lung, and Blood Institute (NHLBI) established the National Cholesterol Education Program (NCEP), which included the Adult Treatment Panel (ATP) and the Laboratory Standardization Panel (6). The Cholesterol Reference Method Laboratory Network for the National Reference System for Cholesterol was established at the CDC in conjunction with NIH (7). Through a coordinated effort all of the diagnostic companies participated in the NCEP standardization program to make sure their cholesterol assays were properly calibrated and traceable to the National Reference System for Cholesterol (8).

Assay Standardization

In subsequent years there were standardization programs for triglycerides, high-density lipoprotein cholesterol, apolipoprotein A-I, and apolipoprotein B (7, 9,10). Creatinine assay standardization was of particular interest to The Rogosin Institute, as a medical research and treatment center specializing in kidney disease, since it allowed for more accurate and precise estimates of kidney function using the Cockcroft-Gault (CG) and Modification of Diet in Renal Disease (MDRD) equations (11). The standardization of lipid and lipoprotein measurements made it possible for NHLBI to effectively implement the NCEP guidelines for healthcare professionals and patients, aimed at reducing risk for and preventing coronary heart disease. Assay standardization ensured that (1) patients were

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classified correctly according to the NCEP guidelines for treatment decisions and (2) epidemiological studies and drug trials were comparable (7). The time has now come for standardization of immunosuppressive drug assays used to monitor therapeutic drug concentrations in solid-organ transplant recipients.

Tacrolimus

Tacrolimus, the most prescribed calcineurin drug inhibitor, is used to prevent allograft rejection in solid organ transplant recipients (12). Guidelines have already been proposed for tacrolimus dosing and testing as a result of the Efficacy Limiting Toxicity Elimination (ELiTE)-Symphony study and the European Consensus Conference on Tacrolimus (13, 14). Both the study and the conference have suggested that tacrolimus dosing should be minimized in order to reduce long-term nephrotoxicity of the drug. The consensus conference proposed that tacrolimus test methods be capable of a Limit of Quantitation (LOQ) of 1 ug/L and use calibrators and controls traceable to certified reference materials (14).

Laboratory Testing for Tacrolimus

Tacrolimus is measured by a variety of methods based upon LC-MS and immunoassays that are all independently calibrated without traceability to an accepted reference LC-MS method or standard tacrolimus reference material. One would suspect that tacrolimus concentration values may not be comparable between methods and/or laboratories globally. To determine whether this was in fact the case, we designed a global proficiency study in 22 laboratories across 14 countries using tacrolimus test methods based on LC-MS and immunoassay technologies (15). The goal of this study was to assess the current comparability of tacrolimus measurements and the need for standardization of assays that measure this critically important therapeutic drug in allograft recipients. The methods chosen, the Abbott ARCHITECT Tacrolimus immunoassay, the Siemens Dade Dimension immunoassay, and a number of laboratory derived LC-MS methods, accounted for approximately 70%– 85% of all methods used for the measurement of tacrolimus globally. In the absence of a standardized tacrolimus test method, the LC-MS/MS assay at the Analytical Unit, St. Georges, University of London was used as the validated comparative test method.

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Comparability Between Tacrolimus Assays

Briefly, here is what we found. Labs using LC-MS methods either made their own calibrator or borrowed one from a commercial assay system like EMIT, Chromsystems, or Waters. A common calibrator did not harmonize LC-MS methods and results between LC-MS methods were not comparable. The Abbott ARCHITECT immunoassay had lower imprecision than LC-MS and Dade methods. The immunoassays were close to target on spiked samples with some positive bias on patient samples due to metabolite detection. Among the methods evaluated, the Abbott ARCHITECT immunoassay results were the most comparable. Clinically significant variability was seen between labs globally in terms of patient classification by drug level (15).

Tacrolimus Study Conclusions

We concluded that: (1) Tacrolimus values were not comparable between laboratories; (2) assay standardization will be necessary to compare patient results between labs; (3) assay standardization must encompass both the extraction and analytical components; and (4), tacrolimus assay standardization is essential to solve the unmet clinical need for comparability of patient results and is an important step toward providing uniform global care for transplant patients. We also suggested that professional associations and academics should take a leadership role and work collaboratively with pharmaceutical and diagnostic companies to fund and promote a standardization effort (15).

A Second Tacrolimus Study

Recently, a second tacrolimus study was conducted that used a commercially available LC-MS method and a similar study design and reference laboratories as ours (16). The authors concluded that it is possible to standardize an LC-MS method. There are now two commercially available methods today which correlate well to the LGC (Teddington, Middlesex, UK) candidate reference method and the LC-MS method at the Analytical Unit, St. Georges, University of London: The Abbott ARCHITECT immunoassay and the Waters Mass Trak LC-MS. While these are important steps that demonstrate standardization of different assays is possible, the goal remains to provide a path forward for all methods to be truly standardized to a reference measurement system. The global proficiency studies demonstrated that the greatest error was between laboratories and not within test methods (15).

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Future Challenges

Due to the 3-year follow-up results of the ELiTE-Symphony study, where patients were maintained largely without complications with tacrolimus blood concentrations below 10 ug/L, much attention has been given to develop tacrolimus dosing minimization strategies (17). Effective clinical use within this range, especially for tacrolimus dose minimization at the low end, requires accurate and precise assays with an LOQ of 1 ug/L or below. In the absence of a test for subclinical rejection, physicians are working within a very narrow range between tacrolimus toxicity and efficacy. The challenge is to minimize tacrolimus dosing, in order to maximize allograft survival, without falling off the immunosuppression cliff into the abyss of allograft rejection. In the absence of standardization, the true tacrolimus blood concentration cannot be determined. We need to apply what we have learned about standardization of other analytes, like lipids and lipoproteins, to the measurement of therapeutic drugs. Standardization of all tacrolimus test methods is essential in order to offer the best standard of care for allograft recipients and is an important step toward providing uniform global care. The time has come for tacrolimus assay standardization.

"We need to apply what we have learned about standardization of other analytes, like lipids and lipoproteins, to the measurement of therapeutic drugs."

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False Advertising of Dose and Effect

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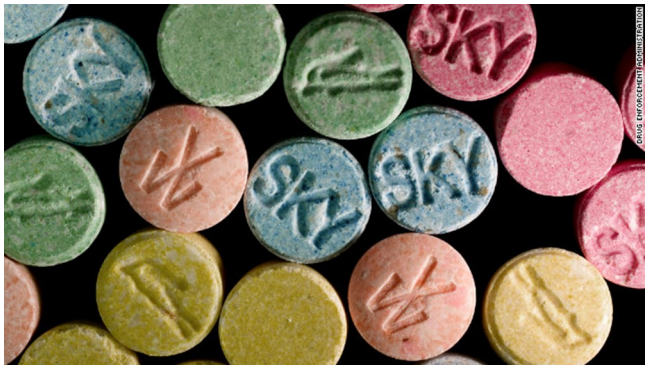
Introduction

Not unsurprisingly, it is difficult for illicit drug users to trust illicit drug distributors. Despite substantial branding efforts by distributors to establish pill colors, shapes, and imprinted symbols (Figure 1) so that product legitimacy and harmlessness are established, the industry often mislabels its products to generate more substantial profits—at the expense of a user's well-being and confidence [1].

This is most prominent in the history of ecstasy branding, distribution and formulation. Originally, a colloquial term for MDMA (3,4-methylenedioxy-N-methylamphetamine), ecstasy formulations morphed into combinations of MDMA with caffeine, ephedrine, DXM (dextromethorphan), or LSD among many other compounds. In order to reinstate the illusion of a safe, pure, and innocent branding of MDMA, distributors utilize the innocuous name of Molly to denote a pure crystalline preparation. These efforts are not done in vain, and have sufficiently enticed people in demographics who may have never experimented with illicit drugs [2]. Ultimately, it appears that Molly may not be as marketed, i.e. pure MDMA, and as many preliminary reports indicate, Molly tablets may not actually contain any MDMA, but instead harbor a cocktail of synthetic cathinones of which there is a paucity of pharmacology and toxicology data.

Figure 1. Molly pills of varying color and unique emblems.



"Molly tablets may not actually contain any MDMA, but instead harbor a cocktail of synthetic cathinones of which there is a paucity of pharmacology and toxicology data."

MDMA and Cathinone Background

The history of MDMA is rife with anecdotes of its early synthesis in 1912 [3] and of additional scientific curiosity in medicinal chemistry, neuroscience, psychology, and toxicology over the last four decades. The political history of its deemed schedule 1 status was controversial in the 1980's [4]; controversy still continues today among medical researchers to determine its potential use in clinical treatment of neurological disorders including post-traumatic stress disorder. Certainly for recreational users, the schedule 1 status ensures for significant risks of self-administration of a drug that does not have oversight regarding its preparation and purity or its pharmacology. Still, most users somehow trust that their pills are consistent in formulation, dose, and effect; although unbeknownst to most, are the facts that these pills are heterogeneous in composition, purity, and dose. Drugs confirmed to be found in Molly pills include cathinones MDPV, Pentedrone, 4-MMC (mephedrone), 4-MEC (4-methylethcathinone), methylone, and MePP; and other stimulants including MDA (methylene dioxyamphetamine), 6-APB (6-(2-aminopropyl)benzofuran), and caffeine.

Dozens of biologically active cathinone derivatives (all harboring a common core structure of a beta-ketone amphetamine, i.e. phenylethylamine) exist, and a number of these are commonly marketed as bath salts and plant food and carry a "not for human consumption" warning label in efforts to evade drug enforcement. While legislation in many states to outlaw these designer drugs limits and exposes their duplicitous marketing, prevalence and use continues to expand. Coordinate with their illegality, increased use, and structural diversity, clinical toxicology labs face an arduous task of deciphering specific drug moieties while mitigating cross-reactivity in immunological-based drug screens.

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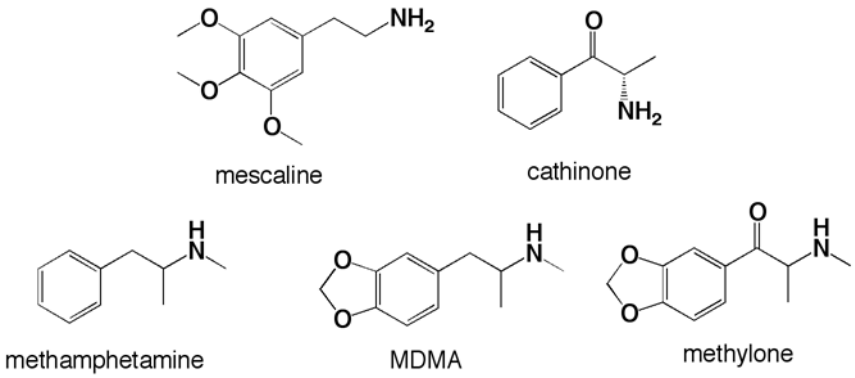
Chemistry and Mechanism of Action

Amphetamines, (including derivatives such as MDMA) and synthetic cathinones are structurally similar to catecholamines (Figure 2) and likely exert their effects in an analogous fashion by increasing synaptic concentrations of norepinephrine, dopamine, and serotonin via inhibition of uptake transporters or stimulation of neurotransmitter release from intracellular stores. Interestingly, the ring substituted methylenedioxy (-O-CH2-O-) functional group found on both amphetamine- and cathinone -based derivatives is structurally congruent with the 3,4-methoxyphenyl groups of mescaline. Consequently, this structure activity relationship induces a hybrid pharmacology resembling that of both amphetamines and mescaline as evidenced in MDMA studies indicating involvement of α_2-adrenergic and 5-HT_{2A} receptors [5, 6]. Users of amphetamine derivatives and synthetic cathinones report acute and chronic toxicities closely similar to amphetamines. Acute desired effects are both physical (e.g. postponement of fatigue, increased wakefulness, sexual arousal) and psychological (e.g. closeness, empathy, euphoria); and orthogonal to these desired effects, are undesired short-term effects (e.g. agitation, restlessness, muscle tension, hyperthermia, nausea, insomnia, etc), withdrawal-like reactions (e.g. anxiety, agitation, delirium) and serotonin toxicity [7, 8].

"Laboratorians tasked with the detection and quantification of amphetamine and cathinone derivatives will continually be in a state of catch-up as dozens of biologically active variants are known or can be readily designed and synthesized, which elude current detection methods."

Figure 2. Structural similarities of amphetamine/cathinone derivatives and mescaline



Conclusion

Significant efforts are made to instill a sense of harmlessness and homogeneity with illicit drug preparations e.g. ecstasy and Molly. In the uncontrolled and unmonitored arena of illicit drug manufacturing and distribution, incongruences in actual drug formulation pose significant risk to those who self-administer. While research to determine mechanistic and toxicological modalities, it suffices to conclude that perhaps the greatest risk associated with these compounds is the complex and not fully understood pharmacology of each compound and their potential synergistic/additive effects and toxicology. Laboratorians tasked with the detection and quantification of amphetamine and cathinone derivatives will continually be in a state of catch-up as dozens of biologically active variants are known or can be readily designed and synthesized, which elude current detection methods.

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"Amphetamines, (including derivatives such as MDMA) and synthetic cathinones are structurally similar to catecholamines (Figure 2) and likely exert their effects in an analogous fashion by increasing synaptic concentrations of norepinephrine, dopamine, and serotonin via inhibition of uptake transporters or stimulation of neurotransmitter release from intracellular stores."

UPCOMING MEETINGS OF INTEREST

MASS SPECTROMETRY APPLICATIONS to the CLINICAL LAB (MSACL)
Annual Meeting
March 1— 7, 2014, San Diego, CA
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