K40 A Systematic Study of the Cellular Toxicity of Common Amphetamine and Ring- Substituted Drugs

Mya Fekry, MSc, Anglia Ruskin University, Mel 210, ARU, East Road, Cambridge, CB1 1PT, UNITED KINGDOM; Michael D. Cole, PhD, Anglia Ruskin University, East Road, Cambridge, CB6 2UD, UNITED KINGDOM; and Beverley R. Vaughan, PhD*, Anglia Ruskin University, East Road, Cambridge, Cambridgeshire CB1 1PT, UNITED KINGDOM*

After attending this presentation, attendees will have an understanding of the effects and comparative toxicity levels between a number of amphetamine type stimulant compounds and the need for such systematic studies

This presentation will impact the forensic science community by informing health care professionals, law enforcement agencies, and those involved in drug administration about the potential dangers of toxicity of a number of established and new stimulant drugs that are increasing in popularity. It will allow attendees to make an informed choice in drug administration and treatment of drug users.

There are now a wide range of amphetamine and 3,4-methylenedioxyamphetamine (MDA) drugs available in the illegal drug market. While amphetamine and methamphetamine were originally used within this context as stimulants, the fact that some users experienced unpleasant side effects let to the development of the ring substituted 3,4-methylenedioxy compounds. These latter compounds, in addition to stimulant effects, are entactogens and facilitate inter-personal communication.

The majority of amphetamine-type-stimulants currently found "on the street" contain one or more of these drugs. MDMA (3,4-methylenedixymethamphetamine), a.k.a. ecstasy, remains a popular contender on the party scene. Globally, there are distinct differences in the usage between countries. In the United Kingdom, the incidence of ecstasy usage has fallen in recent years whilst there has been an explosion of use across Northern America. Its usage is concentrated in younger adults, and in Europe is more commonly abused in males with approximately 2.5 million people using ecstasy in 2009.[1] MDMA is now tightly controlled across the globe but other analogues, including MDA and 3,4-methylenedioxy-N-ethylamphetamine (MDEA) have also been growing in popularity. The legislative control of these compounds, under the Misuse of Drugs Act and their related laws and legislations abroad, often leads to the synthesis of uncontrolled analogues and derivatives of the initial substance in search of similar desired effects.

The mixture of synthetic analogues commonly found in tablets sold as "ecstasy" frequently includes MDMA, MDA, and MDEA. Profiling and analysis of street ecstasy samples via chromatographic methods has often been used to illustrate complexity of the mixture of stimulant compounds present as well as the multiple chemical impurities produced in the synthesis of street samples.[2]

While evidence of the toxicity of these compounds has often been seen in patients admitted to hospital emergency departments, the effects of these compounds and their related analogues on vital organs at a cellular level, is not well defined. Damage to organs involved in the metabolism and excretion of these compounds and their by-products has been reported however, the extent to which toxicity occurs and the mechanisms of action by which this damage is induced are still poorly understood.[3]

Cells were exposed to the drugs at concentrations ranging from 1.1mg/ml to 11mg/ml, after which they were incubated and then assessed morphologically for evidence of cell death in the form of either programmed cell death (apoptosis), uncontrolled cell death (necrosis). Further assessment of cell death was carried out using Annexin V assays in which the presence of apoptosis and propidium iodide (PI) provided evidence of general cell membrane disruption. Samples were analyzed using a flow cytometer, after which results were given according to the expression of annexin V and PI labelling. Results were expressed as the percentage increase in the presence of non-viable cells (Annexin V +/PI +) after correcting for background cell death. This data was confirmed using fluorescent microscopy and immunolabelling of the Annexin V in-situ. The most toxic of the compounds to the liver was amphetamine, with an LD50 of 1.5mg/ml, whilst the least toxic was methamphetamine with an LD50 value of 2.9mg/ml. Conversely in the kidney, amphetamine appeared to be the least toxic compound, while methamphetamine was more toxic. Respective LD50 values were calculated at 4.9mg/ml and 3.9mg/ml.

MDA showed higher levels of toxicity in both the liver and the kidney than both MDMA and MA. LD50 values for this compound were calculated at 2.1mg/ml and 3.7mg/ml respectively, while values for MDMA were 2.5mg/ml and 3.8mg/ml. The effects of all compounds supported both dose and time dependant increases in toxicity. In the process of data analysis, comparisons between amphetamine and MA, and MDMA and MDA were made due to the related nature of their structures.

The data supports published literature that MA and amphetamine become rapidly toxic to the kidney, whilst MDMA and its metabolite MDA are more toxic to the liver. This may be due to rapid accumulation of amphetamine and MA in the kidney as previously described in studies of the pharmacokinetics and distribution of amphetamines in the human body.[4] MDMA is cleared by hepatic metabolism, but with dose dependant increases this rapidly leads to saturation of hepatic clearance and biological toxicity. The profile of ecstasy users however, is all too frequently one of poly-drug abuse, dosing repeatedly in a recreational environment. Therefore, it is essential to perform systematic studies and poly-

drug investigations to understand the profile of the patient presenting to the doctor, and the cold case to the forensic pathologist.

References:

[1] (EDCDDA) EMCDDA Annual Report 2010 – the state of the drugs problem in Europe. Luxenberg: Publication Office of the European Union; 2011.

[2] Gimeno, P. Besacier, F. Chaudron-Thozet, C. Girard, J and Lamotte, A "A Contribution to The Chemical Profiling of 3,4- methylenedioxymethamphetamine (MDMA) tablets. Forensic Science International. 2002 (127) 1-44.

[3] Lyles, J & Cadet, J.L "Methylenedioxyamphetamine (MDMA, Ecstacy) Neurotoxicity: Cellular and Molecular Mechanisms" Brain Research Reviews 2003 (42) 155-168.

[4] Volkow, N.D, Fowler, J.S, Wang, G.J, Shumay, E, Telang, F. Thanos, P.K & Alexoff, D (2010) "Distribution and pharmacokinetics of methamphetamine in the human body: clinical implications." PLoS One 5(12): e1526

MDMA, Ring-Substituted Analogues, Cellular Toxicity

 ** Presenting Author*