K43 The Hidden Dangers of Clandestine Methamphetamine: Synthesis to Cellular Toxicity

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After attending this presentation, attendees will have an understanding of the origins and occurrence of chemical impurities in methamphetamine and appreciation of the potential toxicity of these compounds in a street drug, both alone and in combination.

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 pure stimulant drugs and common impurities. This will allow attendees to make an informed choice in drug administration and treatment of drug users.

Methamphetamine (MA) is a widely abused stimulant, which may be manufactured using a variety of different routes. While amphetamine is the most commonly occurring phenethlyamine of choice in Europe, methamphetamine is more prevalent in North America and the Far East.[1] New data from the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) shows that the geographic concentration and availability of these compounds is responsible for shifts in global user trends.[2] Given the global popularity and accessibility of the Internet, synthetic routes were chosen from this medium for methamphetamine synthesis. One of the most popular and straight-forward routes of synthesis is the reductive amination of benzylmethylketone (BMK), which is commonly employed in Europe.[2] This route involves the reaction of methylamine with BMK in the presence of mercuric chloride and aluminium followed by purification of the crude drug base and subsequent crystallisation of the hydrochloride salt.

Routes such as this lead to complex mixtures of reaction by-products and a significant number of impurities. These compounds may contribute to the toxicity profile of street samples of methamphetamine. To date there had been no systematic study of the drug or its impurities. The aim of such a study was to determine whether these impurities contributed to the overall toxicity of the street methamphetamine samples. Alongside MA's addictive properties, it produces widespread organ toxicity. The most commonly occurring impurities identified in the mixtures, including benzylaldehyde, pheylacetone, 1-phenyl-2-propanol and the related compound ephedrine were subjected to cytotoxicity testing against immortalized human kidney (CAKI-2) and liver (HepG2) cell-line models.

According to the pharmacokinetics of MA, a dosing strategy was developed from the reported distribution of the compound in the body and the recently reported levels of purity in street samples. We focused on vital organs that are involved in the metabolism and excretion of the compound and its by-products. Given that, respectively, 22% and 7% of the ingested compounds are expected to reach the liver and the kidneys, it was possible to assess the cytotoxicity of each individual chemical based on their purity within the sample.[3]

Cells were exposed to the drugs for one hour at concentrations ranges from 1.1mg/ml-11mg/ml, following which they were assessed morphologically for evidence of cell death, either programmed cell death (apoptosis), uncontrolled cell death (necrosis) or no effect at all. To assess for cell death, cells were then labelled with annexin V to evidence the presence of apoptosis and propidium iodide (PI) for evidence of general cell membrane disruption. These samples were then analyzed using a flow cytometer. Results were given according to the expression of annexin V and PI labelling. Results were expressed as the percentage of viable cells (Annexin V -/PI -) veruses the percentage of non-viable cells (Annexin V +/PI +). This data was confirmed using fluorescent microscopy and immunolabelling of the annexin V in-situ.

The most toxic of the compounds was MA, with an LD50 of 3.9mg/ml to the kidney and 8.2mg/ml to the liver. 1-phenyl-2-propanol conversely, produced minimal toxicity with respective LD50 values of 37.1mg/ml to the liver and 111.9mg/ml to the kidney. All other compounds displayed significant toxicity with LD50 values of less than 10mg/ml. Effects of the compounds supported both dose and time dependant increases in toxicity. By flow cytometry, it can be deduced that apoptosis and necrosis are both activated following exposure to these compounds. For further mechanistic confirmation, changes in expression levels of genes associated with these pathways are investigated. This will include Bax (an apoptosis related gene), Beclin (autophagy related) and the Caspase family (apoptosis) on both exposure to impurities and in both dose and time dependant assays.

These data provide evidence of the cellular cytotoxicity of MA and related impurities at the sites of biological purification and excretion. Overall, the liver is more sensitive to these compounds than the kidney, with MA exhibiting the most significant toxicity. This supports published research that there is a complex and highly damaging cellular response to MA and that this drug continues to pose a significant risk to those who fall in its path.

References:

[1] King, L.A – Forensic Chemistry of Substance Misuse: A Guide To Drug Control. RSC Publishing. 2009

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[2] (EDCDDA) EMCDDA Annual Report 2010 – the state of the drugs problem in Europe. Luxenberg: Publication Office of the European Union; 2011.
[3] 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

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