K26 An Investigation Into the Cellular Cytotoxicity of Benzylpiperazine (BZP) and Its Derivatives

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After attending this presentation, attendees will have an understanding of the methods for testing drugs of abuse for *in-vitro* cytotoxicity. Attendees will also have acquired a knowledge of the toxicity of BZP and a number of its major impurities, as well as, be able to relate this to the clinical significance.

This presentation will impact the forensic science community by providing, for the first time, evidence of the toxicity of BZP and its impurities at a cellular level. This allows us to begin to elucidate the mechanism of toxicity and thereby the treatment for those poisoned by these drugs.

The market for clandestine designer drugs has been expanding exponentially over the last decade. Piperazines are a group of psycho- active stimulants including 1-benzylpiperazine (BZP), 1- (trifluoromethyl-phenyl)piperazine (TFMPP), 1-(chlorophenylpiperazine) (CPP) and 1-(methoxyphenyl)piperazine (MeOPP).[1] Of these BZP is the most commonly encountered derivative marketed as a "herbal high"/"legal high" with the street name A2. BZP was originally produced as an anti-helminthic agent for livestock in 1944.[2] Studies on rats have revealed that BZP exerts its effects by elevating levels of serotonin and dopamine by blocking the re-absorption of these at neurological synapses producing the positive psycho-active effect.[1]

Over the last two years, there have been an increasing number of clinical reports published concerning fatalities after ingestion of BZP alone or in combination with other psycho-active agents. BZP is now a controlled substance in many countries. It is a class D drug in New Zealand, class C drug in the United Kingdom, and it is controlled as a schedule 1 drug in the United States.[3] There is very little published in the public domain regarding the toxicity of these drugs at a cellular level. Although there are numerous reports on the renal toxicity and concerns over chronic abuse, the actual activity of the drug has not been studied in cells from sites of biological filtration within the body.

This study examines the effects of short term exposure on immortalized cells derived from the kidney (CAKI-2), the liver (HepG2) and fibroblasts (3T3) to BZP, it's precursor piperazine hexahydrate, and synthetic by-product 1,4-dibenzylpiperzine (DZP).

Cells were exposed to the drugs for 1 hour at concentrations ranging from 0.783mg/ml-3.13mg/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 labeled with annexin V to evidence the presence of apoptosis and propidium iodide (PI) for evidence of general cell death. These samples were analyzed using a BD FACS Calibur Flow Cytometer. Results were expressed according to the degree of annexin V and PI labeling as the percentage of viable cells (Annexin V -/PI -) versus the percentage of non-viable cells (Annexin V +/PI +). This data was confirmed using fluorescence microscopy and immune-labeling of the annexin V *in-situ*.

DZP causes comparatively higher levels of cell death giving LD^{50} values of 2.25mg/kg (HepG2), 1.50mg/kg (CAKI-2) and 1.20mg/kg (3T3). Piperazine hexahydrate resulted in minimal cytotoxicity, being most potent in its activity against HepG2 with an LD^{50} of 1.50mg/kg. BZP was most cytotoxic producing an LD^{50} value of 1.1mg/kg (HepG2), 1.57mg/kg (CAKI-2) and 1.40 mg/kg (3T3). Further to this it is shown that HepG2 cells display a lower threshold for sensitivity to these drugs than CAKI-2 or 3T3.

These data provide clear evidence of the cellular cytotoxicity of BZP and DZP and its synthetic by-products at levels likely to occur following the ingestion of these drugs. Data also indicate that in general the liver, site of primary biological filtration, is most sensitive to the actions of these drugs. This supports the clinical evidence that BZP produces a very real threat of causing hepatic toxicity.

References:

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