Surrogate Markers for Gammahydroxybutyrate (GHB) Exposure In Cell lines

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This thesis is the result of author's original research. It has been composed by the author and has not been previously submitted for the examination which has lead to the award of a degree.

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ABSTRACT

Gamma hydroxybutyric acid (GHB) is a metabolite of gamma-amino butyric acid (GABA) and considered as a natural neurotransmitter found in the brain in low concentrations. GHB has been used in general anaesthesia and is currently used to treat narcolepsy and alcoholism. The abuse of GHB, especially in date rape sexual assaults, has increased in recent years. GHB has a rapid rate of metabolism causing it to disappear quickly making criminal cases often difficult to prosecute. This study is aimed at extending the window of detection of GHB beyond 12 hours through finding robust surrogate markers of GHB exposure in cell lines. Two approaches were used: Agilent SurePrint G3 Human 8x60K arrays were used firstly to detect changes in gene expression in human monocytic leukaemia TH-P1 blood cells after 24hours GHB exposure and the two-dimensional gel electrophores (2-DGE) were used secondly to find proteomic changes in THP-1 and in two brain cell lines (SH-SY5Y and 1321N1 cells) after 24 h GHB exposure. The results show that 900 μM GHB induces an alteration in 2380 genes ($P<0.05$ and a fold change of >2) and the Gene Ontology (GO) enrichment analysis showed that the largest numbers of the altered genes were in the intracellular and membrane parts of the cell. In terms of GO molecular functions, the majority of altered genes code for proteins and nucleotide binding sites, and some of the altered genes encode steroid dehydrogenase enzymes. Furtheremore, the results of 2-DGE revealed that 900 μM GHB in THP-1 cells and 100 μM GHB in brain cells used were induced alteration in proteins after 24h exposure, some of altered proteins were glycolytic enzymes and chaperones. This information may be useful in forensic toxicology.