Salsolinol compounds as biomarkers for human alcohol consumption, disease, and toxicity.

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Abstract:
Alcohol abuse and alcoholism are among the most significant public health issues of our time and is arguably mankind’s oldest drug. The economic cost to Americans is more than $255 billion dollars annually. It is estimated that alcoholics have an average decrease in life expectancy between 12-15 years. Despite the enormous amount of research, there has yet to be a mechanism proposed that accounts for the many different behavioral, neurochemical, or toxicological effects associated with alcohol use and abuse. Upon alcohol administration, ethanol’s primary metabolites, acetaldehyde, readily forms salsolinol and salsolinol-like compounds in the periphery as well as the brain. Alcoholics have elevated levels of salsolinol-like compounds in urine and post mortem brain samples. Moreover, studies have shown that these salsolinol-like compounds can induce Parkinson like neurodegeneration.

Background:
Heavy, chronic consumption of alcohol (ethanol) causes a constellation of adverse effects on health and is associated with a withdrawal syndrome that promotes addiction. Also, motor vehicle accidents and other events associated with alcohol intoxication are a major problem. On the other hand, many people consume alcohol in moderate doses on an occasional basis with no apparent ill effects, presumably because they appreciate its mild euphoric and anecolytic properties, and much evidence supports the view that a glass of red wine a day benefits the cardiovascular system. In spite of decades of research on what is surely mankind’s oldest drug, the exact ways in which alcoholic beverages induce all of their many effects are not completely understood. The direct actions of ethanol on targets such as glutamate and GABA receptors are probably responsible for many of the drug’s psychotoxicity. However, it is possible that metabolites of alcohol are responsible for some of the effects, and alcohol metabolites also have utility as forensic biomarkers of alcohol consumption, that can, for example, help determine responsibility in motor vehicle and industrial accidents. Such metabolites include tetrahydroisoquinoline compounds that are formed from reaction of the proximal ethanol metabolite, acetaldehyde, with monoamines such as dopamine (Figure 1).

While some of these compounds have been the sporadic focus of attention from alcohol researchers over the last half-century, we feel the time is ripe for a reevaluation of their forensic and psychoactive potentials, and have developed an LC/MS/MS system that will permit us to quantify concentrations of tetrahydroisoquinolines and related compounds in animal tissues. We are particularly interested in 3-carboxysalsolinol, the TIQ derived from acetaldehyde and 1-DOPA, and in preliminary work (not shown here) synthesized this compound with and without deuterium substitution. Using the deuterated compound as an internal standard, we demonstrated the presence of SCS and it methoxylated derivative in mouse brain, before embarking on development of the methodology described here.

Figure 1

Method:
Swiss-Webster mice were treated with saline (control), 1-dopa, or ethanol, with or without pretreatment of paroxetine, benserazide, tolcapone, or disulfiram. After times of 5, 10 or 30 mins animals were sacrificed.

Whole brains were removed and dissected on ice into striatum, hippocampus cerebellum, and the rest of the brain sections. Urine, blood, and liver were also collected for analysis. The mice were housed individually in a controlled environment and allowed food and water ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) and the guidelines of the National Institutes of Health were followed.

Results:
- All compounds were determined in fortified samples and were well resolved in this method including R/S chiral enantiomers (Figure 5).
- This method shows good linearity and sensitivity down to 1 ng/ml (Figure 6).
- Several salsolinol-like compounds were determined in the blood, liver and striatal brain sections at 5 and 10 minutes.
- After 30 minutes, these salsolinol like compounds were mostly their methoxy-metabolites.

Conclusions:
- We have developed and validated a method for quantification of salsolinol-like compounds and catecholamines for their determination in urine, serum, liver, and brain tissues.
- This work provides evidence that acetaldehyde derived toxic compounds are present in brain and peripherally for up to 30 minutes after exposure.
- Analysis of these compounds have been shown to be elevated in postmortem samples of neurodegenerative diseases including Parkinson’s.
- This method will be a useful tool to determine the effects of elevated alcohol and its metabolite (acetaldehyde) on the deregulation of catecholamines and determination of the formation of salsolinol-like compounds.

References: