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The neurotoxicity of alcohol

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Patterns of drinking are changing throughout the world and in many countries this will be detrimental to the health and welfare of the local population. Even uncomplicated alcoholics who have no specific neurological or hepatic problems show signs of regional brain damage and cognitive dysfunction. Many of these changes are exaggerated and other brain regions damaged in patients who have additional vitamin B1 (thiamine) deficiency (Wernicke–Korsakoff syndrome). Quantitative neuropathology techniques and improvements in neuroimaging have contributed significantly to the documentation of these changes but mechanisms underlying the damage are not understood. A human brain bank targeting alcohol cases has been established in Sydney, Australia and provides fresh and frozen tissue for alcohol researchers. The tissues can be used to test hypotheses developed from structural neuropathological studies or from animal models and *in vitro* studies. Identification of reversible pathological changes and preventative medical approaches in alcoholism should enhance rehabilitation and treatment efforts, thereby mitigating debilitating morbidities and reducing mortality associated with this universal public health problem. *Human & Experimental Toxicology* (2007) **26,** *251–257*

Key words: alcohol toxicity; Australia; brain banking; brain damage; CNS; cognitive dysfunction; neuropathology; reversible damage; thiamine deficiency; Wernicke–Korsakoff syndrome

Introduction

Alcohol dependence and abuse are costly health and social problems. Patterns of drinking are changing, in a global sense, with dramatic increases in per capita consumption in China[1] and Eastern Europe.[2] Global patterns of drinking were reviewed by Rehm and colleagues who noted that they were most detrimental in the former Socialist economies in the Eastern part of Europe, in Middle and South America and parts of Africa.[2] They predict that the outlook for the future is not favourable, both with respect to average volume and to patterns of drinking. The data for developing countries are less well documented but anecdotal evidence suggests that they will follow a similar but delayed path with increasing use and abuse. In recent years more women and young people have begun drinking heavily.[3,4] Alcohol also contributes to deaths caused by accidental illicit drug overdose. In two forensic studies performed in Australia the most common scenario was a man aged in his 30s who had recently administered heroin (90%) and drunk alcohol (27% or 105 of 293 cases) and died soon afterwards.[5,6]. Thirty one cases (11%) had levels of alcohol greater than 0.15 g/100 mL as well as a toxic level of morphine. Thus, there is a need for increased awareness and further education concerning the dangers of multiple drug use, especially alcohol.

Excessive drinking can lead to impairment of cognitive function and structural brain changes. The latter can be studied using human autopsy material and/or animal models. It should be noted that many animals, and rodents in particular, metabolize alcohol more rapidly than man. Mice can metabolize 600 mg/kg/hour compared to man (100 mg/kg/hour).[7] Human tissues are therefore an important research resource. Autopsy brain tissues from people with alcohol-related disorders are available and can be used for a wide range of structural and molecular techniques.[8] This 'brain bank' was formally established in Sydney, Australia in 1999 with financial support from the National Institute of Alcoholism and Alcohol Abuse and the aim is to provide tissues (fresh-frozen and formalin-fixed) to research groups throughout the world.[9,10] There are now over 200 alcohol and control cases. Approximately 20% of the alcohol cases had either cirrhosis of the liver or associated thiamine deficiency with the Wernicke–Korsakoff syndrome (WKS). Neuroradiological techniques can also be carried out on autopsy brain tissues to obtain high-resolution images that can be used for comparative quantitative neuropathological studies.[11]

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Post-mortem imaging may ultimately serve to identify histological, cellular and molecular biological mechanisms of structural damage pre-existing in genetically disposed groups and damage resulting from neurotoxins and neuropsychiatric disorders *per se*.[12] The success or failure of research using brain bank material largely depends upon careful clinical and pathological characterization of cases, precise matching of control cases and appropriate storage. The future of brain banking is extremely exciting and positive. Donor programmes for alcohol-related disorders have commenced in Australia (www.braindonors.org)[9] and Japan,[13] and these will ensure high quality, prospectively collected clinical information on all cases. A proactive approach to the public is essential to overcome anxieties and negative opinions concerning the use of autopsy tissues for research. Our own recent experience has been extremely positive – in requesting donation for brain banking from next-of-kin in forensic autopsies we receive a positive response in 62% of cases.[14] The rapid progress in molecular techniques is equally exciting as a single brain bank case can now be used for many research projects and the cumulative data can be compared.

Alcohol is absorbed directly into the bloodstream through the stomach and the small intestine. Food in the stomach slows down the rate at which alcohol is absorbed. Alcohol is distributed throughout the water in the body, but not into fatty tissue. The liver breaks down about 90% of alcohol, the first step being oxidation to acetaldehyde, which is catalyzed by alcohol dehydrogenase (ADH). The acetaldehyde is further oxidized to acetic acid (catalyzed by acetaldehyde dehydrogenase, ALDH) and finally CO_2 and water through the citric acid cycle. Alcohol metabolism is a complex process with large individual variations related to absorption, distribution and elimination. Caballería has recently reviewed current concepts and focuses upon the factors that influence these variations.[15] These include genetic factors, especially those related to the different alleles of ADH2 and ALDH2. These alleles are thought to relate to the development of alcohol dependence, particularly in some populations such as those of Asian origin.[16,17] The individual variations in the metabolism of alcohol are responsible for the different toxicity of alcohol in both the liver and other organs like brain. Acetaldehyde and aldehydic products of lipid peroxidation can bind to proteins forming stable adducts in the tissues. Adduct formation has been shown to be associated with the severity of alcoholic liver damage through humoral and cell-mediated immunological responses.[18] In animal models of ethanol toxicity researchers have demonstrated acetaldehyde-protein adduct formation in the white matter and large neurons of the frontal cortex.[19] This suggests the possibility of cell damage caused by the interaction between adducts, cellular structural proteins and immune mechanism. There are many other mechanisms that have been proposed to cause tissue damage in alcoholics; this is beyond the scope of this article, but there are several recent reviews.[20,21]

In the brain some of the damage is permanent and some appears to be reversible. The brain damage relates to lifetime alcohol consumption as well as associated medical complications. The most important of these is the nutritional vitamin deficiency state – WKS, which is caused by thiamine deficiency but is seen most commonly in alcoholics.[22] This disorder is potentially preventable with the simple public health measure of supplementation of the diet by thiamine[23] and an awareness by health professionals to treat patients who are 'at risk' with thiamine.

Impairments of neurological function that are commonly seen in alcohol dependency include deficits in abstract problem solving, visuospatial and verbal learning, memory function, perceptual motor skills and even motor function.[24,25] The pattern of cognitive deficits has generally been considered to be 'frontal' in nature but more recently the possibility that compromised pontocerebellar and cerebellothalamocortical systems might contribute to cognitive and motor impairment has been raised. Sullivan studied each major node of frontocerebellar circuitry using quantitative neuroimaging and showed volume deficits in alcoholics, but noted that each can be independently compromised.[26] Disruption of these circuits might underlie alcoholism-related neuropsychological deficits, either by abnormalities of individual nodes or by disconnection and interruption of selective circuitry. Repeated experience of withdrawal from alcohol (most alcoholic patients have multiple episodes of binge drinking followed by abstinence) is also associated with impaired cognitive function although it appears that this only applies to some specific cognitive tasks.[27] The WKS and hepatic encephalopathy, both commonly seen in alcoholics, also cause cognitive dysfunction and it is important to identify these disorders if studying the effects of alcohol on the brain using clinical, imaging or neuropathological techniques. Harper and Matsumoto recently summarized the extent of damage to different anatomical regions of

the brain in the various groups of alcoholics including cases with WKS.[21]

Results and discussion

Structural changes in brain

Structural and functional abnormalities have been demonstrated in apparently uncomplicated alcoholics who are cognitively impaired.[28] The risks of 'moderate' alcohol consumption are more difficult to assess. Ding and colleagues showed that the more alcohol consumed, the larger the cerebrospinal fluid-filled spaces of the brain became.[29] This is in line with a neuropathological study that showed an increase in the cerebrospinal fluid-filled spaces (pericerebral space) in moderate drinkers.[30]

There are no recent neuropathological data on the subject, which may reflect difficulties with access to pathological material given the worldwide decline in hospital autopsy rates.[31] The brain bank that has been established in Australia will help to address this problem.[10] Neuropathological changes caused by alcohol were reviewed by Harper in 1998 [32] and are summarized here. Alcoholics have a reduced brain weight compared to controls and there is a correlation between the degree of brain atrophy and the rate and amount of alcohol consumed over a lifetime. A reduction in white matter volume largely accounts for the reduction in brain weight and volume. Kril and colleagues noted that the prefrontal white matter was most severely affected in alcoholics with WKS and there was a negative correlation between white matter loss and the maximum daily alcohol consumption.[33] *In vivo* studies have revealed age-related disruption of brain white matter microstructural integrity that varies by region.[34] The mechanism for alcoholism-related white matter volume loss, restoration with alcohol abstinence and disruption of microstructural integrity remains unclear but probably involves changes in both myelination and axonal integrity. Brain bank cases were used for molecular studies to try to identify these pathogenic mechanisms causing reversible 'shrinkage'. DNA microarray technology was used to investigate alterations of gene expression in the white matter of alcoholic cases. Together with several cell cycle genes and neuronal genes, the expression of three genes encoding myelin proteins were found to be decreased in the superior frontal cortex of human alcoholic subjects.[35] These data clearly demonstrate the power of using high-throughput analyses to study the pathophysiology of these complex disorders. More recently, a complete 2D PAGE proteomic map of myelin proteins in mouse CNS was established[36] and the application of a similar methodology to study the neuropsychiatric diseases using human brain samples is now possible.[37]

The identification of these myelin gene changes suggests that some of the pathological changes in the brain in alcoholics could be reversible. This information should be communicated to the public as it could assist in rehabilitation programmes. Moreover, it should also be communicated to the medical community as it could enhance preventative medical approaches and treatment efforts, thereby mitigating the debilitating morbidities and reducing mortality associated with this universal public health problem.

Neuronal loss has been documented in specific regions of the cerebral cortex (superior frontal association cortex), hypothalamus and cerebellum in alcoholics.[38–40] Analysis of the types of neurons lost from the frontal cortex revealed that they were the larger ones with a somal area greater than 90 μm.[41] This population of neurons is also more vulnerable in both Alzheimer's disease[42] and normal ageing.[43] There does not appear to be any link between alcohol-related brain damage and Alzheimer's disease[44] although there is some work that suggests a relationship between alcohol and ageing.[45] No changes were found in basal ganglia, nucleus basalis or raphe nuclei. However, many of these regions are damaged in alcoholics with the WKS.[32] Neuronal dendritic shrinkage has been documented in alcoholics.[46] An important point to note is that this dendritic shrinkage has also been shown to be reversible in an experimental model following a prolonged period of abstinence.[47] This finding has obvious functional implications regarding the documented reversibility of cognitive deficits in abstinent alcoholics.[25]

Using a PCR-based differential display on the human brain bank material, Fan and Depaz and colleagues have identified and cloned a novel human gene with increased expression in both superior frontal cortex and hippocampus of alcoholic subjects.[48,49] Amino acid sequence analysis predicted that hNP22 would possess phosphorylation sites for casein kinase II and protein kinase and two possible calcium-binding sites. There is some homology with calponin and a human protein SM22, both of which can bind elements of the cytoskeleton. Furthermore, hNP22 has been reported to be an actin binding protein. A polyclonal antibody was generated and western blot analysis was conducted, which showed that hNP22 expression was only found in the brain and is specific to neurons.[48] In summary, hNP22 is a brain-specific

hydrophilic, globular, cytoplasmic, putative calcium-binding protein, which may interact with the cytoskeleton. The increased expression in the frontal cortex and hippocampus may reflect an adaptive change to chronic alcohol exposure that may lead to changes of neuronal plasticity.

Atrophy of the cerebellum is commonly associated with alcoholism. Torvik and Torp that 26.8% of alcoholics with WKS had cerebellar atrophy.[50] Neuroimaging *in vivo*[51] and examination of the cerebellum at autopsy reveal shrinkage of the anterior superior cerebellar vermis. Quantitative pathological studies have shown that there is a loss of Purkinje cells in the vermis (reduced on average by 43%), which correlates with clinical ataxia/unsteadiness.[40] There was a 36% loss of Purkinje cells in the lateral lobes of the cerebellum that correlated with 'mental signs'. This is of particular interest given the recent data suggesting that the cerebellum is important in the organization of higher cerebral functions.[26,52] Baker and colleagues showed that there is no consistent correlation in the number of neurons or the structural volume for any of the cerebellar regions in uncomplicated chronic alcoholics.[40] This suggests that chronic alcohol consumption *per se* does not necessarily damage human cerebellar tissue. However, significant changes were noted in alcoholics with the WKS, suggesting that the damage in the cerebellum is caused by thiamine deficiency.

Wernicke–Korsakoff syndrome

There has always been some confusion about the diagnostic categories of Wernicke's encephalopathy and Korsakoff's psychosis. They are often referred to together as Wernicke–Korsakoff syndrome. The confusion arises from the fact that the two disorders are commonly seen in alcoholics and are dramatically different clinically but the neuropathology was thought to be identical for many years.[53] Korsakoff patients have a severe amnesic syndrome with or without the classical signs of Wernicke's encephalopathy (confusion, ataxia and ophthalmoplegia/nystagmus). New criteria for the diagnosis of Wernicke's encephalopathy have been formulated that are more useful. Two of the following should be present: dietary deficiency, oculomotor abnormalities, cerebellar dysfunction and either an altered mental state or mild memory impairment.[54] Many clinicians see the two disorders as a continuum and recent studies would support this hypothesis. However, relatively recent clinicopathological studies with neuronal counts for diencephalic structures have identified the neuroanatomical deficit that appears to account for Korsakoff's psychosis – damage to the anterior nucleus of the thalamus.[55] It should be noted that patients with non-amnesic Wernicke's encephalopathy also have damage in this region but it is less severe. Neuroimaging studies suggest that the amnesia is probably caused by interruption of complex diencephalic-hippocampal circuitry including thalamic nuclei and mammillary bodies rather than a single lesion in the anterior thalamic nucleus.[56]

Prevention of alcohol-related brain damage

Alcohol-related WKS is still a worldwide problem and case reports are seen regularly in medical journals from every country in the world. Many of the cases have a history of alcohol abuse but other cases are caused by poor nutrition, long-term parenteral feeding, gastrectomy, diets, AIDS, hyperemesis gravidarum and other conditions.[57–62] The Royal College of Physicians (London) recommends that patients admitted to A&E who show evidence of chronic alcohol misuse and are suspected of having a poor diet should be treated with B vitamins intravenously or intramuscularly, especially when the clinical signs are initially masked by drunkenness.[63] Regardless of the cause, the prevalence of WKS can also be reduced by the supplementation of our diets with thiamine in stable foods like bread. This has been done in some countries for many years (USA, UK, Australia) with great success[23] and should be considered by all countries where alcohol-related disorders are common.

Conclusion

Alcohol is widely used and enjoyed throughout most societies, and for many people it forms part of an enjoyable and generally healthy lifestyle that should include good diet and exercise. Recent evidence has confirmed that, at low levels, alcohol has health benefits for some people, particularly in contributing to reducing the risk of heart disease from middle age onwards.[64] It also, however, has the potential to cause much harm, and is second only to tobacco as a cause of drug-related deaths and hospitalizations. People who drink regularly at higher levels place themselves at increased risk of chronic ill health and premature death with damage to various bodily organs, particularly the brain and liver. These patterns of drinking also have substantial social and economic implications, not only for individuals, but also for families, workplaces, and society as a whole. The guidelines listed below were published by the Australian Government in 2000

following a review by a National Health and Medical Research Council panel of experts drawn from appropriate Australian academic, professional, community and government organizations. The report comprised an update and summary of the best available evidence regarding low-risk levels of alcohol consumption and its purpose was to provide background documentation and justification for the development of these guidelines.[65]

A standard drink contains 10 g (equivalent to 12.5 mL) of alcohol.

Recommendation for men
- An *average* of no more than 4 standard drinks a day, and no more than 28 standard drinks over a week;
- not more than 6 standard drinks in any one day;
- 1 or 2 alcohol-free days per week.

Recommendation for women
- An *average* of no more than 2 standard drinks a day, and no more than 14 standard drinks over a week;
- not more than 4 standard drinks in any one day;
- 1 or 2 alcohol-free days per week.

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