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Differential protein expression in the corpus callosum (genu) of human alcoholics

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ABSTRACT

Ethanol is an addictive drug that deteriorates different neuronal pathways in the CNS, leading to the induction of cognitive dysfunction. Neuroimaging analyses revealed that alcohol-induced brain damage appears to be region-specific and major dysmorphology has been observed in the prefrontal cortex and the white matter (WM) particularly in the corpus callosum (CC). Recent diffusion tensor imaging (DTI) analysis indicated that microstructural degradation was prominent in the genu followed by the body and the splenium of the CC. Molecular mechanisms underlying these structural changes are largely unknown. In this study, using 2D electrophoresis based proteomics approach, protein expression profiles in 25 genus samples (12 controls, 7 uncomplicated alcoholics and 6 complicated alcoholics with hepatic cirrhosis) were analysed and compared. Image analysis showed that 35 protein spots in the uncomplicated alcoholic and 56 in the complicated group were differentially altered compared to the control ($P < 0.05$; ANOVA). In total of 91 spots, 25 spots were overlapped between two alcoholic groups. When protein expression profile of the genu was compared with those in other WMs [BA9 white matter (WM) and splenium] the highest number of region-specific proteins was identified in the genus indicating that genu might be the most sensitive and/or vulnerable region to chronic alcohol ingestion at least from the aspect of protein expression. Out of total 66 spots (identified as 50 different proteins), 31 spots (identified as 28 different proteins) were expressed only in the complicated group. This result indicates that alcohol-related liver dysfunction has synergetic effects on brain protein expression. It is also interesting to note that abnormality in thiamine-related cascade which was previously found in the BA9 WM was observed in the genu, but not in the splenium. It is therefore suggested that both hepatic and nutritious factors might be underlying the mechanisms of microstructural damage detected by DTI.

1. Introduction

Substance abuse and dependence have been significant burden on many families as well as the societies. Ethanol is an addictive drug with complex mechanisms of action with a variety of pharmacological features and exerts neurotoxic effects after chronic ingestion. It is widely accepted that the chronic use of alcohol induces various biochemicals in the CNS that can lead to the alteration of neuronal plasticity and/or structural damage (Garige et al., 2006; Buckley et al., 2006; Pfefferbaum et al., 2006a,b). These structural changes are indicated to be associated with wide range of cognitive dysfunction such as deficits in abstract thinking, problem solving, spatial and verbal learning, memory function, attention and perceptual motor skills (Harper and Matsumoto, 2005; Olney, 2002). These abnormalities can severely impair the quality of life of the patients and affect the process of recovery during treatment and prognosis. Some of these cognitive changes appear to be reversible after prolonged abstinence of alcohol (O'Neill et al., 2001; Pfefferbaum et al., 2006a,b).

The corpus callosum (CC) was deeply situated WM connecting both brain hemispheres. It consists of 200–250 million contralateral axonal projections and regulates the information possessing mechanisms. Anatomically the CC has five sub-regions (rostrum, genu, body, isthmus and splenium) and each sub-region has their own cognitive functional activities. Higher order sensory and cognitive information from the prefrontal areas and associative temporo-parietal areas are predominantly transmitted through the genu and the splenium, respectively. Visual, auditory, and somatosensory information are primarily transmitted through the fibres with a larger diameter in the body and isthmus (Aboitiz et al., 1992; De Lacoste et al., 1985; Lamantia and Rakic, 1990). Therefore, total functional activities of the CC are dependent on the collective of individual function of each sub-region and abnormalities of any portion of the CC may affect the whole system.

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Neuropathological studies repeatedly suggested that alcohol induces brain 'shrinkage', in particular the reduction of the WM volume (Schweinsburg et al., 2001; Harper and Matsumoto, 2005). It has been thought that structural components of WM are quite homogenous throughout the brain. However, recent diffusion tensor neuroimaging (DTI) studies revealed that WMs are not equally sensitive to alcohol-induced microstructural degradation. Pfefferbaum et al. (1997) have shown that dysmorphology and microstructural degradation are more dominant in the genu followed by the body and the splenium of alcoholic CC (Pfefferbaum et al., 1997, 2006a,b; Pfefferbaum and Sullivan, 2005; Hommer et al., 2001). These abnormalities of the CC have strong correlation with the deficits of cognitive functions such as interhemispheric exchange of sensory and motor activities, attention and executive function (Pfefferbaum et al., 2006a,b). It is therefore suggested that alcohol-induced volume reduction and disruption of the microstructural integrity of the CC could result in slowing of information processing and interhemispheric transfer delays (Pfefferbaum et al., 2006a,b; Pfefferbaum and Sullivan, 2005). Cellular and molecular mechanisms underlying above-mentioned structural changes in the CC are largely unknown.

Proteomics technology is widely used to study the protein expression in biological system (Li et al., 2008; Alexander-Kaufman et al., 2006, 2007; Kashem et al., 2007). Using this technique, we have previously shown that different mechanisms might be involved in alcohol-induced WMs damage in different brain regions (Matsumoto et al., 2007). In the BA9 WM, thiamine shortage/deficiency, but not hepatic factors were suggested to play an important role in alcohol-induced damage (Alexander-Kaufman et al., 2006, 2007). In contrast, in the splenium of the CC, more hepatic effects and possible lipid peroxidation may be contributing to microstructural disturbance (Kashem et al., 2007). These results suggest us each WM has different sensitivity to alcohol-induced changes/damage through different molecular mechanisms. In the present study, we have used the same approach to determine alteration of protein expression profiles in the genu of both uncomplicated and complicated alcoholic CC.

2. Experimental procedures

2.1. Human brain tissue

We have obtained 25 postmortem genu (anterior region of the CC) samples from the NSW Tissue Resource Centre, University of Sydney. Cases were classified as: (A) control (<20 g of ethanol/day, $n = 12$), (B) uncomplicated alcoholics (>80 g of ethanol/day; $n = 7$) and (C) alcoholics with hepatic cirrhosis-designated as complicated (>80 g of ethanol/day; $n = 6$). In the alcoholic groups no indication of Wernicke Korsakoff Syndrome (WKS) was observed, and hepatic cirrhosis in the complicated group was confirmed by postmortem examination. All of the patients had no history, symptoms or signs of any psychiatric or neurological condition except alcohol-associated disorders. Death of the patients was not related to any dysfunction of the central or peripheral nervous system. Alcoholic cases fulfilled the criteria of the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) for alcohol abuse (Anonymous, 1994). Information of lifetime alcohol consumption was obtained from medical records or from reports by the next of kin. Demographics of each case are shown in Table 1. Ethics approval was obtained from the Sydney South West Area Health Service (protocol no. X03-00285) for use of human brain tissue.

Table 1
Patient demographics

Case	Age (year)	Sex	PMI (h)	Brain pH	Cause of death	Type of beverage	Maximun life-time consumption (kg of 100% ethanol)
Control							
1	37	M	21	6.5	Ischaemic heart disease	Nil	0
2	56	M	24	6.53	Cardiac coronary artery atheroma	Nil	0
3	60	F	30.5	6.8	Ischaemic heart disease	Beer	5.11
4	69	M	16	6.6	Ischaemic heart disease	Beer	12.85
5	73	M	10	6.22	Bowel carcinoma, lung and liver metastasis	Nil	4.08
6	58	M	12	6.46	Ischaemic heart disease	Nil	0
7	56	M	37	6.76	Ischaemic heart disease	Beer	4.53
8	82	M	24	6.4	Sepsis	Beer	2.93
9	66	F	6	6.45	Pneumonia	Nil	0
10	48	M	24	6.24	Ischaemic heart disease	Beer	6.72
11	82	M	36	6.24	Cardiac failure	Beer	8.32
Mean ± S.E.M.	62.45 ± 4.16		21.86 ± 3.08	6.47 ± 0.06			4.04 ± 1.25
Uncomplicated alcoholics							
1	56	M	15	6.66	Ischaemic heart disease, and emphysema Steatosis	Spirits	362.08
2	53	M	60	6.75	Emphysema	Combination	204.4
3	67	M	48	6.4	Acute broncopneumonia	Beer	91.98
4	70	M	62	6.82	Cardiomyopathy	Beer	52.56
5	59	M	24	6.57	Cardiomyopathy	Wine	99.28
6	56	M	45	6.51	Bleeding oesophageal varices due to macrovesicular steatosis	Spirits	362.08
7	50	M	17	6.3	Ischaemic heart disease	Beer	54.75
8	81	M	36	6.44		Beer	65.40
Mean ± S.E.M.	61.5 ± 3.66		38.38 ± 6.51	6.56 ± 0.06			161 ± 47
Alcoholics complicated with hepatic cirrhosis							
1	37	M	17	6.33	Acute alcohol intoxication	Spirits	140.16
2	51	M	46	6.3	Pneumonia	Beer	45.55
3	58	F	48	6.4	Liver failure	Beer	57.82
4	46	M	24	6.5	Acute alcohol intoxication	Beer	45.99
5	80	M	36	6.25	Cardiopulmonary arrest	Beer	65.41
6	67	F	68	6.3	Head injury, hepatic and renal failure	Beer	73.58
Mean ± S.E.M.	56.50 ± 6.28		39.43 ± 7.48	6.36 ± 0.04			71.42 ± 14.28