Mechanisms of Cell Death in Cholinergic Basal Forebrain Neurons in Chronic Alcoholics

K.M. Cullen¹,³ and G.M. Halliday¹,²

Received August 30, 1994; Accepted September 26, 1994

Tau immunoreactivity was examined in post mortem tissue from patients in three groups: neurologically-asymptomatic and neuropathologically normal alcoholics, alcoholics with Wernicke's Encephalopathy (WE) and age matched non-alcoholic controls. Tau-positive granular and fibrillary inclusions were frequently observed within the magnocellular neurons of the cholinergic nucleus basalis, within occasional nucleus basalis neurons in non-WE alcoholics, but not in controls. Tau immunoreactivity was not however observed in cortical, brainstem, diencephalic or non-cholinergic forebrain structures. Peroxidase activity was also examined within the nucleus basalis using diaminobenzidine as an indicator. The majority of neurons in the basal forebrain showed increased peroxidase activity in all WE alcoholics and in some nucleus basalis neurons of non-WE alcoholics, but was rarely seen in controls. Neighboring astrocytes also showed increased peroxidase activity. These results suggest a link between peroxidase activity and the abnormal accumulation of phosphorylated tau. The presence of tau in the nucleus basalis of alcoholics with WE suggests a thiamine-dependent mechanism in tau accumulation and cell death in the cholinergic basal forebrain.

Key words: Neurofibrillary tangles, peroxidase activity, nucleus basalis, thiamine deficiency, alcohol

INTRODUCTION

Numerous studies in laboratory animals and post mortem human tissue have shown that there is a regionally specific neuronal loss due to the toxic effects of chronic ethanol intake. Particular subsets of cortical and subcortical neurons are vulnerable in long term alcoholics, for instance frontal cortex and serotonergic brainstem neurons (Kril and Harper, 1989; Halliday et al., 1993). Nutritional and metabolic insults which frequently accompany

¹ Neuropathology Unit, University of Sydney, Sydney, NSW, Australia 2006
² Prince of Wales Medical Research Institute, Prince of Wales Hospital, Randwick, 2031 Australia
³ To whom correspondence should be addressed at Department of Pathology, University of Sydney, Sydney, Australia 2006.

81

0885-7490/95/0300-0081$07.50/0 © 1995 Plenum Publishing Corporation
alcoholism, such as in thiamine deficiency, may subject additional populations of neurons to stress (Halliday et al., 1995). In fact, the periventricular distribution of lesions with neuronal fallout (in particular thalamic and hypothalamic nuclei) are so distinctive as to be diagnostic in Wernicke's encephalopathy (WE) (Harper, 1979). Many studies have explored the pathogenetic mechanisms that may account for selectivity of lesions in WE. For instance, physiological and toxopharmacological studies have located particular brain regions which have ethanol-inducible and thiamine-dependent enzymes or susceptibility to alcohol metabolites (Hansson et al., 1990; Hazell et al., 1993). Degeneration of the cortically-projecting forebrain cholinergic neurons has also been noted in WE alcoholics, and this is thought to account for at least some of the cognitive dysfunction reported in these patients (Halliday et al., 1995). In the present study, we have examined cell death in the cholinergic system in order to reveal the possible pathogenetic mechanism.

In particular, the formation of neurofibrillary tangles (NFT) in the cholinergic nucleus basalis of Meynert (NbM) is evidence that cell loss in this region involves cytoskeletal disruption in some neurodegenerative diseases. Originally described by Alzheimer in 1907, neurofibrillary degeneration is now recognized in several degenerative conditions of the nervous system, such as progressive supranuclear palsy, dementia pugilistica and hydrocephalus, and has been recently associated with the measles virus in young children with subacute sclerosing panencephalitis (McQuaid et al., 1994; Papasozomenos, 1989b). Neurofibrillary degeneration may therefore indicate a general mechanism of cell death rather than a disease specific process. A major constituent of NFT is an abnormally phosphorylated form of the microtubule-associated protein tau (Kosik, 1993). Studies employing antibodies against tau, as well as a variety of histochemical stains (silver impregnation and nickel peroxidase) have identified stages in the formation of the NFT (Bancher et al., 1991; Lovestone and Anderton, 1992; Cullen, 1994), although the pathogenetic trigger in the formation of these abnormal structures is not yet known.

The aim of the present study was to examine tau immunoreactivity in the cholinergic NbM. The immunohistochemical study was supplemented with an analysis of peroxidase activity in these neurons in order to examine one of the possible pathogenetic mechanisms of cell death.

**MATERIALS AND METHODS**

Sixteen cases were selected on the basis of clinical records and detailed neuropathological examination, as previously described (Halliday et al., 1993; Cullen et al., 1995).

Selection criteria for inclusion in this study were as follows:

1. An alcohol consumption of greater than 80 g per day (chronic alcoholics) or less than 20 g per day (non-alcoholic controls) for most of adult life
2. No neurological, psychiatric or neuropathological abnormalities (eg., stroke, Alzheimer's disease, hepatic encephalopathy) other than those associated with chronic alcoholism (i.e., WE)
3. Postmortem delay less than 72 hours
4. For alcoholics with WE, clinical evidence of thiamine deficiency was required.