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Research article

Morphological changes in the cerebellum as a result of ethanol treatment and cigarette smoke exposure: A study on astrogliosis, apoptosis and Purkinje cells

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ABSTRACT

The link between Ethanol (EtOH) and tobacco (TOB) has potentially important implications for people involved in alcohol treatment; many alcoholics smoke, putting them at high risk of tobacco-related complications. The present study investigates the effect of chronic exposure to cigarette smoke, EtOH consumption and the combination of both on astrogliosis and apoptosis in the cerebellum of rats. Adult male Wistar rats were divided into 4 groups (8 animals per group): vehicle (glucose 3%, 10 mL/kg, twice a day), EtOH treated (EtOH 2 g/kg, twice a day), exposure to cigarette smoke (TOB, smoke of 6 cigarettes, twice a day) and a combination of EtOH and cigarette smoke (TOB + EtOH, twice a day). The treatment period was 57 days, after which the animals were euthanized, the cerebellum removed and subjected to immunohistochemical studies focusing on glial fibrillary acidic protein (GFAP), cleaved caspase-3, and S100. We also counted the number of Purkinje cells (PC) present following treatment. The combination of both EtOH and TOB exposure induced an increase in GFAP immunoreactivity, whilst TOB alone increased apoptosis in the white matter of the cerebellum. In addition, EtOH consumption reduced the number of PC and TOB tempered this effect. Overall, the present study opens up relevant perspectives for the consequences on human health of the combined use of alcohol and smoking, by demonstrating the biological mechanisms and cerebellar function vulnerabilities to combined use and dependence of licit drugs.

1. Introduction

Ethanol (EtOH) and tobacco (TOB) are the most highly consumed licit drugs in the world, despite public policies aimed at preventing and limiting their consumption. Over 90% of the population dependent on EtOH are also smokers, usually classified as heavy smokers [1]. The abuse of EtOH and TOB is a major risk factor for the development of pulmonary and heart disease and cancers as well as major changes in the central nervous system (CNS) [1,2]. EtOH impairs the function of neurons and glial cells, affecting a wide range of features, including neuronal survival, cell migration, differentiation, and alterations in motor function [3].

The historical view of the cerebellum as a coordinator of motor functions has changed drastically throughout the past few decades. Neuroanatomical and neuroimaging studies have identified bidirectional pathways that connect the cerebellum to cortical association areas, and the implications of cerebellar neurocognition continue to be investigated; although, little is known about the role of the brainstem in cognitive and behavioral processing. Keschner and colleagues were the first to report behavioral symptoms associated with brainstem lesions [4]. They found that “mental symptoms” such as apathy, drowsiness, torpidity and disturbances of affect frequently occur in patients with tumors of the brainstem [5]. Besides, the cerebellum has also been considered as a primary site of EtOH toxicity because of its atrophy related to loss and alterations of Purkinje cells [6]. Apoptosis triggered by EtOH toxicity is initiated by a cascade of molecular events resulting in caspase-3 activation [7], this activation leads to DNA cleavage and has a crucial role in tissue remodeling during development, contributing to cell loss in neurodegeneration [8]. The relationship between Purkinje cell loss and astrogliosis has also been a focus in understanding the mechanisms involved in EtOH toxicity and cell death in conditions of neurodegeneration [9]. All the changes caused by EtOH can compromise the cerebrocerebellar system and impair the coordinate functions related to non-verbal communication, including

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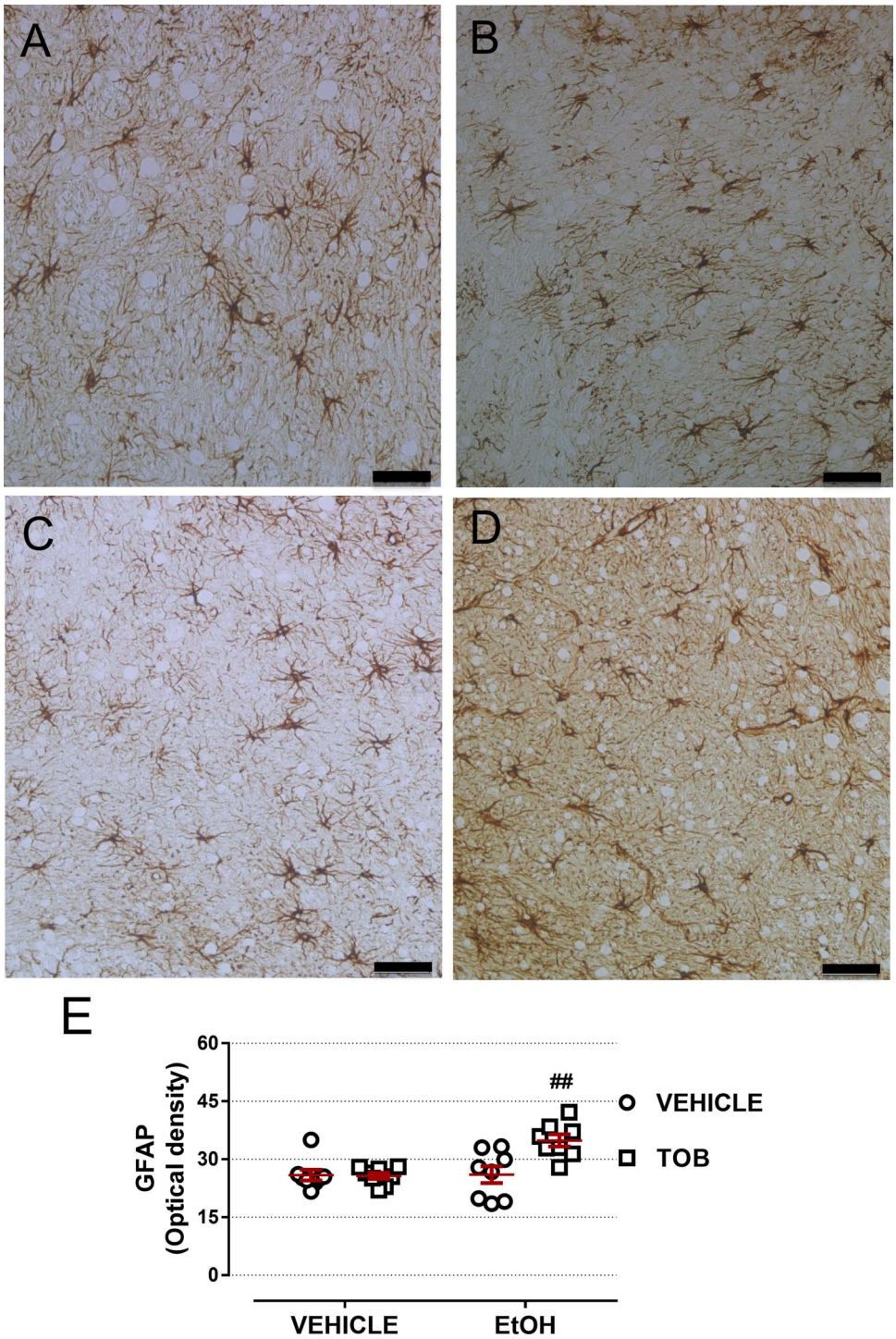

Fig. 1. Effect of exposure to cigarette smoke (TOB), ethanol treatment (EtOH) and association between drugs (TOB + EtOH) on the immunoreactivity of glial fibrillary acidic protein (GFAP, optical density) in the cerebellum of rats. Vehicle (image A, glucose 3%), EtOH (image B, 2 g/kg, twice a day), cigarette smoke exposure (image C, smoke of 6 cigarettes, twice a day) and association of EtOH and TOB (TOB + EtOH, image D). Graph E shows the optical density to GFAP. Black bars represent the size of 50 μm (objective lens 20 ×, field area: 100,000 μm^2). Data analyzed by Two-Way ANOVA and expressed by mean ± SEM (n = 8 animals by group). (## Denotes significant interaction between treatments, $P < 0.01$).

control of motor actions, mood states, and unconscious motivation [10].

Smoking has been described to be contributive and causative for a numerous diseases, many of which lethal. For example lung cancer and stroke. Exposure to cigarette smoke leads to lesions in the airways and increases the levels of inflammatory mediators in the blood stream [11]. Nicotine and its metabolites, present in cigarette smoke, are responsible for the cigarette's psychoactive action [12]. Other constituents, such as isoprene, formaldehyde, acetaldehyde, acetonitrile, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NKK) [13], are present in cigarette smoke and lead to oxidative stress [14], neurotoxicity, and neuroinflammation [15].

Intoxication by cigarette smoke and chronic consumption and ethanol is able to affect neurochemistry and glial functions. Astrocytes or astroglia are the most numerous and versatile type of glial cell and play a central role in building and maintaining the blood-brain barrier to restrict the passage of soluble molecules and toxic substances from the blood to the brain [16,17]. Astrocytes respond to neuronal injury,