Original Article

Potassium Bromate-induced Changes in the Adult Mouse Cerebellum Are Ameliorated by Vanillin*

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Abstract

Objective The current study aimed to elucidate the effect of vanillin on behavioral changes, oxidative stress, and histopathological changes induced by potassium bromate ($KBrO_3$), an environmental pollutant, in the cerebellum of adult mice.

Methods The animals were divided into four groups: group 1 served as a control, group 2 received $KBrO_3$, group 3 received $KBrO_3$ and vanillin, and group 4 received only vanillin. We then measured behavioral changes, oxidative stress, and molecular and histological changes in the cerebellum.

Results We observed significant behavioral changes in $KBrO_3$-exposed mice. When investigating redox homeostasis in the cerebellum, we found that mice treated with $KBrO_3$ had increased lipid peroxidation and protein oxidation in the cerebellum. These effects were accompanied by decreased Na^+-K^+ and Mg^{2+} ATPase activity and antioxidant enzyme gene expression when compared to the control group. Additionally, there was a significant increase in cytokine gene expression in $KBrO_3$-treated mice. Microscopy revealed that $KBrO_3$ intoxication resulted in numerous degenerative changes in the cerebellum that were substantially ameliorated by vanillin supplementation. Co-administration of vanillin blocked the biochemical and molecular anomalies induced by $KBrO_3$.

Conclusion Our results demonstrate that vanillin is a potential therapeutic agent for oxidative stress associated with neurodegenerative diseases.

Key words: Cerebellum; Behavior; Potassium bromate; Vanillin; ATPases; Genes expression

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INTRODUCTION

At the beginning of the 20th century, the cerebellum was thought to solely be a modulator of motor function, including coordination, diadochokinesia, tonus, and motor speech production[1]. Several neuronal systems are involved in body motion, but the cerebellum is specifically implicated in the voluntary movement. The cerebellum controls the activation, timing, and coordination of distinct muscle groups during body motion. The traditional view of the cerebellum solely

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as a coordinator of motor function has been substantially redefined in the past decades. Neuroanatomical, neuroimaging, and clinical studies have found that the cerebellum is further involved in the modulation of cognitive and affective processing. Neuroanatomical studies have demonstrated cerebellar connectivity with supratentorial areas involved in higher cognitive and affective functioning, while functional neuroimaging and clinical studies have provided evidence of cerebellar involvement in a variety of cognitive and affective tasks. Neuroanatomical studies have also revealed that the cerebellum is linked in a reciprocal way to the autonomic, limbic, and associative regions of the supratentorial cortex[2].

The cerebellum was chosen as a region of interest for this study because it has higher susceptibility to oxidative stress than other brain regions[3,4]. In fact, because of its sensitivity to oxidative stress, the cerebellum is known to be vulnerable to damage induced by several chemicals, including xenobiotics. Xenobiotics are important for the production of free hydroxyl radicals in neurodegenerative disorders[5,6]. Exposure to xenobiotics during development has become an important public health concern because of their possible impact on the development and programming of organ functions[7]. In the cerebellum, reactive oxygen species (ROS) are well known for their role in the pathogenesis of primary neurodegenerative diseases, such as amyotrophic lateral sclerosis[8] and Alzheimer's disease[9]. It has been postulated that neuronal death in these diseases may be mediated by oxidative stress caused by the aberrant metabolism of superoxides. Converging evidence suggests that the cerebellum may also be implicated in anxiety disorders. Potassium bromate ($KBrO_3$) is an oxidizing halogen that has long been used as a food additive, mainly for bread making. In addition, $KBrO_3$ is used worldwide as a neutralizer in home permanent cold wave hair kits[10]. Potassium bromate is known to cause severe and irreversible sensorineural hearing loss as well as renal failure. Moreover, it is thought to be a carcinogen, causing chromosome aberrations and 8-hydroxydeoxyguanosine generation, and is capable of both initiating and promoting rat renal tumorigenesis[11]. Although the adverse effect of $KBrO_3$ on auditory function in animals has been well described[10,12], to our knowledge, there are no reports on the effects of $KBrO_3$ on the cerebellum.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is an antioxidant compound isolated from the bean and pod of the tropical vanilla orchid and is widely used in the food and beverage industry[13]. This compound is also used for the synthesis of agrochemicals, antifoaming agents, and pharmaceutical products[14]. Vanillin has been shown to have choleretic, antifungal, antimutagenic, and anticancer effects[15-17] as well as protective effects on the liver and kidney[18,19]. Most of these effects are attributed to the antioxidant activity of vanillin. Recently, it has been shown that vanillin has neuroprotective effects[20]. Therefore, the role of vanillin in the prevention of neurodegenerative diseases is of great interest. As such, the main goal of this study is to assess oxidative imbalance in the mouse cerebellum after two weeks of $KBrO_3$ exposure. Further, we also explore if vanillin can attenuate any of these effects. To do this, we evaluate the protective role of vanillin on oxidative stress regulation, genes expression of select antioxidant enzymes, and we measure the pro-inflammatory cytokines.

MATERIAL AND METHODS

Animal Diet and Tissue Preparation

This study was performed in accordance with the Institute Ethical Committee for the Care and Use of Laboratory Animals guidelines[21]. Adult mice (40 ± 5 g, 4 weeks old, n = 12) were kept in an air-conditioned room (temperature 22 ± 3 °C, relative humidity 40%). They were housed in polycarbonate cages and had a daily standard pellet diet (SNA, Sfax, Tunisia) and water *ad libitum*. The mice were divided into four groups; each group received either no treatment (control group), 2 g/L $KBrO_3$ (Sigma Chemical Co., St. Louis, MO, USA) in drinking water ($KBrO_3$ group), 100 mg/kg body weight vanillin by intraperitoneal injection (vanillin group), or both $KBrO_3$ and vanillin ($KBrO_3$ + vanillin group). $KBrO_3$ and vanillin doses were selected based on previous studies[22,23]. After 15 days of treatment, the animals were sacrificed by cervical decapitation to avoid stressing the animal. All animals were anesthetized with an intraperitoneal injection of chloral hydrate solution (3.6%) before being sacrificed. The cerebellum was quickly excised, rinsed in Tris-HCl buffer, weighed, and then divided into three parts. One part was homogenized in Tris-HCl buffer, as indicated in the procedures of