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Methylmercury affects cerebrovascular reactivity to angiotensin II and acetylcholine via Rho-kinase and nitric oxide pathways in mice

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ABSTRACT

Aims: Methylmercury (MeHg) exposure results in increased risk of hypertension and cardiovascular diseases. In this study, we aimed to investigate whether the in vivo exposure of MeHg in mice affects blood pressure and basilar arterial responses to angiotensin II (Ang II) and acetylcholine (ACh), which are important modulators of cerebrovascular autoregulation.

Main methods: Mice were exposed to MeHg (40 ppm) in drinking water for 21 days. Blood pressure was measured in conscious mice by an indirect tail-cuff method. Functional studies of the isolated arteries' response to vasoactive substances were performed using a micro-organ-bath system.

Key findings: Systolic and mean blood pressures were significantly increased after 2 and 3 weeks of treatment with MeHg, respectively. Ang II-induced contraction in an isolated basilar artery, which is mediated via Rho-kinase activation, was increased in MeHg-treated mice. ACh-induced relaxation, which is dependent on NO production from the endothelial cells, was decreased in MeHg-treated mice. However, alterations of vascular responses to Ang II and ACh were not observed in the isolated thoracic aorta.

Significance: This study demonstrated that the cerebral vasculature appears to be particularly sensitive to in vivo exposure of MeHg. Our results suggest that in vivo MeHg increases blood pressure and causes alterations in the cerebrovascular reactivity in response to Ang II and ACh through enhancement of Rho-kinase activity and inhibition of NO bioavailability, respectively.

1. Introduction

Methylmercury (MeHg) is a potent environmental toxic pollutant that is generated by bacterial methylation of inorganic mercury in the aquatic environment [1]. It is a well-documented neurotoxicant in both humans and experimental animal models [2]. For example, prenatal MeHg intoxication has been associated with neurodevelopmental disorders such as mental retardation and motor and cognitive dysfunction [3]. Experimental animal studies likewise showed deficits resulting from prenatal MeHg exposure in corresponding behavioral domains including deficits in learning, discrimination/transition reversal and working memory, increased perseverative behavior, and increased behaviors interpreted as anxiety [4]. The central nervous system is the main target organ of MeHg toxicity, especially when exposure occurs during the early stages of brain development. Even though the developing brain has been considered the critical target organ of MeHg toxicity in children, recent evidence indicates that the cardiovascular system may be a sensitive organ in adults [5]. In humans, cardiovascular dysfunction by MeHg exposure including myocardial infarction [6], heart rate variability, atherosclerosis, coronary heart disease, and hypertension [7] had also been reported.

Clinical and experimental studies indicate that oxidative stress contributes to the development of hypertension in humans [8] and animals [9]. It has been reported that the harmful effects of mercury are mediated by the excessive release of reactive oxygen species (ROS) and the reduction of antioxidant defenses, resulting in inactivation of important enzymes that are responsible for the body's defenses, including glutathione reductase, glutathione peroxidase, superoxide dismutase, catalase, and glutathione in different organs [10].

In the cardiovascular system, the vascular endothelium is highly sensitive to oxidative stress [11], and is recognized as a fundamental homeostatic organ for the regulation of vascular tone and structure. Under physiologic conditions, endothelial stimulation induces the production and release of nitric oxide (NO), which diffuses to the surrounding tissues and cells. NO exerts its cardiovascular protective role by relaxing smooth muscle cells, preventing leukocyte adhesion and migration into the arterial wall, muscle cell proliferation, platelet adhesion and aggregation, and adhesion molecule expression [12]. Oxidative stress caused by mercury exposure decreases the bioavailability of NO and

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alters the expression of endothelial NO synthase, which result in increased vasoconstriction and the reduction of the endothelial vasodilator response [11,13].

Conversely, activation of Rho-kinase is thought to be a key mechanism of calcium sensitization that regulates vascular responses to several vasoconstrictors [14]. ROS activate Rho-kinase by activation of Rho in the rat aorta in vitro [15], and also increase Rho-kinase activity via a redox-regulated, positive-feedback mechanism in human and rabbit ductus arteriosus [16]. In spontaneously hypertensive rats, augmented contribution of Rho-kinase activity to cerebral vascular tone has been reported [17]. In addition, increased Rho activity and Rho-kinase expression in cultured aortic smooth muscle cells from spontaneously hypertensive rats have been reported [18]. It has been suggested that Rho-kinase pathway negatively regulates NO bioavailability. Upregulation of Rho-kinase activity was accompanied by downregulation of eNOS expression and activity in patients with chronic obstructive pulmonary disease [19]. Together, these studies suggest that ROS play an important role in the regulation of vascular constriction and relaxation through augmentation of Rho-kinase activity and a decrease in NO availability both in vitro and in vivo.

In the present study, we investigated the effect of MeHg exposure on the cardiovascular system, by analyzing blood pressure and vascular responses to angiotensin II (Ang II) and acetylcholine (ACh), using mice treated with MeHg in vivo. Ang II is a potent vasoconstrictor that induces contraction via activation of the Rho-kinase signaling pathway [14] and ACh-induced endothelium-dependent and NO-mediated relaxation [20,21]. In addition, the effect of combined treatment with tempol, an antioxidant, and MeHg were observed.

2. Material and methods

2.1. Animals

Adult male ICR mice (12 week-aged) were used for the study, and were obtained from Kyudo (Kumamoto, Japan). The mice were maintained under controlled environmental conditions (12-h light/dark cycle, 23 ± 2 °C) with access to food and water ad libitum. Mice were divided into four groups: 1) Control (no treatment); 2) MeHg; 3) tempol; and 4) MeHg plus tempol. MeHg (40 ppm) [22] and tempol (2 mM) were administered simultaneously with the drinking water for 21 days. It has been reported that this concentration of temple prevent ROS mediated endothelial dysfunction in rats and mice [23,24]. The body weight and water intake were measured every second day. Significant differences in body weight and visible clinical signs, such as abnormal locomotion or hind limb paralysis, were not observed in the four groups during

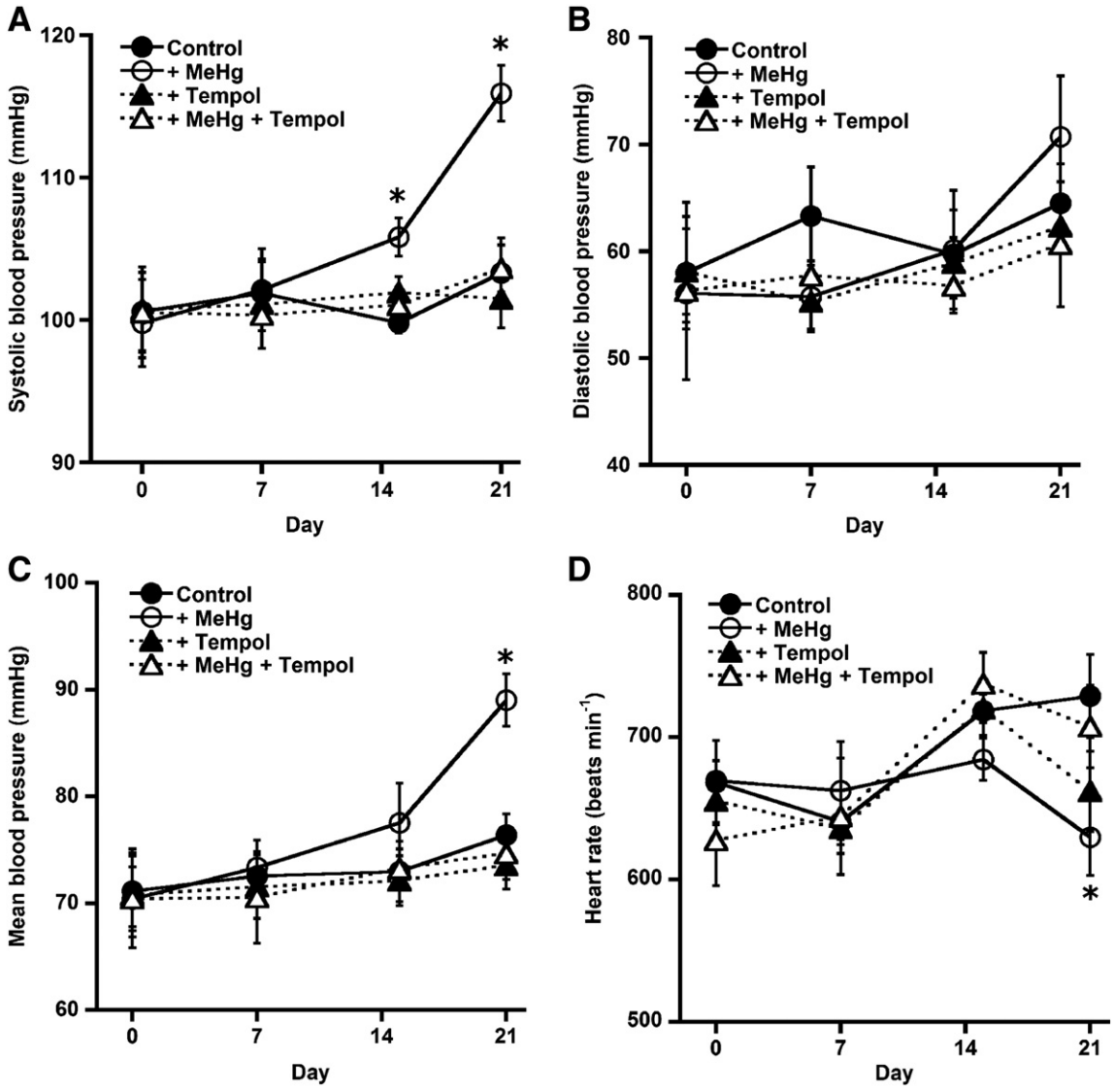

Fig. 1. Effect of in vivo MeHg treatment on blood pressure and heart rate. MeHg or tempol was administered in the drinking water for 21 days. The systolic [A], diastolic [B], and mean [C] blood pressure and the heart rate [D] were measured each week in conscious mice using an indirect tail-cuff method. Each point represents the mean ± SEM of five mice. *P < 0.05 vs. control.