Zn deficiency and hypertension

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Summary

Levels of systolic blood pressure (BP) observed immediately before the start of dietary conditioning were significantly higher in spontaneously hypertensive rats (SHR) than in Wistar Kyoto rats (WKY). However, levels of systolic BP and basal mean arterial pressure (MAP) observed at the end of dietary treatment for 4 weeks were SHR fed a Zn-deficient diet > SHR fed a standard diet > WKY fed a standard diet ≒ WKY fed a Zn-deficient diet. Administration of the nitric oxide synthase (NOS) inhibitor, L-NAME caused an increase in MAP levels in SHR fed a standard or a Zn-deficient diet, demonstrating the involvement of the vasodilator, nitric oxide (NO), in the regulation of systemic BP in a genetically hypertensive state. On the other hand, administration of the superoxide scavenger, tempol, led to a decrease in MAP levels in SHR fed a standard or a Zn-deficient diet, indicating the participation of the oxygen free radical, superoxide, in an increase in systemic BP in a genetically hypertensive state. As reported recently, the mechanism involved may be due to a decrease in the action of the vasodilator, NO, based on the formation of peroxynitrite coming from the non-enzymatic reaction of superoxide and NO. In addition, tempol treatment completely restored MAP levels in SHR fed a Zn-deficient diet to levels comparable to those observed in SHR fed a standard diet, indicating that a further increase in systemic BP levels seen in SHR fed a Zn-deficient v.s. a standard diet may be brought by a reduction in the action of the vasodilator, NO, resulting from an increase in superoxide. The activity of the superoxide scavenger, Cu/Zn-superoxide dismutase (SOD), in the thoracic aorta was significantly decreased in SHR fed a Zn-deficient diet relative to SHR fed a standard diet. It appears that a decrease in the activity of Cu/Zn-SOD observed in the thoracic aorta of SHR fed a Zn-deficient diet at least in part plays a role in an increase in superoxide in this model. Thus, Zn deficiency may be a crucial factor to develop genetic hypertension presumably through the oxidative stress caused by superoxide.

Introduction

Zinc (Zn) as well as copper (Cu) is an essential trace element in humans and animals[1]. Over 300 enzymes require Zn for showing their activities[1]. The superoxide scavenger, Cu/Zn-superoxide dismutase (SOD), is one of the Zn-requiring enzymes[1]. Ordinarily, the activity of Cu/Zn-SOD is therefore decreased in a Zn-deficient state[1]. It is known that a decrease in the activity of Cu/Zn-SOD in the vessel wall results in an increase in superoxide[2]. The increase in superoxide in the vessel wall causes a reduction in the action of the vasodilator, NO, through the formation of peroxynitrite resulting from the non-enzymatic reaction of superoxide and NO, consequently resulting in an elevation in systemic blood pressure (BP) via a decrease in the activity of the soluble guanylate cyclase/cGMP pathway[3]. However, it has not been evident as yet whether Zn deficiency affects systemic BP levels via a fall in the activity of Cu/Zn-SOD.

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The aim of this study was to examine whether Zn deficiency influences systemic BP levels in a genetically hypertensive state. We therefore measured systolic BP, mean arterial pressure (MAP) and the activity of Cu/Zn-SOD in the thoracic aorta using spontaneously hypertensive rats (SHR).

Methods

1. Both Wistar Kyoto rats (WKY) and SHR (8 weeks old) were divided into two groups each and pair-fed either a standard diet containing 0.02% Zn or a Zn-deficient diet with no addition of Zn for 4 weeks[4]. The food consumption was monitored daily over the dietary conditioning. The quantity of diet ingested was comparable in the four groups of rats.

2. Systolic BP was examined in conscious rats that were restrained in a digital thermoholder (37℃) by means of the tail-cuff method using a programmable BP-95A sphygmomanometer (Softron, Tokyo, Japan). Systolic BP evaluated was measured immediately before the start of dietary manipulation (week 0) and at 2 and 4 weeks after the initiation of dietary treatment. Levels of systolic BP were determined by averaging 10 values recorded. The first value measured was not utilized for the determination of mean systolic BP levels.

3. PE-10 Polyethylenecatheters (Beckman Dickson, Spark, MD, USA) filled with heparinized saline were placed around the femoral vein and the abdominal aorta via the femoral artery. MAP was measured through the placed arterial catheter connected to a Nihon Koden pressure transducer (Tokyo, Japan).

Protocol 1. Basal MAP levels were recorded for 30 min. Successively, changes in MAP levels after suppressing the biosynthesis of the vasodilator, NO, were examined for 30 min by injecting 10 mg/kg of the nitric oxide synthase (NOS) inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME), dissolved in saline through the placed venous catheter.

Protocol 2. Using another group of rats, changes in MAP levels after scavenging the oxygen free radical, superoxide, were explored for 30 min following the measurements of basal MAP levels by treating with 100 μmol/kg of the superoxide scavenger, 4-hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl (tempol), dissolved in saline through the placed venous catheter.

4. The activity of Cu/Zn-SOD in the thoracic aorta was determined according to the protocol provided by OXIS Health Products, Inc. (Portland, OR, USA) by measuring at 525 nm the optical density of a chromophore generated by the SOD-mediated autoxidation of the substrate 5,6,6a,11b-tetrahydro-3,9,10 -trihydroxybenzo[c] fluorine.

Results

1. As shown in Fig. 1, levels of basal systolic BP (week 0) were significantly higher in SHR fed a standard (n=12) or a Zn-deficient (n=12) diet than in WKY fed the respective (n=12 in each group) diets. SHR fed a Zn-deficient diet had a progressive increase in systolic BP levels during the dietary conditioning. SHR fed a standard diet showed a significant increase in systolic BP levels above the basal level at 2 weeks following the start of dietary treatment. Thereafter, the rats exhibited no substantial changes in systolic BP levels. Levels of systolic BP obtained at 2 and 4 weeks following the initiation of dietary manipulation were significantly higher in SHR fed a Zn-deficient diet than in SHR fed a standard diet. However, there were no significant differences in systolic BP levels throughout the experimental period between WKY fed a standard and a Zn-deficient diet. Also, the two groups of rats had no substantial

changes in systolic BP levels during the dietary conditioning.

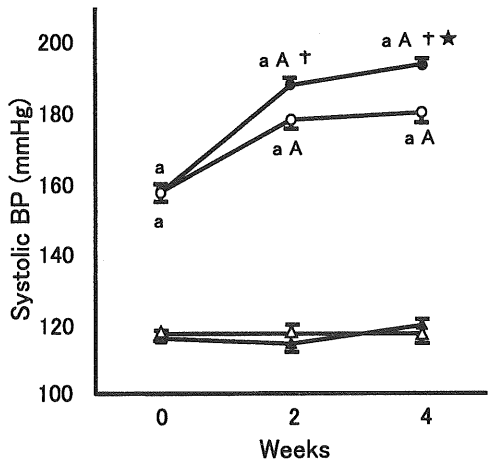

Fig. 1 Time course of systolic BP obtained from WKY and SHR fed a standard or a Zn-deficient diet for 4 weeks (△, WKY fed a standard diet; ▲, WKY fed a Zn-deficient diet; ○, SHR fed a standard diet; ●, SHR fed a Zn-deficient diet). Data reported represent means ± S.E. of the values obtained from twelve rats in each group. Statistical analysis was based on ANOVA with Student's t-test. (a) $P<0.005$ compared with each value of WKY fed a standard or a Zn-deficient diet. (A) $P<0.005$ compared with each basal value measured immediately before the start of dietary treatment (week 0). (*) $P<0.05$ compared with each value measured at 2 weeks following the start of dietary treatment. (+) $P<0.005$ compared with each value of the standard diet group.

2. As shown in Fig. 2A, basal MAP levels were significantly higher in SHR fed a Zn-deficient diet (n=8) than in SHR fed a standard diet (n=8). In the two groups of rats, basal MAP levels paralleled systolic BP levels obtained at 4 weeks following the initiation of dietary conditioning.

Administration of L-NAME significantly augmented MAP levels in SHR fed a standard (n=8) or a Zn-deficient

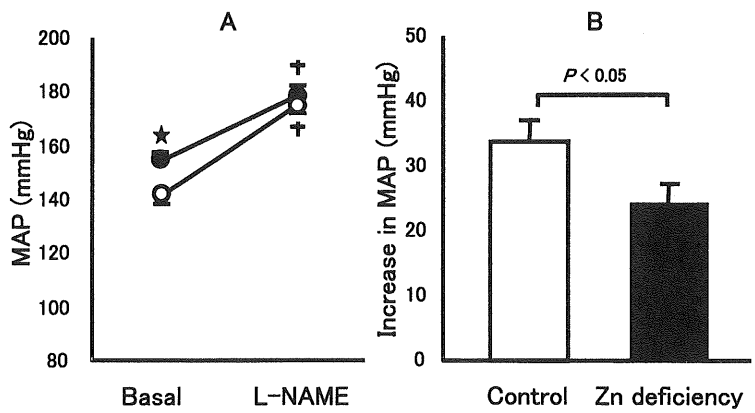

Fig. 2 Effects of L-NAME treatment on MAP seen in SHR fed a standard or a Zn-deficient diet for 4 weeks. The panel (A) presents MAP levels measured before and after administration of L-NAME (○, SHR fed a standard diet; ●, SHR fed a Zn-deficient diet). The panel (B) presents an absolute increase in MAP levels seen after administration of L-NAME (□, SHR fed a standard diet; ■, SHR fed a Zn-deficient diet). Data reported represent means ± S.E. of the values obtained from eight rats in each group. Statistical analysis was based on ANOVA with Student's t-test. (*) $P<0.005$ compared with each value of SHR fed a standard diet. (+) $P<0.005$ compared with each basal value of SHR fed a standard or a Zn-deficient diet.

(n=8) diet. There were no significant differences in MAP levels seen after L-NAME treatment between SHR fed a standard and a Zn-deficient diet (A). Consequently, an absolute increase in MAP levels, showing the difference between the MAP values obtained before and after L-NAME treatment, was significantly greater in SHR fed a standard diet than in SHR fed a Zn-deficient diet (B).

3. As shown in Fig. 3A, the results of basal MAP levels obtained from SHR fed a standard (n=7) or a Zn-deficient (n=7) diet were similar to those presented in Fig. 2A. Tempol treatment significantly decreased MAP levels in SHR fed a standard or a Zn-deficient diet. There were no significant differences in MAP levels seen following tempol administration between SHR fed a standard and a Zn-deficient diet. The degree of an absolute decrease in MAP levels, exhibiting the difference between the MAP values obtained before and after tempol treatment, in the two groups of rats was SHR fed a Zn-deficient diet > SHR fed a standard diet (B).

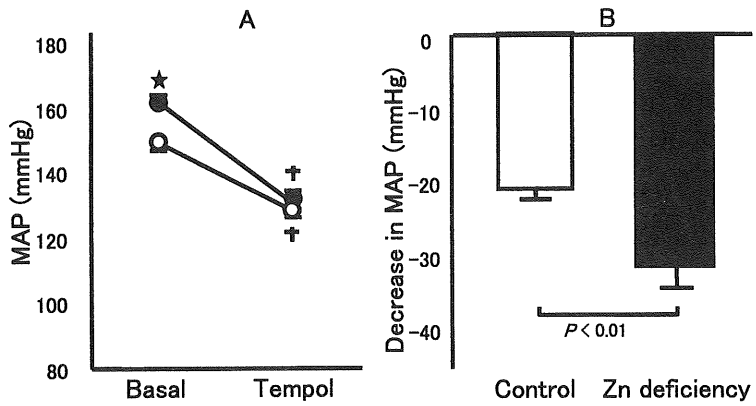

Fig. 3 Effects of tempol treatment on MAP seen in SHR fed a standard or a Zn-deficient diet for 4 weeks. The panel (A) presents MAP levels measured before and after administration of tempol (○, SHR fed a standard diet; ●, SHR fed a Zn-deficient diet). The panel (B) presents an absolute decrease in MAP levels seen after administration of tempol (□, SHR fed a standard diet; ■, SHR fed a Zn-deficient diet). Data reported represent means ± S.E. of the values obtained from seven rats in each group. Statistical analysis was based on ANOVA with Student's t-test. (*) P<0.005 compared with each value of SHR fed a standard diet. (+) P<0.005 compared with each basal value of SHR fed a standard or a Zn-deficient diet.

4. As shown in Fig. 4, the activity of Cu/Zn-SOD in the thoracic aorta was significantly greater in SHR fed a standard diet (n=7) than in SHR fed a Zn-deficient diet (n=7).

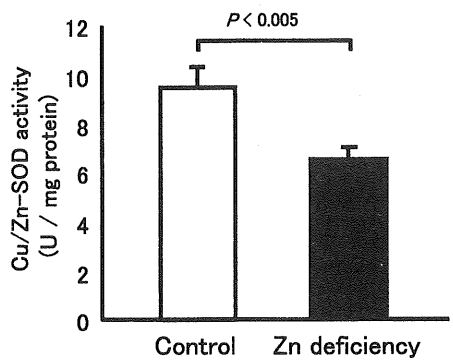

Fig. 4 Activities of Cu/Zn-SOD in the thoracic aorta of SHR fed a standard (□) or a Zn-deficient (■) diet for 4 weeks. Data reported represent means ± S.E. of the values obtained from seven rats in each group. Statistical analysis was based on Student's t-test.

Discussion

1. Unlike SHR fed a standard diet, SHR fed a Zn-deficient diet exhibited a progressive increase in systolic BP levels during the dietary conditioning. Consequently, SHR fed a Zn-deficient diet had a significant increase in systolic BP levels at 2 and 4 weeks following the start of dietary manipulation when compared with SHR fed a standard diet. In two groups of rats, there was a parallel relationship between basal MAP levels and systolic BP levels measured at the end of dietary treatment (Figs. 1, 2A and 3A). These findings indicate that Zn-deficiency exacerbates hypertension in SHR.

2. The vasodilatory gas, NO, derived from eNOS, which primarily exists in vascular endothelial cells, participates in the regulation of systemic BP[5]. In the present study, administration of the NOS inhibitor, L-NAME, led to an elevation in MAP levels in SHR fed a standard or a Zn-deficient diet. This observation demonstrates the involvement of NO in the regulation of systemic BP in a genetically hypertensive state.

3. Tempol, which is known as a genuine SOD-mimetic, is a superoxide scavenger[6]. We showed in the present study that an intravenous injection of tempol caused a fall in MAP levels in SHR fed a standard or a Zn-deficient diet. Surprisingly, tempol treatment completely restored increased levels of MAP in SHR fed a Zn-deficient diet to levels seen in SHR fed a standard diet. The degree of an absolute decrease in MAP levels following tempol administration was SHR fed a Zn-deficient diet > SHR fed a standard. These observations demonstrate that the oxygen free radical, superoxide, may be a modifier of systemic BP in a genetically hypertensive state. The mechanism involved may result from a decrease in the action of the vasodilator, NO, based on the formation of peroxynitrite[3]. In addition, a further elevation in systemic BP levels observed in SHR fed a Zn-deficient v.s. a standard diet may be brought by an increase in superoxide, probably at least in part coming from a fall in the activity of Cu/Zn-SOD caused in the vessel wall by Zn deficiency. Thus, Zn deficiency may be a crucial factor to develop genetic hypertension presumably through an increase in the oxygen free radical, superoxide, and the resultant decrease in the action of the vasodilator, NO.

References

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