

A possible role of plasma aldosterone in hypotension secondary to iron-deficiency anaemia combined with zinc deficiency in rats

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Abstract

Patients with Fe-deficiency anaemia are often afflicted by hypotension. However, the mechanism of secondary hypotension in Fe-deficiency anaemia is unknown. To investigate the pathogenesis of secondary hypotension in Fe-deficiency anaemia, we examined the effects of Fe deprivation on plasma aldosterone concentration and blood pressure in rats. A total of forty 4-week-old male Sprague–Dawley rats were assigned into four treatment groups of ten each for the 4-week study: Fe-deficient group (FD), Zn-deficient group (ZD), Fe/Zn-deficient group (FZD) and control group (CON). At days 26 and 27, blood pressure was measured by the tail-cuff method. Plasma aldosterone concentration was determined by ELISA. The data were analysed by Tukey's multiple comparison test. Rats in the FZD had significantly lower mean blood pressure ($P < 0.01$) and diastolic blood pressures ($P < 0.05$) and plasma aldosterone concentration ($P < 0.01$) compared to the CON. These results suggest that blood pressure is decreased in Fe-deficiency anaemia combined with Zn deficiency partly due to decreased circulating aldosterone concentrations in addition to decreased haematocrit.

Key words: Aldosterone: Blood pressure: Iron-deficiency anaemia: Zinc deficiency

Fe deficiency has been a very important health problem from the nineteenth century^(1–3). Even today, Fe deficiency is the most common nutritional disorder that requires effective measures for prevention⁽⁴⁾. The WHO estimated that two billion people worldwide are suffering from Fe-deficiency anaemia, and four billion people or about 65% of the world population from latent Fe deficiency or Fe deficiency without anaemia⁽⁵⁾. It is well known that Fe deficiency induces lethargy including decreased work performance⁽⁶⁾ and cognitive performance⁽⁷⁾, and deteriorates homeostatic control of heat production^(8–11).

Anaemia is known to cause hypotension⁽¹²⁾. The accepted mechanism of anaemia-induced hypotension in general medical textbooks⁽¹³⁾ is a reduction in peripheral vascular resistance because of the decreased blood viscosity due to the reduced haematocrit based on Poiseuille's (or Hagen–Poiseuille) law^(14,15). However, Whittaker's classic study found that haematocrit less than 40% does not much affect apparent viscosity *in vivo* measured in a canine hindlimb, although haematocrit more than 40% does increase apparent viscosity *in vivo*^(16,17). Hypoaldosteronism generally evokes hypotension^(18,19). However,

plasma aldosterone concentration in Fe deficiency was not reported.

The experiment described here was designed to determine the effect of Fe deficiency on blood pressure and plasma aldosterone concentration in rats, and to test whether aldosterone is involved in the pathogenesis of secondary hypotension in Fe-deficiency anaemia. The combination of Fe deficiency and Zn deficiency was also examined, because the co-occurrence of Fe and Zn deficiencies has been found in infants⁽²⁰⁾, adolescents⁽²¹⁾ and young women^(22,23).

Experimental methods

Animals and diets

A total of forty 4-week-old male Sprague–Dawley rats were purchased from Japan SLC, Inc., Shizuoka, Japan. The animals were randomly assigned into four dietary treatment groups with an equal mean starting body weight (79.7–79.8 g, SEM 0.8 g). All the animals were housed individually in stainless steel cages and given free access to deionised water and diets. The room was

Abbreviations: CON, control group; FD, Fe-deficient group; FZD, Fe/Zn-deficient group; ZD, Zn-deficient group.

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maintained at 22°C and 50% relative humidity with a 12 h light (07.00–19.00 hours)/dark (19.00–07.00 hours) cycle.

Diets were made using milk casein (vitamin-free casein; Sigma-Aldrich Fine Chemicals, St Louis, MO, USA) and were washed with EDTA (Nacalai Tesque, Kyoto, Japan) solution and deionised water (MilliQ; Millipore, Billerica, MA, USA)⁽¹¹⁾. A control diet with ingredients that followed the AIN-93G formulation⁽²⁴⁾ was fed to the control group (CON). Experimental diets were prepared by modifying the AIN-93G formulation. An Fe-deficient diet contained no supplemental ferric citrate and was fed to the Fe-deficient group (FD). A Zn-deficient diet was supplemented with 4.5 mg Zn/kg instead of 30 mg Zn/kg as zinc carbonate in the AIN-93G formula and was fed to the Zn-deficient group (ZD). The Fe/Zn-deficient diet contained no supplemental Fe with supplemental 4.5 mg Zn/kg and was fed to the Fe/Zn-deficient group (FZD). Diets were analysed by inductively coupled plasma-MS. The measured Fe content was 2.9 and 35.7 mg/kg in diets without and with supplemental Fe, respectively. The measured Zn content was 4.5 and 29.1 mg/kg in diets with supplemental 4.5 and 30 mg Zn/kg, respectively.

At days 26 and 27, blood pressures (systolic blood pressure, diastolic blood pressure and mean blood pressure) were measured in the light phase of the light–dark cycle by the tail-cuff method (BP-98A; Softron Company, Tokyo, Japan) by keeping rats at 37°C in a body warmer. In each group, one half of the animals were used to measure blood pressures at day 26, and the other half of the animals were used at day 27. The measured blood pressures at both the days were combined for the statistical analysis.

The present study was approved by the Ethical Committee for the Laboratory Animals of the Seitoku University. All animal care guidelines and animal procedures followed were concordant to Standards Relating to The Care and Management of Experimental Animals (Notification no. 6, 1980, The Japan Prime Minister's Office).

Biochemical analyses

After 4 weeks on the dietary regimens, rats were fasted overnight and anaesthetised with diethyl ether. Blood was collected from the abdominal aorta. The blood was maintained at 4°C and centrifuged at 1000 *g* for 15 min to separate plasma. The plasma was stored at –80°C until assayed for aldosterone by an ELISA kit purchased from Cayman Chemical Company (Ann Arbor, MI, USA).

Statistical analyses

The data were analysed by SYSTAT version 10.2 (SYSTAT software, Inc., Richmond, CA, USA). Statistical analysis was performed by Tukey's multiple comparison test. *P* values less than 0.05 were considered significant.

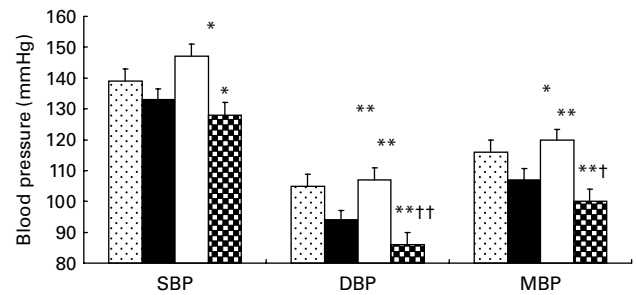


Fig. 1. Systolic, diastolic and mean blood pressures (SBP, DBP and MBP) in rats fed a control, iron-deficient, zinc-deficient or iron/zinc-deficient diet. CON (□), FD (■), ZD (▨) and FZD (▩) denote control group, iron-deficient group, zinc-deficient group and iron/zinc-deficient group, respectively. The column indicates average blood pressures, and the short bar indicates standard errors of the means. Mean values were significantly different from the ZD according to Tukey's multiple comparison test: **P*<0.05, ***P*<0.01. Mean values were significantly different from the CON according to Tukey's multiple comparison test: †*P*<0.05, ††*P*<0.01.

Results

Fig. 1 shows the blood pressures. Systolic blood pressure of the FZD was significantly lower than that of the ZD. Diastolic blood pressure of the FZD was significantly lower than those of the CON and ZD. Mean blood pressure of FZD was significantly lower than those of the CON and ZD.

Table 1 shows the final body weight, total diet intake, relative heart weight, plasma aldosterone concentration and haematocrit. Final body weight and total diet intake of all deficient groups were significantly lower than those of the CON. The relative heart weight of the FD and FZD was significantly higher than that of the CON. Plasma aldosterone concentration of the FD and FZD was significantly lower than that of the CON. The haematocrit data reported previously⁽²⁵⁾ were statistically reanalysed for reference. Haematocrit for the FD and FZD was significantly lower than that for the CON.

Discussion

We found that blood pressures, plasma aldosterone concentration and haematocrit were significantly decreased in Fe-deficiency anaemia combined with Zn deficiency. In Fe-deficiency anaemia only, a decrease in blood pressure was not so marked to yield a statistical significance, while plasma aldosterone concentration and haematocrit were significantly decreased. Total diet intake and final body weight were similarly decreased in the rats fed the Fe-deficient diet, Zn-deficient diet and Fe/Zn-deficient diet. Among these three experimental groups, no significant difference was found in total diet intake and final body weight. Therefore, it is reasonable to assume that the blood pressure of all three experimental groups was similarly affected by diminished final body weight that apparently reflected lower blood pressure in the rats fed the Fe-deficient diet and Fe/Zn-deficient diet.

Table 1. Final body weight, total diet intake, relative heart weight, plasma aldosterone concentration and haematocrit in rats fed a control, iron-deficient, zinc-deficient or iron/zinc-deficient diet

(Mean values with their standard errors)

	CON		FD		ZD		FZD	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Final body wt (g)	272 ^a	7	222 ^b	3	218 ^b	6	205 ^b	4
Total diet intake (g)	443 ^a	13	351 ^{b,c}	5	375 ^b	14	328 ^c	9
Relative heart wt (g/kg)	349 ^a	6	424 ^b	6	341 ^a	7	389 ^b	16
Plasma aldosterone (pg/ml)	128 ^a	22	38 ^b	10	114 ^a	30	20 ^b	5
Haematocrit (%)*	37.9 ^a	0.6	21.1 ^b	0.6	43.0 ^c	0.4	22.2 ^b	0.5

CON, control group; FD, Fe-deficient group; ZD, Zn-deficient group; FZD, Fe/Zn-deficient group.

^{a,b,c}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$) according to Tukey's multiple comparison test. Plasma aldosterone values were logarithmically transformed for statistical analysis.* The haematocrit values reported before⁽²⁵⁾ were statistically reanalysed for reference.

It is generally thought that blood pressure is decreased in anaemia including Fe-deficiency anaemia by a decrease of blood viscosity due to a decrease of haematocrit⁽¹³⁾. Blood pressure equals to cardiac output divided by total peripheral resistance that is proportional to blood viscosity based on Poiseuille's law^(14,15). Thus, blood viscosity is believed to be a major determinant of blood pressure in anaemia. This concept is essentially based on the experiments that used a glass viscometer to measure blood viscosity.

However, Whittaker demonstrated that a change of apparent viscosity was smaller in the range of haematocrit 20–40% than haematocrit 40–60% using a canine hind-limb as an *in vivo* viscometer⁽¹⁶⁾. The mechanism that decreases blood pressure in Fe-deficiency anaemia is not fully understood yet, although several decades have already passed after Whittaker's experiment.

It is well known that Fe-deficiency anaemia causes cardiomegaly and increases cardiac output⁽²⁶⁾ as a compensation of the decreased Hb concentration, i.e. the decreased oxygen-carrying capacity⁽²⁷⁾. The relative heart weight was consistently increased by 11 and 21% in the FZD and FD, respectively, compared to the CON. Although further verification including direct measurement of cardiac output is necessary, the effect of reduction in the apparent blood viscosity *in vivo* on blood pressure may be cancelled by the increased cardiac output in Fe-deficiency anaemia, assuming cardiac output paralleled to heart weight.

Crowe *et al.*⁽²⁾ proposed that release of endothelial vasodilators, such as NO or prostacyclin, might evoke hypotension in Fe deficiency. Plasma NO (nitrate/nitrite) concentration was, however, slightly decreased in Fe-deprived rats⁽¹¹⁾.

Hypoaldosteronism generally evokes hypotension^(18,19). Aldosterone synthase (steroid 11 β -monooxygenase, CYP11 β 2) requires cytochrome P-450 Fe for its activity^(28,29). Therefore, an alternative hypothesis is that decreased aldosterone production in addition to reduced haematocrit is responsible for Fe-deficiency anaemia-induced hypotension.

The mineralocorticoid receptor that binds aldosterone as a physiological ligand is a Zn finger transcription factor⁽³⁰⁾.

Considering the above, effects of decreased circulating aldosterone due to Fe deficiency on blood pressure might be enhanced by co-existing Zn deficiency to induce hypotension, although further studies including analysis of rennin–angiotensin system, aldosterone synthase and mineralocorticoid receptor are necessary to confirm this possibility.

In conclusion, Fe-deficiency anaemia combined with Zn deficiency evoked hypotension, and decreased circulating aldosterone concentrations might be partly involved in the pathogenesis of secondary hypotension in Fe-deficiency anaemia with Zn deficiency.

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