Chronic Treatment With Tin Normalizes Blood Pressure in Spontaneously Hypertensive Rats

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We have reported that short-term treatment of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats with stannous chloride (SnCl₂), which selectively depletes renal cytochrome P450, restores blood pressure to normal in young but not in adult SHR, and is without effect on blood pressure of either young or adult WKY rats. We report in the present study that chronic treatment with SnCl₂, begun at age 5 weeks, prevented the development of hypertension in SHR over a period of 15 weeks at which time they were killed. Suspension of SnCl₂ treatment after 8 weeks (i.e., at age 13 weeks) did not result in return of blood pressure to hypertensive levels in SHR. Age-matched WKY rats were not affected by tin treatment. These findings provide additional evidence that administration of tin, which stimulates heme oxygenase, thereby producing depletion of cytochrome P450, restores blood pressure to normal levels in SHR. (Hypertension 1991;17:776–779)

The kidney, through its endocrine and excretory function, exerts a key role in the regulation of blood pressure. Prostaglandins and other arachidonic acid (AA) metabolites originating within the kidney participate in several mechanisms that can affect blood pressure by contributing to the regulation of extracellular fluid volume and renal hemodynamics.¹² Of the variety of AA metabolites generated by the kidney, those arising from cytochrome P450–dependent monoxygenases also demonstrate the requisite biological activity to influence blood pressure. Several of these eicosanoids generated by a cytochrome P450 monooxygenase system operating intrarenally have been shown to affect vascular tone³⁴ and to be capable of modulating Na⁺,K⁺-ATPase activity.⁵⁶ To define possible relations between the renal cytochrome P450 system and regulation of blood pressure, we studied spontaneously hypertensive rats (SHR), the animal model most resembling human hypertension. In the SHR, renal mechanisms are thought to play a decisive role in elevating blood pressure.⁷⁸

We have obtained evidence to support the hypothesis that eicosanoids generated by the renal cytochrome P450 system participate in the elevation of blood pressure. These renal metabolites are generated in increased amounts only by the young SHR, when compared with either the mature SHR or with age-matched normotensive Wistar-Kyoto (WKY) rats, during the developmental stage of hypertension from the fifth to the 13th week.⁹ We have been able to restore blood pressure to normal levels in young 7-week-old SHR during the developmental stage of hypertension with short-term treatment with stannous chloride (SnCl₂). SnCl₂ induces heme oxygenase activity.¹⁰ Increased activity of heme oxygenase accelerates heme degradation, including that associated with cytochrome P450, thereby impairing the ability of the renal cytochrome P450 system to form eicosanoids¹⁰ and, presumably, to metabolize other local and circulating hormones that could affect blood pressure. We treated 7-week-old SHR for 4 days with SnCl₂, which resulted in reduction of blood pressure to normotensive levels. Tin treatment also decreased the elevated levels of cytochrome P450–dependent AA metabolites that affect vascular tone and salt and water excretion. SnCl₂ was without effect on blood pressure of either 20-week-old adult SHR or 7- or 20-week-old WKY rats. Coadministration of a competitive inhibitor of heme oxygenase prevented the blood pressure–lowering effect of SnCl₂ in
7-week-old SHR, providing additional evidence that the effects were mediated through elevated heme oxygenase activity.

The present study was designed to answer the question: Can long-term SnCl₂ treatment cause prolonged reductions in blood pressure when initiated in the young SHR? With this question in mind, we treated 5-week-old SHR for either 8 or 15 weeks. In either case, blood pressure was recorded until age 20 weeks when the animals were killed.

Methods

SHR (n=11) and WKY (n=10) male rats, obtained from Charles River Laboratories, Wilmington, Mass., were divided into two groups, a control (n=4 SHR; n=4 WKY rats) and an SnCl₂-treated group (n=7 SHR; n=6 WKY rats). SnCl₂ was dissolved in phosphate-buffered saline and pH adjusted to 7.4 by the addition of 0.1N NaOH. The treated groups received 10 mg/100 g body wt SnCl₂ subcutaneously twice weekly from age 5 to age 13 weeks while the control WKY rat and SHR groups received an equal volume of diluent subcutaneously according to the same schedule as the treated SHR. From age 13 to age 20 weeks, when all rats were killed, SnCl₂ treatment was withheld from three rats from each of the treated groups (i.e., three of seven SHR and three of six WKY rats). Systolic blood pressures of conscious unstressed animals were recorded using a plethysmograph-tail method in a constant-temperature room; rats had been previously heated in a constant-temperature cage to 35°C and had become accustomed to the procedure during several training sessions. Measurements of blood pressure were made in triplicate and determined weekly from the beginning of treatment until the 20th week of age in all groups.

Light Microscopy

Rats were anesthetized with sodium pentobarbital (100 mg/kg i.p.) and the abdominal cavity opened after treatment for either 8 or 15 weeks with SnCl₂ as described above. The aorta was tied above the renal arteries, and 0.9% ice-cold saline was flushed through the aorta. Small pieces of kidney, liver, and large and small arteries were taken for examination by light microscopy. All tissues were fixed with 10% formaldehyde and embedded in epoxy resin. One-micron thick vertical sections were cut with a microtome.

Statistical Analysis

Changes in blood pressure over the 15 weeks of the study for SHR and WKY rats, control and treated (including suspended treatment) groups, were compared among groups by two-way analysis of variance with repeated measures and the Bonferroni test using the SAS statistical package (SAS Institute, Cary, N.C.). Body weight differences were also compared by analysis of variance. The null hypothesis was rejected when the value of \( p < 0.05 \).

Results

From ages 6 through 9 weeks, blood pressure (mean systolic blood pressure ±SEM) increased progressively in all groups but was accentuated in the untreated SHR. The striped area of Figure 1 is defined by the systolic blood pressure (mean ±1 SD) of the combined treated and untreated WKY groups as significant differences were not observed between untreated or SnCl₂-treated WKY rats. Thus, at 13 weeks of age systolic blood pressures of untreated SHR did not differ from those previously described in the developmental stage of hypertension. Administration of SnCl₂ (10 mg/100 g body wt), twice a week from age 5 to 20 weeks, prevented the development of hypertension in SHR. The striped area of Figure 1 is defined by the systolic blood pressure (mean ±1 SD) of the combined treated and untreated WKY groups as significant differences were not observed between untreated or SnCl₂-treated WKY rats. Thus, at 13 weeks of age systolic blood pressures of untreated and SnCl₂-treated WKY rats were 132±8 and 138±5 mm Hg, respectively, and at age 20 weeks corresponding values of these WKY rats were 136±5 and 135±5 mm Hg. Changes in blood pressure of the untreated SHR did not differ from those previously described in the developmental stage of hypertension.

Administration of SnCl₂ (10 mg/100 g body wt), twice a week from age 5 to 20 weeks, prevented the development of hypertension in SHR but did not affect systolic blood pressure in age-matched WKY rats (Figure 1). Differences were statistically significant from age 9 weeks onward between treated and untreated SHR (treated SHR versus untreated SHR, \( p < 0.05 \)). In contrast, WKY rats treated with tin did not demonstrate significant differences in blood pressure from untreated WKY rats at any time during the 15-week period of the study. When SnCl₂ treatment was suspended after 8 weeks in the SHR, after they...
had been treated from age 5 to 13 weeks, the blood pressure remained within normal limits for the duration of the study, that is, until age 20 weeks at which time the animals were killed (suspended-treatment SHR versus untreated SHR, p<0.05).

Chronic treatment with tin decreased the growth rate in SHR (Figure 2); by 20 weeks there was a reduction of 20% in the body weight of treated SHR as compared with age-matched control SHR (310±9 g versus 258±3 g, p<0.01 for control and treated 20-week-old SHR, respectively). The decreased body weight seen in SHR treated with tin was also observed in tin-treated WKY rats; at age 20 weeks, weights were 375±14 g and 233±4 g for control and tin-treated groups, respectively. However, if SnCl₂ treatment was terminated at age 13 weeks, the SHR demonstrated a body weight at 20 weeks not different from untreated SHR; namely, 309±10 g versus 310±9 g, respectively, associated with normal blood pressure (Figure 2).

At 20 weeks, the rats were killed and microscopic examination of the kidney, liver, and large and small arteries were performed. All tissues examined were normal by light microscopy. In particular, there was no evidence of renal tubular damage.

**Discussion**

The present study was designed to determine the long-term effects of tin treatment on blood pressure in the young SHR. We have previously reported that SnCl₂ given daily for 4 days produced a selective reduction in blood pressure in the 7-week-old SHR but not in the adult SHR nor in WKY rats of either age. We have shown in the present study that SnCl₂ given to 5-week-old SHR for 15 weeks resulted in sustained lowering of blood pressure. In addition, suspension of SnCl₂ treatment at age 13 weeks (i.e., after 8 weeks of treatment of the 5-week-old SHR) resulted in maintained effects on blood pressure despite discontinuance of tin. The latter group gave us the opportunity to demonstrate that blood pressure-lowering effects of tin treatment could be divorced from growth retardation and whatever consequences resulted from the latter. Thus, SnCl₂, when given over a 15-week period to either 5-week-old WKY rats or SHR, caused significant retardation of growth as evidenced by weight loss. In contrast, discontinuance of SnCl₂ at age 13 weeks allowed the SHR to achieve a normal rate of growth, similar to the untreated age-matched SHR, associated with blood pressures that remained within the normal range.

We have provided evidence that these effects of SnCl₂ on blood pressure of the SHR in a short-term study resulted from depletion of cytochrome P450-containing enzymes consequent to induction of heme oxygenase and attendant enhanced catabolism of heme. It would limit the availability of heme to cytochrome P450–containing enzymes and, thereby, reduce formation of eicosanoids and other products arising from this pathway. In addition to eicosanoids that affect vascular tone and salt and water metabolism, such as 19-hydroxyeicosatetraenoic acid (HETE) and 20-HETE, the production of mineralocorticoids by cytochrome P450 oxygenases should also be affected by SnCl₂ treatment. Inhibition of synthesis of one of these products, 19-nor-deoxycorticosterone, is associated with diminished blood pressure in the SHR. There are other hemoproteins, most notably thromboxane synthase, which also can be affected by SnCl₂ treatment. Thus, the effects of SnCl₂ treatment on elevated blood pressure in the SHR may have several components, including diminished production of mineralocorticoids and thromboxane as well as cytochrome P450–dependent AA metabolites.

The present study provides additional evidence that administration of SnCl₂, which has been shown to target a key component of the cytochrome P450 system, can restore blood pressure to normal levels in SHR. The antihypertensive effect of SnCl₂, which is maintained for 7 weeks after its discontinuance, when the animals were killed, seems puzzling. However, there are reports of prolonged effects of tin on several tissues as well as evidence of its accumulation. The present study addresses several questions raised by the initial report on the antihypertensive effect of SnCl₂ in the SHR, the most important being whether a long-term antihypertensive effect of SnCl₂ treatment is evident when initiated in the young SHR.

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References


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