- Research Letter -

Effects of Lead Nitrate on Liver Weight and Serum Total Cholesterol Amounts in Stroke-Prone Spontaneously Hypertensive and Wistar-Kyoto Rats

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The administration of inorganic lead (Pb) ion to rats is well known to induce liver hyperplasia with liver enlargement and hypercholesterolemia. In the present study, the sensitivities of stroke-prone spontaneously hypertensive rat (SHRSP) and its normotensive control strain, Wistar-Kyoto rat (WKY), to these effects of Pb ion were estimated. Lead nitrate (LN) dissolved in a distillated water for injection was administered to male SHRSP and WKY by a single intravenous injection at a dose of 100 µmol/kg body weight. In WKY, significant increases in the liver weight were observed at 24 and 48 hr after LNadministration, while in SHRSP, no such significant increases were observed up to 48 hr later. On the other hand, increased levels of serum total cholesterol after LN-administration were significantly higher in SHRSP than in WKY at each time, although the constitutive (control) level was the opposite. The present findings suggest that there is different susceptibility between SHRSP and WKY to LN-induced liver hyperplasia and hypercholesterolemia and further indicate that development of hypercholesterolemia is not necessarily correlated with that of liver hyperplasia.

Key words——liver hyperplasia, lead nitrate, hypercholesterolemia, hypertension

INTRODUCTION

Strain differences in rodents often exist in susceptibilities to the toxicities of environmental chemicals. These determinants are most likely to be attributed to genetic factors.

Human essential hypertension tends to aggregate within families, so multiple mutated genes are believed to exist that contribute to its genetic inheritance. The genetic model animals, including spontaneously hypertensive rat (SHR) and strokeprone spontaneously hypertensive rat (SHRSP), are actively used for the identification of such genes. These rats have not only the hypertension trait but also various characteristics such as hyperactivation and hyperinnervation of peripheral sympathetic neurons,¹⁻⁴⁾ vulnerability of central neuronal cells,^{5,6)} and abnormal lipid metabolism.⁷⁻⁹⁾ Although the prototypical Wistar-Kyoto rat (WKY) strain was the origin of both SHR and SHRSP, the current WKY used as a normotensive control for its derived strains might have a genetic abnormality that represses hypertension because of repeated selection for maintenance of the normotensive trait. Some of these pathologic abnormalities are suggestive of the idea that their causative genes may contribute to different susceptibilities to the toxicities of environmental chemicals from those of normal rats. These model rats might therefore be useful in the field of toxicological study. Moreover, such studies might provide us with valuable information regarding the identification of mutated genes associated with the pathologic abnormalities in those rats.

Lead (Pb), one of the toxic metal pollutants, can cause a wide variety of disorders, including blood and brain disorders in humans and animals. Our group has been studying the mechanisms of inorganic Pb ion-induced hepatic hyperplasia and hypercholesterolemia.^{10–13)} As for hepatic hyperplasia, there is accumulating evidence that a single injection (100 μ mol/kg body weight) of inorganic lead ion [*e.g.* lead nitrate (LN) and lead acetate] to rats induces liver cell proliferation and liver weight gain (hepatic hyperplasia) without accompanying signs of liver cell necrosis.^{14–19)} The LN-injection to rats also increases both liver and

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serum total cholesterol (T-CHO) levels.^{10–12)} A single injection of LN induces hepatic expression of c-jun, c-myc, and c-Ha-ras genes, which are transcriptionally activated in response to various mitogens, and tumor necrosis factor- α gene, which has the ability to induce hepatic cell proliferation. $^{19-24)}$ It is of interest how these changes play a role in Pb-induced liver hyperplasia. Moreover, we have reported that LN-administration induces the gene expression of cholesterol synthesis enzymes, including 3-hydroxy-3-methylglutaryl CoA reductase and lanosterol 14α -demethylase [cytochrome P450 (CYP) 51], and decreased gene expression of a cholesterol metabolic enzyme, cholesterol 7α hydroxylase (CYP7A1), in the livers of rats and mice. $^{10-12}$ It is easily understood that these expressional changes lead to the development of hypercholesterolemia. However, it remains unclear what sort of early changes act as a trigger of Pb-induced hepatic hyperplasia and/or cholesterol synthesis.

The SHRSP has significantly lower serum T-CHO than WKY when both strains are maintained on a normal diet.^{7,8)} However, feeding of a high-fat and high-cholesterol diet leads to higher cholesterol levels in SHRSP than those in the WKY.⁹⁾ Therefore, SHRSP has been recognized to be a unique model of hypercholesterolemia as well as hypertension and stroke. These features of SHRSP imply the existence of genetic factor(s) involved in the pathogenesis of hypercholesterolemia and, indeed, progress is made in quantitative trait locus analysis for the hypercholesterolemia.^{25,26)} Such a genetic factor(s) might influence the sensitivity to the effects of some chemicals, including Pb, on cholesterol and/or lipid synthesis/metabolism. In other words, comparative study using SHRSP and WKY might provide valuable information for clarifying the mechanisms of these effects and identifying the genetic factors responsible for the pathologies of SHRSP and WKY.

In the present study, we attempted to evaluate whether there are differences in liver enlargement and serum T-CHO levels in SHRSP and WKY after LN-injection.

MATERIALS AND METHODS

Treatment of Rats with Lead Nitrate — Male WKY/Izm and SHRSP/Izm strains were supplied by the Disease Model Cooperative Research Association, Japan, and were used at 8 weeks of age.

Rats were kept in plastic cages in an air-conditioned room with a 12-hr light/12-hr dark cycle, and given a basal diet, MF (Oriental Yeast, Co., Tokyo, Japan), and water ad libitum. Experimental protocols were approved by the Animal Experimentation Ethical Committee at the University of Shizuoka. LN was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and dissolved in a distillated water for injection. Rats were administered a single dose of LN [100 µmol/kg body weight, intravenous drip (i.v.)]. All rats used in the present experiments were sacrificed by decapitation 0 (untreated, control), 3, 6, 12, 24, or 48 hr after administration. Each group contained four animals. Livers were removed from individual rats. Blood was also collected from individual rats. After clotting at room temperature, the serum was separated by centrifugation and stored at -80°C until use.

Serum T-CHO Levels — The serum T-CHO content in individual rats was measured with a 7170S Automatic Analyzer (Hitachi, Tokyo, Japan). Statistical Analyses — Values are expressed as the means \pm S.D. Statistical significances between LN-treated groups and the corresponding control (0 hr) in each strain of rats were determined using one-way analysis of variance (ANOVA), followed by Dunnett's test. Statistical significance between SHRSP and WKY rats at the same time point was evaluated by Student's *t*-test. All statistical analyses used a software, JMP version 7 (SAS Institute Japan, Inc., Tokyo, Japan).

RESULTS AND DISCUSSION

The liver weights (g per 100 g body weight) and their relative ratio to control (0hr) after LNadministration are shown in Fig. 1. The liver weight of the LN-administered WKY was slightly higher (10% increase) than that of the control WKY (0 hr) at 24-48 hr after LN-administration. The increases at 24 and 48 hr were statistically significant in relation to the control WKY. No such significant increases were observed up to 48 hr later in SHRSP. In addition, the liver weight (g per 100 g body weight) was significantly higher in SHRSP than in WKY up to 12 hr after LN-administration, and 24 hr later, their liver weights were almost the same. Furthermore, the liver weights relative to the corresponding controls were significantly increased in WKY, but not SHRSP. 24 and 48 hr after LN-administration.

The amounts of T-CHO in serum and their rela-



Fig. 1. Changes in the Liver Weights of WKY and SHRSP after LN-administration

The bottom graph (panel B) represents the ratio to the corresponding control rats. Data points represent the means \pm S.D. (N = 4). **p < 0.01, significant differences from the corresponding controls (0 hr). #p < 0.05, ##p < 0.01, ###p < 0.001, significant differences between WKY and SHRSP at the same time point.

tive ratios to the corresponding controls (0 hr) after LN-administration are shown in Fig. 2. The amount of T-CHO in the control SHRSP was significantly lower than that in the control WKY. The administration led to significantly higher amounts of T-CHO in both strains after 12–48 hr when compared with those of the corresponding controls, although the amounts in both strains after 24–48 hr were nearly equal. The relative ratios of the T-CHO amounts to that of the control (0 hr) in SHRSP were significantly higher than those in WKY at 12–48 hr after LN-administration.

Many previous studies using male Wistar and Spraque-Dawley (SD) rats have indicated that liver weight is increased by more than 50% at 48 hr after LN-administration at a dose identical to that used in the present study (100 μ mol/kg).^{14, 19)} However, the present study showed that the liver weight gain in WKY was only 10% and no such significant increases were observed in SHRSP. Therefore, both WKY and SHRSP strains are most likely to have



After LN-administration (hr)

Fig. 2. Changes in the Serum T-CHO Levels of WKY and SHRSP after LN-administration

The bottom graph (panel B) represents the ratio to the corresponding control rats. Data points represent the means \pm S.D. (N = 4). *p < 0.05, **p < 0.01, ***p < 0.001, significant difference from the corresponding controls (0 hr). #p < 0.05, ##p < 0.01, significant differences between WKY and SHRSP at the same time point.

very low sensitivity and resistance to the effects of Pb on liver enlargement. These results set up the hypotheses that genetic abnormality might exist in some sort of cellular factor involved with the formation of Pb-induced liver enlargement in SHRSP and WKY and that its abnormality might be more severe in SHRSP than in WKY.

The present study has demonstrated that, under the normal condition (normal diet), SHRSP has lower serum T-CHO level than WKY, as previously observed.^{7,8)} The amounts of serum T-CHO in SHRSP and WKY were significantly increased 12–48 hr after LN-administration, suggesting that both strains can also respond to the inducing effects of LN on the T-CHO levels. However, the rate of its variability indicated that SHRSP (Max. \approx 1.70 times) is more sensitive to LN than WKY (Max. \approx 1.37 times). We and another group have previously observed that serum T-CHO levels rise approximately 50–70% in male SD and Wistar rats at 12–72 hr after LN-administration.^{10, 11, 27)} Therefore, WKY might be less sensitive to the effects of LN than SHRSP, SD and Wistar rats, and might have a genetic abnormality in some sort of cellular factor playing an important role in the generation of LN-induced hypercholesterolemia. In addition, considering the present results regarding the measurement of liver weights, it is considered that development of LN-induced hypercholesterolemia is not necessarily correlated with that of liver hyperplasia.

The present study was performed based on our interest in how the genetic backgrounds of SHRSP and WKY affect the toxicities of environmental chemicals, including medicinal drugs. The LNadministration actually demonstrated the possibility that the susceptibilities of both strains to the Pbeffects differed from each other and/or from other rat strains. We therefore propose herein that it is crucially important in the toxicological field to examine the susceptibilities of WKY-derived strains, including SHRSP, to the toxicities of various chemicals and to determine their mechanisms based on the identification of relevant genetic factors. These trials would be of assistance in understanding the pathogenesis of human diseases including hypertension and stroke, which these strains can mimic. Moreover, we could move forward in a new field to assess the health hazards of environmental chemicals to persons having a genetic background for those diseases.

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