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Blood Pressure Reduction by CCl_4 in the Spontaneously Hypertensive Rat

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It has been established that the spontaneously hypertensive rat (SHR) presents an experimental model whose pathogenesis resembles that of essential hypertension in man (Okamoto 1969). A great advantage of this model is that the entire life history of this disease is compressed within a time frame of two years. Many antihypertensive agents have been found effective in reducing blood pressure in SHR animals (Freis et al. 1972). Carbon tetrachloride (CCl_4) treatment has resulted in blood pressure reduction and subsequent elevation after discontinuing treatment in Grollman renal hypertensive rats (Loyke et al. 1960; Loyke 1964b) and in endocrine hypertensive rats (Loyke and Mackrell 1961). The purpose of this study was to determine whether hypertension in the spontaneously hypertensive rat (SHR), could be modified by CCl_4 treatment and to evaluate its effects on kidney and liver tissue.

MATERIALS AND METHODS

Observations were made on a group of 12 genetically selected SHR of the Okamoto Aioki strain rats obtained from Taconic Farms. For comparative study, a group of five Sprague-Dawley rats without genetic selection obtained from Beaumanor Farms became spontaneously hypertensive (SH) with systolic blood pressures of over 160mm Hg for two months. Thirteen Sprague-Dawley normotensive rats served as normotensive controls. All rats were fed Purina Chow, given tap water ad lib, and housed in individual cages. Initially, all rats weighed approximately 120g. All animals were weighed twice weekly and warmed to approximately 40°C for systolic blood pressure measurements by the tail microphonic method (Friedman and Freed 1949) without anesthesia. When hypertension persisted for two months, injections of 0.15ml undiluted analytical reagent grade CCl_4 were given subcutaneously twice a week for a total of 15 doses to five SH, six NT, and five SHR

rats. Seven SH and seven SHR rats served as untreated controls. The SH blood pressures were also measured after stopping CCl_4 injections. At the end of the experiment, all rats were sacrificed and kidney and liver tissue removed and stained with hematoxylin and eosin for light microscopic histological study. Liver tissue damage was graded as either absent, mild to moderate, or severe with respect to the degree of fatty metamorphosis or fibrotic changes.

RESULTS AND DISCUSSION

Blood pressures were compared between SHR and SH animals before and after CCl_4 treatment. The data was analyzed to determine the mean and standard deviation (SD) of each group's blood pressure. Statistical significance was determined by use of Student's t test.

The pre-treatment mean systolic pressure of non-genetic SH animals reached a level of 173 ± 5.2mm Hg compared to a mean pressure of 138 ± 5.4mm Hg in normotensive controls (Table 1). After 15 CCl_4 injections, the SH group averaged 148 ± 5.4mm Hg ($P<.001$). Twenty-five days following cessation of CCl_4 injections, the mean blood pressure in SH animals had returned to its

Table 1. Systolic blood pressure studied before and after CCl_4 treatment in Sprague-Dawley and spontaneously hypertensive rats.

Type	Strain	Group	#	Systolic Blood Pressure mm Hg Pre-Treatment Period	Post Treatment Period	CCl_4
Genetic	Okamoto Aioki	SHR	5	178 ± 4.2	152 ± 8.3	+
			7	149 ± 4.7	173 ± 3.7	0
Non-Genetic	Sprague-Dawley	SH	5	173 ± 5.2	145 ± 5.4	+
		NT	7	138 ± 5.4	143 ± 5.1	0
		NT	6	131 ± 4.5	136 ± 4.0	+

Spontaneously hypertensive rat (SHR), spontaneous hypertension (SH) and normotensive rats (NT). Carbon tetrachloride (CCl_4) given (+) not given (0)

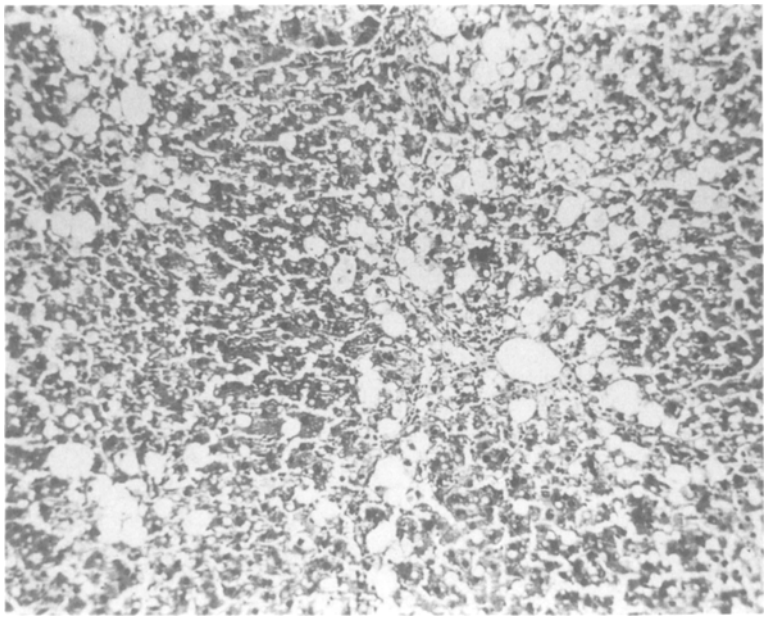

Figure 1. Liver x 90 from spontaneous hypertensive (SH) Sprague-Dawley CCl_4 treated animal showing vacuolization of cytoplasm.

pre-treatment hypertensive level of 175±4.3mm Hg. The genetic SHR pre-treatment blood pressure was 178±4.2mm Hg, and after 15 CCl_4 injections, the pressure was reduced to 152±8.3mm Hg ($p<.001$). The blood pressure of untreated control SHR animals remained hypertensive (173±3.7mm Hg) (Table 1). The NT animals treated with CCl_4 and their untreated controls had similar blood pressure: 136±4.0 mm Hg and 138±5.4 mm Hg and 138±5.4 mm Hg (Table 1).

Weights of the SH treated and untreated animals were comparable (245 and 242 gms) and in similar time periods the genetic SHR CCl_4 treated animals weighed 248 grams ±8.3g and the untreated weighed 243 grams ± 7.5g. The normotensive treated animals weighed 257±12g and the untreated weighed 265±9g.

Histological examination of liver tissue by light microscopy from the SH CCl_4 treated group (Figure 1) revealed moderate to mild changes of fatty metamor phosis and one animal had minimal fibrotic changes. All of the untreated animals' liver tissue was without fatty changes and reported as normal. The genetically SHR CCl_4 animals had normal liver tissue and two had changes of a mild degree of fatty metamorphosis. All of the untreated animals had normal liver tissue. The kidneys had the usual architecture in the SHR and SH

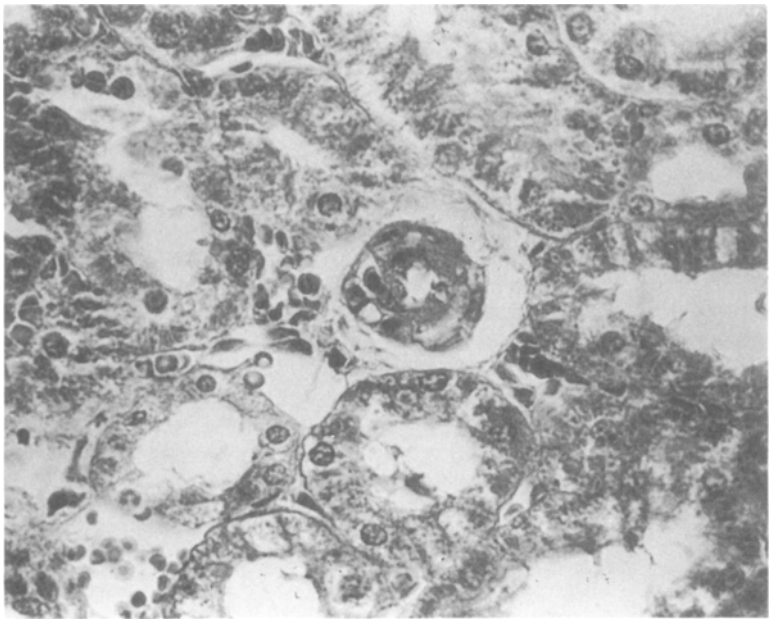

Figure 2. Cross section of renal arteriole from SH Sprague-Dawley (x724) marked thickening of wall, diminution of lumen size, medial muscular hypertrophy with prominent nuclei, and slight hyalinization.

animals. The small arteries and arterioles in the SH animals varied from having walls of normal thickness to occasional ones which had greatly thickened walls and small lumens, due to hypertrophy of the medial vasculature (Figure 2). No arterial or arteriolar changes were observed in any of the genetic SHR kidneys.

These findings demonstrate that blood pressure can be reduced by CCl_4 treatment in non-genetically and genetically spontaneous hypertensive rats. The blood pressure reduction was comparable to that previously demonstrated in both renal (Loyke et al. 1960), and DOCA hypertensive animals (Loyke 1964a), following CCl_4 treatment. The blood pressure reduction in the SH and SHR animals was statistically significant, but normotensive levels were not reached. This same observation has been made in the renal and DOCA hypertension animals. Components of the renin- angiotensin-aldosterone system which have been ruled out as being related to the blood pressure change are: a decrease in angiotensinogen (Loyke 1964b), an increase in angiotensinase (Loyke 1964c), a change in aldosterone (Loyke 1964a), and a decrease in renin production (Loyke 1981).

Saline injections given as a control for CCl_4 did not alter blood pressure (Loyke 1983). Discontinuing CCl_4 treatment resulted in a return to hypertensive blood pressure levels in the non-genetic SH group. The genetic SHR group of animals were not tested for this phenomenon. Although the blood pressure was significantly lowered with CCl_4 treatment (150 mm Hg), the pressure did not compare with normotensive animals (135 mm Hg). Both CCl_4 treated and untreated spontaneous hypertensive animals gained weight with normal growth. The normotensive treated animals weighed more than their untreated controls, demonstrating that CCl_4 treatment did not inhibit normal growth.

Since Ng, and Vane in 1967 reported that considerable converting enzyme activity occurred in the lung and this reaction takes place in seconds, the place for liver converting enzyme was questioned. The conversion of angiotensin I to II occurs in plasma (Friedman and Freed 1949) or serum (Loyke 1970a) after more than one hour. If the lung activity is accomplished in seconds, then how can serum from CCl_4 treated animals contain vasoinactive angiotensin I. This question was answered by injecting AI into hypertensive animals made normotensive by CCl_4 treatment and finding that AI was rapidly converted to AII by lung action as exhibited by a pressor rise. This means that there was no interference by CCl_4 treatment on lung converting enzyme activity, and that the lung probably is not involved in the blood pressure reduction in this experimental condition. The position of liver converting enzyme seems to be paramount in the CCl_4 blood pressure lowering effect.

The hepatotoxic changes, in response to subcutaneous CCl_4, in all of the SH animals, were primarily of mild to moderate degrees of fatty metamorphosis and one showed early cirrhotic changes, similar to that found in the renal hypertensive animals (Loyke 1964b). Confirmatory findings were reported by Cikrit, et.al. (1975) that CCl_4 injections given twice a week (1 mg/Kg) for three months to rats resulted in only a small part of the CCl_4 exposed animals exhibiting a picture of cirrhosis while the majority of the others merely had fatty metamorphosis of liver tissues. These non-cirrhotic changes in liver tissue may be partly due to the low dose of injected CCl_4. Hepatotoxic changes in the SHR animals were devoid of cirrhotic changes and only two of the five treated animals had minimal fatty deposits.

Similarly, no renal arterial lesions were found in the SHR; however, the SH animals had a greater degree of hypertrophied small arteries. The observance of hyper-

trophied small arteries and arterioles in the kidneys of these animals has a questionable significance. Lundh (1964) has described similar findings of thickened arterioles in response to CCl_4 injections in normotensive animals. This introduces a likelihood that sequelae of arteriolar damage may lead to thickening of those arterioles. It is, therefore, unclear whether there are any histologic changes present in the non-genetic SH animals which may serve to separate these from genetic SHR and normotensive animals.

Exploring the mechanism of action of CCl_4 in reducing blood pressure, primarily angiotensin I has previously been identified in renal and DCA hypertensive animals, (Loyke 1964a). The presence of vasoinactive angiotensin I in the experimental hypertensive CCl_4 treated animals seems to reflect an interference with the ability of angiotensin converting enzyme to convert the vasoinactive angiotensin I to vasoactive angiotensin II (Loyke 1965). Since the blood pressure reduction by CCl_4 is not to normotensive levels, this may be a partial explanation for the fall in blood pressure. Another possible mechanism for the CCl_4 blood pressure reduction may be the presence of a vasodepressor substance, since a repeatable vasodepressor substance has been demonstrated with blood from a CCl_4 treated rat into a hypertensive rat (Loyke and Hoobler 1982).

Patients who have well established hypertension and later develop cirrhosis of the liver with serum protein abnormalities (hyperglobulinemia), have a reversal of their hypertension to normotensive, (Loyke 1955, Loyke 1962). A partial explanation of the findings in man (Loyke 1970b) for blood pressure changes in patients with cirrhosis may be due in part to the inhibition of angiotensin converting enzyme, and/or the effect of a vasodepressor substance.

The arrest in the development of hypertension in the genetic SH and SHR animals with CCl_4 administration adds to the group of drugs which are able to alter the course of this form of hypertension.

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REFERENCES

Cikrit M, Tichy M, Holusa R (1975) Biliary Excretion of Cu, Zn, and Hg in the Rat with Liver Injury Induced by CCl_4. Arch Toxicol 34:227:36

Freis E, Ragan D, Pillsbury III H, Mathews M (1972) Alteration in the Course of Hypertension in the Spontaneous Hypertensive Rat. Circ Res 31:1-7

Friedman M, Freed S (1949) Microphone Manometer for Indirect Determination of Systolic Blood Pressure in the Rat. Proc Soc Exp Biol Med 70:670-2

Loyke HF, Plucinsky JS, Crawford TL (1960) Effect of Liver Damage on Experimental Renal Hypertension in the Rat. Circ Res 8:535-7

Loyke HF (1964a) Experimental Hypertension Treated with CCl_4: Measurements of Adrenal Function, Vascular Responsiveness, Angiotensinase and Converting Enzyme. Pro Soc Exp Biol Med 115:1035-40

Loyke HF, Mackrell SJ (1961) Effect of Liver Damage on Experimental Desoxycorticosterone Hypertension in the Rat. J Lab Clin Med 58:871-5

Loyke HF (1970a) Converting Enzyme in Rat Serum. Proc Soc Exp Biol Med 134:248-251

Loyke HF (1964b) Angiotensinogen Effect of CCl_4 Treated Experimental Hypertension. Am J Med Sci 247:177-181

Loyke HF (1964c) Experimental Hypertension Treated with CCl_4. Proc Soc Exp Biol Med 115:1035-40

Loyke HF (1981) Effect of Converting Enzyme and Angiotensin II Inhibitors in Chronic One Kidney Bipolar Ligated Hypertension. Pharmacol Res Commun 13:501-509

Loyke HF (1983) Effect of Indomethacin on Rats with Grollman Hypertension. Pharmacol 27:325-9

Loyke HF (1965) Converting Enzyme in Experimental Hypertensions. Am J Med Sci 250:53-57

Loyke HF, Hoobler S (1982) Presence of a Circulating Depressor Substance by Rat Cross Perfusion after CCl_4 Treatment. Pharmacol Res Comm 14:621-627

Loyke HF (1955) The Relationship of Cirrhosis of the Liver to Hypertension. Am J Med Sci 230:627-32

Loyke HF (1962) Reduction of Hypertension after Liver Disease. Arch Intern Med 110:83-87

Loyke HF (1970b) Liver Enzymes in Hypertension. Gastroenterology 53:339-51

Lundh HAB (1964) Sequence Comparison Between Kidney and Liver. J Occup Med 6:123

Ng K, Vane J (1967) Conversion of Angiotensin I to Angiotensin II. Nature 216:726

Okamoto K (1969) Kidney and Liver Spontaneous Hypertension in Rats. Int Rev Exp Pathol 1:227-70

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