Cadmium Deposition in Canine Heart and Major Arteries Following Intravascular Administration of Cadmium Chloride

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INTRODUCTION

The many potential sources of environmental contamination by cadmium compounds have been reviewed extensively (FLICK et al. 1971). The exposure of mammals to environmental cadmium is probably ubiquitous since it appears as a trace contaminant of food, water, and air (DUGGAN and CORNELIUSSEN 1972, LIEBER and WELSCH 1954, SCHROEDER 1970).

PERRY and ERLANGER (1971) have suggested that vascular tissue has a high affinity for cadmium. The first comprehensive study of relative cardiovascular deposition of administered cadmium was reported by FISCHER and THIND (1971) who measured the metal content in heart, pulmonary and mesenteric arteries, and aortic segments from cadmium-dosed rabbits. In view of the known association between cadmium administration and the induction of experimental hypertension (SCHROEDER and VINTON 1962), further study of cadmium distribution within vascular tissue is warranted.

Studies of whole-body distribution of injected cadmium radioisotopes in mice indicated that appreciable amounts are retained in the heart (LUCIS and LUCIS 1969). SCHROEDER et al. (1965) have found measurable amounts of the metal in the hearts of cadmium-treated rats with one effect of prolonged cadmium treatment being left ventricular hypertrophy. Cardiomegaly also was observed after oral administration of cadmium to rabbits (STOWE et al. 1972).

SIMON et al. (1947) have summarized the two types of reactions cadmium may undergo with tissue proteins: (1) it may combine with carbonyl groups to form insoluble metal proteinates, or (2) it may react with sulfhydral groups to form stable metal mercaptides. Within the latter category are the cadmium metalloproteins specifically identified as mettallothioneins. These proteins are confined to the intracellular compartment and have been isolated from the cytoplasmic soluble fraction of a variety of tissue sources (FRIBERG et al. 1971), but to our knowledge, metallothionein has not been reported in cardiovascular tissue.

One objective of the present study was to determine the relative distribution and deposition of cadmium within the heart

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and selected major arteries of cadmium-dosed dogs. In addition, the cytoplasmic soluble portion of aorta was examined to determine whether cadmium-binding proteins (Cd-BP) similar to those reported in other mammalian tissues are also present in canine vascular tissue.

MATERIALS AND METHODS

Experimental animals

Eight male and two female mongrel dogs in apparent good health and ranging from 6 to 23 kg were held for nine days prior to the beginning of the experimental regime to allow familiarization and health check. They were housed in approved metal cages and exercised every other day. A mixed diet of high protein dog meal and canned meat dog food was provided daily; water was available ad libitum.

Experimental design

The experimental design was three-fold in nature: (1) to develop a comparative study of arterial cadmium deposition versus vessel size and proximitry to the heart; (2) to determine cadmium deposition in the left and right ventricles; and (3) to examine selected cardiovascular tissue for the presence of a cadmium-binding component similar to metallothionein.

Nine dogs were assigned randomly to one of three groups (Group I - III) with one male serving as the control. All nine experimental dogs received three cephalic vein injections of cadmium chloride at a dosage level of 1 mg Cd/kg body weight per injection. Sterile procedure was observed and one day elapsed between injections.

Group I, II, and III dogs were sacrificed and examined at 1, 3, and 5 weeks, respectively, after the last cadmium injection. The control dog was examined at the same time as the Group I dogs (i.e., after three weeks in our facility). Selected cardiovascular tissue were excised following intravenous injection of sodium barbital to a plane iii level of anesthesia, and examined for cadmium deposition. Aortic segments between the heart and diaphragm of Group II dogs were examined for cadmium-binding protein (Cd-BP).

Tissue selection and preparation

Ten representative arterial segments and square sections weighing 1 - 2 grams from left and right ventricles were obtained from each animal. The description and source of these tissue samples are given in Table 1. These specimens were excised, chilled on ice, cleared of blood and connective tissue, rinsed with distilled water, blotted dry, and then weighed. Samples were stored at -18 C until analyzed for cadmium content.

TABLE 1

Anatomical description and source of dog arterial and ventricular segments.

Arterial Specimen	Description and Source
ascending aorta	the section from 2 cm above the valves to the innominate artery.
descending aorta	a 2-3 cm segment below the left subclavian artery.
thoracic aorta	a 2.5-3 cm segment of aorta just above the diaphragm.
abdominal aorta	the last 3-3.5 cm portion terminating at the iliacs.
left carotid	that portion between carotid bifurcation and the larynx.
left subclavian	from the aortic arch to the axillary artery origin.
renal arteries	from the aorta to the renal hilus.
pulmonary trunk	from 1 cm above valves to the second bifurcation on each side.
left femoral	a 4-5 cm segment from the region of the femoral triangle.
popliteal	a 1.5-3 cm segment between the femoral artery and tibial bifurcation.
right ventricle	a 1-2 gm square from right lateroanterior including epi-, myo-, and endocardium.
left ventricle	a 1-2 gm square from left lateroanterior including epi-, myo-, and endocardium.

Metal extraction and analysis

Cadmium tissue levels were determined by atomic absorption spectrophotometry after a preparative process which combined and modified the wet digestion method of ADRIAN (1971) and the organic solvent extraction technique of BERMAN (1967). Tissue samples were placed in polyethylene bottles containing 5 ml concentrated HNO_3 and 2 ml of 70% $HClO_4$, tightly capped, and left

overnight at room temperature. On the following day the capped bottles were placed in a 70 C water bath for 3-4 hours and then cooled. Following addition of 5 ml distilled water to each, they were returned uncapped to the water bath for another 4 hours. When cool, the clear digests were adjusted to pH 6.5-7.5 with NaOH, then transferred to a separatory funnel along with 1 ml of 1% aqueous sodium diethyl dithiocarbamate and, depending upon cadmium concentration, 5 to 25 ml methyl isobutyl ketone. The funnel was shaken for 2 minutes, the phases allowed to equilibrate, and the upper organic layer assayed immediately for cadmium.

Cadmium was quantitated with a Perkin-Elmer model 305A Atomic Absorption Spectrophotometer supplied with an air-acetylene flame. A blank and cadmium metal standards were prepared each time with the tissue samples. Analysis of the discarded aqueous phase at cadmium concentrations up to 0.20 µg/ml revealed no detectable cadmium.

Isolation of cadmium-binding protein

The aorta was examined for Cd-BP by a modification of the procedure of PULIDO et al. (1966). The cytoplasmic soluble component was obtained by first homogenizing the minced tissue with cold 0.25 M sucrose (20% w/v) with a Potter Elvehjem device and then centrifuging the homogenate for 1 hour at 29,000 x g in a refrigerated centrifuge. Three to five milliliters of the supernatant were applied to a 41.5 x 2.5 cm Sephadex G-75 column and eluted with 0.001 M tris-HCl buffer (pH 8.6). The sedimented pellets were retained and later assayed for metal content. Column effluent was monitored for UV absorbance at 250 and 280 nm and fifteen minute fractions (4,5-5 . 5 ml) were collected. Each fraction was subjected to direct atomic absorption analysis and compared to 0.01-0.50 µg Cd/ml aqueous standards. The correlation between absorbance and concentration was linear under these conditions to 2 µg Cd/ml.

RESULTS AND DISCUSSION

Results from the atomic absorption analyses of 108 tissue specimens representing ten arterial and two cardiac sites in nine dogs are presented in Table 2 and illustrated in Figure 1.

The heart cadmium content was greater at all three time periods than any arterial specimen within that same group. Mean tissue levels for the left ventricle were slightly greater than from the right ventricle. The control dog heart showed intrinsic cadmium deposition in both left and right ventricles (0.149 and 0.058 µg Cd/gm, respectively). Heart cadmium in the experimental animals showed a consistent, significant ($P < 0.05$) decrease from the first to fifth week.

All ten arterial specimens showed an affinity for cadmium which was rather constant with time. Of the Group I arteries, the

renals had the greatest concentration of cadmium and the femorals and popliteals had the least. In Group II the abdominal aorta had the highest levels while the femorals and popliteals again had the lowest cadmium levels. Of the Group III arteries, the renals once again had the greatest cadmium content and the femorals had the least.

TABLE 2

Dog arterial and cardiac tissue cadmium levels. Each value represents the mean + S.E. for three animals expressed as micrograms cadmium/gram whole tissue.

Source	Group I	Group II	Group III
ascending aorta	1.055±0.167	0.749±0.060	0.893±0.166
descending aorta	1.099±0.167	1.013±0.108	0.891±0.137
thoracic aorta	0.877±0.070	0.707±0.076	0.713±0.100
abdominal aorta	0.871±0.186	1.020±0.081	0.700±0.054
left subclavian	0.857±0.132	0.706±0.090	0.694±0.084
renal arteries	1.593±0.346	0.891±0.046	1.320±0.292
popliteal	0.786±0.210	0.528±0.106	0.767±0.034
pulmonary trunk	1.107±0.133	0.832±0.168	0.668±0.126
left carotid	0.925±0.070	0.694±0.154	0.623±0.138
left femoral	0.765±0.064	0.484±0.145	0.456±0.167
right ventricle	2.330±0.173	1.672±0.141	1.403±0.157
left ventricle	2.526±0.139	1.748±0.194	1.473±0.162

An inspection of Fig. 1 reveals no consistent pattern of change within the different arterial classifications with respect to time. Only the left subclavian, pulmonary, and left carotid display what appears to be a steady trend of decreasing accumulation with time, but this was not significant ($P > 0.05$). In fact, none of the ten arterial specimens including the renals showed a significant difference in metal accumulation among the different times ($P > 0.05$).

A plot of cadmium content versus vessel size (rank order) shows the general relationship that the larger, more proximal arteries accumulate cadmium more than the smaller, more distal

arteries. However, a distinct exception is the renal artery accumulation. The renal artery mean cadmium content for all nine experimental animals irrespective of time was 1.268±0.166 µg Cd/gm tissue. This was greater than the total mean value of any other artery examined. Analyses of control dog arteries revealed renal artery deposition (0.242 µg Cd/gm tissue) as well as deposition in the ascending aorta, left subclavian artery, and thoracic aorta (0.009, 0.034, and 0.036 µg Cd/gm tissue, respectively).

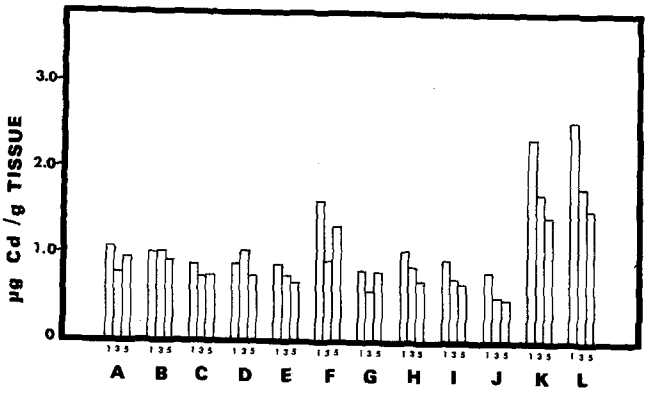

Figure 1. Cardiac and arterial tissue cadmium levels 1, 3, and 5 weeks after cadmium exposure. Each value represents the mean for 3 animals. Numbers along the abscissa refer to weeks post-injection. A = ascending aorta, B = descending aorta, C = thoracic aorta, D = abdominal aorta, E = left subclavian, F = renal arteries, G = popliteal artery, H = pulmonary trunk, I = left common carotid, J = left femoral, K = right ventricle, L = left ventricle.

These findings suggest that the canine renal artery has the greatest affinity for cadmium of the ten major arteries examined, and that this accumulation is persistent. THIND and PETERSON (1968) have demonstrated that the intrarenal arterial injection of cadmium prior to angiotensin, epinephrine, and norepinephrine administration resulted in a dose-dependent, reversible inhibition of the vasopressor-induced renal vasoconstriction in dogs without concurrent systemic hemodynamic changes. FISCHER and THIND (1971) have further suggested that the presence of cadmium in vascular tissue at a concentration of about 1.0 µg Cd/gm tissue may play a role in the pathophysiology of cadmium hypertension. If the predilection of the renal arteries for cadmium deposition shown in this study extends to the renal arterioles as well, the intra-renal vascular tissue may play a different and more sensitive

role in cadmium hypertension than the peripheral vascular tissue. The possible nature of this role, however, cannot be determined from the present study.

The analysis by gel filtration of homogenate prepared from segments of aorta from Group II dogs failed to disclose the existence of a soluble Cd-BP as found in equine kidney(MARGOSHES and VALEE 1957). Traces of cadmium just above the detection limit of instrumental analysis were detected between the relative elution volumes (V_e/V_o) of 1.02-1.34 which coincided with the major protein peaks, and between V_e/V_o = 1.74-1.83. On the basis of this information, it appears that most of the arterial cadmium is confined to the fibrous or particulate portion of the artery.

SUMMARY

The examination of ten arterial specimens from nine experimental dogs shows a generally persistent accumulation of cadmium in the blood vessels following acute cadmium exposure. Gel filtration analysis of the aorta revealed that this vascular cadmium is largely confined to the insoluble cellular compartment. The left and right ventricles of cadmium-treated dogs showed a cadmium deposition which significantly decreased over the five week period subsequent to exposure. Within all three time intervals, heart cadmium exceeded the level in any of the arteries examined. Control heart, renal and subclavian arteries, and ascending and thoracic aorta were all found to contain intrinsic cadmium. Of the arterial specimens examined, cadmium deposition was generally greatest in the renal arteries and lowest in the femoral and popliteal arteries.

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