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Prevention of Hereditary Cardiomyopathy in the Hamster by Verapamil and Other Agents[1] (38771)

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Hereditary cardiomyopathy in hamsters is manifested by multifocal degenerative changes, mainly comprising early myocytolysis and myofibrillar calcinosis, which develop without any morphologic evidence of impaired microcirculation (1, 2). The increase in myocardial calcium observed during the prenecrotic stage of the cardiac disease (3) is, perhaps, due to a primary defect in the cellular organelles that regulate calcium homeostasis (4), or it may be derived from altered enzyme activities in plasma membranes controlling calcium transport (5). Whichever mechanism is responsible for the augmented cellular calcium uptake, it often coincides with an endogenous release of catecholamines in the heart (6, 7). The present report deals with the development and progression of heart and muscle lesions in myopathic hamsters, as well as with the influence of: (1) drugs acting on calcium influx into muscle fibers (8); (2) a calcium-deficient diet; and (3) injections of calcium gluconate.

Materials and Methods. Male and female 20- to 30-day-old hamsters of the UM-X7.1 line, maintained under controlled housing conditions, were given free access to Purina Lab Chow and tap water.

Verapamil[2] (Knoll E. G., Ludwigshaffen, W. Germany) was administered at dose levels of 0.5 mg during the first week and 0.75 mg until the 28th day of the experiment. Prenylamine[3] (Hoechst, Montreal) and Cinnarizine[4] (Janssen Pharmaceutical, Beerse, Belgium) were given in constant doses of 2.5 mg and 1 mg, respectively. The drugs were solubilized in distilled water (Verapamil) or in their own vehicles to obtain a final volume of 0.1 ml, and injected twice daily. Irritation was minimized by giving the morning injection intraperitoneally and the afternoon injection subcutaneously, at different sites. Control animals were similarly treated with distilled water alone.

In another experiment, male and female hamsters of the same strain were either maintained on Purina laboratory chow and tap water or on a calcium-deficient diet (supplied in pellet form by General Biochemicals, Chagrin Falls, Ohio) and water. Some of these animals were given 10 mg (0.1 ml) of calcium gluconate (Sandoz, Montreal), twice daily, ip, for 28 days.

On the 29th day, all the hamsters were killed by aortic exsanguination under ether anesthesia. Their sera were analyzed individually for inorganic phosphorus according to the method of Dryer *et al.* (9), for calcium by atomic absorption spectrophotometry (10), and for creatine phosphokinase (CPK) according to a technique described by Nuttal and Wedin (11). The gross pathologic findings were recorded at autopsy to appraise the extent of necrotic changes in the entire body musculature; the heart and biceps femoris were then removed, fixed in neutral formol, and processed for routine histologic studies. Other muscles, such as the lattissimus dorsi and diaphragm, were often taken for a more accurate assessment of the severity of the disease. Sections were stained with hematoxylin, phloxine-saffran, celestine blue, or by a modified cobalt substitution technique for the demonstration of calcium in muscle fibers (12).

The severity of cardiac and skeletal muscle lesions (myolysis and/or necrosis) was assessed in an arbitrary scale of 0 to 3, in which 0 = no change, 1 = occasional, isolated fiber degeneration, 2 = moderate damage in the form of small necrotic foci, and 3 = severe fiber destruction in large

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[2] 5-[(3,4-Dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile.

[3] *N*-(3′-Phenyl-2′-propyl)-1,1-diphenyl-3-propylamine.

[4] 1-Cinnamyl-4-diphenylmethylpiperazine.

TABLE I. EFFECT OF CALCIUM ANTAGONISTIC DRUGS ON THE DEVELOPMENT OF CARDIAC AND SKELETAL MUSCLE LESIONS AND ON SERUM ELECTROLYTES AND CREATINE PHOSPHOKINASE IN HAMSTERS WITH HEREDITARY POLYMYOPATHY.

Treatment	Final body weight (initial 55 ± 3.0)	Myocardial lesions		Skeletal muscle lesions		Serum electrolytes (mg/100 ml)		Serum CPK (IU/liter)
		Severity (Grade 0–3)	Incidence (%)	Severity (Grade 0–3)	Incidence (%)	Ca	P	
Distilled water (10)	88 ± 1.8	2.9 ± 0.1	100	2.5 ± 0.14	100	11.3 ± 0.3	7.26 ± 0.18	11,050 ± 740
Verapamil (12)	70 ± 1.7	0.08 ± 0[a]	8	2.3 ± 0.17	100	10.3 ± 0.2[b]	7.75 ± 0.39	10,240 ± 2,480
Prenylamine (15)	46 ± 3.6[a]	0.36 ± 0.09[a]	60	1.6 ± 0.22[c]	93	10.0 ± 0.1[a]	6.73 ± 0.23	2,140 ± 235[a]
Cinnarizine (10)	74 ± 1.1	1.3 ± 0.4[a]	70	2.6 ± 0.14	100	11.3 ± 0.5	7.36 ± 0.28	9,440 ± 635

[a] $P < 0.001$ (as compared to solvent-treated controls).
[b] $P < 0.05$ (as compared to solvent-treated controls).
[c] $P < 0.01$ (as compared to solvent-treated controls).
Figures in parentheses indicate number of animals.

areas. The means and standard errors of these microscopic readings are listed in the tables with the percentage frequency of the lesions. Body weight changes and the just-mentioned data were subjected to variance analysis, and the significance of the chemical values was calculated by Student's *t* test (13).

Results. Effect of calcium antagonists on cardiac and skeletal muscle lesions. Control myopathic hamsters showed cardiac lesions of maximal severity by the 58th day of age (Table I). The myocardial changes varied in size at different stages and covered the entire wall of both ventricles, tending to be more confluent in the interventricular septum. Cellular infiltration and mineralization were intense within the majority of degenerating foci. Verapamil (Group 2) completely prevented the development of cardiomyopathy, except in one hamster which showed a few isolated, degenerated fibers. The integrity of the cardiomyocytes was generally well preserved, a finding which was confirmed by subsequent electronmicroscopy. Prenylamine (Group 3) was almost as effective as Verapamil in reducing the severity of the heart lesions; it restricted them to myolytic changes in 9 out of 15 hamsters. There was a relative decline of body weight presumably because of the irritative properties of this drug. The effects of Cinnarizine (Group 4) were less spectacular in that the severity of the myocardial lesions was reduced by more than 50%. However, the calcific changes in this group indicated that the drug was not very potent as a specific inhibitor of calcium entry into damaged myofibers.

Verapamil and Cinnarizine, unlike Prenylamine, had little or no influence on the progression of the skeletal muscle lesions. Characteristically, there were focal areas of necrosis with variations in fiber diameter, and central nuclear rowing in all sections examined. Since these lesions developed earlier than those in the heart, the antagonistic effect of the two drugs could not have been manifested unless they had been given prior to the occurrence of muscle cell damage. This consideration does not necessarily apply to Prenylamine, which significantly reduced the severity of the myopathy in animals that showed a substantial loss of body weight. The CPK values were lower than those of the other groups, confirming that alterations in the muscle fibers were less severe. Serum calcium and phosphorus levels remained within the normal range, suggesting that the three drugs acted at the cellular level.

Effect of low calcium intake and calcium gluconate on cardiac and skeletal muscle lesions. Restriction of dietary calcium (Group 2) interfered with the progression of both cardiac and skeletal muscle lesions (Table II). In fact, there were only a few myolytic foci in the myocardium. The skeletal muscle lesions exhibited a different pattern from those observed in normally fed controls. They appeared predominantly as patchy areas of coagulation necrosis, without such reactive phenomena as phagocytic cell infiltration and sarcolemma nuclei proliferation. Serum calcium was markedly depleted and the inorganic phosphate levels were elevated in this group. Unexpectedly, the serum CPK concentrations were disproportionally high despite a relatively low necrotic index.

Calcium gluconate (Group 3) markedly reduced the severity of the myocardial lesions, but this effect was not related to pronounced changes in serum electrolyte levels. The CPK values fell sharply in spite of the rather striking skeletal muscle damage.

Combination of calcium gluconate treatment with low calcium intake (Group 4) considerably decreased the cardiac lesions. The serum calcium values remained low despite parenteral administration of the calcium salt and, unlike group 2 animals (in which calcium was similarly depleted), there was no increase in serum CPK.

Discussion. These findings strengthened our belief that, in the hamster, cardiomyopathy and polymyopathy are intimately related to some genetically determined molecular defect of muscle cell membranes. Similar views have been expressed by a number of investigators (5, 14) with reference to a decrease in myocardial adenyl cyclase activity that precedes the development of the cardiac lesions.

Recently, it was shown that, in isolated heart preparations, Verapamil prevents the movement of superficially located calcium across heart sarcolemma (15). Cardiomyocyte contractility is known to require calcium

TABLE II. EFFECTS OF LOW CALCIUM INTAKE AND CALCIUM GLUCONATE ON THE DEVELOPMENT OF HEART AND SKELETAL MUSCLE LESIONS AND ON SERUM ELECTROLYTES AND CREATINE PHOSPHOKINASE IN HAMSTERS WITH HEREDITARY POLYMYOPATHY.

Treatment	Final body weight (initial 29 ± 5.0)	Myocardial lesions		Skeletal muscle lesions		Serum electrolytes (mg/100 ml)		Serum CPK (IU/liter)
		Severity (Grade 0–3)	Incidence (%)	Severity (Grade 0–3)	Incidence (%)	Ca	P	
Purina lab chow (10)	74 ± 2.2	2.18 ± 0.28	100	2.36 ± 0.20	100	10.3 ± 0.2	8.17 ± 0.31	12,900 ± 2,070
Low calcium diet (10)	64 ± 2.4	0.20 ± 0.10[a]	20	1.50 ± 0.14[b]	100	6.2 ± 0.14[a]	11.92 ± 0.53[a]	25,060 ± 7,800
Purina lab chow + calcium gluconate (8)	59 ± 1.3[a]	0.18 ± 0.00[a]	38	1.87 ± 0.10	100	11.2 ± 0.08[a]	8.76 ± 0.14	1,517 ± 126[a]
Low calcium diet + calcium gluconate (8)	51 ± 1.0[a]	0.00 ± 0.00[a]	0	2.00 ± 0.17[c]	100	7.5 ± 0.3[a,c]	8.50 ± 0.52[d]	921 ± 252[a,e]

[a] $P < 0.001$ (in comparison with hamsters kept on Purina Laboratory Chow).
[b] $P < 0.005$ (in comparison with hamsters kept on Purina Lab Chow).
[c] $P < 0.05$ (as compared to calcium-deficient animals).
[d] $P < 0.01$ (as compared to calcium-deficient animals).
[e] $P < 0.025$ (as compared to calcium-deficient animals).
Figures in parentheses denote number of hamsters.

(16), and adrenergic substances may cause calcium overload in muscle cells (17, 18). It is noteworthy, in this respect, that adrenergic nerve activity is augmented during the early phases of cardiomyopathy in hamsters (6), as evidenced by an elevation in norepinephrine synthesis and turnover with an increase of histochemically demonstrable amines in adrenergic nerve terminals. Furthermore, mitochondrial oxydative phosphorylation is defective in myopathic hamsters (19, 20), which are especially susceptible to catecholamine-induced myocardial lesions (8), a phenomenon that may be ascribed to a disturbance of the intracellular ionic milieu.

The beneficial effect of calcium deficiency on hamster myopathy underlines the role of abnormal cellular calcium metabolism in this hereditary disease. It is likely that a drop in serum calcium results in a diminished influx or greater efflux of ions from muscle fibers, thereby preventing the degenerative changes elicited by calcium overaccumulation. At physiologic or slightly above physiologic calcium levels, the inward movements of electrolytes can only be facilitated by a defect in plasma membranes whereas, at lower levels, the phenomenon tends to be reversed. No explanation can yet be offered for the rise of serum CPK in calcium-deficient, myopathic hamsters, since there is no evident aggravation of the pathologic process. This elevation is probably related to altered plasma membrane permeability and, apparently, calcium gluconate could counteract it as indicated by the decrease of serum CPK.

As mentioned earlier, the skeletal muscle changes appeared before initiation of treatment with Verapamil or the other drugs. Since the effects of calcium antagonists are more preventive than curative in nature, their efficacy could be demonstrable only if given prior to the development of cellular damage in the skeletal muscles. The preventive nature of these drug effects is also suggested by the fact that the degenerative changes in the heart soon reappear after termination of treatment with Verapamil and, within 2 wk, become as severe as those seen in untreated myopathic hamsters (21).

Summary. Verapamil, Prenylamine and, to a lesser extent, Cinnarizine were highly efficient in preventing the development or reducing the severity of heart lesions in cardiomyopathic hamsters of the UM-X7.1 line. The calcium antagonistic compounds did not protect against the skeletal muscle changes already present at the time when treatment was initiated. The cardiac lesions were also significantly diminished in frequency and severity by a low calcium diet and, in apparent contradiction, by parenteral administration of calcium gluconate. The relation of these protective effects to changes in the values of electrolytes and creatine phosphokinase is not yet fully understood but suggests that a primary defect in muscle cell membranes may be responsible for hereditary cardiomyopathy in hamsters.

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