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Effect of Orotic Acid and Magnesium Orotate on the Development and Progression of the UM-X7.1 Hamster Hereditary Cardiomyopathy

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Summary. **This study deals with the potential therapeutic effect of orotic acid (OA) and Mg Orotate (MgO) on myocardial degeneration and the development of congestive heart failure in cardiomyopathic (CM) hamsters of the UM-X7.1 line. Two major age groups (group I, < 30 days and group II, >180 days old) were used in these experiments, which lasted 30 and 50 days, respectively; the orotic salts were incorporated (10%) into Purina Lab Chow given ad libitum. Macroscopic and microscopic assessment of pathologic changes together with ECG recordings revealed that MgO treatment significantly reduces myocardial damage, especially the severity of calcific changes. ECG recordings clearly demonstrated a significant shortening of QTc and PR intervals, resulting in partial electrical stabilization of failing hearts, with a significant delay in systemic congestive changes. The prevention of heart lesions was less evident in animals receiving OA, but both preparations proved to be equally efficient in prolonging survival of the CM hamsters.**
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Key Words. **hamster cardiomyopathy, cardioprotection calcification, magnesium orotate, ECG**

The beneficial effects of orotate salts on various in vitro and in vivo experimental models of heart ischemia, as well as the substantial improvement observed in patients with heart hypoxia, have often been reported [1–3]. The therapeutic action of orotic acid (OA) is thought to consist of an improvement in heart contractility due to activation of anerobic metabolism by maintenance of glycogen stores or to increased release of endogenous catecholamines [2,4] and a significant increase in protein synthesis [5] and nucleotide metabolism [6]. It has also been claimed that OA exerts an antihypoxic effect upon the myocardium [7] and prevents the degradation of sarcolemmal glycoproteins in cardiomyopathic (CM) hamster cardiomyocytes [8]. Previous electrophysiological studies carried out in our and other laboratories suggest defective transmembrane ion movement in hamster myopathic hearts. Cardioprotection in these myopathic hamsters may be achieved by a broad spectrum of different pharmacological agents, namely, certain calcium blockers [9], beta adreno receptor agonists [10] and antagonists [11], and, as recently shown, by some K^+_{ATP} Channel openers [12]. In this respect, patch-clamp studies carried out by Bkaily have revealed abnormal Na^+ and Ca^{2+} membrane currents in isolated hamster cardiomyocytes [13,14].

While there is little doubt that K^+ occupies a pivotal position in the maintenance of normal cardiac activities, we decided to investigate further the possible therapeutic effect of an electrolyte dietary supplement of an Mg^{2+} salt preparation on early myocardial calcific changes and the development of congestive heart failure. By grading the severity of histopathologic changes and ECG recordings, we estimated whether OA and MgO, given as dietary supplements, have any preventative actions on the necrotization process and on the development of heart failure. Syrian cardiomyopathic hamsters from the UM-X7.1 line were used in these studies because the myopathic process in this highly inbred strain is highly predictable, and over 50% of the animals die prematuraly within 250 days from circulatory failure [9].

Materials and Methods

As mentioned previously, these studies were carried out with the UM-X7.1 line of CM hamsters. Animals were divided into two major age groups according to the stage of disease. Group I consisted of 38 young male and female hamsters (28 ± 1 days) in the prenecrotic stage of cardiomyopathy, and group II consisted of 64 older animals (185 ± 5 days) of either sex that had reached the moderate stage of heart failure. Males and females in these two major groups were subdivided into three subgroups and received, respectively, regular Purina Laboratory Chow or the same pelleted diet supplemented with 10% OA or MgO, as outlined in the tables. Experiments at the prenecrotic stage and at the

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heart failure stage lasted 30 and 50 days, respectively. All animals had free access to tap water and chow and were housed at 24°C and under a 12:12 hour light: dark cycle. The animals were cared for in accordance with the Canadian Council on Animal Care 1993 guideline Food consumption and growth were estimated twice weekly by weighing the residual chow and recording the body weight gain.

Before sacrifice, blood was taken from the jugular vein under light chloral hydrate (250 mg/kg) anesthesia to assay the serum creatine kinase (CK) activity using the Boehringer Mannheim Diagnostica UV test. At autopsy the animals were skinned to make an overall quantification of the disseminated heart and skeletal muscle necrotic foci visible by the naked eye [9]. In the older animals in group II, the degree of heart failure was classified by referring to liver congestion, and pulmonary edema, and the severity of anasarca [15].

Microscopic studies were carried out on freshly dissected specimens arising from the myocardium, tongue, and liver. All tissue samples were fixed in neutral formol (10%), embedded in paraffin, and sectioned with a microtome. Serial 4-μm sections, selected at three different levels of the myocardium, were stained with hematoxylin-phloxine-saffron (HPS) and Von Kossa stains for demonstration of tissue calcium [16]. The microscopic readings were computed following a double-blind procedure using an arbitrary scale of 0–3 (with half-marks when necessary) to determine the severity of ventricular and skeletal muscle lesions and liver sclerosis [9,15]. The maximum score of 3.0 indicates that the damage exceeded 50% of the entire myocardium, whereas lower scores corresponded to moderate and mild changes. Cardioprotection becomes significant when the mean of the readings for a given group is lower than 1.4 in $\leq 80\%$ of animals.

ECG recordings

ECG recordings were carried out on 28 untreated, OA-treated, and MgO-treated CM hamsters of group II, as indicated in Table 3, and on 8 healthy normal hamsters of comparable age, which served as absolute controls. Electrographic tracings of 230 ± 5-day-old animals were obtained using a computer-averaging technique under anesthesia [17] induced by sodium pentobarbital (45 mg/kg ip) and were registered in the natural prone position. Each of four limbs was introduced into small copper tubes filled with a conductive gel (EKG Sol Graphic Controls, Canada), which served as electrodes. These bipolar electrodes were connected to a bioelectric amplifier (Nikon Kohden, Tokyo, Japan) to measure simultaneously the three standard leads I, II, and III [15]. The numerical readings were transferred to computer memory and processed on-line. After an equilibration period of (5–10 minutes), 200–400 beats were recorded, aligned, and averaged to eliminate noise from muscle contractions. The intervals RR, PR, QT, and QRS (in ms) were computed. The QT interval and QRS width are referred as such, even though the Q wave is absent in the hamster ECG. The QT interval was measured from the R wave to the apex of the T wave, because of difficulty identifying the termination of the T wave, and was corrected for RR variations according to formula Q_a $T_c = Q_a$ T/square root [RR]. The R, S, and T wave amplitudes were determined by measurement of the average signal in millivolt deflection from the isoelectric line. To verify the reproducibility of the technique, the ECGs of normal healthy animals were repeated on different days; no appreciable changes were noted.

Two nonparametric tests were used to determine the statistical significance of the microscopic readings: the Kruskal-Wallis test, a one-way analysis of variance by rank, and the Mann-Whitney U-test, a nonparametric equivalent of the impaired t-test, when analysis of variance indicated the presence of significant changes among the CM-untreated, OA-treated, and MgO-treated groups [18]; Student's test was used for the remaining data. The differences were considered nonsignificant at the $P > 0.05$ level versus the untreated CM group or healthy controls (ECG recordings). The OA and MgO were supplied by Worwag Pharma GmBH (Stuttgart, Germany); these salts were incorporated in Purina pellets (Tekla Comp., Wisconsin).

Results

Group I

Table 1 summarizes the pertinent findings on the effect of MgO and OA on gross morphology and microscopic changes. A sustained oral administration of OA or MgO, at the 10% level into the chow, retarded by 10% and 5%, respectively, the body weight gain despite the fact that the animals consumed 12% more daily chow. The relative ventricular weight, however, was unaffected by either treatment. On naked eye examination, there were less evident heart lesions in MgO-treated hearts than in OA-treated and untreated hearts. Under the microscope, numerous disseminated heart necrotic lesions of different size were seen in serial staged sections of all untreated and OA-treated animals (Figure 1B); the myocardial degenerative changes are characterized by coagulation necrosis, calcification, and myofibrolysis. Treatment with MgO, on the other hand, was efficient in partially preventing necrotic calcific lesions, whereas only moderate dispersed myolytic changes were observed (Figure 1A). Interestingly, the microscopic tongue lesions were equally less severe after MgO treatment. The high serum CK activity (>20,000 U/l), however, remained unchanged in all groups regardless of the treatment. As shown in Table 1, a significant cytoprotection was achieved only in the MgO-treated group.

Group II

Table 2 summarizes the pathologic findings after 50 days of treatment with OA or MgO during moderate

Table 1. *Pathological changes after treatment of young UM-X7.1 cardiomyopathic hamsters treated with orotic acid and Mg orotate (group I)*

Treatment (no. animals)	Untreated (12)	Orotic acid (12)	Mg orotate (14)
Purina consumption (g/animal/d)	7.6 ± 0.1	8.5 ± 0.3	8.5 ± 0.3
Dose (mg/animal/d)	0	850 ± 27	850 ± 28
Body weight gain (g) (initial = 47 ± 1 g)	52 ± 2	43 ± 2	48 ± 5
Ventricle (mg)/BW (g)	3.14 ± 0.10	3.31 ± 0.12	3.10 ± 0.05
Microscopic heart lesions			
Severity (0–3)	2.22 ± 0.11	2.08 ± 0.09	1.23 ± 0.10*[a]
Incidence (%)	100	100	80
Microscopic tongue lesions			
Severity (0–3)	1.76 ± 0.11	1.54 ± 0.10	0.78 ± 0.12*[a]
Incidence (%)	100	100	100
Serum CK activity (U/l)	20,193 ± 3,939	18,089 ± 3,686	23,124 ± 4,133

[a] Mean ± SE.
*$P < 0.001$ significantly different from the untreated CM hamsters.
CM hamsters aged 28 ± 1 days were treated over 30 days.

and severe heart failure (Figure 2). In all surviving CM hamsters, their body weight gain and the relative ventricular weight remained constant for the duration of the experiment, except for animals that died prematurely in a state of cachexia or anasarca. Like animals in group I, the consumption of chow supplemented with OA and MgO was 15–20% greater than in the control group. Both OA and MgO prolonged the lifespan, with 20% improvement of mortality, including CM hamsters in a state of anasarca. Surprisingly, OA-

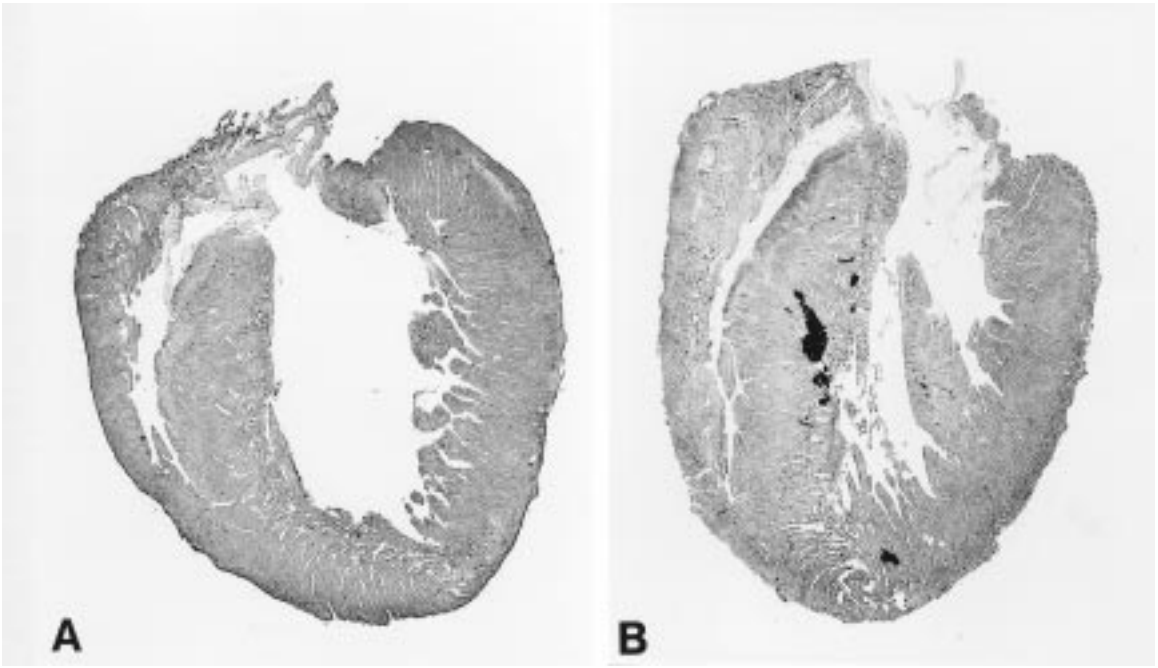

Fig. 1. *Prevention by MgO of myocardial lesions occurring in CM hamsters between 30 and 60 days of age.* A: *Transection of a 60-day-old hamster heart following administration of MgO in the diet.* B: *Untreated heart in a CM hamster of the same age with multiple disseminated necrotic foci (Von Kossa × 25).*

Table 2. *Pathological changes during moderate to severe stages of heart failure in cardiomyopathic hamsters treated with orotic acid and Mg-orotate (group II)*

Treatment (no. animals)	Untreated (21)	Orotic acid (22)	Mg orotate (21)
Purina consumption (g/animal/d)	7.8 ± 0.2	9.0 ± 0.3	8.6 ± 0.2
Dose (mg/animal/d)	0	900 ± 31	860 ± 25
Body weight gain (g) (initial = 132 ± 2 g)	8 ± 1	5 ± 2	3 ± 1
Mortality (%)	62	45	43
Ventricle (mg)/BW (g)	3.51 ± 0.06	3.71 ± 0.09	3.56 ± 0.07
Microscopic heart lesions			
Severity (0–3)	2.68 ± 0.05	2.50 ± 0.08	2.50 ± 0.10
Incidence (%)	(100)	(100)	(100)
Microscopic liver changes			
Severity (0–3)	1.42 ± 0.20	1.20 ± 0.2	1.15 ± 0.19
Incidence (%)	(60)	(40)	(60)
Pulmonary edema			
Severity (0–3)	1.42 ± 0.13	1.66 ± 0.08	1.39 ± 0.18
Incidence (%)	(90)	(100)	(100)
(%) of anasarca: incidence	15	15	10

Mean ± SE.
CM hamsters aged 185 ± 5 days were treated over 50 days.

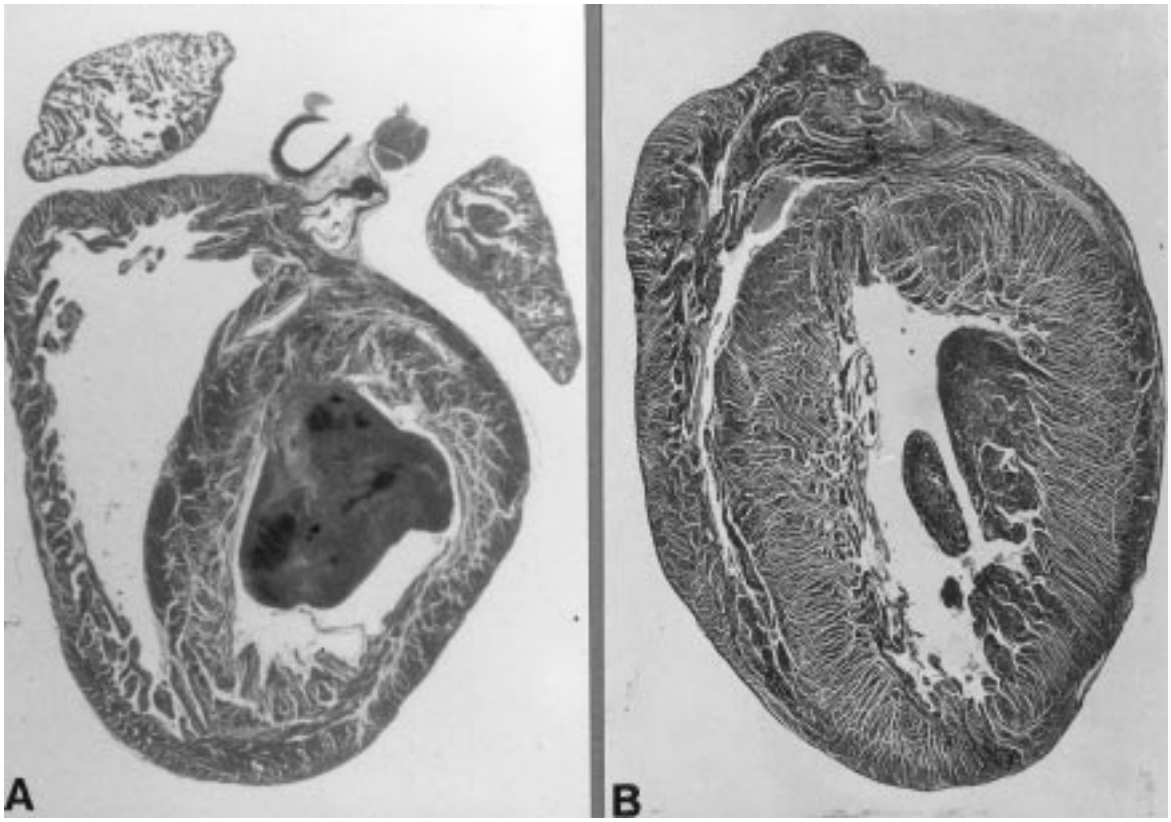

Fig. 2. *Transection of a myopathic heart during the advanced stage of heart failure.* A: *Note the presence of a mural thrombus in the right ventricle of the untreated myopathic hamster.* B: *Protection afforded by the MgO diet. (Reprinted with permission of Nagano et al. [15].)*

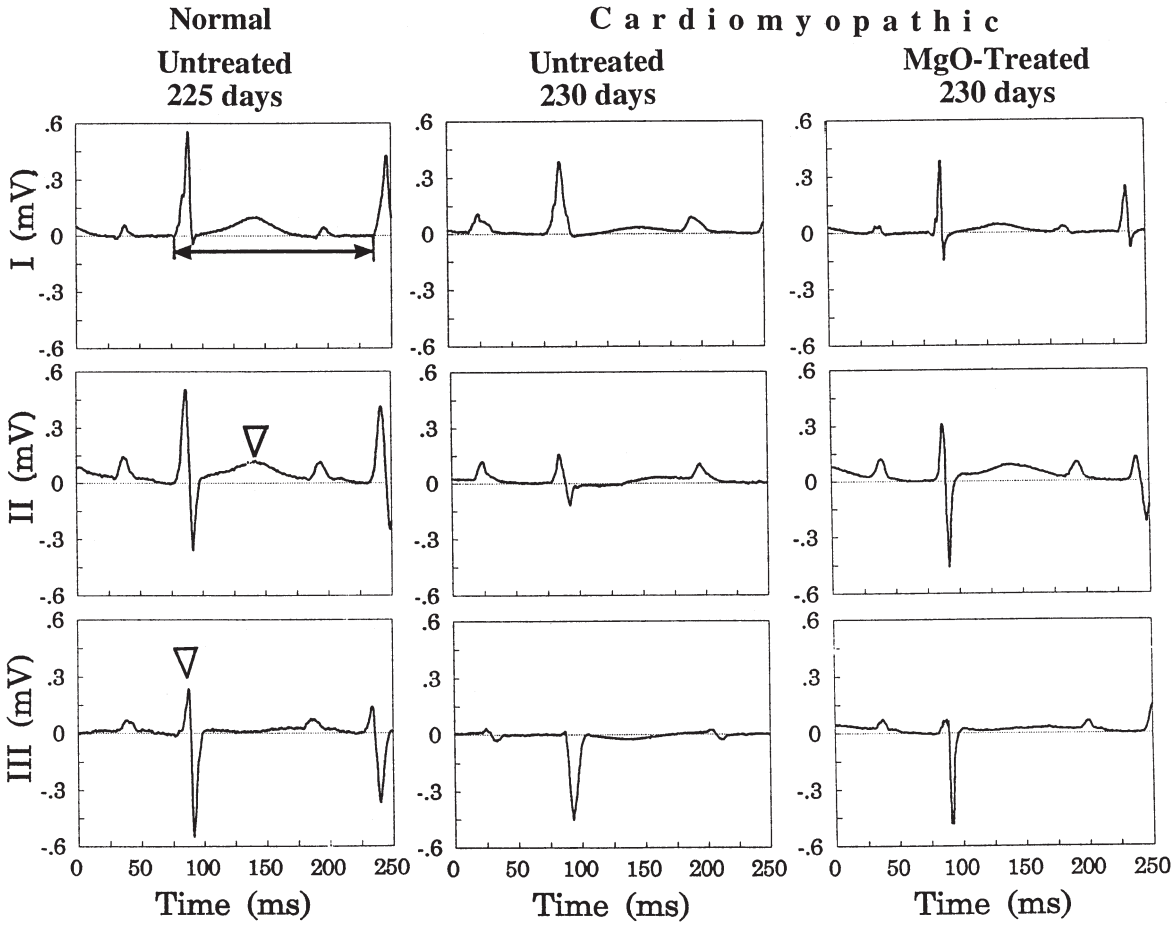

Fig.3. *Comparative ECG tracings in the untreated and MgO-treated CM hamster at 230 days of age. Standard-lead (I, II, III) ECG in untreated CM hamsters indicated the RR interval is wider than in the MgO-treated group. Similarly, MgO treatment increased the depressed T- and R-wave amplitudes.*

treated females survived 2–3 weeks longer with their giant edema. However, neither macroscopic assessment nor histological findings demonstrated any structural improvement in the treated hamsters.

Electrocardiographic assessment

Representative ECG computerized tracings of CM untreated and MgO-treated hamsters as compared with normal healthy animals of similar age, are shown in Figure 3. After MgO treatment, the electrical activity in myopathic heart was improved, as revealed by the shorter duration of QTc and PR intervals and the greater amplitude of the T wave in lead II, changes that indicate a myopathic process. Table 3 summarizes the ECG readings: The data indicate that MgO contributed to electrical stabilizing of myopathic hearts and to a delay in the development of congestive heart failure. A significant shortening of PR and almost normalization of the QTc intervals tempered the propensity to lethal ventricular arrhythmia, and improved atrioventricular conductivity was observed despite no appreciable differences in the duration of the QRS complex among all groups investigated. Flat and sometimes inverted T waves, readily seen in untreated CM animals, were increased by 100% and 60% after treatment with MgO and OA; in edematous animals, however, the R-wave amplitude decreased (Table 3).

Discussion

These studies were conducted because of growing interest in the therapeutic use of OA in clinical and experimental cardiology [19]. The studies of Altura [20] and others [21,22] led us to believe that the Mg^{2+} salt of OA might further improve the energy-coupling process in heart mitochondria, possibly by reducing the conspicuous high Ca^{2+}/Mg^{2+} ratio found in CM hamster hearts [23]. Moreover, modulating the Mg^{2+} effect on cardiac ryanodine receptors [24], the density of which is elevated in CM hamster hearts [25], can account for the less

Table 3. *Heart rate, electrographic intervals, and wave amplitudes of 235-day-old CM hamsters after 50 days of treatment with orotic acid and Mg Orotate*

Group	HR (min^{-1})	QTc	QRS (ms)	PR (ms)	T_{II} (mV)	R_{III} (mV)
Cardiomyopathic						
Untreated (12)	315 ± 7[b]	5.30 ± 0.31[b]	21.2 ± 1.9	60.9 ± 3.4[b]	0.021 ± 0.004[b]	0.058 ± 0.015[b]
Acid orotic (8)	312 ± 13[b]	5.86 ± 0.32[b]	23.2 ± 0.9	54.8 ± 3.2[b]	0.034 ± 0.007[b]	0.045 ± 0.010[b]
Mg orotate (8)	320 ± 10	4.68 ± 0.28[a]	23.8 ± 1.4	48.2 ± 1.8[b]	0.041 ± 0.010[b]	0.046 ± 0.020[b]
Healthy controls (8)	351 ± 10	4.52 ± 0.30	22.1 ± 2.5	36.6 ± 1.2	0.104 ± 0.031	0.195 ± 0.135

Mean ± SE.
$P < 0.05$ statistically different from CM [a] untreated and [b] healthy controls.

severe necrotic heart lesions in the Mg^{2+} salt of OA-treated animals. Our microscopic findings corroborate the above-mentioned assumption because only MgO significantly protected CM hearts by reducing the severity of necrotic and calcific changes. These findings are consistent with the hypothesis that MgO can modify Ca^{2+} influx; interestingly, partial heart protection and reduction of the myocardial Ca^{2+} overload was shown previously with in vivo treatment with Mg aspartate in CM hamsters [26]. Under the present experimental conditions, the beneficial effects of OA seemed less obvious than those resulting from MgO treatment.

As revealed by the ECG tracings, the beneficial effects of MgO in aging myopathic hearts (group II) consisted mainly of stabilizing electrically failing hearts; MgO treatment shortened the PR interval and practically normalized the QTc interval. Thus its effects may be ascribed to the lower frequency of lethal arrhythmia and the diminished severity of atrioventricular block in CM hamsters. The clinical implication of QT shortening achieved by MgO might be that an inherited disorder such as the long QT syndrome [27] can be improved by an orotate salt. Attenuation of the R-wave amplitude, allegedly low in CM hamster hearts, seems to be related to the extensive anasarca that occurs at the end stage of the disease [28]. The presumed nonspecificity of flat or inverted T waves observed in various forms of muscular dystrophies [29], just as in CM hamsters [15], possibly deriving from a primary cardiac pathology, were increased by either treatment, thus suggesting an improvement in the depolarization activity and ischemia in myopathic hearts. It is inferred that MgO acts as a natural Ca antagonist due to the presence of Mg^{2+} [30], in addition to improving cardiac performance and bioenergetics.

Conclusions

The effects of a Purina Laboratory Chow diet supplemented with 10% OA or MgO were on the occurrence of heart necrotic changes and the severity of heart failure were investigated in UM-X7.1 cardiomyopathic hamsters. Our findings were as follows:

1. The growth of treated animals (OA, MgO) was 10% and 5% lower, respectively as evidenced by differences in body weight gain; conversely, the consumption of chow supplemented with OA and MgO was 12% higher.
2. The absolute and relative ventricular weights were unaffected by either treatment.
3. MgO-treated CM hamsters developed 40% less severe heart lesions. Moreover, the diaphragm and tongue were relatively protected; the severity of lesions was reduced by 30% and 60%, respectively.
4. On the other hand, OA did not afford cardioprotection or diminish the severity of skeletal muscle lesions.

Additional studies are required to determine whether a higher concentration of orotate given during a sufficiently long period could interfere with the development and severity of myopathic changes in the heart and skeletal muscle.

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