Interdependence of potassium and magnesium in the pathogenesis and healing of myocardial necrosis*) **)

By *K. Sarkar* and *H. A. Heggtveit*

Department of Pathology, School of Medicine, University of Ottawa, Ottawa, Ontario, Canada

Zusammenfassung

Es wurde der Mechanismus der gegenseitigen Abhängigkeit von K und Mg untersucht, der postuliert wird, da sich Myocardnekrosen dann entwickeln, wenn ein Mangel an einem der beiden Elektrolyte vorliegt. Hierzu wurden 30 Tage alte männliche Sprague-Dawley-Ratten mit einem durchschnittlichen Körpergewicht von 105 g in vier Gruppen aufgeteilt. Gruppe I erhielt K- und Mg-haltiges Futter; Gruppe II eine K- und Mg- freie Diät; Gruppe III eine K-freie und Gruppe IV eine Mg-freie Diät. Alle Ratten wurden nach vier Wochen getötet, bis auf 2 Drittel der Tiere aus Gruppe II. Die Hälfte dieser Ratten aus Gruppe II erhielt jetzt KCl (Gruppe IIa), die andere Hälfte $MgCl_2$ (Gruppe IIb), während jeweils einer Woche. Es zeigte sich, daß bei gleichzeitigem Mangel an Mg und K mehr Myocardnekrosen entstehen als bei Mangel an nur einem Element. Der Abheilungsprozeß setzt ein, wenn den Tieren eines der beiden Salze wieder zugeführt wird. Die histologischen Bilder des Abheilungsprozesses sind einander ähnlich, und Narbenbildung ist minimal.

Summary

To elucidate upon the postulated mechanism that some relationship might exist between potassium and magnesium when myocardial necrosis is induced by depleting either of these two electrolytes, 30 day old male Sprague-Dawley rats with an average weight of 105 mg were divided into 4 groups. Group I ate a diet containing potassium and magnesium, Group II ate a potassium and magnesium free diet, Group III ate a potassium free diet and Group IV ate a magnesium free diet. All the rats, except 2/3 of Group II, were killed after 4 weeks. One-half of Group II was repleted with KCl (Group IIa) and the other half (Group IIb) with $MgCl_2$ for one week and killed. We found that the degree of myocardial necrosis is more widespread by simultaneous magnesium and potassium depletion than with that caused by one of the electrolytes. The healing of the lesion can be initiated when animals are repleted with either of the two electrolytes. The historival patterns of healing are similar and scarring is minimal.

Résumé

Afin d'élucider le mécanisme hypothétique selon lequel une certaine relation peut exister entre le K et le Mg quand une nécrose cardiaque est induite par la déplétion de l'un ou l'autre de ces deux électrolytes, des rats mâles Sprague-Dawley de 30 jours, d'un poids moyen de 105 g ont été répartis en 4 groupes. Le groupe I a consommé un régime contenant du potassium et du magnésium, le groupe II a consommé un régime dépourvu de potassium et de magnésium, le groupe III a consommé un régime dépourvu de K, et le groupe IV a consommé un régime dépourvu de Mg. L'ensemble des rats, à l'exception de 2/3 du groupe II a été sacrifié au bout de 4 semaines. Une moitié due groupe II, a été soumise à une réplétion par KCl (groupe IIa) et l'autre moitié (groupe IIb) par $MgCl_2$ pendant une semaine et ils ont été sacrifiés. Nous avons trouvé que le degré de la nécrose du myocarde est plus étendu par la déplétion simultanée du Mg et du K que celui provoqué par un seul des électrolytes. La guérison de la lésion peut être induite quand les animaux sont soumis à une réplétion par l'un ou l'autre des électrolytes. Les caractéristiques histologiques sont semblables et la cicatrice résiduelle est minime.

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Introduction

Potassium (K) and magnesium (Mg) are the two most important intracellular cations, and it is believed that a metabolic interrelation exists between them [13]. During Mg deficiency, there is a concurrent loss of tissue K even when dietary K intake remains adequate [1]. In the heart, focal myofibrillar degeneration takes place in both Mg and K deficiency [3, 4], and the lesions produced by one deficit closely resemble those caused by the other. Simultaneous Mg and K depletion increases the extent of myocardial lesions in the rat [12].

The present morphological study was undertaken in the rat to further our understanding of the interpendence of Mg and K not only in the process of induction of myocardial lesions but in their healing as well.

Materials and methods

Male Sprague-Dawley rats, weighing about 100 g, were obtained from the Canadian Breeding Farm, St. Constant, Quebec. After acclimatization to our laboratory, the rats were separated into 4 groups. One group ate a K-free diet, the second group a Mg-free diet and the third group a combined K and Mg-free diet. The last group served as control, eating the same diet as Group 3 but having both of the electrolytes added to the water.

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The diets were supplied by Teklad Test Diets, Madison, Wisconsin. The K-free diet consisted of the following ingredients in 10 Kg:

Basal diet:	9,971.8 g
Na_2CO_3:	4.06 g
NaCl:	8.24 g
$4MgCO_3 \cdot Mg(OH)_2 \cdot nH_2O$	15.94 g

The basal diet (TD 78093) contains casein and corn starch as its principal ingredients with salts and vitamins, the details of which have been previously reported [10].

The Mg-free diet (TD 76446) had the following g/Kg constitutents:

Casein:	250.0
Corn starch:	100.0
Sucrose:	535.6
Corn oil:	70.0
Vitamin mix:	10.0
$CaCO_3$:	6.3
$CaHPO_4$:	10.0
K_2HPO_4:	5.6
NaCl:	9.0
KCl:	2.8
Ferric citrate (16.7 % Fe):	0.2994
KIO_3:	0.0004
$MnSO_4 \cdot H_2O$:	0.1538
$ZnCO_3$:	0.0268
$CuSO_4$:	0.025

The combined K and Mg-free diet was composed of (in g/Kg):

Casein, high protein:	200.0
DL-methionine:	3.0
Sucrose:	697.6499
Corn oil:	70.0
Vitamin mix:	10.0
$CaHPO_4$:	14.539
$CaCO_3$:	1.79
NaCl:	2.59
$MnSO_4 \cdot H_2O$:	0.179
$FeSO_4 \cdot 7H_2O$:	0.175
$ZnCO_3$:	0.056
$CuCO_3$:	0.0105
$NaIO_3$:	0.0003
$Na_2SeO_3 \cdot 5H_2O$:	0.0003
$CrCl_3 \cdot 6H_2O$:	0.01

It is estimated by the manufacturer that the levels of Mg and K in the diet are < 10 ppm and < 20 ppm respectively. Both the animals on double electrolyte depletion and their controls ate the same diet; the former drank distilled water while the latter received salts of $MgCl_2$ (200 mg/100 ml) and KCl (1.11 g/100 ml) in water. After 4 weeks, all of the first two groups and a part of the third group of depleted animals were killed along with the controls. The rest of the animals with double electrolyte depletion were divided into 2 groups: one was given $MgCl_2$ (200 mg/100 ml of water) while their K deprivation was continued. The other group had KCl (1.11 g/100 ml of water) while they continued being Mg deficient. After one week, both of these partially depleted groups were killed.

For light microscopy, hearts were fixed in 10 % neutral buffered formalin. Sections from paraffin-embedded tissues were stained by hematoxylin and eosin, phosphotungstic acid hematoxylin and trichrome methods.

Electron microscopical examination was done only on heart tissues obtained from double electrolyte depleted rats, their controls and from partially repleted rats. The animals were anesthetized by an intraperitoneal injection of nembutal. The heart was either fixed in vivo with halfstrength *Karnovsky's* fixative by intraarterial perfusion or by immersion while still beating into the same fixative. A central coronal section of the myocardium was cut into small pieces and the fixation was continued for a further period of 2 hours at 4° C. After post-fixation in 1 % osmium tetroxide, the pieces were dehydrated in graded ethyl alcohol and propylene oxide and embedded in eponaraldite. Thin sections were stained with uranyl acetate and lead citrate.

Results

After 4 weeks, the group depleted of K and that depleted of both K and Mg had a negligible gain in weight. The Mg depleted rats gained about 60—80 g, but that gain was far below that of the control animals which had more than 3 times their starting weight. When rats with double electrolyte depletion were repleted, one group with K and the other with Mg, the former began to gain some weight almost immediately while the latter failed to do so.

Clinically, the Mg-deficient animals demonstrated the well known signs of erythema of ears, foot pads and tail. These changes were absent when rats were depleted of K simultaneously with Mg.

When the hearts of 3 groups of depleted animals were examined after 4 weeks, it was evident

that the lesions were consistently more widespread in double electrolyte depleted animals than in those with either K or Mg depletion. The Mg-depleted rats were the least constant in developing significant myocardial lesions within this period.

The light microscopic appearance of lesions in all 3 groups, however, was more or less similar. The areas of necrosis were focally distributed, had irregular shape and size, and showed a slight predilection for the right ventricle and the endocardial regions. They were well vascularized and consisted of mononuclear cells of which a large number were macrophages and only a few appeared to be acute inflammatory cells. Myofibers seldom persisted within the lesions.

Electron microscopy of myocardial lesions from the double electrolyte depleted rats showed that the cells were predominantly macrophages with ruffled cell surface and containing prominent phagolysosomes (Fig. 1). Small degenerative fragments of myofibers were scattered within the lesions. The most severe changes in adjacent myofibers consisted of simplification of myofilamentous distribution with loss of the sarcomeric pattern and sarcoplasmic organelles (Fig. 2). In some other myofibers, fibrils were randomly separated, mitochondria were swollen and vacuoles or lipidic inclusions were frequent. There was partial widening of many intercalated discs. Fibroblastic cells were few and capillaries appeared generally unremarkable.

After 1 week of K repletion, necrotic lesions were sparse and small. Scarring was minimal in and around these lesions. In contrast, lesions after 1 week of Mg repletion, although somewhat less in size and extent than after 4 weeks of double electrolyte depletion, were still conspicuous. Many of the cells in the lesions showed significant basophilia, and mitotic figures were occasionally found in relation to the lesions.

Ultrastructurally, the characteristics of the lesions after either K or Mg repletion, were broadly similar. The most abundant constituents were anucleated fragments enveloped by a basal lamina; these resembled myofibers which lacked organization of filaments and showed incomplete forms of intercalated discs (Fig. 3). Among the nucleated cells, there were undifferentiated cells in which the nucleus occupied a major part of the cellular volume. Many of these were surrounded by a basal lamina, but they could be distinguished from the pericytes which were often hypertrophied and sometimes detached from the vessels while retaining their morphological features. The nuclei of undifferentiated cells without basal lamina were mostly euchromatic, imparting a blastoid appearance (Fig. 4). Fibroblastic cells with well-

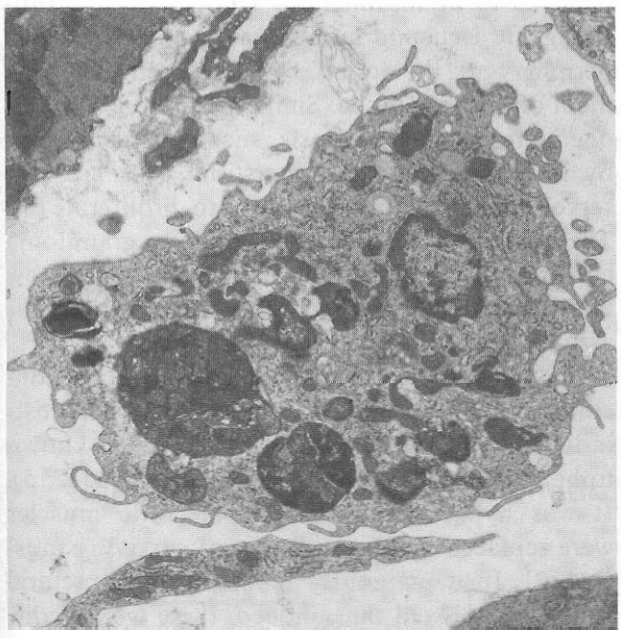

Fig. 1: Macrophage with ruffled cell surface and containing phagolysosomes. Uranyl acetate (UA) and lead citrate (LC). × 10,800.

Fig. 2: Dedifferentiating myofiber with a degenerative vacuole. UA and LC. × 10,700.

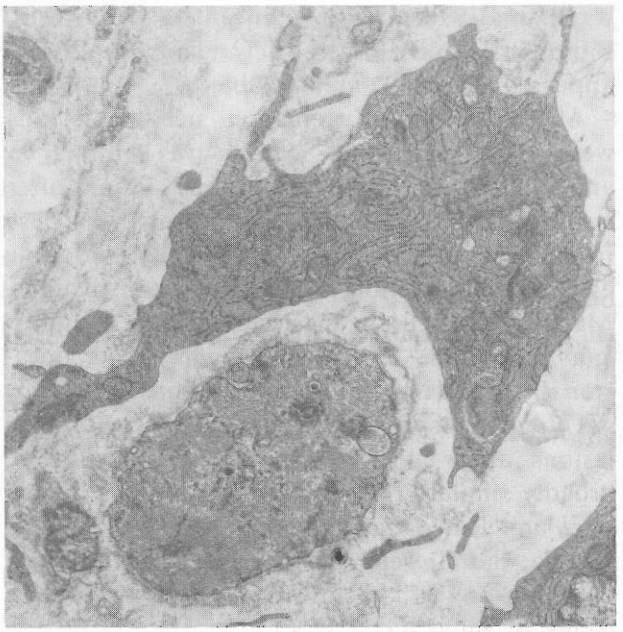

Fig. 3: Myofiber fragment with incomplete intercalated disc along with a fibroblastic cell. UA and LC. × 13,000.

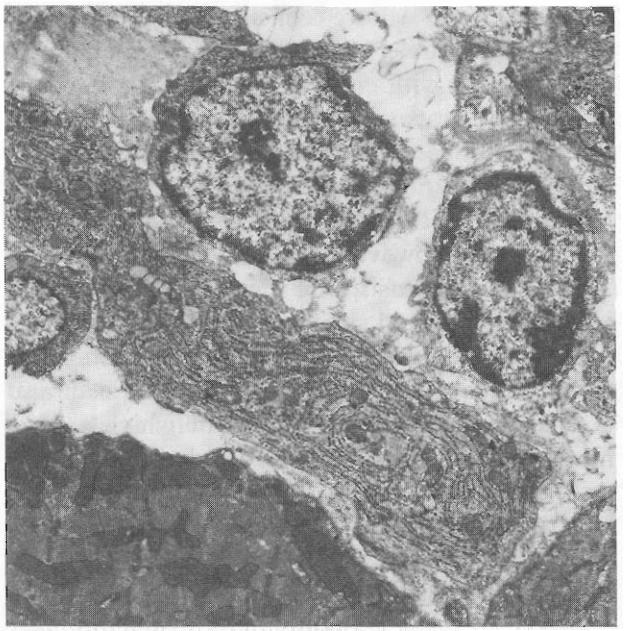

Fig. 4: Fibroblastic cell along with small cells which have a blastoid appearance. UA and LC. × 8,900.

developed rough endoplastic reticulum (Fig. 4) were abundant after Mg but not so much after K repletion. Infrequently, there were recognizable early myocytes with scattered aggregates of myofilaments.

Discussion

It appeared from our study that growth is much more adversely affected by K than by Mg depletion alone. Thus, the impairment of growth in double electrolyte depleted animals could be attributed primarily to the lack of K rather than Mg. It is worth noting, however, that the decrease in serum Mg was more severe with the magnesium deficient diet than with the double electrolyte depleted diet, although the manufacturer claimed that the magnesium content of both diets was similarly negligible. The importance of K in relation to growth was further evidenced during the period of partial repletion. With K repletion, rats resumed growing to some extent almost immediately but failed to do so after Mg repletion.

In the induction of myocardial lesions, a simultaneous depletion of K and Mg was considerably more effective than deficiency of either of those two electrolytes alone. With partial repletion, however, the lesions were more responsive to K than to Mg.

The ultrastructural pattern of the healing lesions was broadly similar with either K or Mg repletion despite the tardiness of the process in the latter. The most outstanding aspect of healing was the relative absence of scarring which usually occurs in the repair of both coronarogenic and non-coronarogenic cardiac necroses. The regenerative capacity of the vertebrate myocardium is believed to be minimal [6]. The healing of coronarogenic ischemic infarcts takes place from the periphery of the lesion by fibroblastic replacement of necrotic tissue. Similarly, in non-coronarogenic myocardial necrosis, such as that induced by isoproterenol, some interstitial cells are preserved which retain the ability to proliferate [8]. The initial phase of repair resembles a granuloma consisting of fibroblasts, histiocytes and a few multinucleated giant cells. The granulomatous area is gradually replaced by a fibrous scar [7].

In contrast to repair by scar tissue, the non-coronarogenic form of myocardial necrosis induced by K depletion in rats, has been known to heal without scarring when K is restored [2, 5]. It was hypothesized that the necrotic muscles were replaced by „hypertrophy of surviving muscle" [2]. In a previously reported ultrastructural study, we showed that, indeed, there were dedifferentiated fragments of myofibers remaining in the cardiac lesions of rats chronically depleted of K. These fragments were found to be rapidly

redifferentiating when the lesions were examined sequentially during the stage of K repletion [11].

In the present study, the healing lesions not only contained redifferentiating fragments of myofibers, but also undifferentiated, mononucleated cells with a primitive blastoid appearance that might play a role in the reparative mechanism. We were not able to examine the ultrastructure of mitotic cells, which we found after Mg repletion, because of their rarity. Recent studies in rats and frogs have shown that cardiac myogenesis may occur by mitosis after injury [9].

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(For the authors: Dr. Kiriti Sarkar, Department of Pathology, University of Ottawa, 275 Nicholas Street, K1N 9A9, Ottawa, Ontario, Canada)