Original Article

Experimental, electronomicroscopical and biochemical researches about disseminated hemodynamic myocardial infarctions by beta-adrenergic stresses.

The efficiency of therapy with magnesium ascorbate

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Abstract

The nonatherosclerotic hemodynamic ischemic cardiomiopathies were induced on the guinea pigs and Wistar rats, by beta-adrenergic aggressions of Isoprenalin (ISOP). This it was made for an elctronomicroscopical and biochemical thoroughgoing study of the hypoxic coronary spastic lesions.

Ultrastructurally, they were noticed some hypoxic catecholaminic lesions, until they were got disseminated myocardial infarctions. Biochemical, ATP and magnesium depletion, hydroelectrolitic perturbations and increase of serum LDH levels were predominantly.

In case of the lots which got both injectable ISOP and Magnesium Ascorbat with myocardial protector substances- Magnobil (Merck), the results were benefic. The ultrastructural lesional images didn't outrun the limits of reversibility and biochemical, the ATP and Mg^{2+} levels returned to the control levels.

The efficiency of this medication was certified at the patients with myocardial ischemias by improvement of clinical condition and EKG images.

Keywords: *experiment, beta-adrenergic aggressions, Isoprenalin, electronic microscopy, biochemistry, disseminate hemodynamic infarctions, therapy with Magnesium Ascorbat.*

Introduction

In the last decades, after Rona and co-workers' studies [1], it appeared a series of papers [2, 3, 4, 5, 6, 7, 8, 9] about the influence of some beta adrenergic substances, especially Isoprenalin (ISOP). Because we wanted to know much better about the pathogenesis of atherosclerotic nonobstructive disseminated myocardial infarctions, daily we watched the evolution of lesions determined by ISOP administration, used for this ultrastructural and biochemical examinations.

These experiments showed resemblances with some aspects from human pathology, determinated by recurrent long stresses and led to ischemic cardiomyopathy, and also showed resemblances with the cathecolaminic pheochrocytomas cases.

Because, the Magnesium Ascorbat, (which was synthesized at the Industry of Medicines, Bucharest), contains the same as Magnobil (Merck), 10% myocardial-protective substances: Ascorbic Acid and Magnesium, we experimented the efficiency of Magnesium Ascorbat simultaneous administrated with Isoprenalin (ISOP), to reveal his protective role against aggressive character of the beta adrenergic substances.

Material and methods

Experimental methods:

We used for this study a lot of 120 guinea pigs, with average weight between 450 and 550g and a lot of 100 Wistar rats with average weight between 170 and 240g, both sex.

The rats were grouped in 4 lots:

1. control lot;
2. lot of rats, daily subcutaneous injected with Isoprenalin (ISOP), one dose, 10 mg/kg body, 1-7 days, sacrificed through bleeding, at an hour after the last injection.
3. lot of the rats, subcutaneous injected with ISOP, a dose of 50-70 mg/kg body, 1-7 days;
4. lot of rats protected with Magnesium Ascorbat, a dose of 13-26 mg/kg body given with an hour before ISOP injected and sacrificed after 1-7 days.

The guinea pigs were grouped in 4 lots too:

1. control lot;
2. lot of guinea pigs, subcutaneous injected with 10 mg/kh body ISOP, sacrificed after an hour.

3. lot of guinea pigs, subcutaneous injected with Magnesium Ascorbat 13-26 mg/kg body, different doses and after an hour, subcutaneous injected with ISOP 10 mg/kg body, sacrificed after an hour.
4. lot of reduced number of guinea pigs, with the same experiment as before, but daily injected and a subgroup injected for 4-7 days and another injected for 1-3 months.

There were made some morphological techniques:

Anatomical techniques:

- some intracardiac injections with China ink made by N.M.Constantinescu, U.M.F. Carol Davila, to highlight the coronary tree situation. After that, some fragments were drawn from each myocardic zone, fixed in formaldehyde 10% and cut in serial sections of 7-50-100 microns.

Histological techniques:

- we used the stains: Hematoxylin-Eosin (HE), Van Gieson (VG), PAS-Alcian Blue, Gomori.

Histoenzymological techniques:

- we used them to making evident LDH (lactate dehydrogenase), ATP-ase of membrane, Acid Phosphatase (ACP).

Electronomicroscopical techniques:

- the fragments of 1 mm diameter were fixed in glutam-aldehyde 2,5%, embedded in Epon 812, cut in semifine and fine sections and examined to the electron and optic microscope.

Biochemical techniques:

- they were made in the Pathology and Chemistry laboratory of the "Victor Babes" National Institute, lead by Dr. S. Constantinescu. The ATP levels were investigated on the fresh tissue by the spectrophotometric method with hexokinase and also, the Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} endogenetic levels were investigated by the spectrophotometric method in UV (ultraviolet).

Electrocardiographical techniques:

- they were made by an electrocardiograph of the Politehnic Institute, Bucharest.

Results:

The anatomical studies were made on the animals which got ISOP, by intravenous injections with China ink. The studies showed that, the penetration of China ink was more difficult than the control animal. The China ink penetration was difficult, in the left ventricle, especially intraventricular septum and apex and in this way, showed the vessels of capillary network poor injected, as isolated islands-like. The small number of the arterioles and venules showed the spastic conditions of vessels after the high doses of ISOP were injected [10].

The histological studies made on the lots of animals which got only ISOP, showed different dystrophic lesions of the cardiomyopathies and perivascular and interstitial edema. The Lie stain (figure **1A**) shows the most precocious beginning of the cardiomyocytes' lesions, by variable intensity of eosinophilia, consecutive to acidification of sarcoplasmic medium, which also can be seen with Alcian Blue stain (**Figure1B**).

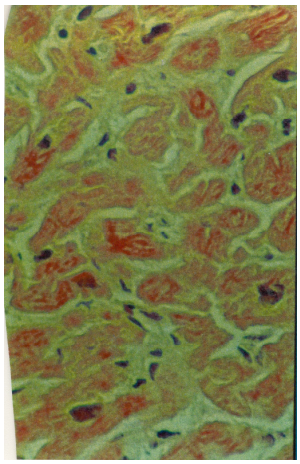

Figure 1A: *rat which got daily injections with Isoprenalin (ISOP), 70 mg/kg body, 2 days; Lie stain (400 x): advanced cardiomyocytar lesions with varied eosinophilia reactions and interstitial edema.*

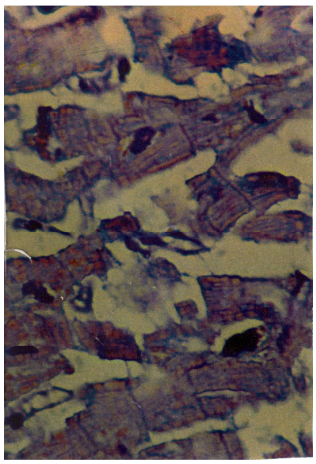

Figure 1B: *idem, Alcian Blue stain (400x): intense alcianophilia, shown acidophilia of sarcoplasm.*

On these levels, LDH reaction decrease gradually until demise, in the same time with development of irreversible lesions. In these situations, the vacuolations spread, the striations start to vanish and appear sarcoplasmic homogenizations. After 2 days of it, the infiltrates with leucocytes and mononuclears appear and substitude the disintegrated myocites focuses with formation of the fibrosis scar in the end.

The electronomicroscopical studies: at the animals which got only ISOP, the mitochondrial lesions appeared gradually, as rarefactions of ridges, vacuolations, tendency to swelling (Figure **2A**, **2B**) and calcium granules depositions (Figure **2C**), visible especially on the mitochondrial suspensions (Figure **2D**). Also, they were seen gradually dilations of sarcoplasmic reticulum (Figure **7, 8 B**), with constitution of plexiform images (Figure **3**), subsarcolemmal balonizations, the accentuation of Z lines, the presence of contraction bands (Figure **5**), gradual glycogenic depletions, increased the volume and number of lysosomal structures, the appearance of phagolysosomes (Figure **6**).

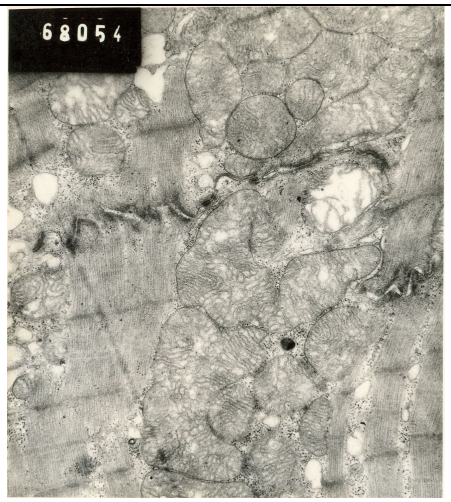

Figure 2A - ***rat injected with ISOP 10 mg/kg body, 2 days: mitochondrion with varied lesions of the crests and with tendency to sweeling.***

Figure 2B - ***rat injected with ISOP, 3 days: mitochondrial lesions with sweeling, dilations of sarcoplasmic reticulum, contraction bands;***

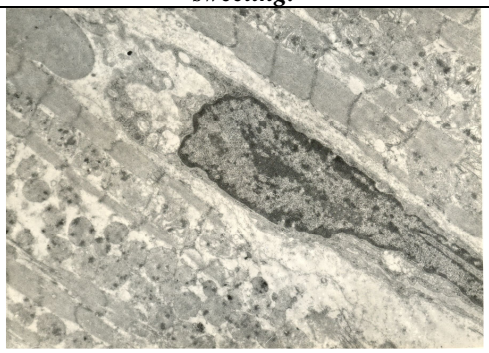

Figure 2C - ***myocardium; rat injected with ISOP, 4 days: mitochondrial necrosis with calcium granules depositions, capillaries with membranal lesions;***

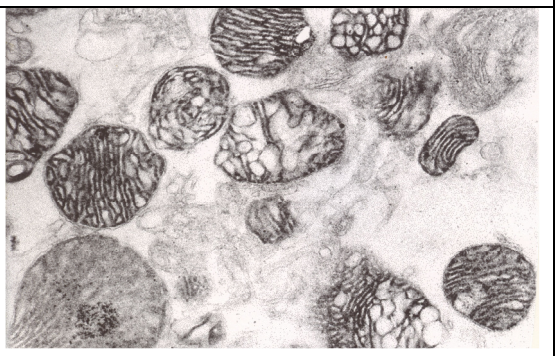

Figure 2D - ***myocardium; mitochondrial suspension: rat injected with ISOP 2 days: the different lesions with tendency to vacuolation* (12.000x);**

Figure 3 - ***myocardium; rat injected with ISOP, 3 days: many dilatations of sarcoplasmic reticulum, made plexiform confluent images*** (9500x).

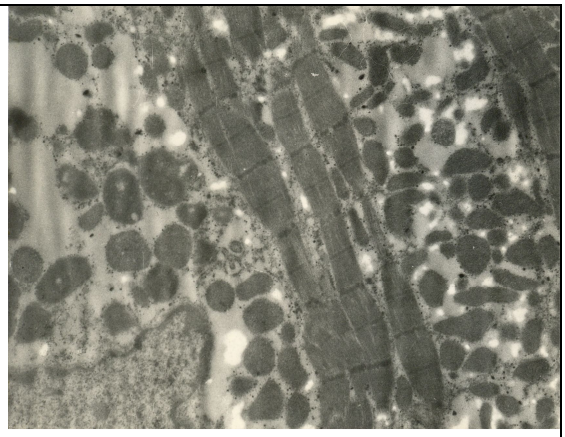

Figure 4 - ***myocardium; rat injected with ISOP, 3 days: lysosomal and phagolysosomal hyperplasias, which replaced the disintegrated structures of sarcomeres*** (9000x);

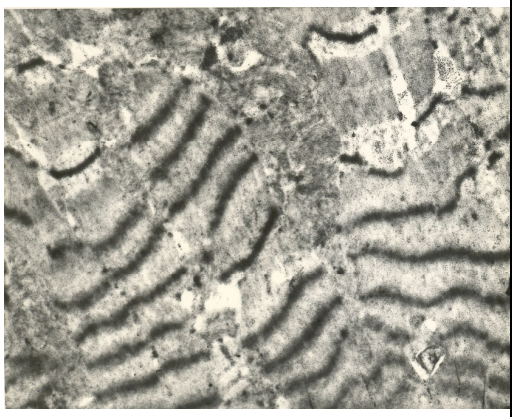

Figure 5 - ***myocardium; rat injected with ISOP, 4 days: contraction bands, myofilamental alterations*** (4500x);

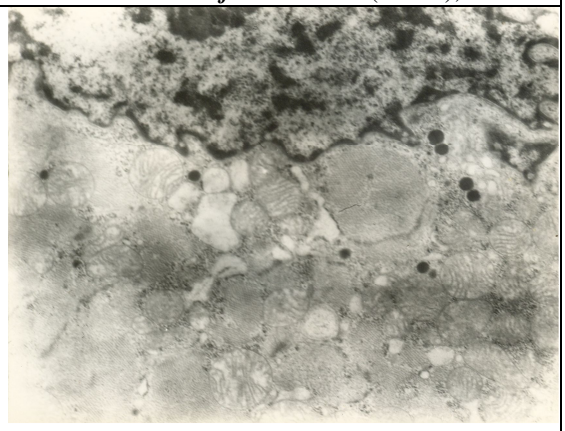

Figure 6 - ***rat injected with ISOP, 7 days: nucleus with the fission and marginalism of the chromatin, the disappear of the sarcomeres structures, with vacuolations, homogenizations, severe mitochondrial lesions, the presence of sodium granules*** (9100x);

The capillary vessels had free lumen but narrowed, festooned, with many small vesicles on the basal membranes and perivascular edema, which increased gradually and spreads intersarcoplasmic. The apparition of the irreversible lesions it is marked by the fission of sarcomeres through intracellular edema, which dissociated the myofilaments, by the homogenizations of some areas from sarcomeres and also by big increased of phagolysosomes, total deplation of

glycogen, nucleuses with position and chromatin structures altered, especially their marginations. On the 4th day, the cytolysis focuses were predominantly, with cellular detrituses, activated lymphocytes, macrophages, fiboblastes and synthesis of collagen. The treatment with Magnesium Ascorbat improved the intencity of these lesions.

The biochemical studies: (**table 1 -** serum LDH, electrolytes and H_2O from myocardium, in conditions of acute, beta adrenergic aggression and treatment with Magnesium Ascorbat) showed that, the ISOP injected to guinea pigs, 10 mg/kg body established at the cardiomyocytes' level, an intracellular influx of Ca^{2+} and Na^{+} and a marked efflux of Mg^{2+} and K^{+}. The LDH serum level was constantly high.

Table 1: Serum LDH, electrolytes and H_2O in myocardium in conditions of acute beta adrenergic aggression by ISOP and treatment with Magnesium Ascorbat;

Tip de experiment	LDH seric moli NADH ml ser	Ca^{2+} in tissue moli/100g dry tissue	Mg^{2+} in tissue moli/100g dry tissue	Na^{+} in tissue moli/100g dry tissue	K^{+} in tissue Moli/100g dry tissue	H_2O in tissue g/100g dry tissue
Control	0,31+/-0,039 n = 7	0,47+/-0,034 n = 4	16,85+/-1,309 n = 3	78,64+/-2,606 n = 4	70,39+/-18,36 n = 4	76,28+/-1,111 n = 4
ISOP 70 mg/kg.corp	$0,53^{d}$+/-0,104 n = 3	0,73+/-0,211 n = 3	$2,54^{a}$+/-0,168 n = 7	80,48+/-2,010 n = 3	64,69+/-2,589 n = 3	76,91+/-0,573 n = 7
ISOP 50 mg/kg.corp	$0,87^{d}$+/-0,169 n = 6	$1,79^{a}$+/-0,043 n = 4	$5,93^{a}$+/-0,178 n = 4	$104,82^{a}$+/-2,451 n = 4	71,65+/-1,380 n = 4	78,81+/-0,799 n = 6
ISOP 3x25mg/kg.corp	$1,00^{b}$+/-0,208 n = 3	$2,45^{c}$+/-0,788 n = 3	$7,44^{b}$+/-2,47 n = 3	$123,94^{a}$+/-7,882 n = 3	$92,52^{d}$+/-8,100 n = 3	$82,16^{d}$+/-1,115 n = 3
Magnesium Ascorbat 32,5mg + ISOP 70 mg/kg.corp	$0,71^{c}$+/-0,137 n = 9	$1,34^{c}$+/-0,210 n = 7	$16,31^{b}$+/-0,953 n = 8	$93,88^{b}$+/-3,117 n = 8	$78,77^{c}$+/-2,055 n = 8	77,81+/-0,366 n = 8
Magnesium Ascorbat 19,5mg +ISOP 70mg/kg.corp	0,65+/-0,852 n = 4	$2,000^{a}$+/-0,047 n = 3	$6,39^{b}$+/-0,577 n = 3	96,59+/-9,983 n = 3	80,65+/-4,929 n = 3	79,22+/-1,957 n = 3
Magnesium Ascorbat 11,35mg +ISOP 50mg/kgcorp	0,51+/-0,029 n = 4	$1,59^{a}$+/-0,084 n = 4	$5,49^{a}$+/-0,135 n = 4	$110,36^{a}$+/-0,757 n = 4	73,29+/-1,213 n = 4	78,47+/-0,183 n = 4

a = p < 0,001; b = 0,001 > p > 0,01; c = 0,02 > p > 0,01; d = 0,05 > p > 0,02

The ATP level decreased more than 40% and the same, the phosphocreatine level (**table 2 -** myocardial ATP level and serum LDH level in the same experimental conditions). The H_2O penetration into cardiomyocytes got the maximum level after administration of 3,25 mg/kg body ISOP. We noticed that

the rats were more resisting to the aggressive action of ISOP than guinea pigs, and this allowed to increase the dose of ISOP from 10 mg/kg body to 70 mg/kg. body administrated an one injection or 3 injections of 25 mg/kg. body on 24 hours. The administration of Magnesium Ascorbat established a considerable decrease of the lesional modifications intensity.

Table 2: The myocardial ATP level and serum LDH level in conditions of acute beta adrenergic aggression with ISOP and the treatment with Magnesium Ascorbat.

Experiment Type	ATP mols/wet tissue	LDH mols NADH/ml serum
Control	2,98 +/- 0,113 n = 10	0,38 +/- 0,046 n = 11
ISOP 70mg/k.corp	1,84[a] +/- 0,147 n = 6	0,60[a] +/- 0,105 n = 6
ISOP 3x25mg/kg.corp	1,65[a] +/- 0,199 n = 3	0,87[a] +/- 0,035 n = 4
Magnesium Ascorbat 32,5mg Mg + ISOP 70mg/kg. body	2,31[a] +/- 0,167 n = 9	0,71[b] +/- 0,096 n = 9
Magnesium Ascorbat 81,25mg Mg + ISOP 70mg/kg. body	1,83[b] +/- 0,151 n = 4	0,47 +/- 0,089 n = 4
Magnesium Ascorbat 0,81mg Mg + ISOP 70mg/kg. body	1,75[b] +/- 0,304 n = 3	1,18[a] +/- 0,187 n = 3
Magnesium Ascorbat 19,5mg Mg + ISOP 70mg/kg. body	2,06[a] +/- 0,107 n = 3	2,16[a] +/- 0,331 n = 3

***a* = *p* < 0,001; *b* = 0,01 > *p* > 0,001**

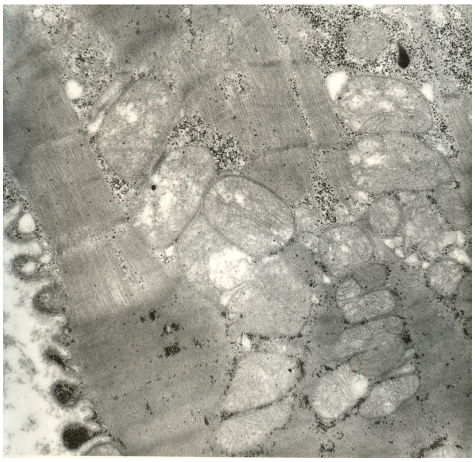

Figure 7 - ***rat injected with ISOP, 2 days, preceded by Magnesium Ascorbat with an hour before, 20 mg/kg body: subsarcolemmal dilatations, reversible mitochondrial lesions*** (9500x);

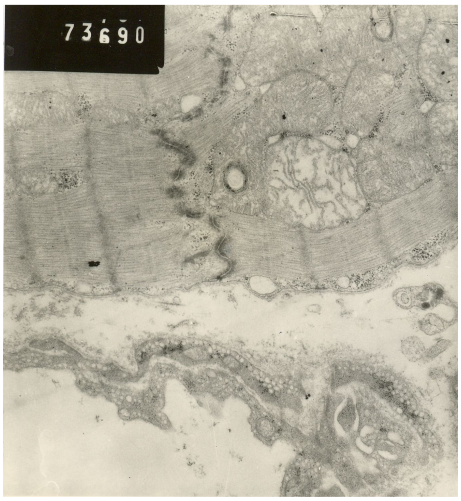

Figure 8A - ***idem: vesicules of pinocytosis in capillary membranes and sarcolemma too, perivascular and pericellular edema, dehiscences of the intercalary disk, minimum mitochondrial modifications, with one exception with tendency to vacuolation*** (9500x);

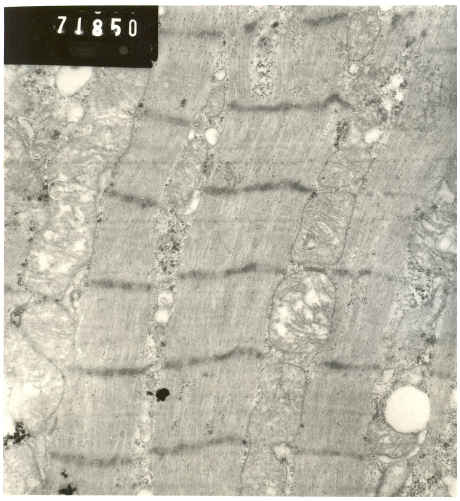

Figure 8B - ***identical experiment, but 3 days injections with ISOP and Magnesium Ascorbat: mitochondrion with tendency to the reversible vacuolations;***

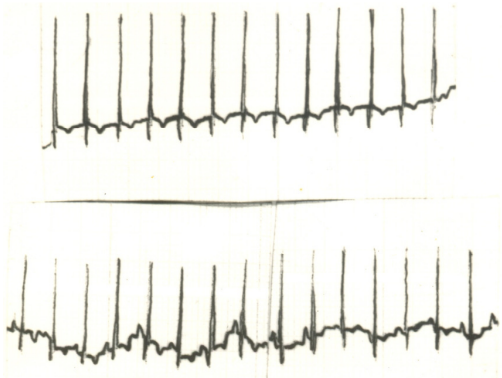

Figure 9A - ***EKG; guinea pig injected with ISOP, 10 mg/kg body, preceded by daily injections, 3 months with Magnesium Ascorbat, 20 mg/kg body: ischemic EKG aspect with inversion of T-wave.***

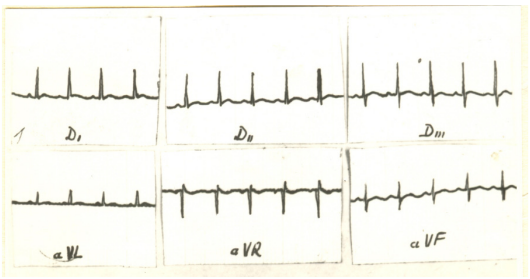

Figure 9B - ***rat injected 3 months with ISOP 70 mg/kg body associate with Magnesium Ascorbat for myocardial protection: normal EKG aspect.***

So, electronomicroscopical, we didn't find disseminated necrosis, but only simple or moderate reversible lesions as: dilatations of the sarcoplasmic reticulum, parcelar interstitial edema, myofilamentes with superficial modifications, glycogen well represented in the granules shape, often around mitochondrion, large lysosomes but with distict membranes. The hydration of the myocardial tissue decreased after the association with Magnesium Ascorbat and the same, the Ca^{2+} level of the myocardium.

Biochemical, the favorable, protective action of Magnesium Ascorbat on the serum LDH level, electrolytes and water from the tissue is shown in the 1 and 2 tables (ATP, LDH levels).

The EKG examinations: made on the rats and guinea pigs lots as on the control animals lots too, showed 2 categories of EKG alterations after ISOP administration: the inversions of T waves that show the ischemic character and cardiac rhythm disorders, like sinus tachycardia.

At the animals treated with Magnesium Ascorbat, we noticed improvements of the EKG tracts, although at some animals, the cardiac rhythm disorders persisted with ventricular extrasystoles and at the others, these disorders missed with images almost normaly. The similar benefic modifications appeared at the animals treated with Magnobil (Merck), proofed in this way, the good quality of Romanian pharmaceutical product.

Discussions

Our experimental model represent a suitable mode to study the pathogenesis of the hemodynamic infarctions met in the human pathology in some

circumstances as: recurrent long stresses, pheochromacytomas where the catecholamines are liberated suddenly and also in some treatments cases for long low blood pressure and states of shock. For these reasons, some authors classified the myocardial necrosis cases happened in the absence of coronary arteries diseases but caused by the high levels of catecholamines, in the category of catecholamine cardiomyopathy [11]. Such lesions were also got experimental with noradrenaline injections [12].

Isoprenalin is a synthetic simpaticmimetic amine, alike norepinephrine, with beta adrenergic effect. At the cardiac level, it induce tachycardia, the increase myocardial contraction force, the intensification of the metabolic processes and increase the oxygen consumption. The high concentrations of Isoprenalol establish exhaustions of the energetic reserves, vasoconstrictions and focal coronary ischemias, followed by hypoxias and disseminated necrosis in the end.

Our anatomical researches with China ink injections plead for focal coronary vasoconstrictions on left ventricle, to explain diminution and disappearance of the vascular network images. The results of focal hypoxias could be visualized by us because of the cardiomyocites lesions until reduced necrosis, especially subendocardial and myocardial level. They weren't finding at subepicardial level where exist an abundant vascularization and many anastomosis.

For the hypoxic character of the lesions, also plead the characteristic of the morphological and biochemical descriptions, which superpose on the morphological and biochemical descriptions met by us in our previous studies of the chronic transitory ischemias [13, 14, 15].

In the metabolic disorders category, we underline the precocious depletion of phosphocreatine and ATP level (through inhibition of ATP synthesis, because of both hypoxia and ATP consumption) by intense beta adrenergic stimulation [6, 7, 16, 19].

The acidification of sarcoplasmic medium by hypoxia was revealed even histological, by eosinophillia variability at the Lie stain. The permeability disorders of cellular membranes systems are also because of beta adrenergic catecholamines. The beta adrenergic catecholamines establish severe alterations of the hydroelectrolytic balance because of hypoxia too. In this way, it is explained, K^+ and Mg^{2+} decrease from cells by their extracellular migration and H_2O and Na^+ influx, especially Ca^{2+}, which appears in mitochondrion, sarcoplasmic reticulum, myofilaments. These informations are mentioned in some publications [5, 9, 17, 18]. The ultrastructural lesions intensified by these established metabolic disorders are particularly the lesions of sarcolemmas, the dilatations of sarcoplasmic reticulum, the gradual mitochondrial lesions until swelling, interfibrillar edema which dissociate the myofillaments, the activation of

lysosomes and formation of phagolysosomes, the nuclear alterations and appearance of cytolysis focuses in the end. Concomitant with these ultrastructural lesions, there are biochemical lesions too, at the organoids level, with decreases of the control mitochondrial lesions, increase of the cytolytic lysosomal enzymes and increase of LDH serum level.

The introduction in the case of these beta adrenergic aggressions of some protector myocardial factors comprised in the Magnesium Ascorbat had benefic effects.

Thus, Ascorbic Acid has an important role in the cellular respiration processes, in the intermediary metabolism of the proteins, glucides and lipides, in reglation of capillary permeability, been partial responsible for the O_2 radicals decrease, which appear in a series of biological processes as lipids peroxidation with consecutive lysis of the mithocondrial membranes.[20]. Ascorbic Acide speed up the degradations of the catecholamines and toxic products from the catecholamines catabolism. It has a general protective effect on the cells and membranes. Also, it increases the resistance of muscular tissue at hypoxia. The shockproof action of Ascorbic Acid is also owing to his synergyc effect with glucocorticoid hormones and catecholamines to maintain the vascular tonus.

The Magnesium role is demonstrated by numerous publications [9,17,21,22], been important for the myocardial fibre homeostasis. It intervenes to protection and reglation of cellular pump function, near other ions.[23]. The Mg^{2+} intracellular decrease establishes the lysis of sarcolemma and contributes to dilatation of sarcoplasmic reticulum. The disorders of Mg^{2+} metabolism induce increases of the catecholamines concentration. Mg^{2+} is an important activating agent of many enzymatic systems with an important role in the cellular energetic metabolism.

We underline the fact that, Mg^{2+} ion opposes to coronary spasm induced by catecholamines and protects the cardiomyocytes against electrolytic lack of poise, especially K^+ loss.[24].

In the hypoxia conditions, the antagonism Ca^{2+}-Mg^{2+} is one of the most important pathogenic factors. It is known, that in hypoxia is happened the massive penetration of the Ca^{2+} in the cell, especially in the mitochondrion, in the same time with the cellular depletion of Mg^{2+} and in the final, with the installation of cellular necrosis.

The association of Mg^{2+} with Ascorbic Acid can reduces the high reactive O_2 and H_2O radicals, which are responsible factors for the destruction of the cellular membranes. The benefic action of the Magnesium Ascorbat is demonstrated by the great improvement of the electronomicroscopical picture, comparative with the animals subdued only to beta adrenergic aggression.

Concomitantly with these morphological improvements, biochemical it is seen the ATP recovery and the Mg^{2+} level returns at the levels from the control animal, although, the LDH level maintains at the high levels, the hydroelectrolitic lack of poise is significant improved.

The EKG aspects are improved, but in some cases appeared monofocal ventricular extrasystoles.

Our investigations made on the patients with ischemic cardiomyopathy plead for the fact that Magnesium Ascorbat exerts a favourable effect on the heart rhythm disorders and angina symptomatology.

Conclusions

The experimental investigations about the pathogeny of hemodynamic beta adrenergic myocardial infarctions with Isoprenalin (ISOP) in high repeatable doses showed ultrastructural gradual hypoxic lesions until disseminated necrosis, consecutive coronary branches spasms.

Biochemical, it was specified the ATP and Mg^{2+} depletion in cardiomyocytes consecutive with the increase of Ca^{2+}, endogen Na^{+} and LDH serum level.

The pathogeny of these lesions was similar with those related in the human pathology from the cases with repeatable stresses and pheochromocytomas with abrupt release of catecholamine.

It was demonstrated the benefic, protective role of Magnesium Ascorbat which because of his myocardial protective components, decreases the severity of cardiomyocytes lesions which don't outrun the reversible limits. Also, it contributes to the recovery of ATP reserves and to the reestablishment of Mg^{2+} concentration in the limits of the control levels and also to the remaking EKG tracts, in the most part.

Legend of pictures:

Figure 1A - rat which got daily injections with Isoprenalin (ISOP), 70 mg/kg body, 2 days; Lie stain (400 x): advanced cardiomyocytar lesions with varied eosinophilia reactions and interstitial edema.

Figure 1B - idem, Alcian Blue stain (400x): intense alcianophilia, shown acidophilia of sarcoplasm.

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Figure 2B - rat injected with ISOP, 3 days: mitochondrial lesions with sweeling, dilations of sarcoplasmic reticulum, contraction bands;

Figure 2C - myocardium; rat injected with ISOP, 4 days: mitochondrial necrosis with calcium granules depositions, capillaries with membranal lesions;

Figure 2D - myocardium; mitochondrial suspension: rat injected with ISOP 2 days: the different lesions with tendency to vacuolation (12.000x);
Figure 3 - myocardium; rat injected with ISOP, 3 days: many dilatations of sarcoplasmic reticulum, made plexiform confluent images (9500x).
Figure 4 - myocardium; rat injected with ISOP, 3 days: lysosomal and phagolysosomal hyperplasias, which replaced the disintegrated structures of sarcomeres (9000x);
Figure 5 - myocardium; rat injected with ISOP, 4 days: contraction bands, myofilamental alterations (4500x);
Figure 6 - rat injected with ISOP, 7 days: nucleus with the fission and marginalism of the chromatin, the disappear of the sarcomeres structures, with vacuolations, homogenizations, severe mitochondrial lesions, the presence of sodium granules (9100x);
Figure 7 - rat injected with ISOP, 2 days, preceded by Magnesium Ascorbat with an hour before, 20 mg/kg body: subsarcolemmal dilatations, reversible mitochondrial lesions (9500x);
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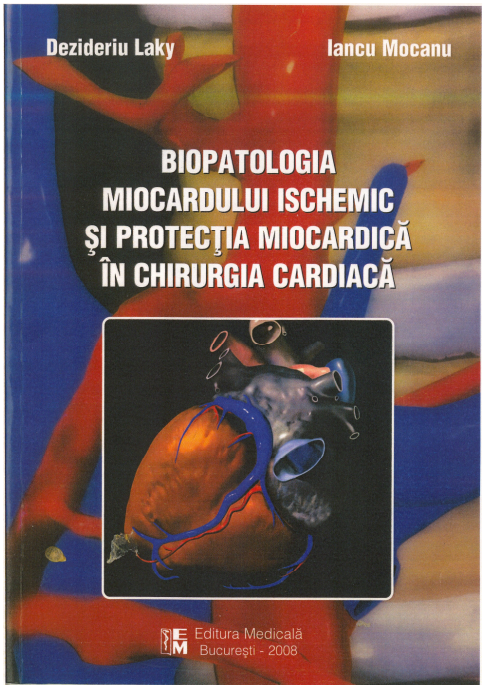
Figure 8B - identical experiment, but 3 days injections with ISOP and Magnesium Ascorbat: mitochondrion with tendency to the reversible vacuolations;
Figure 9A - EKG; guinea pig injected with ISOP, 10 mg/kg body, preceded by daily injections, 3 months with Magnesium Ascorbat, 20 mg/kg body: ischemic EKG aspect with inversion of T wave.
Figure 9B - rat injected 3 months with ISOP 70 mg/kg body associate with Magnesium Ascorbat for myocardial protection: normal EKG aspect.

References



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