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Histological and ultrastructural changes in cross-striation muscle cells, under the influence of atorvastatin-reductase HMG-CoA inhibitor

Drugs from the atorvastatin group, such as Sortis and Lipitor are often used in the prevention and treatment of hypercholesterolaemia, and hypertriglyceridaemia.

The purpose of this experiment was to estimate the benefits or risks of utilizing these pharmaceuticals. Striated muscle cells were chosen as experimental material and their ultrastructure was observed.

Atorvastatins (Sortis, Lipitor) are reductase HMG-CoA inhibitors. The drugs are referred to as statins and enter the cell synthetically (5, 13). After oral administration, maximum concentration in blood is obtained quickly, within 1–2 hours. Systemic bioavailability is 12% (10). More than 98% of a given dose is bound to plasma protein. This high bonding can lead to the reduction of side-effect, and to low distribution within the organism, with distribution limited above all, to the liver, spleen and adrenal glands (8). The half-period in the blood is about 14 hours, and the half-period of HMG-CoA reductase enzyme blocking is 20–30 hours, and is due to the result of activating metabolites which constitute about 70% of this drug's activity (4, 8, 10). Because of its strong activity in increasing chloresterol LDL level (from 25 to 60%), and increasing triglyceride (from 10 to 40%), Actovastatin is used in the treatment of hypercholesterolaemia and hypertriglyceridaemia mixta, with distinct increase of cholesterol level and moderate increase of triglycerides (5). This and other statins used to be administered to patients after cardiac infarct and after operations on coronary vessels, in insulin-dependent diabetes, and for nephrotic syndromes (6). Their effective use was also documented in the prevention and treatment of antherosclerosis (5).

The contraindication of atorvastatin use is the potential hypersensitivity to this drug. Effects include active illness of liver parenchyma, and/or increased activity of aminotransferase in serum. Administration of this drug is not advised during pregnancy and breast feeding, and for children (5).

MATERIAL AND METHODS

The investigation was carried out on 15 Wistar rats – males weighing about 200 mg each. The animals were divided into three groups: one control group and two experimental groups, with five animals in each. In the control group the animals received granulated fodder and water *ad libitum*. In experimental group I the animals received emulsion of atorvastatin in distilled water at the therapeutical dose of 80 mg/kg of body mass, by stomach tube for 6 weeks. In experimental group II the animals received atorvastatin at the maximal dose of 800 mg/kg of body mass, in the same manner as in experimental group I. The animals were killed by decapitation and then skeletal muscle samples were taken.

For preparation for light microscope examination, skeletal muscle samples were fixed in Baker's solution (formalin, calcium chloratum, and distilled water), dehydrated in alcohol with increased concentration, soaked in xylene and then embedded in paraffin. The samples were then sliced in a microtom to obtain paraffin sections 5 nm thick. Observations were made and photographs taken using a JenaMed light microscope made by Carl Zeiss Jena. For preparation for electron microscope examination, the skeletal muscle samples of 0.5 cm^3 were fixed with 4% glutaraldehyde in 0.1 M phosphatic buffer – pH – 7.5, for 4 hours. The samples were then washed in phosphatic buffer at the same pH and were fixed in 1% OsO_4 for one hour. The samples were then dehydrated in a series of alcohol concentrations, then embedded in "Spurr" resin. The samples were polymerized in 60° degree temperature, then sliced using a Reichert Ultracut S ultramicrotom. Ultrathin sections were contrasted with uranyl acetate and lead citrate according to Reynolds. Observations and photographs were made using a Tesla BS-500 electron transmission microscope, in the Electron Microscopic Laboratory of the Department of Histology and Embryology, Medical University of Lublin.

RESULTS

Skeletal muscles of experimental animals (rats) after 6 weeks' administration of atorvastatin in therapeutical and maximal dosages did not show any essential differences in comparison with the control group, when examined under light microscope. Degenerative changes were observed after atorvastatin administration, when examined under electron microscope. These changes were dependent upon dosage and were directly proportional to dosage rate.

Six weeks' administration of atorvastatin in the therapeutical dose (80 mg/kg b.m.) produced invagination of the nuclear envelope into the cell nucleus, and within the cytoplasm, numerous vacuoles – some of which included the myelin structures – were evident.

Atorvastatin administration in maximal dosage (800 mg/kg) under electron microscope examination, showed the following differences: the appearance of numerous vacuoles in the perinuclear spaces, and between myofibrils; dilation of mitochondria; disintegration of sacomers; fibrinosis within the intercellular spaces.

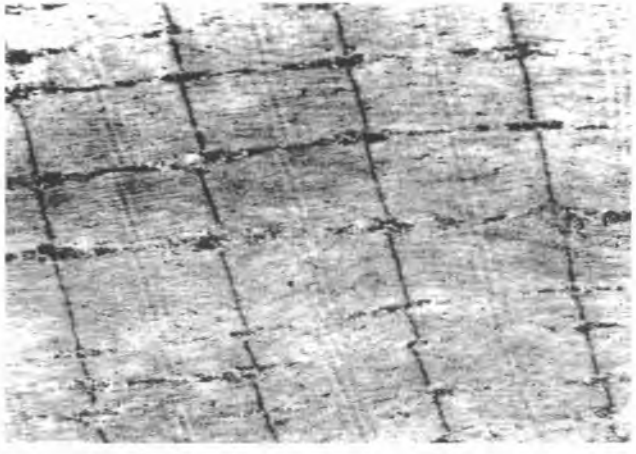

Fig. 1. Control group. Magn. 10 000x

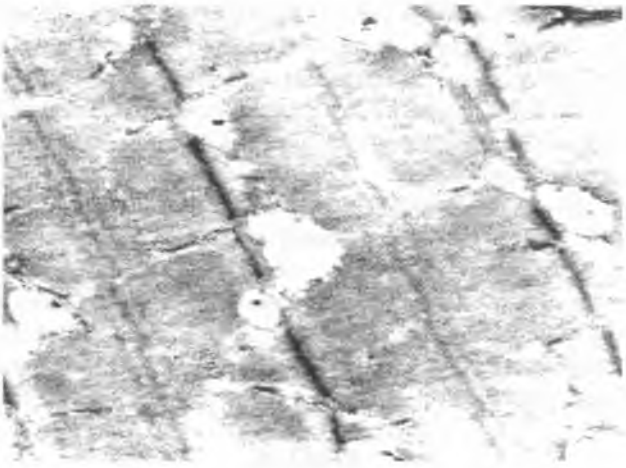

Fig. 2. Experimental group I. Magn. 14 000x

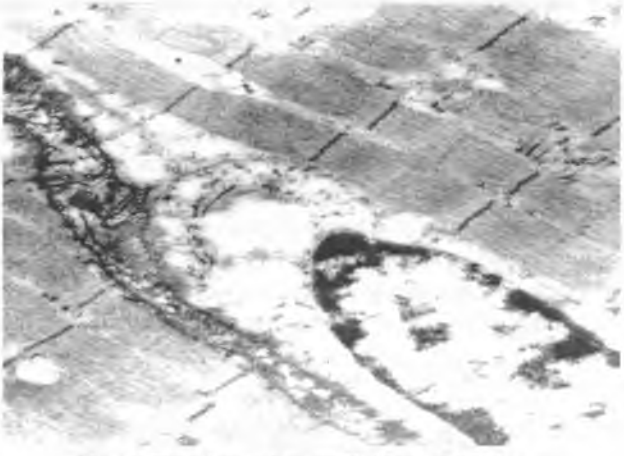

Fig. 3. Experimental group II. Magn. 10 000x

Fig. 4. Experimental group II. Magn. 10 000x

DISCUSSION

HMG-CoA inhibitors belong to drugs which most strongly decrease cholesterol level. They are well tolerated by patients and rarely produce side-effects. Among these side-effects are: increased liver enzyme activity, myopathy, rhabdomyolysis, gastro-intestinal disturbances and central nervous system effects. The most frequent complication appearing during statin treatment is myopathy (2, 5, 12), appearing in monotherapy within 0.1–0.2% of the patients, with intensification dependent on the dosage (14). Symptoms of myopathy manifest themselves as weakening of muscles or as muscle pain. Phosphokinase creatinine concentration may also increase (5).

In this experiment, after six weeks' administration of Atorvastatin, at the therapeutical dose, in rat muscle tissue, no essential changes were observed in comparison with the control group. What was different was the presence of muscle cells with the increased number of cell nuclei. A similar picture, but with dilated cellular spaces, was observed after administration of the maximal dose. Wicher--Muniak et al. observed greater changes after administration of simvastatin at the dose rate of 20 mg/24h for patients after cardiac infarct (14). After two months, these researchers observed proliferation of perimysium cells, neutrophils infiltration, oedema, *focalis necrosis*, increase in cell size and increase in the number of cell nuclei. This picture could however, have been influenced by the additional administration of hypotensive drugs which can interact with statin drugs. In addition, simvastatin has a greater lipophilic effect than does atorvastatin, and this lipophilic effect could have a large effect upon the myopathy mechanism. It should be considered that statins which have hydrophilic construction are hepatocytes and influence the metabolism of chloresterol only within the liver. Lipophilic substances, however, are not selective only for distribution within the liver and enter respective tissues through simple diffusion. This was verified in experiments by Reijneveld et al. They observed numerous changes within rat tissue after simvastatin administration (15 mg/kg) and lovastatin (43–55 mg/kg) administration – both of which are lipophil statins. The observed effects include: growth set-back of young rats; increased creatine kinaze activity; evidence (under histological analysis) of excessive contraction and necrosis of muscle fibers. These researchers also found when pravastatin was administered (a hyperphil drug administered in the dose of 8–55 mg/kg), no myopathy signs were observed (11). In our observations using the electron microscope, we did however, observe degenerative changes.

The changes observed in experimental group II were similar to the changes evident in experimental group I, but were more intense. Evident in experimental group I were: invagination of nuclear membrane into the cell nucleus; sacomer disintegration; the appearance of myelin figures, and numerous vacuoles within the cell cytoplasm. The appearance of numerous vacuoles is probably due to increased calcium ion concentration within the cytosol (9), whereas the presence of myelin structures indicates pathological processes occurring within the cell. The mass appearance of myelin structures leads to the complete disintegration of the cell (3). The observed changes are similar to IV types in the classification system of Bohan and Peter, of idiopathic myositis (in modification of Cronin). We observed inclusion body myositis in cell cytoplasm, and rounded vacuoles under light microscope examination, and polymorphic inclusions in cytoplasm and nucleus, in electron microscope examination. We also saw degenerative changes, necrosis and muscle cell interstitial fibrosis, as well as mononuclear cell infiltration (1). These observations were also recorded by Wicher-Muniak et al.

In experimental group II, besides the changes described above, we observed dilated mitochondria and fibrosis in intercellular spaces. Dilation of mitochondria can lead to parenchymatous degeneration, which is one of the most visible forms of the effects of intensive physiological stimuli, usually due to pathological situations. The processes that lead to mitochondrial dilation are induced by disturbances of cellular oxidation and energy processes. Such disturbances can lead to the incompetent activity of the cell membrane as a water barrier. As the cell swells, the mitochondrial matrix becomes dilated.

Vacuolar changes are dependent on a dilated cisterna of the sarcoplasmic reticulum which usually accompanies the incompetent activity of the cell membrane, while mitochondrial changes are reversed until cytoplasmic membranes are continuous (3). The myopathy mechanism could be a result of cell membrane damage. In these experiments, myopathy was induced in rats after lovastatin administration, and morphological changes in organella, mitochondrium, and sarcoplasmic reticulum membranes occurred. In addition, microvacuoles were formed and muscle tissue was constricted (7). This constriction can be triggered by dysfunction of mitochondria and hypoexcitation of muscle cell membranes (7), resulting in increased calcium ion concentration (7, 9).

REFERENCES

1. Biemat E. et al.: Uszkodzenie mięśni indukowane najprawdopodobniej simvastatyną u chorej z ksantomatozą w przebiegu hiperlipidemii typu Ha. Reumatologia, 33, 3, 314, 1995.
2. Cybulska B. et al.: Leczenie hipercholesterolemii. Efekty uboczne farmakoterapii. Wiad. Lek., 23, 941, 1995.
3. Groniowski J.: Patomorfologia. PZWL, Warszawa 1981.
4. Kantola T. et al.: Effect of itraconazole on the pharmacokinetics of atorvastatin. Clin. Pharmacol. Ther., 64, 1, 58, 1998.
5. Kłosiewicz-Latoszek L.: Statyny. Kardiol. Pol., 47, 339, 1997.
6. Kostowski W.: Farmakologia. Podstawy farmakoterapii. PZWL, Warszawa 1998.
7. Kuriyama N. K. et al.: Myopathy induced by HMG-CoA reductase inhibitors in rabbits: a pathological, electrophysiological, and biochemical study. Toxic. Appl. Pharm., 152, 1, 1998.
8. Malinowski J. M.: Atorvastatin: a hydroxymethylglutaryl-coenzyme A reductase inhibitor. Am. J. Health Syst. Pharm., 55, 21, 2253, 1998.
9. Pierno S. et al.: Effects of HMG-CoA reductase inhibitors on excitation-concentration coupling of rat skeletal muscle. Eur. J. Pharmacol., 364, 1, 43, 1999.
10. Podlewski J. K. et al.: Leki współczesnej terapii. Split Trading, Warszawa 1999.
11. Reijneveld J. C. et al.: Differential effects of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors on the development of myopathy in young rats. Pediatr. Res., 39, 6, 1028, 1996.
12. Ucar M. et al.: HMG-CoA reductase inhibitors and myotoxicity. Drug Saf., 22, 6, 441, 2000.
13. Ulotka preparatu Sortis: PARKE-DAVIS 1996, GmbH Berlin.
14. Wicher-Muniak E. et al.: Simvastatin-induced myopathy in a patient treated for hypercholesterolemia. Morphological aspects. Pol. J. Pathol., 48, 1, 69, 1997.

SUMMARY

The investigation was carried out on 15 Wistar rats – males weighing about 200 mg each. The animals were divided into three groups: one control group and two experimental groups, with five animals in each. In experimental group I the animals received emulsion of Atorvastatin in distilled water at the therapeutical dose of 80 mg/kg of body mass, by stomach tube for 6 weeks. In experimental group II the animals received atorvastatin at the maximal dose of 800 mg/kg of body mass. Skeletal muscles of experimental animals (rats) after 6 weeks' administration of atorvastatin in therapeutical and maximal dosages did not show any essential differences in comparison with the control group, when examined under light microscope. Degenerative changes were observed after Atorvastatin administration, when examined under electron microscope. These changes were dependent upon dosage and were directly proportional to dosage rate. Six-week administration of Atorvastatin in the therapeutical

dose (80 mg/kg b. m.) produced invagination of the nuclear envelope into the cell nucleus, and within the cytoplasm, numerous vacuoles, some of which included the myelin structures, were evident. Atorvastatin administration in maximal dosage (800 mg/kg) under electron microscope examination, showed the following differences: the appearance of numerous vacuoles in the perinuclear spaces, and between myofibrils; dilation of mitochondria; disintegration of sacomers; fibrinosis within the intercellular spaces.

Histologiczne i ultrastrukturalne zmiany mięśni poprzecznie prążkowanych pod wpływem atorwastatyny - inhibitora reduktazy HMG-CoA

Badania przeprowadzono na piętnastu szczurach – samcach rasy Wistar o masie ciała około 200 g. Zwierzęta podzielono na trzy grupy: kontrolną i dwie grupy doświadczalne. W grupie doświadczalnej I szczury otrzymywały atorwastatynę przez 6 tygodni w dawce terapeutycznej równej 80 mg/kg m.c. W grupie doświadczalnej II szczury otrzymywały dawkę maksymalną leku równą 800 mg/kg m.c. Mięśnie szkieletowe zwierząt doświadczalnych (szczurów) po 6-tygodniowym podawaniu atorwastatyny w dawce terapeutycznej i maksymalnej nie wykazywały istotnych zmian w obrazie mikroskopu świetlnego w porównaniu z grupą kontrolną. W obrazie mikroskopu elektronowego występowały zmiany degeneracyjne po podaniu atorwastatyny w nasileniu zależnym od dawki w stosunku wprost proporcjonalnym. 6-tygodniowe podawanie atorwastatyny w dawce terapeutycznej (80 mg/kg m.c) wywołuje: wpuklenie błony jądrowej do jądra komórki, obecność w cytoplazmie licznych wakuoli, niektórych z figurami mielinowymi. Podawanie atorwastatyny w dawce maksymalnej (800 mg/kg m. c) wywołuje następujące zmiany: pojawienie się licznych wakuoli w przestrzeniach okołojądrowych oraz między włóknami kurczliwymi, poszerzenie mitochondriów, dezintegrację sarkomerów i zwłóknienie w przestrzeniach międzykomórkowych.