Statin-Induced Myopathy in Muscles of Lower Limb with special reference to Gastrocnemius Muscle in Albino Rats

Thesis

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Contents

List of Abbreviations

Abbrev.	Meaning

CVD = cardiovascular disease

HMG-CO = Hydroxy-methyl-glutaryl-coenzyme A

LDL =Low density lipoprotein

ASCVD =Atherosclerotic cardiovascular disease

CHD= coronary heart disease

SINAM =statin induced necrotizing autoimmune myopathy

CK = creatine kinase

CoQ10=coenzyme Q10

SAS =statin associated symptoms

SAMS =Statin-associated muscle symptoms

L. tendo calcaneus = calcaneal tendon

L. sura = triceps surae = calf

Sarcolemma = the cytoplasm is the sarcoplasm

Endoplasmic reticulum = the sarcoplasmic reticulum

CAD = coronary artery disease

AKI = acute kidney injury

4S = Scandinavian Simvastatin Survival Study

RCT = randomized controlled trials

T2DM = type 2 diabetes mellitus

NODM = New-onset type 2 diabetes mellitus

Hx&E = Hematoxylin and Eosin

PBS = phosphate buffer saline

LS = longitudinal section

TS = Transverse section

Introduction

Atherosclerosis is considered a chronic inflammatory disease and it represents one of the most common causes of morbidity and mortality worldwide. Atherosclerosis is characterized by a progressive accumulation of cholesterol, oxidized lipids and fibrous elements in the large arteries.

This will lead to development of plaques, which later harden and narrow the arteries leading to reduced supply of oxygen rich blood to organs and other parts of the body. The loss of heart and brain function as a result of reduced blood flow is termed cardiovascular disease. Low density lipoprotein (LDL) is a vehicle for the delivery of cholesterol. Increased LDL level is one of the most important risk factors for the initiation and progression of atherosclerosis. Thus, management of plasma LDL levels has been considered as a good therapeutic strategy for the treatment of atherosclerosis **(Kang et al., 2015).**

Statins or Hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors reduce low-density lipoprotein (LDL)-cholesterol concentrations **(Taylor et al., 2015).**

Statins are safe in majority of patients, however, they have still some side effects that limit their usage,

resulting in discontinuation in 5 to 20% of patients. Among these side effects are muscle-related adverse events. **(Babu and Yuebing. 2015).**

Although treatment with statins may cause muscle - related symptoms in 10 to 20% of patients, yet these symptoms usually resolve within weeks after discontinuation of the medication. In rare instances, however, the medication causes statin-triggered autoimmune myopathy. It is a condition characterized by muscle weakness, prominent necrosis of muscle fibers (detected on biopsy), elevated serum levels of creatine kinase (CK), and the presence of autoantibodies that recognize 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. **(Kesselheim and Avron. 2015).**

Statin-associated autoimmune myopathy is an exceptionally rare side effect of statin use. Its incidence is not known with certainty, but it is estimated to occur in approximately 2 or 3 of 100,000 patients treated with statins **(Mammen, 2016).**

The cause of muscle damage in statin-triggered autoimmune myopathy is not understood. Presence of few infiltrating lymphocytes and membrane attack complex on non-necrotic muscle-cell membranes raises the possibility that anti HMG-CoA reductase autoantibodies are

pathogenic. This hypothesis is supported by the observation that autoantibody levels are correlated with both creatine kinase levels and the degree of muscle weakness. **(Mammen, 2016).**

Clinical trials commonly define statin-induced toxicity as myalgia or muscle weakness with CK levels greater than 10 times the upper normal limit **(Tomaszewski et al., 2011).**

Symptoms of statin-induced myopathy include any combination of myalgia, muscle tenderness or weakness. Patients describe an aching or cramping sensation in their muscles. Tendon pain and nocturnal leg cramps may also occur **(Tomaszewski et al., 2011).**

Treatment involves the discontinuation of the offending statin, which often results in the resolution of the myopathy process **(Haman et al., 2013).**

Aim of the Work

1- To observe the histological and immunohistochemical changes of rosuvastatins on gastrocnemius muscle of adult male albino rat.

2- To observe the histological and immunohistochemical changes on gastrocnemius muscle after discontinuation of rosuvastatins.

Review of Literature

Gastrocnemius muscle

Anatomy of gastrocnemius muscle in man

Posterior compartment of leg:

Kulkarni (2012) demonstrated that the posterior compartment of leg is the largest osteofascial one. Superiorly it is continuous with the popliteal fossa and inferiorly it is continuous with the sole of foot deep to the flexor retinaculum.

Singh (2014) added that It is subdivided by two strong transverse fascial septa (superficial and deep) into three parts: superficial, middle, and deep. The superficial transverse septum is attached *medially* to the medial border of the tibia and *laterally* to the posterior border of the fibula. The deep transverse septum is attached *medially* to the proximal part of the soleal line and vertical ridge on the posterior surface of the tibia, and *laterally* to the medial crest of the fibula. The Superficial part (between superficial transverse septum and deep fascia) contains gastrocnemius, soleus, and plantaris. The Middle part (between superficial and deep

transverse fascial septa) contains flexor digitorum longus, flexor hallucis longus, and posterior tibial nerve and vessels. The Deep part (between deep transverse fascial septum and posterior surfaces of interosseous membrane, tibia, and fibula) contains tibialis posterior.

Standring (2016) added that the superficial part is large in size compared to quadrupeds and this is definined as a human characteristic, which is related to the upright stance and bipedal locomotion of the human**.**

Furthermore gastrocnemius and soleus form a powerful muscular mass in the calf. They share a common tendon, the calcaneal tendon (L. tendo calcaneus, Achilles tendon), which is attached to the calcaneus. Collectively, these two muscles form the three-headed triceps surae (L. sura, calf) **(Moore et al., 2015).**

Gastrocnemius has two heads that form the inferolateral and inferomedial boundaries of the popliteal fossa and then merge at the inferior angle of the fossa **(Moore et al, 2015).**

The large medial head arises by a broad flat tendon from the posterosuperior aspect of the medial

condyle of the femur behind the adductor tubercle and adjoining part of the posterior surface of the shaft of the femur. While the Small lateral head arises by a broad flat tendon from the lateral surface of the lateral condyle of the femur above the lateral epicondyle and adjoining part of the lateral supracondylar line **(Singh, 2014)**.

The fleshy part of both two heads converge to lie side by side and the larger medial head extends to a lower level than the lateral head. The broad bellies of the muscle insert into a dense aponeurosis on their anterior surfaces, together with soleus muscle forming the tendocalcaneus, which is inserted into a smooth transverse area on the middle third of the posterior surface of the calcaneus (**Sinnatamby, 2011)**.

Both heads are supplied by the tibial nerve in the popliteal fossa **(Singh, 2014).**

The medial and lateral heads receive separate branches from the tibial nerve in the popliteal fossa **(Kulkarni, 2012)**.

The muscle performs many actions. It is the chief plantar flexor of the foot at the ankle when the knee

is extended. And It is also a flexor of the knee. It provides rapid movements of the foot during running and jumping **(Singh, 2014)**.

Histology of skeletal muscle:

Marieb and Keller (2017) explained that there are three types of muscle tissue: *skeletal*, *cardiac*, and *smooth*. Each type can be characterized by two distinctive features: (1) the presence or absence of light and dark stripes, called *striations*, in the muscle cells; and (2) whether control of contraction is *voluntary* or *involuntary*.

For skeletal Muscle tissue, More than 600 skeletal muscles make up the muscular system, and technically each one is an organ. It is composed of skeletal muscle tissue, connective tissue, and nervous tissue. Each muscle also has a particular function, such as moving a finger or blinking an eyelid **(De Graaff et al., 2001).**

Marieb and Keller (2017) found that skeletal muscles make up 40% of body weight. The elongated, cylindrical skeletal muscle cells are called muscle fibers. Histologically the skeletal muscle cells are striated muscle showing dark and light stripes extending transversely across its muscle cells.

Skeletal muscle is innervated by the voluntary division of the nervous system and is subjected to conscious control.

The masses of fibers that make up the various types of muscle are arranged in regular bundles surrounded by the epimysium, an external sheath of dense connective tissue surrounding the entire muscle. From the epimysium, thin septa of connective tissue extend inward, surrounding the fascicles or bundles of fibers within a muscle. The connective tissue around each fascicle is called the perimysium. Each muscle fiber is itself surrounded by a more delicate connective tissue, the endomysium **(Mescher, 2015).**

The epimysium becomes a part of the fascia, a layer of fibrous tissue that separates muscles from each other (deep fascia) and from the skin (superficial fascia). Collagen fibers of the epimysium continue as a strong, fibrous tendon that attaches the muscle to the periosteum of the bone **(Mader, 2004).**

Muscle fibers are long, cylindrical multinucleated cells with diameters of 10–100 μm. Multinucleation results from the fusion of embryonic mesenchymal cells called myoblasts. The long oval

nuclei are usually found at the periphery of the cell under the cell membrane. This is in contrast with cardiac and smooth muscle which have centrally located nuclei **(Mescher, 2015).**

Seidman (2013) noted that, the muscle fibers are of relatively uniform size and shape.

Mader (2004) explained that, a muscle fiber contains the usual cellular components, but special names have been assigned to some of these components. The plasma membrane is called the sarcolemma; the cytoplasm is the sarcoplasm; and the endoplasmic reticulum is the sarcoplasmic reticulum. A muscle fiber also has some unique anatomical characteristics. One feature is its T system; the sarcolemma forms T tubules that penetrate, or dip down, into the cell so that they come into contact—but do not fuse—with expanded portions of the sarcoplasmic reticulum. The expanded portions of the sarcoplasmic reticulum are calcium storage sites. Calcium ions (Ca2) are essential for muscle contraction. The sarcoplasmic reticulum encases hundreds and sometimes even thousands of myofibrils which are the contractile portions of the muscle fibers. Other organelles, such as mitochondria, are located in the sarcoplasm