"Histological and Immunohistochemical study on the possible role of suramin in the healing of experimentally induced laceration of skeletal muscle"

Thesis

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By

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List of Abbreviations

α -SMA	Alpha smooth muscle actin
A bands	Anisotropic bands
ab	Abjunctional stump
ad	Adjunctional stump
BM	Basal membrane
BMPs	Bone morphogenetic proteins
cz	Central zone
DG	Dystroglycans
ECM	Extracellular connective tissue matrix
FDA	Food and drug administration
FGF	Fibroblast growth factor
FGF-2 and -6	Fibroblast growth factor-2 and 6
GFAP	Glial fibrillary acidic protein
GM	Gastrocnemius muscle
H&E	Hematoxylin and eosin
HGF	Hepatocyte growth factor
I bands	Isotropic bands
IF	Intermediate filament
IFP	Intermediate filament protein
IGF	Insulin-like growth factor
INF-γ	Interferon gamma

MCK	Muscle creatine kinase
MCN	Myocyte nucleus
MDSCs	Muscle-derived stem cells
MHC	Myosin heavy chain
MMP-2 and -9	Matrix metalloproteinases-2 and -9
MRFs	Myogenic regulatory factors
MSTN	Myostatin
MTJs	Myotendinous junctions
MYF5	Myogenic factor 5 protien
MYOD	Myogenic differentiation protien
NMJs	Neuromuscular junctions
NSAIDs	Non-steroidal anti inflammatory drugs
Pax3	Paired box 3 gene
Pax7	Paired box 7 gene
PBS	Phosphate buffered saline
PDGF-AA and BB	Platelet-derived growth factor-AA and –BB
RICE	Rest, ice, compression and elevation principle
rz	Regeneration zone
SCs	Satellite cells
SG	Sarcoglycans
SHH	Sonic hedgehog protien
SP cells	Side population cells

SR	Sarcoplasmic reticulum
SYN	Syntrophins
sz	Survival zone
T tubules	Transverse tubules
TGF-α	Transforming growth factor-α
TGF-β1	Transforming growth factor–β1
TN-C	Tenascin-C
V phenotype	Vimentin phenotype
VAD phenotype	Vimentin, α -SMA, and desmin phenotype
VADM phenotype	Vimentin, α -SMA, desmin and myosin heavy chains phenotype
VD phenotype	Vimentin and desmin phenotype
VEGF	Vascular endothelial growth factor
WNTS	Wingless/integrated protien

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Abstract

Background:

Skeletal muscle injuries are one of the most frequently occurring injuries in sports medicine. Although these injuries are capable of healing, incomplete functional recovery often occurs due to excessive scar tissue formation.

Hypothesis:

Suramin enhances muscle healing by both stimulating muscle regeneration and preventing fibrosis in lacerated skeletal muscle.

Study Design:

Controlled laboratory study.

Methods:

In vivo: a single concentration of suramin was injected in the gastrocenimus muscle of mice at variable timing after muscle laceration injury. Muscle regeneration and scar tissue formation were evaluated by histologic and immunohistochemical analysis.

Results:

Suramin treatment significantly promoted muscle regeneration, decreased fibrosis formation when introduced after laceration injury.

Conclusion:

Intramuscular injection of suramin after a laceration injury in a multiple dose manner improved overall skeletal muscle healing in comparison to single dose administration

Clinical Relevance:

These findings could contribute to the development of biological treatments to aid in muscle healing after experiencing a muscle injury.

Keywords:

Muscle laceration injury, Suramin, TGFβ1, Myostatin, Muscle regeneration, Fibrosis

Introduction

Skeletal muscle injuries, including contusion, strain, or laceration, are the most common injuries occurring in sports, with an incidence varying from 10% to 55% of all the sustained injuries **(Beiner and Joki, 2001).** The healing of an injured skeletal muscle follows a fairly constant pattern irrespective of the underlying cause, such pattern has been described to occur in three phases:1. *Destruction phase:* characterized by the rupture and consequent necrosis of the myofibers, the formation of a hematoma between the ruptured muscles stumps and the inflammatory cell reaction, 2. *Repair phase*: consisting of the phagocytosis of the necrotized tissue, the regeneration of the myofibers, and the concomitant capillary ingrowth, as well as the production of a connective tissue scar into the injured area; 3. *Remodeling phase*: a period during which the maturation of the regenerated myofibers, the contraction and reorganization of the scar tissue, and the recovery of the functional capacity of the muscle occur. The latter two phases, *repair* and *remodeling*, are usually closely associated or overlapping**(Järvinen et al ., 2005)**.

However, the healing process is slow and often incomplete because of the over deposition of collagen leading to significant fibrous scarring that frequently results in functional and structural deficits, contraction injury, muscle atrophy and pain**.** Therefore a major concern of researchers is how to improve the healing process in the direction of minimizing scar formation in order to overcome these complications **(Huard et al., 2002).**

Growth factors are small peptides that bind to membrane receptors to influence the various steps of the growth and development of cells through several signaling pathways **(Menetrey et al ., 2000).** A large number of growth factors and cytokines are known to be expressed in the injured skeletal muscle, such as the members of fibroblast growth factor**(FGF)**, insulin-like growth factor**(IGF)**, and transforming growth factor–β1**(TGF-β1)** families **(Bunn et al ., 2004).**

Some of them are powerful stimulators for myogenic precursor cells differentiation and the later fusion of myotubes into multinucleated mature myofibers during the regeneration process, while others such as **(TGF-β1)** are inhibitory for both differentiation and fusion of myoblasts **(Menetrey et al ., 2000).**

Many authors reported that the overproduction of **(TGF-β1)** in response to injury and disease triggers fibrosis via the activation of extracellular matrix production and connective tissue proliferation both in animals and humans **(Li et al ., 2004)**.

In light of the apparent role of **TGF-β1** in skeletal muscle fibrosis, **Li and Haurd (2002)** succeeded to improve muscle structure and function by the direct injection of the **TGF-β1** antagonists, as decorin and gamma interferon, into the lacerated muscle blocking its fibrotic effect. However side effects are usually observed with gamma interferon, also decorin is not a clinically available drug. Suramin is a food and drug administration **(FDA)** approved drug that appears to be a readily available medication, since it has anti fibrotic properties, as it prevents **TGF-β1** from initiating its effect on the fibroblasts by competitively binding to its receptors **(Foster et al ., 2003 ; Li et al ., 2004).**

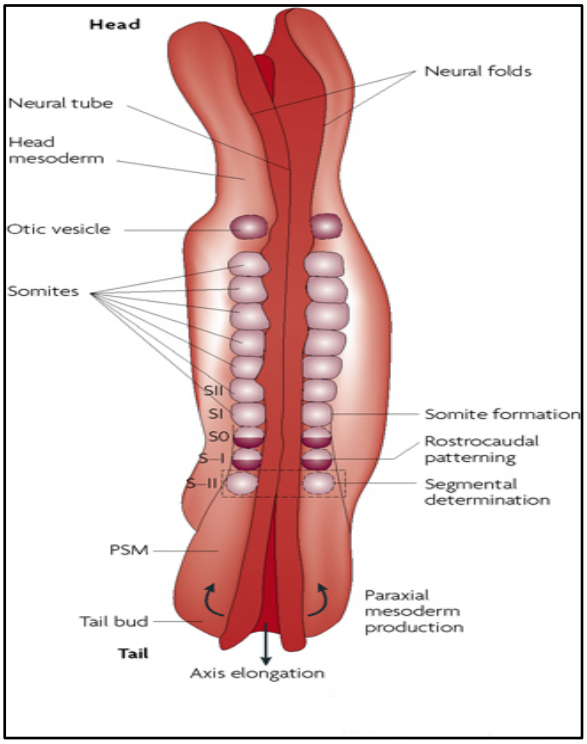
Aim of work

Based on the prior literature, the aim of this study was to investigate and clarify the possible role of suramin on the healing process of skeletal muscle after induced laceration injury in a mice-model using histological, immunohistochemical and morphometric methods.

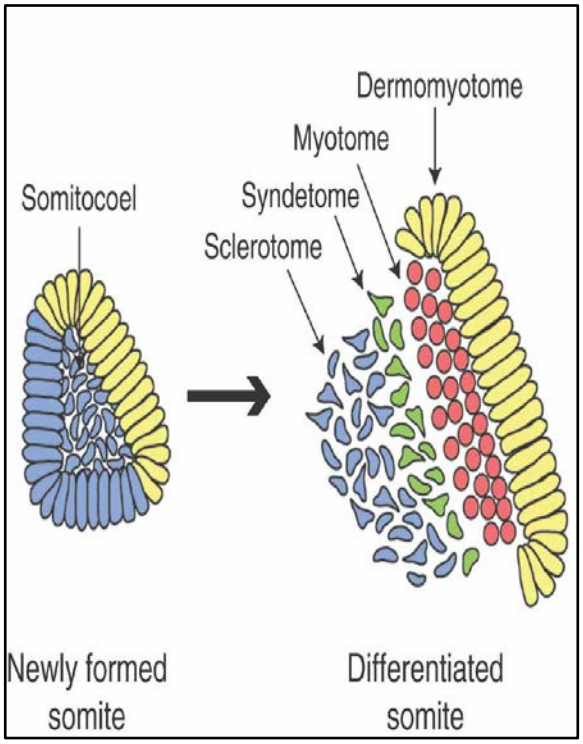
Skeletal Muscle

Skeletal Muscle Development (myogenesis)

All vertebrate skeletal muscles (apart from head muscles) are derived from mesodermal precursor cells originating from the somites **(Diagram 1)** (transient epithelial spheres that pinch out of the paraxial mesoderm lining both sides of the neural tube), to be more specific, skeletal muscle progenitor cells arise from the dermomyotome **(Diagram 2)** (an epithelial layer located in the dorsal compartment of the somite) **(Asakura and Rudnicki, 2002).**



Diagram(1):A schematic presentation of a 4-week old human embryo showing somites **(Dequéant and Pourquié , 2008)**.



Diagram(2):A schematic presentation showing the maturation and compartmentalization of somites **(Eckalbar et al ., 2012)**

During embryonic development, specification of mesodermal precursor cells to the myogenic lineage (committed myoblasts) is regulated by several signaling proteins (proteins that pass signals from outside of a cell through cell surface receptors to the inside of the cell) such as wingless/integrated protien **(WNTS)**, Sonic hedgehog protien **(SHH)**, Noggin and bone morphometric proteins **(BMP4) (Diagram 3A)**.This specification to the myogenic lineage requires the up-regulation of the primary myogenic regulatory factors **(MRFs):** myogenic differentiation protien **(MYOD)** and