BENEFICIAL EFFECT OF TETRACYCLINE THERAPY ON MUSCLE IN IMMOBILIZED KNEE OF RATS

FARELERİN İMMOBİLİZE DİZLERİNDE KASLAR ÜZERİNE TETRASİKLİN TEDAVİSİNİN YARARLI ETKİLERİ

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SUMMARY

Aim: Immobilization increases the expression of reactive oxygen species (ROS) in skeletal tissues. As a defense strategy against ROS, it was shown that enzymes like matrix metalloproteinase type 2 (MMP-2), superoxide dismutase (SOD), and heat shock protein 70 (HSP-70) are induced in the muscle. Tetracycline was used to reduce tissue degradation in skeletal muscles exposed to immobilization.

Methods: The right knee of Wistar rats was immobilized by a rigid external fixator device for 1, 2 , 3, and 4 weeks. Aqueous Tetracycline solution was administrated 3 times a week, starting 2 days following the External Fixation (EF) was constructed. Control group 1 was immobilized for 3 weeks, did not receive tetracycline but did received saline injection, and control group 2 only received tetracycline for 3 weeks. MMP-2, total SOD, and HSP-70 protein and mRNA levels in the gastrocnemius, quadriceps and soleus muscles were analyzed at the molecular level by RT-PCR and the protein level using SDS-PAGE gels and western blots.

Results: We have shown that rats treated by Tetracycline reduce the MMP-2 expression and HSP-70, while trigger the activity of SOD. Theses changes mainly occurred in type IIb muscle fibers.

Conclusion: Tetracycline administration has beneficial effect on expression of antioxidant enzyme (i.e. SOD), and reduces expression of enzymes involved in protein degradation. This may suggest a protective effect on protein degradation during immobilization.

ÖZET

Amaç: İmmobilizasyon iskelet dokularında reaktif oksijen türlerinin (ROT) ekspresyonunu arttırır. ROTa karşı bir savunma stratejisi olarak kasta matrix metalloproteinase type 2 (MMP-2), superoxide dismutase (SOD), ve heat shock protein 70 (HSP-70) gibi enzimler indüklenir. Tetrasiklin immobilizasyona maruz kalan iskelet kaslarında doku yıkımını azaltmak için kullanılmıştır.

Method: Wistar sıçanlarının sağ dizi rijit eksternal fiksatör cihazı ile 1, 2 , 3, ve 4 hafta süreyle immobilize edildi. Eksternal Fiksasyon uygulandıktan sonra 2. gün başlayarak Aqueous Tetracycline solüsyonu haftada üç kez uygulandı. Kontrol grubu 1, üç hafta süresince immobilize edildi, tetrasiklin almadı ama salin solüsyonu aldı, kontrol grubu 2 üç hafta süresince sadece tetrasiklin aldı. Gastrocnemius, kuadriceps ve soleus kaslarında MMP-2, total SOD, ve HSP-70 protein ve mRNA düzeyleri RT-PCR yöntemi ile moleküler düzeyde, SDS-PAGE jel ve western blots yöntemi ile protein düzeyinde analiz edildi.

Bulgular: Tetrasiklin ile tedavi edilen sıçanlarda MMP-2 ekspresyonu ve HSP-70 azaldı, SOD aktivitesi arttı. Bu değişiklikler özellikle type IIb kas liflerinde görüldü.

Sonuç: Tetracycline uygulamasının antioksidan enzim (SOD gibi) ekspresyonu üzerine olumlu etkileri vardır ve protein yıkımında sorumlu enzimlerin ekspresyonu azalır. Bu durum immobilizasyon sırasında protein yıkımı üzerine koruyucu bir etki sağlayabilir.

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INTRODUCTION

Muscle atrophy occurs in numerous pathologies such as cancer, sepsis, and prolonged periods of muscle inactivity (1-3). It is well established that muscle atrophy occurs in prolonged bed rest, limb immobilization or unloading the diaphragm via mechanical ventilation (4, 5). Among the mechanisms suggested to contribute to local catabolism and progressive skeletal muscle atrophy is activation of systemic and local markers of inflammation (e.g. TNF-α, IL-1 and IL-6) (6), matrix remodeling (3, 6-9) and increased levels of oxidative stress (10-12). As defense strategy, muscle tissues of human and animals, were shown to induce enzymes like matrix metalloproteinases (MMPs); (13, 14), superoxide dismutase (SOD) (15) and the heat shock protein -70 (HSP-70) (16).

MMPs are a family of about 24 proteolytic enzymes that belong to a large group of zinc enzymes, and are crucially involved in the turnover of extracellular matrix (ECM) components (17). MMPs play an important role in the homeostasis of the extracellular matrix (ECM) in skeletal muscle (14). The ECM surrounding muscle fibers provides structural support and protection, and is important in maintaining functional integrity of the fibers. In particular, type IV collagen is a major component in the basement membrane and plays an important role in the cellular arrangement of skeletal muscle fibers. Changing demands in skeletal muscle (e.g. increased or decreased contractile activity) promotes remodeling of the extracellular matrix. Although several MMPs are expressed in muscle, two MMPs are thought to play an important role in skeletal muscle adaptation to changing contractile demands and to response to injury are MMP-2 (also known as Gelatinase A) and MMP-9 (also known as Gelatinase B). MMP-2, for example, was shown to play a key role in maintaining the structure and activity of basement membrane components of atrophied muscles such as in inflammatory myopathies (18, 19) and immobilization (20, 21). Both of these MMPs degrade type IV collagen and belong to a group of calcium and zinc endoproteinases that have important functions in homeostasis of the extracellular matrix during morphogenesis, proliferation, and cell apoptosis in a wide range of tissues (22, 23). Activation of MMP-2, MMP-9 has also been shown to be involved in various myopathic and inflammatory-induced changes in skeletal muscle (19, 24). Expression of MMP-2 in skeletal muscle was increased following administration of chronic electrical stimulation (25), and expression of MMP-9 exposed to a chronic increase in blood flow (26).

As one of the major antioxidant enzymes, superoxide dismutase (SOD) plays an important role in catalyzing the dismutation of superoxide radicals (O2·?) to hydrogen peroxide (H2O2), thereby preventing the dangerous Haber-Weiss reaction which generates ·OH (27). In mammals, two forms of SOD co-exist, a tetrameric mitochondrial enzyme containing manganese (Mn SOD), and a dimeric cytosolic enzyme containing both copper and zinc (CuZn SOD). Although catalytic mechanisms appear the same, gene expression, protein turnover and regulatory properties of the two isoenzymes are known to be quite different (28).

Free radicals and reactive oxygen species (ROS) are produced in inactive and contracting skeletal muscles (29). When oxidant production in skeletal muscle exceeds the antioxidant capacity to buffer oxidants, oxidative stress occurs. Oxidative stress was implicated as a potential contributor of disuse atrophy although it is unknown which ROS-producing pathways is responsible for this observed oxidative injury within inactive skeletal muscles (11, 12, 30). In addition to oxidative damage present during muscle disuse, antioxidant enzymes respond in a manner that suggests elevated free radical content (31).

The expression of Heat shock proteins (HSP) is increased when cells are exposed to elevated temperatures or other type of stress. This increased expression is transcriptionally regulated. The HSPs are named according to their molecular weights. HSPs include Hsp90, Hsp70, Hsp25, and crystalline, (32, 33) and play essential roles in refolding, as chaperones, in regulation of protein degradation, and in protection against oxidative stress (32,34,35). Impaired protection of muscle cells against atrophy and reloading-induced damage may be related to lower levels of heat shock proteins (HSPs), an important family of protective stress proteins (Schlesinger, 1990).

Severity of muscle atrophy is related to the duration and magnitude of the activity limitation. Extended periods of disuse induce the specific loss of proteins associated with contraction and cytoskeletal structure (36, 37). If left unchecked, this muscle wasting can lead to long-term sequelae, including impaired functional capacity and permanent muscle damage (38). Therefore there is a need to find ways not only to improve but also to accelerate muscle recovery. Tetracycline may hold promise, unrelated to their antimicrobial properties, in this regard.

Tetracycline was previously proposed as a therapeutic approach to combat deleterious effects of inflammation processes of connective tissues contain

collagen (39). Moreover, it was also shown to inhibit, by several non-antimicrobial mechanisms, the activity of several host-derived matrix metalloproteinases (MMPs) responsible for connective tissue breakdown (40, 41) and was thus used to reduce tissue degradation in maladies such as periodontal disease and arthritis (42, 43).

Little is known about changes of MMP-2, SOD and HSP-70 level in the different hindlimb muscles following immobilization and tetracycline supplementation.

As previously reported (44) there are no immobilization-related changes in fiber type distribution in aged rats. In normal skeletal muscle MMP-2 levels are low in the ECM and its expression is tightly regulated by cytokines and growth factors such as capillary growth factor. Generally, immobilization-induced MMP-2 and HSP-70 in skeletal muscles occurs in situation with myopathies and congenital dystrophies (45).

Although production of proteolytic enzymes is known to be associated with various myopathies and inflammatory conditions (46, 24), their involvement in changes in different fiber types under various conditions of loading stimulation has been little investigated. Excessive acute or chronic muscle use, such as in intensive sporting activities, may lead to structural damage involving protein degradation, myopathy, and muscle dysfunction (19).

The purpose of this study was to determine the effects of tetracycline on SOD, heat shock proteins and MMPs in slow and fast muscles of rats with external fixation during the early onset of muscle disuse. It was hypothesized that tetracycline administration would orchestrate downregulation of MMP and SOD levels and upregulation of heat shock proteins and that this will result in faster and better muscle recovery.

MATERIAL AND METHODS

Animals

Wistar rats (20 months-old, body weight ranged 260-320g) were maintained under constant conditions of room temperature (22°C) and humidity (40%) with a 12/12 hour light-dark cycle. Rats were fed on standard rat chow and water ad libitum. All animals were maintained according the principles of laboratory animal care formulated by Tel Aviv University, (no 11-04-031).

Animals were randomly assigned to either of the 6 following groups (n=5 each): four immobilization groups that each was immobilized for 1,2,3, and 4 weeks. Tetracycline was administered 3 times a week. Two control groups (n=5 each) comprised of: 1. rats that were immobilized for 3 weeks and injected with saline without tetracycline; and 2. a group that was injected with tetracycline only for 3 weeks.

Immobilization

Rats were anesthetized by intra muscular injection of 60 mg/kg Ketamine HCl, and 70 mg/kg Cefamizine (70 mg/kg) 40 mg/kg Nembutal intra peritoneal (IP) injection. Immobilization was done as previously published (47). Briefly, rigid external fixation (EF) was achieved by inserting two 0.8 mm diameter Kirschner wires through the lateral plane of the femur and tibia. Wires were then connected by two threaded brass rods to a rigid frame. The rods were 4.8 mm in diameter and 33 mm in length and had a 13 mm slot cut longitudinally from both ends to contain the wires. The overall fixation device weighed approximately 12 g. The right knee was immobilized in 45º flexion position.

Rats were anesthetized by IP injection of pentobarbital sodium (200mg/kg), 1wk, 2 wks, 3 wks, and 30 days post-fixation. After reaching a surgical plane of anesthesia (stage III, plane 3, resulting in paralysis of muscles and absence of lid, corneal, and skin reflex) the surgical procedure of carefully removing the EF and the right and left hindlimb muscles i.e., gastrocnemius (Gast.), Soleus (Sol.), and Quadriceps (Quad.) was carry out. Then, animals were sacrificed with an over dose of pentobarbital. Muscles were weighed and frozen in isopentane chilled by liquid nitrogen (-192ºC).

Tetracycline treatment

Two days following external fixation, aqueous Tetracycline solution (Engemycine 10% Oxythetrcybclinum LA) was injected intra peritonealy, 3 times a week, at a dose of 1ml/1Kg which is the minimal effective dose to abolish MMP and HSP activity (Roach et al., 2002).

Molecular analysis

Total RNA was isolated from 30mg muscle tissue using SV total RNA isolation kit. (Promega Z3100). RNA was used as a template for RT-PCR reaction (Access QuickTM RT-PCR system, Promega A1702). 50 ng of cDNA was used as template for PCR amplification using touch-down program: 94°C for 1 min, 30 cycles of 94°C for 5 sec, and 72-68 C for 3 min. Annealing and primer extension were done at 72, 70, and 68°C during 1-5, 6-10, and 11-30 cycles, respectively. The PCR products were separated on 1.2 % agarose gel electrophoresis and ethidium bromide staining for visualization.

Specific primers were used for PCR:

MMP-2: Forward CCATCAAACGGGTATCCATC

Reverse GTCGGACCTCTCAGGGTTCT.

HSP-70: Forward: TCGGGAACCATGAATAGAGG

Reverse: TTTGGAGAAAGGAGCAGCAT.

Alpha (?) Tubulin:

Forward: ATTGACATCTTTGGGGACCA

Reverse: ATCACAGGCAAGGAAGATGC (Sigma).

For negative control, RT reactions using 1 μg of total RNA from each tissue with no reverse transcriptase (No-RT control).

Biochemical Studies

SDS-PAGE gels and Western blot analysis

100 mg muscle tissue was homogenized (20 sec homogenization and 10 sec pause x 3 times) in cold buffer containing 42mM Trizma base, 0.3M KCl, 2.5mM MgCl, 0.1% Triton x-100 and protease inhibitor cocktail (P-8340, Sigma) and centrifuged (10,000 x g for 10 min at 4°C) and supernatant was collected. Total protein concentration was measured using Bradford reagent (cat. 500-0006, Bio-Rad, Hercules, CA). Equal amount of protein was 50μg in sample buffer containing 5% beta-mercaptoethanol. Samples were then vortexed, boiled and centrifuged. Proteins were separated on 10% SDS-PAGE, and then transferred to nitrocellulose membranes. Blots were blocked with 2.5% skim milk (cat.170-6404, Bio-Rad) in PBST (PBS containing 0.05% Tween 20) for 1 hr. the following primary antibodies were added for 1 hour in room temperature: MMP-2 goat polyclonal antibody (sc-6838, Santa Cruz Biotechnology, CA), alpha-tubulin specific mouse monoclonal IgG2a antibody (sc-5286, Santa Cruz Biotechnology, CA), mouse anti-HSP-70 monoclonal antibody (Stressgen, Victoria BC Canada). Secondary antibodies were bovine anti-goat IgG-HRP (sc-2350, Santa Cruz Biotechnology, CA) or donkey anti-mouse IgG-HRP (sc-2314, Santa Cruz Biotechnology, CA). Autoradiographs were developed using Super Signal West Pico chemiluminescent substrate (cat. 34080, Pierce Chemical Co., Santiago, Chile) followed by exposure to X-ray films (Fuji). Quantification of MMP-2 and HSP-70 were performed using the Scion Image Version 4.0.2 beta, Scion Cooperation.

SOD Assay

This assay utilizes the reduction of cytochrome c via superoxide anions produced via xanthine oxidase reaction. SOD catalyses the reaction below by producing hydrogen peroxide and molecular oxygen from superoxide and hydrogen ions: $2O2^- + 2H^+ \xrightarrow{XOD} H2O2 + O2$ Xanthine oxidase produces superoxide through the following reaction:

Xanthine + O2 $\xrightarrow{XOD}$ urate + O2- Superoxide will reduce cytochrome c producing a change in absorbance at 550nm. SOD can slow down the reduction of cytochrome c. One unit (U) of SOD activity is defined as the amount of SOD required for a 50% decrease in cytochrome c reduction rate. Activity is expressed in U/gww or U/mg protein.

Statistical Analysis

t-test for dependent samples (paired t-test) was used unless noted otherwise. When multiple t-test comparisons were used, a Bonferroni correction was applied. P values less than 0.05 were considered significant.

RESULTS

Body and muscle weights

Body weights of all animals are shown in Table 1. Body weight did not significantly differ between groups. Weights of the three studied muscles are

Tablo I

The body-weight mean (±SD) of immobilized leg, and after administration of tetracycline.

	3 wk of EF		*1* and 3 wk of EF + Tetracycline	
	Before	After	Before	After
Body-weight (g)	268±57	250± 65	*261± 62* 269±59	*252± 53* 248±60
Mean difference (g)		-18	-9	-21
95% confidence	-2.571 to 4.029		-2.445 to 3.807	- 2.360 to 3.500
Interval of difference change (%)		-3.8	-2	-4.5
p value		NS		NS

Tablo II

The mean (±SD) change in the muscle weight (mg) of three hindlimb muscles after 3 weeks of external fixation (EF) (control 1), 3 weeks of tetracycline administration without EF (control 2), and in comparing to1,2,3,4 weeks of EF plus administration of tetracycline.

	Contralateral Control	Control 1	Control 2	EF + tetracycline			
				1 wk	2 wks	3 wks	4 wks
Gastrocnemius	***2998*** ± 261	2343±301	2295± 288	2303 ± 271	2285± 305	***2280***± 299	2288± 273
*Mean difference						-630	
*95% change of difference						-1105 to -627	
*Change (%)		-22				-2.6	
Soleus	***435*** ± 41	357 ±54	369± 50	341± 38	340± 45	***323***± 42	309± 37
*Mean difference						-122	
*95% change of difference						-92 to-51	
*Change (%)		-18				-9.5	
Quadriceps	***3995*** ± 330	3021± 360	2993± 311	3200± 346	3009± 337	***3027***± 362	2995± 328
*Mean difference						-74	
*95% change of difference						-1393 to -826	
*Change (%)		-24				-2.4	

* values are between contralateral leg of group 1 and 3 weeks of EF + tetracycline

depicted in Table 2. Following three weeks of immobilization, there was a 6% decrease in muscle weights compared to control group 1 (18% and -24%, respectively). However, there was no difference in Gast. and Quad. muscle weight between immobilized with tetracycline to non-immobilized controls . Sol. muscle weight, however, was significantly reduced despite the administration of tetracycline ($p<0.05$).

Molecular and Biochemical analysis

To write separate the gene from proteins

The content of MMP-2, SOD and HSP-70 was measured in three muscles throughout 4 weeks of immobilization and non immobilization condition. There was a significant increase in the MMP-2 and HSP-70 (Figures 1 and 2), protein and mRNA levels, in type IIb fibers (fast glycolytic, FG) in the immobilized animals (Figure 3). In contrast, levels of the aforementioned factors did not change in the immobilized animals that were treated with tetracycline. The levels of MMP-2 and HSP-70 in type I (Sol.), IIa (Quad.) and IIb (Gast.) in the contra lateral leg in the immobilized group was equivalent and comparable to sedentary control levels as well as to control-2 group. All three muscles in control group 2 were not affected by tetracycline treatment. Mean and ±SD activities for total SOD in three muscles is depicted in Table 3.

Total activity of SOD is depicted in Table 3. SOD activity in Gast. and Quad. muscles significantly decreased after three weeks of immobilization, whereas there was no change in SOD activity in Sol muscles. In the tetracycline treated animals the level of SOD

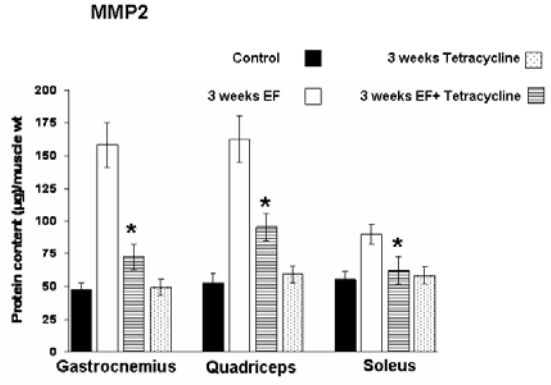

Figure 1. Content of MMP-2 protein (ì g) relative to muscle weight (mg) for three different muscles following immobilization and tetracycline administration.

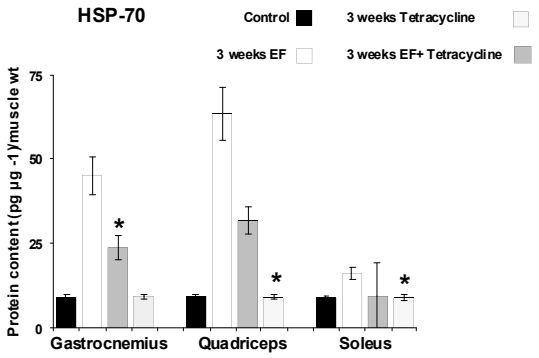

Figure 2. Content of HSP-70 protein (pg ì g-1) relative to muscle weight (mg) for three different muscles following immobilization and tetracycline administration.

Tablo III

Total Activity of SOD (unit per mg protein)

Muscles	Control	3 weeks of EF	p	3 weeks of EF with tetracycline	p
Gastrocnemius	11.2±3.1	7.2±2.3	0.05	9.1±1.7	0.05
Soleus	11.5±4.9	11.8±1.8	NS	11.2±2.4	NS
Quadriceps	10.2±2.5	7.7±2.4	0.05	9.8±1.7	0.05

*p <0.05

activity resembled (<0.05) that of the non-immobilized control group.

DISCUSSION

In this study we used tetracycline in skeletal muscle subjected to immobilization in order to investigate its effect. Our findings suggest that tetracycline participates in minimizing the damage occurred to the muscle fibers due to the immobilization and thus protects muscle protein degradation.

The protein content of MMP-2 and HSP-70 were increased after immobilization but their level remain low and constant following administration of tetracycline without appreciable changes in mRNA abundance, suggesting that post-transcriptional regulation plays an important role in these proteins adaptation (48). There are differences in the composition of the extracellular matrix surrounding myofibers of differing functional types. Slow-twitch muscles of rats contain more collagen in the ECM than fast-twitch muscles (49). It is conceivable that the adaptation responses of muscles with predominantly slow-twitch fibers will differ from those of fast-twitch fibers and this may be reflected in changes in expression of MMPs.

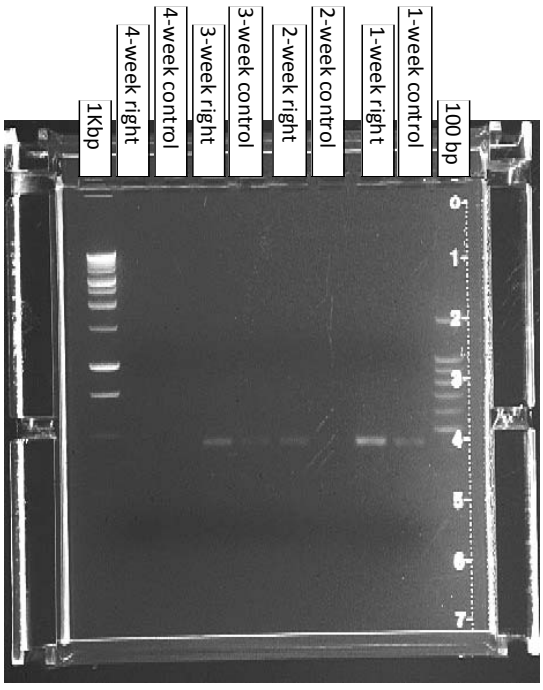

Figure 3. mRNA MMP-2 levels, in type IIb fibers (fast glycolytic, FG) in the immobilized animals.(The volume of the loaded samples was 110ng following RT-PCR. All samples had the same concentration of RNA during the beginning of the RT-PCR reactions. MMP-2 was running in agrose gel of 2% at 120 voltage for 90 minutes)

The results of our study suggest that disuse-related change effects differently the three muscles studied. The current study was therefore designed to investigate the possible effect of tetracycline on different skeletal muscles (i.e., Gastrocnemius is well known as type IIb muscle fibers, Soleus as type I muscle fibers, and Quadriceps is composed of a mixed fiber composition (type IIa), (50), that immobilized for short and longer period of time. The repairing potential of tetracycline due to immobilization was successfully demonstrated rather than its potential to prevent muscle atrophy.

Our novel study is of interest both from a basic science perspective as well as from a clinical angle. Investigating the kinetic of protein metabolism in skeletal muscles following disuse is important to understand how to minimize the disuse muscle atrophy and the 'sarcopenia' phenomenon. It is possible to look at protein metabolism (i.e. synthesis and degradation) systems within the myofiber (i.e., HSP-70, SOD) and out the myocell (i.e., MMP-2), and to study their interrelationships. Additionally, this experiment was performed to test the therapeutic efficacy by using tetracycline in reducing the pathological effects of immobilization in MMP-2, SOD and HSP-70 levels.

The increase of HSP-70 mRNA abundance correlates with previous investigations indicating elevation of HSP-70 mRNA content against fatigue-induced injury (48). The protein content of MMP-2 and HSP-70 were increased after immobilization but their level remain low and constant following administration of

tetracycline without appreciable changes in mRNA abundance, suggesting that post-transcriptional regulation plays an important role in these proteins adaptation. There are differences in the composition of the extracellular matrix surrounding myofibers of differing functional types. Slow-twitch muscles of rats contain more collagen in the ECM than fast-twitch muscles (49). It is conceivable that the adaptation responses of muscles with predominantly slow-twitch fibers will differ from those of fast-twitch fibers and this may be reflected in changes in expression of MMPs.

The results of the present study showed that knee immobilization resulted with muscle atrophy and higher expression of the inactive precursor, or zymogen, (pro MMP-2, 72 kDa), indicating accelerated activities of the active form of MMP-2 and increased capacity for ECM degradation. Following EF the relative increase in gelatinolytic activity of MMP-2 was higher in the fast-twitch muscle fibers than in the slow-twitch fibers. These results agree with the viewpoint that only under extreme or abnormal conditions of muscle use, possibly following injury-related over use or inflammation-related disuse, MMP-2 is expressed conceivably by leucocytes and macrophages. We recently showed that MMP-2 may be inhibited by tissue inhibitors of metalloproteinases 1 and 2 (TIMP-1, -2), which are secreted by the same myofibers as MMP-2 (51).

Moreover, in the current study, we demonstrated that different muscle fiber types show differing response patterns to EF and to administration of tetracycline. We found that type IIb muscle fibers were more susceptible both to disuse and tetracycline than type I muscle fibers. From previous reports, it has been shown that disuse and overuse lead to muscle tissue damage followed by functional decline (52). The significant changes in type IIb fibers were observed particularly following 3 weeks of immobilization. Both in Gast. and Quad. muscles, net tissue degradation was observed when related to soluble protein concentration, suggesting a higher rate of protein degradation in slow and fast oxidative glycolytic (IIa and IIb) muscle fibers than in (type I) slow-twitch muscle fibers. Therefore, our findings certainly cause us to believe that fast fibers are more responsive to disuse and to tetracycline.

There are two possible explanations regarding the differing proteins expression in the various muscle fiber types. In rat skeletal muscles, type IIB fibers are markedly larger and stronger than type I fibers and appear to be associated with larger amounts of extracellular collagen (53). It is possible that fast-twitch fibers require a different molecular mechanism to maintain their structural integrity than slow-twitch fibers (54). Moreover, "white fibers" demonstrate better muscle plasticity than "red fibers" and adapt faster to mechanical disuse or overuse (55). It is also possible that as a result of immobilization fast fibers (low oxidative, type IIb) may undergo a transition to slow-fiber types (high oxidative, type IIa) with corresponding changes in the composition of the extracellular matrix. The results of the present study suggest that immobilization may affect the overall balance of protein turnover in skeletal muscle fibers, and that changes also involving degradation and synthesis of extracellular matrix, are more distinct in type II muscle fibers than in type I fiber. Therefore, it is safe to conclude that the anaerobic type II muscles fibers may be more susceptible to oxidative stress due to immobilization, and less able to cope with the increased energy and oxidative demands. Our study supports the hypothesis that in order to maintain efficiency with elevated energy requirements for long periods, type II fibers show both intracellular and extracellular adaptation responses.

Our findings show that 3 weeks of EF reduced SOD activity only in type II muscle fibers. Yet, increased SOD activity following administration of tetracycline provides protection against oxidative stress. Our data agree with previous findings that tetracycline serves as a trigger for SOD elevation which is an essential defense mechanism. Fast twitch fibers contain lower SOD than the slow-twitch fibers, possibly indicating that the SOD enzyme system is more susceptible to undergo changes in type II muscle fibers. From our study it seems that in order to maintain their efficiency requires producing energy for long period of time, type II fibers underwent some intra and extra cellular adaptation.

Despite of convincing results the major limitation in our animal-based study is the exist differences between human and animals skeletal muscle biology. The differences are both in the molecular profile and histo-morphology components i.e., fiber types and fiber dominant. Therefore, our outcomes should be translated to human with some degree of caution and critics.

Conclusions

Tetracycline, found to abolish the anti MMP and HSP activity, and at the same time set-off the SOD activity and therefore participates in minimizing the damage occurred to the muscle fibers due to the immobilization and thus protects muscle protein degradation.

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