Cellular adaptations of skeletal muscles to cyclosporine

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Biring, Manmohan S., Mario Fournier, David J. Ross, and Michael I. Lewis. Cellular adaptations of skeletal muscles to cyclosporine. J. Appl. Physiol. 84(6): 1967–1975, 1998.—The aim of this study was to evaluate the cellular response of the diaphragm, extensor digitorum longus (EDL), and soleus (Sol) muscles to clinically relevant doses of cyclosporine administered to male rats over 4 wk. Control rats were provided with vehicle only. Muscle fiber types, cross-sectional areas, indexes of capillarity, and succinate dehydrogenase (SDH) activity were determined by quantitative histochemistry. Myosin heavy chain isoforms were identified by SDS-PAGE, and their proportions were measured by scanning densitometry. Serum cyclosporine level, 20–24 h after the last dose of cyclosporine, was 145 ± 81 ng/ml. Final body weight and muscle mass were similar between the cyclosporine and control groups. In the diaphragm, EDL, and Sol, no differences were observed between the groups with regard to fiber type proportions, fiber cross-sectional areas, and proportions of myosin heavy chain isoforms. In the EDL, reductions, both in SDH activity in type I, IIX, and IIB fibers (−26 to −37%) and in indexes of capillarity (−18 to −37%), were noted. In the Sol, SDH activity and capillarity were similar between the groups. In the diaphragm of cyclosporine-treated rats, there was significant reduction in the number of capillaries around individual fibers (−5%), whereas levels of SDH activity tended to be lower. This suggests that activation history may in part determine muscle-specific responses to cyclosporine. We speculate that reduced oxidative activity and capillarity of some limb muscles contribute to reduced exercise capacity and the “deconditioned state” observed in patients receiving cyclosporine after successful solid-organ transplantation.

A number of case studies have reported possible adverse effects of cyclosporine on skeletal muscle, including clinical observations of myopathy that reversed after cessation of cyclosporine, with exacerbation of muscle weakness on reinstitution of therapy (2, 10, 17, 21, 22, 36). Furthermore, muscle fiber atrophy as well as ultrastructural abnormalities have been reported in specimens from both necropsy and clinical biopsies (17, 21, 36). Because of the complex polypharmacy of patients on cyclosporine, however, it was difficult to exclude other medications, such as corticosteroids, as causative or potentiating factors.

Cardiopulmonary exercise studies in patients after either cardiac or lung transplantation have revealed significant abnormalities despite marked clinical improvement, rehabilitation programs, and an active nonsedentary lifestyle. After cardiac transplantation, significant reductions in exercise capacity and maximum oxygen consumption (VO2max) have been consistently reported compared with age- and activity-matched controls (26, 29, 39), as well as reduced ventilatory threshold and increased blood lactate levels with exercise (26, 29). Although exercise rehabilitation resulted in some improvement, VO2max remained significantly reduced compared with age-matched control subjects (29). Similarly, after lung transplantation, exercise capacity and VO2max remain well below predicted levels despite significant improvements in pulmonary function tests (19, 34, 39). Ross and colleagues (38) also reported reduced exercise, no ventilatory limitation to maximum exercise, and a reduced lactate threshold after lung transplantation. They postulated whether the exercise characteristics could be explained by a “deconditioned state” or peripheral factors impairing adequate oxygen supply to the exercising muscles [e.g., problems at a microcirculatory or cellular level (38)]. Of interest, a recent study in heart-transplant patients reported reduced capillarity-to-fiber ratio and capillary density in the vastus lateralis muscle of these patients compared with control subjects (31). Because these patients received cyclosporine as well as other immunosuppressive agents (i.e., azathiaprine, prednisone), it is difficult to ascribe these effects solely to cyclosporine.

Evaluations of the effects of cyclosporine in animal studies or isolated cellular systems are useful in that they enable one to control for the singular effects of the drug. These studies strongly suggest a distinct impact of cyclosporine at a cellular level. For example, oxidative phosphorylation in isolated kidney mitochondria from both rat and human sources was reduced by cyclosporine (27, 28). Inhibition of respiration has been reported in mitochondria isolated from homogenates of several rat hindlimb muscles when the animals were exposed to cyclosporine (24). In addition, reduced treadmill exercise capacity was reported in rats treated with supratherapeutic doses of cyclosporine for 2 wk (33). A strong correlation between reduced exercise time and the degree of mitochondrial inhibition was noted (33).

The aim of this study was to evaluate the impact of prolonged administration of cyclosporine on the histochemical, morphometric, and biochemical properties of the rat diaphragm and limb muscles (both fast and slow) by using a clinically relevant dosage regimen. We postulated that cyclosporine in doses producing clinical therapeutic levels would impair oxidative capacity and capillarity of individual muscle fibers. In addition, we wished to test whether cyclosporine alone induces fiber atrophy, as reported in clinical case studies (17, 21, 36). Furthermore, we specifically wished to examine whether respiratory or appendicular muscles with different properties would be similarly influenced by cyclosporine.
correspondence between the mATPase-based classification and muscle fiber type immunohistochemically, with 95% or more in previous studies, in both hamsters and rats, we verified representative of the entire profile of the whole muscle or segment. various fields within the entire cross section and were repre-filled at pH 6. Animals and Drug Administration Studies were performed on 3-mo-old adult male American Cancer Institute (ACI) rats (Harlan, Indianapolis, IN) with an initial body weight of 218 ± 7 g. The animals were randomly divided into two groups: 1) a cyclosporine group (n = 6) in which cyclosporine (Sandimmune IV; Sandoz, Basel, Switzerland) was administered by an oral-gavage technique over the 4-wk experimental period by using a clinical dosage regimen of 7 mg·kg⁻¹·day⁻¹ (diluted in olive oil to produce a final volume of 0.1 ml/100 g body wt) and 2) a control group (n = 6) in which vehicle only was administered daily (0.1 ml/100 g body wt). All animals were provided with food and water ad libitum (Purina rat chow: 56% carbohydrate, 23% protein, 4.5% fat, 6% fiber, and 10.5% ash minerals). The animals were housed in individual cages under controlled temperature (22°C) and light conditions (i.e., 12:12-h light-dark cycle). The research protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Cedars-Sinai Medical Center Burns and Allen Research Institute.

Histochemical Procedures

Fiber type proportions and cross-sectional areas. With the animals under deep general anesthesia (100 mg/kg ketamine and 7.5 mg/kg xylazine ip), two adjacent strips of the midcos-tal diaphragm (respiratory mixed muscle), as well as appendicular predominantly slow- and fast-twitch muscles [i.e., the soleus and the extensor digitorum longus (EDL)] from both limbs, were dissected. This procedure will address the question not only of possible differences in the responses of respiratory and nonrespiratory muscles to cyclosporine administration but also of whether marked differences in muscle composition and activation history alter the impact of the drug. The first segment of diaphragm as well as the soleus and EDL from one limb were frozen in liquid nitrogen and used for biochemical analysis. The second strip of diaphragm was stretched to 1.5 × excised length, a value previously demonstrated to approximate diaphragm optimal length (L₀) for force (37); mounted on cork; and then rapidly frozen in isopentane that had been cooled to its freezing point by liquid nitrogen. The limb muscles were stretched to in situ resting length (i.e., muscle length when both ankle and knee joints are at 90°, which also approximates L₀, unpublished observations), mounted, and frozen as described above. Serial cross sections of the muscle segments were cut at 10-µm thickness, by using a cryostat (model 2800E; Reichert-Jung) kept at −20°C.

Muscle fibers were classified on the basis of difference in staining intensity for myofibrillar ATPase (mATPase) after alkaline (pH = 9.0) and acid (pH = 4.3 and 4.55) preincubations (6, 15). One additional serial section was fixed in 2% paraformaldehyde at pH = 7.4 for 2 min at room temperature and then preincubated at pH = 10.4 [modification by Fournier and Lewis (18) of method by Guth and Samaha (23)]. These various staining procedures allow the classification of fibers into several types, i.e., types I, IIA, IIB, IIX, and IIC (18; see also Ref. 20). In each muscle, fiber-type proportions were determined from a sample of 200–300 fibers selected from various fields within the entire cross section and were representative of the entire profile of the whole muscle or segment. In previous studies, in both hamsters and rats, we verified muscle fiber type immunohistochemically, with 95% or more correspondence between the mATPase-based classification and the major isoform of myosin heavy chain (MHC) expressed in single muscle fibers (18).

Muscle fiber cross-sectional area was determined from microscopic images of digitized muscle sections, by using a computer-based imaging-processing system. The latter is composed of a Leitz Laborlux S (Leica) microscope, charge-coupled device video camera system (model V1-470, Optronics Engineering, Goleta, CA), high-resolution Trinitron color video monitor (model PVM-1343MD, Sony), 486 DX-50-MHz personal computer with a Targa® imaging board (Truevision), and Mocha image-analysis software (version 1.20; J andel, San Rafael, CA). A microscope-stage micrometer was used to calibrate the imaging system for morphometry. The cross-sectional areas of those individual fibers used in the analysis of fiber proportions were determined from the number of pixels within manually outlined fiber boundaries.

Fiber succinate dehydrogenase (SDH) activity. Fiber oxidative capacity was determined by quantifying the activity of SDH (a key enzyme in the Krebs cycle) in individual muscle fibers. The methodology used to quantitate SDH activity has been described in detail in previous reports (3, 4, 15, 41). Briefly, in the histochemical reaction for SDH, the progressive reduction of nitroblue tetrazolium (NBT) to an insoluble colored compound (a diformazan) is used as a reaction indicator. The reduction of NBT is mediated by H⁺ released in the conversion of succinate to fumarate. In a series of 6–sections, the incubation medium contained a large quantity of succinate (60 mM), and thus the SDH reaction was not substrate limited. In other sections, succinate was absent from the incubation medium so that the reduction of NBT in these sections was nonspecific. These sections are referred to as tissue blanks.

The concentration of NBT-diformazan (NBT-dfz) deposited within a muscle fiber was calculated by using the Beer-Lambert equation

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\text{[NBT-dfz]} = \frac{\text{OD}}{K \times L}
\]

where OD is the optical density of the muscle fiber measured at 570 nm (the peak absorbance wavelength for NBT-dfz), K is the molar extinction coefficient for NBT-dfz (26,478 mol/cm) and L is the path length (i.e., 6-µm section thickness) for light absorbance. The OD of muscle fibers was determined by using a microdensitometric procedure implemented on the computer-based image-processing system. The video image was then digitized (8-bit gray-level resolution) into a matrix of 1,024 × 1,024 pixels (picture elements). The gray levels of the video scanner were calibrated for photometry (OD units) by using a series of neutral-density filters (0.004–2.00 OD unit, Melles Griot). We have previously demonstrated that, during the SDH reaction in the cat and rat, the formation of NBT-dfz in diaphragm fibers increases linearly over a period of at least 9 min (3). In reactions in which succinate was absent from the reaction medium, there was measurable staining (i.e., reduction of NBT), but the OD did not change significantly across the same time periods. The tissue-blank OD also corresponded to the OD measured at time 0 in reactions in which succinate was present in the medium. On the basis of these data, we justified the use of a single-end-point measurement of OD, with a reaction time of 5 min. From these end-point measurements, a rate of SDH reaction was interpolated. Mean SDH activity of individual diaphragm muscle fibers was determined by averaging the OD of all pixels within outlined muscle fibers. To correct for the nonspecific formation of NBT-dfz, the tissue-blank OD for each fiber was subtracted from the OD measured when substrate was added.
to the incubation medium. From the Beer-Lambert equation, the mean SDH activity of each fiber was expressed as millimoles fumarate per liter tissue per minute. Approximately 200–300 fibers were sampled from each muscle.

Measurements of fiber capillarization. An acidic ATPase reaction was used to visualize surrounding capillaries (15, 43). This technique has been validated by our group against other commonly used methods [i.e., amylase-periodic acid-Schiff (42) and alkaline phosphatase (Fournier and Lewis, unpublished results; also Ref. 43)]. This ATPase method identifies only the arterial end of the capillary bed by staining the capillary endothelium. Thus the methodology used in capillary analysis is important in comparing results across studies. For example, fixation methods identify all microvessels (arterial and venous) and, with tissue shrinkage due to fixation, may result in an overestimation of capillary density compared with techniques such as those used in the present study. In addition, strain differences may also play a role, because preliminary data from our laboratory suggest lower capillarization (up to 20%) in the diaphragm of ACI vs. Sprague-Dawley rats. Furthermore, the ATPase technique used in this study does not distinguish between perfused and nonperfused vessels. Three indexes of capillarity were determined: 1) the capillary-to-fiber ratio, i.e., the total number of capillaries divided by the total number of fibers within the muscle section; 2) the average number of capillaries surrounding each fiber; and 3) the capillary density, defined as an expression of the number of capillaries per mean cross-sectional area of muscle fibers.

Electrophoretic Identification of MHC Isoforms

For the myofibril extraction (44), 10-mg muscle samples were homogenized by hand on ice in 20 vol of cold buffer containing 250 mM sucrose, 100 mM KCl, 20 mM Tris, and 5 mM EDTA, pH at 6.8. The homogenate was centrifuged at 1,000 g at 4°C for 10 min. The supernatant was discarded, and the pellet was resuspended in 20 vol of a washing buffer containing 175 mM KCl, 2 mM EDTA, 20 mM Tris, and 0.5% Triton X-100, pH at 6.8. After centrifugation the final pellet was resuspended in 10 vol of a buffer containing 150 mM KCl and 20 mM Tris, pH at 7.0. Myofibrillar content was determined by using a micro-bicinchoninic acid protein assay kit (Pierce) and quantified by using an ELISA reader at 550 nm. Samples of purified myofibrils were diluted 1:8 in a denaturing sample buffer in which the final concentration was 1.5% dithiothreitol, 2% SDS, 10% glycerol, 0.012% bromophenol blue, and 80 mM Tris (pH 6.8). Final myofibrillar protein concentration was ~0.125 µg/µl. Proteins were denatured in the same buffer by boiling samples for 2 min before loading.

The determination of the MHC composition was performed by using a SDS-PAGE separation technique (45). The separating gels were composed of 30% glycerol, 8% acrylamide/bis (50:1), 0.2 M Tris (pH 8.8), 0.1 M glycine, and 0.4% SDS. The stacking gels were composed of 30% glycerol, 4% acrylamide/bis (50:1), 70 mM Tris (pH 6.7), 4 mM EDTA, and 0.4% SDS. Polymerization of the gels was achieved with 0.1% ammonium persulfate, and 0.05% TEMED. Each well was loaded with 0.7–0.9 µg of protein extract. Electrophoresis was performed by using a Bio-Rad Mini-Protein II System with a power supply for a duration of 25 h at constant 80 V with running buffers kept at 4–7°C by placing the gel unit in a box containing ice and/or cold packs. The upper buffer was composed of 0.1 M Tris, 150 mM glycine, and 0.1% SDS. The lower buffer contained 50 mM Tris, 75 mM glycine, and 0.05% SDS.

The separating gels were stained with silver nitrate (BioRad Silver Stain Plus kit). Dried stained gels with duplicate samples were scanned twice by using an Ultra-Violet Products Image Store 5000 System and densitometric measurements performed with its Gel Documentation and Software System. After background subtraction, the relative contribution of each band within a gel was determined by the ratio of the total gray level within the area of a specific band to that of the cumulative gray level of all the bands present in a sample. The specificity of each band has been demonstrated on the basis of immunoblotting identification after electrophoretic transfer (30, 40). This SDS-PAGE method allows the clear separation of MHC 1 (β/β), 2A, 2B, 2X, embryonic, and neonatal isoforms from denatured myofibrils of skeletal muscle. In adult rat muscle, the fastest migrating band corresponds to MHC 1 (β/β), followed in order by MHC 2B, MHC 2X, and MHC 2A. The densitometric analysis of each migrated band corresponding to the identified MHC isoforms was performed in duplicate on two samples for each muscle, and the average relative content of each MHC isoform was estimated.

Cyclosporine Analysis

Blood samples were taken from the animals 20–24 h after the last dose of cyclosporine. Samples were obtained from the inferior vena cava before diaphragm and limb muscle removal. Levels were assayed by using a whole-blood fluoroscence polymerization immunoassay (Abbott Diagnostic).

Statistical Analysis

The distribution of all data was tested for normality before parametric analyses. Statistical analysis of group means was then performed (GraphPad Instat, GraphPad Software, version 2.04), by using an unpaired Student's t-test. An alpha level of 0.05 was used to compare differences in independent groups and to determine overall significance. All data are represented as means ± SD.

RESULTS

Body and Muscle Weights

During the experimental period, there were similar body weight increments in the two groups, with the final mean body weight being 276 ± 12 g for the cyclosporine group and 278 ± 1 g for the control animals. The muscle weight-to-body weight ratios for the soleus and EDL were similar in the treated and control groups (i.e., soleus: 0.048 ± 0.002 vs. 0.048 ± 0.001%; EDL: 0.051 ± 0.002 vs. 0.052 ± 0.001%). The total muscle weight of the whole diaphragm was not obtained because of technical limitations related to other tissue sampling for a concurrent study.

Cyclosporine Levels

The mean cyclosporine level in the cyclosporine-treated rats was 145 ± 81 ng/ml. This represents a trough value 20–24 h after the last dose of cyclosporine. Cyclosporine was undetectable in the control group as expected.

Histochemical Data

Fiber type proportions and cross-sectional areas. The proportions of muscle fibers in the soleus, diaphragm, and EDL muscles were unaffected by the provision of cyclosporine for 4 wk (Fig. 1, A–C). Similarly, the mean
cross-sectional areas of individual muscle fibers in the diaphragm and limb muscles tested were unaffected by the provision of the immunosuppressive agent (Fig. 1, D–F).

Oxidative capacity of muscle fibers. SDH activity in the EDL was significantly reduced in types I (P = 0.02), IIx (P < 0.05), and IIb (P < 0.02) fibers (Fig. 2C). Although not statistically significant, there was a definite trend for type IIA fibers to also be reduced (P = 0.06).

In the diaphragm, there was a tendency toward reduction in SDH activity in types I (P = 0.10), type IIa (P = 0.12) and type IIx (P = 0.09) muscle fibers (Fig. 2B). In the soleus, there was complete preservation of SDH activity in all fiber types (Fig. 2A).

Muscle fiber capillarity. In the EDL of cyclosporine-treated animals, significant reductions in all three indexes of capillarity were observed compared with control rats (P < 0.05; Fig. 3). In the diaphragm of the cyclosporine group, the mean number of capillaries...
Fig. 2. Mean fiber succinate dehydrogenase (SDH) activity of soleus (A), diaphragm (B) and extensor digitorum longus (C) muscles in control and cyclosporine-treated animals. Note significant reduction in SDH activity of types I, IIx, and IIb fibers in extensor digitorum longus of cyclosporine-treated rats, compared with control animals. Values are means ± SD. *Significant difference from control, $P < 0.05$.

Fig. 3. Capillary-to-fiber ratio (A), mean number of capillaries (Cap) around each fiber (B) and capillary density (C) of soleus (SOL), diaphragm (DIA), and extensor digitorum longus (EDL) muscles in control and cyclosporine-treated animals. Note significant reduction in all 3 indexes of capillarity in extensor digitorum longus of cyclosporine-treated rats, compared with control animals. Values are means ± SD. *Significant difference from control, $P < 0.05$. 
around each fiber was slightly but significantly reduced (P < 0.05; Fig 3B), whereas there was a tendency for reduced capillary density in these animals (P = 0.15; Fig. 3C). By contrast, all three indexes of capillarity were preserved in the soleus of cyclosporine-treated animals.

Proportions of MHC Isoforms

The proportions of MHC isoforms in all muscles tested were similar between cyclosporine-treated and control animals, as might have been anticipated from the histochemical data on fiber proportions and cross-sectional areas (Fig. 4). The slight discrepancy in the results between histochemical and biochemical techniques likely reflects the coexpression of MHC isoforms within single fibers or small amounts of MHC isoforms below the resolution of the electrophoretic procedures used. For example, the relatively small amount of MHC 2B in the diaphragm is likely coexpressed with MHC 2X within type IIx fibers identified histochemically by the mATPase technique.

DISCUSSION

The major finding of this study is that prolonged administration of cyclosporine reduced the oxidative capacity of types I, IIx, and IIb muscle fibers of the EDL, a limb muscle, by using clinical dosage regimens. In addition, indexes of capillarization were significantly reduced in the EDL in cyclosporine-treated animals. Although there were trends for similar changes in the diaphragm regarding oxidative activity and capillarity, no effects were observed in the soleus muscle of treated animals. Furthermore, cyclosporine had no impact on muscle fiber size, the proportion of fiber types, or MHC isoforms in respiratory and limb muscles of animals receiving the immunosuppressive agent.

Muscle Fiber Size

A number of case reports have documented a possible association between cyclosporine and myopathy in patients after either heart or kidney transplantation (17, 21) or in the management of Grave's disease (36). Deltoid or quadriceps biopsies revealed atrophy of type I and/or type II muscle fibers (17, 21, 36). Inferences on the role of cyclosporine were made on the basis of absence of other drugs that could be implicated, discontinuation of corticosteroids for several months before onset of muscle weakness, and reversible changes on muscle biopsy and/or clinical improvement with dosage reduction or discontinuation of cyclosporine (17, 21, 36). In the present study, the final body and limb muscle weights of cyclosporine-treated and control animals were similar. Furthermore, cyclosporine had no impact on fiber size in any of the muscles examined. This, together with preservation of fiber type proportions and densitometric proportions of MHC isoforms, mitigates strongly for the preservation of fiber-specific force (i.e., force per unit cross-sectional area) and thus whole-muscle strength. Thus, in contrast to the clinical case reports cited above, we were unable to document the presence of myopathic changes morphometrically or infer loss of force-generating capacity in the respiratory or limb muscles examined in our animal model.

Fig. 4. Proportions of different myosin heavy chain (MHC) isoforms in soleus (A), diaphragm (B), and extensor digitorum longus (C) muscles of control and cyclosporine-treated animals. Note that no differences were observed in MHC proportions among the 3 muscles studied. Values are means ± SD.
Muscle Fiber Oxidative Capacity

An adverse impact of cyclosporine on mitochondrial function was initially suggested by experiments on isolated kidney mitochondria from rats and humans, showing inhibition of glutamate/malate- and succinate-supported respiration (27, 28). In addition, it has recently been demonstrated (24, 33) that cyclosporine decreased coupled and uncoupled oxidative phosphorylation of isolated mitochondria from rat hindlimb muscle homogenates, which included the EDL and soleus (32, 33). Our results suggest a more specific effect on the EDL with complete preservation of the oxidative capacity in the soleus. The effect on the diaphragm appeared to be intermediate between that on the EDL and soleus. The dose of cyclosporine used by Mercier and colleagues (33) was 20 mg·kg⁻¹·day⁻¹ for a 14-day period, which is far greater than dosage regimens used clinically. The dose used in the present study is more in line with maintenance doses used in clinical practice over a more prolonged time period. This yielded mean blood levels of cyclosporine at the lower aspect of the clinical therapeutic range, 20–24 h after the last administration of the drug. The rationale for using cyclosporine once daily was based on data from Didlake and coworkers (14), who reported clearance rates of cyclosporine in Wistar rats that were approximately one-half that in humans.

The mechanisms underlying the reduced oxidative capacity of muscle fibers of the EDL are unclear. Although not the focus of this study, a number of potential mechanisms may be postulated. These include effects on the integrity of the inner membrane of mitochondria mediated by the ability of cyclosporine to inhibit opening of a Ca²⁺-dependent pore in the membrane. This would lead to reduced efflux of mitochondrial Ca²⁺ with subsequent mitochondrial dysfunction and reduced respiration (11–13, 24, 25, 35). It is also conceivable that cyclosporine could mediate its effects by inhibiting key enzyme complexes involved in the electron-transport chain (24, 25, 27).

Muscle Fiber Capillarity

Cyclosporine may functionally and anatomically reduce microcirculatory networks within muscle. For example, cyclosporine may induce the release of endothelin, a potent vasoconstrictor (8, 9), and has recently been reported to reduce capillary density and the capillary-to-fiber ratio in the vastus lateralis muscle of heart-transplant patients (31). In the present study, similar findings were evident in the EDL, also a mixed “fast” muscle like the vastus lateralis, whereas no changes were seen in the soleus of cyclosporine-treated animals. In the diaphragm, only one of three indexes of capillarity was reduced. Overall the changes observed in capillarity in all three skeletal muscles tested appeared to parallel those described above for oxidative capacity. It is unclear whether these pathophysiological effects are linked. For example, does a reduced capillary network diminish oxygen delivery to an extent impairing mitochondrial oxidative phosphorylation? By contrast, are the mechanisms resulting in a reduced capillary network and oxidative capacity independent of each other, and are their physiologically adverse effects additive or synergistic? The precise mechanisms responsible for reduced capillarity are unclear. It is unlikely that “deconditioning” is an important factor in both our animal model and clinical studies. In the present study, however, precise monitoring of animal or muscle activity was not performed, although no apparent differences in general cage activity were observed.

Muscle-Specific Influences

An interesting aspect of our data relates to the discordant effects between muscles with clear differences in activation pattern and history (e.g., Ref. 1). The soleus is an important postural muscle that is chronically and tonically active and exhibits increased phasic activity with locomotion (1). By contrast, the EDL is a locomotory muscle with less total phasic activity than the soleus, and it remains quiescent for long periods (1). The diaphragm is somewhat intermediate between the two, in that, although it is phasically active throughout life, only ~25% of its constituent muscle fibers are recruited during normal resting ventilation (42). It would be of interest to speculate on the influences of cyclosporine in other important limb muscles. These include other extensors such as the plantaris and the medial gastrocnemius that exhibit some background tonic activity during standing but to a lesser extent than the soleus (1). We postulate that the response of these muscles to cyclosporine would likely be intermediate between those of the EDL and soleus muscles, but still somewhat reduced, which would have important functional implications. Thus a continuum in the response to cyclosporine may be evident in a variety of limb muscles depending on their specific activation histories.

The explanations underlying these differential influences of cyclosporine on SDH activity and capillarization are not clear. We speculate simplistically that some activity-related factor may attenuate the impact of cyclosporine on local microcirculation and/or SDH activity, whereas reduced muscle activation (i.e., EDL) provides no protection against the adverse influences of cyclosporine. It is well known that exercise conditioning and training may augment muscle oxidative capacity and capillarity, whereas inactivity may reduce the levels of oxidative enzymes and the capillary network (e.g., Refs. 5, 32). Whatever the explanation, it appears that the muscle exhibiting the lowest overall activity is least protected against the adverse effects of cyclosporine. These observations at the level of the muscle, however, fall short at the level of individual muscle fibers. For example, all fiber types in the EDL appeared to be affected, including those belonging to motor units that would rarely be recruited in a relatively sedentary animal (e.g., type IIX, IIB (1), 42)). The fact that fibers of different types often share common capillaries means that the presence of fewer capillaries surrounding the muscle fibers is likely to impact nonselectively on the surrounding fibers.
Clinical Implications

The clinical implications of these findings may assist to some extent in explaining exercise limitation in transplant patients. In lung-transplant patients, Ross and co-workers (38) concluded that the reduced exercise capacity was not limited by ventilatory factors and was associated with an abnormally low lactate threshold. Other studies in lung-transplant patients have also confirmed decreased VO_{2max} with features compatible with a cardiovascular limitation to exercise (19, 34, 46). Similar effects have been reported in heart-transplant patients (7, 26, 46). A recent study by Evans and co-workers (16) evaluated quadriceps muscle phosphorylation pH by magnetic resonance spectroscopy in lung-transplant patients (receiving cyclosporine, azathioprine, and prednisone) and healthy controls. In the lung-transplant patients, quadriceps muscle intracellular pH was more acidic at rest compared with that of controls and fell further during exercise at much lower metabolic rates than in controls. The metabolic rate at which the intracellular pH fell correlated closely with VO_{2max} (16). These data support metabolic aberrations at the level of the limb muscle, contributing to reduced exercise tolerance, peak oxygen consumption, and lactate threshold (38). Recognizing cyclosporine as a potential important factor in reduced exercise capacity in patients after transplantation imposes important therapeutic questions. For example, do the newer immunosuppressive agents such as FK-506 (tacrolimus) and rapamycin also cause reduced exercise tolerance and, if so, by which mechanism? Thus further important basic and clinical research studies on immunosuppressive agents are dearly indicated.

In conclusion, cyclosporine administered to male ACI rats produced a reduction in the oxidative capacity of type I, IIx, and IIb fibers of the EDL, as well as reduced indexes of capillarity. These effects were observed at therapeutic levels of cyclosporine. We speculate that the reduced oxidative capacity and capillarity of some limb muscles may contribute to the reduced exercise capacity, lowered ventilatory and lactate thresholds, and “deconditioned state” seen in patients receiving cyclosporine after successful organ transplantation.

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