Contrast Agent-Enhanced Magnetic Resonance Imaging of Skeletal Muscle Damage in Animal Models of Muscular Dystrophy

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Membrane lesions play an early role in the pathogenesis of muscular dystrophy. Using a new albumin-targeted contrast agent (MS-325), sarcolemmal integrity of two animal models for muscular dystrophy was studied by MRI. Intravenously injected MS-325 does not enter skeletal muscle of normal mice. However, mdx and Sgca-null mutant mice, animal models for Duchenne and sarcoglycan-deficient limb-girdle muscular dystrophy, respectively, showed significant accumulation of MS-325 in skeletal muscle. The results suggest that contrast agent-enhanced MRI could serve as a common, noninvasive imaging procedure for evaluating the localization, extent, and mechanisms of skeletal muscle damage in muscular dystrophy. Furthermore, this method is expected to facilitate assessment of therapeutic approaches in these diseases. Magn Reson Med 44:655–659, 2000. © 2000 Wiley-Liss, Inc.

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Accumulating data suggest that one of the initial events in the degeneration of skeletal muscle fibers in Duchenne muscular dystrophy patients is the breakdown of the sarcolemma (1–3). It is assumed that the structural weakness of the sarcolemma and the increase in its permeability occurs when the dystrophin-glycoprotein complex (DGC) mediated linkage between the cytoskeleton and the extracellular matrix is disrupted (4–6). One characteristic feature of muscular dystrophies arising from primary defects in the DGC is that damaged fibers accumulate in clusters (7). The formation of these clusters, which consist mainly of necrotic fibers, is not clearly understood. We and others have previously demonstrated that these clustered fibers take up membrane impermeant tracer molecules like procion orange and Evans blue dye (EBD), indicating damage to the sarcolemma (8–11). Currently, detailed analysis of membrane damage in dystrophic muscle requires microscopic examination of tissues obtained from biopsies. Our objective is to study impaired membrane permeability in dystrophic skeletal muscle in vivo using noninvasive techniques. To accomplish this we have employed an albumin-targeted contrast agent, MS-325 (Epix Medical, Cambridge, MA) (12–15) to examine membrane integrity in living mice via MRI.

Both the dystrophin-deficient mdx (16,17) and the recently characterized α-sarcoglycan deficient mouse (Sgca-null) (11), an animal model for limb-girdle muscular dystrophy 2D (18–20), show disruption of the DGC, progressive muscular dystrophy, and profound damage of the sarcolemma (10,11). We imaged these two animal models before and after administration of MS-325 to evaluate the applicability of MRI for the in vivo assessment of muscle damage in muscular dystrophy.

MATERIALS AND METHODS

Animals

Normal control (C57/BL 10f), the mdx mutant, and Sgca-deficient mice were bred at the University of Iowa (11). For MRI we studied six mice of each group, all matched by age and gender. The age range of the mice was 8–10 weeks. Animals were kept in the animal care unit of the University of Iowa College of Medicine according to animal care guidelines. The animal care use and review committee of the University of Iowa and of the Medical College of Wisconsin authorized all animal studies.

For i.v. catheterization we anesthetized animals with ketamine (90 mg/kg, i.p.) with ace promazine (1.5 mg/kg, i.p.) and surgically instrumented with jugular vein catheters for administration of MS-325. Sterile, heparinized saline-filled (25 U/ml) catheters were inserted into the right jugular vein (polyethylene tubing, PE-10), tunneled subcutaneously, exteriorized, and sutured in place between the scapulae.

EBD was prepared as previously described (10) and injected through the central vein catheter at a concentration of 0.5 mg EBD/0.05 ml/10 g body weight. Three hours after injection the mice were euthanized by cervical dislocation. Skeletal muscle samples were taken from muscles that showed contrast enhancement on the MR images. Specific muscles were sectioned according to their relative position to specific anatomical structures, e.g., the femur or the base of the tail. Serial sections were cut until the region of interest was in the plane of section. Muscle sections were prepared and analyzed by hematoxylin and eosin (H&E) staining and for EBD uptake as described previously (10). Sections were observed under a Zeiss Axioplan fluorescence microscope (Carl Zeiss, Thornwood, NY).
MRI

All experiments were performed on a 3 T Bruker (Billerica, MA) Medspec MR Imaging System located at the Medical College of Wisconsin. A 10 cm diameter, three-axis local gradient coil, together with a 2 x 4 cm quadrature transmit/receive radiofrequency surface coil were used. After obtaining sagittal scout images, five to seven 2 mm axial slices were chosen to image the proximal limb muscles (e.g., gluteal and femoral quadriceps muscles). Next, $T_1$-weighted spin-echo images (TR/TE = 5400 ms / 19 ms, FOV = 3.2 cm, matrix = 256 x 256 giving an in-plane image resolution of 125 microns) were acquired before and after 5, 15, 30, 45, and 60 min after administration of 0.1 mM Gd/kg MS-325.

The mean signal intensity was determined from a region of interest (ROI) of 12 x 12 voxels (1.5 x 1.5 mm). Both the pre- and postcontrast mean signals ($S_{pre}$, $S_{post}$) were extracted from the same ROI. The percent enhancement was then determined by using Eq. [1]:

$$\frac{(S_{post} - S_{pre}) \times 100\%}{S_{pre}}.$$  \[1\]

An unpaired $t$-test was used to determine whether the signal enhancement was significantly different between the control and mdx groups at the 0.05 significance level.

RESULTS

MS-325 in Skeletal Muscle of Control Mice

Muscles of pelvic girdle are among the most affected tissues in Duchenne and sarcoglycan-deficient muscular dystrophy. We therefore studied muscles of these anatomic regions in control and diseased mice by MRI. Because of its efficient protein-binding capabilities, MS-325 can remain at high concentration throughout the vascular system for up to 50 min before being excreted through the kidneys (14). In the absence of MS-325, control mice displayed a homogenous structure within their skeletal muscles (a). Fifteen minutes after MS-325 injection (d-f) no change in contrast agent levels in skeletal muscle of control mice (d) was found. In dystrophin-deficient mdx mice (b,e), intravenously administered MS-325 resulted in dramatic uptake of the contrast agent in the muscle tissue. As in mdx mice, signal enhancement was consistently observed in muscles of the $\alpha$-sarcoglycan null mutant mice 15 min (c,f) after MS-325 injection.
Following MRI, we intravenously injected the mice with EBD (10). We analyzed skeletal muscle sections of the imaged muscles for EBD uptake and by H&E staining to correlate and confirm fiber lesions seen by MS-325 enhancement in the MRI. Microscopic inspection of muscle sections from control animals did not reveal fiber damage by means of intracellular tracer accumulation or muscle histology (Fig. 3a,b,g,h).

**Mdx and Sgca-Null Mutant Mice Show Influx of MS-325 Into Skeletal Muscles**

Compared to control animals mdx and α-sarcoglycan deficient mice occasionally showed structural changes that were detected by MRI even prior to contrast agent administration (Fig. 1b,c). These foci of higher intensity in images of mdx and α-sarcoglycan deficient mice corresponded to dystrophic lesions observed in the histologic sections of the same muscles (21). In the dystrophic mice, i.v. administered MS-325 resulted in dramatic localized increases in the signal intensity of the muscle tissue (Fig. 1e,f). The signal intensity remained elevated for at least 60 min after injection (Fig. 2).

Although there was always a clear uptake of MS-325 in skeletal muscle, the distribution of lesions varied between individual mdx and Sgca-null mice and was not necessarily symmetrical. The contrast agent was incorporated particularly in the gluteal and thigh muscles (Fig. 1e,f). Together, these results indicate that loss of sarcolemmal integrity in mdx and Sgca-null mice allows influx of MS-325 into skeletal muscle fibers.

To provide verification that the intramuscular MS-325 corresponded to damaged muscle fibers, we examined tissues sections of MS-325-injected animals histologically, and also for uptake of EBD. Areas of intense MS-325 signaling coincided with clusters of damaged skeletal muscle fibers as visualized by both uptake of EBD (Fig. 3c,f) and H&E staining (Fig. 3i,l).

**DISCUSSION**

In our study we have demonstrated that the commonly used noninvasive imaging technique MRI, in combination with the new contrast agent MS-325, can be used to examine hallmark pathological features of muscular dystrophy in living animals. We evaluated the intramuscular accumulation of MS-325, a gadolinium-based MRI blood pool

**FIG. 2.** The diagram shows the increase in signal enhancement in skeletal muscle lesions as a mean enhancement percent calculated from the mean signals in a region of interest before and after i.v. injection of MS-325. The mean enhancement in mdx (gray columns) and Sgca-null (white columns) mice was significantly higher during all four analyzed time points compared to control (black columns) mice (*P ≤ 0.001; †, P ≤ 0.017). The signal enhancement in control mice was due to MS-325 in the vessels and reflected the long blood half-life of the contrast agent.
agent, in dystrophin-deficient mdx and α-sarcoglycan-de-
deficient mice compared to control mice. We and others have
shown that muscle damage in muscular dystrophy is often
associated with an influx of extracellular fluid containing
albumin into the damaged muscle fibers (10,22–24). MS-
325 efficiently binds to albumin and remains at high con-
centrations throughout the vascular system for up to 50 min
(12,14,15). This binding causes increased concentration
and retention of this MRI contrast agent in the specific
tissues and fluids that contain targeted albumin molecules.
Muscular dystrophies therefore seemed to be good candid-
ate diseases for the application of this new albumin-
targeted MRI contrast agent. Moreover, contrast-enhanced
MRI has been previously used to indicate the extent and
severity of myocardial injury (25).

The blood half-life of MS-325 is longer in humans com-
pared to rodents (14,26). Longer circulation times may
result in more of the agent entering the lesions and there-
fore even greater enhancement in patients with muscular
dystrophy. On the other hand, we know that there is al-
ways a small amount of free tracer present in the circula-
tion due to the reversibility of the binding of both EBD and
MS-325 to albumin (14,15,27,28). Therefore, the early en-
hancement (5 min postinjection) that we observed may be
at least partially explained by unbound agent diffusing
into the permeable muscle cells. This may result in less
signal enhancement in dystrophic lesion of human skeletal
muscle, where at the anticipated concentration of 0.6 mM,
MS-325 is 90% bound compared to less than 80% in
rodents (27,28). Our observations do not exclude the pos-
sibility that unbound EBD, unbound MS-325, and albumin
diffuse separately into damaged fibers and bind intracel-
lularly to each other. However, in previous investigations
we never saw EBD-positive/albumin-negative fibers (10),
which one would expect to see at least occasionally if free
dye crosses the membrane without albumin.

Previous MRI studies of skeletal muscle entailed mea-
suring tissue $T_2$ relaxation times. In these studies changes
in $T_2$ were associated with muscular dystrophy (29), exer-
cise, and sports-related injuries (30). Although such mea-
surements provide good spatial information with regard to
injury or disease location, the changes may reflect a variety
of normal and pathologic changes such as muscle edema,
fatty infiltration, and/or a modified chemical environment
as observed in recovering muscles (30). Therefore, the
measurement of $T_2$ is unlikely the optimal approach for
the specific evaluation of cell permeability changes. Also,
the percent change in $T_2$ can be relatively small (29),
necessitating precise optimization of the measurement
procedure. And, due to the multi-exponential nature of $T_2$
decline in skeletal muscle the measurement can be very
sensitive to the choice of measurement parameters (i.e.,
number and range of echo times).

Alternatively, dynamic contrast agent-enhanced MRI
studies have been performed to evaluate vascular perme-
ability changes associated with myocardial injury (31). In
these studies the general approach has been to use the
slope of the enhancement curve as an indicator of perme-
ability changes. Although this approach has enabled dif-
ferentiation of diseased from normal myocardium, it pro-
vides only an indirect measure of vascular permeability
changes since the rate of signal enhancement is also de-
pendent on perfusion and tissue compartment sizes (32).
Furthermore, this approach does not enable evaluation of the cellular
membrane changes.

In this study, the use of a long-lived albumin contrast
agent under steady-state conditions enabled highly sensi-
tive measurements that should more directly reflect cell
membrane permeability changes. The difference images,
calculated from the pre- and postcontrast images, are in-
dependent of any $T_2$ changes. And since the measure-
ments are made under steady-state conditions, the mea-
surement is not confounded by regional or global differ-
ences in muscle perfusion.

The noninvasive, in vivo method of investigation has
several important implications. While conventional skel-
etal muscle MRI allows the identification of dystrophic
muscle (21), the combination of this technique with the
intravenous application of MS-325 makes it possible to
study mechanisms of fiber damage. So far, the successes of
gene transfer into skeletal muscle fibers, whether via i.m.
injection or by systemic application of the gene, has been
evaluated through examination of biopsy samples. Such
an approach allows one to look only at a small portion of
the tissue of interest and, especially in patients, may not
always be representative of the condition of the entire
tissue. Furthermore, the invasiveness of a muscle biopsy
does not allow one to take consecutive samples, which are
necessary to evaluate the course of a disease or success of a
therapeutic approach. Since biopsies are restricted to
certain easily accessible muscle groups, they are most
often taken from the femoral quadriceps, gastrocnemius,
biceps, deltoid, or pectoral muscles. Muscles of the deeper
layers such as the quadratus lumborum and the ilioctoas
cannot be looked at by conventional biopsy techniques.

Our results demonstrate that contrast agent-enhanced
MRI can be used as an important diagnostic tool in the
assessment of skeletal muscle fiber lesions. Consequently,
this imaging procedure should prove very useful in eval-
uating the disease course and therapeutic efficacy in mus-
cular dystrophy, as well as other instances such as muscle
trauma and exercise-induced damage.

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