J Neurol (1996) 243 : 410–416

ORIGINAL COMMUNICATION

S. C. J. M. Jacobs
A. L. Bootsma
P. W. A. Willems
P. R. Bär
J. H. J. Wokke

Prednisone can protect against exercise-induced muscle damage

Received: 6 April 1995
Received in revised form:
18 September 1995
Accepted: 6 November 1995

S. C. J. M. Jacobs (✉) · A.L. Bootsma
Department of Cell Biology,
Medical School, Utrecht University,
Heidelberglaan 100, 3584 CX,
AZU H02.314, Utrecht, The Netherlands
Tel.: 31–30–2534337,
Fax: 31–30–2541797

P. W. A. Willems · P. R. Bär
J. H. J. Wokke
Department of Neurology,
Utrecht University, Utrecht,
The Netherlands

Abstract In an experimental animal exercise model we tested whether daily administration of prednisone prevents the development of mechanically induced muscle fibre damage. Six-week-old rats were treated with different doses of prednisone ranging from 1 to 50 mg/kg body weight per day or with placebo, for 8 days. On day 6 of treatment the rats were forced to run for 2 h on a level treadmill. Two days after exercise morphological damage in the soleus muscles was quantified using light microscopy and a semi-automatic image analysis system. Creatine kinase (CK) activity was measured before exercise (day 5) and directly after exercise (day 6). The expression of dystrophin in a placebo group and in a group that received 5 mg prednisone/kg body weight per day with and without performing exercise was studied with Western blotting. The effect of prednisone on fibre type distribution was determined with an antibody against fast myosin and the effect of prednisone on the proliferative activity of muscle satellite cells was studied using bromodeoxyuridine (BrdU) immunohistochemistry. Exercise-induced muscle fibre damage varied in a dose-dependent way. In the placebo group the mean (SEM) damaged muscle fibre area was 4% (1%). The groups that received low doses of prednisone, 1 or 2.5 mg/kg per day, showed a similar level of muscle damage. However, with 5 mg prednisone/kg per day the amount of muscle fibre damage [mean (SEM)] was significantly reduced to 1.4% (0.5%) ($P \leq 0.05$, Student's t-test). High doses of prednisone had no protective effect. Directly after exercise the CK activity was increased two-fold, except in the group that received 50 mg prednisone/kg body weight per day. No changes in the amount of dystrophin were found after densitometric analysis of the Western blots. Prednisone did not affect the fibre distribution or the labelling index of satellite cells. We conclude that prednisone, given in an appropriate dose, protects muscle fibres against the development of mechanically induced damage, possibly by stabilizing the muscle fibre membranes. This action may explain the beneficial effect of prednisone observed in Duchenne muscular dystrophy patients.

Key words Prednisone · Exercise · Muscle damage · Dystrophin · Creatine kinase activity

Introduction

Prednisone improves strength in patients with Duchenne muscular dystrophy (DMD) [5, 16, 30], by an increase in muscle mass [15, 30] and a fall in the rate of muscle degradation [32]. In clinical trials improvement was demonstrated from as soon as 1 week after the start of treatment [16, 30]. Recently a double-blind controlled trial with prednisone and azathioprine demonstrated that azathioprine did not have a beneficial effect on muscle strength in DMD patients [16]. Azathioprine-treated patients showed histological changes in muscle that were similar to those in prednisone-treated patients [23], which may imply that the clinical improvement after prednisone treatment does not result from an immunosuppressive action [16, 23].

Muscle damage in DMD probably results from mechanical instability of the membrane as a result of a deficiency of the membrane-associated cytoskeletal protein dystrophin [7, 27, 29, 36, 42]. Thus, a positive effect of prednisone may result from an increase in the stability of the muscle fibre membrane in DMD. Level exercise in rats leads to disturbances of the normal muscle fibre structure [2, 4, 19, 25], probably resulting from mechanically induced, small membrane disruptions of the sarcolemma which rapidly heal [19, 29]. Healthy muscle may react to level exercise in a similar way as do dystrophin-deficient muscle fibres from DMD patients to the strain of normal daily activity. Therefore, we tested whether prednisone protects normal muscle fibres against mechanically induced muscle damage. We treated rats with different doses of prednisone, let them perform exercise and measured the post-exercise plasma creatine kinase (CK) activity, the amount of muscle fibre damage at 2 days after exercise and the number of type II muscle fibres in the soleus muscle from placebo-treated rats and rats that received 5 mg prednisone/kg per day. In addition, the expression of dystrophin was determined in muscle samples from a placebo group and a group that received 5 mg prednisone/kg per day, both after exercise and without performing exercise. Finally, a possible effect of prednisone on the activity of muscle precursor cells was studied using bromodeoxyuridine (BrdU) immunohistochemistry.

Materials and methods

Experimental protocol

Male Wistar rats (U:WU, GDL, The Netherlands) were housed in macrolon boxes (2 or 3 together). They had water ad libitum and were fed commercially obtained rat chow (Hope Farms, Woerden, The Netherlands). A total of 49 6-week-old animals were divided into eight groups of at least five animals. These groups had approximately the same mean body weight (190 g) at the start of the study. The body weight was measured daily and the mean weight gain and SEM of the groups of animals was calculated. Group I (n = 9) received placebo orally (olive oil). Groups II (n = 6), III (n = 7) and IV (n = 7) received 1, 2.5 or 5 mg prednisone/kg per day respectively (Upjohn, Milan, Italy). Groups V and VI (n = 5) received 25 and 50 mg/kg per day respectively. Group VII (n = 5) received placebo (olive oil) and group VIII (n = 5) received 5 mg prednisone/kg per day. Groups VII and VIII did not perform exercise. To obtain different dosages, 0.2, 0.5, 1, 5 or 10 mg prednisone was dissolved per millilitre of olive oil, so the rats received the same amount of oil per kilogram of body weight each day. The rats were treated for 8 days. On day 6 all rats, except those in groups VII and VIII, were forced to run for 2 h on a level treadmill, as described previously [2, 19]. One day before, and directly after exercise, animals were anaesthetized with diethylether and blood samples were taken from the tail artery and collected in heparinized cups. Previous experiments have shown that the serum CK activity is only increased immediately after exercise and decreases to normal levels within a short period [2, 4, 19]. Also from groups VII and VIII blood samples were taken on days 5 and 6. After centrifugation (900 g) the plasma was separated, stored on ice and the CK activity was measured within 2 h. All rats were killed by cervical dislocation at day 8. Previous experiments have shown that exercise-induced muscle fibre damage is maximal at 2 days after exercise and most pronounced in the proximal part of the soleus muscle [19]. Morphological muscle fibre damage was therefore quantified at this time in longitudinal sections of the proximal part of the left soleus muscle. The number of type II fibres was determined in cross-sections of the soleus muscles from placebo-treated rats and rats that received 5 mg prednisone/kg per day. The soleus muscles of the right hindlimbs of the rats from groups I, IV, VII and VIII were deeply frozen in liquid nitrogen for dystrophin analysis. Finally, the rats from groups VII and VIII received a single intraperitoneal injection of BrdU (100 mg/kg body weight) 2 h before killing, to label cells synthesizing DNA, and the effect of prednisone on the proliferative activity of muscle satellite cells was analysed.

Histology

Immediately after the animals were killed, the soleus muscles of the left hindlimbs were dissected and carefully fastened to small pieces of wood by the tendinous ends of the muscle in order to avoid crinkling. Next they were fixed in periodate-lysine-paraformaldehyde [28]. The muscles were cut into a proximal and a distal half. The proximal parts were embedded in glycol methacrylate (GMA; Technovit 7100, Kulzer, Wehrheim). Longitudinal sections 3 μm thick were prepared and stained with a mixture of toluidine blue with 1% borax and studied with an image analysis set-up, using the image processing software package TIM (DIFA, Breda, The Netherlands). From each muscle part three sections were selected, separated by a mutual distance of at least 60 μm. In every section five optical windows were investigated (magnification 160 ×, total fibre surface 220×10^3 μm^2). In these windows the area of abnormalities in muscle fibre structure and the total muscle fibre area were measured as described in a previous paper [19]. Abnormalities included focal disarray of A and I bands, fading or even complete loss of cross-striation, streaming or disruptions of Z discs and supercontracted sarcomeres. The damaged muscle fibre area was expressed as a percentage of the total muscle fibre area measured.

Immunostaining procedure

From the belly part of the soleus muscle, embedded in GMA, cross-sections 3 μm thick were prepared. A mouse monoclonal antibody against rabbit skeletal fast myosin (Sigma, St. Louis, Mo., USA) was used to discriminate between type I and type II muscle fibres in these sections. The cross-sections were incubated with 1% periodic acid for 30 min at 60°C and thereafter rinsed 3 times

with phosphate-buffered saline (PBS). The sections were incubated overnight with the mouse monoclonal antibody against fast myosin (dilution 1:1000 in PBS). After rinsing with PBS, sections were incubated for 1 h with a biotinylated horse anti-mouse IgG antibody (Vector, Burlingame, Calif., USA; 1:110 diluted in PBS with 2% rat serum), followed by a rinse with PBS and incubation with an avidin-biotinylated horseradish peroxidase solution (ABC kit, Vector) for 1 h. The immune complex was visualized by incubation for 15 min in DAB (3,3′-diaminobenzidine tetrahydrochloride) as chromogen with nickel and cobalt as intensifying agents [1]. The number of type I and II fibres was counted in cross-sections from rats ($n = 3$) that received placebo or 5 mg prednisone/kg per day with and without performing exercise.

BrdU immunohistochemistry

To visualize nuclei that had incorporated BrdU during the S phase, an antibody against BrdU (Monoclonal; Becton and Dickinson, Mountain View, Calif., USA) in combination with immuno-gold-silver staining was used [19]. For an optimal discrimination between satellite cells and endomysial cells in close proximity with muscle fibres, BrdU immuno-histochemistry was combined with a PAS reaction that stains the basement membranes of the muscle fibres [39]. Cross-sections from the belly of the soleus muscles, embedded in GMA, were used for counts of labelled and unlabelled satellite cells. A microscope was used in which the slide could be studied with Nomarski interference, epipolarization and conventional light microscopy [19]. Counts were performed at a magnification of 1000 ×. In this way data were obtained on the number of labelled satellite cells/total number of satellite cells, expressed as the labelling index (LI%).

CK assay

The plasma CK activity was measured according to the regulations of the International Society for Clinical Chemistry and expressed as units/litre [mean (SEM)] per group of animals.

Western blotting

Frozen samples of the soleus muscles from the right hindlimbs of a placebo group that performed exercise (group I), a placebo group that did not exercise (group VII), a group that received 5 mg prednisone/kg per day and performed exercise (group IV) and a non-exercising group that received 5 mg prednisone/kg per day (group VIII) were dissolved and homogenized in SDS-PAGE treatment buffer [33]. Homogenates were boiled and spun down. The supernatant was used for SDS-PAGE. Samples were loaded onto the stacking gel (3%) on a resolving gel with a linear gradient of 4–7%. Electrophoresis was carried out during 4 h at a constant current. The proteins were blotted onto 0.45-µm nitrocellulose membranes (Bio-Rad) using a Bio-Rad transblot apparatus (Bio-Rad Laboratories, Richmond, Calif., USA) and transfer buffer (24 mM TRIS, 192 mM glycine). Fifteen hours at 50 V provided optimal transfer. Nitrocellulose filters were rinsed in distilled water and blocked with 0.1% PBS Tween-20 and 3% bovine serum albumin. Blots were incubated with a dystrophin monoclonal antibody [MANDYS8 (Sigma) 1:1000 diluted in 0.1% PBS Tween-20 (Sigma)] during 1 h. Thereafter the blot was incubated for 1 h with a biotinylated horse anti-mouse IgG (1:220 diluted in 0.1% PBS Tween-20), followed by incubation with an avidin-biotinylated horseradish peroxidase solution for 1 h (ABC peroxidase kit, Vector) and finally stained with DAB-Co-Ni solution [1]. On the Western blot and on the corresponding Coomassie brilliant blue stained gel the dystrophin and the myosin bands were scanned [33]. The intensity of the bands was analysed with an Image Quant program (densitometric analysis, Molecular Dynamics). Results are expressed as a ratio (scan intensity of dystrophin bands obtained by Western blot/scan intensity of total myosin protein stained with Coomassie brilliant blue in the corresponding lane). The values are expressed as mean units (SD).

Statistics

All data on muscle damage are presented as the mean (SEM) percentage muscle fibre damage of all rats in one group. The Student's t-test was used to analyse differences in muscle damage between groups and LI, expressed as percentage (SEM). ANOVA and Bonferroni tests were used for statistical analysis of the body weight [mean (SEM)] and percentage (SEM) of type II fibres. For analysis of differences in plasma CK activity paired Student's t-tests were applied, using values before and after exercise. Western blot results are expressed as arbitrary units (SD) and the Student's t-test was used to analyse differences between the two groups. Test results are considered significant if $P \leq 0.05$.

Results

Weight

Treatment with prednisone resulted in a decline in weight gain (Fig. 1). In the groups receiving 25 or 50 mg prednisone/kg per day the differences from the placebo group were statistically significant from day 2 after the start of the experiment; in the group receiving 2.5 mg prednisone/kg per day a significant decline in weight gain was

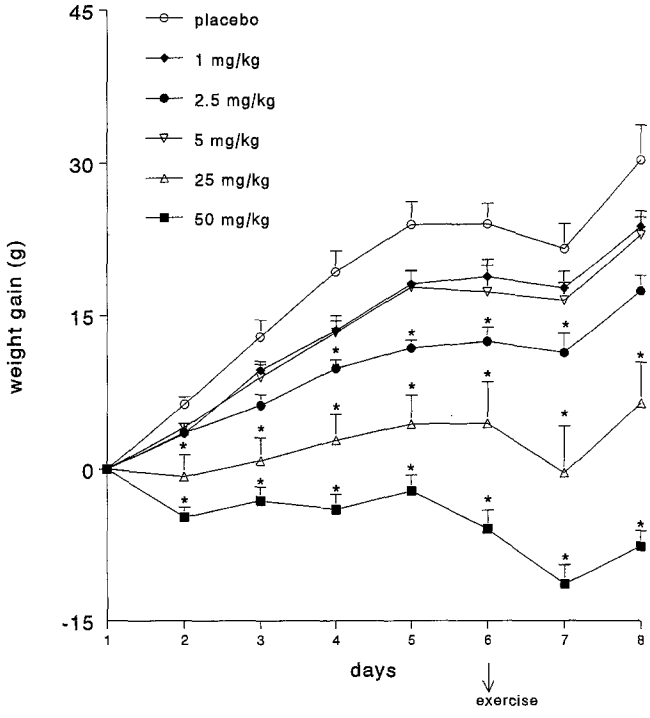

Fig. 1 Mean (SEM) weight gain (g) per group of animals over time. * Significantly different from the placebo group ($P \leq 0.05$)

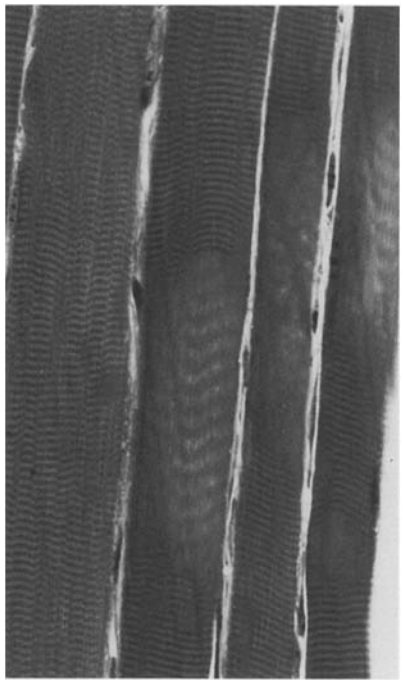

Fig. 2 LM picture showing a longitudinal section with muscle fibre structure at 48 h after exercise. × 400

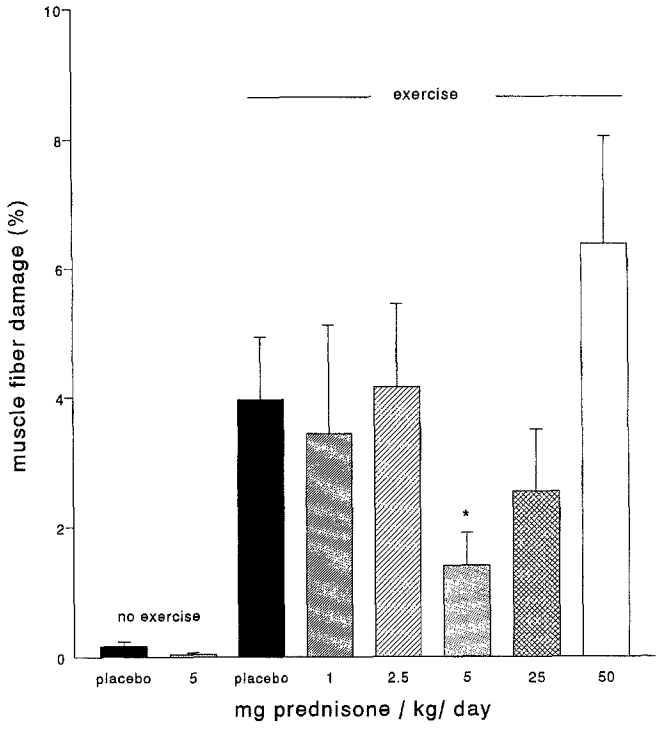

Fig. 3 Damaged muscle fibre area (%) per group in longitudinal sections of the soleus muscle at 48 h after exercise. The values are expressed as mean (SEM) per group. * Significantly different from the placebo group ($P \leq 0.05$)

measured from day 4 on. The other dosages did not induce a significant decrease in weight gain. The rats finished the exercise session on day 6 without problems. All rats that performed exercise showed an extra decrease in weight gain on day 7 as a result of this exercise.

Muscle damage

After exercise all rats showed morphological alterations of normal muscle fibre structure to some degree (Fig. 2). In all groups there was a large variation in the amount of damage between animals. Nevertheless, significantly less muscle damage was measured in the group that received 5 mg prednisone/kg per day (Fig. 3). At 2 days after exercise this group showed 1.4% (0.5%) damaged fibre area compared with 4.0% (1.0%) in the placebo group ($P \leq 0.05$). The groups that received other dosages of prednisone, up to 25 mg/kg per day, showed no significant differences in muscle fibre damage compared with the placebo group. Only in the group that received the highest dose of prednisone, 50 mg prednisone/kg per day, was the muscle damage significantly increased compared with the group that received 5 mg prednisone/kg per day.

The groups that received placebo or 5 mg prednisone/kg per day (groups VII and VIII) and did not perform exercise showed almost no muscle damage (Fig. 3).

Table 1 Mean (SEM) creatine kinase (CK) values (units/l) per group before and after exercise

Prednisone dose (mg/kg/day)	Before exercise	After exercise
Placebo	78 (4)	192 (31)*
1	111 (6)	220 (30)*
2.5	87 (12)	154 (17)*
5	97 (5)	208 (55)*
25	111 (34)	240 (55)
50	101 (29)	127 (17)
No exercise	CK activity at day 5	CK activity at day 6
Placebo	89 (11)	75 (3)
5	90 (8)	81 (3)

* Significantly different from the CK value before exercise

Type II muscle fibres

Soleus muscle from rats that were treated with placebo and did not perform exercise contained 27.9% (1.4%) type II fibres. After exercise 28.2% (1.4%) type II muscle fibres were present. In the prednisone-treated groups similar values were found: 30.7% (1.4%) without exercise and 26.3% (0.8%) after exercise.

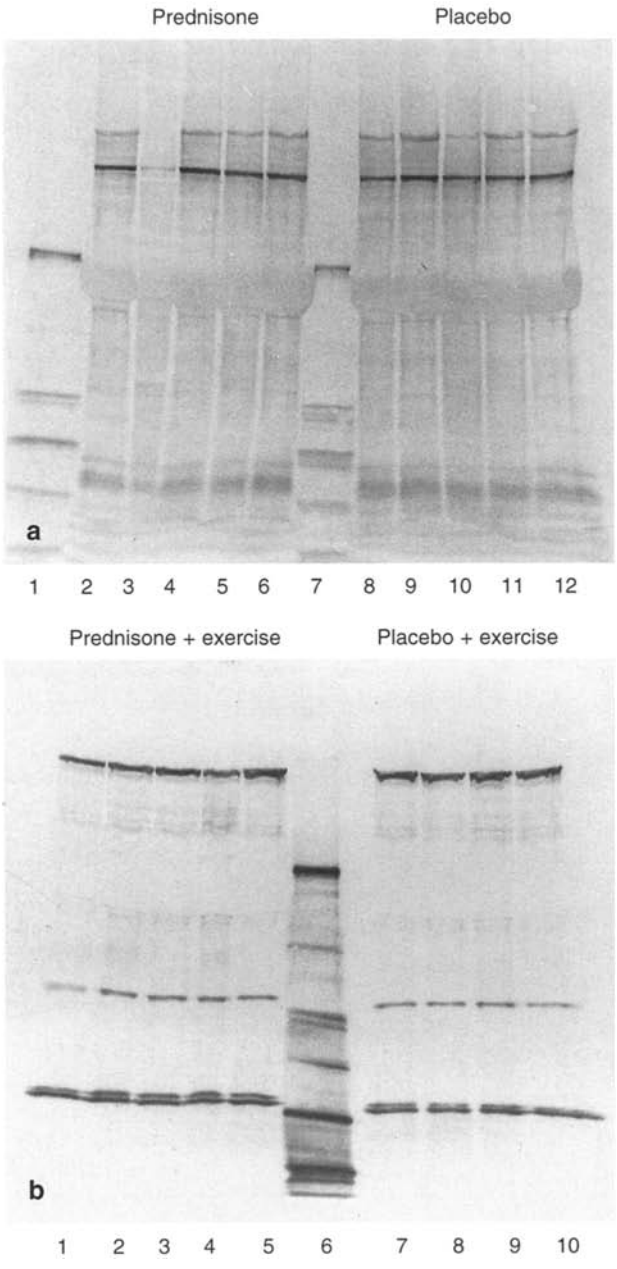

Fig. 4a, b Western blot demonstrating a 427-kDa dystrophin band. **a** *Lanes 1 and 7* High molecular weight marker. *Lanes 2–6* Samples of soleus muscle from rats treated with 5 mg prednisone/kg body weight per day that did not exercise. *Lanes 8–12* Samples of the soleus muscle of placebo-treated rats which did not perform exercise. In *lane 3* less SDS-PAGE treatment buffer was loaded onto the stacking gel, resulting in less myosin in the Coomassie blue stained gel and dystrophin on the Western blot. **b** *Lanes 1–5* Samples of soleus muscle after exercise from rats treated with 5 mg prednisone/kg per day. *Lane 6* High molecular weight marker. *Lanes 7–10* Samples of soleus muscles from placebo-treated rats after exercise

Labelling index

In cross-sections of muscles from rats that were treated with placebo the LI was 16% (5%). Treatment with prednisone resulted in an almost similar value 13% (5%).

CK enzyme measurements

The plasma CK activities are shown in Table 1. Mean CK activity before exercise was ± 100 units/l and was not affected by prednisone. In all groups the CK activity was increased immediately after exercise. However, this increase was only significant in controls and in the groups that received up to 5 mg/kg per day prednisone. Plasma CK activities from groups VII and VIII were not increased.

Analysis of Western blots

The samples of muscle from both the placebo groups and the groups treated with 5 mg prednisone/kg body weight per day gave reproducible bands across the blot. With a monoclonal antibody against dystrophin, a 427-kDa protein band was visualized (Fig. 4). After densitometric analysis of the Western blot and the corresponding Coomassie brilliant blue stained gel, no change in the amount of dystrophin could be measured. Treatment with 5 mg prednisone for 8 days resulted in 0.48 (0.10) arbitrary units compared with 0.42 (0.05) units in the placebo group (Fig. 4a). After exercise 0.54 (0.22) units were present in the placebo group compared with 0.43 (0.10) units in the group that received 5 mg prednisone/kg per day (Fig. 4b).

Discussion

The main finding of this study is a dose-related protective effect of prednisone against exercise-induced morphological muscle fibre damage, determined at 48 h after exercise. From a previous study [19] it appeared that level exercise induces far more muscle fibre damage in the soleus muscle, consisting predominantly of type I fibres, than in the extensor digitorum longus muscle, which consists mainly of type II fibres. The morphological damage is maximal at 48 h after exercise [19]. From the literature it is known that prolonged steroid treatment affects type II fibres [21]. Our study shows that prednisone, given in an appropriate dose, can protect a muscle with mainly type I fibres against damage induced by mechanical forces without changing the fibre type distribution. Prednisone did not, however, prevent leakage of CK from the muscle into the blood immediately after exercise. The CK activity of the prednisone-treated rats rose to the same levels as ob-

served in the control animals, except in the group that received 50 mg prednisone/kg per day, although these rats performed the exercise session without problems. In previous experiments we demonstrated that the CK activity in rats is only significantly increased immediately after exercise [2, 4, 19]. This means that CK activity is back to normal values when morphological damage develops. This underlines the notion that enzyme markers for muscle damage, such as CK activity [2, 4, 19, 24, 29] or myoglobin [10], follow a different time course than morphological muscle damage, leading to apparent discrepancies in time appearance.

The protective dosage of 5 mg prednisone/kg per day for rats, calculated in relation to body surface area according to Paget and Barnes [35], is comparable with a dosage of 0.75 mg prednisone/kg per day for humans. This dosage was also used in the clinical studies of Griggs et al. [16] and Kissel et al. [23], who demonstrated a beneficial effect of prednisone on muscle strength in DMD patients and concluded that the immunosuppressive action of the drug was not likely to be the primary mechanism of the clinical improvement. In our experiments, a decline in weight gain occurred during treatment with increased doses of prednisone. This may be due to inhibition of growth resulting from a decreased appetite [21] or from an inhibition of insulin-like growth factor (IGF-I) and growth hormone gene expression [26], or both. The data on weight gain and fibre damage after the very high dose of 50 mg prednisone/kg per day further suggest that very high doses of prednisone may render the fibres more vulnerable towards mechanical forces instead of protecting them (Fig. 3). This confirms the observations of Kelly et al. [21] that very high doses of steroids have catabolic effects on muscle and induce changes in the fine structure of striated muscle fibres.

The mechanism by which appropriate doses of steroids improve muscle strength and functional abilities in DMD is puzzling [22]. In view of the absence of any effect of prednisone on the proliferative activity of satellite cells, a role of these cells in the improvement of muscle function seems unlikely. Some authors suggest that there is a prednisone-induced stabilization the muscle fibre membrane via an increased dystrophin expression [17, 36, 37]. In patients with DMD, the absence of dystrophin and the consequent loss of the dystrophin-associated proteins cause disruptions of the linkage between the subsarcolemmal cytoskeleton, the sarcolemma and the extracellular matrix [12, 18, 34]. This may render the sarcolemma vulnerable to physical and mechanical stress [20, 27, 34, 36, 42]. Consequently, the frequently occurring necrosis of dystrophin-deficient fibres could be attributed to mechanical weakening of the plasma membrane [31, 42], and to an alteration of specific Ca^{2+}-regulatory mechanisms resulting in an excessive influx of Ca^{2+} ions [11, 38]. The musculoprotective effect of dantrolene sodium, which blocks Ca^{2+} flux over the sarcoplasmic reticulum, suggests such a role for Ca^{2+} after exercise [3]. Sklar and Brown [37] and Hardiman et al. [17] observed that methylprednisolone augments the expression of dystrophin in muscle cultures from healthy humans. However, in the soleus muscles of our animals we could not detect a prednisone-induced higher amount of dystrophin (Fig. 4). We can not exclude the possibility that prednisone administration has an effect on the expression of dystrophin, as it may be that the method used in our study is not sensitive enough to detect increases in the normal levels of dystrophin in muscle dissected ex vivo. An alternative explanation for a prednisone-induced reinforcement of muscle fibre membranes may be a decrease of phospholipase A_2 activity. Several authors have found a glucocorticoid-induced lipocortin synthesis [13, 14], resulting in a decreased phospholipase A_2 activity [6, 8, 9, 13], which can lead to a decrease in the hydrolysis of membrane phospholipids [13, 41], decreased membrane fluidity [40], and a decreased Ca^{2+}influx and muscle damage [13].

Whatever the mechanism, the finding that prednisone, given in an appropiate dose, protects normal muscle fibres against mechanically induced damage, suggests that the basis of the clinical improvement of DMD patients lies in a prednisone-induced reinforcement of muscle fibre membranes.

References

1. Adams JC (1981) Heavy metal intensification of DAB-based HRP reaction products. J Histochem Cytochem 29: 775–780
2. Amelink HGJ, Bär PR (1986) Exercise-induced muscle damage in the rat. Effects of hormonal manipulation. J Neurol Sci 76: 61–68
3. Amelink HGJ, Van der Kallen CJH, Wokke JHJ, Bär PR (1990) Dantrolene sodium diminishes exercise-induced muscle damage in the rat. Eur J Pharmacol 179: 187–192
4. Bär PR, Rodenburg AJB, Koot RW, Amelink HGJ (1994) Exercise-induced muscle damage: recent developments. Basic Appl Myol 4: 5–16
5. Brooke MH, Fenichel GM, Griggs RC, Mendell JR, Moxley RT, Miller JP, Kaiser KK, Florence JM, Pandya S, Signore L, King W, Robinson J, Head RA, Province MA, Seyfried W, Mandel P (1987) Clinical investigation of Duchenne muscular dystrophy: interesting results in a trial of prednisone. Arch Neurol 44: 812–817
6. Chang J, Musser JH, McGregor H (1987) Phospholipase A_2: function and pharmacological regulation. Biochem Pharmacol 36: 2429–2436
7. Cullen MJ, Johnson MA, Mastaglia FL (1992) Pathological reactions of skeletal muscle. In: Mastaglia FL, Lord Walton (eds) Skeletal muscle pathology. Churchill Livingstone, London, pp 123–184

8. Davidson FF, Dennis EA, Powel M, Glenney JR Jr (1987) Inhibition of phospholipase A_2 by lipocortins: an effect of binding to phospholipids. J Biol Chem 262: 1698–1705
9. Dennis EA, Davidson FF (1990) Phospholipase A_2 and Lipocortin effects. In: Meli M, Parente L (eds) Cytokines and lipocortins in inflammation and differentiation. Progress in clinical and biological research, vol 349. Wiley-Liss, New York, pp 47–54
10. Driessen-Kletter MF, Amelink GJ, Bär PR, Van Gijn J (1990) Myoglobin is a sensitive marker of increased muscle membrane vulnerability. J Neurol 237: 234–238
11. Duncan CJ (1989) Dystrophin and the integrity of the sarcolemma in Duchenne muscular dystrophy. Experientia 45: 175–177
12. Ervasti JM, Ohlendieck K, Kahl SD, Gaver MG, Campbell KP (1990) Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle. Nature 345: 315–319
13. Flower RJ (1990) Lipocortin. In: Meli M, Parente L (eds) Cytokines and lipocortins in inflammation and differentiation. Progress in clinical and biological research, vol 349. Wiley-Liss, New York, p 25
14. Flower RJ, Rothwell NJ (1994) Lipocortin-1: cellular mechanism and clinical relevance. Trends Pharmacol Sci 15: 71–76
15. Griggs RC, Moxley RT, Mendell JR, Fenichel GM, Brooke MH, Pestronk A, Miller JP and the Clinical Investigation of Duchenne Dystrophy Group (1991) Prednisone in Duchenne dystrophy. A randomized controlled trial defining the time course and dose response. Arch Neurol 48: 383–388
16. Griggs RC, Moxley RT, Mendell JR, Fenichel GM, Brooke MH, Pestronk A, Miller JP, Cwik VA, Pandya S, Robinson J, King W, Signore L, Schierbecker J, Florence J, Matheson-Burden N, Wilson B (1993) Duchenne dystrophy: randomized, controlled trial of prednisone (18 months) and azathioprine (12 months). Neurology 43: 520–527
17. Hardiman O, Sklar RM, Brown RH (1993) Methylprednisolone selectively affects dystrophin expression in human muscle cultures. Neurology 43: 342–345
18. Ibraghimov-Breskovnaya O, Ervasti JM, Leveille CJ, Slaughter CA, Sernett SW, Campbell KP (1992) Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. Nature 355: 696–702
19. Jacobs SCJM, Wokke JHJ, Bär PR, Bootsma AL (1995) Satellite cell activation after muscle damage in young and adult rats. Anat Rec 242: 329–336
20. Karpati G, Carpenter SC (1988) The deficiency of a sarcolemmal cytoskeletal protein (dystrophin) leads to necrosis of skeletal muscle fibers in Duchenne-Becker dystrophy. In: Sellin LS, Libellius R, Thesloff S (eds) Neuromuscular junction. Elsevier, Amsterdam, pp 429–436
21. Kelly FJ, McGrath JA, Goldspink DF, Cullen MJ (1986) A morphological/biochemical study on the actions of corticosteroids on rat skeletal muscle. Muscle Nerve 9: 1–10
22. Khan MA (1993) Corticosteroid therapy in Duchenne muscular dystrophy. J Neurol Sci 120: 8–14
23. Kissel JT, Lynn DJ, Rammohan KW, Klein JP, Griggs RC, Moxley RT, Cwik VA, Brooke MH, Mendell JR (1993) Mononuclear cell analysis of muscle biopsies in prednisone- and azathioprine-treated Duchenne muscular dystrophy. Neurology 43: 532–536
24. Komulainen J, Vihko V (1994) Exercise-induced necrotic muscle damage and enzyme release in the four days following prolonged submaximal running in rats. Pflugers Arch 428: 3–4
25. Kuipers H, Drukker J, Frederik PM, Geurten P, Van Kranenburg G (1983) Muscle degeneration after exercise in rats. Int J Sports Med 4: 45–51
26. Luo VJ, Murphy LJ (1989) Dexamethasone inhibits growth induction of insulin-like growth factor (IGF-I) messenger ribonucleic acid (mRNA) in hypophysectomized rats and reduces IGF-I mRNA abundance in the intact rat. Endocrinology 125: 165–171
27. Matsumura K, Campbell KP (1994) Dystrophin-glycoprotein complex: its role in the molecular pathogenesis of muscular dystrophies. Muscle Nerve 17: 2–15
28. McLean IW, Nakane PK (1974) Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. J Histochem Cytochem 22: 1077–1083
29. McNeil PL (1993) Cellular and molecular adaptations to injurious mechanical stress. Trends Cell Biol 3: 302–307
30. Mendell JR, Moxley RT, Griggs RC, Brooke MH, Fenichel GM, Miller JP, King W, Signore L, Pandya S, Florence J, Schierbecker J, Robison J, Kaiser K, Mandel S, Arfken G, Gilder B (1989) Randomized, double-blind six-month trial of prednisone in Duchenne's muscular dystrophy. N Engl J Med 320: 1592–1597
31. Menke A, Jockusch H (1991) Decreased osmotic stability of dystrophinless muscle cells from mdx mouse. Nature 349: 9–71
32. Moxley RT, Lorenson M, Griggs RC, Mendell JR, Fenichel GM, Brooke MH, Miller JP, and CIDD group (1990) Decreased breakdown of muscle protein after prednisone therapy in Duchenne dystrophy (DD). J Neurol Sci 98: 419
33. Nicholson LVB, Johnson MA, Gardner-Medwin D, Bhattacharya S, Harris JB (1989) Heterogeneity of dystrophin expression in patients with Duchenne and Becker muscular dystrophy. Acta Neuropathol (Berl) 80: 239–250
34. Ohlendieck K, Matsumura K, Ionasescu VV, Towbin JA, Bosch EP, Weinstein SL, Sernett SW, Campbell KP (1993) Duchenne muscular dystrophy: deficiency of dystrophin-associated proteins in the sarcolemma. Neurology 43: 795–800
35. Paget GE, Barnes JM (1964) Toxicity test. In: Bacharach L (ed) Evaluation of drug activities: pharmacometrics. Academic Press, London, pp 161
36. Pasternak C, Wong S, Elson EL (1992) The mechanical function of dystrophin in muscle cells. Mol Biol Cell 192a: Suppl 192a : 1111
37. Sklar RM, Brown RH (1991) Methylprednisolone increases dystrophin levels by inhibiting myotube death during myogenesis of normal human muscle in vitro. J Neurol Sci 101: 73–81
38. Turner PR, Westwood T, Regen CM, Steinhardt RA (1989) Increased protein degradation results from elevated free calcium levels found in muscle from MDX mice. Nature 335: 735–738
39. Van de Kant HJG, De Rooij DG (1992) Periodic acid incubation can replace hydrochloric acid hydrolysis and trypsin digestion in immunogold-silver staining of bromodeoxyuridine incorporation in plastic sections and allows the PAS reaction. Histochem J 24: 170–175
40. Van Kuijk FJGM, Sevanian A, Handelman GJ, Dratz EA (1987) A new role for phospholipase A_2: protection of membranes from lipid peroxidation damage. Trends Biochem Sci 12: 31–34
41. Vane J, Botting R (1987) Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J 1: 89–96
42. Weller B, Karpati G, Carpenter S (1990) Dystrophin-deficient mdx muscle fibers are preferentially vulnerable to necrosis induced by experimental lengthening contractions. J Neurol Sci 100: 9–13