

The efficacy of antisense oligomer mediated exon skipping is enhanced by concurrent administration of prednisolone in the *mdx* mouse

G.J. Pinniger,¹ S. Fletcher,² A.M. Adams,² R.D. Johnsen,² K. Greer,² H.M. Moulton,³ T. Lavin,¹ A.J. Bakker¹ and S.D. Wilton,² ¹School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia, ²Centre for Neuromuscular and Neurological Disorders, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia and ³Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331, USA.

Duchenne amuscular dystrophy (DMD) is a progressive, fatal muscle wasting disorder with a predictable course and limited treatment options. Recent advances in clinical care and management have increased the life expectancy of affected boys, but do not address the primary etiology of DMD, the loss of dystrophin. Corticosteroids represent the best treatment option currently available for a large proportion of DMD patients. However, molecular therapies are now becoming available, and it is important to establish the effectiveness of these treatments when combined with corticosteroids. Antisense oligomer-mediated splicing manipulation can bypass dystrophin gene lesions, resulting in functional dystrophin expression, and is showing promise as a therapy for DMD. We evaluated the combined administration of oral methyl prednisolone and peptide-conjugated phosphorodiamidate antisense oligomers (*PPMO*) targeting dystrophin exon 23 in the *mdx* mouse dystrophinopathy model.

All experiments performed on animals were approved by the University of Western Australia, Animal Experimentation Committee. PMOs targeting mouse dystrophin exon 23 conjugated to either peptide K (*PPMok*) (Fletcher *et al.*, 2007) or peptide B (*PPMob*) (Jearawiriyapaisarn *et al.*, 2008) were supplied by AVI Biopharma Inc. (Bothell, Oregon). Sham treatments contained saline (vehicle) only. *Mdx* and C57BL/10ScSn mice in the treatment groups were injected (IP, 10 mg/kg) once-weekly for five weeks with peptide-conjugated PMOs and/or given oral methyl prednisolone (SOLU-MEDROL Sterile Powder System, Pfizer, West Ryde, Australia) (1 mg/kg/day), beginning at one month of age. One week after the final treatment (10 weeks of age) animals were euthanized (sodium pentobarbitone, 40 mg/kg, IP) and the *extensor digitorum longus* (EDL) and strips of diaphragm muscle were dissected and mounted in an *in vitro* muscle test system containing physiological saline solution. Physiological evaluation of contractile function included measures of maximum specific force and susceptibility to contraction-induced muscle weakness. Other tissues, including *tibialis anterior* and remaining diaphragm were frozen in liquid nitrogen-cooled isopentane for analysis of dystrophin expression.

Oligomer administration induced dystrophin expression at near normal levels in diaphragm and a corresponding significant (~85%) reduction in contraction-induced muscle weakness. In EDL, there was a significant (~50%) increase in maximum specific force in addition to a significant (~65%) reduction in contraction-induced muscle weakness. In both EDL and diaphragm muscles, *PPMok* induced more consistent dystrophin expression and higher levels of exon 23 skipping than *PPMob* as determined by western blotting and dystrophin immunofluorescence. Prednisolone alone did not affect dystrophin expression or contractile function. However, exon skipping and dystrophin expression were enhanced by concurrent peptide-conjugated PMO and methyl prednisolone administration. The additional benefits conferred by these combined treatments were reflected in further improvements in contractile function in the EDL, but not diaphragm muscle.

We conclude that effectiveness of peptide-conjugated phosphorodiamidate morpholino oligomer treatment is not attenuated by concurrent prednisolone administration in *mdx* mice and that exon skipping, dystrophin expression and contractile function are in fact enhanced by the combined treatment.

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Jearawiriyapaisarn N, Moulton HM, Buckley B, Roberts J, Sazani P, Fucharoen S, Iversen PL, Kole R. (2008) *Molecular Therapy*, **16**: 1624-1629.

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