MMP2 and MMP9 in wild-type and mdx mice with taurine supplementation

X. Ren,[1] R.G. Barker,[1] G.D. Lamb[2] and R.M. Murphy,[1] [1]La Trobe Institute for Molecular Science, La Trobe University, Bundoora, VIC 3086, Australia and [2]School of Life Sciences, La Trobe University, Bundoora, VIC 3086, Australia.

Duchenne muscular dystrophy (DMD) is a severe and progressive muscle wasting disorder which leads to early death (Bonilla *et al.*, 1988; Hoffman, Brown, & Kunkel, 1987). The mdx mouse, which is an established animal model for DMD, similarly lacks dystrophin and displays a peak in muscle cell necrosis at approximately 3-4 weeks of age, however in direct contrast to DMD, mdx mice experience muscle regeneration into adulthood (>6 weeks) (Bonilla *et al.*, 1988; Cullen & Jaros, 1988; Spencer, Croall, & Tidball, 1995). The amino acid taurine has been shown to increase muscle strength in peak damage 28 day mdx mice (Barker, Horvath, van der Poel, & Murphy, 2017). Matrix metalloproteinase 2 (MMP2) and MMP9 reportedly play a critical role in differentiation and regeneration of skeletal muscle fibres through processing extracellular substrates (Chen & Li, 2009) and MMP9, but not MMP2, has been implicated in the pathology of the mdx mouse. In this study we examined the gelatinolytic activity and abundance of MMP2 and MMP9 in 28 day (D28) and D70 wild-type (WT) and mdx mice, and in D28 mdx mice with or without pre-natal taurine (tau) supplementation.

All procedures were approved by La Trobe University Animal Ethics Committee. Male mdx and WT mice (C57/BL10ScS) (D28 and D70) were anesthetized with an intraperitoneal injection of 10 μL/g Nembutal (Sodium Pentobarbitone) prior to euthanasia by heart excision, then *gastrocnemius* muscle was collected. Muscle samples from WT and mdx mice at D28 and D70, and mdx taurine mice (D28 mdx tau) were analysed using both zymography (to determine gelatinolytic activity of both pro and active forms of MMPs) and Western blotting (to determine total MMP protein contents).

Summaries of the results are shown in the Tables.

Table A. *Protein abundance and activity of pro- and active-MMP2 and proMMP9 in D28 and D70, WT and mdx mice, by zymography and Western blotting. Mean ± SD, *p<0.05 compared to D70 WT, #p<0.05 compared to D28 mdx mice, one way ANOVA, Holm-Sidak's multiple post-hoc test.*

	ProMMP2		ActiveMMP2	ProMMP9	
	Zymography	Western	Zymography	Zymography	Western
D28 WT (n = 5)	1.2 ± 0.6 *****	1.4 ± 0.6 * #	0.5 ± 0.4 * #	1.7 ± 1.6 *	1.6 ± 0.6 *
D70 WT (n = 6)	0.2 ± 0.1	0.3 ± 0.2	0.05 ± 0.02	0.4 ± 0.4	1.0 ± 0.2
D28 mdx (n = 6)	1.0 ± 0.5	2.4 ± 0.4	1.0 ± 0.4	1.0 ± 0.4	1.3 ± 0.2
D70 mdx (n = 6)	0.6 ± 0.3 *	1.5 ± 0.4 * #	0.4 ± 0.2 * #	0.5 ± 0.2	1.0 ± 0.3

Table B. *Effect of taurine supplementation on MMP2 and MMP9 activity by zymography, student t-test.*

D28 mdx with or without Taurine (Tau) - Zymography			
	ProMMP2	ActiveMMP2	ProMMP9
− Tau (n = 5)	1.0 ± 0.3	1.0 ± 0.4	1.0 ± 0.1
+ Tau (n = 5)	0.8 ± 0.2	0.5 ± 0.1 #	0.8 ± 0.2 #

The data indicated that the amount of MMP2 is greater in the necrotic peak phase (D28) of mdx mouse (Table A), suggesting that MMP2 may play an important role in the necrosis of skeletal muscle fibres in muscular dystrophy. This is supported by the observation that the improved muscle function seen with Tau supplementation was accompanied by a decrease in the gelatinolytic activity of active MMP2 (Table B). MMP9 showed age-related differences in WT mice, but not in mdx mice, suggesting that it may play a role during the development of skeletal muscle but is not crucial for the degeneration / regeneration cycles occurring in the mdx mouse model.

Barker RG, Horvath D, van der Poel C, Murphy RM. (2017). *PLoS currents* doi: 10.1371/currents.md.9a3e357a0154d01050b591601cbd4fdb

Bonilla E, Samitt CE, Miranda AF, Hays AP, Salviati G, Dimauro S, Kunkel LM, Hoffman EP, Rowland LP. (1988). *Cell* **54**, 447-452.

Chen X, Li Y. (2009). *Cell Adhesion Migration* **3**, 337-341.

Cullen MJ, Jaros E. (1988). *Acta Neuropathol* **77**, 69-81.

Hoffman EP, Brown RH, Jr., Kunkel LM. (1987). *Cell* **51**, 919-928.

Spencer MJ, Croall DE, Tidball JG. (1995). *J Biol Chem* **270**, 10909-10914.