Inhibition of NF-κB signaling prevents the development of DMD-associated cardiomyopathy in *mdx:Utrn*$^{+/-}$ mice

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Background

In DMD, loss of dystrophin within cardiomyocytes results in cell death leading to myocardial fibrosis and cardiomyopathy. There are no definitive therapies to effectively induce reverse cardiac remodeling and improve overall cardiac function in DMD patients. NF-κB is a key driver of skeletal muscle and cardiac pathogenesis in DMD. Edasalonexent (CAT-1004) is an oral small molecule NF-κB inhibitor currently in development for the treatment of DMD. We tested the ability of CAT-1004 to prevent the development of DMD-associated cardiomyopathy using the *mdx:Utrn+/-* mouse model which displays DMD-like skeletal muscle and cardiac pathology, including hypertrophy, fibrosis and left-ventricular dysfunction.

NFκB signaling in the heart

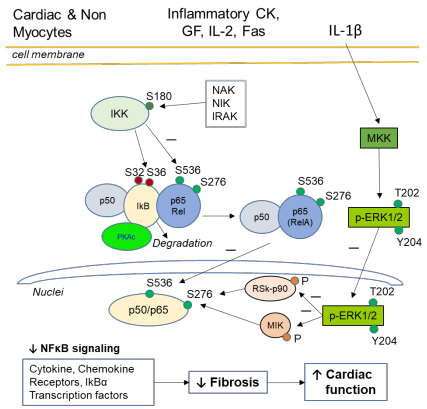

Figure 1. The proposed mechanisms of CAT-1004 within the heart. CAT-1004 prevents cardiac dysfunction through two proposed methods leading to inhibition of NF-κB signaling: ***1.*** Reduction of phosphorylation of p65 at the site of serine 536, and ***2.*** Reduction of phosphorylation of p65 at the site of serine 276 through inhibition of p44/42 ERK signaling. Minus signs (–) indicate potential points of inhibition by CAT-1004.

CAT-1004 downregulates p44/42 ERK signaling in DMD*mdx:Utrn+/-* hearts

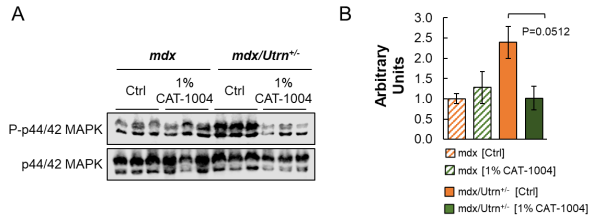

Figure 2. CAT-1004 treatment reduces ERK phosphorylation in DMD*mdx:Utrn+/-* mice. (A-B) Western blots show decreased phosphorylation levels of p44/42 ERK in the hearts from DMD*mdx:Utrn+/-* mice fed with high dose CAT-1004 (1%) compared with the control *mdx/Utrn*$^{+/-}$ group (1.02±0.30 vs 2.09±0.40, *P=0.051*), whereas phosphorylation of p44/42 ERK in the hearts from DMD*mdx* mice treated with the same CAT-1004 diet did not change compared with the control *mdx* group. The samples were extracted from whole ventricles homogenized in RIPA buffer. (N=3 per group).

CAT-1004 prevents the development of DMD-associated cardiomyopathy in DMD*mdx:Utrn+/-* mice

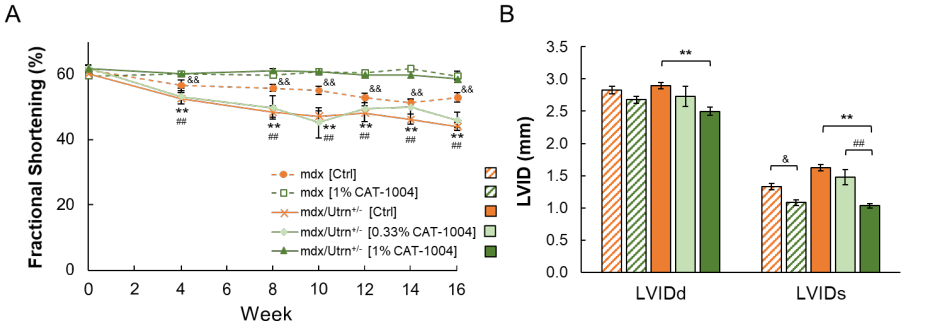

Figure 3. CAT-1004 (1%) treatment prevents left-ventricular dysfunction in both DMD*mdx:Utrn+/-* and DMD*mdx* mice. (A) Mice were placed on either a control or CAT-1004 diet starting at 4 weeks of age. Fractional shortening, as assessed by echocardiography, is maintained within normal ranges in both mouse models fed high dose CAT-1004 (1%), while it is significantly decreased in both mouse models after 12 weeks on either control diet or low dose (0.33%) CAT-1004 diet. **(B)** LVIDd decreased in DMD*mdx:Utrn+/-* mice on high dose diet. LVIDs is also reduced significantly in both models on 1% CAT-1004 diet. [*N*=10-13 for each treatment group. ** *P*<0.01, 1% CAT-1004 group compared to control diet group (*mdx/Utrn*$^{+/-}$ mice); ## *P*<0.01, 1% CAT-1004 compared to 0.33% CAT-1004 (*mdx/Utrn*$^{+/-}$ mice); & *P*<0.05, && *P*<0.01, 1% CAT-1004 group compared to control group (*mdx* mice)] LVIDd, and LVIDs are left ventricular internal diameters at diastole and systole, respectively.

CAT-1004 treatment reduces cardiac hypertrophy and fibrosis in DMD*mdx:Utrn+/-* mice

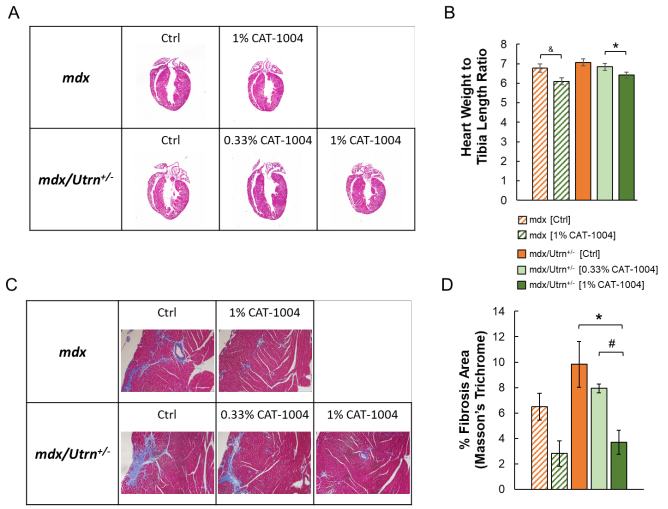

Figure 4. Changes in cardiac morphology, histology, and fibrosis. (A) The representative 4-chamber views of H&E staining are shown for all groups. **(B)** High dose CAT-1004 reduced cardiac hypertrophy as revealed by the ratio of heart weight to tibia length. **(C)** The representative images for Masson's trichrome staining (cardiac fibrosis) are shown. **(D)** Quantification of fibrosis area revealed less cardiac fibrosis in *mdx/Utrn*$^{+/-}$ mice fed the CAT-1004 diet. [*N*=3-4 in each group. * *P*<0.05, 1% CAT-1004 group compared to control diet group (*mdx/Utrn*$^{+/-}$ mice); # *P*<0.05 1% CAT-1004 compared to 0.33% CAT-1004 (*mdx/Utrn*$^{+/-}$ mice); & *P*<0.05, 1% CAT-1004 group compared to control group (*mdx* mice)] Scale bar: 200 µm.

CAT-1004 reduces skeletal muscle hypertrophy

A

Mouse Genotype	Diet	Skeletal Muscle Weight to Tibia Length Ratio			
		Diaphragm	Tibialis Anterior	Gastrocnemius	Soleus
mdx	Ctrl	7.66±0.23	7.17±0.20	18.67±0.55	1.45±0.05
	1% CAT-1004	7.46±0.25	6.76±0.12	16.88±0.39[&]	1.39±0.04
mdx/Utrn$^{+/-}$	Ctrl	7.77±0.19	7.41±0.22	17.87±0.56	1.57±0.05
	0.33% CAT-1004	7.76±0.26	7.66±0.30	18.29±0.62	1.57±0.03
	1% CAT-1004	6.71±0.14[**#]	7.18±0.26	16.72±0.59	1.40±0.02[**##]

* P<0.05, ** P<0.01 compared with *mdx/Utrn*$^{+/-}$ [Ctrl] diet group; # P<0.05, ## P<0.01 compared with *mdx/Utrn*$^{+/-}$ [0.33% CAT-1004] diet group; & P<0.05, compared with *mdx* [Ctrl] diet group. N=6-13 for each diet group.

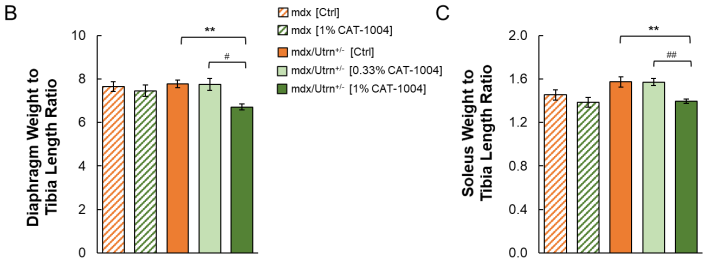

Figure 5. CAT-1004 reduces skeletal muscle hypertrophy. (A-C) 1% CAT-1004 diet significantly reduced the ratios of diaphragm to tibia length in DMD*mdx:Utrn+/-* mice compared to control diet. Soleus weight to tibia length ratios were also significantly decreased. The ratio of gastrocnemius muscle to tibia length ratios were significantly reduced in DMD*mdx* mice fed 1% CAT-1004 diet compared to the control diet. [*N*=6-13 for each treatment group. * *P*<0.05, ** *P*<0.01 1% CAT-1004 group compared to control diet group (*mdx/Utrn*$^{+/-}$ mice); # *P*<0.05, ## *P*<0.01 1% CAT-1004 compared to 0.33% CAT-1004 (*mdx/Utrn*$^{+/-}$ mice).

Conclusions

Collectively, the data suggest 1% CAT-1004 (edasalonexent) prevents cardiac dysfunction in a murine model of DMD. The amelioration of myocardial fibrosis through the inhibition of NF-κB signaling (presumably via the inhibition of phosphorylation of p44/42 ERK signaling) leads to preserved cardiac function in *mdx/Utrn*$^{+/-}$ mice. Further studies are ongoing to investigate the precise molecular mechanisms leading to the decrease in myocardial fibrosis and preservation of cardiac function.

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