Natural xanthines rescue myotonic dystrophy-like phenotypes in Drosophila and increase MBNL expression levels

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Abstract: Muscle mass wasting is one of the most debilitating symptoms of myotonic dystrophy type 1 (DM1), ultimately leading to immobility, respiratory defects, dysarthria, dysphagia and death in advanced stages of the disease. Malignant heart arrhythmias constitute an additional medical concern. Since the majority of current drugs ultimately derive from natural compounds here we sought to discover natural xanthines able to improve DM1-like phenotypes in a Drosophila model of the disease.
Method: INSR:luc spliceosensor flies were used to screen natural compounds for an increase in the reporter activity. Initial hits were validated in additional Drosophila phenotypes and in patient-derived fibroblasts.

Results, conclusions and funding:

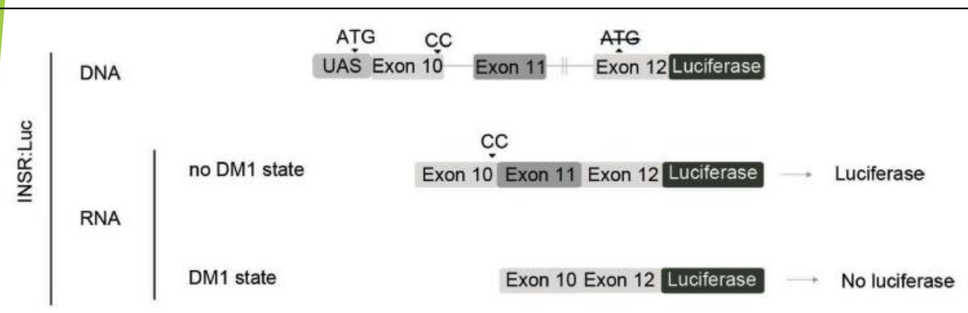

Fig 1: Design of spliceosensor flies that identified caffeine as a hit.

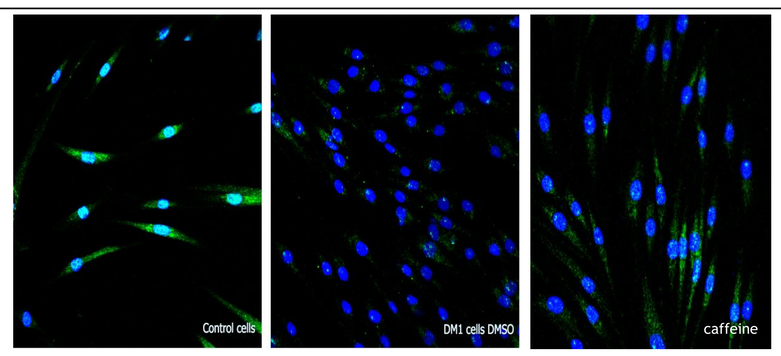

Fig 2: Caffeine increases free MBNL1 in DM1 fibroblasts.

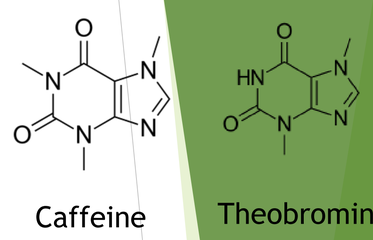

Fig 3: Caffeine (C) and Theobromine (T) are different types of xanthines

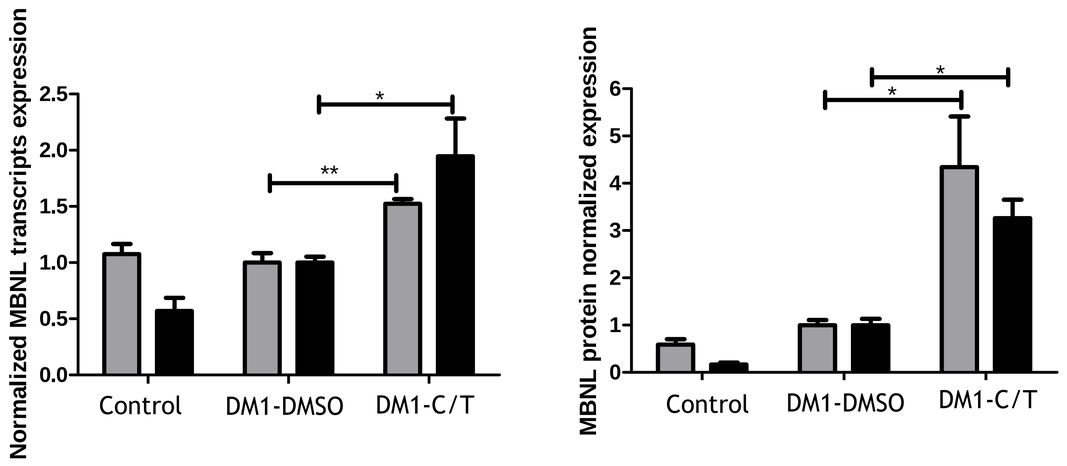

Fig 4: A C/T mixture (1/1.85, w/w) increased MBNL1 and MBNL2 expression level in human DM1 cells. (A) Bar graphs showing the mean ± sem of the expression levels of MBNL1 (grey) and MBNL2 (black) transcripts. GAPDH was used as endogenous control. (B) Bar graphs showing the mean ± sem of the expression levels of MBNL1 (grey) and MBNL2 (black) proteins. B- Actin was used as endogenous control. In both graphs, results were normalized to the DM1-DMSO, which were given the value of 1. *p<0.05, ** p<0.01, ***p<0.001 in Student's t test.

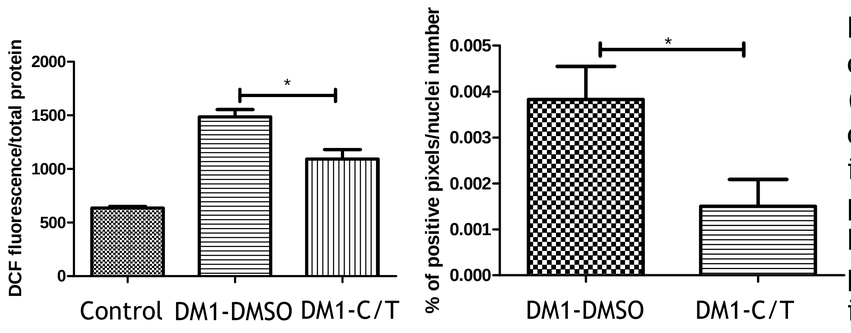

Fig. 5: Quantification of DCF fluorescence (left), a general oxidative stress indicator, and percentage of Lysotracker-positive pixels per nuclei (right) in untreated DM1 cells and cells treated with the C/T mixture.

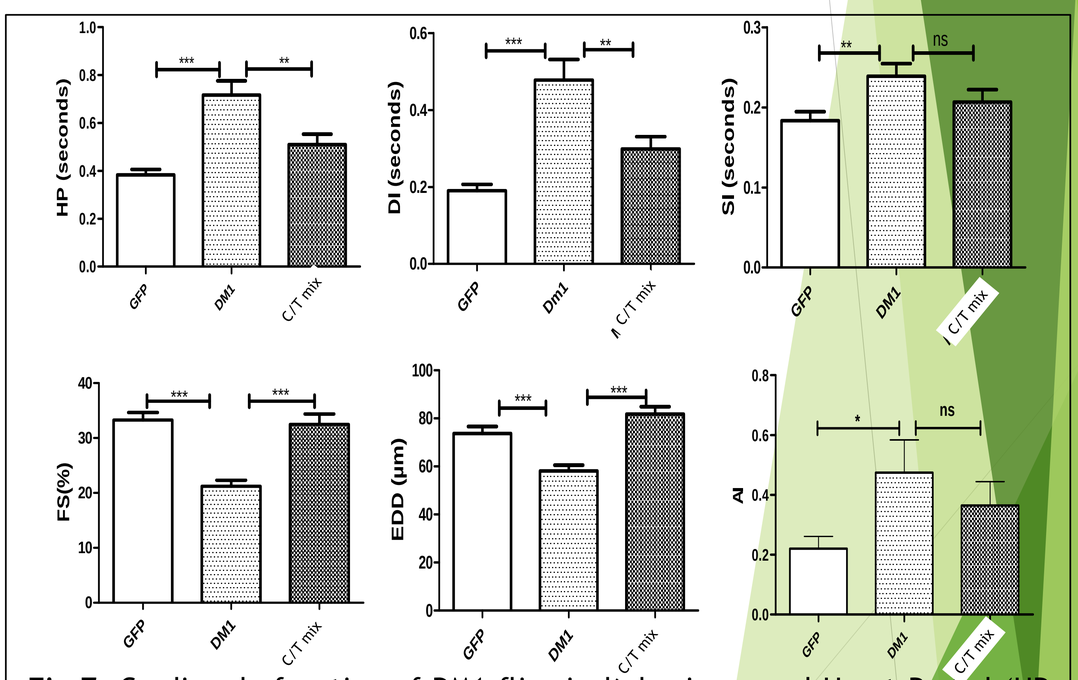

Fig 7: Cardiac dysfunction of DM1 flies includes increased Heart Period (HP, in A), due to increased Diastolic and Systolic Intervals (DI and SI, in B and C), reduced Fractional Shortening (FS%, in D), due to a reduction of End Diastolic Diameter (EDD, in E), and increased Arrhythmicity Index (AI, in F). Feeding of adult flies with 0.75% C/T mixture for 7 days rescued DI, %FS and EDD compared to DM1 model flies fed with DMSO (DM1). Bars show mean values and their SEM. *$p < 0.05$, **p<0.01, ***$p < 0.001$.

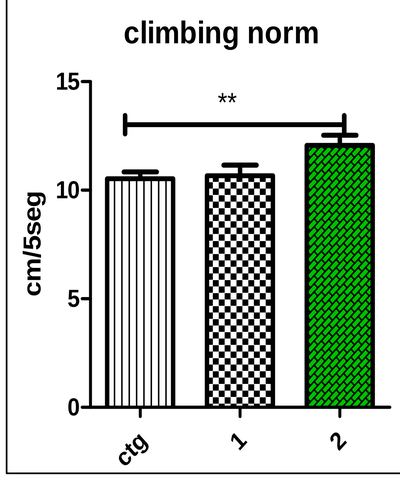

Fig. 6. Bar graphs showing:
- the climbing speed (left) as the mean speed ± *SEM* of 7-day-old disease flies fed with standard food (CTG and ctg) or food with just Caffeine (1) and the C/T mixture (2).
- the average landing height (right) data ± *SEM* of 7-day-old control (yw) or disease flies (CTGs) with Caffeine and the C/T mixture.

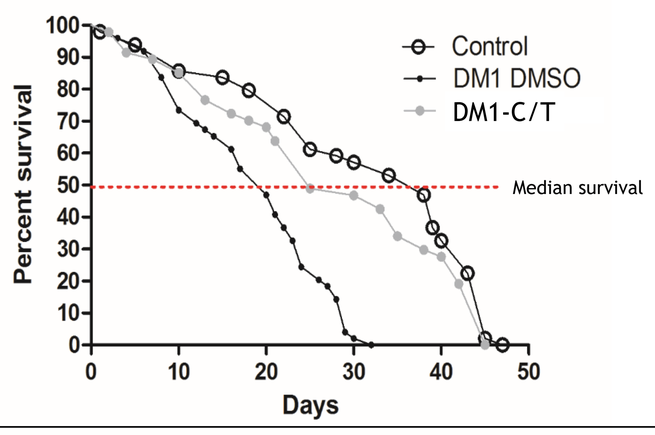

Fig 8: Average percentage of live flies versus age (in days). The GMH5-Gal4 driver was used to induce the expression of the inocuos reporter GFP (control) or the 250CUG repeats (DM1) to Drosophila cardiomyocytes ($p < 0.0001$, log-rank test).The C/T mixture induce a significant rescue of lifespan and median survival of DM1 model flies.

Conclusions: Our data support a desired effect of a C/T mixture on DM1 phenotypes in disease models. These effects stem, at least partially, from enhancement of MBNL1 expression levels.

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