Tamoxifen: a potential new drug for Duchenne

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Why tamoxifen in DMD?
A Treatment of mdx mice for 15 months
Performance
Diaphragm
Cardiac fibrosis
B Treatment of adult mdx mice (2 months at “mid-life”)
Dose – effect
Muscle contractibility
Membrane permeability
C Tamoxifen
Mechanism of action
Plasma levels of cytokines
Mdx cardiomyocytes
Summary of effects
Pro’s & Con’s
Why evaluate tamoxifen?

A Physiology of estrogens
• Female *mdx* mice have stronger muscles than male ones.
• Force is increased when plasma estrogen levels are high.
• Estrogens improve muscle resistance to fatigue.
• Estrogens increase myofiber regeneration.
• Estrogens increase muscle mass recovery from disuse atrophy.

B Why tamoxifen?
• Tamoxifen has estrogen-like activity.
• Tamoxifen is an antagonist in the mammary gland – used to treat breast cancer and an agonist in bone (and maybe in muscle).
• Over 30 years of clinical experience with tamoxifen.

Note: Tamoxifen is a pro-drug: metabolized into 4-hydroxy amoxifen – 100 times more potent.
Major signalling events in the dystrophic pathogenesis

LACK of DYSTROPHIN

- kinase activity
- Ca\(^{2+}\) influx
- oxidative stress
- membrane fragility

multiple dysfunctions of muscle cell homeostasis
- protein function
- FORCE GENERATION
- metabolism
- mitochondrial function
- ATP deficit

muscle cell death

- regeneration
- fibrosis/adiposis
- inflammation

muscle wasting

DEATH
A. Treatment of \( mdx^{5Cv} \) mice with tamoxifen for 15 months: Experimental design

- **Birth**
- **4-5 weeks**
- **6-8 weeks**
- **16 months**
- **Death**

**TAM, 10 mg/kg/day via food for 15 Mo**

- body weight, food consumption
- wire test
- force recording
- postmortem analyses
  - Histology
  - Western blots
  - gene expression

**Groups**
- **Dys:** untreated mdx males
- **TAM:** TAM-treated mdx males
- **wt:** untreated wildtype males
- **FEM:** untreated female mdx males

**The mdx mouse**

(From SCIENCEphotoLIBRARY)
A. Treatment of $mdx^{5Cv}$ mice with tamoxifen for 15 months – Performance & CK

Performance at the wire test

Creatine kinase levels

TAM treatment for 15 months increases motor performance and reduces CK blood levels
A. Treatment of $mdx^{5Cv}$ mice with tamoxifen for 15 months: Diaphragm morphology

TAM treatment for over a year makes diaphragm bigger, less fibrotic, and normalizes myofibre size.
A. Treatment of $mdx^{5Cv}$ mice with tamoxifen for 15 months – Cardiac fibrosis

TAM treatment for 15 months reduces cardiac fibrosis
B. Treatment of adult *mdx* mice for 2 months: experimental design

Alm: effect on the low intensity stage of the disease – “mid-life”

- TAM, 10 mg/kg/day for 15 Mo
- TAM (mg/kg/day)
- raloxifene (RAL), fulvestrant (Faslodex)
- weekly grid tests
- locomotor activity
- force recording
- tissue collection
- plasma CK
- postmortem analyses

The *mdx* mouse (muscular dystrophy, Xlinked)
B. Treatment of adult *mdx* mice for 2 months: dose - effect

Grid test

**TAM treatment of adult mice dose-dependently ameliorates motor performance**
In vivo muscle contraction test

- Stimulator
- Intensity
- Duration
- Frequency
- Mouse
- Force
- Transducer
- Data acquisition and analysis

**single twitches / phasic contractions**

**forcefrequency curve / tetanic contractions**

**muscle fatigue**
B. Treatment of adult *mdx* mice for 2 months: Isometric muscle contractibility

TAM treatment of adult mice dose-dependently causes muscle to contact stronger and slower.
B. Treatment of adult *mdx* mice for 2 months: Membrane permeability

**Evans blue dye uptake**

**diaphragm**

- Dys
- TAM
- RAL
- Poloxamer
- wt

**gastrocnemius**

- Dys
- TAM

A mouse 18h post EBD injection

**Creatine kinase activity (U/mL)**

- TAM
- RAL
- P188
- wt

**% EBD positive fibres**

- TAM
- Dys
- RAL
- Poloxamer
- wt

TAM treatment of adult mice dose-dependently stabilizes myofibre membranes
C. Mechanisms of action of tamoxifen on dystrophic muscle

Antagonism of TAM effects by pure antiestrogen fulvestrant / Faslodex

Data suggest that TAM acts, at least partly, via estrogen receptors:
TAM effects are antagonized by fulvestrant
TAM is potent: low nanomolar concentrations in plasma and tissues

TAM effect is antagonized by the pure antiestrogen fulvestrant
C. Tamoxifen alters plasma levels of cytokines

**TAM** ↓ TGF, PDGF & osteopontin but strongly ↑ IGF-1 levels
C. Tamoxifen reduces stress-induced calcium elevations in *mdx* cardiomyocytes

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**Figure 1:** Intracellular Ca$^{2+}$ response of ventricular myocytes from *mdx*SCV mice (n=6) and TAM treated *mdx*SCV mice (n=6) during the osmotic shock protocol. (A) Images of Ca$^{2+}$-related fluorescence (fluo-3) at 0s (a), 30s (b) and 60s (c). Top: *mdx*SCV mice, Bottom: TAM mice. Scale bar: 10 μm. (B) Mean values of fluorescent intensity averaged for each cell from the *mdx*SCV and tam group between 60 and 100 seconds. (C) Time courses of normalized fluorescence (±SEM) averaged for each cell from the *mdx* and tam group for the entire protocol. P<0.01: ***. N=1 mouse, n= number of cells.

Results by Emmanuel Lauber, Charlotte Lorin & Ernst Niggli - University of Berne
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DEATH
Tamoxifen – Pro’s & Con’s

Pro’s

1. **Mimics natural physiological pathway** – females, estrogen action
2. **Well known profile** – 30 years of experience, incl. children
3. **Exon-independent**
4. **Results validated in another lab**
5. **Cost** – £ 1000 - 5000/year

Con’s

1. **Not done according to GLP rules**
2. **Not a cure**
3. **Mechanism not yet fully clarified**
4. **No patent** – difficulty in large funding

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Thank you for your attention

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