MUSCLE DISEASE

1. Pharmacological Therapies

Assessments to evaluate therapeutic efficacy

- **Role of Dystrophin**
  - Dystrophin is critical for the architecture of the muscle and is a shock absorber, lack of dystrophin leads to gradual but remorseless degeneration of skeletal muscle
    Dystrophin-Glycoprotein Complex (DGC)
    - Long stringy protein - It is tightly associated with a series of transmembrane proteins
    - Holds contractile fibres to basal lamina via laminin - Sarcoglycan complex, dystroglycan complex - both link externally to laminin, a component of the basal lamina
    - Interaction of dystrophin with cytoskeleton is mediated via binding to F-actin
    - Receiver and transducer of signals - essential for stability of sarcolemma during muscle contractions and muscle growth
      - *Physiology - DGC forms rib-like lattices on cytoplasmic face of sarcolemma - costameres. Mechanical links between sarcomeres within fibre and laminin in ECM. Stabilises sarcolemma during muscle contractions, especially when activated and stretched forcibly. Lack of dystrophin disorganises costameres and enhances membrane leak with inappropriate cytosolic Ca2+ and ROS generation. Increased ECM deposition surrounding myofibres also occurs and perturbs membrane integrity.*
- DMD (Duchen) - complete disruption
- BMD (Becker) - with some working DGC complex
- **Repair strategies**
  - Install new shock absorber
  - Try and repair defective one
  - Find something else to take its place
  - Try to stabilise defective unit

Evaluating therapeutic efficacy for muscle disorders - how to test it?

Therapeutic Tests

- Make DMD patients more like BMD
- Preserve muscle function and maintain/increase mass - to maintain quality of life!
- Good animal models of human diseases, complementary physiological, histological and molecular biochemical assessments

- Tests
  - **Whole body functional tests** - non or minimally invasive, overall health or functional capacity of the animal, assess/monitor treatment effects during period of administration, track performance (e.g. running, swimming, climbing
  - **Vertical Hang Test** - timing the latency to fall onto padded mats, assess overall muscle endurance (and strength), perform measurements before, during and after treatment.
    - Pros: natural exercise, simple to perform, can assess motivational aspects too
    - Cons: can't assess specific muscles, fairly crude assessment of strength and endurance
  - **Rotarod Test** - assessing co-ordination and fatigability

        - Pros: simple, non-invasive assessment, can track performance regularly, provides assessment of coordination motivation, fatigue
        - Cons: difficult to identify muscle specific effects
    - **Grip strength test** - allows basic assessment of strength throughout treatment, screen compounds for fast or delayed response. Seated row to assess strength.
        - Pros: simple, non-invasive assessment of strength, can assess regularly and track performance
        - Cons: crude measure of strength, requires volition/motivation for proper assessment, considerable variation between investigators performing the task, biochemical advantages can affect reliability
- Non-invasive tests provide important information but are not definitive compared with functional tests on isolated muscles or the assessment of cellular functions using single muscle fibres.

- <u>Evaluation of whole muscle functions</u>

Parameters: max force/strength and power, contraction and relaxation times, shortening/lengthening/isometric, muscle fatigue, contraction-induced injury, structure-function correlations

- **In vitro** - remove muscle from body and string it up. Measuring the max force (shift in activation frequency may reflect shift in fibre type proportions)
    - Pros: can assess muscle parameters of muscle directly, free from influence of nerve or blood supply
    - Cons: less physiological as nerve and blood supply are removed, need to ensure all motor unites activated for accuracy of force measures.
        - *Diaphragm muscle strips - since this is used to inflate the lungs, evaluation of this muscle function is very important - evaluating efficacy of interventions for MD using mdx or dko mice where muscles of breathing are severely affected, delaying time for requiring ventilator is clinically relevant*
- **In situ** - dead mouse with muscle still in body, preferred method
    - Pros: preservation of nerve and blood supply - can stimulate isolated nerve muscle or via electrodes into the muscle, can assess properties of single muscle and its specific adaptations to interventions
    - Cons: more technically challenging, time consuming - limits number of preparations that can be assessed
- **In vivo** - assess with invasive surgery
    - Pros: can be done in minimally invasive manner, able to assess whole muscle groups, can use for training/conditioning programs lengthening/eccentric protocols in controlled manner
    - Cons: more technically difficult, equipment can be expensive, cannot isolate effects to specific muscle

- <u>Evaluation of single muscle fibre function</u>

Isolate 1 fibre by removing sarcolemma.

- **Mechanically skinned fibres** - can study excitation-contraction coupling, SR Ca2+ release, Ca2+ uptake rates SR function.
  Chemically permeabilised fibres - contractile apparatus, force-pCa/force-pSr, Vmax: velocity of shortening, contraction-induced injury.
    - Pros: can study cellular level for mechanistic understanding
    - Cons: technically difficult, requires expensive equipment
- Other analyses

- **Structure-function assessments** - need to perform complementary histological, immunohistochemical and molecular biochemical analyses. Look at EC coupling, Vmax and contractile injuries
- **Look at CK levels** (muscle damage marker), CSA and Synth/degradation - assessment of muscle fibre size - biochemical pathway analyses (anabolic vs catabolic processes, inflammatory markers)

- Muscle Damage hypothesis
  - **Dystrophic muscles more susceptible to contraction-induced injury**
    - mdx mice (mice with DMD) have greater sarcolemmal fragility of fibres, greater susceptibility to rupture following osmotic shock (cell membranes more easily ruptured in mdx vs wild type), greater susceptibility to injury following stretch (greater force deficits).
      - Contraction-mediated damage contributes to the aetiology of the dystrophic pathophysiology (shown in graph)
      - Mechanism of contraction-induced injury in dystrophic and control muscles - initial injury - damage to membranes in muscles of dystrophic but not control mice, membrane disruption allows influx of calcium - hypercontraction, necrosis.
      - Also showed greater susceptibility to damage in muscles following repeated lengthening muscle actions, high incidence of muscle fibre branching is also likely to contribute to susceptibility to stretch-induced injury, branch points are potential areas of injury susceptibility.
        - *Loss of DGC -> no force transmitter -> damage occurs*
  - **Calcium hypothesis of myofibre death in muscular dystrophy**
    - Influx of Ca2+ into cytosol overwhelms muscle cell's ability to maintain physiologic Ca2+ levels. Elevated levels of Ca2+ causes programmed cell death via activation of proteases (e.g. calpains).
      - *Increase in calcium -> activates proteases -> cell death*
  - **Curse of fibrosis**
    - Abnormal and unresolvable, chronic increase in extracellular connective tissue that interferes with functions.
    - Replaces contractile material and creates a physical barrier that limits efficacy of drug, cell and gene therapies. This alters contractile function.
      - *Inflammatory cytokines -> Increase fibrosis -> stiffer muscles and impaired contractility, decreases muscle regeneration*
        Must treat ASAP for greatest efficacy

Pharmacological Therapies

1. Gene therapy - corrects genetic defect - replaces defective gene or protein
2. Cell therapies - myoblast transfer therapy, stem cell therapies
3. Pharmacological therapies - usually prevention of secondary consequences of phenotypic defect

Therapy	Rationale/ Proposed Action	Drawbacks
Anabolic steroids	↑ muscle fibre size and strength, improved muscle fibre regeneration	may ↑ muscle fibre susceptibility to injury
β_2-agonists	↑ muscle fibre size and strength, improved muscle fiber regeneration	tremor and palpitations (adrenaline-like effects), cardiac hypertrophy. May ↑ fibre susceptibility to injury
Insulin-like growth factor-I (IGF-I)	↑ muscle fibre size and strength, improved muscle fibre regeneration	promote growth of all cells incl. tumour cells?
Myostatin inhibitors	↑ muscle fibre size and strength, improved muscle fiber regeneration	limited effectiveness once dystrophic pathology takes hold
Proteasome inhibitors/ ubiquitin conjugating enzymes	interference with the ubiquitin-proteasome (mechanism of muscle wasting)	can be cytotoxic in high concentrations
Membrane sealants	e.g. Poloxamer P-188; incorporate into cell membrane – 'plug' tears to attenuate muscle degradation; improve force	unknown if toxic at high levels; chronic treatment required
Vitamin E	antioxidant properties, scavenger of free radical-mediated muscle damage	generally ineffective, potentially toxic in very high doses
Ca^{2+} channel blockers	↓ cytosolic Ca^{2+} levels and prevent initiation of muscle degradative pathways	↑ potential for cardiac arrhythmia in some patients
Heat shock protein induction (e.g. Hsp70)	preservation of SERCA (sarco-endoplasmic reticulum Ca^{2+}-ATPase) activity – improve Ca^{2+} handling to prevent degradation	unknown at this stage

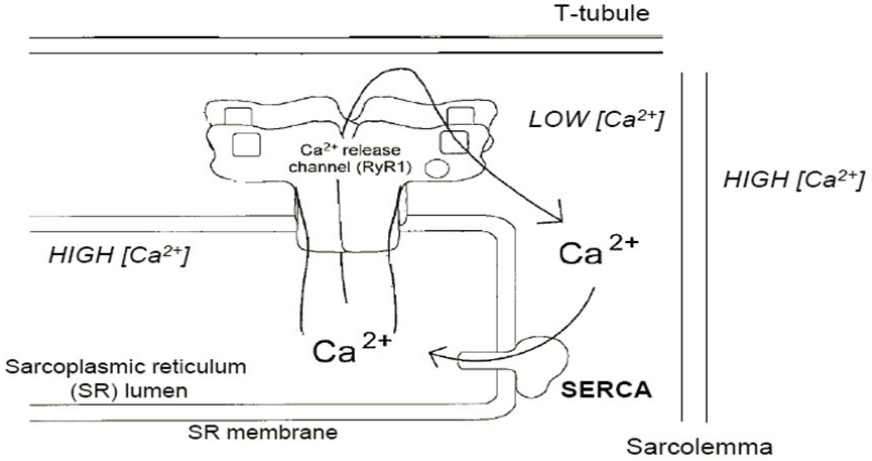
Treatments

- **Corticosteroids**
  (e.g. prednisolone or deflazacort)
  - Maintain size and improve strength by decreasing inflammation/muscle fibre size. Used to counter effects of chronic inflammation and preserve existing muscle fibres, hence the observed increase in strength
  - Mechanism of action is not clear- slow invasion of fibrosis into muscle - anti-inflammatory, smaller fibre sides - so less susceptible to contraction-induced injury, increase compensatory proteins to replace dystrophin, modify muscle fibre phenotype.
  - Golden standard for intervention for preserving muscle function in DMD, but with side effects - weight gain, fluid retention, high blood pressure, ulcers, growth inhibiting.
- **Anabolic agents**
  (e.g. anabolic steroids, growth hormone, B2-agonists, IGF-1, myostatin antibodies)
  - Increases protein synthesis. Increases muscle mass. Reduces degradation.
  - Myostatin blocker - therapy for muscle wasting diseases (genetic manipulation, neutralising antibody) - increases mass and force, and decreases degradation.
    - *Myostatin is a negative regulator of muscle mass, so keeping it low will increase muscle mass.*
- **Insulin-like growth factor (IGF-1)**

    - Important in normal muscle growth and repair levels of IGF-1 after injury and overload, formation of new fibre growth of existing fibres. Stimulates muscles cell - proliferation and differentiation
    - Increase muscle mass and strength, as well as function (in laminin deficient mdx that exhibit muscle wasting and weakness). Decreases fibrosis.
    - Safety concerns - new tumour formation, growth of existing tumours.
- **B2-Agonists**
    - Used for treating asthma initially as bronchodilators, has powerful effects on muscle, taken in higher doses and systematically that inhalation, potential to reverse muscle atrophy
    - Increase muscle mass and fibre size, increases force, increases fast fibres with reduced force deficiency and decreases susceptibility to injury (enhances muscle repair after damage)
    - No difference in strength or manual muscle tests
- **Exercise training**
    - Dystrophin deficient muscles are more vulnerable to injury and less able to sustain muscle repair. Due to ongoing degeneration, the risk of overwork and weakness is great. So exercise needs to be prescribed carefully
    - mdx mice showed EDL and soleus muscles being weaker and damaged after exercise.
    - Low impact forms of training is beneficial e.g. swimming shows to improve effects on metabolic enzyme activities, improved cardiovascular performance
        - Swim - no eccentric, inspiratory muscle training can improve in ventilation strength and endurance
        - Low resistance - moderate increase in strength, no side effects
    - High resistance and eccentric muscle training - increases muscle injury and myofibre death - should be avoided.
    - Immobilisation - conflicting evidence - reducing damage vs quality of living.
- **Polaxmer-188**
    - Plugs hole in cell membrane to stop calcium influx, prevents cardiac failure in mice.

<u>mdx mice</u>

- **Genotypic model for DMD**
    - Lacks dystrophin, compensatory utrophin, limb muscles undergo severe degeneration but regenerate successfully, muscles highly susceptible to contraction damage, diaphragm has severe pathophysiology
- **SERCA pump (which removes calcium) is compromised**



- **Heat Shock Proteins (HSP) binds to SERCA**