Receptor classification

- framework for understanding & developing drugs
- methods – structure using molecular biology
  - ligand binding
  - function (agonist / antagonist potency order)

Receptor classification by agonists

- rat aorta – contraction
  - NA >> potent than adrenaline
  - α – adrenoreceptor
- Guinea-pig bronchus – relaxation
  - isoprenaline >> potent than NA
  - β-adrenoreceptor

Agonist classification

- Drug X – high potency as relaxant of guinea pig bronchus, low potency on rat aorta
- conclude, agonist at β-adrenoreceptors
- BUT assuming that, conc. of drug at site in proximity to a receptor = conc. at bathing medium

Antagonism: the biological effect of 2 drugs is smaller than the expected sum of their individual effects

Pharmacokinetic antagonism

↳ The concentration of agonist at site of action is reduced by the antagonist

eg: $Mg(OH)_2$ antagonism on tetracyclines → effects of drugs on the body

Pharmacodynamic antagonism

- Non-competitive / allosteric antagonism

↳ antagonist binds to a site, distinct on the receptor from agonist recognition site

↳ antagonists prevent receptor activation by allosteric / other mechanisms

Pharmacodynamic antagonism

↳ The concentration of agonist at the site of action is NOT reduced by antagonist

eg: atropine antagonism of Ach

- Competitive antagonism

↳ antagonist bind to the same recognition site on the receptor as agonist

↳ antagonist competes w the agonist for binding to the recognition site

High throughput screening capacity

Upto mid 1990s

- 96-well screening
- 10,000 compounds tested per target per week

Late 1990s / now

- 384-well screening
- 10s of thousands of compounds per target per day

Now / future

- 384-well plates, 1536-well plates and beyond
- 100s of thousands of compounds per target per day

Assay and screen design

- what assay / screening strategy to use?
- set up assay
  - demonstrate appropriate biological activity
  - establish proof of assay principle
  - develop and optimise the final method
  - validate the method as 'fit for purpose'

Screen validation

- demonstrate signal reproducibility within and between plates
- demonstrate biological activity is reproducible within and between experiments
- test runs at the level of throughput needed
- confirm correlation between test runs
- demonstrate long-term reliability
- demonstrate screen is robust

Compound screening

initial detection of activity
↓
confirmation of activity
↓
determination of activity
↓
demonstration of cellular activity
(phenotypic / mode of action endpoint)
↓
initial physiochemical properties
↓
kinetics and selectivity

Compound libraries
↓
actives
↓
hits
↓
leads

Lead Optimisation

- involves synthetic modification of a biologically active compound in order to fulfil all stereoelectric, physicochemical, pharmacokinetic and toxicological properties required for clinical usefulness
- to eliminate or at least diminish the biological deficiencies in the lead compound from lead identification

Target Product Profile

- safe and effective
- unmet medical need (or best in class)
- route of administration and formulation best for patients
- dose in man (potency)
- ease of manufacture (cost of goods)
- freedom to operate (patent status)

Candidate Drug Target Profile

- potency against target
- efficacy against target
- selectivity for target
- duration of action
- pharmacokinetics
- toxicity profile
- patent position
- scale up and manufacturing
- formulation & route of administration

Lead optimisation Goals

1) To achieve in vivo efficacy in a disease and/or pharmacodynamic model, predictive of human disease, using a dose and schedule consistent with the proposed clinical use in humans → ideal administration is once/day (oral)

- high potency against molecular target
- good efficacy
- activity against clinically relevant biomarkers in pre-clinical species
- good free drug exposure levels in pre-clinical species
- predicted PK in humans meet clinical / commercial targets
- acceptable IP risks and cost-of-goods
- pharmaceutical properties - solubility, crystalline form, photo-stability
- route of administration, usually oral (once/twice daily)

Why are animals used in research?

1. To advance scientific understanding
2. as models to study disease
3. to develop and test potential forms of treatment
4. To protect the safety of people, animals and the environment

What can be done without in vivo studies?

Molecular biology can
↳ identify genetic cause of disease
↳ identify new targets for drug action ~30,000 genes
↳ develop screens for high throughput screening

Computer modelling can
↳ identify target: bioinformatics
↳ model the handling of drugs by the body
↳ estimate safety of new molecules

In vitro biological systems can
↳ measure affinity of drugs for targets
↳ measure potency and selectivity of drugs for targets
↳ measure metabolism of drugs
↳ suggest toxicity

What only in vivo studies can do?

1) Determine effects in the whole organism/body
2) Determine long-term effects
3) determine pharmacokinetics
4) reveal the unexpected
5) assess safety and toxicology
6) set the clinical dose range

procedure = anything that can give pain to animals

Animal Research in the UK
- regulated by Animal Scientific Procedures Act (ASPA), 1986 and EU2010-63
- ASPA controls any experimental or other scientific procedure applied to a 'protected animal'
- Protected animals are defined as all living vertebrate animals, except man plus all cephalopods (octopus, squid, cuttlefish ~~cephalopods (including foetal, larval/embryonic forms that have reached specific stages in their development)~~
immature forms: mammals, birds reptiles from 2/3 through the gestation/incubation period
: fish, amphibia, and ~~cephalo~~ cephalopods from the time at which they become capable of independent feeding
- All research using animals must be authorised by Home office

All animal research in UK requires 3 separate licenses
1) certificate of Designation for the premises (establishment licence)
2) individual project license for the research
3) personal license for the individuals working on the project

* animal facilities are ~~rigidly~~ visited regularly and inspected by Home Office inspectors (qualified vets experts & unannounced visits. or doctors)

Examples of Refinement

1) using non-invasive techniques

2) using appropriate ~~anaesthetics~~ anaesthesia & analgesia

3) training animals to co-operate in certain procedures eg: taking blood samples

So the animals are less stressed

4) ensuring that accomodation meets the animals' needs

5) environmental enrichment to improve living conditions for research animals

⇒ Automated blood sampling

- greater throughput
- more reproducible data
- ↓ stress to animals
- dramatic ↓ in animal numbers.

* transgenic technology lead to ↑ in no. of animals used for research

Secretary of State will NOT grant a ~~project~~ licence unless he is satisfied

1) that the aims cannot be achieved by another method not entailing the use of protected animals

2) that the regulated procedures to be used are those which:

- use the minimum no. of animals, with appropriate experimental design
- use the species with the lowest degree of neurophysiological sensitivity
- cause the least pain, suffering, distress or lasting harm
- are most likely to produce satisfactory results

Animals models ~~for~~ of disease : An example

- Parkinsons' disease (PD) is the most common neurodegenerative disorder (>1% population over 60)

It is chronic and progressive

- Symptoms include : tremor, rigidity, slowness of movement, postural instability, cognitive ~~for~~ problems
- a distinct anatomical circuit, the dopaminergic nigrostriatal pathway is preferentially damaged
- Levodopa, dopamine agonists & MAO B inhibitor alleviate symptoms but do not stop the advance of illness
- the lack of a neuroprotective agent is the major unmet therapeutic need in PD

The targets shortlist

1) enzymes involved in FA synthesis
2) class II glutamyl-tRNA synthetase
3) protein involved in tRNA splicing
4) " " " sphingolipid biosynthesis

If encoding genes are essential for growth in A. fumigatus, are their homologues in C. albicans essential too?

To test this, we generated conditional null mutants of C. albicans (diploid)

1. The ORF of one allele was replaced w heterologous DNA sequence (selection marker)
2. The promoter of the 2nd allele was replaced w that of MET3 (promoters that down regulates / abolishes transcription of adjacent gene in Met / Cys)

One target was taken forward (Ppt 2)

- It is encoded by an essential gene in both A. fumigatus & C. albicans
- It has homologues in a wide range of fungal pathogens — potential to be broad spectrum target
- Its human homologue is a diff. class — potential for selective inhibitors
- The encoded protein is an enzyme involved in mitochondrial FA synthesis — potential for assay development
- It is a novel target with no prior history of patent protection

Factors associated w assay development

- The enzyme (Ppt 2) catalyses the transfer of the [illegible]phosphopantetheinyl group from CoA to a receptor protein known as Acyl Carrier Protein (ACP)
- enzyme requires Mg^{2+}
- This assay would require the enzyme, the receptor protein ACP (Ppt 2) and Mg^{2+}

We set out to produce the required proteins in a heterologous system

- Diff host systems are available for heterologous protein production
  - E. coli chosen for ease of manipulation
- Diff. expression vectors are available for use with E. coli
  - pET vector system (T7 RNA polymerase based) chosen because it is selective, efficient and high-yield
- A range of host E. coli strains are available (help solve diff. problems)

Neuropathic pain

↳ pain caused by a lesion / disease of the somatosensory nervous system (International Association for the Study of Pain (IASP))

↳ common symptoms

- allodynia = pain caused by a stimulus that does not normally provoke pain
- hyperalgesia = ↑ response to a stimulus that is normally painful
- anaesthesia dolorosa = pain felt in a numb area
- sensory gain / loss

Neuropathic Pain – Impact on Healthcare

- prevalence estimated : 1-9%
- health-related quality of life (HR-QOL) is substantially impaired
- inability to work, reduced mobility
- ↑ patient and healthcare resource expenditure

Consequences
- impaired HR-QOL
- ↓ personal productivity
- anxiety disorders
- sleep disturbances
- decreased motility
- depression

(- flares - sharp, localised pain (myelinated) burning

Nociception

- The sensory [illegible] associated with [illegible] small diameter nociceptive afferents
- activated by diff. stimuli : chemical, mechanical, thermal

Examples of How Cortical Pain is measured

- verbal numerical scale (0-10)
- word scale
- visual analogue scales

Current medications

- Paracetamol / NSAIDs / Opioids (Not effective)
- tricyclic antidepressants
- selective 5HT reuptake inhibitors
- 5HT-NA reuptake inhibitors
- antiepileptic (anti convulsant drugs)

Scenario:

1) the enzyme CYP2D6 is responsible for metabolism of codeine → morphine

2) patient X is poor metaboliser of CYP2D6

CYP2C9 phenotype

- CYP2C9 contributes ~20% of total hepatic P450 content and metabolises ~20% of all drugs
- metabolises: S-warfarin, phenytoin, NSAIDs
- known variants - CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu)
  - with ↓ hydroxylation of S-warfarin
  - VKORC1 1173C>T; VKORC1 1639G>A
  - thus, ↓ clearance
  - higher risk of internal bleeding
  - require lower doses of warfarin

VKORC: vitamin K epoxide reductase

Metabolism: N-acetyltransferase (phase II)

- 2 forms: NAT1 & 2
- NAT2 polymorphisms: fast and slow acetylators
- slow acetylators - reduced metabolism
  - thus plasma [drug] ↑
- fast acetylators - drug efficacy

Eg: isoniazid, hydralazine, procainamide

Consequences

- extended pharmacological effect
- ADRs eg: succinyl choline
- lack of pro-drug activation eg: codeine & CYP2D6
- drug toxicity eg: warfarin
  - ↑ effective dose
  - metabolism by alternative pathways which maybe toxic

identifying genetic variations

- International HapMap project
  - the 1000 Genome Project (International)
  - the 100,000 ... (NHS)
- candidate genes: using a known pathway of metabolism/action at receptor as starting point
- genome-wide association studies (GWAS)
  - identify common genetic variants associated with disease/drug response by testing 100,000 SNPs in large population samples
- next-generation sequencing
  - examination of whole genome as well as targeted genes

The perfect drug

1) well absorbed
2) not rapidly metabolised
3) gets to site of action

→ high affinity binding to target receptor the block of which gives clinical efficacy

→ no binding to other proteins, the modulation of which could have safety implications

Process

Find target → validate target → Find lead (10^6) ↔ optimise lead (10^3) : development → phase I → II → III → NDA

: IND

Find target : find a molecular target (receptor/enzyme/ion channel) the blockade of which you believe may lead to desirable therapeutic effect

Validate target : do some experiments that confirm and ideally build belief in your hypothesis

Find lead : find some low MW compounds that have some affinity for your target protein (via HTS)

optimise lead : improve the affinity of your compounds for the target protein via synthesis–screening cycles, also try to optimise in vitro drug metabolism and safety profile

Phase I : administer best compound to small groups of healthy volunteers
↳ test safety and drug metabolism properties

In oncology, phase I → patients due to toxicity of drugs

Phase II : administer drug to small group of patients – look for evidence of clinical efficacy

Phase III : administer drug to large group of patients – look for evidence of clinical efficacy

Process : central role of synthesis–screening cycle

chemical leads → modify compounds → test activity in a rapid assay for affinity, drug metabolism, safety

towards end lead optimisation phase best compounds from in vitro assay test in vivo to assess : efficacy, drug metabolism and safety

What is NOT big pharma?

- Specialty pharma companies
  - ↳ usually operate in one major geographic region
  - ↳ involved in 2 or ~~more~~ 3 therapeutic areas at most
  - ↳ focus mostly on sales & marketing
  - ↳ outsource the other key functions
- Regional pharmaceutical companies
  - ↳ look like smaller versions of Big Pharma
  - ↳ typically only operate locally ~~or~~ w little if any global presence
- Generic Pharmaceutical companies
  - ↳ no R & D
  - ↳ create copies of already marketed drugs that have lost market exclusivity (ie: patent expired)
  - ↳ use price as the main marketing tactic
- Biotech companies

Biotechnology-derived drug types:

- plasma-derived proteins
- tissue-derived proteins
- vaccines
- recombinant peptides & proteins
- antibodies
- products derived from transgenic animals / plants
- gene therapy and related tissue or cell therapy products
- antisense drugs
- DNA type / no. of bases, drug-like small molecules ↑

Comparison of low MW drugs & biologics

Drug-like small molecules	Biologics
Usually synthetic, organic compounds	Usually protein / carbohydrate based
Well-defined structures and relatively stable	Complex physio-chemical characteristics and heat-sensitive
MW < 700Da and low # atoms	MW > 700Da eg: peptides ~1kDa, recombinant protein ~30kDa, Antibodies ~~150kDa~~ 150kDa
Typically given orally	Typically given ~~orally~~ parenterally
potential for off-target activity	high selectivity & specificity
metabolised	catabolised

Placebo control

- environmental / random effects occur
- in the absence of placebo, all AEs are more likely to be considered drug related
- recommend placebo always except when study has very limited objectives eg: kinetic only

What should be measured?

Tolerability – human toxicology
- AEs, biochem, haematol
- ECG, BP, urine
- this data alone will lead to MTD being subsequently used

Kinetics – may have a plasma concentration ceiling from pharmacology / toxicology data
- may need kinetic feedback during study especially non IV route
- advances in bioanalysis such as LC / MS / MS allow on the fly analysis
- mainly in terms of exposure AUC

Dynamics – does the compound show evidence of desired effects? unwanted effects?

↳ may last shorter eg: tolerance or longer (active metabolite / tissue / receptor binding) than plasma concentration

↳ assist in narrowing the range of doses for phase 2 and beyond MTD

- desired effect may be measured directly
eg: anticoagulant antiplatelet, BP, direct HR bronchodilation
where this is NOT possible consider a surrogate marker / model

Surrogate Marker & Models

- A model is an experimental system

Biomarker : a characteristic that is objectively measured and evaluated as an indicator of normal biological process, pathogenic process or pharmacological responses to a therapeutic intervention

clinical endpoint : a characteristic / variable that reflects how a patient feels / functions or how long a patient survives eg: heart attack, death or particular disabilities

Surrogate endpoint : A biomarker intended to substitute for a clinical endpoint. A clinical investigator uses epidemiologic, therapeutic, pathophysiological or other scientific evidence to select a surrogate endpoint that is expected to predict clinical benefit, harm or lack of benefit / harm

The patients

- It is impossible to treat all patients w a given condition – a sample is used
- Do this sample is ever a random ~~process~~ sample of the population and care is needed in extrapolation to all patients

Factors commonly affecting response to treatment
- age
- gender
- severity
- duration of disease
- previous / current therapy

Power and study design → bigger difference, smaller n

- The power of the study is its ability to detect the ~~worthwhile~~ worthwhile difference between the treatments you are hoping for

Why do clinical trials fail?

- trying to answer too many questions
- not speaking to statisticians early
- wrong investigator
- failure to produce / deliver drug
- wrong patients
- poor recruitment
- poor compliance
- wrong no. of subjects

Single blind – patient X know
Double blind – patient / doctor X know

Seeing who's blind

- 'matched' treatments differ
- well matched ~~but~~ differently
- patients & doctors try to break code

~~Physiological effect of~~ the treatments may alter the course of disease

Randomisation → to avoid bias and to ensure that all patients have an equal chance of being allocated to either of any group

Crossover trials

- All subjects receive all treatments during the study – differences due to individual differences is thought to be cancelled
- impact of time related difference is substituted
- difficult if treatment alters disease (hopefully for the better)

Toxicity

- similar to outcome biomarkers but for negative implications rather than +ve
- used for "Stop" decision for candidate compound
- QT prolongation and hERG channel blockade
  - Torsade de pointes and sudden cardiac arrest
  - liver, kidney

Pharmacogenomic

- informative for the clinical setting
- identifying cohorts of patients — similar responses
  — lack of responses
  - ~~drug target~~ possess / lack target
  - metabolism of drugs

diagnostic

- primarily used in clinical setting
- can also be used in preclinical in vivo setting
- identify whether disease present or not
  risk of disease
  progression / regression of the disease
  disease before manifestation of symptoms

Benefits

- Better inform next stage of development
  ↳ ↓ risk (financial and patient)
  ↓ cost , ↓ attrition
- All drug development projects have a biomarker strategy

Where are we now?

- only a handful of biomarkers are approved by FDA
- biomarker must have a clear and convincing scientific evidence that it consistently predicts clinical outcome
- if there is a correlation, then can be used as a surrogate endpoint

Suitability of Biomarkers for Drug Development

(X)

- Difficult to measure
- expensive to implement
- require long experiments
- difficult to reproduce

(✓)

- easily measured
- time-efficient
- quantitative
- objective
- highly reproducible
- clinical relevance and reliability across heterogeneous patient population

Biomarkers can be ....

Complex

- imaging (CT, PET, SPECT)
- analytical biochemical tool (mass spectrometry)
  - gene expression patterns
  - protein expression patterns

Simple

- HR
- BP
- body temperature
- sleep induction
- [glucose] in plasma