CASE 1

DOSE AND DOSING INTERVAL DETERMINE THERAPEUTIC AND TOXIC EFFECTS OF A BIOLOGICALLY ACTIVE COMPOUNDS

PS: Pharmacokinetics (look at ADME in general and apply it to alcohol and paracetamol)

BS: Peter swallowed 26 paracetamol tablets (500 mg x 26 = 13 000 mg or 13 g) 13 000/mg divided by 70 kg BW = 185 mg/kg – severe liver damage follows.

LG:

1. What is the ADME of alcohol?

→ Absorption:

Absorption is quick and almost complete after oral intake. Absorption occurs via GI system, primarily in the duodenum and the remainder in the small intestine (approx. 80%) and stomach (20%). When the stomach is empty, peak blood ethanol levels are reached between 30 and 90 minutes after ingestion. Food and particularly carbohydrates retards absorption: blood concentration may not reach a quarter of those achieved on empty stomach. Absorption also takes place through the skin and by inhalation.

Absorption occurs by **passive diffusion,** down its concentration gradient.

→ Distribution:

Alcohol molecule is a small polar molecule with lipophilic and hydrophilic characteristics.

Vd 0.5-0.7 L / kg, alcohol is completely soluble in water and thus has a similar volume of distribution to total body water.

and watching for adverse reactions, but mainly by obtaining phenytoin plasma concentrations.

3. How can drugs bind to plasma proteins? How does this affect the action of the drug in the body?

→ Plasma protein binding (PBB):

The distribution of drug from plasma to target tissues can be affected by number of factors out of which plasma protein binding is the most important. **Compounds that are extensively bound to plasma proteins will have a low volume of distribution, long plasma half-lives and low clearance by hepatic and renal routes.** High plasma protein binding may also have an impact on efficacy since it is usually the free fraction of drug that is responsible for the pharmacological action. Thus, the therapeutic response should be related to the level of unbound drug in plasma rather than to the total drug concentration.

The more lipophilic compounds display greater plasma protein binding. Majority of small molecules bind to plasma proteins with many being bound to be more than 90% bound

Many drugs circulate in the blood-stream bound to plasma proteins. **Albumin** is a major carrier for acidic drugs, **alpha1 acid glycoprotein** binds basic drugs. If albumin becomes saturated, these drugs will bind to **lipoprotein.** The binding is usually reversible.

Albumin – most abundant protein in human blood plasma (3.5 to 5.0 g/dL), comprises about half of the blood serum protein. Plasma albumin may act as a **drug reservoir** (as the concentration of the free drug decreases due to elimination of by metabolism or excretion, the bound drug dissociates from the protein).

Alpha-1-acid glycoprotein – has a normal plasma concentration between 0.04-0.1 g/dl (1-3% of plasma protein). It is negatively charged at physiological pH and interacts mainly with basic drugs.

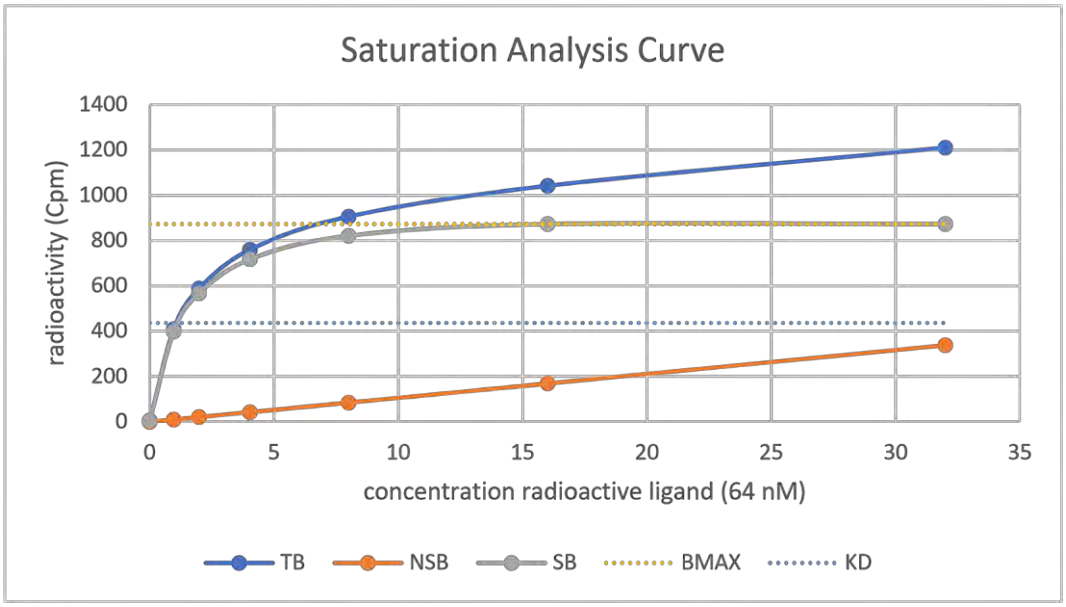
We do this experiment only once and can test lot of compounds in our second and third experiments.

→ Slope

We take the last two points because we assume that the increase is due to non-specific binding in our total binding curve.

Multiply the concnetration times the slope. This is because the last two points are due to increase in non-specific binding so we multiply our concentration times the slope.

The specific binding is determined by subtracting nonspecific binding from total binding.

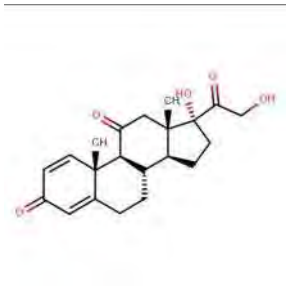


Bmax = 873.08 cpm

Kd (isoprenaline) = 1.2 – 1.3 nM (50% of the receptors are occupied at this value)

Experiment 2 – competition (inhibition or displacement) assay

Aim: We want to evaluate the ability of our test compound to displace the radioactive ligand from the beta-2 receptors.

	Prednisone is the most commonly prescribed corticosteroid used to treat allograft rejection, asthma, SLE and many inflammatory states. → **Side effects** Myopathy, weight gain, depression, pancreatitis, high blood pressure.
Indication	For the treatment of drug-induced allergic reactions, perennial or seasonal allergic rhinitis, serum sickness, giant cell arteritis acute rheumatic or nonrheumatic carditis, severe psoriasis…
Mechanism of action	The anti-inflammatory actions of corticosteroids are thought to involve **phospholipase A2 inhibitory proteins,** lipocortin, which control the biosynthesis of potent mediators of inflammation such as **prostaglandins and leukotrienes.** Prednisone can stimulate secretion of various components of gastric juice. Suppression of the production of corticotropin may lead to suppression of endogenous corticosteroids. **Prednisone has slight mineralocorticoid activity, whereby entry of sodium into cells and loss of intracellular potassium is stimulated. This is particularly evident in the kidney, where rapid ion exchange leads to sodium retention and hypertension.**

	(10%) respectively. UDP-glucuronosyltransferase (UGT) 2B7 and 2B4 are the major metabolic enzymes mediating the glucuronidation of codeine to the metabolite, codeine 6 glucuronide. Cytochrome **P450 2D6** is the major enzyme responsible for the transformation (codeine is demethylated to morphine) of codeine to morphine and **P450 3A4** is the main enzyme mediating the conversion of codeine to norcodeine. Morphine and codeine are then further metabolized by conjugation with glucuronic acid. The glucuronide metabolites of morphine are **morphine-3-glucoronide (MG3**) and **morphine-6-glucoronide (M6G). MG3** does not have any role in pain relief. **MG6** analgesic potency 4x to 6x that of its parent compound (morphine). **Ndemethylation** to normorphine is considered a minor pathway and catalyzed mostly by **CYP3A4.** The opioids are converted in large part to polar metabolites (glucuronides) which are often readily excreted by the kidneys.
Excretion	About 90% of the total dose of codeine is excreted by the kidneys. Approximately 10% of the drug excreted by the kidney is unchanged codeine. The majority of the excretion products can be found in the urine within 6 hours of ingestion, and 40-60% of the codeine is excreted free or conjugated. Approximately 5 to 15% as free and conjugated morphine and approximately 10-20% free and conjugated norcodeine. Glucuronide conjugates are also found in bile, but enterohepatic circulation represents only a small portion of the excretory process.

In addition to the passive diffusion into the aqueous phase, lipid-soluble drugs such as citalopram may have co-secretion by dissolution in the fat droplets of milk. In practical terms, this may not be of concern.

→ **Maternal pharmacogenomics**

A growing understanding of the influence of pharmacogenomics is well exemplified with **codeine which is variably metabolized to morphine by the cytochrome P450 (CYP) 2D6 enzyme.** The ultra-rapid metabolizer phenotype occurs in up to 10% of Western Europeans and up to 30% of North Africans. Repeated codeine doses in these women produce significant amounts of morphine. Rapid transfer from maternal plasma to the milk may result in central nervous system depression and potentially infant death. Codeine should be avoided during breastfeeding and alternative analgesics are recommended, such as paracetamol or ibuprofen.

→ **Oral bioavailability**

The drug‟s presence in breast milk does not necessarily lead to significant exposure for the baby. The infant gut may degrade or destroy a drug, for example omeprazole (for which the standard formulation is enteric-coated). Gentamicin is given intravenously to the mother. As it is poorly absorbed orally by the baby, drug concentrations will not be reflected in infant plasma.

6. Differences in metabolism between neonates and adults?

→ Due to the difficulties of conducting traditional pharmacokinetic studies in children and neonates extrapolation of dosing information from adult studies has persisted, despite increasing evidence that this is both inappropriate and at times ineffective.

→ Infants, particularly neonates are not merely small adults requiring smaller dosages, but a unique patient group.

→ **Absorption:** The developmental differences in chemical, mechanical and physical barriers between neonates and adults can change the rate and extent of drug absorption. Bioavailability is also affected by the development of intestinal CYP enzymes and Pglycoprotein efflux pumps.

CASE 8 – SYSTEMS BIOLOGY APPROACHES FOR PRECISION MEDICINE IN COPD

1. What is COPD?

Chronic obstructive pulmonary disease is characterized by persistent airflow limitation that is usually progressive and that is caused by an enhanced chronic inflammation response in the airways and the lung to noxious particles or gas. There is partial or complete obstruction at any level from the trachea and larger bronchi to the terminal and respiratory bronchioles.

- Shortness of breath, cough, persistent airflow limitation on spirometry in the absence of any other major lung conditions with at least 10 pack-years of smoking history or equivalent biomass exposure.

Emphysema and chronic bronchitis are grouped together and are referred to as COPD, since the majority of patients have features of both. **Emphysema is an irreversible enlargement of the airspaces distal to the terminal bronchiole, accompanied by destruction of their walls without obvious fibrosis. Chronic bronchitis** is defined as clinically persistent cough with sputum production for at least 3 months in at least 2 consecutive years, in the absence of other identifiable cause.

Risk of developing COPD is related to following factors:

- Tobacco smoke, indoor air pollution, occupational exposures, outdoor air pollution, genetic factors, age and sex (female sex), lung growth and development, socioeconomic status, asthma and airway hyper-reactivity, chronic bronchitis, infections.

COPD is a major global burden, affecting more than 300 million people worldwide and accounting for 2.9 million deaths annually. By 2040 this number will increase by 32% to 4.4 million deaths annually, making it the fourth leading cause of mortality, trailing only ischemic heart disease, stroke and pneumonia. This increase will be driven largely by the ageing populations around the world, decreasing mortality rates of competing causes of deaths, increased exposure to ambient indoor and outdoor pollution, and poor lifestyle choices including tobacco smoking. According to WHO there are 4.2 million deaths per year attributed to ambient air pollution and 3.8 million deaths per year from biomass exposure from dirty stoves and fuels.

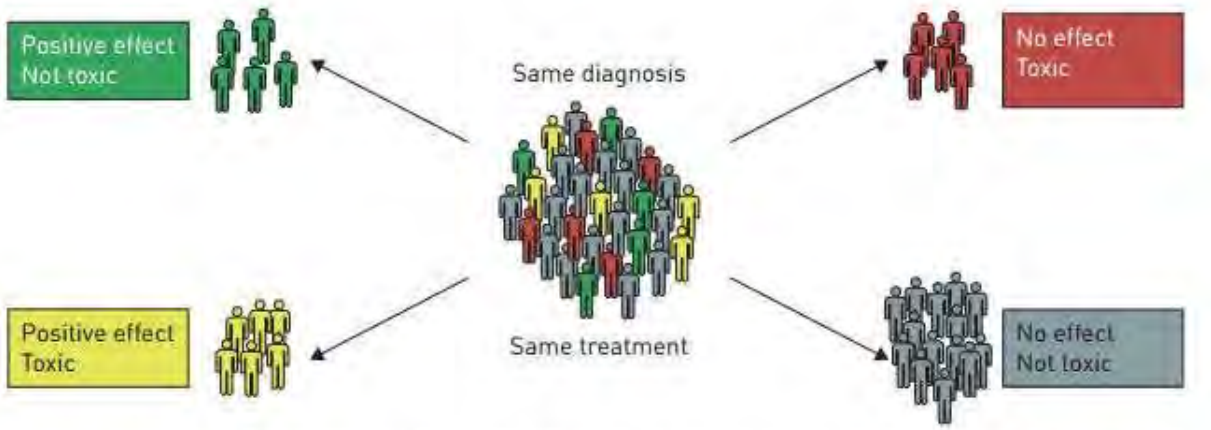


FIGURE 1 Principles of precision medicine that illustrate the heterogeneity of any human disease and the potential impact of stratifying the population appropriately. Adapted from Chakma Justin (*Journal of Young Investigators*, 2009, Vol 16)

Systems biology – system approaches stem from the premise that separate analysis of information gathered from different elements, compartments or levels of a dynamic system cannot yield appropriate understanding prediction of the global behavior of the system (so called emergent properties, which are implicit in nonlinear systems) nor allow it to fix it found globally away from an homeokinetic state.

- Systems biology departs from the reductionist approach followed by traditional biomedical research by integrating (rather than taking apart) different biological levels (genes, molecules, cells, organs and the environment) and mechanisms, and shares a very similar goal with integrative physiology: to better understand holistically the systemic dynamic state of individuals.

- In such context, systems biology is nothing more than physiology which has always meant to be multiscale and integrative.

- Today's availability of new tools, high-throughput technologies and computing power allows, for the first time, real physiology to be performed.

- Systems biology includes many different biological dimensions.

Omics data include (epi)genomics, transcriptomics, proteomics, metabolomics, microbiomics, single-cell analyses, phenotypic assays, extensive medical records and an endless list of environmental factors (exposome) such as smoking, exercise, diet and pollution among others.

- **Genomics:** identify nucleotide variants (SNPs) in the whole genome in associated GWAS. platform – genotyping arrays, whole exome sequencing.

- **Transcriptomics:** quantify expression levels of cellular transcripts (e.g. mRNA). Expression arrays, RNA sequencing.

- **Proteomics:** characterize protein expression levels of cells/samples.