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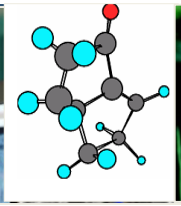
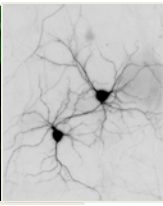




Toxicology – Future Trends

(toxicogenetics, pharmacogenomics, metabolomics)
in a regulatory context

Brian C. Foster, Ph.D.

CAPRA
October 2012

My hope today is to:

- overview of the basics rather than specifics
- ambiguities, *etc*.
- challenges
- some examples
- summation
- try to answer your questions……

Health Products: Biologics, Drugs, Functional Foods, Natural Health Products.

- **Health products (HPs) contain medicinal and non-medicinal substances.**
- **All substances have risk; some more than others.**
- **A pharmacodynamic effect is NOT required to have an interaction ….**
- **The regulator will always want more (better) information ….**

Disclaimer: I'm "retired", the opinions expressed in this presentation are mine and may not reflect those of the

“If it were not for the great variability among individuals, Medicine might be a Science, not an Art”

……Sir William Osler

“The Art of Medicine is, in part, the ability to reduce observations from population samples to a single, individual patient.”

……undocumented

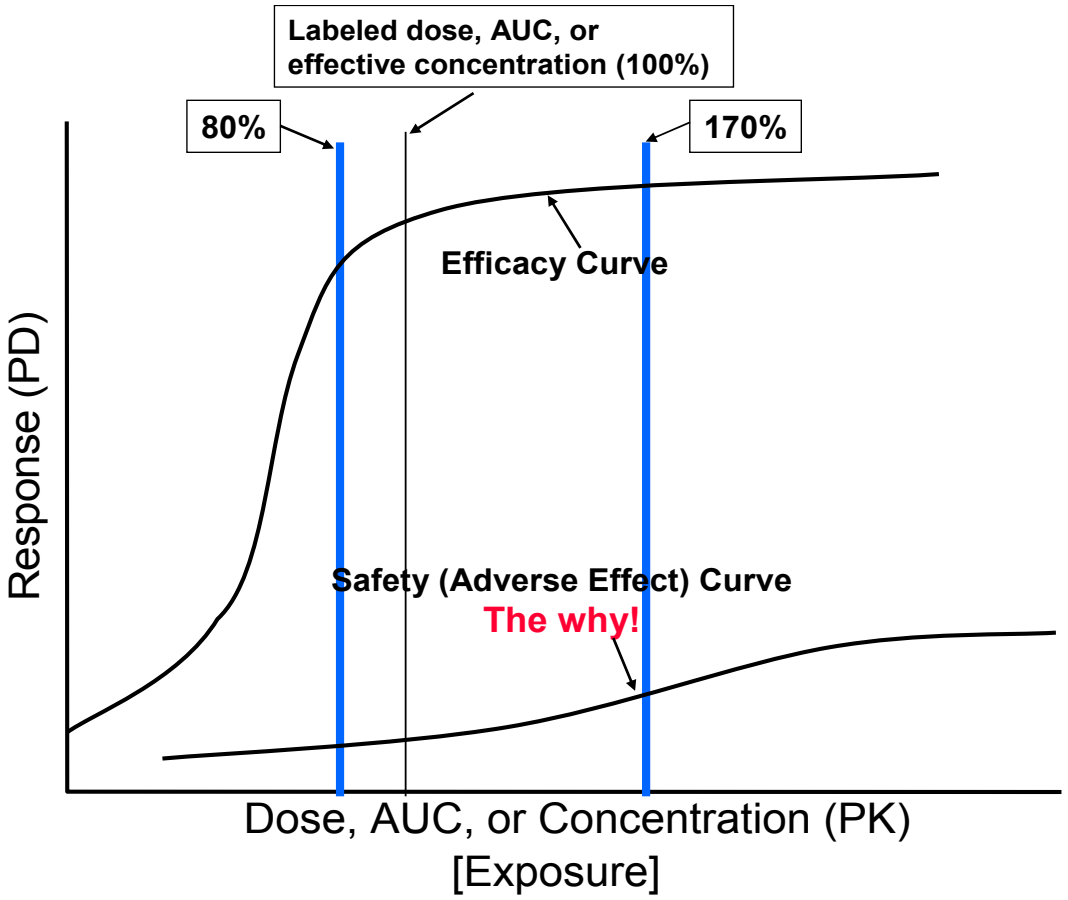
Personalised Medicine:

"The tailoring of preventative, diagnostic or therapeutic interventions to the characteristics of an individual or population."

[Shortened HC Working Definition]

PM ≠ PGx

PM Niches: Diagnostics, Prevention, Treatment, ADR Mechanisms



Depends on drug (pro-drug, etc.), host & disease

Adapted from Dr. S-M. Huang, US FDA

Toxicity Testing in the 21st Century: A Vision and a Strategy (2007)

a transformative paradigm shift is needed:

- provide broad coverage of substances, mixtures, outcomes, and life stages,
- reduce the cost and time of testing,
- use fewer animals and cause minimal suffering in the animals used, and
- develop a more robust scientific basis for assessing health effects of environmental agents.

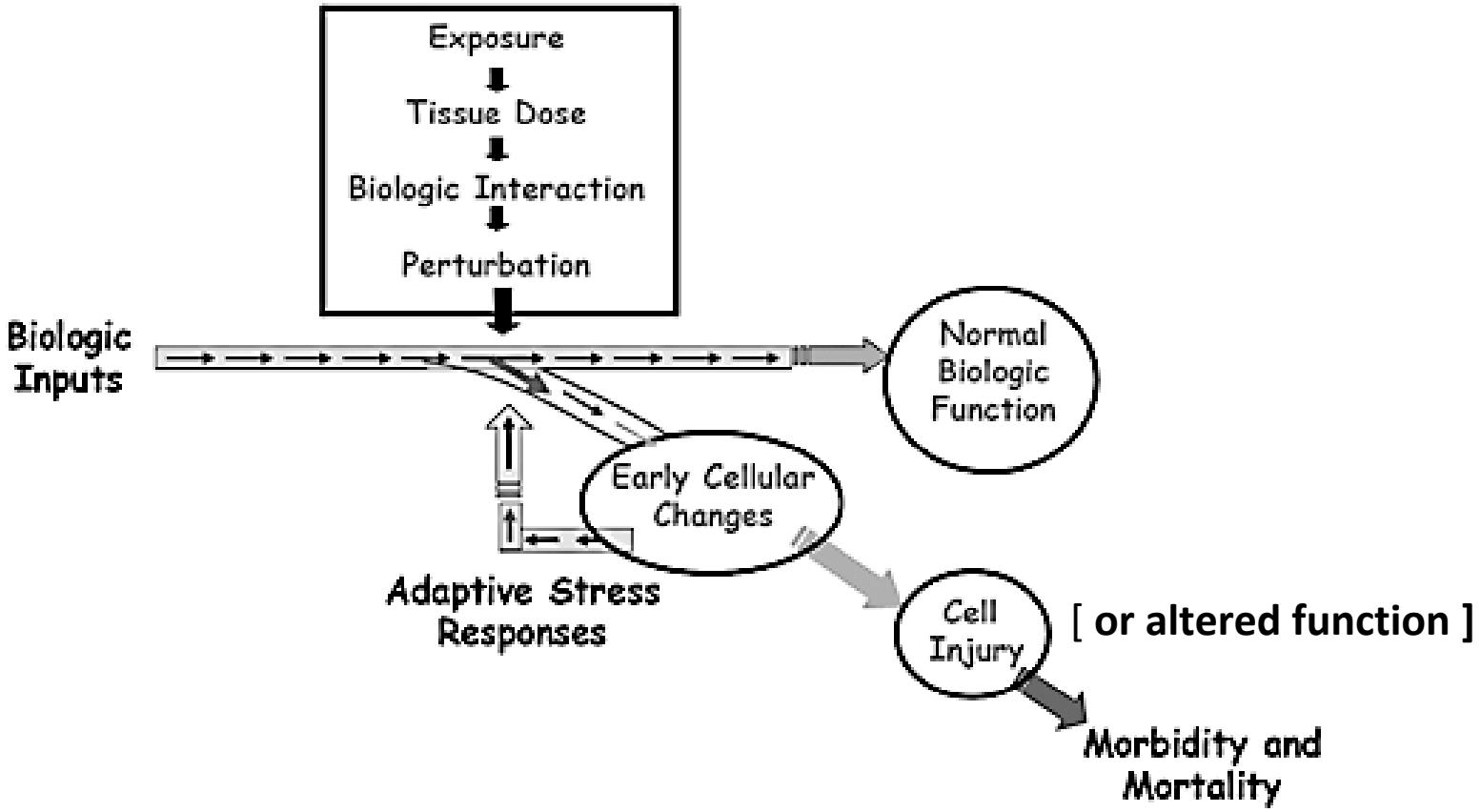
Advances

- in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on *in vitro* methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin;
- *but maybe not*

Ambiguities

- **A particular dragon protects the gold therein: the dragon is the "curse of dimensionality" and its formidable fire weapon, which is burning researchers, is the "combinatorial explosion".**
  - arises because many genomic, proteomic, clinical, and lifestyle factors may interact and cannot necessarily be considered on a simple pair wise or additive basis (Robson B. 2004).
- Algorithms - ***not*** validated; need more advanced algorithms that can take into account additional genetic predictors and confounding factors
- Translation is everything *but* easy

Response: intersection of exposure and biologic function



Adapted from Andersen, Dennison, Thomas, and Conolly. New directions in incidence-dose modeling. Trends Biotechnol. 23(3):122-127. 2005.

Drug Disposition Inter-relationships

- Intrinsic (Genetic): gender, race, polymorphisms, genetic disease. (Physiological): age, organ/tissue function, clinical status - disease state {co-morbidities}, pregnancy
- Extrinsic (Environmental): climate, sunlight, culture {adherence, education – awareness}, socio-economic {nutrition}, diet, alcohol, smoking, stress, exposure - single or repeated dosing, loading
- Pre-systemic and systemic

Populations at Risk

will vary in sub-populations along physical and cognitive dimensions of health; risk ↑ number of risk factors; life stages:

- **Foetus**
- **Neonate**
- **Infant**
- **Elderly**
  - **elderly (≥ 65 yrs) and very elderly (> 80 yrs); and**
  - **frail and non-frail.**
- **Individuals with:**
  - **hormonal changes due to pregnancy, malnutrition, obesity, diabetes mellitus, systemic inflammation (tissue damage, burns, trauma, tumours and autoimmune disease);**
  - **infection; and**
  - **increasing number and prior exposure to licit and illicit products.**

Female, 53 yr, asthma & menopause

hospitalized: fatigue, liver necrosis

- Budesonide/formoterol, 200/6 µg BID
- Progesterone, 100 mg
- Extradiol, 50 µg patch
- Venlafaxine, 150 mg
- Lorazepam, 1 mg OD
- Varenicline, 0.5 mg OD
- Tobradex, eye drops

- Congugated linoleic acid, 1 gm, TID
- Probiotic, 3 caps, TID
- Methosulfonylmethane, 1 gm, 3-9/d
- NutriMin C, 2 caps, BID
- Fish oil, 1 gm
- Electrolytes, 1-3 caps/d
- Phaseolamin, 540 mg, 4-6/d
- Vitamin D3, 1000 IU, OD
- GHR
- Alcohol

Note: Vit D3 used in Caco-2 cell culture to ↑ CYP metabolism & P-gp.

SONAR

Inflammation, Infection & Stress

Renton KW, Expert Opin Metab Toxicol 1:629-40, 2005.

- expression and activity of P450 is altered
- most of the major P450s affected
- decreased capacity to handle xenobiotics and some endogenous compounds
- results from production of cytokines and modulation of transcription factors that control P450 expression
- critical and narrow therapeutic index (warfarin) products may have aberrant drug handling & ADRs

Phenotypes

In general: 4 phenotypes with some populations skewed to one side of the bell curve

1. poor (PMs, lack functional alleles),
2. intermediate - slow (IMs, heterozygous with 1 functional allele). Can ↑ expression result in EM?
3. extensive - fast (EMs, homozygous with 2 normal alleles),
4. ultra-rapid (UMs, multiple copies, ↑ transcription).

Is this really just limited to metabolism - transporters, receptors, ion channels?

Equivocal Genotype (phenotype)

Nebert DW, Tox Appl Pharmacol. 207:S34-42, 2007

- Genocopy – same SNP, different trait (outcome);
- Phenocopy – different SNPs, same trait;
- Penetrance – proportion with same SNP and trait if <100%;
- Non-penetrance – failure of trait when SNP present;
- Expressivity – var. in expression, degree, severity of trait;
- Epistasis – interaction ≥2 genes, dominance of another;
- Epigenetics – not classical Mendelian genetics;
- Dynamic genome;
- Gene-gene interactions;
- Molecular or meiotic drive;
- Gene conversion or silencing; and
- 19 other factors that each can override the importance of any one SNP and its association with a trait.

ADME - complexity

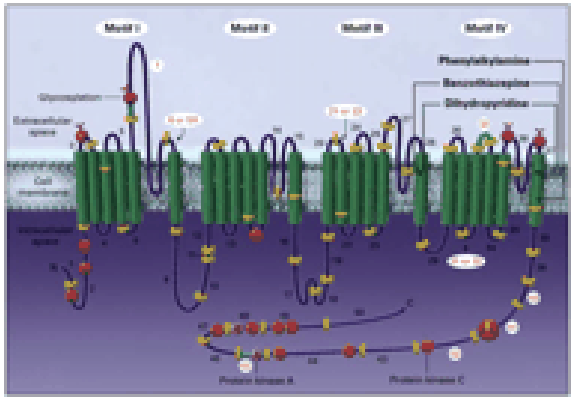
> 300 human ADME genes

- broad specificity (more than one pathway), atypical kinetics, formation of multiple products, PK promiscuity
- expression is cell & tissue dependant
- most polymorphic and inducible
- linked to immune pathways

the major pathways include (but not limited to):

- nuclear regulators: farnesoid X-receptor (FXR), liver X-receptor (LXR), pregnane X-receptor (PXR) and peroxisome proliferator-activated receptor-alpha (PPAR-alpha)
- metabolic activation: > 30 Phase I enzymes such as CYP450 (1A2, 2B6, 2C9, 2C19, 2D6, 2E1, 2J2, 3A4/5/7/43, 4, 19), CES, FMO, & MAO
- metabolic detoxification: Phase II enzymes such as NAT2, GSTM1, GSTT1, SULT, UGT1A1, UGT1A3, UGT1A9 & UGT2B7
- transport: Major Facilitator Superfamily (MFS), ATP-binding Cassette Superfamily (ABC); more than just P-glycoprotein

Transporter Proteins



International Transporter Consortium – several out of 1000s are *or* could be important:

- **P-glycoprotein (P-gp, *ABCB1*)*,**
- **breast cancer resistance protein (BCRP, *ABCG2*)*,**
- organic anion transporter (OAT, *SLC22A6-11*),
- organic cation transporter (OCT, *SLC22A1-3*),
- organic anion transport protein (OATP, *SLC01-5*),
- multiple resistance proteins (MRPs, *ABCC2-6*), &
- multi-drug and toxic compound extrusion proteins (MATEs, *SLC47*).

Health Product Absorption

Absorption/transport - major rate limiting factor

- Route of administration (enteral, parenteral, topical)
- Concentration (enzyme & substrate competition)
  - physical state of formulation & dissolution rate
- Area of absorbing surface
- Vascularity & blood flow
- Movement
- Gastric motility & emptying
- Solubility & binding
- Donnan effect (presence of charged molecules on one side of semi-permeable membrane)
- Vehicle, absorption adjuvants & excipients
- Previous and concomitant health product exposure

A Simple Comparison

- **Human**
  - **57 putatively functional CYP genes ~ half involved in xenobiotic metabolism; 58 pseudogenes**
- **Mouse**
  - **102 putatively functional CYP genes; 88 pgenes**

CYP: Family > 40% identical (CYP1), Subfamily > 55% identical (CYP1A)

- **Variances in transporters and regulators**

more human *in vitro* and and clinical studies

Microflora

- Human body has 10 microbes for every human cell and 100 microbial genes for every unique human gene.
- Microflora affects immunity, inflammatory disease, obesity and probably others.
- Horizontal gene transfer (HGT) facilitates adaptation to drugs and other perturbations.

Chimeric Mouse

- FDA Guidance for Industry: safety testing of metabolites present in greater than 10%
  - human hepatocytes and liver preparations, static
  - no preset limit
- Chimeric mice carrying human livers (autologous organ: donor hepatocytes ***or*** from embryonic or adult stem cells to repopulate a scaffold of animal liver)
  - does not model extra-hepatic ADME, pathways or networks

Your substance needs activation..

Zhao YN *et al*. 2012.

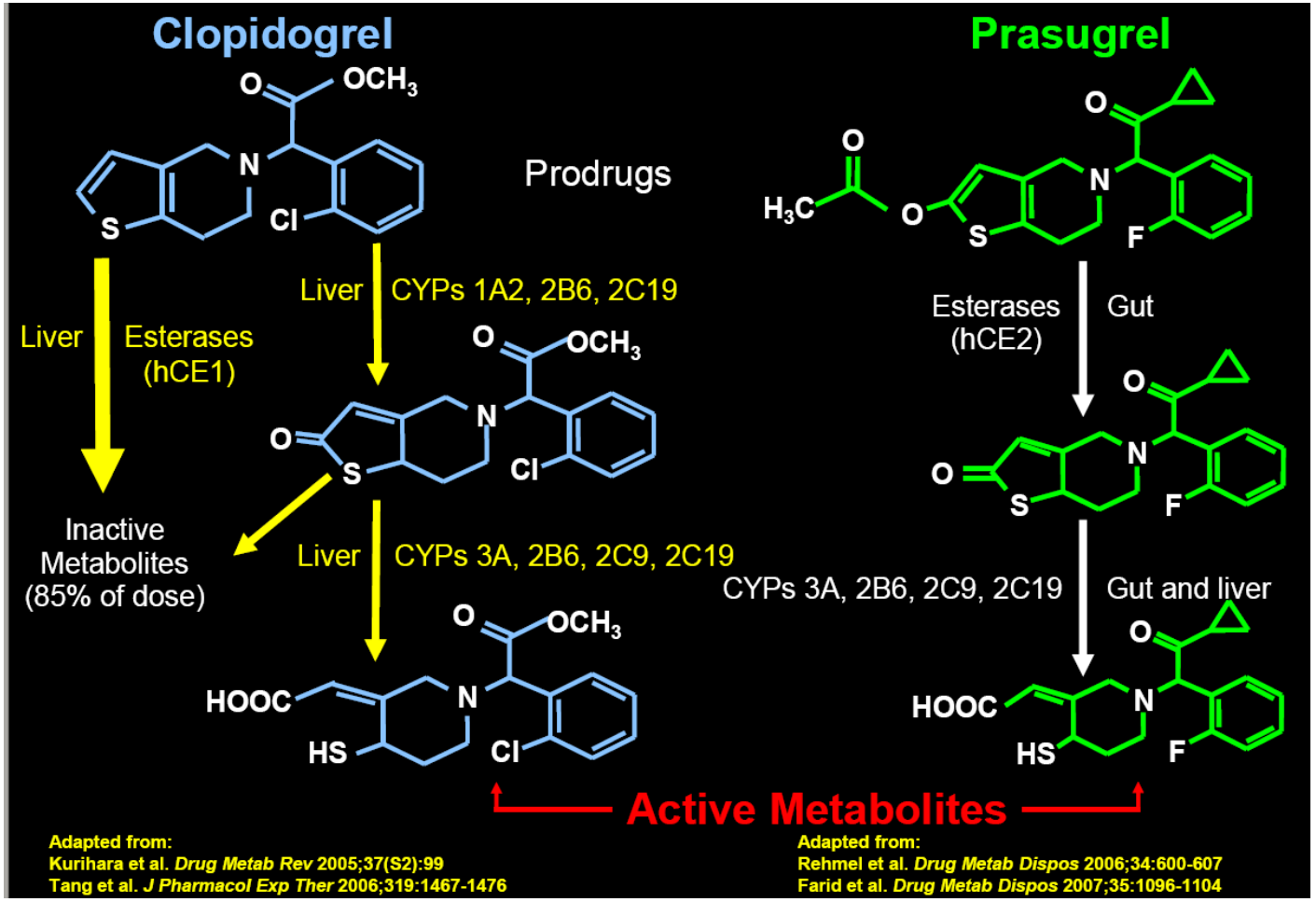
Chinese women: mRNA in breast cancer and (adjacent) tissue relative to hepatic

- CYP1A1 ↓81.8% (↓27.2%)
- CYP1B1 ↓77.5% (↓38.8%)
- CYP3A4 ↓85.6% (↓51.3%)
- COMT ↑6.9x (↑6.4x)

will require tissue (cell)-specific information

http://www.carolsutton.net/download_pink-ribbon.html

Clopidogrel *vs* Prasugrel



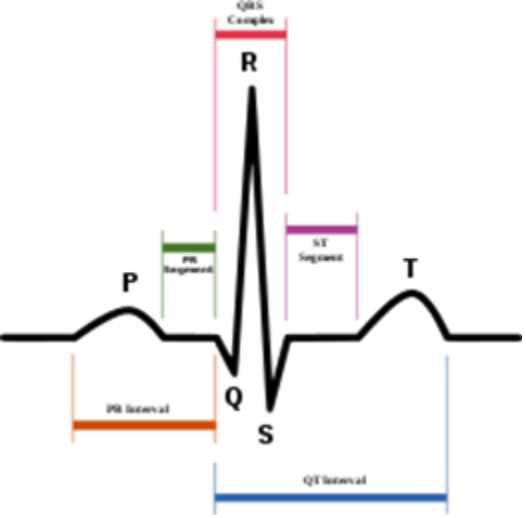
Also a (possible) role for: paraoxonase-1 (PON1) and P-glycoprotein (ABCB1)

modified from Close *et al.* , courtesy M. Phillips

Paraoxonase-1 is Not a Major Determinant of Stent Thrombosis in a Taiwanese Population

Chen *et al*. PLoS One. 7(6), 2012.

- 0.64% incidence of ST in 4964 patients (~32 cases).
- Assessed genetic polymorphisms in 20 ST subjects and in 40 age- and sex-matched controls.
- Reported relative % of each allele; variations in *PON1* and *ABCB1* showed no significant association with ST.
- *CYP* 2C19*2 allele was significantly associated with ST (*P*=0.031).
  - but, no differences in stages of onset
- **Some concerns - questions:**
  - [clopidogrel] plasma?, and
  - if 2C19*2 is significant, why was the ST incidence rate << 12%
  - did not show haplotype for these 20 subjects; hence no conclusive data



Torsades des pointes

- **Drug interactions may lead to QT prolongation**
  - **associated with some Class 1A, 1C and II anti-arrhythmics, anti-psychotics, tricyclic/tetracyclic anti-depressants, macrolide and quinolone antibiotics, etc.**
- **So may NHP and food interactions: Grapefruit and tonic (quinidine) a deadly combination in a patient with long QT.**
- **US herbal/supplement sales in 2011**

> $5.3 bn US Sales 2011

HerbalGram, Sept 2012

Food, Drug Market

- Cranberry
- Soy
- Saw palmetto
- Garlic
- Ginkgo
- Milk thistle
- Echinacea
- Black cohosh root
- St. John's wort
- Ginseng

Natural, Health Foods

- Flax seed/oil
- Wheat/barley
- Tumeric
- Aloe
- Milk thistle
- Spirulina
- Saw palmetto
- Elderberry
- Echinacea
- Garlic

Overtreatment – Implications

Fiona Godlee, editor BMJ, October 10, 2012

Due to:

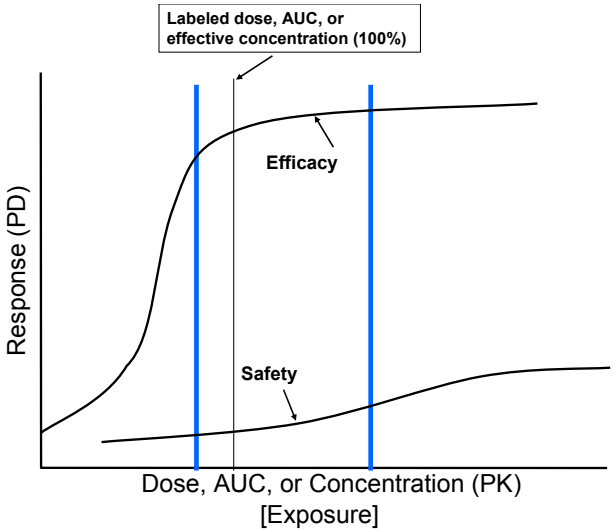
- fear of malpractice lawsuits,
- supply driven demand,
- knowledge gaps,
- biased research,
- profit seeking,
- patient demand,
- financial conflicts of guideline writers,
- failure to fully inform patients of the potential harms of elective treatments, and
- physician fee for service.

Inactive but not Inert

- Wright (2010) noted that chemical detritus of antibiotics may be **inactive** but yet capable of affecting a physiological – pharmacological response.
- Substances (non-medicinal) that lack the desired PD activity may affect other physiological processes, thereby altering PD or PK of the active.

Type of Interaction : + *or* – outcome
Inhibitor – Inducer (or both)

- **Kinetics: a**  **b**
  - **competitive**
  - non-competitive
  - un-competitive



- **Direction: a**  **b**
  - **reversible**
  - quasi-reversible: tight binding
  - irreversible: inactivation (mechanism-based, covalent binding, suicide-substrate, time-dependent, metabolism-dependent)

Inter-individual Variations

- Past and current exposures to xenobiotics
  - "Environment often trumps genetics. Even if you've been dealt a bad hand of genes, it's not a life sentence for most people," The Globe and Mail quoted Hegele as telling delegates to the Canadian Cardiovascular Congress in Edmonton.(Oct 2009) …

Pharmacogenetic and toxicogenetic factors rarely act alone; they produce a phenotype in concert with other variant factors – often tissue specific & polygenic which can change expression and catalytic function

Genotype ≠ Phenotype

- **both need to be detailed**
- **need to work closer with clinicians**

Protein Therapeutics

- still a relatively new class of drugs (*e.g.*, Herceptin);
- knowledge of protein drug distribution remains limited;
- determination of four critical parameters: clearance, volume of distribution, half-life, and bioavailability.
- PK issues: distribution of biomolecules depends on the molecule (*e.g.*, charge, size, binding affinity, and dose), target tissue characteristics (*e.g.*, vascular and interstitial volumes, vascular permeability, target expression level, and pressure differences between vascular and interstitial spaces).

Nanotherapeutics

- complex multi-component products may be susceptible to greater PK variability due to variations resulting from manufacturing, intended use, localized toxicity, biodistribution and clearance mechanisms that can affect the pharmacological action of the active substance;
- full understanding of the active and non-medicinal ingredients and their interactions is critical to establishing the safety and efficacy of these products;
- particularly important in products where proteins or nucleic acids, which may be sensitive to the manufacturing process, form an integral part of the product.

Pharmacoenhancement / Combination products

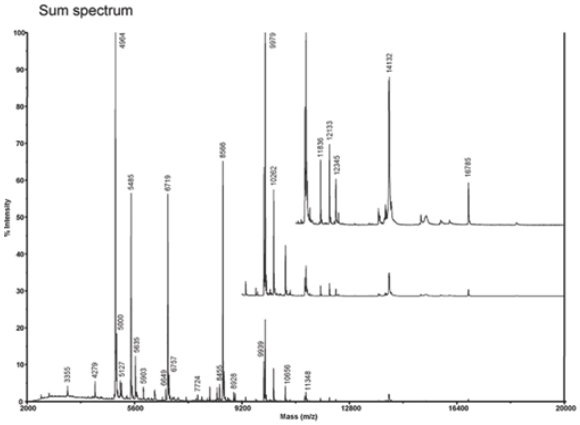
- Interactions intentionally explored to boost, enhance or spare the plasma or intra-cellular PK levels or parameters of one or more substances.
  - interactions with hepatic, intestinal and microbiome metabolic enzymes and transporters.
- Boosting substance should have a PK profile similar to that of the boosted substance.
  - The lowest dose of each substance to achieve the required PD effect;
  - Plasma levels may be gauged by pre-dose or trough levels, which most closely approximate C_{min}.
  - Safety depends on the tolerability of the boosted substance and the new PK profiles of both substances throughout the combination period to ensure stability without major fluctuations which may further exacerbate bioavailability or resistance.

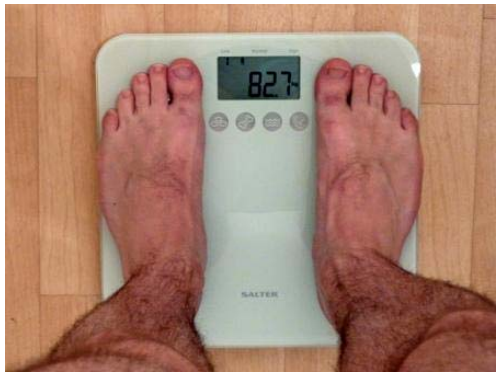
Toxicity Testing

- *in silico,* biomarkers, surrogate assays, the ‘omics, ***etc.***

useful only if it can be used to facilitate more informed and efficient responses to the public-health concerns (*i.e.* limited resources, reduce clinical & societal harm)

too much information: clinical utility – relevance?





PM: beyond ICH E8 - 1

- **Basic clinical trial issues**
  - **better definition of patient:**
    - **clinical disease (geno- and phenotype)**
    - **local or global market – extrapolation to larger markets**

  - **Clinical effectiveness – comparative effectiveness**
    - **acute &/or chronic studies (sequence: prior, concomitant, after)**
    - **current RCTs are insufficient, observational also required**
    - **multiple goals – require clinical trial redesign**
      - **arms: who are the test and control subjects (-/-, +/-, +/+ symptomatics / asymptomatics)?**
      - **comparator treatment &/or population level intervention, reduced morbidity & mortality**
      - **pharmaco-economics (including cost of screening)**

PM: beyond ICH E8 - 2

Systems biology - organism analysis: demonstrate biologic plausibility rather than spurious association in multi-allelic disease or therapy

Standards & guidelines for power, consideration for confounding factors, revisit the statistical methods

- individual (& sub-population) assessment to factor/marker/haplotype ($p < 10^{-6}$ - 10^{-7})
- much more data (incl. more subject information)
  - large, statistically valid, multi-centered, multi-ethnic, multi-factorial studies are required (real world inclusivity, *i.e.* incl. First Nations),
  - may need national health care collection & documentation of drug response phenotypes
  - 4 PM niches: one size does NOT fit all

Future Interaction Clinical Trials

Clinical utility – relevance to the patient population

Highest approved or proposed dose with the shortest dosing interval (*i.e.* OD, bid, tid, qid) to maximize the possibility of an interaction

- **initial studies with strong inhibitor and inducer;**
- **where possible, use substances likely to be co-administered.**

statistical significance ≠ clinical relevance

Interaction Reporting
(& publications)

- **PK Interactions: AUC, Cmax, Tmax, (Cl, Vd, *etc*.) for <u>both</u> substances;**
- **90% confidence intervals around the geometric mean ratio of the PK measures of (S+I) and (S alone)**
  - clinical importance (*e.g.* potency), dosing adjustments, contraindications, monitoring, precautions or warnings; and
- **no effect boundaries &/or clinical equivalence intervals,**
    - interval – time within which a change in systemic exposure is not considered clinical significant; and
    - do not overly state the limitations of the studies…..

Thank you

Merci

Megwetch

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