

Pharmacokinetic and Pharmacodynamic Considerations in
the Development of Macromolecules
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OUTLINE OF LECTURE TOPICS

Macromolecules

Interspecies Scaling

Pharmacokinetic Characteristics
Scientific Issues

Pharmacodynamics

Monoclonal Antibodies

REPRESENTATIVE MARKETED MACROMOLECULES

<u>Macromolecule</u>	<u>Trade Name</u>
Erythropoietin	Epogen (Amgen)
Growth Hormone	Nutropin (Genentech)
G-CSF	Neupogen (Amgen)
IL-2	Proleukin (Chiron)
IL-11	Neumega (GI)
Factor IX	BeneFIX (GI)
rt-PA	Alteplase (Genentech)

APPROVED MONOCLONAL ANTIBODIES

Name	Approval	Indication
Avastin Bevacizumab	Feb, 2004	First line (with 5-FU) in metastatic colon CA
Erbitux Cefuximab	Feb, 2004	Alone or in combination in metastatic colon CA
Raptiva Efalizumab	Oct, 2003	Moderate to severe psoriasis
Xolair Omalizumab	June, 2003	Asthma
Humira Adalimumab	Dec, 2002	Prophylaxis of acute organ rejection
Campath Alemtuzumab	May, 2001	Second line treatment of β -cell CLL in patients

ASSAYS FOR MACROMOLECULES

Immunoassays

ELISA (Enzyme-Linked Immuno-sorbent Assay)

RIA (Radioimmunoassay)

IRMA (Immunoradiometric Assay)

RRA (Radioreceptor Assay)

INTERSPECIES SCALING OF MACROMOLECULES

Factors to Consider

- Species specificity
- Glycosylation and sialation
- Binding proteins
- Size, shape and charge
- Relative abundance of tissue receptors

ALLOMETRIC EQUATIONS FOR SOME MACROMOLECULES

Macromolecule	Allometric V_1	Equations CL
Factor IX	87 $W^{1.2626}$	14 $W^{0.68}$
Factor VIII	44 $W^{1.04}$	10 $W^{0.69}$
IL-12	65 $W^{0.85}$	8 $W^{0.62}$
GH	68 $W^{0.83}$	7 $W^{0.71}$
Rt-PA	91 $W^{0.93}$	17 $W^{0.84}$

INITIAL COMPARTMENT VOLUME
PREDICTED BY ALLOMETRIC SCALING COMPARED WITH OBSERVED
 V_1

	[mL]	[mL]
FIX	18,380	10,150
Factor VIII	3,617	3,030
IL-12	2,406	3,360
GH	2,243	2,432
rt-PA	5,814	4,450

ELIMINATION CLEARANCE
PREDICTED BY ALLOMETRIC SCALING
COMPARED WITH OBSERVED CL

Macromolecule	Human Parameter: Predicted [mL/hr]	CI Observed [mL/hr]
FIX	248	434
Factor VIII	195	174
IL-12	113	406
GH	148	175
rt-PA	646	620

ALLOMETRIC EQUATIONS for
EGF Mab PK PARAMETERS

Parameter (Y)	Coefficient (a)	Exponent (b)	r
V_d (mL)	219	0.84	0.92
CL (mL/hr)	4.07	0.85	0.94

COMPARISON BETWEEN the PREDICTED EGF PK PARAMETERS and OBSERVED PK PARAMETERS

<u>Parameter (Y)</u>	<u>Predicted PK Parameter Estimate</u>	<u>Observed PK Parameter in Cancer Patients</u>
V _d (L/kg)	0.01	0.04
CL (mL/hr/kg)	0.22	0.98

PHARMACOKINETIC CHARACTERISTIC OF MACROMOLECULES

Endogenous concentrations

Absorption

Distribution

Metabolism

Elimination

THE PROBLEM OF ENDOGENOUS CONCENTRATIONS OF MACROMOLECULES

Endogenous concentrations - What do you do with them?

Two examples

Growth Hormone

Erythropoietin

Growth Hormone

Three plots – the first is Plasma GH (mU/L), the second is Plasma GH (mU/L), and the third is GH secretion (U/h) and all three are over clock time (hours). The second plot also shows GH secretion (U/h) and the third plot also shows cumulative GH secretion (U/24 h).

Albertsson-Wikland K, et al. Am J Physiol 1989;257:E809-14.)

ERYTHROPOIETIN

Plot showing the mean serum EPO concentration, mIU/mL of Erythropoietin over time, study days. The plot shows the levels after the placebo, 150 IU/kg t.i.w., 450 IU/kg, 900 IU/kg, 1350 IU/kg, and 1800 IU/kg EPO over time, days.

Cheung et al CPT 1998; 64:412-423

ABSORPTION OF MACROMOLECULES

Flip-flop model

Site of administration

RELATIONSHIP BETWEEN MW AND LYMPHATIC ABSORPTION OF WATER SOLUBLE COMPOUNDS

Plot showing this relationship with Inulin, Cytochrome C, and IFN-alpha-2b, and lymph recovery (% of dose), from 0 to 80, over molecular weight (kDa) from 0 to 20.

Supersaxo A et al. Pharm Res 1990; 7:167-169

COMPARISON OF ABSORPTION AND ELIMINATION RATE CONSTANTS

<u>Macromolecule</u>	<u>Route of Administration</u>	K_a (hr ⁻¹)	K_e (hr ⁻¹)
GH	SC	0.23 ± 0.04	0.43 ± 0.05
	IV		2.58
IFN- α -2b	SC	0.24	0.13
	IV		0.42
Erythropoietin	SC	0.0403 ± 0.002	0.206 ± 0.004
	IV		0.077

SITE OF INJECTION EFFECTS ON EPO ABSORPTION

Two plots which show this absorption from the femur and abdomen by s-EPO (U/t) from 0 to 350 over time (h).

Jensen JD et al Eur J Clin Pharmacol 1994; 46:333-337

DISTRIBUTION OF MACROMOLECULES

Volume of Distribution

Binding Proteins

DISTRIBUTION VOLUMES OF REPRESENTATIVE MACROMOLECULES

<u>Macromolecule</u>	<u>MW (kDa)</u>	<u>V₁ (mL/kg)</u>	<u>V_{ss} (mL/kg)</u>
Inulin	5.2	55	164
Factor IX	57	136*	271*
IL-2	15.5	60	112
IL-12	53	52	59
G-CSF	20	44	60
rt-PA	65	59	106

* Calculated from
literature

PHARMACOKINETICS of MARKETED MONOCLONAL ANTIBODIES

Mabs	Molecular Weight (kD)	$T_{1/2}$^a (Days)	V_I^a (L)	V_{ss}^a
Avastin	149	13-15	3	3.5-4.5 L
Erbitux	152	ND ^b	2.7-3.4	2-3 L/m ²
Raptiva	150	6-7.5 ^c	NR ^d	9 L ^e
Humira	148	12-18	3	5 L
Campath	150	1-14 ^f	NR ^d	7-28 L

EFFECTS & RELEVANCE OF MACROMOLECULE BINDING TO α_2 - MACROGLOBULIN

<u>Macromolecule</u>	<u>Effect</u>	<u>Relevance</u>
NGF		Assay interference
IL-1	Regulation of Proliferation of thymocytes	Regulatory protein
IL-2	Impaired proliferation of T-cells	Inactivation
TGF β	Growth of kidney fibroblasts	Clearance

HYPOTHETICAL MODEL of the BINDING EFFECTS of IGF-1

IGF-I gives rise to IGF-II, which binds to IGFBP-2 – Free IGF-II crosses the vascular endothelium to reach the extravascular space.

METABOLIC EFFECTS OF MACROMOLECULES

Effects on P450s

EFFECTS OF MACROMOLECULES ON P450 CYP ENZYMES

<u>Macromolecule</u>	<u>Isoenzyme</u>	<u>Effects</u>
IFN- γ	CYP2C11	Decreased mRNA and enzyme levels
IL-1	CYP2C11	Decreased mRNA and enzyme levels
	CYP 2D	Decreased mRNA and enzyme levels
IL-2	CYP2D1	Increased mRNA and enzyme levels
IL-6	CYP2C11	Decreased mRNA and enzyme levels
TNF	CYP2C11	Decreased enzyme levels

EXCRETION OF MACROMOLECULES

Contributions of kidney and liver

CHO vs E. Coli produced

Receptor mediated clearance

RELATIONSHIP BETWEEN MOLECULAR WEIGHT AND ELIMINATION CLEARANCE

CL (mL/min/kg), from 0 to 7, over molecular weight (kd) from 0 to 80.

There is no apparent correlation between MW and CL.

IL-2, IGF-1, IL-12 and rt-PA shown as examples.

LIVER CELL SURFACE RECEPTORS FOR CLEARANCE OF CARBOHYDRATES & MONOSACCHARIDES

<u>Specificity</u>	<u>Cell Type</u>
Gal/Gal/NAc	Liver parenchymal cells
Gal/GalNAc	Liver Kupffer and endothelial cells Peritoneal macrophages
Man/GlcNAc	Liver Kupffer and endothelial cells Peritoneal macrophages
Fuc	Liver Kupffer cells

DIFFERENCES BETWEEN rhEPO AND NESP
(NOVEL ERYTHROPOIESIS-STIMULATING PROTEIN)

rhEPO	NESP
165 normal amino acid sequence	5 amino acid exchanges
Up to 40% carbohydrate	Up to 52% carbohydrate
3 N-linked sugar chains	5 N-linked sugar chains
Up to 14 sialic acids	Up to 22 sialic acids
30.4 Kd	38.5 Kd
Plasma $T_{1/2}$ = 4-8 hrs	Plasma $T_{1/2}$ = 24 hrs

SERUM CONCENTRATION-TIME PROFILES FOR CHO VS. E. Coli PRODUCED GM-CSF

Graph showing concentration of GM-CSF ng/ml over time/hours

<u>Type</u>	<u>C_{max}</u>	<u>t_{1/2α}</u>	<u>t_{1/2β}</u>
CHO	138	20	68
E. Coli	57	8	75

Mortensen HD et al. Eur J Haematol 1993; 50:32-36

SERUM CONCENTRATION-TIME PROFILES FOR NON-GLYCOSYLATED VS. GLYCOSYLATED G-CSF

Graph showing Serum G-CSF ng/L from 0 to 14000 over time (hours) for non-glycosylated G-CSF (filgrastim) and Glycosylated G-CSF (lenograstim). Their profiles are similar although at approximately 4.2 hours non-glycosylated G-CSF (filgrastim) reaches a higher peak at 14000 compared to glycosylated G-CSF (lenograstim) peak at approximately 11090 at the same time.

Watts et al. Br J Haematol 1997; 98:474-479

RELATIONSHIP BETWEEN G-CSF CLEARANCE AND ABSOLUTE NEUTROPHIL COUNT

Plot of rhG-CSF plasma clearance (ml/h/kg) from 0 to 12 over absolute neutrophil count-ANC (cells/ μ l) from 0 to 7000.

Ericson SG et al. *Exper Hematol* 1997; 25:1313-1325

MONOCLONAL ANTIBODY PRODUCTION

Graphic illustration of hybridomas.

HUMAN IgG

Molecular model

© 1996 Mike Clark

IgG and SINGLE-CHAIN Fv

Graphic illustration

CONCEPT OF ANTIBODIES

Murine, chimaeric, humanised, and human antibodies

PROPOSED HUMAN PLASMA CLEARANCE of DIFFERENT ANTIBODY
MOLECULES

<u>Antibody Molecule</u>	<u>Molecular Weight (kD)</u>	<u>Relative Plasma Clearance (Cl)</u>
Native intact human IgG	150	≈ 21 days
Fully human/humanized	150	
Chimeric human-mouse IgG	150	
Whole mouse IgG	150	
F (ab)₂	110	
Fab'	50	
Single chain FV (scFV)	25	≈ 1 day

DESIGN OF ANTIBODIES

Molecules that can be attached:

Enzymes

Toxins

Viruses

Cationic tails

Biosensors

CHARACTERISTICS THAT AFFECT THE PHARMACOKINETICS OF MACROMOLECULES

Physical characteristics

Post-translational modification

Binding

Route of administration

Duration of administration

Frequency of administration

PATIENT CHARACTERISTICS THAT AFFECT PHARMACOKINETICS OF MACROMOLECULES

Age

Gender

Disease

Concurrent drugs

EFFECTS OF GENDER ON GROWTH HORMONE PK/PD

Daily rhGH dose/kg required to normalize IGF-1 response in GH deficient women is higher than in men

Estrogen replacement also significantly increases rhGH dose requirement

Drug-Drug Interactions

The Journal of Clinical
Pharmacology
<http://www.jclinpharm.org>

Drug Interaction Studies of Therapeutic Proteins or Monoclonal Antibodies
Iftekhar Mahmood and Martin David Green
J. Clin. Pharmacol. 2007; 47; 1540 originally published online Oct 25, 2007;
DOI: 10.1177/0091270007308616

The online version of this article can be found at:
<http://www.jclinpharm.org/cgi/content/abstract/47/12/1540>

PHARMACODYNAMICS OF MACROMOLECULES

Important considerations

Regimen dependency

Endpoints

Models

REGIMEN DEPENDENCY OF IL-12 PHARMACOKINETICS AND IFN- γ STIMULATION

Two graphs showing this with graph A showing IL-12 (pg/mL) from 0 to 48 hours and graph B showing IFN γ (pg/mL) from 0 to 96 hours) over hours.

Motzer RJ et al. Clin Cancer Res 1998;4:1183-1191

PHARMACODYNAMIC ENDPOINTS

Easy - replacement proteins

rFIX

Difficult- cascade of events

IGF-1

RELATIONSHIP BETWEEN rFIX CONCENTRATION AND ACTIVITY

Plot showing factor IX activity (%) over factor IX concentration (ng/mL).

34.5 ng/mL for 1% FIX activity

Schaub et al. Seminars in Hematology 1998; 35:28-32

PK-PD MODEL OF rhGH WITH
MEASURED VS. PREDICTED [IGF-1] AFTER SINGLE AND DAILY SC
rhGH INJECTIONS

Model of rhGH pharmacokinetics

Indirect Response Model of IGF-I induction by rhGH

Sun YN et al. JPET 1999; 289:1523-1532

PHARMACODYNAMIC ENDPOINTS

Omalizumab: Free IgE levels
Clinical outcomes

Basiliximab: Soluble IL-2 receptor
CD25+ T lymphocytes 1%

Summary

Use scientific judgment and good sense in the interpretation of PK/PD results with macromolecules

Application of PK principles that have been developed work with macromolecules

Difficult to select the most appropriate pharmacodynamic endpoint

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