



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	$87 W^{1.26}$	$14 W^{0.68}$
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

Macromolecule	Human Parameter: Predicted (mL)	V_1 Observed (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)	Cl 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

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



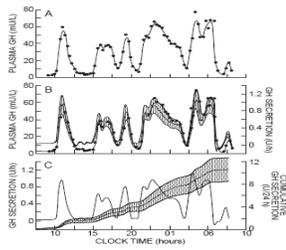
PHARMACOKINETIC CHARACTERISTIC OF MACROMOLECULES

- Endogenous concentrations
- Absorption
- Distribution
- Metabolism
- Elimination

THE PROBLEM OF ENDOGENOUS CONCENTRATIONS OF MACROMOLECULES

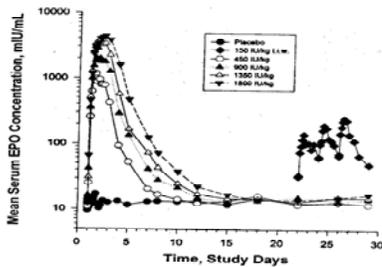
- Endogenous concentrations - What do you do with them?
- Two examples
 - Growth Hormone
 - Erythropoietin

Growth Hormone



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

ERYTHROPOIETIN

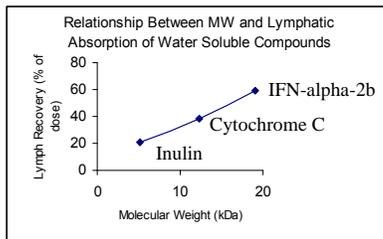


Cheung et al CPT 1998; 64:412-423

ABSORPTION OF MACROMOLECULES

- Flip-flop model
- Site of administration

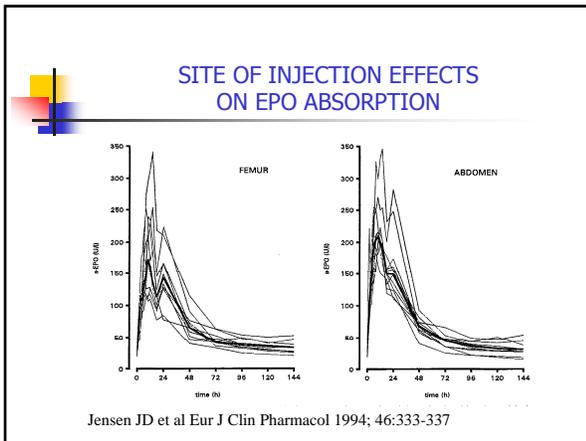
RELATIONSHIP BETWEEN MW AND LYMPHATIC ABSORPTION OF WATER SOLUBLE COMPOUNDS



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

COMPARISON OF ABSORPTION AND ELIMINATION RATE CONSTANTS

Macromolecule	Route of Administration	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



- ### DISTRIBUTION OF MACROMOLECULES
- Volume of Distribution
 - Binding Proteins

DISTRIBUTION VOLUMES OF REPRESENTATIVE MACROMOLECULES

Macromolecule	MW (kDa)	V_1 (mL/kg)	V_{ss} (mL/kg)
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

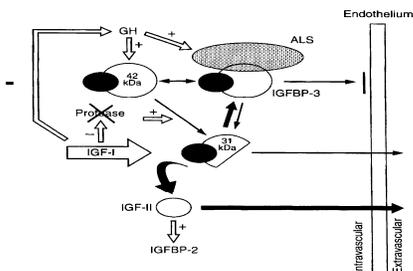
PHARMACOKINETICS of MARKETED MONOCLONAL ANTIBODIES

Mabs	Molecular Weight (kD)	T _{1/2} ^a (Days)	V _f ^a (L)	V _{ss} ^a
Avastin	149	13-15	3	3.5-4.5 L
Erbix	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 α₂-MACROGLOBULIN

Macromolecule	Effect	Relevance
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





METABOLIC EFFECTS OF MACROMOLECULES

- Effects on P450s



EFFECTS OF MACROMOLECULES ON P450 CYP ENZYMES

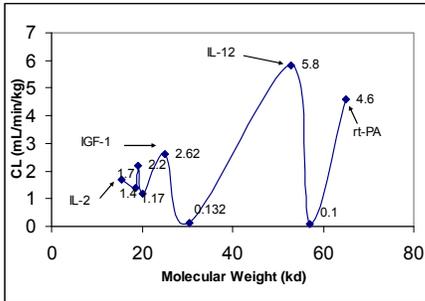
Macromolecule	Isoenzyme	Effects
IFN- γ	CYP2C11	Decreased mRNA and enzyme levels
IL-1	CYP2C11	Decreased mRNA and enzyme levels
	CYP 2D	Decreased mRNA and enzyme levels
IL-2	CYP2D1	Increased mRNA and enzyme levels
IL-6	CYP2C11	Decreased mRNA and enzyme levels
TNF	CYP2C11	Decreased enzyme levels



EXCRETION OF MACROMOLECULES

- Contributions of kidney and liver
- CHO vs E. Coli produced
- Receptor mediated clearance

RELATIONSHIP BETWEEN MOLECULAR WEIGHT AND ELIMINATION CLEARANCE

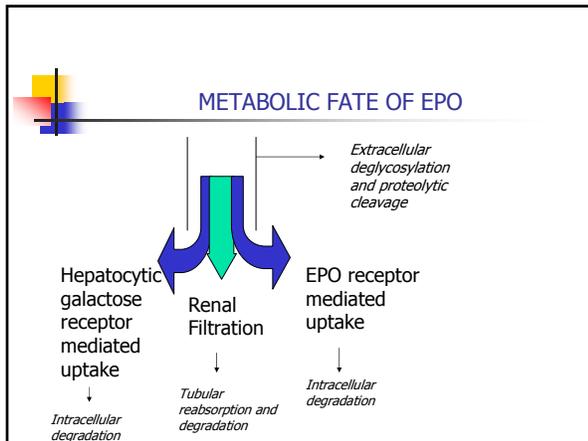


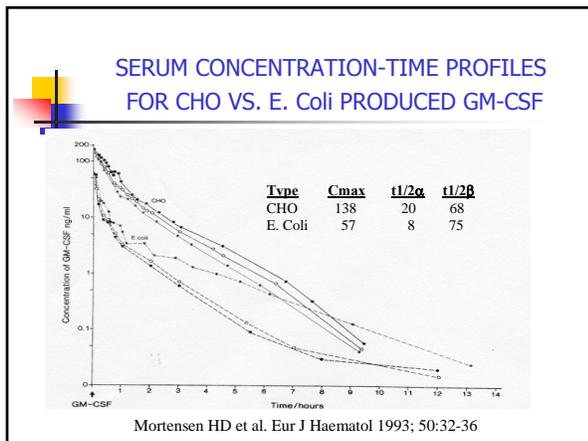
LIVER CELL SURFACE RECEPTORS FOR CLEARANCE OF CARBOHYDRATES & MONOSACCHARIDES

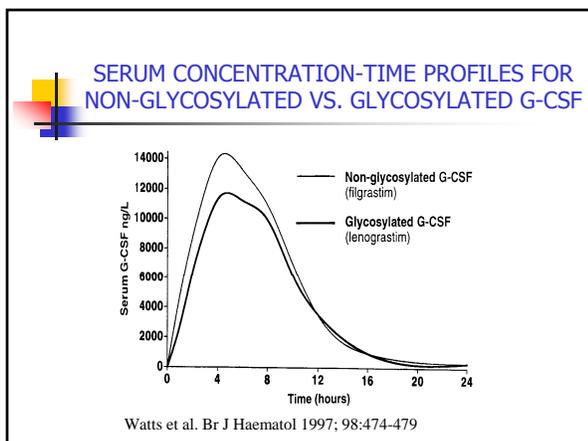
Specificity	Cell Type
Gal/Gal/NAc	Liver parenchymal cells
Gal/GalNAc	Liver Kupffer and endothelial cells Peritoneal macrophages
Man/GlcNAc	Liver Kupffer and endothelial cells Peritoneal macrophages
Fuc	Liver Kupffer cells

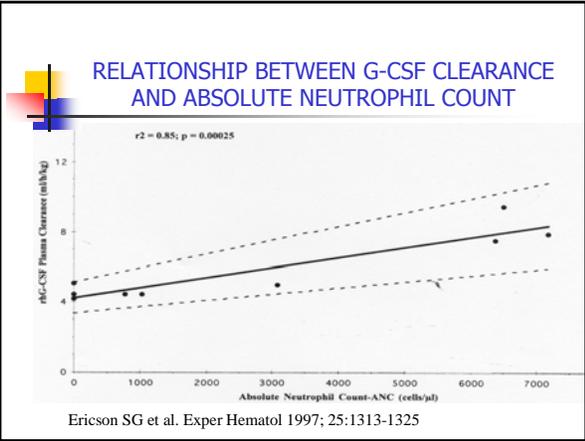
DIFFERENCES BETWEEN rhEPO AND NESP (NOVEL ERYTHROPOIESIS-STIMULATING PROTEIN)

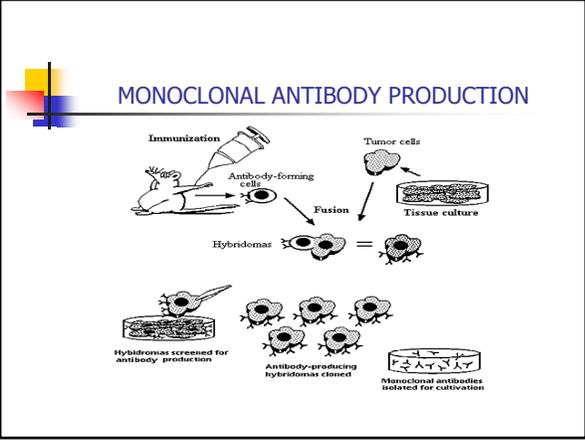
- | | |
|---|---|
| <ul style="list-style-type: none"> ■ rhEPO <ul style="list-style-type: none"> ■ 165 normal amino acid sequence ■ Up to 40% carbohydrate ■ 3 N-linked sugar chains ■ Up to 14 sialic acids ■ 30.4 Kd ■ Plasma $T_{1/2}$ = 4-8 hrs | <ul style="list-style-type: none"> ■ NESP <ul style="list-style-type: none"> ■ 5 amino acid exchanges ■ Up to 52% carbohydrate ■ 5 N-linked sugar chains ■ Up to 22 sialic acids ■ 38.5 Kd ■ Plasma $T_{1/2}$ = 24 hrs |
|---|---|

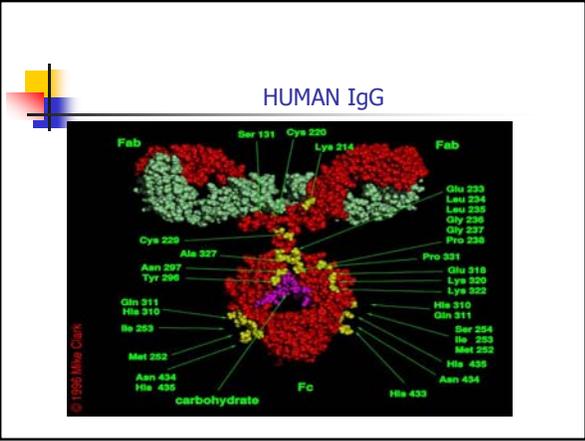


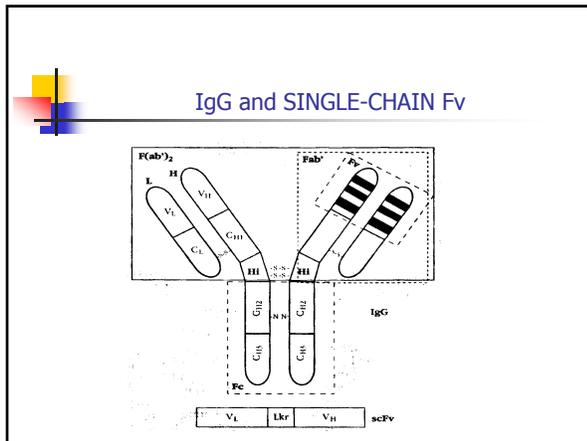


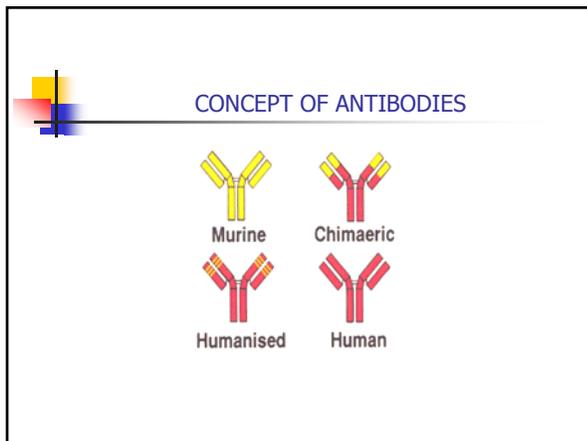












PROPOSED HUMAN PLASMA CLEARANCE of DIFFERENT ANTIBODY MOLECULES

Antibody Molecule	Molecular Weight (kD)	Relative Plasma Clearance (CI)
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.jclpharm.org>

Drug Interaction Studies of Therapeutic Proteins or Monoclonal Antibodies
Richard Malmgren 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.jclpharm.org/cgi/content/abstract/47/12/1540>

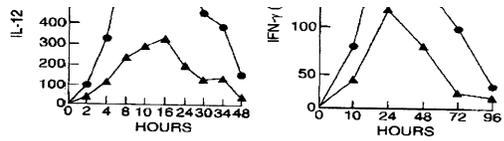


PHARMACODYNAMICS OF MACROMOLECULES

- Important considerations
 - Regimen dependency
 - Endpoints
 - Models



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



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

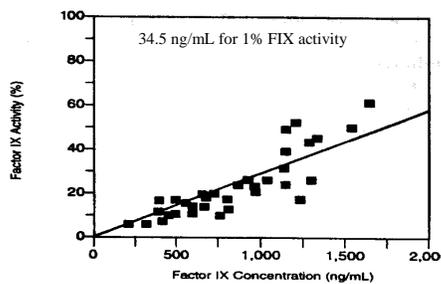


PHARMACODYNAMIC ENDPOINTS

- Easy - replacement proteins
 - rFIX
- Difficult- cascade of events
 - IGF-1



RELATIONSHIP BETWEEN rFIX CONCENTRATION AND ACTIVITY



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

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graph LR
    IS((Injection Site)) -- k1 --> C((Central))
    C -- k12 --> P((Peripheral))
    P -- k21 --> C
    C -- CL --> E((Elimination))
    
```

Indirect Response Model of IGF-1 Induction by rhGH

$$k_{in} \left(1 - \frac{S_{IGF1}}{S_{max} + S_{IGF1}} \right) - k_{out} [IGF-1]$$

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 \square 1%

Summary

- Use scientific judgement 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



Acknowledgements

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