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Skin Metabolism of Biotherapeutics as a Limiting Factor for Subcutaneous Bioavailability

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Pfizer
WORLDWIDE RESEARCH & DEVELOPMENT
Pharmacokinetics, Dynamics & Metabolism – NBE

Outline

- Introduction of bioavailability
- Introduction of peptide PK
- Overview of technologies for peptide half-life extension

- Case Study of Understanding Low SC F% of a Peptide
  - Introduction of Oxynmodulin
  - Problem
  - Methods
  - Results

Bioavailability

➢ Fraction of the dose of a drug contained in any dosage form that reaches the systemic circulation in unchanged or active form administered through any route is known as bioavailability.

➢ Drugs injected using intravenous route of administration have 100% bioavailability, while others have much less bioavailability, because:

- ❑ All of the drug may not be adsorbed
- ❑ Metabolism of the drug might occur before reaching the site of action



Challenges to Predict Subcutaneous Bioavailability of Biotherapeutics in Human

	Name	Description	Bioavailability (%F)		
			rat	NHP	human
Therapeutic protein	Aranesp	darbepoetin alfa			37%
	Betaseron	IFNβ-1b	**16%**	**12-38%**	**6-62%**
	Cimzia	PEG-anti-TNFα Fab	**24-34%**		76-88%
	Epogen	epoetin	50%	**26-100%**	**15-35%**
	Kineret	rhIL-1R antagonist	62%	88%	95%
	Mircera	PEG-EPO	31-45%		54-62%
	Neulasta	PEG-GCSF	5-23%	**49-68%**	**5-15%**
	Somavert	PEG-hGH		**70-81%**	49-65%
	Pegasys	PEG-IFNα-2a			61-80%
	PEG-Intron	PEG-IFNα-2b	43-51%	57-89%	
mAb/Fc fusions	Amevive	LFA3-Fc			57%
	Arcalyst	(IL-1RI/IL-1RAcP)-Fc	60%	**70%**	43%
	Embrel	TNFR-Fc		73%	58%
	Humira	Anti-TNFa mAb		89%	64%
	Ilaris	Anti-Il1β mAb		60%	63-67%
	Raptiva	Anti-CD11a mAb		36%	50%
	Simponi	Anti-TNFa mAb		77%	53%
	Stelara	Anti-p40 mAb		97%	**24-95%**
	Xolair	Anti-IgE mAb		90%	53-71%

- Wide range of bioavailability (%F) and high degrees of variability
  - Especially for therapeutic proteins
- Lack of preferred animal model or scaling strategy

The prefered %F is > 60%

Source: FOI and McDonald et al., (2010) Curr Opin Mol Ther 12:461-470

Peptide Usually Pertains Short Half-Life ($t_{1/2}$)

Table 2 Molecular Size and Glomerular Filtration. The Glomerular Sieving Coefficient (GSC) Is Calculated as the Ratio of Concentration in the Ultrafiltrate to the Plasma Concentration. From Rowland and Tozer (121).

Protein	Molecular Weight (kD)	Glomerular Sieving Coefficient (GSC)
Insulin	6	0.89
Lysozyme	14	0.80
Myoglobin	16.9	0.75
Growth hormone (GH)	20	0.72
Superoxide dismutase (SOD)	32	0.33
Bence Jones	44	0.08
Albumin	69	0.001
IgG	160	0.000

Peptide with size <20 kD has $t_{1/2}$ ranging from minutes to hours, rending BID or QD dosing

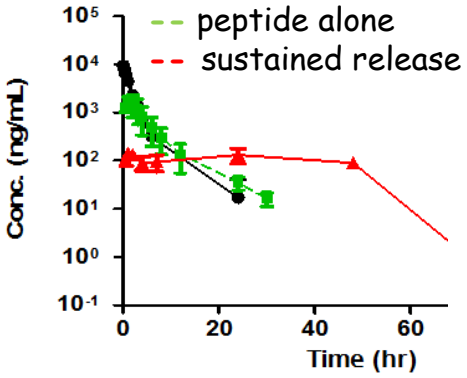
Ways to Extend Peptide Half-Life

Conjugation:

- Pegylation
- Others such as Hesylation etc.

Sustained Release:

- transdermal patches
- polymeric micelles
- Complexation, injectable depots such as those in which the drug is embedded in
  - polymeric matrix, vesicles (e.g. liposomes)
  - biodegradable microspheres, hydrogels
  - formation of a reversible, water insoluble complex of the protein with divalent metal cations
  - combination of the above

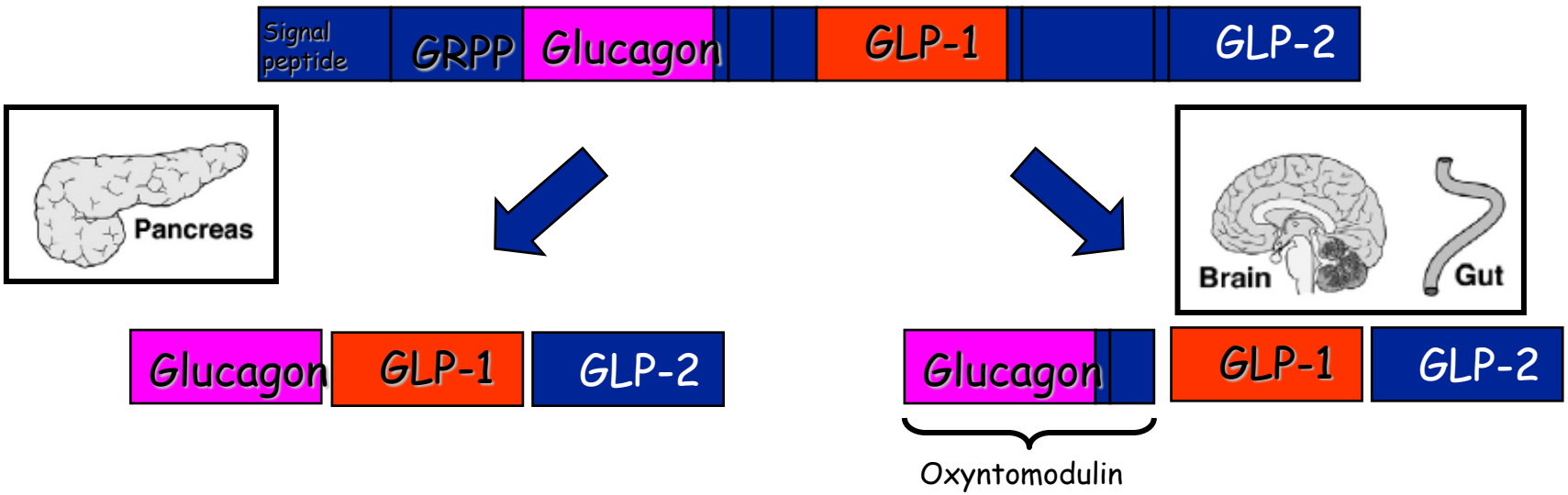


In addition to improving half-life, the sustained-released formulations can also help to reducing the fluctuation between peak and trough concentrations and maintain the serum concentration relatively constant within the therapeutic window, in contrast to the PK profiles obtained in soluble solutions

Oxyntomodulin as a Target for Type 2 Diabetes and Obesity

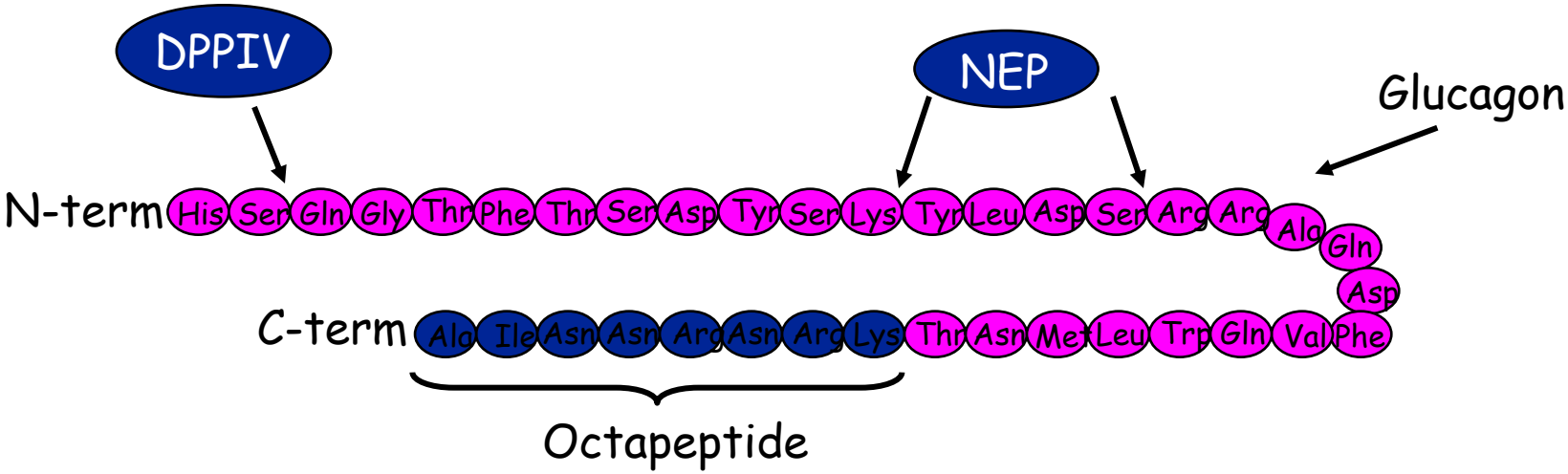
- Oxyntomodulin is a 37 amino acid peptide secreted by the L-cells of the small intestine

- It is derived from the processing of the preproglucagon gene by prohormone convertase (PC)



Oxyntomodulin is Cleaved by Two Major Peptidases

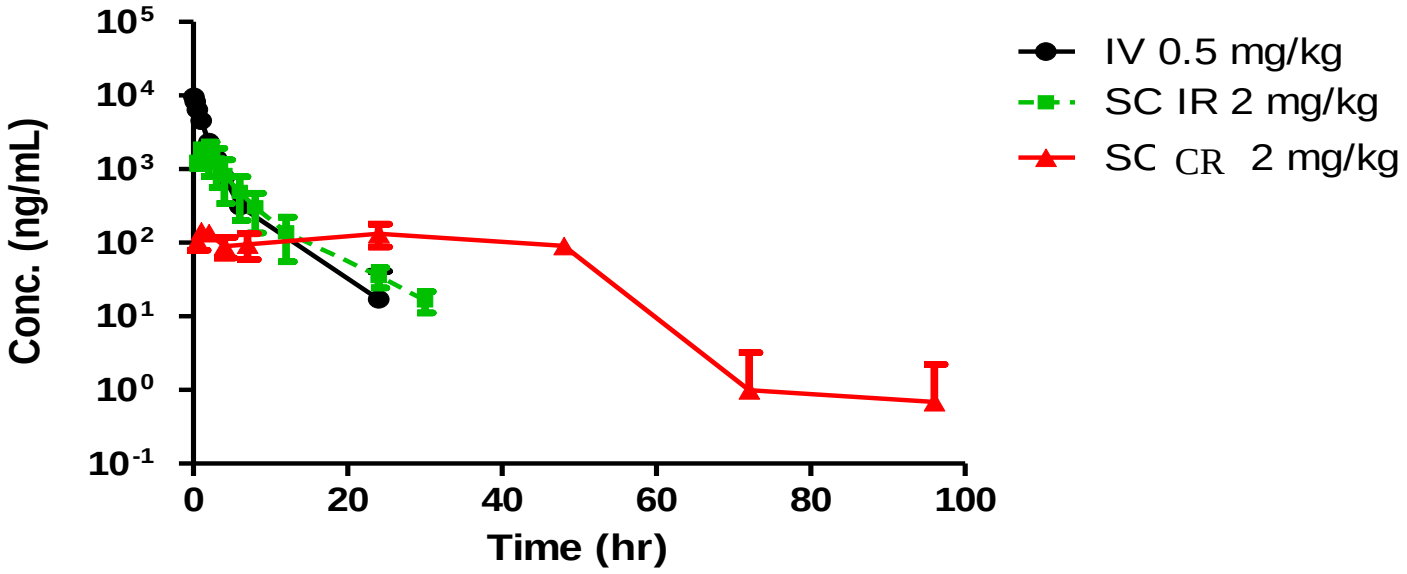
- Oxyntomodulin contains entire glucagon sequence plus 8 amino acids (octapeptide)
- 100% homologous between human, mouse, rat
- Cleaved by 2 major peptidases: DPPIV and neutral endopeptidase (NEP)
- Oxyntomodulin is not an appropriate therapeutic due to proteolysis and poor PK properties.



Generation of OXM Analog: Peptide A

- Peptide A is an OXM analog that was designed to be stable against DDPIV and NEP
- Resulting peptide is resistant to proteolysis (DPPIV and NEP degradation) with an in vivo half-life of 3 hrs in humans (versus 10 minutes for oxyntomodulin).
- Optimal short-term weight loss observed in human after SC injection of Peptide A.
- Two formulations (IR: instant release; CR: controlled release) have been evaluated in animals with the primary objective of evaluating CR formulations intended to provide prolonged Peptide A exposure compared with the IR formulation

Decreased Exposure with CR Formulation Was Observed in Rats



Route	Dose	C_{max}	T_{max}	$t_{1/2}$	AUC_{last}	CL	V_{ss}	F
	(mg/kg)	(ng/mL)	(hr)	(hr)	(hr*ng/mL)	(ml/hr/kg)	(mL/kg)	(%)
IV	0.5	9638	NA	2.9	15757	30	68	NA
SC	2, IR	1835	1.3	4.7 (n=1)	9653	NA	NA	15
SC	2, CR	160	10	10 (n=1)	5506	NA	NA	9

Wang et al., J Bioequiv Availab 2012, 4:5

Why Low SC BA of IR Form, and Even Lower for CR Form

Possibilities:

Where does the drug (IR and CR Form) go - why lower BA):

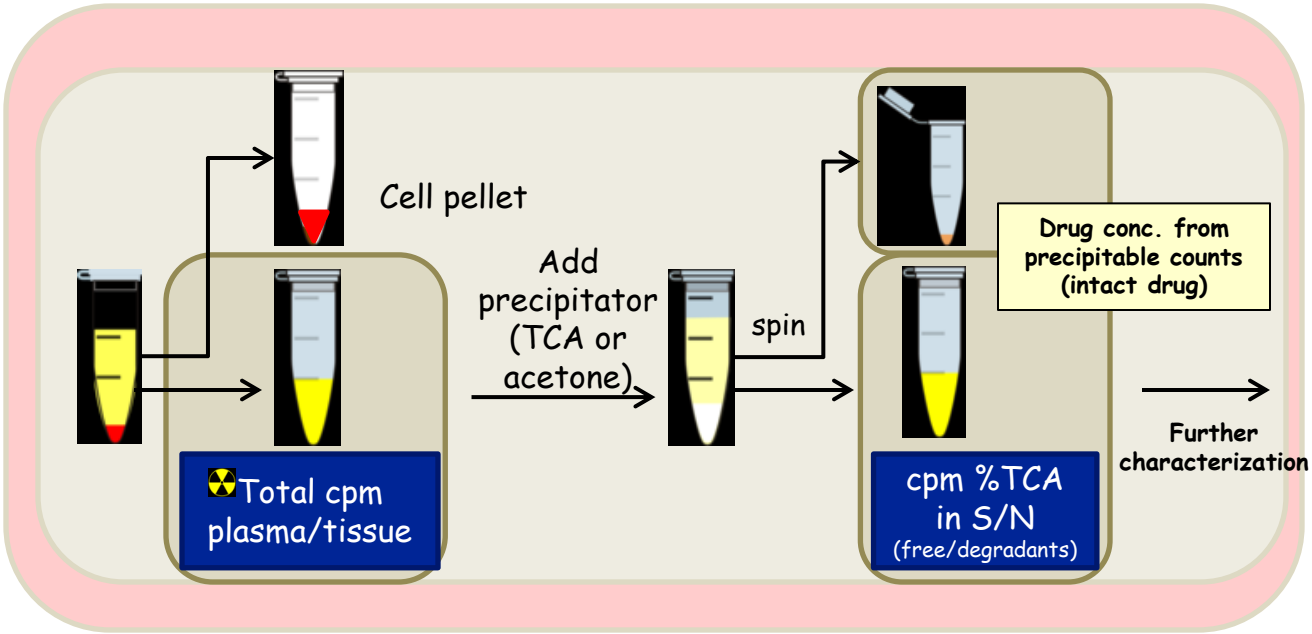
does the drug stay in skin or degraded in the skin or something else?

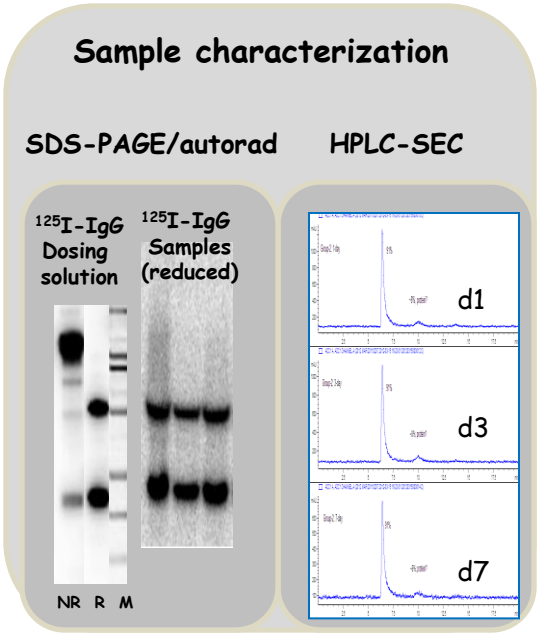
Plans:

- Dose recovery study (where does the drug go)
- *In vitro* stability study in skin
- Metabolite investigation (*in vitro* and *in vivo*)

Analysis of Drug Disposition in Plasma/Tissue

•Measuring total drug counts together with precipitable drug counts indicates degree of free radiolabel or proteolytic drug degradation (ie too small to precipitate)



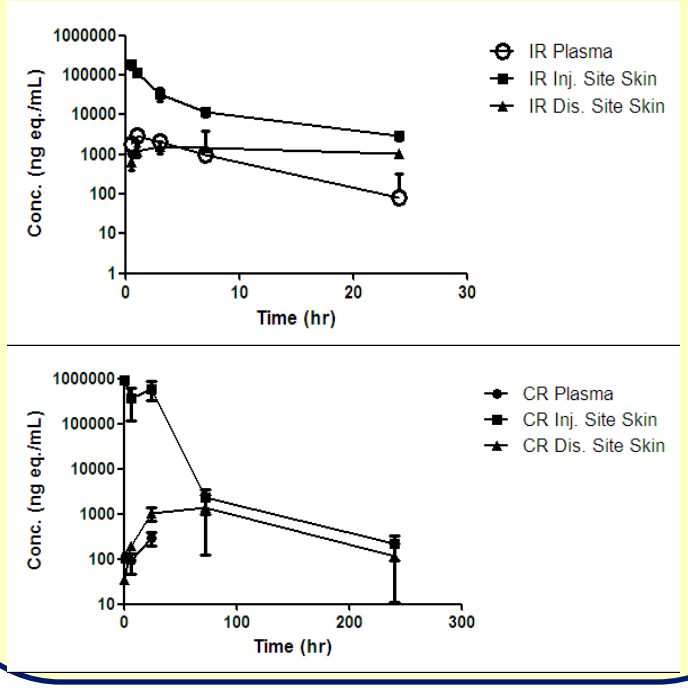


Good Recovery of ^{125}I Radioactive Dose Injected in Rats
---- Radiolabled dose was well absorbed from injection site

Groups	Route	Formulation	Dose	Conc.	Dose vol.
			(mg/kg)	(mg/mL)	(mL/kg)
1	IV	IR	0.5	5	0.1
2-7	SC	CR	2	5	0.4

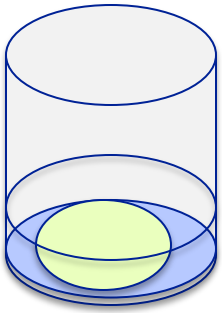
- SD rats, N=3, serum, tissues, urine and feces were collected at various time points up to 10 days.

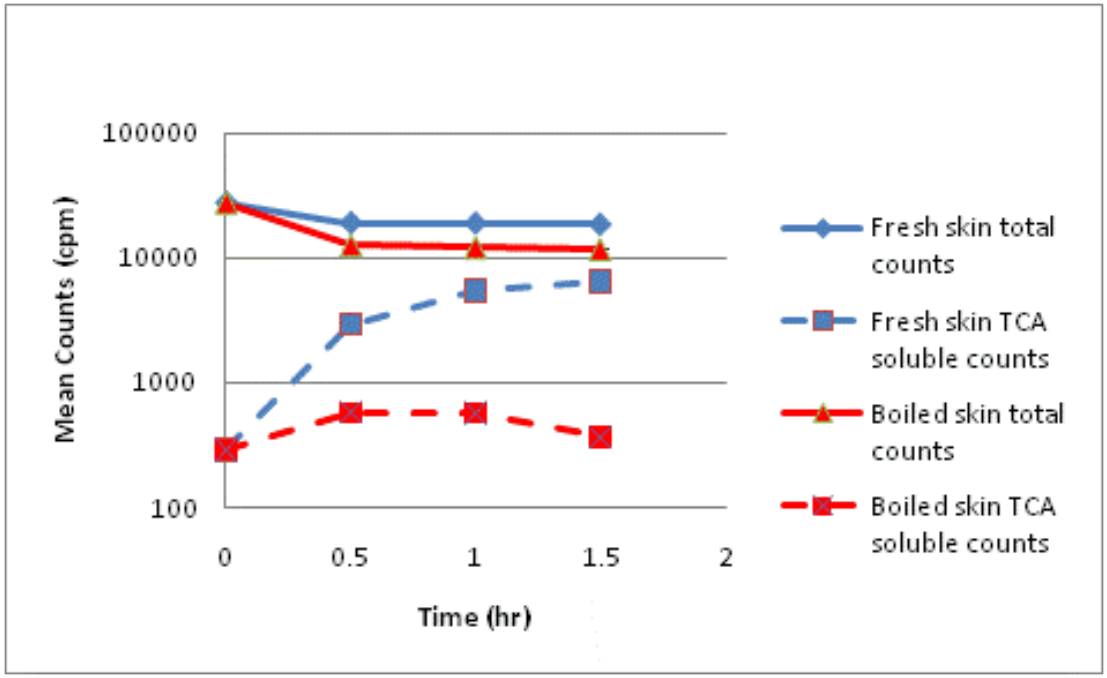
%Dose Recovery	IV group 0.5 mg/kg		SC group 2 mg/kg	
	Ave.	Stdev	Ave.	Stdev
Tissue	2	1	1	1
Urine	85	7	95	16
Fece	2.5	5.4	0.0	0.1
Total	**90**	6	**97**	16



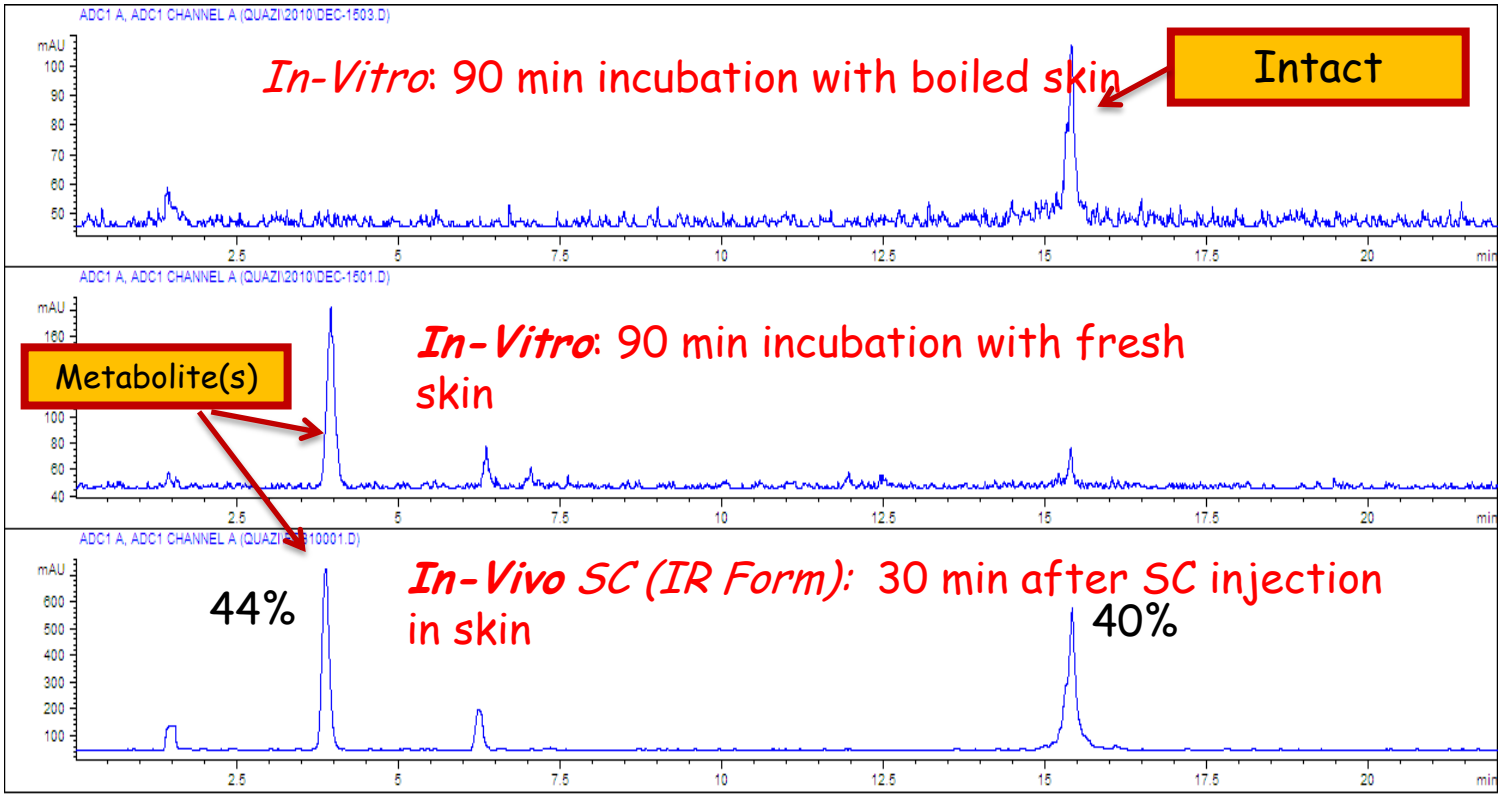
Metabolism was Observed in Skin – In Vitro Study

Skin sample (1 inch in diameter, freshly cut or boiled) was incubated with 2 μg/mL [^{125}I]Peptide A in PBS at 37°C (total incubation volume: 1 mL). The incubation solution was analyzed for total and TCA soluble counts)



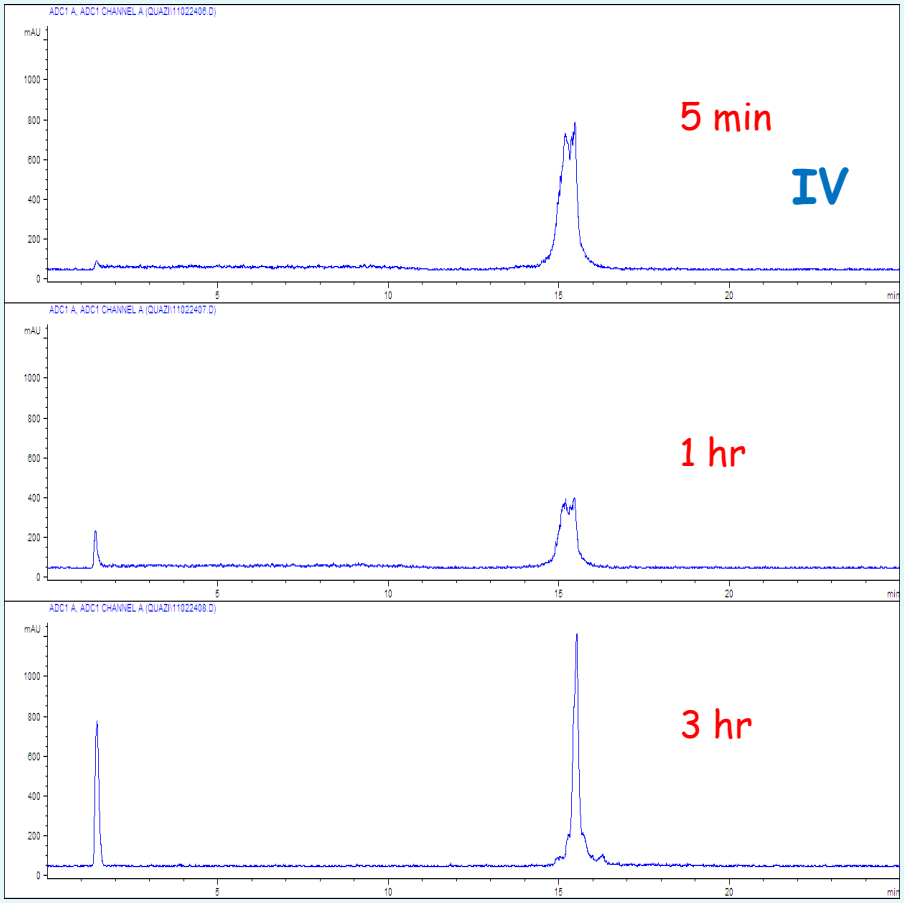


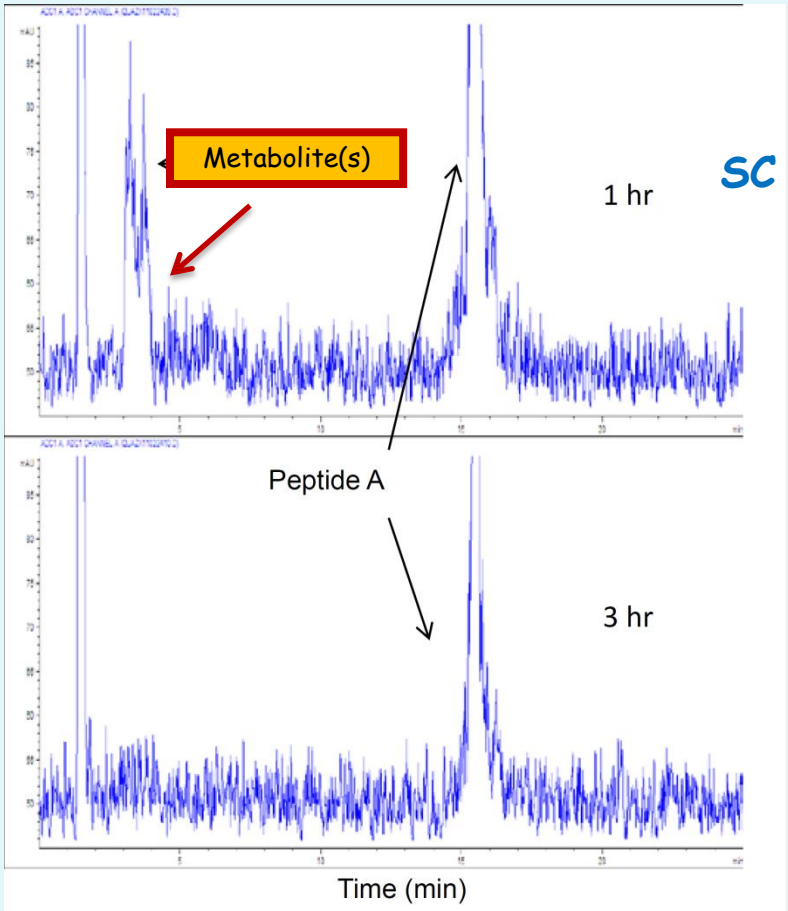
Metabolites Observed in Skin from In Vivo and In Vitro Studies



LC-Radio-chromatogram of Peptide A Metabolism in Skin

Skin Mediated Metabolite(s) Observed in Plasma after SC Administration





(LC-Radio-chromatogram after IV and SC Dosing of Peptide A in IR Form)

Conclusions

- The dose recovery data from radiolabeled PK studies in rats revealed that Peptide A related **radioactivity was completely absorbed** into systemic circulation after SC injection.

- *In vitro* study showed increased formation of degradants of [^{125}1]Peptide A during the incubation with fresh rat skin but not with boiled skin. A major metabolite peak was detected in the skin incubation sample using the HPLC radiochromatography.

- The metabolite peak at the same retention time was also observed in plasma of rats after SC adiminstration of [^{125}1]Peptide A, but not in samples after IV dosing.

- Results from this study demonstrated that while CR formulation prolonged the systemic $t_{1/2}$ of Peptide A, **metabolism of the peptide in skin** during its prolonged residence could potentially offset its systemic exposure. Thus, caution needs to be taken to balance the increase of half-life and the decrease of exposure while choosing a right formulation.

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