Workshop Japan-Egypt

“Pharmacognosy and Traditional Medicine”

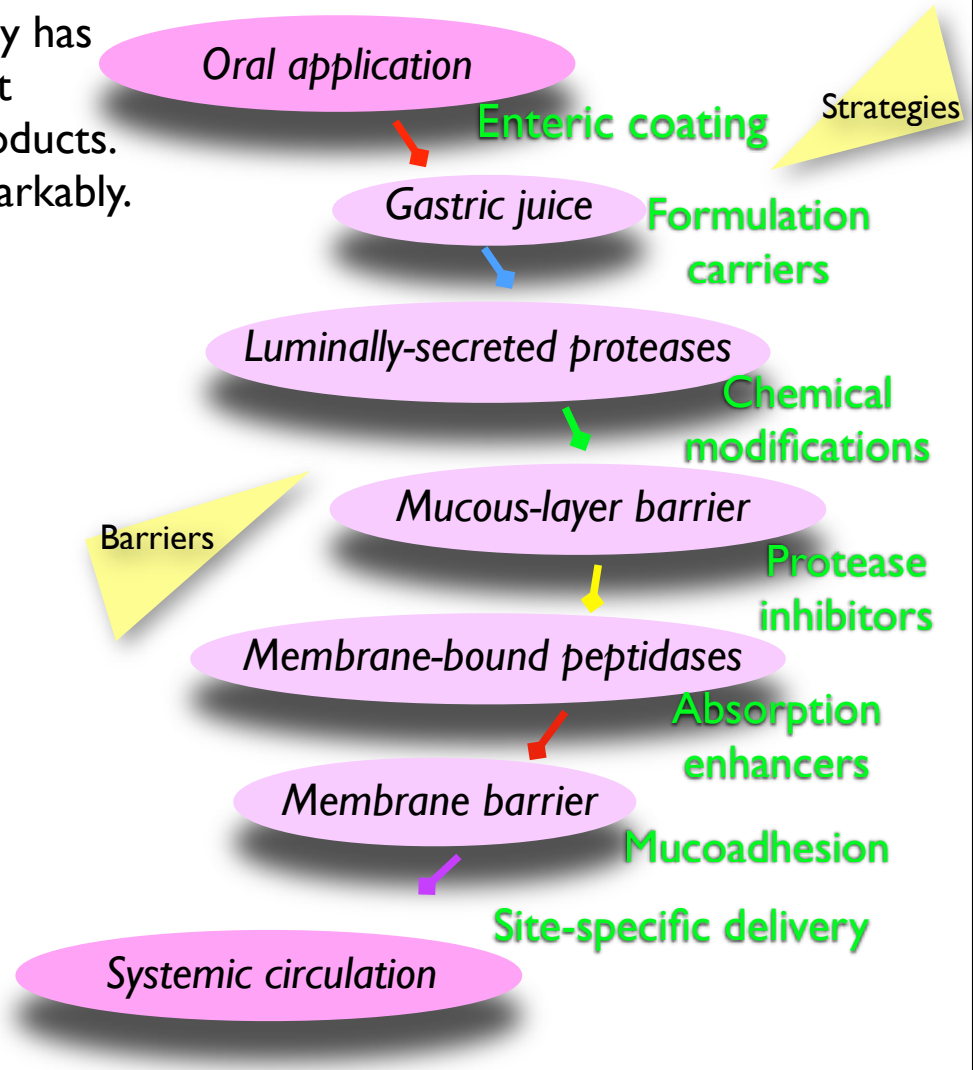
July 20-23 2010, Tokyo, Japan

Maximizing Peptide and Protein Absorption via Noninvasive Route of Administration

Over the past several decades, biotechnology has developed extensively and led to a significant increase in the number of bioengineered products. Now, the market is growing rapidly and remarkably.

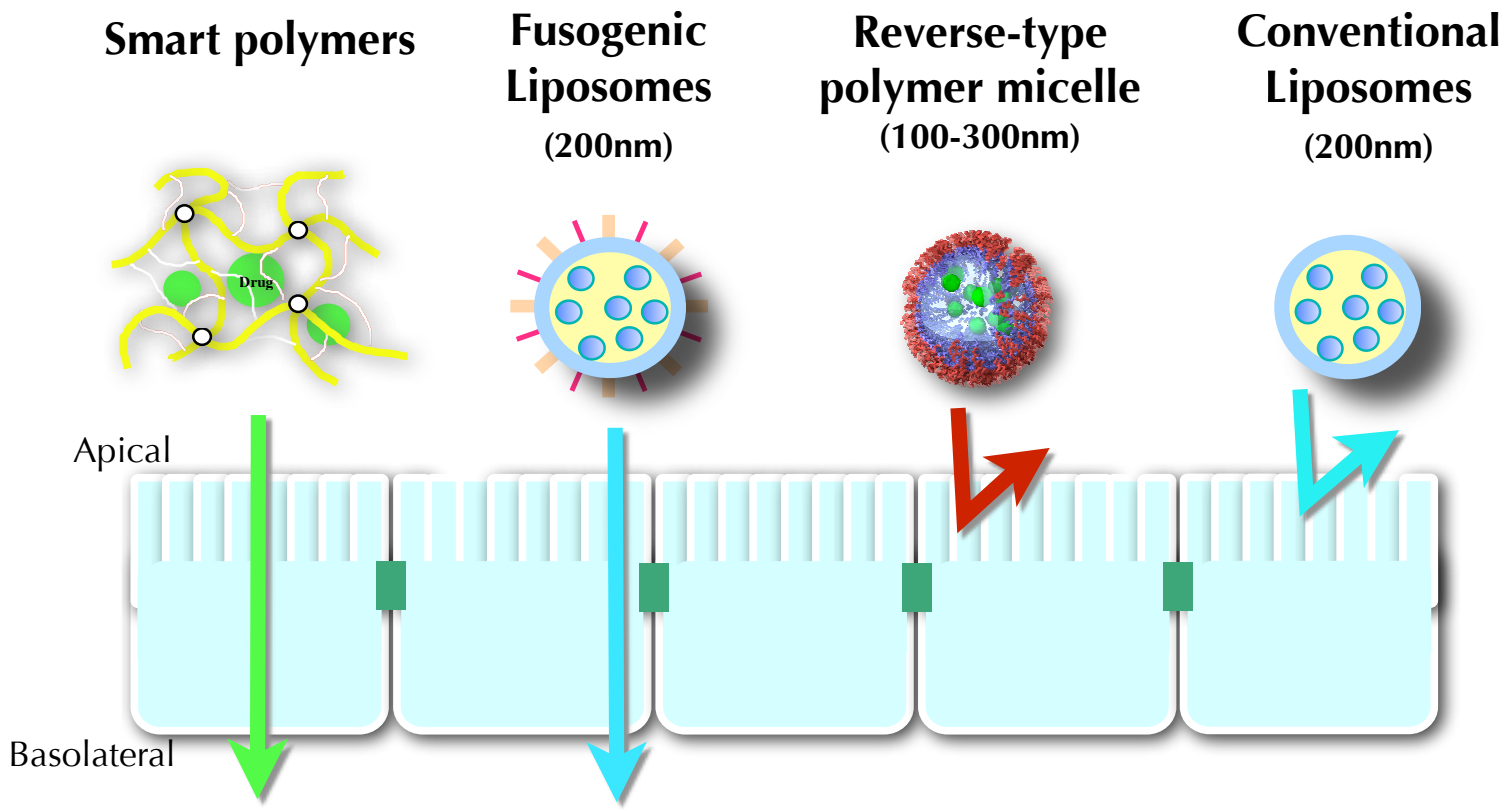
Ranking	Drug
4	Rituximab
5	Etanercept
6	Infliximab
9	Erythropoietin α
10	Bevacizumab
11	Trastuzumab
15	Adalimumab
25	Insulin Glargine
28	Darbepoetin α
29	Pegfilgrastim

2008 world drug market



Various strategies have been pursued over the past few decades to develop safe and effective noninvasive delivery systems for biodrugs. However, the success in the clinical application is limited.

Carrier Development for Biodrugs



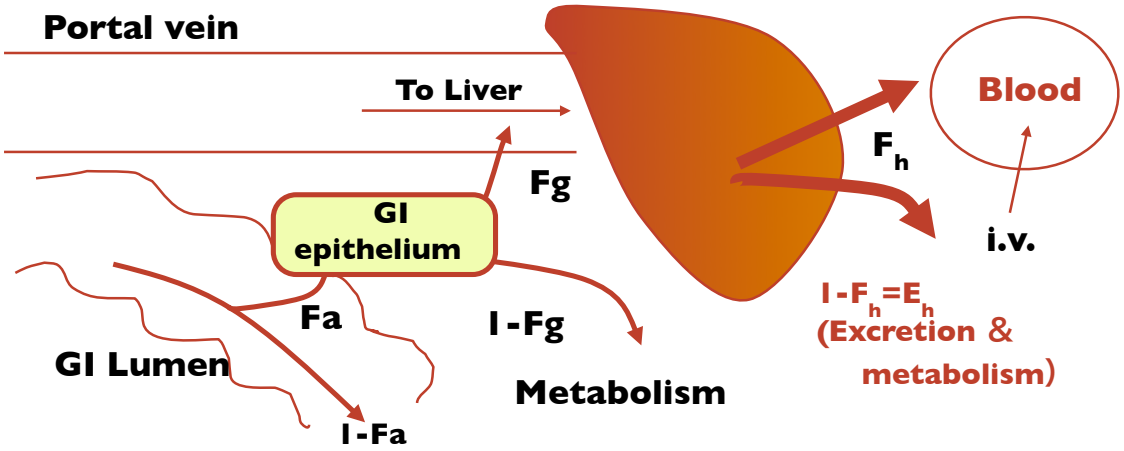
BA(oral) = 7.4%

M. Morishita et al., **JCR**, 2006

BA(colonic) = 7.3%

T. Goto et al., **Pharm Res**, 2006

How Can We Increase the BA of Biodrugs from Noninvasive Route of Administration ?



Oral Bioavailability

$F = Fa \cdot Fg \cdot Fh$

F_a : Fraction of absorbed

F_g : Fraction escaping gut-wall elimination

F_h : Fraction escaping hepatic elimination

Drug amount (reached to the blood) $= D F_a F_g F_h$

What is the most practical approach?

The key to make the delivery succeed is to maximize **Fa**

Modifying the physicochemical nature of macromolecules **Fa ↑**
(hydrophobicity↑、 stability to enzymes↑)

Adding novel functionality to macromolecules **Fa ↑**
(use of endogenous cellular transport systems)

Development of superior delivery carriers **Fa ↑ Fg ↑**

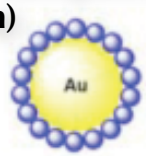
M. Morishita and N.A. Peppas, Drug Discovery Today, 2006

Materials Delivered Inside Cells by CPPs

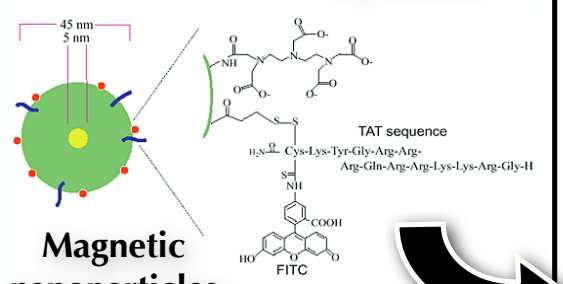
Particulate Carriers

Proteins and Nucleic acids

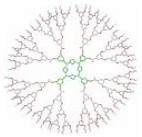
Gold nanopaticles (20nm)



Liposome



Magnetic nanoparticles

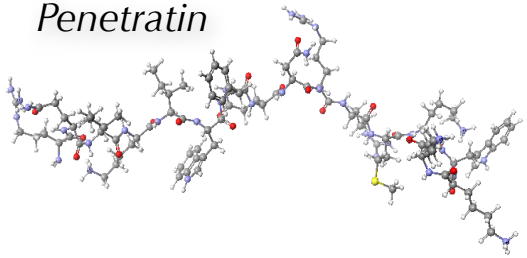


Dendrimers

Cell Penetrating Peptide (CPP)

(arginine rich cationic peptide)

Penetratin



Beta-galactosidase
Horseradish peroxidase
Green fluorescent protein
Antibody
p53
Ribonuclease
siRNA

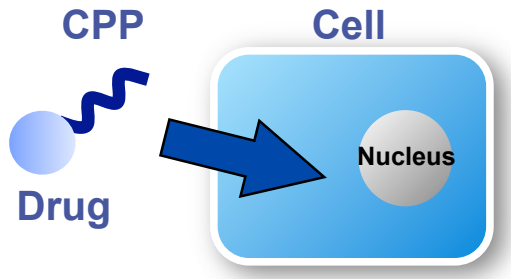
Chemical conjugation

Chemical conjugation
Fusion protein
Physical mixture

Cell

M. Morishita and N.A. Peppas, Drug Discovery Today, 2006

Can we use CPP Strategy for the noninvasive biodrug delivery systems ?



If so, which is more useful strategy, covalent or noncovalent?

The CPP Strategy by Chemical Conjugation

Leuprolide

pGlu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-$NHCH_2CH_3$

Leuprolide-D-R6 conjugate

pGlu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-**Arg-Arg-Arg-Arg-Arg-Arg**-D-Lys(FITC)-NH_2

D-form

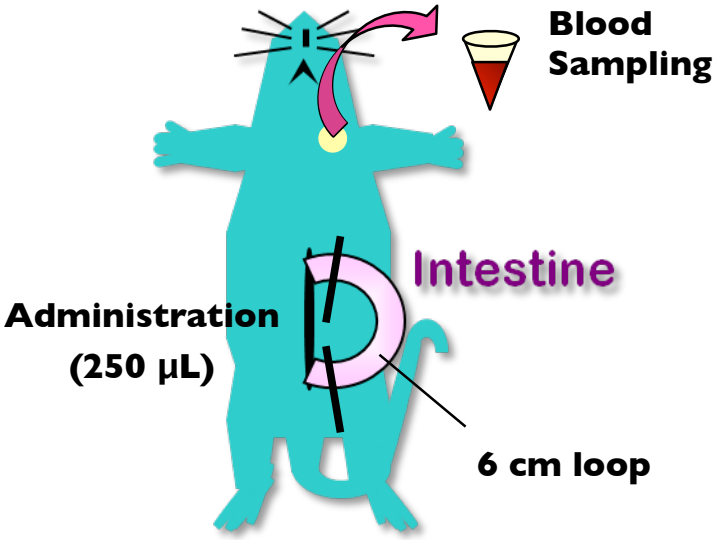
In situ loop absorption study

Animal: Male Wistar rats (weight 180-220 g)

Segment: Ileum

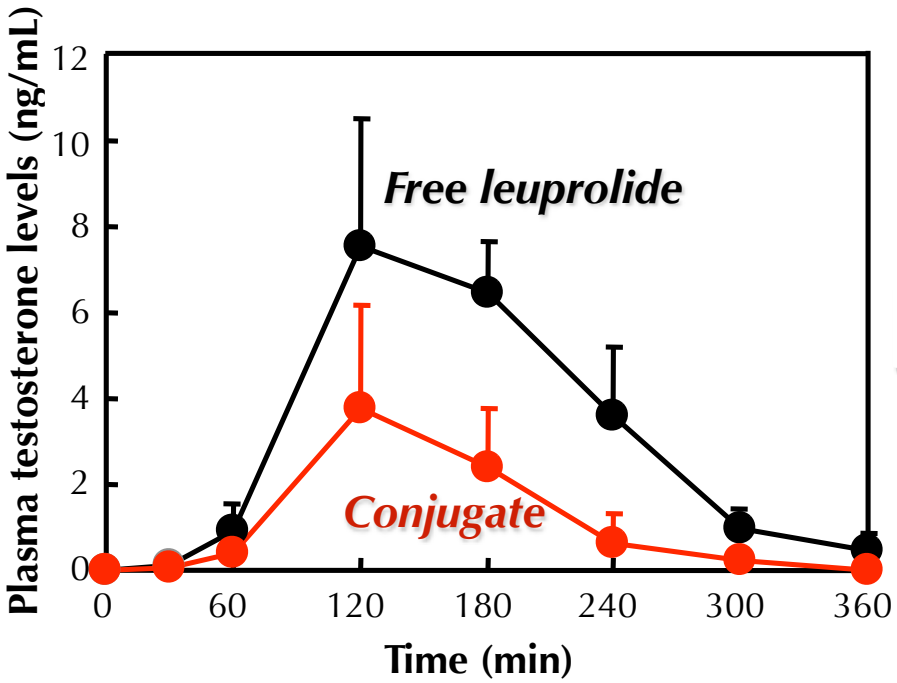
Analysis: Plasma testosterone concentration (Enzyme immunoassay method)

Replication: n=3 (mean ± S.D.)



Effectiveness of Covalent Strategy

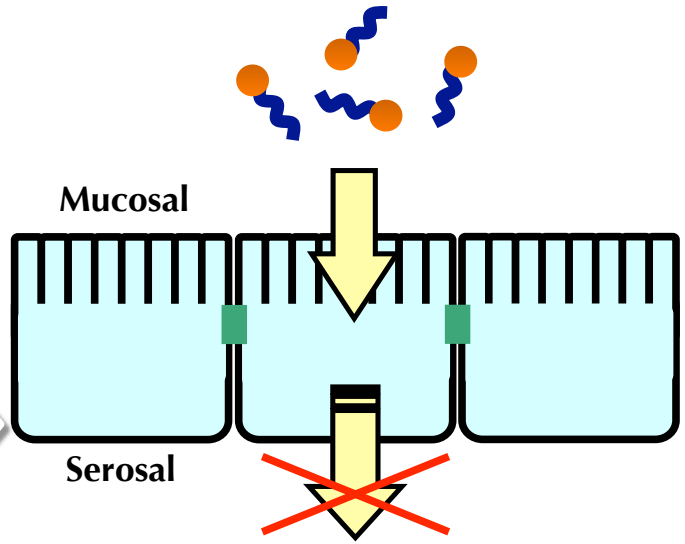
Plasma testosterone concentration - time profiles



In situ ileal loop absorption study in rats

N. Kamei et al., **JCR**, 2008

Leuprolide-D-R6 (10mM)



No improvement of intestinal peptide absorption was observed by conjugating with D-R6

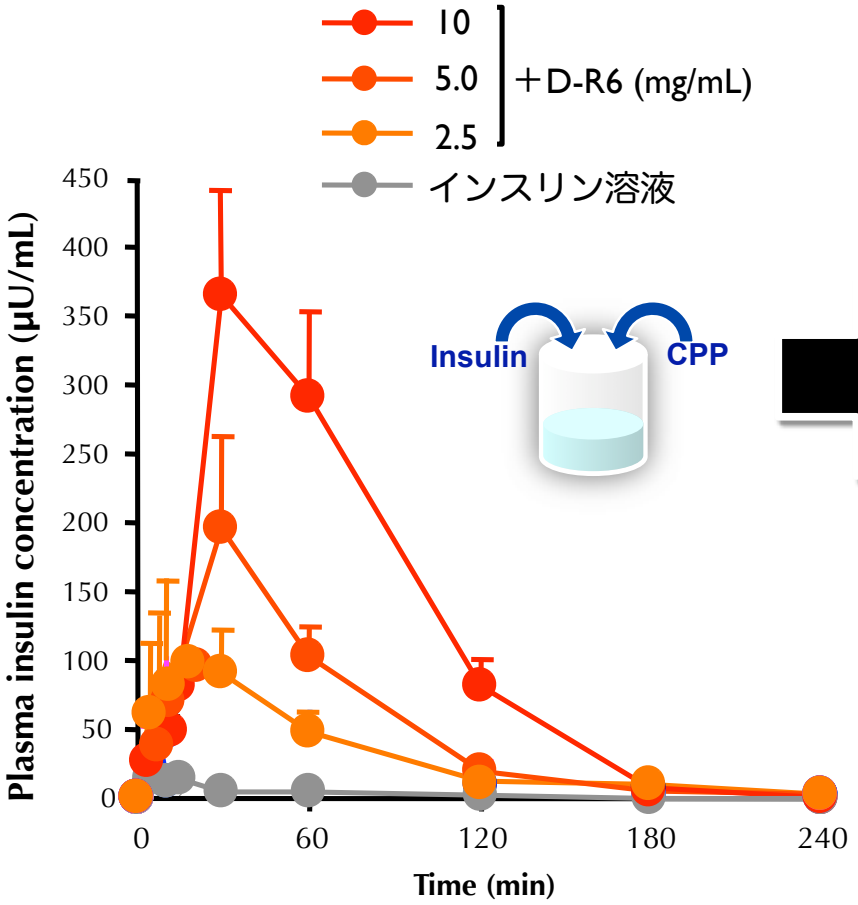
increase in MW

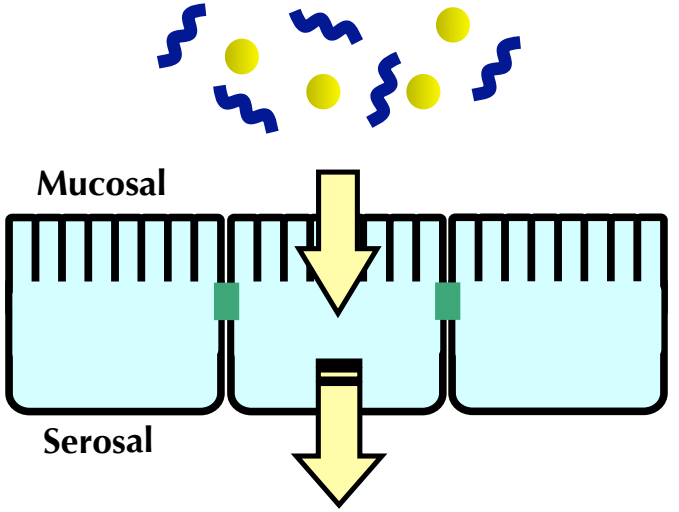
increase in hydrophilicity

decrease in pharmacological activity (36%)

Effectiveness of Noncovalent Strategy

Plasma insulin
concentration -time profiles





Remarkable improvement of intestinal peptide absorption was attained by physical mixture of D-R6

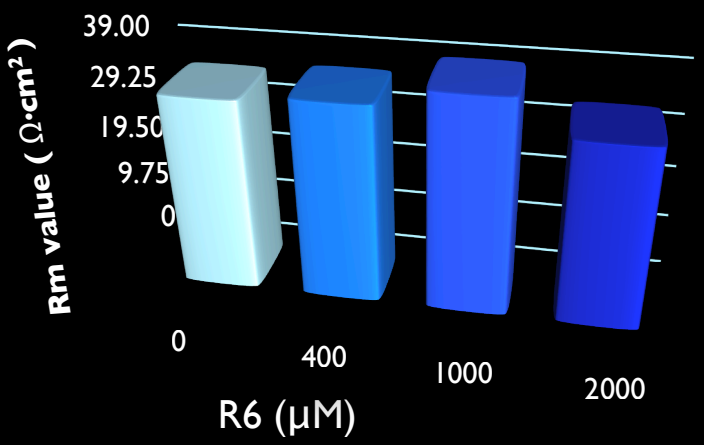
Advantage of physical mixture:

- preservation of pharmacological activity
- retention of physicochemical characteristics
- ease of the preparation

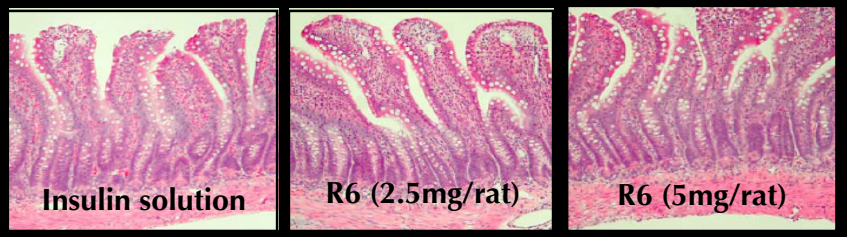
M. Morishita et al., **JCR**, 2007

Safety of Noncovalent Strategy

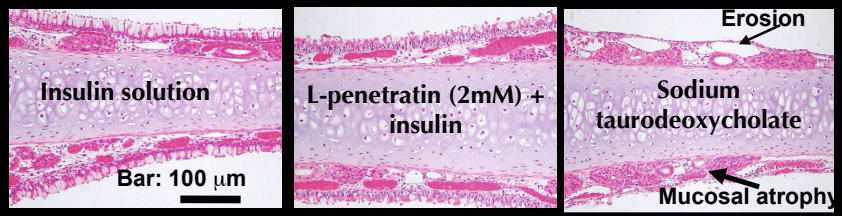
TEER Values of intestine



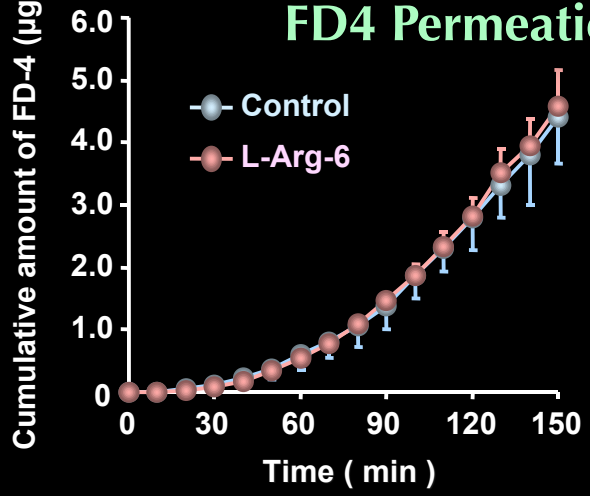
Histopathology of Intestinal Mucosa



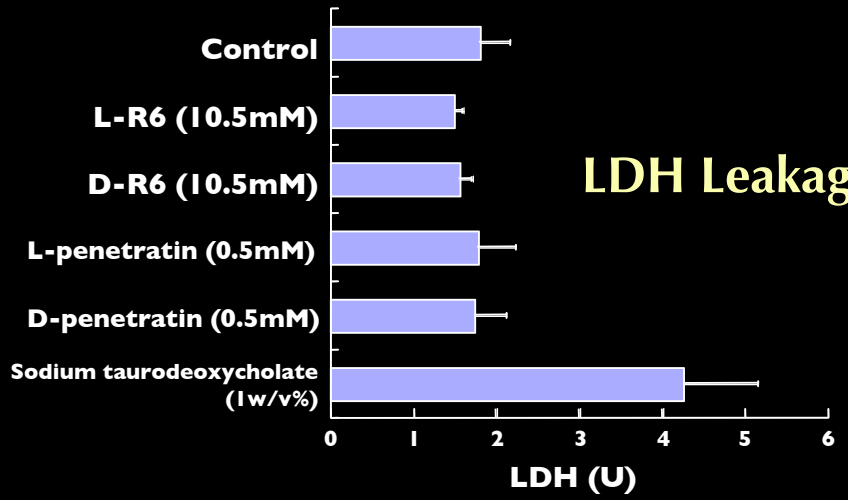
Histopathology of Nasal Mucosa



FD4 Permeation



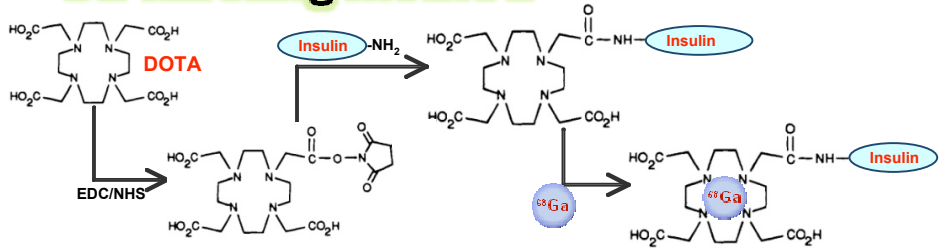
LDH Leakage



Suggesting mucosal membrane integrity was maintained following the application of CPP

Molecular Imaging Analysis of Intestinal Insulin Absorption Boosted by CPP

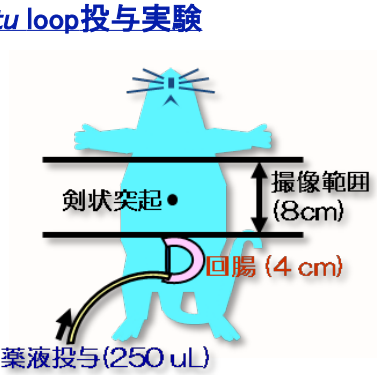
^{68}Ga-labeling method

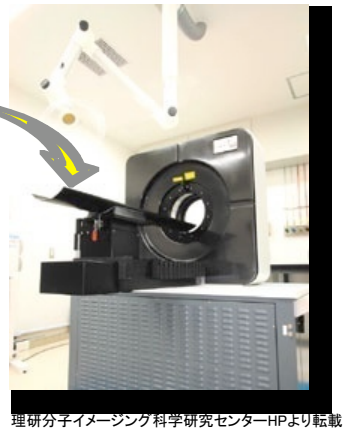


Intestinal absorption and subsequent distribution of insulin were quantitatively analyzed using molecular imaging technique based on positron emission tomography (PET).

Experimental method

In situ loop投与実験



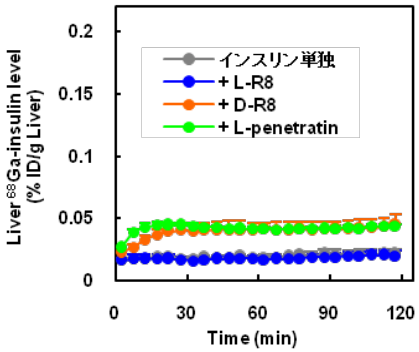


理研分子イメージング科学研究センターHPより転載

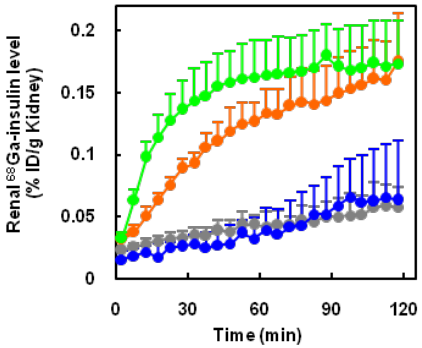
動物: SD系雄性ラット (体重: 180–220g)
固定: 背位固定
薬物: インスリン (cold, 132 μM) に ^{68}Ga–DOTA–インスリン (約4 μM) を添加
CPP: L–R8, D–R8 and L–penetratin (1000 μM)
投与方法: 回腸ループ内に薬物–CPP混合溶液を投与
前処理: 大豆トリプシンインヒビター (10 mg/mL)
血液採取: 尾静脈採血
検出方法: PET (microPET® Focus 220, Siemens社製)

Molecular Imaging Analysis of Intestinal Insulin Absorption Boosted by CPP

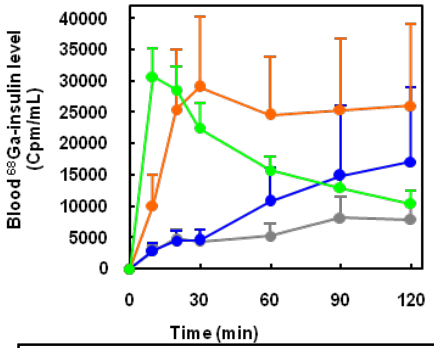
liver ^{68}Ga-DOTA-insulin



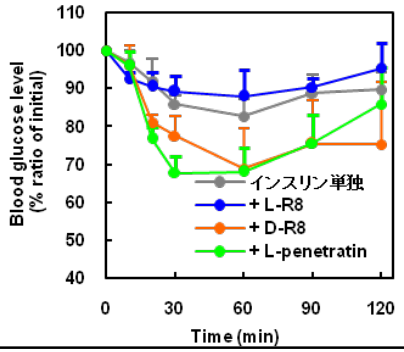
Kidney ^{68}Ga-DOTA-insulin

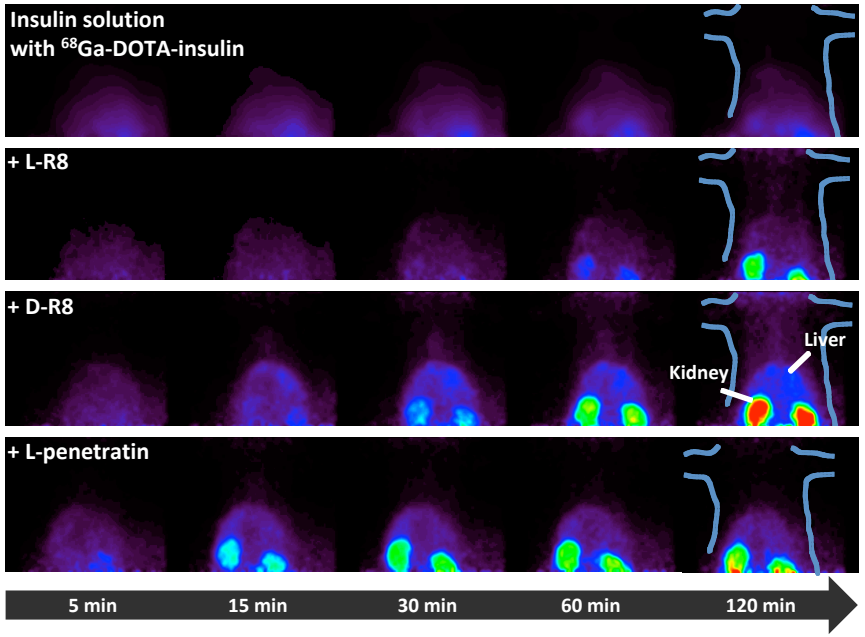


Blood ^{68}Ga-DOTA-insulin



Blood glucose level



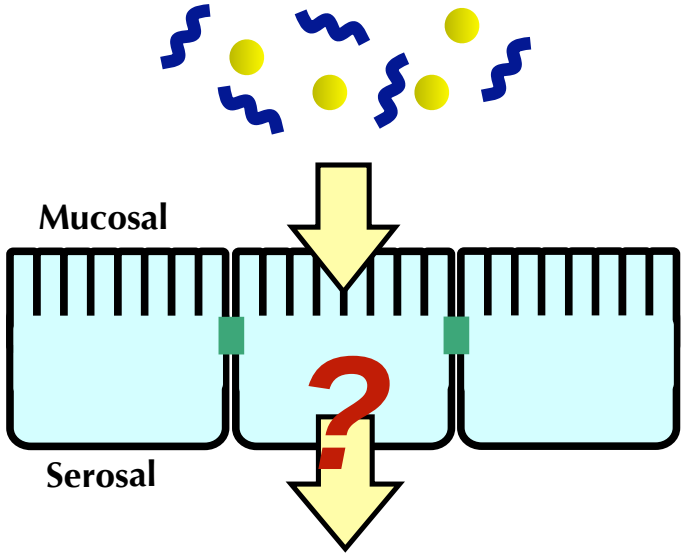


Time course of maximum intensity projection based on the PET imaging

^{68}Ga-DOTA-insulin after administration from the intestine was rapidly passed through the liver and significantly accumulated into the kidney. By co-administrating with D-R8 and L-penetratin, ^{68}Ga-DOTA-insulin levels in the liver, kidney and circulation was significantly increased, and the resultant hypoglycemic effect was observed.

➡ This is the first PET imaging study of intestinal insulin absorption in the presence of CPP.

What is the mechanism for improving biodrug absorption?



- universal vector ?-

Key is intermolecular interaction

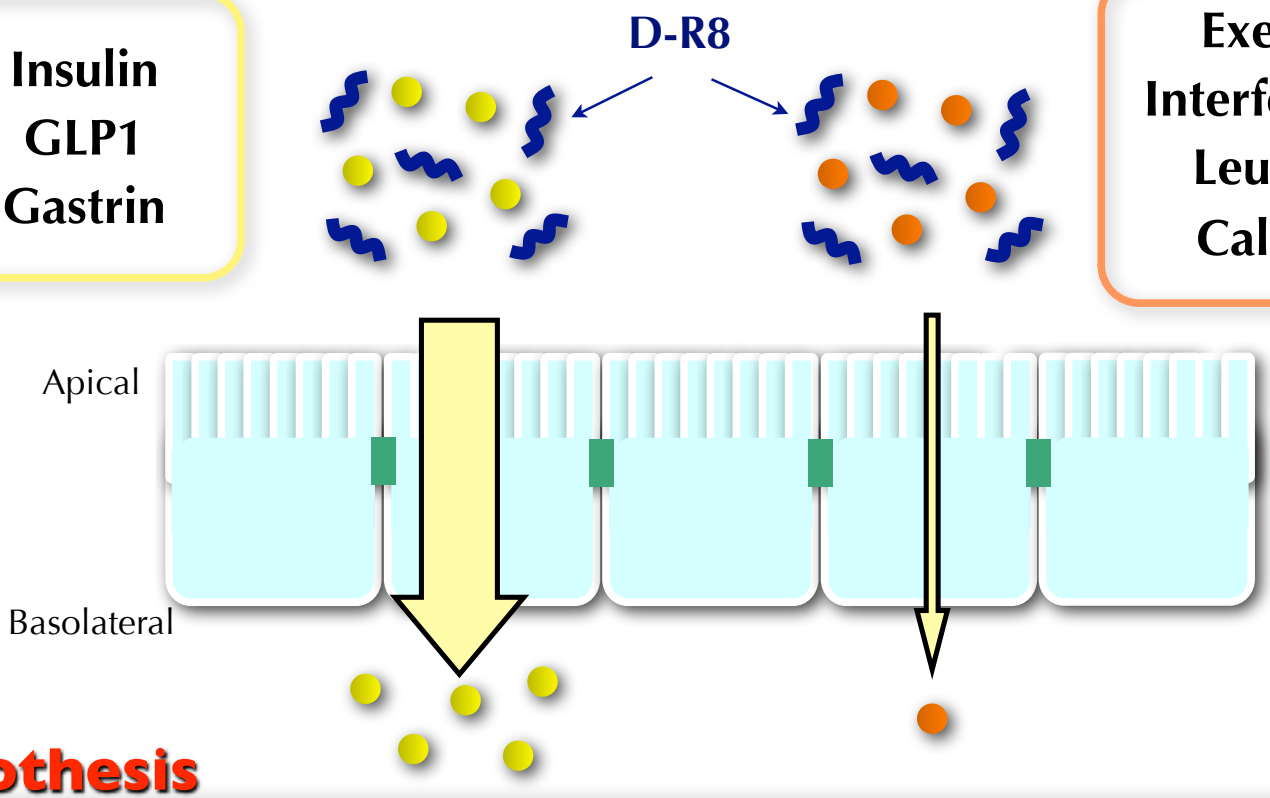
Intermolecular Interaction

Significant improvement

Insulin
GLP1
Gastrin

Weak improvement

Exendin-4
Interferon beta
Leuprolide
Calcitonin



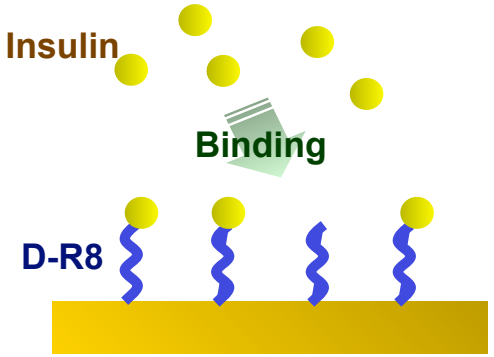
Hypothesis

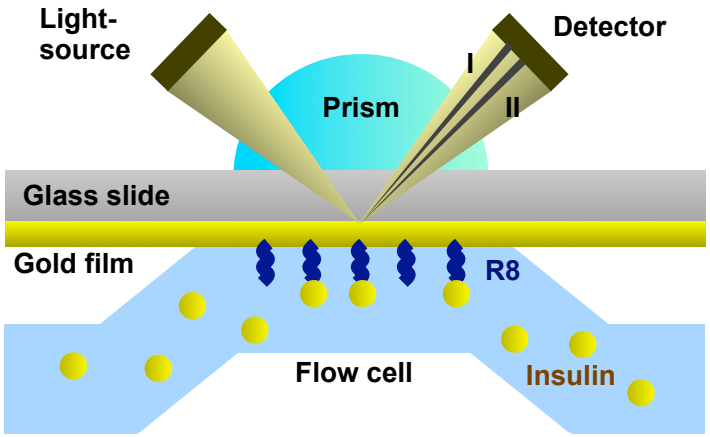
"Electrostatic interaction" between drug and CPP may be related to intestinal drug absorption enhancing effects.

Method for Binding Characteristics of Biodrug to D-R8

Surface plasmon resonance (SPR)

Apparatus: BIAcore 2000
Sensorchip: Streptavidin-immobilized chip
Carboxylmethyldextran chip
Immobilized ligand: Biotinylated D-R8, insulin
Analyte: D-R8, Gastrin, insulin, calcitonin, etc.
pH: 6.0





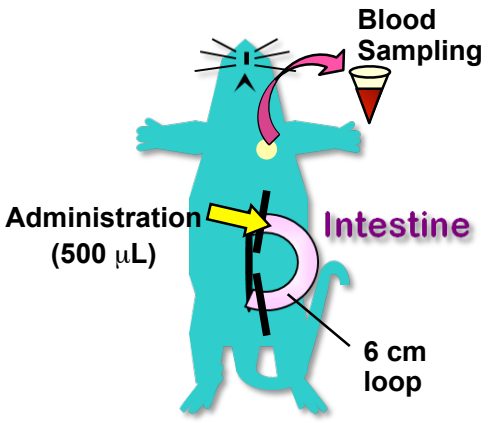
~Calculation of bound concentration~

$$Analyte_{total} = Analyte_{free} + \frac{B_{max} \cdot Analyte_{free}}{KD + Analyte_{free}}$$

$$B_{max} = Ligand_{total} \times \text{Binding ratio (analyte/ligand)}$$

In situ loop absorption study

Animal: Male SD rats (weight 180-220 g)
Segment: Ileum
Drug: Insulin, gastrin, exendin-4, calcitonin (132 μM)
CPPs: D-R8 (50-2620 μM)
pH: 6.0
Replication: N=3-5 (mean ± S.E., *p<0.05 vs. control)

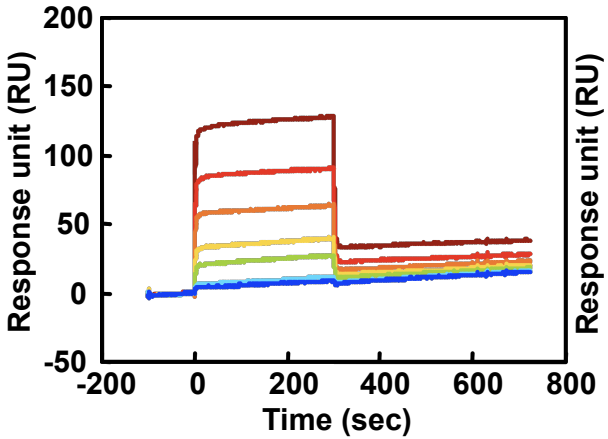


Binding Characteristics of Biodrug to D-R8

Characteristics of Biodrugs

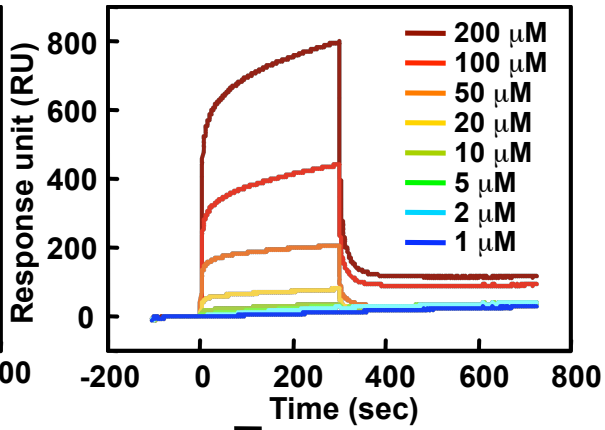
	Mw	pI
Gastrin	2098	2.77
Exendin-4	4187	4.49
Oxytocin	1007	5.20
Insulin	5808	5.3
GLP-1	3298	5.46
Leucine Enkephalin	556	5.68
TRH	362	7.85
Angiotensin	1296	7.96
[Arg^8]-Vasopressin	1084	8.28
Calcitonin	3492	9.03
Somatostatin	1638	9.16
Leuprolide	1209	9.65
LH-RH	1182	9.65
Mastoparan	1479	10.85
ACTH	2933	11.05
Dynorphin A	2147	11.45

Gastrin



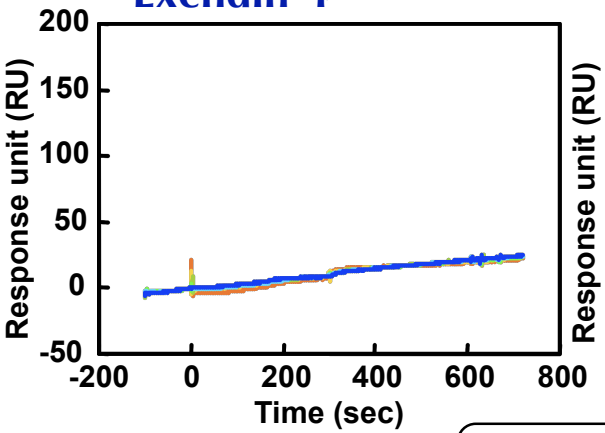
Bound gastrin 59.4%

Insulin

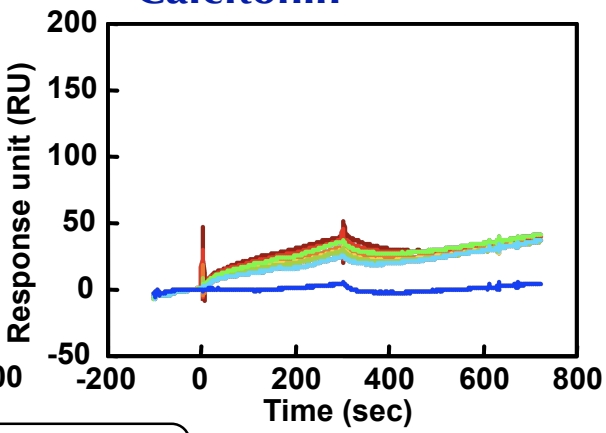


Bound insulin 58.7%

Exendin-4



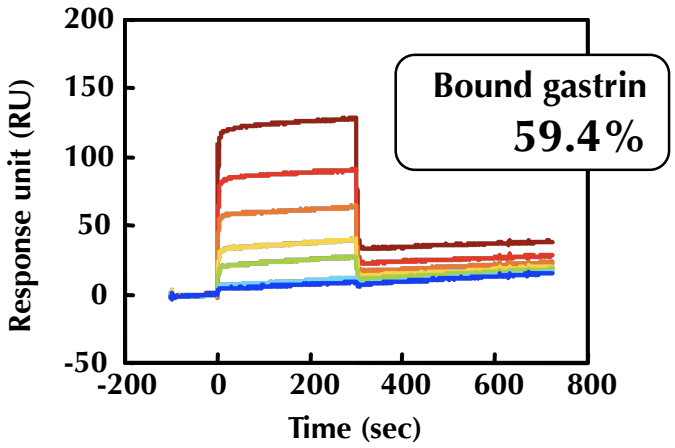
Calcitonin

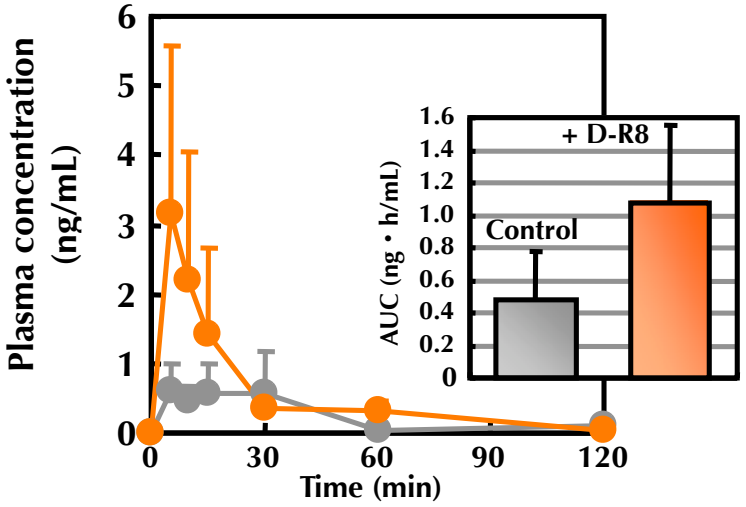


No interaction was observed

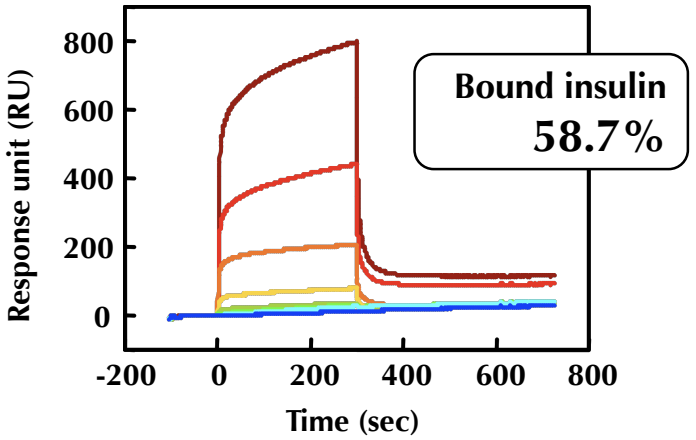
Effect of D-R8 on the Ileal Absorption of Biodrugs

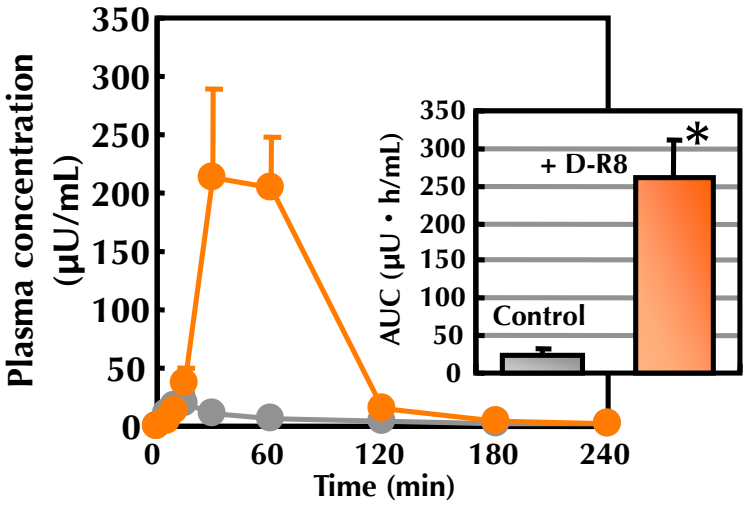
Gastrin





Insulin

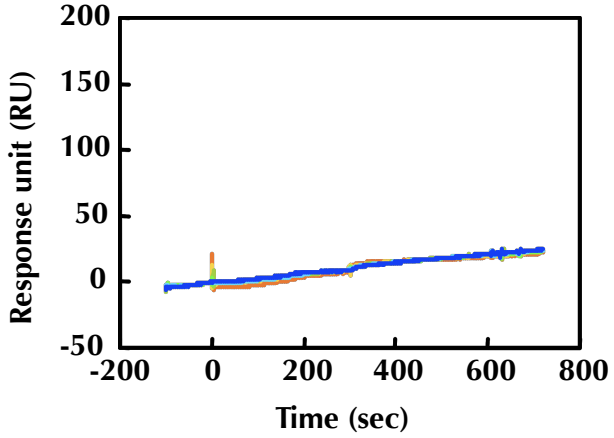


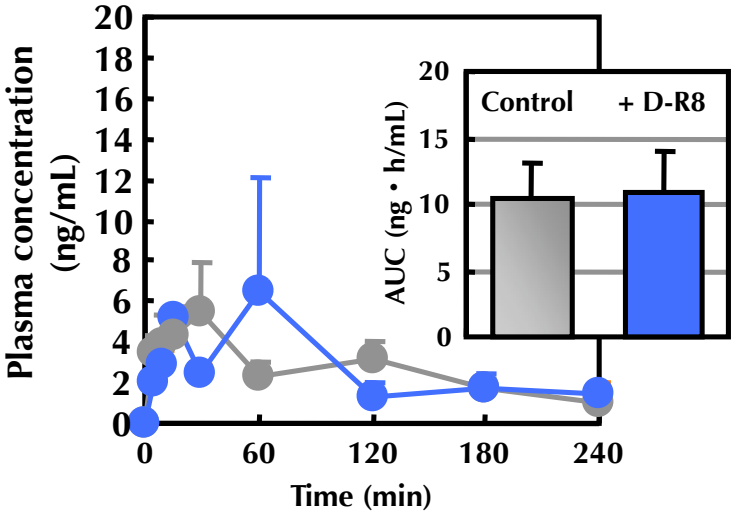


N. Kamei et al., **JCR**, 2009

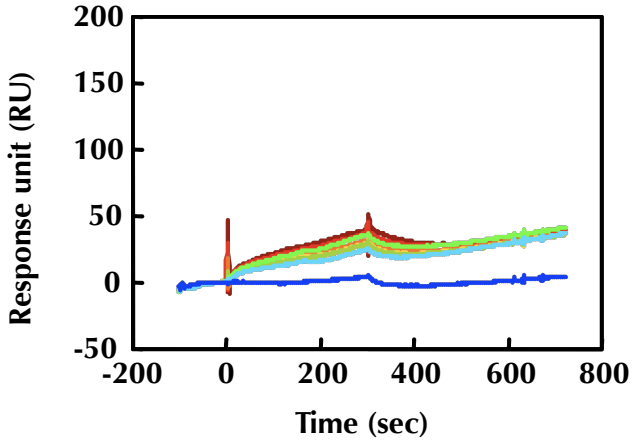
Effect of D-R8 on the Ileal Absorption of Biodrugs

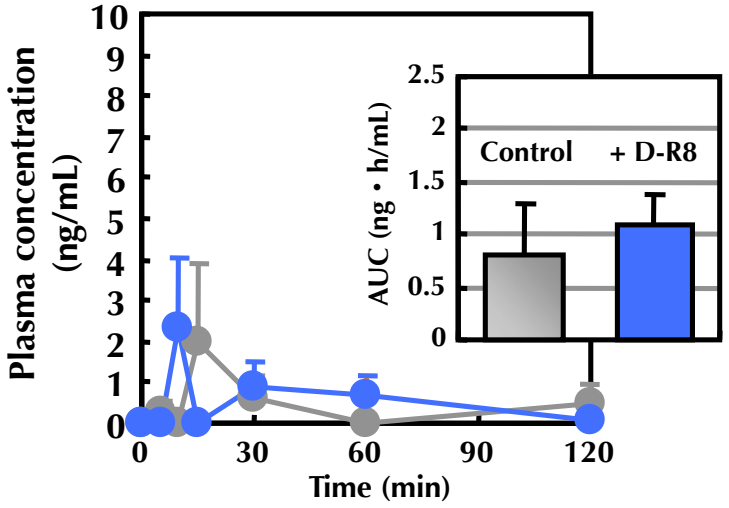
Exendin-4





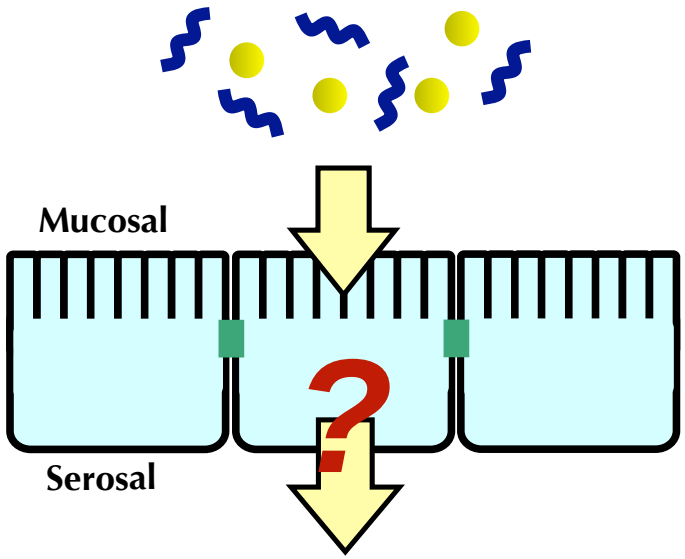
Calcitonin





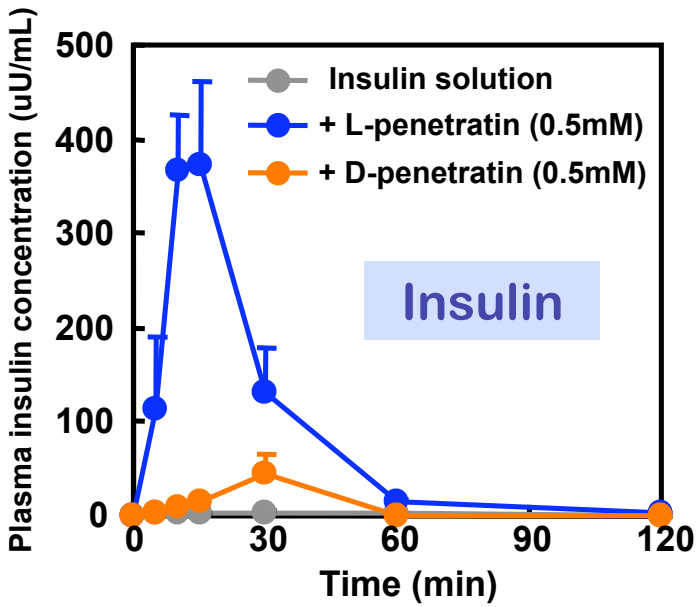
Suggesting that intermolecular binding between drug and CPP is an important factor governing the enhancing effect of the CPP on their intestinal absorption.

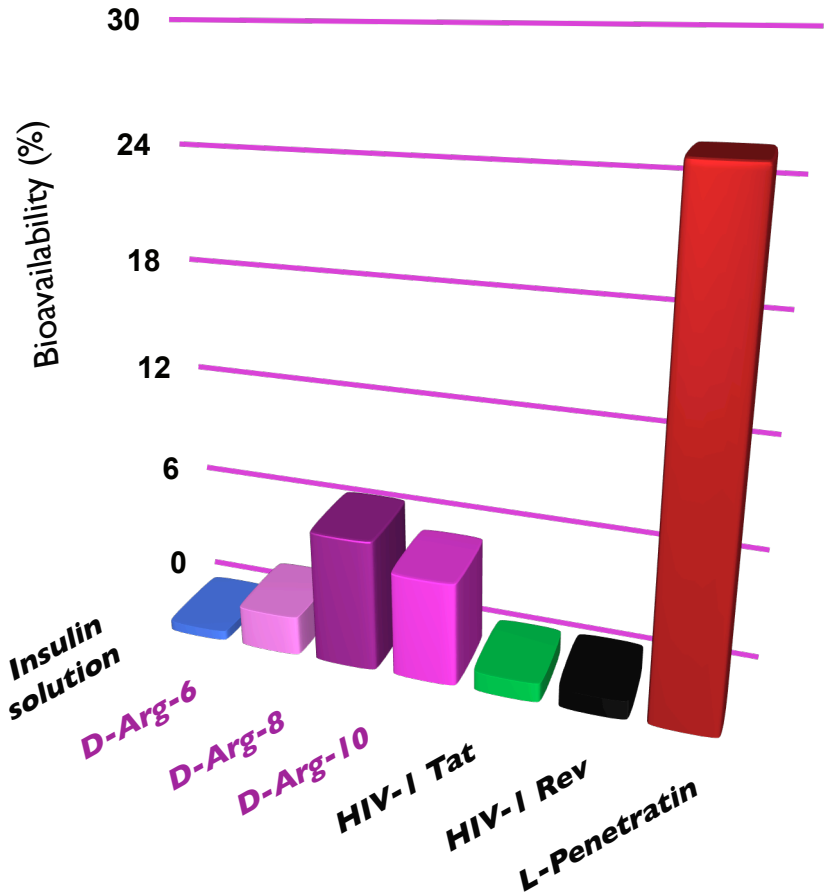
What kind of CPP is safe and useful?



Effect of Different CPP on the Ileal Insulin Absorption

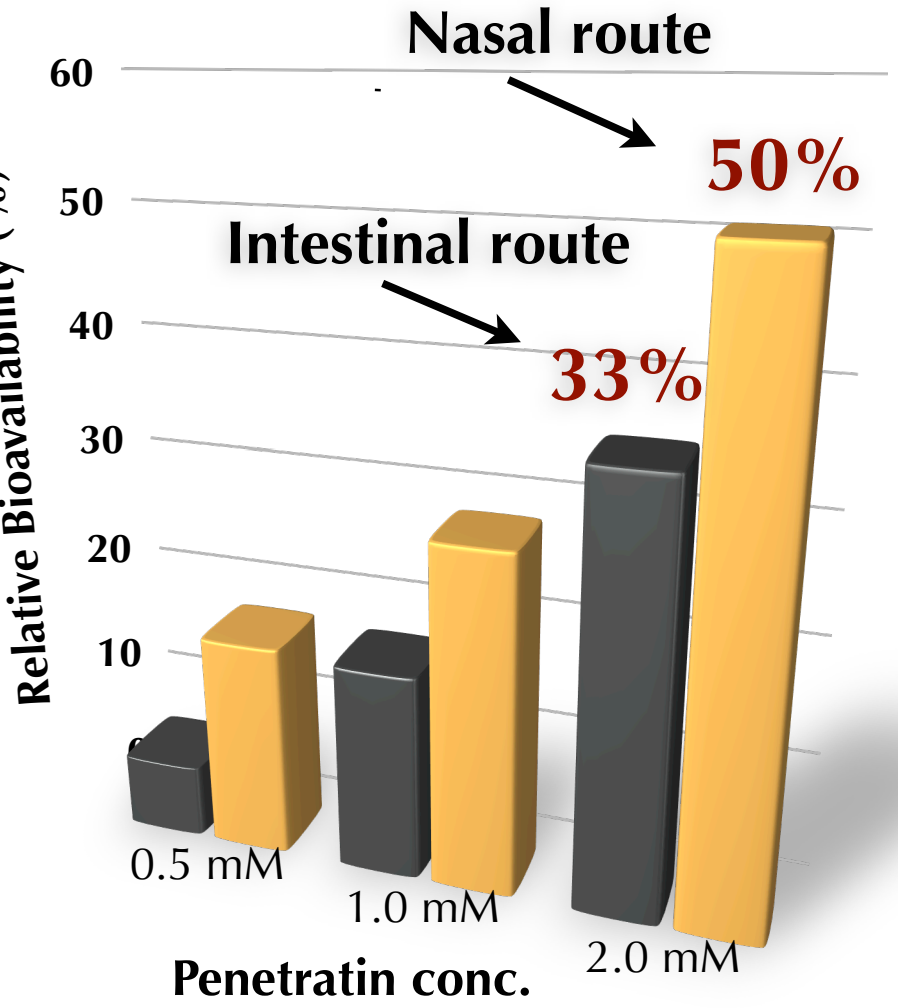
CPP	Amino acid sequence
Penetratin	RQIKIWFQNRRMKWKK
pVEC	LLIILRRRIRKQAHAHSK
RRL helix	RRLRRLLRRLRRLLRRLR
Rn	Rn (n=6, 8, 10)
HIV-1 Tat	GRKKRRQRRRPPQ
HIV-1 Rev	TRQARRNRRRRWRERQR

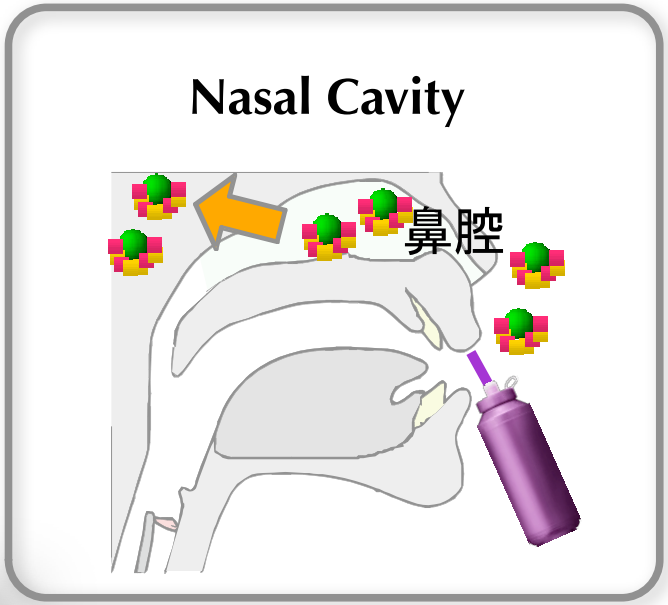




M. Morishita et al., J Control. Release, 2007

Perspectives



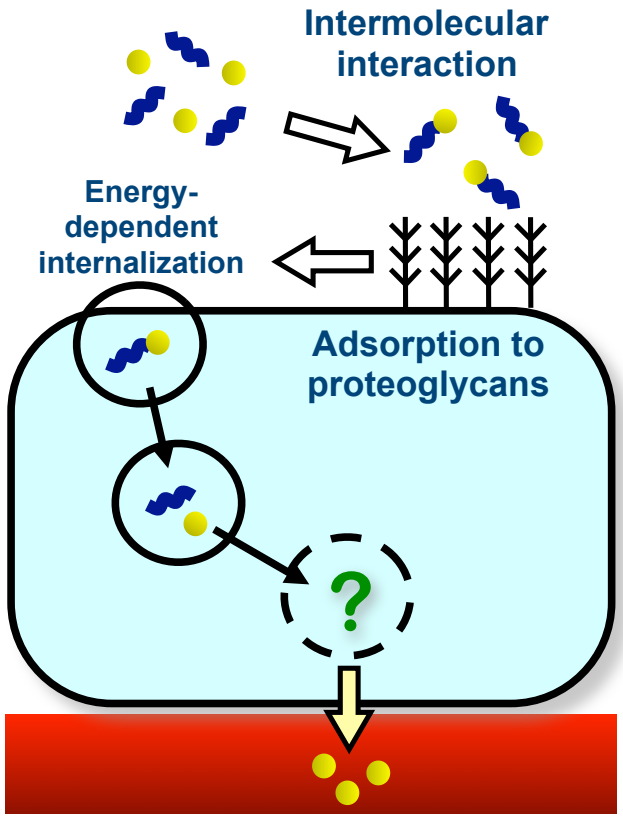


El-Sayed Khafagy et al., **JCR**, 2009

Conclusions and Perspectives

- Is the CPP useful as a promoting tool of noninvasive biodrug absorption?

YES



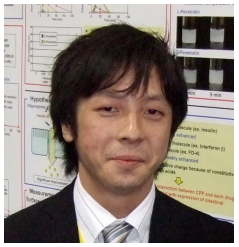
Several CPPs significantly enhanced intestinal and nasal absorption of biodrugs. The most significant enhancement effect was observed with penetratin analogs.

The absorption enhancement effects were demonstrated by physical mixture and does not need chemical conjugation. In addition, the enhancement was not associated with cellular damage and diffusion.

Therefore, CPP are likely to become powerful tools for overcoming the low permeability of biodrugs through the epithelial cell membranes: the major barrier to macromolecular drug delivery.

Collaborators

Prof. Takayama and lab members



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