Neuropeptide Receptor Targeted Therapeutics for the Treatment of Endocrine Diseases and Cancers

Gonadotroph and GnRH Neuron Targeted Approaches for Nonsurgical Sterilization of Cats and Dogs

The Hypothalamic-Pituitary-Gonadal (HPG) Endocrine Axis

GnRH Therapeutics
- Breast/Prostate Cancer: eliminate gonadal steroids
- Endometriosis/Fibroids: Prevent high mid-cycle estrogens
- Infertility: Prevent premature LH surge
- Precocious Puberty: block LH/FSH pulsatility

Sterilization Strategies
- GnRH Neuron Ablation

Hypothalamus
- Kisspeptin Neurons
- GnRH Neurons
- Optic Chiasma
- Pituitary
- Gonadotroph Ablation

Gonads
- Testosterone
- Estrogen
- Sperm
- Eggs
Hormones and Receptors in the Reproductive Endocrine System

GnRH Targeted, Gated-Entry Toxins

GnRHR Expression in Human Reproductive Tumors

Gonadotrophin Neuron
GnRH
Kisspeptin
Gonadotropins
GnRH Receptor
Kisspeptin Receptor
Gonadotropin Receptors
Gonadal Cells
Sperm
Eggs
Testosterone
Estrogen

Tumor Type
Tumor Samples Expressing GnRHR
Method (Reference)

Positive
Negative

PCR
(Wilkinson 2008)

Immunohistochemistry
(Wilkinson 2008)

Ligand Binding
(Srkakovic 1998)

Ligand Binding
(Srkakovic 1990)

Ligand Binding
(Fukada 1989)

AN-152 (Peptide-Chemotherapeutic)

GnRH-PAP (Peptide-Protein)

Pokeweed Toxin

[DLys₆]GnRH-Pokeweed toxin maintains high affinity GnRHR binding

Yang et al., Endocrinology(2003) 125, 801-6
Nagy et al., PNAS (1996) 93, 7269-73

DNA Intercellular Target Escape Cell Death

Concentration (nM) % Binding

D-Lys6-GnRH
GnRH-PAP
PAP
Unanswered Questions

1. Were gonadotrophs ablated?
   • Time course more consistent with GnRH immunization.
2. Why did reproductive function return?
   • If gonadotrophs were killed, do they regenerate?
3. What limits efficacy of first generation GnRH-toxins?

GnRH Peptide Targeted Toxins: Limitations

1. Peptide rapidly degraded
2. Can’t access intracellular receptors
3. Agonists down-regulate receptors
4. Single shot specificity

Effect of [DLys⁶]GnRH-Pokeweed toxin in adult male dogs

Cell Viability (% of control)

Cell Viability (% of control)

[DLys⁶]GnRH-Pokeweek toxin conjugate selectively kills GnRHR⁺ cells

Effect of [DLys⁶]GnRH-Pokeweek toxin in adult male dogs

Sabeur et al., Reproduction (2003) 125, 801-6

Yang et al., Endocrinology (2003) 125, 801-6

Effect of [DLys⁶]GnRH-Pokeweek toxin in adult male dogs

Sabeur et al., Reproduction (2003) 125, 801-6

Yang et al., Endocrinology (2003) 125, 801-6
Pharmacokinetics of \([\text{DLys}^6]\text{GnRH}\)-Pokeweed toxin in sheep
Rapid degradation of \([\text{DLys}^6]\text{GnRH}\)-PAP conjugates in sheep


Orally available, small molecule GnRH antagonists

Sufugolix/TAK-013 (Takeda)
elagolix (Neurocrine-Abbott)
IN3/indoles (Merck)
Furamides (Agouron/Pfizer)
Benzimidazole-sulfonamides (Bayer Yakuhin)

Hypothesis: Stably linked, small molecule GnRH antagonist conjugates

Nonpeptide GnRH antagonist bound to the GnRHR receptor
Stable Linker
Small Molecule Effector

Griffith Receptor Binding
Intracellular Targets

One Molecule Two Activities

“Concentrate and Kill” model
Theoretical Improvements
Stable ligand maintains specificity
Concentrated by mass action to receptor expressing cells
No signals for receptor down regulation
Intracellular receptors accessible (99% GnRHR is internal)

Risks
Conjugate can access cytoplasm in non-target cells
Traditional in vitro selectivity assays may be misleading
Medicinal Chemistry approach to drug discovery

- Targeting options
- Design, synthesize conjugates

- Receptor expressing cell lines (rat, dog, cat)
- Pharmacology on cells in vitro
- Feedback to guide design
- Safety and efficacy in rats
- Safety and efficacy in dogs & cats

Screening Small Molecule Library for Potent Gonadotroph Toxins

Screened at 1 µM for toxicity in both mPit12-3 (gonadotroph) and GH4 (somatotroph) cells for 72h

Most Conjugates Maintain GnRH Receptor Binding

- CRN00019 EC_{50} = 4.8 nM
- CRN00028 EC_{50} = 7.9 nM

Some Conjugates Lose Cytotoxicity

- CRN00107 EC_{50} = inactive
- CRN00109 EC_{50} = 1.6 nM

Inhibition of GnRH agonist stimulated inositol phosphate production

- CRN00019
- CRN00028

Cytotoxic Activity

- CRN00107
- CRN00109

ACC&D 5th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control
CRN-0108 Is Both a Potent GnRHR Antagonist and Toxin

Inhibition of GnRH-stimulated IP production

GnRH antagonist Conjugate

Toxin Conjugate

Vehicle

CRN-0119

CRN-0110

CRN-00108

CRN-00119

CRN-00110

CRN-00108

No obvious toxicity/unplanned deaths

Effects of single dose CRN-0108 in intact male rats

CRN-0108 PK in rats following iv administration of CRN-0108 (1.7 mg/kg)

CRN-0108

Vehicle

% inhibition (of full:2:1)

Timecourse by Group

0

1

2

3

4

5

Vehicle

CRN-0108

Time (hrs)

Testosterone (% T=0.25)

Inhibition (of t=0.25)

8

16

24

32

40

48

56

64

72

80

88

96

104

112

120

0

50

100

150

200

Vehicle

CRN-0108

[CRN-0108] (ng/mL)

Time (d)

LH (%)

[CRN-0108] (ng/mL)

Vehicle

CRN-0108

Conclusions so far

• Small molecule GnRH antagonists can be used to target GnRHR expressing cells
• Multiple small molecule toxins can be targeted without loss of cytotoxic activity
  - Impaired cytoplasmic access is a common issue
• CRN-0108: Occupies GnRH receptors in vivo with minimal gonadotroph cytotoxicity
  - Insufficient intracellular delivery in vivo?
  - Ineffective toxin for quiescent eutopic gonadotroph?

Questions

• Are GnRH agonists more effective for intracellular delivery of small molecule cytotoxins?
• Are there more effective toxins for quiescent eutopic gonadotrophs?
Next Steps: Controls and more controls

CRN-00108

CRN-00121

Optimized peptide agonist

CRN-00177

Define single attachment site

Inactive point mutant control available

CRN-0203

CRN-00121

CRN-00177

Chemotherapeutic

Stable

GnRH Agonist

Chemotherapeutic

Stable

GnRH Agonist

Cleavable

Inactive point mutant control available

CRN-177 appears to be selective for GnRHR expressing cells

RBL Cells

Log[Compd], M

% Survival

CRN-00121

CRN-00177

CRN-0203

CRN-0203

EC50 = 11 nM

Recombinant Toxin P

EC50 = 18 nM

Second generation GnRH agonist targeted protein toxins

Agonist activity in aT3 cells

Comparing transfected to untransfected cells is inappropriate for determining GnRHR receptor dependent toxicity (for some toxins)

RBL Cells

rGnRHR Transfected

% Survival

Log[Compd], M

EC50 = 11 nM
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