FSH Receptor Ligand-Cytotoxin Conjugates: Potential for Permanent Chemosterilization – *Ja*

The overpopulation of homeless dogs and cats is a growing tragedy that results in the suffering and death of millions of animals annually. A single-dose, injectable drug for sterilization would eliminate costly procedures such as spaying and neutering, and would greatly help control feral populations. Cytotoxins that target follicle-stimulating hormone receptor (FSHR), a protein found in specific cells of the male and female reproductive systems that are crucial for fertility, may act as potential chemosterilants. Drug candidates will be screened for potency on cell culture models of the targeted tissues. Toxicity profiles will be determined by assaying tissue slices of target and non-target organs. Our results will provide the preliminary data and establish the tools necessary for studies on live animal models to determine the maximum safe dosages, immunogenicity, and potency of potential drugs as chemosterilants.

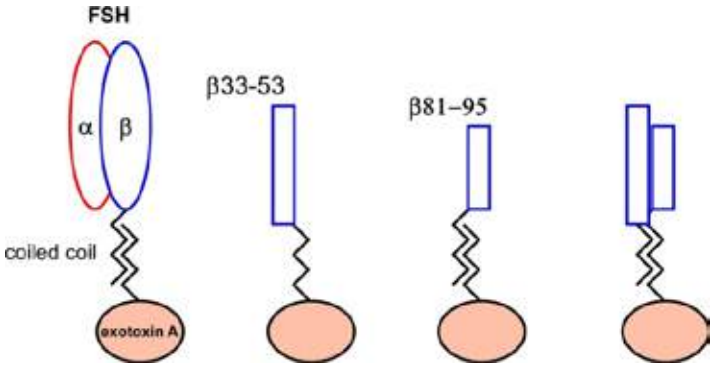
Background and research strategy

Immunotoxins are antibody-cytotoxin drug conjugates that target cell-surface markers and deliver a toxic payload to cells. These compounds have shown great promise as cancer therapeutics.[1, 2] Of crucial importance is the identification and targeting of cell-surface proteins that are strongly represented in specific cells. We plan to use a similar approach to ablate testicular and ovarian cells required for fertility.

Sertoli and granulosa cells play crucial roles in mammalian sperm and egg development, respectively. Aberrant function in either of these cell types causes infertility.[3-6] Hence, drugs that lead to Sertoli and granulosa cell death would be potential chemosterilants. Importantly, the G protein-coupled receptor, FSHR, is expressed specifically in male testicular Sertoli cells[7, 8] and in female ovarian granulosa cells.[9] FSHR ligands will be fused to a cytotoxin that is only active within the cell to produce lead compounds that selectively destroy FSHR-expressing cells, with minimal or no toxicity to non-target cells. Using an E. coli expression system, we will purify the cytotoxic fragment of the deadly Pseudomonas aeruginosa exotoxin A,[10, 11] fused to the acidic peptide half of a heterodimerization domain. This peptide "Velcro" facilitates the conjugation of cytotoxin to proteins or molecules that contain the basic peptide half of the heterodimerization domain.[12, 13] Full-length FSH and several short peptide mimetics will be constructed as fusions to the basic peptide partner. These ligands will be conjugated to exotoxin A to produce potential drug candidates that will be tested for cytotoxicity in vitro. Our system will allow the rapid testing of a variety of potential drugs, as we can produce alternative targeting ligands (e.g., recently developed small molecule FSH agonists) and toxins (e.g., ricin or maytansine) fused to the heterodimerization domain and quickly "mix and match" compounds to assay their potencies.

The major advantage of our approach is that a single cytotoxin can be produced in high purity and conjugated to a variety of ligands to rapidly test multiple candidates. Other cytotoxins, including recently described variants of exotoxin A that exhibit reduced animal toxicity, increased protease-resistance, and decreased immunogenicity,[14-16] can be easily produced as fusion proteins containing the heterodimerization domain and conjugated to the desired FSHR-targeting ligands. Thus, "upgraded" or alternative cytotoxins can be produced and tested quickly without redesigning the ligands to which they are coupled.

Potential chemosterilants will be tested in vitro on cell culture models. Studies using organ or tissue slices have been useful in establishing models to study drug toxicity.[17] Cells in tissue slices can react in an environment that is most alike to their natural in vivo embedding. The FSHR ligand-cytotoxin conjugates will be applied to determine dose effects on cell death in different organ and tissue slices. Minimal cytotoxicity on non-target tissues would greatly support the use of these drug candidates in live animal studies.



Examples of fusions of full-length FSH (a heterodimeric protein) or FSH-derived peptides with exotoxin A via a coiled coil dimerization domain. These compounds are designed to require only a few components that can be "mixed and matched" via the coiled coil domain to test a variety of potential drug candidates. For example, the FSH-derived peptides can be quickly assayed individually or together to observe synergistic effects.

References

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