LEADING ARTICLE

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Anti-Gonadotropin Releasing Hormone Vaccines and Their Potential Use in the Treatment of Hormone-Responsive Cancers

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Abstract

Gonadotropin-releasing hormone (GnRH) and its analogues have been used clinically to treat a range of hormone-dependent conditions. It is often necessary for large, toxic and expensive drug doses to be administered. Improvements in drug delivery have necessitated new developments in formulation, but these in turn can induce new adverse effects. Immunological neutralisation of GnRH has been examined as a less toxic and cheaper replacement therapy, and has been studied closely in different animal species. However, only a few clinical trials have been carried out with respect to hormone-dependent cancers. Based on clinical trials of the free peptide drug in cancer patients, it would appear that there is an increasing trend towards using GnRH and its analogues in adjuvant therapy and that antibody-based GnRH neutralisation will have a role in this treatment regimen.

Gonadotropin releasing hormone (GnRH, a decapeptide, glu-his-trp-ser-tyr-gly-leu-arg-pro-gly-NH_2) or luteinising hormone releasing hormone (LHRH) (fig. 1) stimulates the release of luteinising hormone (LH) and follicle-stimulating hormone (FSH) from gonadotropic cells in the pituitary, into the general circulation.[1,2] In turn, these heterodimeric glycoproteins regulate steroidogenesis and gamete maturation, in both males and females.[3,4] Shortly after the isolation and structural characterisation of porcine GnRH in 1971,[5,6] synthetic GnRH became available[7] and research began on its use in the treatment of hypogonadal disorders.[4,8,9] Numerous GnRH analogues were developed for their enhanced stability and longer duration of action. However, it was observed that supraphysiological stimulation increased analogue binding to GnRH receptors and this led to pituitary desensitisation,[10,11] down-regulation of the gonadotropins[12] and ablation of the reproductive axis. Thus, the direction of research changed, and many agonists and antagonists of varying potency, specificity and stability were (and continue to be) developed for use in the treatment of clinical disorders requiring modification of gonadal function.[4,13,14]

1. Clinical Applications of Gonadotropin Releasing Hormone (GnRH) Analogues

Antagonists are thought to act by competitively blocking the GnRH receptor and preventing endogenous GnRH from binding.[4] Agonists, on the other

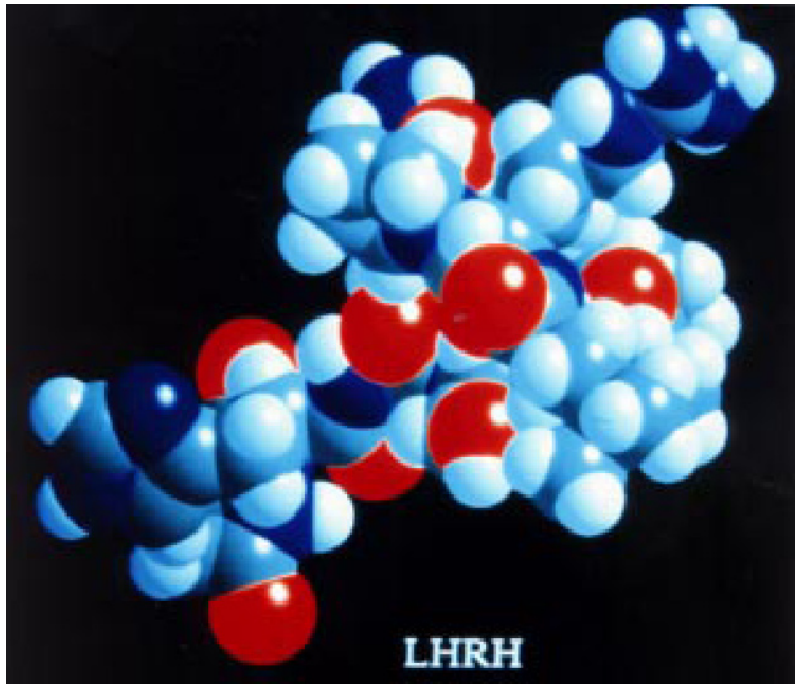

Fig. 1. A space-filled model of gonadotropin-releasing hormone (GnRH), also known as luteinising hormone releasing hormone (LHRH).

hand, provoke initial gonadotropin release,[4,12] resulting in hormonal flare and an exacerbation of symptoms, prior to pituitary desensitisation and gonadotropin ablation. Both agonists and antagonists provide clinicians with the tools to effect treatments in several broad categories of conditions (reviewed in Conn and Crowley[4] and Henzl[14]). One important category includes situations where gonadal steroid secretion exacerbates a disease; for example, prostate cancer,[15-18] endometriosis,[19,20] pre-menstrual syndrome[21,22] and uterine fibroids.[21,23-26] Another covers situations where interference in reproductive cycling is desired, such as in contraception,[27,28] before gynaecological surgery[29,30] or during *in vitro* fertilisation.[8,31-33] A third category covers suppression of a pathological function of the pituitary-gonadal axis, as in polycystic ovarian disease,[32,34] or in the treatment of precocious puberty.[35] Analogues are also used increasingly in adjuvant therapy of hormone-dependent gynaecological cancers[17,36-41] and in treating neoplasias exhibiting GnRH receptors.[39,42-44]

In women, complete and prolonged suppression of pituitary gonadotropin hormones induces symptoms of oestrogen deficiency such as hot flushes, vaginal dryness, dyspareunia and decreases in bone density over time (reviewed in Lemay[45]). Supplementation with oestrogens in severe cases has resulted in irregular menstrual bleeding and endometrial proliferation, as has the use of lower doses of analogues.[4] In men, greater success has been achieved using this medication as a type of chemical castration; benefits have included the relief of bone pain, decreases in acid and alkaline phosphatase levels and remission of metastatic disease.[46-49] Furthermore, greater long term survival can also be achieved using additional anti-androgen therapy and suppression of adrenal androgen synthesis.[50] However, adverse effects in males, such as loss of

libido, still cause problems with patient compliance (reviewed in Fraser[51]).

Other disadvantages associated with analogue treatment relate to the route of administration: orally, the analogues are inactivated and large doses are essential; intranasally, analogue absorption is low; and subcutaneously, the duration of absorption is limited.[12] Although newer slow release subcutaneous formulations appear to be more effective,[52,53] efficacy is counterbalanced by adverse effects such as headaches, nasal irritation, local inflammatory reactions, urticarial reactions at the injection site and sometimes anaphylactic reactions,[53,54] together with a growing awareness that small doses of analogue may stimulate short term growth of cancer cells *in vitro*.[55]

Research continues into improvements in delivery mechanisms, such as the development of vaginal suppositories.[4] Nevertheless, a therapy which is reliant on an individual's response to dose, route of administration, and frequency and duration of treatment, as well as the potency of a particular analogue, can be rather 'hit or miss' from a clinical, if not patient, perspective. Furthermore, as more and more elaborate designs are required to increase potency and decrease adverse effects, analogues have become increasingly expensive.[4,14,56] Thus, an alternative approach with equal efficacy to overcome many of these problems is evolving.[57,58] This approach is still in its infancy in terms of clinical studies, but it has been examined exhaustively in animal models. Consequently, although there is one anti-reproductive veterinary GnRH vaccine on the market, no clinical vaccines are available as yet.[59]

2. Immunoneutralisation of GnRH

Reports of early attempts to generate antisera for use in specific radioimmunoassays for endogenous GnRH were accompanied by descriptions of gonadal atrophy in the immunised animals.[60] Such reports stimulated interest in the use of immunisation to investigate selective neutralisation of GnRH, both as a means of studying the mode of action of the endogenous hormone and as an alternative to surgical castration. This led to the development of both passive[61,62] and active immunisation experiments.[63-67]

Because of its low molecular weight, GnRH is non-immunogenic; however, this can be remedied by increasing the size of the molecule[68] or by adsorbing it onto large inert particles.[68-70] The consistency of response is improved with the use of carriers such as keyhole limpet haemocyanin, thyroglobulin, gamma globulin, bovine serum albumin[65,71-75] and adjuvants such as Havlogen, dimethyldioctadecylammonium bromide, non-ionic surfactant vesicles, alum, Freunds' adjuvant, N-acetylmuramyl-L-alanyl-D-isoglutamine.[63,64,76] Freunds' adjuvant is commonly used in animal studies as it evokes high-level and long-lasting immunity, but its use is limited because of local and systemic toxicity.[77] Aluminium hydroxide has approval for clinical use; however, problems associated with it are also well known.[78,79] Thus, the development of novel, nontoxic, effective adjuvants is critical and is a progressive area of research (reviewed in Gupta and Siber[80]). Types of new adjuvants include vesicles and liposomes,[77,81] biodegradable microspheres,[82-86] immune stimulating complexes – ISCOMS,[87,88] lipids,[89,90] peptides[63] and glycopeptides.[91] The development of new types of delivery systems, in addition to enhancing immunogenicity, reduces the need for frequent immunisations.[83,84,86,92,93] Our group is working on the use of GnRH-containing vesicles entrapped in slow release polymer molecules.[94]

Other formulation considerations include the dosage, with effective experimental doses ranging from 10 to 600μg peptide[76,95,96] and carrier : peptide conjugation ratios varying between 2 and 20.[65,76] It has also been found that the specificity and efficacy of the antibodies raised are dependent on the site of conjugation of the peptide to the carrier molecule[64,96] and the class and subclass of the antibodies.[97] The delay of antibody buildup to effective levels (in clinical trials approximately 8 weeks) is seen as a disadvantage of immunisation,

and studies have begun to examine means of inducing earlier responses.[85]

3. Effects of Active Immunisation on Reproductive Physiology

Active immunisation against GnRH[73,96,98] and its analogues[36,75,99,100] has a marked physiological effect on the impairment of fertility in both males and females, as has been demonstrated in a variety of animal species. This takes place because of the resulting reduction in sex steroid levels, arrest of gametogenesis and gonadal atrophy through the induction of high titres of antibody.[101] GnRH antibody titres can be measured easily by enzyme-based or radiolabelled immunoassays. The titres are generally undetectable until after the first booster immunisation and thereafter they increase following successive administrations.[76,102]

Immunisation against GnRH in male animal studies has resulted in decreased gonadotropin and testosterone levels, gonadal atrophy and arrest of spermatogenesis. These studies have been carried out in a wide range of species including rodents,[75,96,103-109] sheep,[98,110,111] cattle,[110-114] pigs,[114,115] dogs,[27] rabbits,[96] horses[116,117] and monkeys[118] to name but a few. Treatment-related changes were observed as marked reductions in the size of the testes, epididymides, seminal vesicles and prostate. Histologically, the testes show marked reductions in spermatogenesis, with azospermia and a marked depletion of Leydig cells. Azospermia is accompanied by the pre-pubertal appearance of the epididymal tubes, seminal vesicles and prostate gland.[75,76] FSH levels and spermatogenesis return with the use of testosterone implants[66,104,105] or after antibody levels fall significantly and fertility, as determined by the production of offspring, has returned to normal.[75]

Studies carried out in a variety of female animal species have shown that active immunisation disrupts the oestrous cycle and that there is a correlation between antibody titre and the reproductive state of the animal. In the rat, animals with low antibody titres showed prolonged periods of oestrus and dioestrus[106,107] while high titres induced constant dioestrus,[102,108,109] as determined by vaginal cytology. However, in monkeys[118] and the ewe,[98] cycling stopped as soon as specific antibody levels became detectable.

Disruption of cycling can also be seen through a change in morphology of the reproductive tract and accessory sex organs, with an established correlation between the antibody titre and atrophy. In the rat, low titres resulted in ovaries with luteinised follicles and large numbers of antral follicles.[106] The uteri showed squamous-cell metaplasia and fibrosis of the endometrial stroma. On the other hand, high antibody blood levels resulted in small immature follicles without luteal tissue,[102] and caused severe atrophy of the uteri with cystic glandular dilation,[109] reduction of the intercellular matrix and condensation of nuclei; the endometrial glands were tubular or slightly coiled and contained a hyperchromatic cuboidal or columnar lining; in extreme cases the uterus was diminished in size.[102]

Ovarian and uterine weights were significantly reduced by active immunisation,[98,102,106,109,118] indicating that gonadotropin and steroid secretion reduced drastically. The vaginal and cervical epithelia also showed changes – stratification and keratinisation. These changes were unlike those seen in aging animals, the epithelia more closely resembled those seen in juvenile animals. Once the antibody titres declined, cycling was re-established, but could be stopped again with booster injections.[118] However, treatment reversibility, as determined by the ability to produce offspring, was dependent on the route of immunisation and the vaccine formulation. If a vaccine containing Freunds' adjuvant or alum was given by intraperitoneal injection, adhesions and irreversible tissue damage occurred,[102] although neither Freunds' adjuvant nor the intraperitoneal route would be used in a clinical situation.

4. Experimental Tumour Studies

In an extensively used animal model, the Sprague-Dawley rat, spontaneous, multiple mammary adenocarcinomas can be induced by the single administration of the polycyclic hydrocarbon 7,12-dimethylbenz[a]anthracene (DMBA).[119] These tumours are considered to be hormone dependent, as they regress following ovariectomy[120] or after the administration of various steroids[119,121] and antihormones.[120,122] Tumour suppression and regression by GnRH neutralisation has been demonstrated in this experimental model.[100] A GnRH analogue, GnRH-glycys,[95] was conjugated to a carrier protein derived from tuberculin (PPD) and female rats bearing DMBA-induced tumours were immunised. The primary observation was that serum oestradiol concentrations were substantially reduced but not totally suppressed. This may have been due to the production of oestrogen by other tissues (skin, adipose, adrenal glands) not necessarily under the control of GnRH.[123] Animals showing extreme gonadal atrophy and changes in histology did not exhibit compound mammary tumours. Similar results have been observed using GnRH conjugated to bovine serum albumin.[71]

A common experimental male tumour model is the Dunning R3327 rat prostate adenocarcinoma.[124] These tumours are implanted subcutaneously in the flank of male Copenhagen-Fischer rats.[125-127] There are various sublines, including androgen-sensitive Dunning R3327-PAP and androgen-independent R3327-AT2.1. Active immunisation with GnRH conjugated to diphtheria toxoid has been shown to inhibit the growth of R3327-PAP by suppression of cell division as opposed to an increase in cell death.[128] Even in the androgen-insensitive R3327-AT2.1 there is a slight reduction in the volume of the tumour, which is believed to indicate a local stimulatory GnRH effect on this subline.[128] In another study, active immunisation against GnRH was carried out alone or in combination with a GnRH-antagonist, compared with GnRH-antagonist treatment only.[129] Tumour growth was inhibited in all the treatment groups; however, the combination treatment of immunisation and antagonist resulted in the greatest suppression.

5. Clinical Studies

Prostate cancer represents a heterogeneous disease, associated with varying degrees of tumour behaviour and aggressiveness, patterns of metastasis and responses to therapy. Metastasis commonly occurs in the regional lymph nodes and/or the bony skeleton and contributes significantly to the death of the patient. The major difficulty in treating prostate cancer is the lack of diagnostic tools to accurately predict the behaviour of a given tumour. For example, although many prostatic cancers are clinically insignificant, others will result in considerable morbidity and mortality. The use of androgen ablation in the treatment of prostate cancer was first demonstrated in 1941,[130] using orchidectomy or oestrogen therapy, and many new classes of drugs have since been introduced.[131] In addition, indirect evidence was obtained by observations that men with 5α-reductase deficiency and young castrated men did not develop prostate cancer.[132-134]

Total androgen ablation by means of castration, antiandrogens or GnRH analogues remains the standard systemic treatment for this disease and it is estimated that 70 to 80% of patients benefit from this therapy.[135] However, responses are short-lived in most patients with progression of hormone-refractory disease being inevitable over the course of 2 to 3 years.[136,137] To date, there has been little evidence to support the routine use of nonhormonal chemotherapeutic agents over symptomatic treatment only.[138] Chemoprevention is most effective in the early stages of tumour formation where reversibility may be feasible, but once stromal invasion occurs disease progression is not easily preventable. However, approximately one-third of patients diagnosed with prostate cancer will present with advanced disease and, for these, more radical treatment is required.

A number of GnRH vaccine preparations are reported to have been tested in men, in several coun-

tries.[101,139] One GnRH vaccine has been tested in Phase I and II clinical trials in advanced metastasising carcinoma, with reportedly encouraging results.[140-143] Where antibodies against GnRH were induced, the LH, FSH and testosterone levels declined to near castration levels, accompanied by a reduction in prostate-specific antigen and acid phosphatase. Shrinkage of the prostate gland was clearly demonstrated and nephrostograms showed clearance of urinary passages in most of the patients.

Prostatic tumour cells are often responsive to androgen ablation, but eventually they fail to respond (become resistant) and continue to survive and spread in the absence of androgenic stimulation. Following orchidectomy, 30 to 40% of patients develop resistant clones within 12 months, 5 to 15% live for approximately 10 years, 50% live less than 2 years and 10% live under 6 months.[144]

Although the effects of steroid ablation have been known for over 50 years,[130] little headway has been made into the understanding of the mechanism of formation of resistant clones.[143-145] Several hypotheses exist, among them that various peptide growth factors, receptors[145] or ligands are implicated in androgen-independent cell survival,[26,146] or that the expression of the protein caveolin,[143,147,148] which is thought to be controlled by LDL cholesterol and a high fat diet,[148,149] is involved. In our experience with the Dunning prostatic cancer cell lines, the characteristics of the tumours changed rapidly following anti-GnRH treatment, leading to hormone-independence. At the time (in the early 1990s), we believed this to be a fault of the tumour model. However, in retrospect, it was probably a reflection of what is being observed now in clinical studies. It is becoming evident that androgen signal transduction pathways have an essential role in tumour progression, even in the absence of androgen, and patients continue to benefit from androgen withdrawal despite the presence of androgen-resistant clones.[150]

Similar observations with hormone-resistant clones have also been made with the DMBA-induced mammary tumours.[100] In breast cancer, hormonal therapy began over a hundred years ago with the observation that in some pre-menopausal patients, bilateral oophorectomy caused tumour regression.[151] Various means have been used to disrupt ovarian function, but irrespective of the type of therapy, regression rates have never exceeded 35%. Nevertheless, adjuvant chemotherapy in conjunction with hormonal therapy and radiation is used to significantly prolong survival, while maintaining a reasonable quality of life.[41,152]

Investigation of one anti-GnRH vaccine in postmenopausal patients[153] resulted in the conclusion that active immunisation is a safe option and an affordable therapeutic agent for use in some hormone-dependent clinical conditions. Three women, with a mean age of 60 years, 5 years' amenorrhoea, severe hypoestrogenism and elevated serum LH and FSH levels, were immunised twice with 300μg GnRH, at monthly intervals. After 60 days there was an increase in antibodies to GnRH, followed by a decrease in gonadotropins. After 180 days, in the absence of booster injections, these effects were reversed. These results are comparable to our animal studies[100] but, in view of the irreversible tissue changes, a cautionary note should be attached to clinical studies intending to use premenopausal women who wish the option of childbearing.

6. Future Work

Release factors other than GnRH, including hormones and cytokines, have been implicated in the control of gonadotrophin secretion.[154] Furthermore, it is clear that removal of the GnRH stimulus has more profound inhibitory effects on LH than on FSH.[155] FSH secretion can remain for some time after withdrawal of GnRH and can persist for several days from pituitaries *in vitro*. Short term suppression of GnRH removes LH secretion, while FSH persists,[156] but longer term suppression removes FSH secretion.[157] One approach worth considering would be to target FSH or LH directly to reduce some of the adverse effects of total gonad-

otropin ablation and maintain better control.[158] This may be particularly relevant in light of recent data which implicates FSH as a ligand in the regulation of the growth of hormone-refractory prostate cancers.[159]

Another consideration is that there is growing evidence for more than one GnRH form in the primate,[160] each with different functions.[161] It may be possible to target these separately by using bioeffective monoclonal antibodies, developed against GnRH.[142,162,163] The advantage of these is that they can be humanised and produced cost-effectively in bacteria and plants, thus paving the way for passive use of such antibodies for immunotherapy of hormone-dependent cancers.

7. Conclusion

As a result of animal experimentation, it has become obvious that the effects of GnRH neutralisation on the female system are far more complex than in the male. Translated to human terms, this could result in unnecessary complications in women. The ease of treatment reversibility in men indicates a greater potential for this type of therapy.[164] In recent years, there has been an increase in the number of studies examining the use of different unconjugated GnRH analogues (reviewed in Jackson et al.[135]) in conjunction with radiotherapy and surgery, as neoadjuvant (short term) therapy for prostate cancer.[15-17,47-49] Although no agreement as to the benefits has come from these studies,[18,165,166] endocrine manipulation, whether directly by chemical means or indirectly by the immunological approach, appears to have a part to play in the initial treatment of steroid-dependent cancers in terms of delay in the progression of the disease, but not as a cure.[49,149,167-171]

The evidence presented in this review indicates that the main advantage of using vaccines as opposed to the free peptide drug lies in the reduction of adverse effects. In terms of increasing health costs, vaccines of this nature use small quantities of analogue, as opposed to large, expensive doses, with equal efficacy. It seems reasonable to conclude, therefore, that significant advances will continue to be made in terms of improvements in delivery, vaccine formulation and manufacturing processes,[80,85,92,172,173] and that these vaccines will play a major role in cancer adjuvant chemotherapy to facilitate, rather than replace, more drastic therapies. Furthermore, a growing interest in the development of anti-cancer vaccines using non-hormone antigens[174-177] could have large implications for GnRH vaccines.

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