Analysis of an Anti-GnRH Chimeric Antibody Purified from Tobacco Leaves

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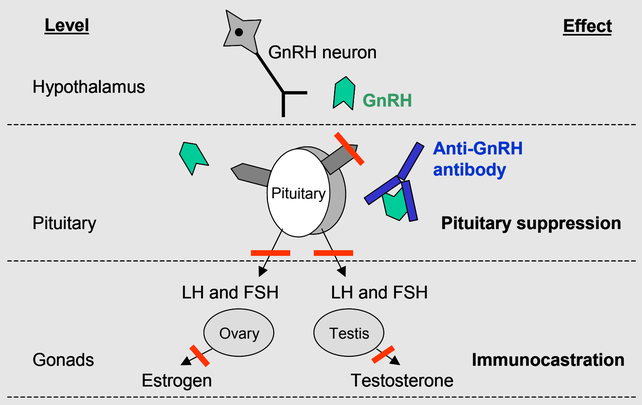
Introduction

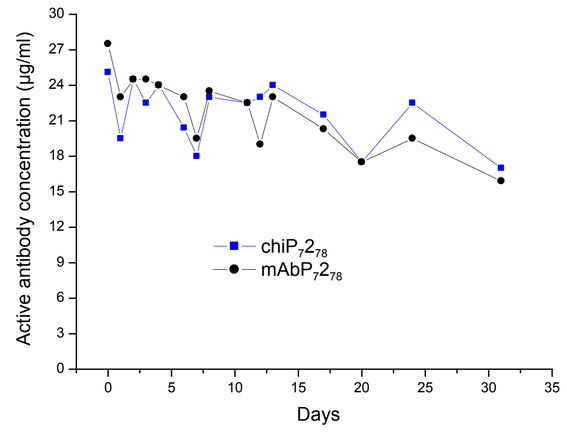
Gonadotropin releasing hormone (GnRH), a decapeptide neurohormone, is the key regulator of the reproductive hormone cascade. The effect of immunoneutralisation of GnRH is equivalent to the GnRH antagonists that are widely used for various clinical conditions, like:-

- Prostate cancer
- Breast cancer
- Endometriosis
- Precocious puberty
- ART (artificial reproductive technology)



Presented here is the characterisation of a mouse-human **chimeric antibody (chi$P_7 2_{78}$)** recognizing GnRH, transiently expressed in tobacco leaves by vacuum assisted agroinfiltration. The variable domains of the chi$P_7 2_{78}$ were cloned from a well characterized **monoclonal antibody (mAb$P_7 2_{78}$)** having high affinity for GnRH.

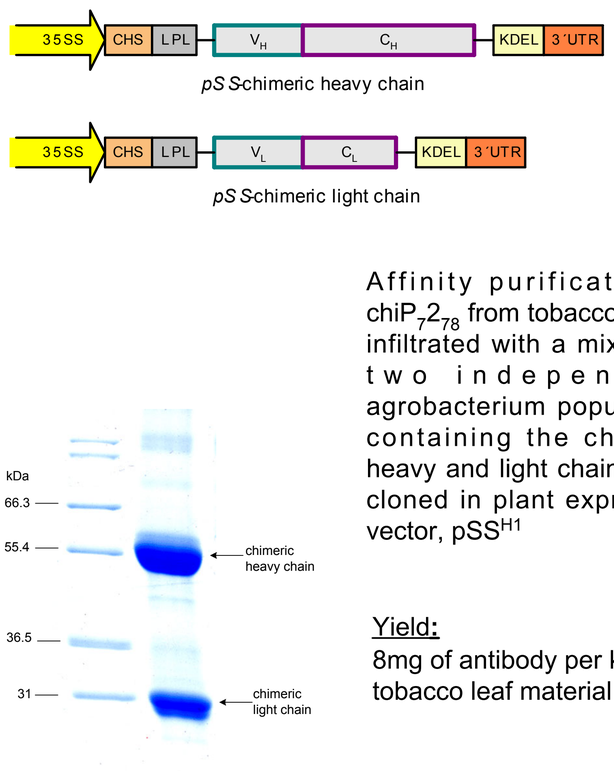
Stability of mAb$P_7 2_{78}$ and chi$P_7 2_{78}$ in human serum at 37°C in vitro



Antibody activity after 32 days at 37°C in human serum
chi$P_7 2_{78}$ – 70%
mAb$P_7 2_{78}$ – 60%

Determination of active antibody concentration (at each time point) by BIAcore

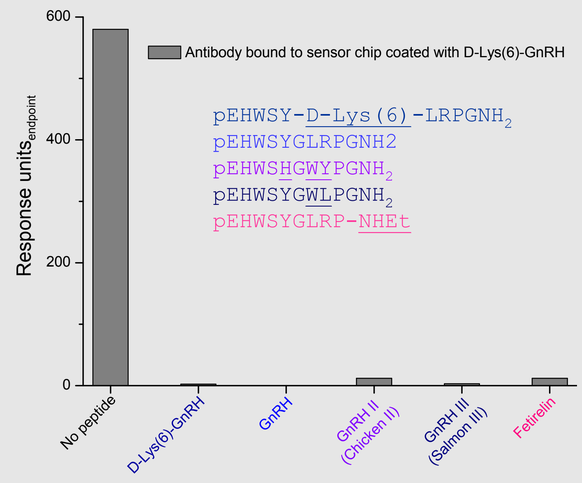
Transient expression and purification of chi$P_7 2_{78}$



Affinity purification of chi$P_7 2_{78}$ from tobacco leaves infiltrated with a mixture of two independent agrobacterium populations containing the chimeric heavy and light chain genes cloned in plant expression vector, pSS^{H1}

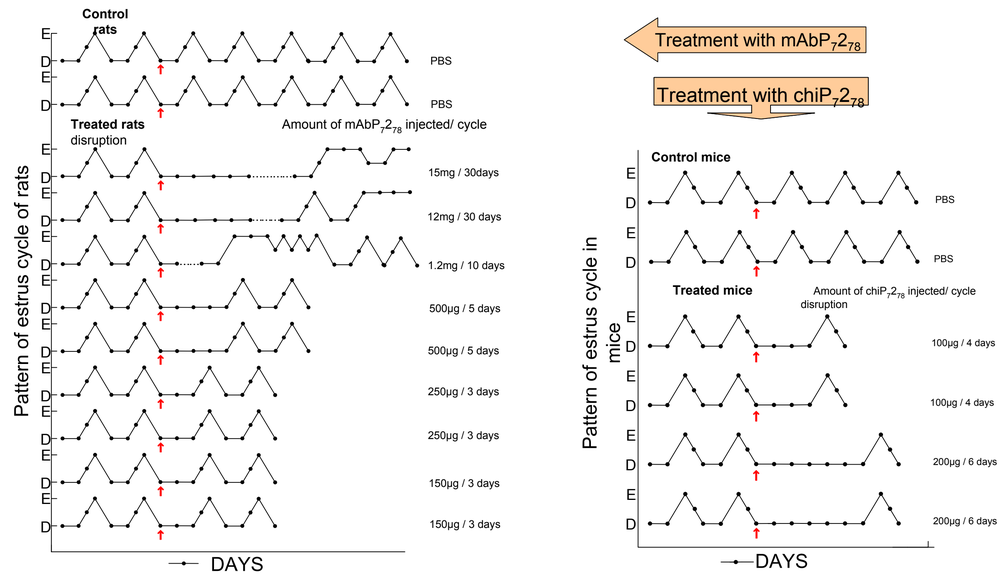
Yield:
8mg of antibody per kg of tobacco leaf material

Chi$P_7 2_{78}$ recognises GnRH II and GnRH III, the two recently discovered isoforms in humans



Incubation of Chi$P_7 2_{78}$ with a 1000 fold excess of the peptides completely inhibits the antibody from binding to the D-Lys(6)-GnRH coated chip on the BIAcore.

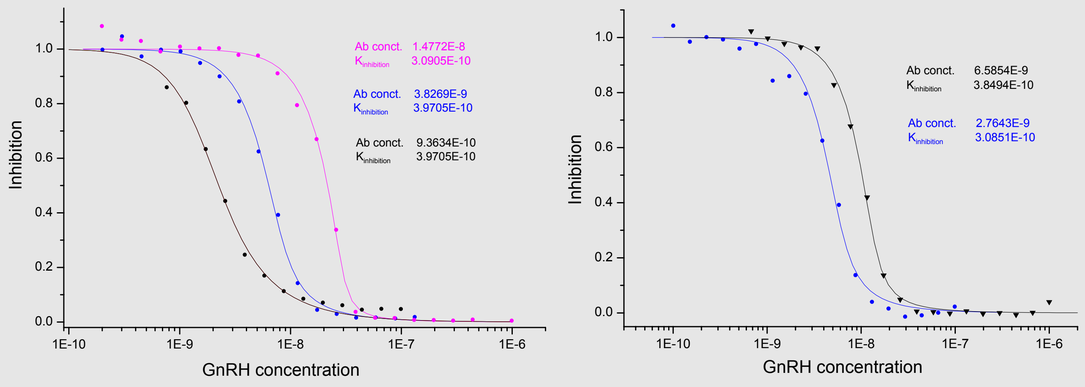
Bioneutralisation of LHRH causes estrus suppression in rodents



E – estrus D - diestrus ↑ - day of injection
Estrus cycle monitored by vaginal cytology study

Intra-peritoneal injection of GnRH specific antibodies given to regularly cycling rodents causes a dose dependent suppression of the estrus cycle, a consequence of the disruption of hypothalamic-pituitary-gonadal axis.

Indistinguishable affinities of chi$P_7 2_{78}$ (3.4×10^{-10} M^{-1}) and mAb$P_7 2_{78}$ (3.5×10^{-10} M^{-1})



The molecular interaction between GnRH and the antibodies was analysed using the BIAcore system using a homogenous phase assay. Experiments were carried out under mass transport limitation (MTL = 0.86) measuring concentrations of antibodies having one or both binding sites free. The inhibitory constant Ki and the concentration of the antibody were determined by non-linear least square fit using the given equation:

$$m = m^0 \cdot \left(1 - \frac{1}{16 \cdot C_{total}^{\ 2}} \cdot \left\{ \left[K_I + 2 \cdot C_{total} + L_{total}\right] - \sqrt{\left(K_I + 2 \cdot C_{total} + L_{total}\right)^2 - 8 \cdot L_{total} \cdot C_{total}} \right\}^2 \right)$$

KI = Inhibitory constant; Ctot = total antibody concentration; Ltot = total GnRH concentration; m = observed binding rate; m0 = observed binding rate without peptide

Conclusion and future perspectives

The plant expressed chi$P_7 2_{78}$ was shown to have inditinguishable affinities from the parental antibody, highly stable and capable of neutralizing GnRH in vivo to cause a bio-effect, demonstrating its ability to disrupt the hypothalamo-pituitary-gonadal axis.
The anti-GnRH antibodies have the potential for use as a natural, convenient and non-surgical mode of therapy for many clinical conditions that will benefit from the down regulation of the reproductive hormones. Molecular farming of the recombinant antibody will enable production of large amounts of the antibody, a prerequisite for the development of any drug.
The ability of the antibody to bind to the GnRH isoforms presents exciting possibilities. The antibody can be used to study the role and localisation of the peptide variants. Protein engineering might be used to evolve derivatives of $P_7 2_{78}$ that can discriminate between the different isoforms fascilitating the research.