ISSN: 2475-6296

Review Article

Journal of Clinical & Experimental Immunology

A New Monoclonal Antibody-Based Biosimilar GnRH Antagonist

Gregory Lee

UBC Centre for Reproductive Health, Vancouver, Canada

***Corresponding author**
Dr. Gregory Lee, UBC Centre for Reproductive Health, 9117 Shaughnessy Street Vancouver, BC, V6P 6R9, Canada, E-mail: leecyg@gmail.com

Submitted: 05 Nov 2018; **Accepted**: 13 Nov 2018; **Published**: 20 Nov 2018

Abstract

GHR106 is a monoclonal antibody generated against an oligopeptide corresponding to that in the extra cellular domains of human GnRH receptor. The humanized forms of GHR106 exhibit almost identical biological properties to those of decapeptide GnRH antagonists such as Antide and Cetrorelix. The Fc region of humanized GHR106 has been replaced with IgG4 subtype to eliminate activities of effector function. Therefore, the newly humanized GHR106-IgG4Fc can be used clinically as biosimilar GnRH antagonists of higher molecular size, and longer half-life (hrs. vs. days) for therapeutic treatments of fertility-related health conditions without complications arising from the effector functions of immunoglobulins.

Keywords: GHR106 Monoclonal Antibodies, Biosimilar GnRH Antagonist, Long Acting GnRH Antagonist GHR106 (IgG4-Fc)

Abbreviations

Antide: peptide GnRH antagonist
ALP: alkaline phosphatase
Cetrorelix: an injectable gonadotropin-releasing hormone (GnRH) antagonist
C-fos: onco gene
Cycling D1: A 36-kD protein which regulates cycling-dependent protein kinase activity
EGF: Epitermal growth factor
Fab: Varian domain fragments of immunoglobulins
Fc: Constant domain fragments of immunoglobulins
GHR106: monoclonal antibody generated in mice immunized against N1-29 amino acid residues
HGHR106: Humanized forms of GHR106 monoclonal antibody
GnRH: GnRH receptor
IgG1/IgG4: immunoglobulin G with subtypes G1 and G4 in the Fc domains, respectively
L37: ribosomal protein
Luprorelin: peptide GnRH agonist oligopeptide of human GnRH receptor
Po: ribosomal protein
P1: ribosomal protein
P21: cell cycle regulator
ScFv: Single-chain variable fragment of IgG
Trastuzumab/Beracizumab/Panitumumabe: Human or humanized monoclonal antibodies (antibody drugs for clinical uses)

GHR106 is a Biosimilar GnRH Antagonist

GHR106 is a monoclonal antibody generated in mice against an oligopeptide corresponding to N1-29 amino acid residues in the extra cellular domains of human GnRH receptor found mainly in the anterior pituitary or reproductions-related tissues as well as cancers [1-4]. The initial biological and immunological studies revealed that GHR106 has affinity and specificity similar to those of decapeptide GnRH antagonists such as Antide and Cetrorelix [5, 6]. Functional assays such as induced apoptosis and complement-dependent cytotoxicity reactions were also performed to indicate that GHR106 and decapeptide antagonist, Antide are biosimilar in their respective biological/immunological actions to culturing cancer cells [5, 6]. Effects of GHR106 and Antide on gene regulations of cancer cells are virtually identical on the molar basis [6]. Therefore, it has become apparent that GHR106 or its humanized forms are first-in class monoclonal antibodies which are biosimilar to decapeptide GnRH antagonists, except that the former has such a larger molecular size (80 kDa) and longer circulations half-life (5-21 days or 120-500 hrs).

In view of the biosimilarity, GHR106 and its humanized forms can serve as long-acting GnRH antagonists for treatment of numerous indications for fertility-related human health/disease conditions such as ovulations inhibition, endometriosis, ovarian cysts, uterine fibroids, precocious puberty, premenstrual syndrome as well as cancer of many tissue origins [7-9].

Biosimilarity Based on Binding Affinity and Specificity

Binding immunoassays were performed with well-coated cancer cell extract or with synthetic N1-29 oligopeptide on the extracellular domains of GnRH receptors, GHR106 and its humanized forms including hGHR106 (IgG1-Fc) and hGHR106 (IgG4-Fc) were employed for dose-depending binding assays. Goat anti-mouse IgG-ALP (alkaline phosphatase-labeled) and goat anti-human IgG-ALP were used as the enzyme-labeled detecting antibodies for determination of affinity contents (KD in NM) for specific

binding. It was generally concluded that the Kids of GHR106 and its humanized forms (IgG1Fc or IgG4F4 subtypes) had been determined to be between 2-5 NM, which are comparable to those of GnRH antagonists such as Antide (0.5-1 NM).

In a separate experiment, binding assays were performed with well-coated N1-29 oligopeptides derived separately from human, monkey and mouse species for comparisons of binding specificity. It was generally observed that GHR106 or hGHR106 showed similar degree of binding affinity to either human or monkey peptides coated on micro wells, but not to the mouse one. This is not unexpected, since a high degree of sequence homology was observed between these human and monkey oligopeptides (≥ 94%), but not with those of mouse (≤ 79%) [10, 11].

Biosimilarity based on Functional Assays of GHR106 and Peptide GnRH Antagonists

The Biosimilarity between GHR106 and peptide GnRH antagonists, can also be demonstrated by induced apoptosis of treated cancer cells. Results of comparative TUNEL apoptosis assays revealed that GHR106 (in 1 or 10 μg/ml) and Antied, GnRH antagonist (1 μg/ml) were shown to induce apoptosis to a similar extent on the molar basis (80 kDa vs. 1.5 kDa) [12]. Therefore, GHR106 and Antide are biosimilar in terms of functional properties of these two GnRH antagonists.

Biosimilarity Based on Similar Patterns on Gene Regulations of Affected Cancer Cells

Biosimilarity between GHR106 and GnRH peptide antagonist, Antide was demonstrated by gene regulations studies. Expressions of a number of selected genes involved in proliferations or survival of cancer cells were investigated and compared. They are listed as follows: GnRH, GnRHR, P0, P1, L37, and EGF, c-fos, P21 and cycling D1 (5, 6). Upon respective ligand treatments, GHR106 and Antide were found to up-regulate GnRH expression (≥ 50%), while that of GnRH receptor remained unchanged. EGF and Cyclin D1 are both down regulated upon treatments of either ligand to cancer cells [6]. The observations of identical gene regulation pattern changes by either ligand are consistent with the similar molecular mechanisms of actions of these two different ligands upon interaction with cancer cells [13-15].

Half-life of GHR106 and its Antibody Fragments as well as Decapeptide GnRH Antagonists

Compared to decapeptide GnRH antagonist such as Antide which has only hours of circulation half-life, GHR106 has a relatively long half-life of 5-21 days. Upon fragmentations of GHR106 into IgG fragments such as $(Fab)_2$, Fab and ScFv (single chain fragments of variable regions) their circulation half-lives can change drastically from 120-500 hours to less than 12 hours [16, 17]. At the same time, the molecular size of GHR106 antibodies is reduced from 160 kDa for IgG and 25 kDa for ScFv fragment. Therefore, the half-lives of GHR106 antibody-based GnRH antagonists can be adjusted from days to hours through the process of fragmentations or molecular size reductions, if circulation half-life of GHR106-based antagonists needs to be optimized during clinical applications. The half-life analyses of GHR106 and related IgG fragments are presented in (Table 1) together with those of selected GnRH decapeptide analogs as well as clinically used humanized antibodies drug.

Table 1: comparisons of circulation half-life of GHR106, Fab fragments, GnRH peptide analogs and other clinically used mabs

Drug candidates	Molecular species (molecular weight)	Estimated half-life (hrs.)
GHR106 anti-GnRH receptor Mab (IgG4)	IgG4 (humanized) (160 kDa)	120-500
	(Fab')2 (110 kDa)	12-20
	Fab (55 kDa)	12-20
	ScFv (25 kDa)	≤ 12
Trastuzumab	Humanized (160 kDa)	24.2
Beracizumab	Humanized (160 kDa)	480
Panitumumab	Human (160 kDa)	180
GnRH decapeptide analogs	Cetrorelix (Antagonist) (1.5 kDa)	10-63
	Luprorelin (1.5 kDa)	3
	Native GnRH (1.5 kDa)	0.03-0.06

Note: GHR106 in IgG4 subtype can be reformulated as active Fab fragments with shortened half-life

Conclusions

In this short review, we have been able to show that through biological and immunological studies, GHR106 and its humanized forms of IgG, in the Fc domain can be utilized as biosimilar GnRH antagonists for future clinical applications. The major benefit of these antibody-based antagonists may arise from their relatively long half-life during clinical application for treatment of many fertility-related health conditions or diseases [16, 17]. Comparative clinical studies of antibody-based GnRH antagonists would be required for the efficacy of these biosimilar GnRH antagonists [13-15].

Acknowledgement

The research project describe in this review was supported in parts by NRC-IRAP (#821-901) of Canada. Helpful comments and suggestions by Dr. Joseph Han are acknowledged.

Conflict of Interest

The author of this review is co-founder of Vancouver Biotech Ltd. In Vancouver No conflict of interest regarding the content is involved.

References

1. Chi L, Zhou W, Prikhozhan A, Flanagan C, Davidson JS, et al. (1993) Cloning and characterization of human GnRH receptor, Mol. Cell Endocrinal 91: R1-R6.
2. Clayton RN (1989) Gonadotropin-releasing hormone: its action and receptors. J Endocrinal 120: 11-19.
3. Karande A, Rajeshwari K, Schol DJ, Hilgers JH (1995) Establishment of immunological probes to study human gonadotropin-releasing hormone receptors, Mol. Cell Endocrinal 114: 51-56.
4. Lee G, Ho J, Chow SN, Yasojima K, Schwab C, et al. (2000) Immunoidentification of gonadotropin releasing hormone receptor in human sperm, pituitary and cancer cells. Am J Reprod Immunol 44: 170-177.
5. Lee G, Ge B (2010) Growth inhibition of tumor cells in vitro by using monoclonal antibodies against gonadotropin-releasing hormone receptor. Cancer Immunol Immunother 59: 1011-1019.
6. Lee G, Zhang H, Tang Y (2013) Anti-GnRH receptor monoclonal

antibodies as bioequivalent analogs of GnRH. In: Sills ES, editor. Gonadotropin-Releasing Hormone (GnRH): Production, Structure and Function. Los Angeles: Nova Biomedical 10: 157-174.
7. Tur-Kaspa I, Ezcurra D (2009) GnRH antagonist, cetrorelix, for pituitary suppression in modern, patient-friendly assisted reproductive technology. Exper Opinion on Drug Mutable and Repro Technol 5: 1323-1336.
8. Copperman AB, Benadiva C (2013) optimal usage of the GnRH antagonists: a review of literature. Reprod Biol and Endocrinal 11: 20.
9. Engel JB, Rieqer L, Dietl J, Hönig A (2007) The GnRH antagonist cetrorelix: established indications and future potential. Expert Reviews of Obstetrics and Gynecology 2: 431-440.
10. Reinhart J, Mertz LM, Catt KJ (1992) Molecular cloning and expression of cDNA encoding the murine gonadotropin releasing hormone receptor. J Biol Chem 267: 21281-21284.
11. Santra S, Rao VS, Shanker YG, Rao AJ (2006) Cloning and characterization of bonnet monkey GnRH receptor. Mol Human Reprod 6: 415-427.
12. Lee G, Chow SN, Chien CH, Liu S (2015) Anti-GnRH receptor monoclonal antibodies, first-in-class GnRH analog. J Gynecol Res 1: 102.
13. Hayden C (2008) GnRH analogs: applications in assisted reproductive techniques. Enr J Endocrino 159: 517-525.
14. Huirne JA, Lambalk CB (2001) Gonadotropin-releasing-hormone receptor antagonists. Lancet 358: 1793-1803.
15. Olivennes F, Cunha-Fiho JS, Fanchin R, Bouchard P, Frydman R (2002) the use of GnRH antagonists in ovarian stimulation, Hum Reprod Updated 8: 279-290.
16. Adams R, Griffin L, Cornpson JE, Jairaj M, Baker T, et al. (2016) Extending the half-life of a fab fragment through generation of a humanized anti-human serum albumin Fv domain: an investigation into the correlation between affinity and serum half-life. Mabs 8: 1336-1346.
17. Bazin-Redureau MI, Renard CB, Schermann JM (1997) Pharmako kinetics of heterologous and homologous immunoglobulin G, F(ab')2, and Fab after intravenous administration in the rat. J Pharm Pharmacol 49: 277-281.