Abstract

Pegvisomant is the pegylated form of mutant growth hormone (B2036). B2036 has increased affinity in one binding site and lowered affinity in its second binding site, it has been shown that this molecule still enables dimerisation of the growth hormone receptor at the cell surface but does not allow the necessary conformational changes for signalling. Pegylation decreases the antagonistic activity of B2036, however the rate of clearance of the pegylated B2036 is greatly reduced compared to the unpegylated form. Even though the antagonistic activity of pegvisomant is lower than B2036, the reduced rate of clearance makes it an effective clinical drug for the treatment of conditions such as acromegaly.

Introduction

The growth hormone (GH) antagonist, pegvisomant, is the first specific growth hormone receptor antagonist to be developed for the treatment of acromegaly (Trainer et al. 2000, van der Lely et al. 2001). In this review, the relationship between pegvisomant structure and function is discussed.

Growth hormone signals by binding two molecules of its receptor (GHR) at the cell surface. As a consequence there are two receptor binding sites on the GH molecule (Fig. 1). The affinity of site 1, for the receptor, is greater than that of site 2.

The core molecule in pegvisomant is B2036, which is GH with a number of mutated amino acids. A G120K mutation at binding site 2 greatly reduces the binding affinity of this site. Additionally, B2036 also contains mutations in binding site 1, which increase the affinity of this binding site. These mutations enhance binding to growth hormone binding protein (GHBP, derived from the extracellular domain of the GH receptor) but as our data suggests they do not change affinity for the cell surface receptor.

To generate pegvisomant, 4–6 moities of polyethylene glycol (PEG) 5000 are then conjugated to this core B2036 molecule. Pegylation has little effect on the affinity of pegvisomant for GHBP but reduces affinity for the cell surface receptor. Therefore, there is a high dose requirement for pegvisomant in clinical practice; however pegylation has a benefit in that it increases the half life of clearance.

Background

Site 2 binding

The discovery of the GH site 2 mutant antagonist was serendipitous. The original observation came whilst groups were looking for mutations that might increase agonist activity. A mutation at G119A in the bovine GH molecule suppressed the growth of transgenic mice (Chen et al. 1990). Subsequent research demonstrated that a similar mutation at G120R in the human molecule also acted as a GH antagonist (Chen et al. 1994). With the characterisation of the crystal structure for GH binding to a homo-dimer of its receptor (de Vos et al. 1992), it was demonstrated that the mutation at G120R prevented effective binding of the second GH receptor molecule. This single mutation at site 2

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binding in the GH molecule is the basis of the GH antagonistic activity of pegvisomant.

**Site 1 binding**

Using monovalent phage display, various residues in the GH molecule have been demonstrated to be critical in binding at site 1. By mutating these residues, it has been demonstrated that the affinity of site 1 for GHBP can be increased (Lowman & Wells 1993). This potential increase in site 1 affinity could be an advantage in an antagonist, which had a mutated site 2 (Cunningham et al. 1998). In B2036, there are 8 mutations at binding site 1 (H18D, H21N, R167N, K168A, D171S, K172R, E174S and I179T). In practice, these mutations at binding site 1 increase the affinity of B2036 for recombinant GHBP by

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Figure 1 The crystal structure of growth hormone complexed to the extracellular domains of its receptor. Growth hormone (red) can bind two molecules of its receptor (blue). Thus the growth hormone has two binding sites, binding site 1 (high affinity) and binding site 2 (low affinity). (Crystal structure determined by de Vos et al. 1992, Protein Database (PDB) code: 3 HHR.)
four- to five-fold compared with GH (Goffin et al. 1999, Ross et al. 2001). However, binding affinity to cell surface receptor in stable cell lines expressing the human GH receptor is equivalent between B2036 and GH (Ross et al. 2001). The results on binding studies suggest that although the site 1 mutations create an increased affinity for GHBP, they do not change the affinity for the dimeric complex at the cell surface.

**GH antagonists and receptor trafficking**

There is now evidence that the B2036 antagonist binds to a receptor dimer but does not induce conformational changes required for signalling (Ross et al. 2001). This evidence comes from two sets of experiments. In Western blotting experiments, there was evidence that the antagonist might associate with a receptor dimer in a 2:2 molecular configuration (Harding et al. 1996). In recent experiments, we have demonstrated that GH and B2036 have similar numbers of binding sites at the cell surface and that the number of binding sites is doubled in the presence of a dimerisation blocking antibody (Ross et al. 2001). This strongly suggests that the antagonist, B2036, binds to a receptor dimer. It is clear that the antagonist, B2036, prevents JAK2 phosphorylation and STAT5 signalling (Maamra et al. 1999). However, the antagonist receptor complex is still rapidly internalised into cells expressing the human GH receptor (Maamra et al. 1999). This internalisation is associated with a down-regulation of GH binding and signalling with a refractory period of around 2–3 h (Ross et al. 2001). This is consistent with recycling and the presence of new receptor at the cell surface.

**Pegylation**

The half-life of native GH is short, as is that of B2036. In order to develop an effective treatment, it was recognised that an antagonist with delayed clearance would have to be generated. Pegylation of GH reduces its affinity for the receptor but
delays its clearance (Clark et al. 1996). If the molecular ratio of PEG to GH is optimised, a pegylated molecule with increased bioactivity can be generated. PEG conjugates to lysines and the N-terminus, increasing the molecular size and hydrodynamic volume of a protein. Pegvisomant is B2036 with 4–6 PEG moieties covalently conjugated.

It was recognised early on that there was a lysine in binding site 1 of GH and, was therefore, mutated to prevent pegylation in site 1. In addition, to increase the antagonist activity, the mutation generated at site 2 was a lysine, G120K, thus increasing the probability of pegylation at site 2. Comparing binding of site 2 only mutated GH (pegylated GH.G120K) with pegylated B2036, there is a four-fold greater binding affinity for pegylated B2036 (Ross et al. 2001). This is presumably explained by the lack of pegylation in binding site 1 of B2036 compared with the probability of pegylation of site 1 in the GH.G120K. Thus, in the pegylated antagonist, there is an advantage to having the site 1 mutations. However, pegylation does considerably reduce the binding affinity for pegvisomant at the cell surface. There is only about a four- to five-fold reduction in binding of pegvisomant to GHBP but there is a much greater reduction of around 20-fold when binding is studied at the cell surface receptor. This is presumably explained by stearic hindrance.

Even though the activity of pegvisomant is reduced due to pegylation, its efficacy as a clinical drug is derived from its highly reduced rate of clearance, which is a consequence of pegylation.

Conclusions

Figure 2 shows a hypothesis for the interaction of GH, B2036 and pegvisomant with the GHR. The site 2 mutation prevents a conformational change required for signalling but does trigger internalisation of the antagonist receptor complex. The site 1 mutations enhance binding to GHBP but do not change affinity at the cell surface. Pegylation reduces the affinity of the antagonist for the cell surface receptor because of stearic hindrance, but a high concentration is still effective in completely blocking GH signalling. The benefits of pegvisomant compared with B2036 is that the former has a greatly reduced rate of clearance, which facilitates clinical treatment using this molecule.

References


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