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Discovery and uses of pegvisomant: a growth hormone antagonist

Odkrycie i zastosowanie pegwisomantu: antagonisty hormonu wzrostu

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Abstract

Growth hormone (GH) is a well established participant in several complex physiological processes including growth, differentiation, and metabolism. Recombinant human GH is a drug that has been approved for use for several clinical conditions where the action of GH is diminished or completely lacking. Thus there is considerable interest in developing novel drugs that modify the function of GH. Only in the last several decades have the detailed structural features of GH along with its interaction with its receptor been elucidated. In this review we summarise the basic structural and functional properties of GH, its receptor and their interaction. In addition, we discuss the discovery and development of an effective GH receptor antagonist, pegvisomant, and summarise potential therapeutic uses of this drug.

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***Key words**: growth hormone, growth hormone receptor, growth hormone receptor antagonist*

Streszczenie

Hormon wzrostu (GH, *growth hormone*) uczestniczy w wielu fizjologicznych procesach dotyczących wzrastania, różnicowania i metabolizmu. Leczenie rekombinowanym ludzkim GH jest akceptowane w wielu schorzeniach wiążących się z całkowitym brakiem lub zmniejszeniem działania GH. Wynika stąd znaczne zainteresowanie rozwojem nowych leków mogących modyfikować czynność GH. Dopiero niedawno wyjaśniono dokładną strukturę GH i jego interakcje z receptorem. W niniejszej pracy autorzy podsumowują wiedzę dotyczącą podstawowej budowy GH, jego receptora i interakcji między nimi. Ponadto, omówiono odkrycie i rozwój skutecznego antagonisty receptora GH, pegvisomantu i przedstawiono potencjalne możliwości zastosowania terapeutycznego tego leku.

(Endokrynol Pol 2007; 58 (4): 322–329)

***Słowa kluczowe**: hormon wzrostu, receptor hormonu wzrostu, antagonista receptora hormonu wzrostu*

Introduction

The functions of growth hormone (GH) are pervasive, having a direct or indirect impact on most tissues in the body. To exert its biological effect, GH interacts with specific GH receptors (GHRs) on the surface of target tissues. GHRs have been detected in a variety of tissues, including liver, adipose tissue, muscle, lymphocytes, prostate, kidney, placenta, heart, brain and mammary gland [1–5]. Binding of GH to GHRs on target tissues activates proteins involved in the Janus kinase/signal transducer and activator of transcription (JAK-STAT) signal transduction pathway, as well as other pathways [6]. In addition to having a direct impact on target tissues, GH stimulates the synthesis and release of insulin-like growth factor-1 (IGF-1). Since IGF-1 has many distinct metabolic effects, GH has the ability to alter tissue function, both directly and indirectly, via IGF-1 production. Thus GH, along with IGF-1, is considered

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to have dual effects on target tissues [7, 8] with the initiator of this cascade being the interaction of GH with the GHR.

Disorders in growth, either via GH deficiency (GHD) or by production of elevated levels of GH such as in acromegalic individuals, have resulted in a variety of treatment modalities. For deficiency states rhGH has been approved by the FDA for treatment of several growth retardation conditions in children including GHD, Turner syndrome, chronic renal disease, Prader-Willi syndrome and intrauterine growth retardation and for children born small for gestational age (SAGE) or with idiopathic short stature. In adults, rhGH has been approved for GHD associated with a history of hypothalamic and/or pituitary disorders and, more recently, for human immunodeficiency virus (HIV)-associated wasting. The guidelines for rhGH use in children and adults have been thoroughly reviewed elsewhere in more detail [9–11]. Recently, recombinant IGF-1 has been approved for children resistant or insensitive to GH treatment [12]. For conditions of elevated GH (gigantism and acromegaly) drugs that lower GH secretion (somatostatin analogues) or inhibit GH activity (GH antagonists) are currently used. Thus by altering the GH/GHR interaction both GH and IGF-1 activities will be affected. In the future the ability to uncouple the action of GH from that of IGF-1 will certainly result in the discovery of new therapeutic targets.

GH and GHR structure

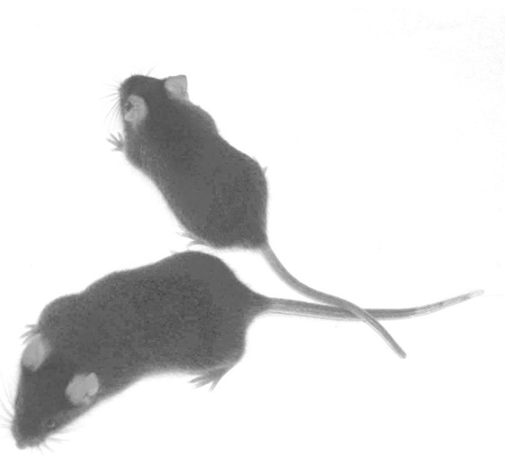
The primary sequence of GH from many species, as well as the crystal structure of both porcine (p) and human (h) GH, has provided significant insight as to the structurally significant regions of this hormone. Although somewhat variable according to the species, the main secreted form of GH is composed of ~191 amino acids. Analysis of the three-dimensional structure of pGH [13] and hGH [14] has revealed that both are globular proteins which contain four highly conserved cysteine residues. These cysteine residues form both a large and a small disulfide bridge with the large bridge being important for GH activity [15]. Approximately one half of the amino acid residues in GH reside in four distinct alpha helices. These four anti-parallel helices connect in an "up-up-down-down" pattern with the core of the four-helical bundle consisting of mostly hydrophobic residues, which presumably function to hold the helices in a specific packed configuration [14]. Relevant to this review, a tryptic peptide of GH containing helix 3 was previously shown to have significant growth-promoting activity [16], although this was not documented to be critical for GHR recognition.

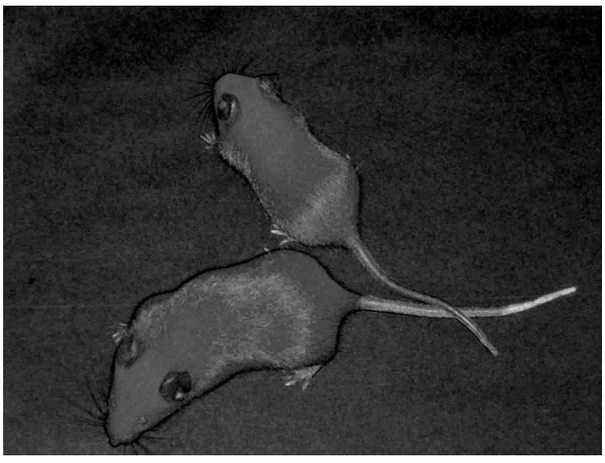
The GHR belongs to the cytokine receptor superfamily, which also includes the receptors for granulocyte-colony stimulating factor, leptin and prolactin as well as other cytokines [17]. There are common features and motifs among this receptor family. In particular, receptor family members contain several specific disulfide bonded Cys residues and a distinct WSXWS-like (Trp, Ser, any amino acid, Trp and Ser) motif near the cell membrane. The GHR is composed of approximately 620 amino acids. The N-terminus contains the extracellular hormone-binding region (~245 amino acids), followed by the 24 amino acid hydrophobic transmembrane region and the C-terminal domain (~350 amino acids), which contains motifs important in intracellular signalling [18]. On the basis of analysis of the crystal structure of human GHR, the extracellular region contains two distinct yet similarly designed domains (termed 1 and 2), each composed of seven beta strands divided into two anti-parallel beta sheets [14].

Interaction of GH with the GHR

The sensitivity of a tissue to GH is partly dependent on the number of cell-surface GHRs in that tissue. The stoichometry of the ligand:receptor complex is 1:2, based on a number of biophysical methods and later confirmed through X-ray crystallography data [14, 19]. Thus a single GH molecule interacts with a homodimer of the GHR. Several studies have revealed that GHR exists as a preformed homodimer [20–22], which undergoes a conformational change in the intracellular signalling region of the receptor, initiating the signalling cascade [23]. This account of the heterotrimeric GH:GHR interaction is depicted in Figure 1.

The regions of GH responsible for receptor binding have been exhaustively studied [24–29]. These studies identified a patch of three regions of GH that come into close proximity in the three-dimensional structure, which are responsible for the high affinity binding of GH to its receptor [24, 25]. These three regions of GH, collectively referred to as Site 1, include the N-terminal portion of helix 1, a portion of the connection between helices 1 and 2, and the C terminal portion of helix 4. Yet, GH forms a hGH:GHR2 complex and is an asymmetric protein, suggesting that an additional site within GH (first suggested by Chen et al [30]) was responsible for binding the second GHR monomer. This additional site was later found in helix 3 of GH and is called Site 2. Because two physically separate sites of a single GH protein are responsible for binding to the GHR, it may not be surprising that Site 1 of GH interacts with higher affinity to GHR than Site 2 [14]. Described sequentially, Site 1 of GH is thought to interact with higher





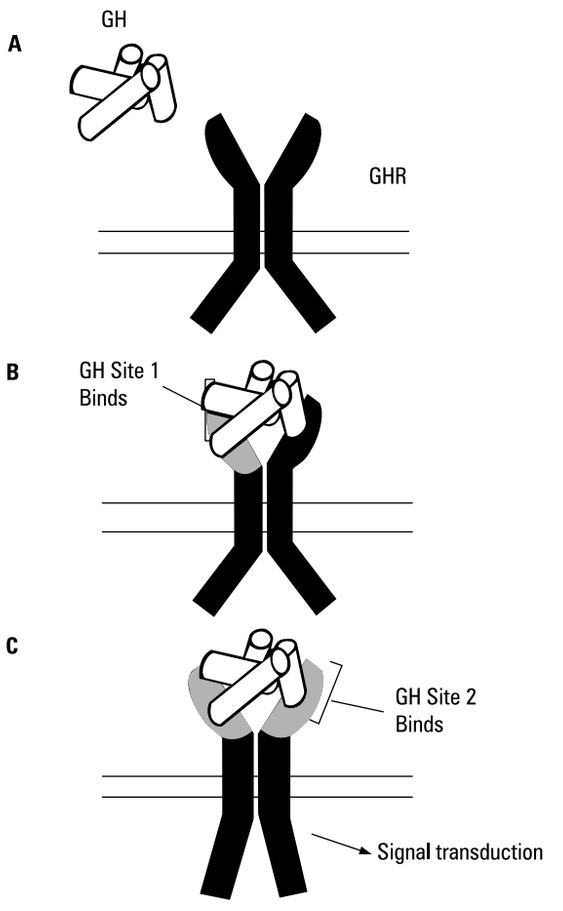


Figure 1. *Model for the formation and signal transduction of the heterotrimeric complex between GH and 2GHR.* **A**. *The preformed dimer of GHR is shown embedded in the lipid bilayer and GH is in the extracellular space.* **B**. *Site 1 (within GH) binds with high affinity to a monomer of the preformed GHR dimer.* **C**. *Site 2 (within GH) subsequently binds the second GHR, resulting in signal transduction. (Reprinted with permission from Cold Spring Laboratory Press [91]*

Rycina 1. *Schemat powstawania i przekazywania sygnału w obrębie kompleksu heterotrimerycznego między cząsteczkami GH i 2GHR.* **A.** *Wbudowany w dwuwarstwę lipidową preformowany dimer GHR; GH znajduje się w przestrzeni zewnątrzkomórkowej.* **B.** *Miejsce 1. (w obrębie GH) wiąże się z dużym powinowactwem do jednego z monomerów proformowanego dimeru GHR.* **C.** *Miejsce 2. (w obrębie GH) wiąże się następnie z drugim monomerem dimeru GHR, co powoduje przewodzenie sygnału*

affinity to the first GHR monomer, followed by the binding of Site 2 of GH to the second GHR monomer with lower affinity.

Discovery of the GH antagonist

Detailed focus was placed on the 3rd α-helix of GH due to the growth-promoting abilities [16, 30, 31] observed to be specific to that helix. Mutations at 3 amino acid sites within bovine (b) GH helix 3 were engineered to provide an amphipathic formation hypothesised to further enhance the growth-promoting activity of this helix. Specifically, the substitutions were Glu-117 to Leu, Gly-119 to Arg, and Ala-122 to Asp in bGH. This GH analogue bound to the GHR's with the same affinity as wild-type GH [30]. Surprisingly, this GH analogue antagonised the action of wild-type GH in transgenic mice, resulting in a dwarf phenotype [30–32]. This result represents the first discovery of a GH antagonist. Further investigation of each individual substitution revealed that the specific replacement of Gly-119 with Arg promoted the GH antagonist effect [29]. This single Gly-119 amino acid substitution is sufficient to promote a dwarf phenotype in mice transgenic for the GH antagonist (Fig. 2) [31].

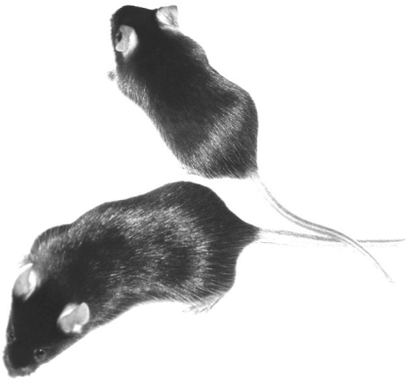
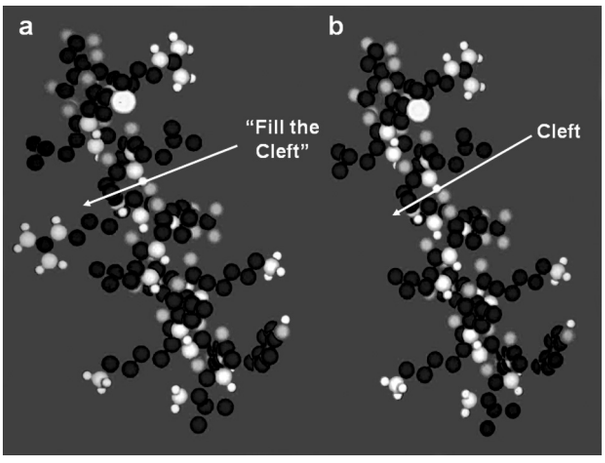


Figure 2. *Photograph showing size comparison of control (bottom) and GH antagonist transgenic mice (top). Shown are 6-week old male mice*

Rycina 2. *Na fotografii przedstawiono różnice w wielkości między kontrolną (u dołu) i transgeniczną myszą po zadziałaniu antagonistów GH (u góry). Przedstawione myszy to 6-tygodniowe osobniki męskie*

Interestingly, the Gly at this position is conserved in all members of the GH family. The GH antagonist is able to bind with high affinity to the preformed GHR dimer while blocking subsequent signal transduction (Fig. 3) [30, 31, 33]. Gly's side chain is made up of a single hydrogen atom, which, in the context of other amino acids in the vicinity, creates a cleft in a region of the 3rd helix (Fig. 4). The substitution of this Gly with an amino acid containing a bulky side chain fills this gap, which ultimately generates the GH antagonist [29]. It is important to note that these types of GH antagonist bind to the GHR with affinities similar to wild-type GH and do not inhibit GHR dimerisation but perturb proper or functional GHR dimerisation.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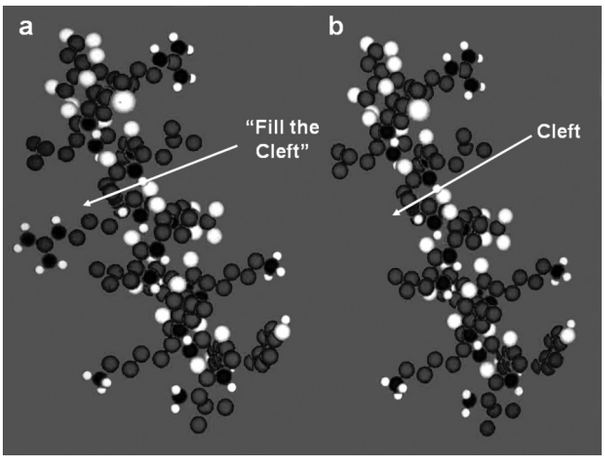
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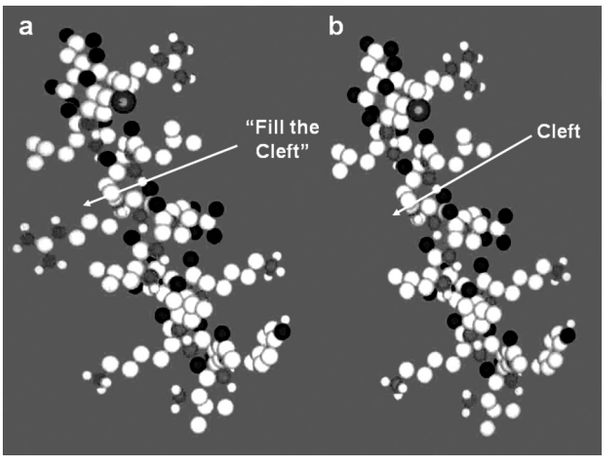
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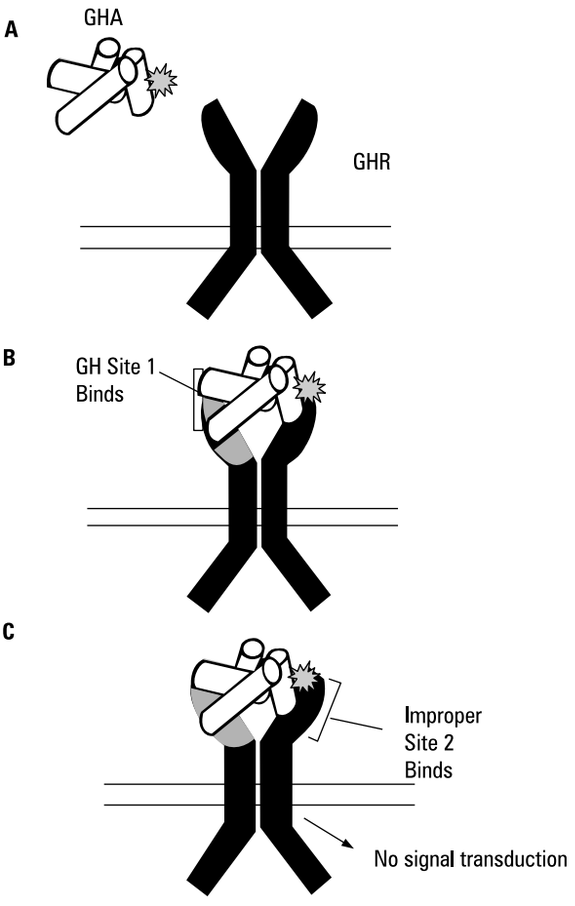

Figure 3. *Model representing the interaction of the GH antagonist with the receptor.* ***A.*** *A preformed dimer of GHR is shown embedded in the lipid bilayer with the GH antagonist in the extracellular space.* ***B****. Site 1 within the GH antagonist binds with high affinity to one monomer of the preformed GHR dimer.* ***C****. Improper binding at Site 2 within the GH antagonist blocks subsequent intracellular signal transduction. (Reprinted with permission from Cold Spring Harbor Laboratory Press [91])*

Rycina 3. *Na schemacie przedstawiono oddziaływanie między anta-gonistą GH a receptorem.* ***A.*** *Wbudowany w dwuwarstwę lipidową preformowany dimer GHR; antagonista GH znajduje się w przestrzeni zewnątrzkomórkowej.* ***B.*** *Miejsce 1. (w obrębie GH) wiąże się z dużym powinowactwem do jednego z monomerów proformowanego dimeru GHR.* ***C.*** *Nieprawidłowe wiązanie miejsca 2. (w obrębie cząsteczki antagonisty GH) do drugiego monomeru GHR blokuje późniejsze wewnątrzkomórkowe przewodzenie sygnału*

Development of a long-acting, effective GH antagonist

Owing to GH's relatively short half life (30 minutes), it has proved challenging to create a GH antagonist molecule that was an effective therapeutic agent. In order to counteract kidney excretion of low molecular weight GH, the addition of polyethylene glycol (PEG) was used to significantly increase the molecular mass of the

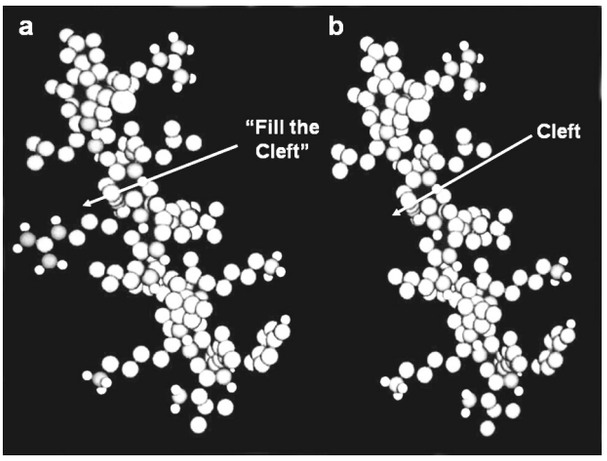

Figure 4. *Partial space filling model of the third alpha helix of bGH and bGH-G119R.* **A**. *Structural representation of the third alpha helix when the glycine is substituted with an arginine.* **B**. *Structural representation of the wild-type helix. The position of the cleft is indicated. (Reprinted, with permission, (38) [Ó The Endocrine Society])*

Rycina 4. *Częściowy model przestrzenny trzeciej alfa helisy bGH i bGH-G119R.* ***A.*** *Struktura trzeciej alfa helisy po podstawieniu glicyny argininą.* ***B.*** *Struktura helisy typu dzikiego. Zaznaczono pozycję szczeliny*

protein [34]. This technology was adapted for the GH antagonist. The PEG addition decreased the affinity of the GH antagonist for its receptor but still proved an effective antagonist because the serum half-life was improved [21]. Furthermore, in an attempt to improve the affinity of the pegylated GH antagonist for its receptor, 8 amino acid substitutions were generated at Site 1, each of which had previously been shown to improve the affinity for GH binding protein [35]. This 8 amino acid substituted and pegylated antagonist (containing lysine at Gly 120) had improved binding affinity for membrane receptors as compared to the pegylated Gly 120K antagonist, resulting in a more effective molecule [21].

This pegylated GH antagonist has been termed pegvisomant and the approved marketed name is Somavert ® (pegvisomant for injection). Many papers have documented the clinical efficacy of pegvisomant and these will not be further reviewed here. However, readers should visit the following papers and reviews for specific details concerning the many clinical trials [36–45].

Pegvisomant and diabetes

Although it has been known for many decades that GH inhibits insulin's action[46–49], the mechanism responsible for this effect has remained elusive. Recent data have started to illuminate possible mechanisms. For example, a recent link between a specific GHR poly-

morphism and resistance to Type 2 diabetes (T2DM) has been presented [50]. Furthermore, mice transgenic for bGH are insulin resistant, while mice that lack GH signalling are insulin sensitive despite their obesity [51–53]. In terms of intracellular signalling events that account for GH-induced insulin resistance, disruption of p85alpha, a subunit of PI 3-kinase, will increase insulin sensitivity, while elevated p85alpha levels are associated with insulin resistance [54–57]. A recent study by del Rincon et al. reports that GH up-regulates expression of p85alpha in white adipose tissue and suggests this may be responsible for alterations in insulin sensitivity seen in mouse models of altered GH action [58]. A similar situation also occurs in muscle [56]. Thus the diabetogenic effect of GH may be due to "cross-talk" between the GH and insulin signalling pathways.

The established impact of GH on insulin sensitivity led researchers to monitor parameters of insulin action in human subjects given pegvisomant. Healthy subjects given pegvisomant for 7 days did not show altered glucose tolerance or stimulated insulin secretion [59]. As pegvisomant began to be used to treat acromegaly, a disease often accompanied by insulin resistance and diabetes, clinicians were able to examine the effect of this drug on insulin sensitivity and other measures of diabetes. In 2002 Rose and Clemmons reported that treatment with pegvisomant lowered fasting insulin, glucose and haemoglobin A(1)C levels in patients with acromegaly [60]. Later studies have further confirmed an improvement in insulin sensitivity following pegvisomant treatment of patients with acromegaly [61–63].

Clearly, pegvisomant can improve insulin and glucose levels in patients with acromegaly, but what about patients with other insulin-related conditions? Williams et al. treated young, Type 1 diabetic adults with 5 or 10 mg/day of pegvisomant for 3 weeks [64]. No changes in insulin sensitivity under hyperinsulinaemic euglycaemic clamp conditions were observed; however, both doses of pegvisomant decreased the amount of insulin required overnight to maintain euglycaemia. Thus although there has been limited research to date, pegvisomant shows promise for treating not only acromegalics with insulin resistance but also young adult patients with Type 1 diabetes. Further research is required to determine if pegvisomant treatment might benefit patients with type 2 diabetes as well.

Pegvisomant and nephropathy

Long and short term renal changes can be caused by GH and IGF-1. Transgenic mice expressing GH antagonist are dwarf and have reduced circulating IGF-1 levels [30, 32]. When GH antagonist mice are made diabetic, they are protected from renal damage [65]. In addition, treatment of control and diabetic mice with GH antagonist protects them from renal damage [66, 67] and prevents compensatory renal growth in uni-nephrectomised mice [68]. The mechanism in which GH antagonist protects the kidney has not been determined, but studies point to several possibilities. When exogenous GH antagonist is administered in increasing doses to adult female Balb/C mice, there is a dose-dependent decrease in hepatic and serum IGF-1 levels, no effect on hepatic or renal IGFBP-1 and 3 levels, and an increase in hepatic and circulatory IGFBP-4 levels [69]. In effect, this would create a significant decrease in IGF-1 bioavailability. Additionally, variable concentrations of pegvisomant have a significant impact on the GHR/GHRBP gene transcription in stable cell lines of T-SV40 immortalised glomerular mesangial cells [70, 71]. Interestingly, GH antagonist has been reported to inhibit GHR/GHRBP gene transcription directly at the cellular level in human mesangial cells at all concentrations of pegvisomant tested [72]. Collectively, this data indicates that pegvisomant administration may influence kidney function.

Pegvisomant and retinopathy

The role of GH in the development of retinopathy was first described after ablation of the pituitary gland resulted in reduction of the disease [73, 74]. This result, coupled with the fact that diabetic dwarfs do not develop retinopathy [75], suggests that the use of GH antagonists for the treatment of diabetic retinopathy may be beneficial. Furthermore, results using mice expressing a GH antagonist to study non-diabetic ischemia-induced retinal neovascularisation showed an inhibition of neovascularisation despite elevated levels of vascular endothelial growth factor receptor [76]. Pegvisomant treatment of diabetic patients with severe retinopathy ensued. In this 12-week study, where type 1 and type 2 diabetic patients were treated daily with pegvisomant, no regression of retinopathy was seen [77]. However, considering the short length of the study as well as the advanced retinopathy of the subjects, further studies are warranted.

Pegvisomant and cardiovascular disease

Acromegaly has been shown to be associated with an increased cardiovascular risk. Thus it is not surprising that CRP (C-reactive protein) levels, a common marker for cardiovascular risk, were found to be lower with the administration of pegvisomant in humans [78]. Since pegvisomant blocks GHR activation and decreases IGF-1 production, the effects observed on CRP could be mediated both by the decrease in IGF-1 and the direct effect of GHR blockade. GH antagonist treatment in patients with acromegaly is

also known to induce a reduction in diastolic blood pressure in hypertension and improve glucose metabolism [62]. A recent study by Pivonello et al. also showed that pegvisomant can reverse left ventricle hypertrophy and progressively improve left ventricular diastolic and systolic performance in acromegalics [79]. Thus long term treatment with pegvisomant has positive effects on cardiovascular function and may prevent the development or progression of cardiac insufficiency, at least for acromegalics.

Pegvisomant and cancer

The IGF-1/GH axis has been implicated in contributing to the growth and formation of many different cancers [80, 81]. IGF-1 has been shown to be a growth factor for numerous types of cancer and neoplastic growth [82]. Additional studies have also shown that some neoplasms are also capable of producing autocrine and/or paracrine IGF-1 [82]. Transfection of MCF-7 cells with the hGH gene showed that these cells synthesised and secreted hGH into the media, and these cells were found to have higher levels of STAT5-mediated transcriptional activation than controls [83]. The disruption of excess GH stimulation and therefore reduction in IGF-1 levels may therefore be useful in the treatment of numerous cancers.

Multiple studies using both animals and humans have attempted to show the beneficial use of GH antagonist to prevent or slow the growth of various tumours. GH antagonist mice were found to have lower IGF-1 levels and a decreased mammary tumour incidence in relation to litter-mate controls when exposed to a chemical carcinogen [84]. Additional studies using GHR/-and C3(1)/Tag mouse models showed an inhibition of oestrogen-independent mammary carcinogenesis [85]. Recently a study using the spontaneous dwarf rat (an animal known to have lower levels of GH and IGF-1) injected with GH showed that these animals were more vulnerable to mammary carcinogenesis with increasing levels of circulating IGF-1 and GH [86]. Pegvisomant administration to virgin female mice caused a 70–80% reduction in serum IGF-1 levels and a 30% reduction in the volume of MCF-7 xenografts [87]. In mice the growth of human meningioma xenografts significantly decreased following pegvisomant treatment, and in some cases tumour regression was observed [88]. Additional studies xenografting human colorectal cancer lines into female nude mice with subsequent pegvisomant treatment reported a 39% reduction in tumour volume with a reduction in both IGF-1 and IGFBP-3 levels [89]. Studies involving GHR disrupted and Tag mice suggest that the disruption of GH signalling may also reduce prostate carcinogenesis [90]. These results indicate a potential therapeutic use of pegvisomant in the prevention and treatment of certain cancers.

Conclusion

Since the initial discovery of a growth hormone antagonist [30] both basic and clinical studies have advanced. In terms of human use, the growth hormone antagonist Somavert® (pegvisomant for injection) has been approved for lowering IGF-1 levels in acromegalic individuals. Further studies are likely to provide insight into its therapeutic potential for the treatment of diabetes, diabetic complications and cancer indications. Finally, the growth hormone antagonist is now a commonly used reagent that specifically antagonises the effects of growth hormone in many basic research scenarios. In the future, the growth hormone antagonist will also assist researchers in uncoupling the biological effects of growth hormone from those of IGF-1.

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