Antagonists of growth-hormone-releasing hormone: an emerging new therapy for cancer

Andrew V Schally*, Jozsef L Varga and Jörg B Engel

SUMMARY

This article reviews the potential clinical uses of antagonists of growth-hormone-releasing hormone (GHRH) for tumor therapy. GHRH antagonists suppress the growth of various human cancer lines xenografted into nude mice; such tumors include breast, ovarian, endometrial and prostate cancers, lung cancers (small-cell lung carcinomas and non-small-cell lung carcinomas), renal, pancreatic, gastric and colorectal carcinomas, brain tumors (malignant gliomas), osteogenic sarcomas and non-Hodgkin's lymphomas. The antitumor effects of GHRH antagonists are exerted in part indirectly through the inhibition of the secretion of GH from the pituitary and the resulting reduction in the levels of hepatic insulin-like growth factor I (IGF-I). The main effects of the GHRH antagonists are, however, exerted directly on tumors. GHRH ligand is present in various human cancers and might function as an autocrine and/or paracrine growth factor. Pituitary-type GHRH receptors and their splice variants are also found in many human cancers. The inhibitory effects of GHRH antagonists seem to be due to the blockade of action of tumoral GHRH. Antagonists of GHRH can also suppress cancer growth by blocking production of IGF-I and/or IGF-II by the tumor. Further development of GHRH antagonists that are still-more potent should lead to potential therapeutic agents for various cancers.

KEYWORDS cancer, GHRH antagonists, GHRH receptors, tumor growth inhibition

REVIEW CRITERIA

We searched for original articles that focused on GHRH and GHRH antagonists in MEDLINE and PubMed published between 1982 and 2007. The search terms we used were "GHRH" and "GHRH antagonists". All papers so identified were English language full-text papers. We also searched the reference lists of identified papers for further review articles.

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INTRODUCTION

Growth-hormone-releasing hormone (GHRH) was first demonstrated in the 1960s.[1,2] It is a peptide hormone secreted by the hypothalamus. Upon binding to GHRH receptors on somatotrophs in the anterior pituitary, GHRH regulates the synthesis and secretion of GH. The breakthrough for the identification of GHRH was provided by Frohman and Szabo,[3] who demonstrated ectopic production of GHRH by carcinoid and pancreatic tumors. The 44-amino-acid and 40-amino-acid forms of GHRH were then first isolated and characterized from human pancreatic tumors that caused acromegaly, and GHRH was only subsequently identified in human and animal hypothalami.[4,5]

GHRH is structurally related to vasoactive intestinal peptide and secretin. The full intrinsic biological activity of GHRH is retained by the amino-terminal sequence of 29 amino acids, termed GHRH(1–29)NH_2 (Figure 1).[5] A plethora of studies established the endocrine effects of GHRH in the regulation of GH secretion in animals and humans (for reviews, see Muller *et al.*[6] and Gelato[7]). Despite the fact that GHRH was first identified from tumor tissue,[3–5] however, few groups have investigated the likely role of this neuropeptide in carcinogenesis.

One of the aims of this article is to review the significant information collected so far on antagonists of GHRH as potential agents for therapy of cancer. The case for the development of GHRH antagonists has been recently greatly strengthened by new evidence on the important role of GHRH as a growth factor in various cancers and the discovery of tumoral GHRH-receptor splice variants (SVs); therefore, these findings will also be summarized.

GHRH IN HUMAN CANCERS

The presence of GHRH and its receptors, and the expression of the GHRH gene in several extrahypothalamic tissues, including placenta, ovary, testis, digestive tract[8] and tumors, suggests that GHRH might have a role in these tissues,

Amino acid residue		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
hGHRH (1–29)NH_2	H-	Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly	Gln	Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg–NH_2
Robberecht's antagonist	Ac-	▪	*D*-Arg	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪–NH_2
MZ-4-71	Ibu-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	▪	▪	▪	▪	▪	▪	▪	Abu	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	Nle	▪	Agm
MZ-5-156	PhAc-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	▪	▪	▪	▪	▪	▪	▪	Abu	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	Nle	▪	Agm
JV-1-36	PhAc-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	▪	Arg	▪	▪	▪	▪	▪	Abu	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	Nle	*D*-Arg	Har–NH_2
JV-1-38	PhAc-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	▪	Har	Tyr(Me)	▪	▪	▪	▪	Abu	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	Nle	*D*-Arg	Har–NH_2

Figure 1 Comparative structures of the bioactive amino-terminal 29-amino-acid core fragment of human growth-hormone-releasing hormone—hGHRH(1–29)NH_2—and early antagonists of GHRH that were synthesized. Amino acid residues identical to those of hGHRH(1–29)NH_2 are denoted by dots. Where applicable, standard three-letter abbreviations are used for coded amino acids. Noncoded amino acids and *N*-acyl moieties are abbreviated as follows: Abu, α-aminobutyric acid; Ac, acetyl; Agm, agmatine; Har, homoarginine; Ibu, isobutyryl; Nle, norleucine; PhAc, phenylacetyl; Tyr(Me), O-methyltyrosine.

independent of the regulation of GH secretion. Much evidence has now accumulated on the involvement of GHRH and its receptors in carcinogenesis. The presence of mRNA for GHRH has been demonstrated in surgical specimens of human prostate, breast, ovarian, endometrial, adrenal, and pancreatic cancers.[9–14] In addition, various human cancer lines express mRNA for GHRH peptide; these cell lines include ones derived from breast, endometrial, ovarian, prostate, pancreatic, gastric, colorectal, lung (both small-cell lung carcinomas [SCLC] and non-SCLC) and brain tissue, bone sarcomas, lymphomas and renal-cell carcinomas.[11–25]

GHRH peptide, first found in pancreatic and carcinoid tumors,[3–5] was subsequently also detected in breast, endometrial, ovarian, colorectal, gastric, pancreatic and lung cancers (both SCLC and non-SCLC), and lymphomas.[9,12,13,20,26] Human cancer lines that produce GHRH peptide include breast, endometrial, SCLC, non-SCLC and prostate cancers, osteosarcomas, and lymphomas.[12,15–17,20,21,23,25]

The proliferation *in vitro* of many cancer cell lines including ones derived from prostate, mammary, endometrial, ovarian, gastric, pancreatic, colorectal and lung tissue, and osteosarcomas, is stimulated by exogenous GHRH and, conversely, inhibited by GHRH antagonists or antisera to GHRH.[14,15,18,23,25,27,28]

The presence of biologically or immunologically active GHRH and mRNA for GHRH in various cancers supports the hypothesis that locally produced GHRH might function as an autocrine growth factor in proliferation of these tumors.[12] A significantly higher expression of mRNA for GHRH was also found in cell lines derived from immune-cell tumors,[22] as compared with normal tissues and cell lines. These observations suggest that a deregulation of GHRH gene transcription might contribute to the pathogenesis of some of these malignancies.

GHRH RECEPTORS IN HUMAN CANCERS

The pituitary GHRH receptor (pGHRH-R) is a seven-transmembrane-domain receptor, with considerable homology to the receptors for vasoactive intestinal peptide, calcitonin and others (reviewed in Rekasi *et al.*[29]).

Our group has identified tumor receptors that mediate the effects of GHRH and its antagonists.[29] The isolation and sequencing of complementary DNA molecules (cDNAs) corresponding to the tumoral GHRH-R mRNAs revealed that they are SVs—isoforms of the pGHRH-R.[29] The major part of the cDNA sequence of SV1 is identical to the corresponding sequence of pGHRH-R cDNA, but the first 334 nucleotides of SV1 are different.[29] The deduced protein sequence of SV1 differs from that of pGHRH-R only in the amino-terminal extracellular domain,[29] where a 25-amino-acid sequence replaces the first 89 amino acids of pGHRH-R. The mRNA sequence of SV1 and the observed molecular mass of the receptor protein from western-blot assays are consistent with a seven-transmembrane-domain receptor that has the third intracellular loop critical for the interaction with G proteins.[29–31]

Reverse transcriptase PCR (polymerase chain reaction) analyses and western blots using specific antisera revealed the expression of SVs in several cancers, including prostate,[11,24] colorectal, gastric, pancreatic,[14] and renal cancers,[32] SCLC

and non-SCLC,[33] breast carcinomas,[34] ovarian and endometrial carcinomas,[17] bone sarcomas,[15] glioblastomas[20,35] and lymphomas.[21]

In a study published in 2005, we investigated whether human tumors can also express pGHRH-R. By using real-time PCR and western blots with antibodies specific for pGHRH-R, we showed that the pituitary-type receptor is present in human lymphoma, glioblastoma and SCLC cell lines and in surgical specimens of human lung cancers.[20] Both types of GHRH receptors should, therefore, be considered as potential targets for anticancer therapy with GHRH antagonists.

Our findings on GHRH receptors in tumors have been confirmed by several groups. Chopin and Herington[16] found the presence of SVs in human prostate cancers. Schulz *et al.*[36] investigated, using immunocytochemistry, the distribution of GHRH receptors in 69 human tumors and showed that GHRH receptors were frequently expressed in breast, ovarian and prostate carcinomas. In western blots of membranes prepared from human tumors, an antibody to GHRH receptors detected a protein band with a molecular mass of 40,000 Da, which corresponded to SV1. Freddi *et al.*[10] assessed, by nested PCR, the expression of SVs in 45 human adrenocortical tumors and found SV1, SV2 and SV4. All carcinomas that expressed SVs were also positive for the presence of GHRH mRNA.

To investigate the role of SV1 in tumorigenesis, we have expressed full-length pGHRH-R or SV1 in MCF-7 human breast cancer cells.[37] Cells transfected with pGHRH-R or SV1 had the ability to respond to GHRH by proliferating, with SV1 being more potent than pGHRH-R.[37] MCF-7 cells transfected with SV1 proliferated more quickly than the nontransfected controls, even in the absence of exogenously added GHRH, suggesting the existence of intrinsic, ligand-independent activity of SV1.[37] These findings show that the expression of SV1 confers oncogenic activity and provide further evidence that GHRH operates as a growth factor.

SYNTHESIS AND EVALUATION OF GHRH ANTAGONISTS

Both insulin-like growth factor I (IGF-I) and IGF-II are implicated in the malignant transformation of cells, tumor progression, and the metastases of various cancers.[28,38,39] The clinical need for GHRH antagonists was first advocated by Pollak *et al.*[40,41] in the early 1990s on the grounds that somatostatin analogs do not adequately suppress GH and IGF-I levels in patients with neoplasms potentially dependent on IGF-I. Previously, Robberecht *et al.*[42] reported that replacement of Ala at position 2 of GHRH(1–29)NH_2 with D-arginine produces antagonists. The GHRH antagonist [N-Ac-Tyr^1, D-Arg^2]GHRH(1–29)NH_2[42] (Figure 1) was, therefore, able to suppress GH release in rats. There was, however, little activity in this field until our development of more-potent GHRH antagonists[28,43,44] with increased antitumor activities.[28,45,46] Inhibitory effects of these GHRH antagonists have been evaluated *in vitro* and in a variety of human experimental cancer models in nude mice.[28,45,46]

Early GHRH antagonists MZ-4-71 and MZ-5-156 (Figure 1) were about 7–19 times more potent than the antagonist of Robberecht[42] and were longer acting.[43,44,47] Subsequently, more-potent antagonists including JV-1-36 and JV-1-38 (Figure 1) were prepared, containing arginine, D-arginine or homoarginine residues in positions 9, 28, and 29.[48]

We later synthesized antagonists containing other substitutions at positions 8, 9, and 10, such as JV-1-63 with a *para*-amidino-phenylalanine substitution at position 10, as well as JV-1-65 and JV-1-68, which contain a *para*-amidino-phenylalanine modification at position 9 (Figure 2).[49] Antagonists JV-1-63 and JV-1-36 exhibited potent endocrine activity.[49] More recently, we prepared GHRH antagonists such as MZ-J-7-110 and MZ-J-7-114, which contain fatty acyl moieties at their amino terminus (Figure 2).[50] We also replaced arginine and lysine residues in positions 11, 12, 20, and 21 of the GHRH peptide with histidine and ornithine.[45,46] GHRH antagonists with histidine and ornithine replacements including MZ-J-7-118, MZ-J-7-138, and JMR-132 (Figure 2) exhibit some of the most potent antitumor effects reported so far.[45,46,51–53]

In tests on the inhibition of serum GH and IGF-I levels *in vivo*, antagonists including JV-1-65, MZ-J-7-110, MZ-J-7-118, MZ-J-7-138, and JMR-132 have weaker endocrine activities than JV-1-63, MZ-4-71 or MZ-5-156, but exhibit greater anticancer potency.[17,19,21,49,52–54] This indicates that the oncological activity of GHRH antagonists is not necessarily correlated with endocrine effects, but is based on other mechanisms such as direct inhibitory effects on cancer cells (see below).[45,46] Antagonists MZ-J-7-118, MZ-J-7-138, and JMR-132 can thus inhibit the growth of various cancers in nude mice in doses

Amino acid residue		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
hGHRH (1–29)NH_2	H-	Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly	Gln	Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg–NH_2
JV-1-63	PhAc-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	▪	Har	Amp	▪	▪	▪	▪	Abu	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	Nle	*D*-Arg	Har–NH_2
JV-1-65	PhAc-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	▪	Amp	Tyr(Me)	▪	▪	▪	▪	Abu	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	Nle	*D*-Arg	Har–NH_2
JV-1-68	PhAc-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	▪	Amp	▪	▪	▪	▪	▪	Abu	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	Nle	*D*-Arg	Har–NH_2
MZ-J-7-110	HOOC-$(CH_2)_{12}$-CO-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	▪	Amp	Tyr(Me)	▪	▪	▪	▪	Abu	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	Nle	*D*-Arg	Har–NH_2
MZ-J-7-114	CH_3-$(CH_2)_6$-CO-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	▪	Amp	Tyr(Me)	▪	▪	▪	▪	Abu	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	Nle	*D*-Arg	Har–NH_2
MZ-J-7-118	CH_3-$(CH_2)_6$-CO-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	Ala	His	Tyr(Et)	His	▪	▪	▪	Abu	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	Nle	*D*-Arg	Har–NH_2
MZ-J-7-138	CH_3-$(CH_2)_6$-CO-	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	Ala	His	Tyr(Et)	His	Orn	▪	▪	Abu	▪	▪	▪	▪	His	Orn	▪	▪	▪	▪	▪	Nle	*D*-Arg	Har–NH_2
JMR-132	PhAc	▪	*D*-Arg	▪	▪	▪	Phe(4-Cl)	▪	Ala	Har	Tyr(Me)	His	▪	▪	▪	Abu	▪	▪	▪	▪	His	▪	▪	▪	▪	▪	▪	Nle	*D*-Arg	Har–NH_2

Figure 2 Comparative structures of the most potent antagonists of human growth-hormone-releasing hormone (hGHRH). Amino acid residues identical to those of the bioactive amino-terminal 29-amino-acid core fragment, hGHRH(1–29)NH_2, are denoted by dots. Where applicable, standard three-letter abbreviations are used for coded amino acids. Noncoded amino acids and *N*-acyl moieties are abbreviated as follows: Abu, α-aminobutyric acid; Amp, *para*-amidino-phenylalanine; Har, homoarginine; Nle, norleucine; Orn, ornithine; PhAc, phenylacetyl; Tyr(Et), O-ethyltyrosine; Tyr(Me), O-methyltyrosine.

as low as 2.5–5.0 μg/day, whereas antagonists such as MZ-4-71 or MZ-5-156 require doses of 20–80 μg/day.[45,46,53] Continuing synthesis of GHRH antagonists should lead to compounds that are even more potent.

MECHANISM OF ACTION OF GHRH ANTAGONISTS

IGF-I and IGF-II seem to influence the growth of various human cancers including breast, prostate, colorectal and lung cancer by endocrine, autocrine and/or paracrine mechanisms.[28] Our initial investigations of the oncological activities of GHRH antagonists were based only on the assumption that the blockade of the axis between pituitary GH and hepatic IGF-I might inhibit the growth of IGF-I-dependent cancers.[28] We subsequently discovered, however, that GHRH antagonists can also suppress the proliferation of diverse tumors that are influenced by autocrine and/or paracrine production of IGF-I and IGF-II.[28,45,46,55] Then it became apparent that GHRH antagonists can inhibit growth of some cancers such as SCLC or prostate and breast cancers by blocking the action of autocrine and/or paracrine GHRH.[23]

The indirect mechanism of inhibition of tumor growth by GHRH antagonists (Figure 3A) is important for those cancers that depend on IGF-I as a growth factor. A strong positive association was reported between plasma IGF-I levels and the risk of prostate, breast and colorectal cancers.[28] Inhibition of the release of GH from the pituitary results in a suppression of hepatic IGF-I production (Figure 3A). In some studies in nude mice bearing various cancers in which tumor inhibition was accompanied by a decrease in levels of serum IGF-I, the antiproliferative effect of GHRH antagonists could be ascribed in part to the suppression of the GH–IGF-I endocrine axis (Figure 3A).

In other cancer models, however, GHRH antagonists did not cause significant inhibition of serum IGF-I levels; moreover, GHRH antagonists inhibit the proliferation of various cancer cell lines *in vitro*, where the involvement of the GH–IGF-I axis is clearly excluded.[55] These direct inhibitory effects seem to be mediated by tumoral SVs and can be dependent or independent of IGF-I and IGF-II. GHRH and SVs are present in a variety of human cancers and might form an autocrine and/or paracrine stimulatory loop[28,45,46] (Figure 3B). In the case of H-69 SCLC and other cancers, tumor inhibition was not associated with a suppression of production of IGF-I and IGF-II, but seemed to be due to the blockade by GHRH antagonists of the stimulatory action of tumoral autocrine GHRH on pGHRH-R or SVs in tumors.[23,28,45,46]

In many human cancer lines studied *in vitro* and *in vivo*, including prostate, renal, pancreatic and colorectal cancers, glioblastomas, ovarian cancers, and non-SCLC, GHRH antagonists inhibited the production of IGF-I and IGF-II and the expression of IGF-II mRNA.[45,46,55]

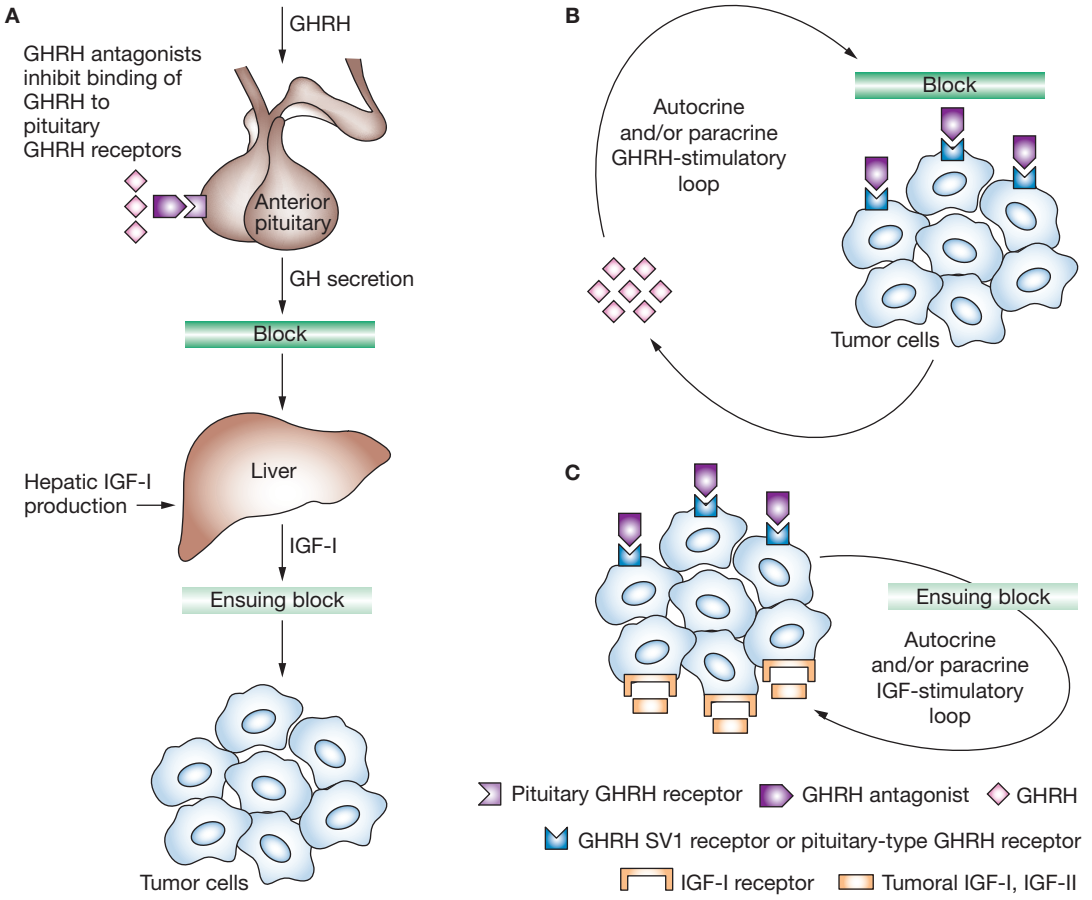

Figure 3 Schematic representation of the three potential mechanisms through which growth-hormone-releasing hormone (GHRH) antagonists mediate the inhibition of tumor growth. (**A**) An indirect action mediated through the suppression of the axis between pituitary GH and hepatic IGF-I. GHRH antagonists block the secretion of GH from the pituitary gland by inhibiting the binding of hypothalamic GHRH to GHRH receptors on pituitary somatotrophs. GH activates liver IGF-I gene transcription and could directly stimulate IGF-I production in some tumors. Inhibition of GH results in a decrease of the production of hepatic IGF-I and the lowering in the levels of circulating IGF-I, ultimately leading to inhibition of tumor growth. (**B**) Direct action. GHRH antagonists competitively inhibit the binding of tumoral autocrine or paracrine GHRH to pituitary-type GHRH receptors or GHRH-receptor splice variants on tumors. This inhibition blocks the stimulatory effect of locally produced GHRH on tumor growth, probably without the involvement of the IGF system. (**C**) GHRH antagonists can also directly inhibit tumor growth by suppressing the autocrine and/or paracrine production of IGF-I or IGF-II by the tumor. This effect is probably mediated through pituitary-type GHRH receptors or GHRH-receptor splice variants on tumors. Locally produced IGF-I and IGF-II activate cell proliferation by binding to type I IGF receptors on tumors. In some tumors GHRH antagonists can also block the stimulatory effect of local GH on tumor proliferation (not shown). The cell cycle is arrested at the G2-M point, which triggers apoptosis.[58] Abbreviations: GH, growth hormone; GHRH, GH-releasing hormone; IGF, insulin-like growth factor; SV1, GHRH-receptor splice variant 1.

This action is apparently exerted through SVs (Figure 3C). Because IGF-I and IGF-II are potent mitogens for many cancers,[38,39] suppression of their production would inhibit tumor growth. In some tumors, more than one of these mechanisms might operate. The relative importance of these mechanisms also varies in different tumors.

The evidence supporting the concept of multiple mechanisms of action of GHRH antagonists was derived from a large number of studies (reviewed by Schally and Varga[28,45,46]). The inhibition of tumor growth of human osteogenic sarcomas, prostate, renal and lung cancers, and lymphomas in nude mice by high doses (40–80 μg/day) of early antagonists such as MZ-4-71 and MZ-5-156 was linked with an inhibition of serum GH and IGF-I levels.[21,28,56] Lower doses of these antagonists, or more-recently

synthesized analogs with decreased endocrine inhibitory effects, such as JV-1-65, MZ-J-7-118, and MZ-J-7-138 did, however, suppress the growth of pancreatic, colorectal, prostate, breast, ovarian, endometrial, lung cancers, and lymphomas in the absence of any significant effects on serum IGF-I.[17,21,23,25,53,57] This finding suggests that the direct inhibitory effects of GHRH antagonists on cancer cells are more important for tumor inhibition than their endocrine activities on GH and IGF-I.

The tissue levels of IGF-I and/or IGF-II in tumors, and the expression of their mRNAs, were reduced by GHRH antagonists in pancreatic, colorectal, renal, prostate, lung, ovarian, breast, and bone tumors, and glioblastomas.[19,25,28,35,45,46,56,58,59] This reduction is apparently because of direct effects of antagonists on tumor tissue. In H69 SCLC, MDA-MB-435 breast cancers, LNCaP prostate cancers, and in HEC-1A endometrial cancers, however, tumoral IGF-I and IGF-II levels were not decreased, indicating that GHRH antagonists can inhibit tumor growth without the participation of the tumoral IGF system.[17,34,45,46,59] In MXT mouse mammary cancers, tumoral GH and GH-receptor levels were greatly diminished after treatment with GHRH antagonists.[58] The concentration and mRNA levels of tumoral angiogenic growth factors—vascular endothelial growth factor and its receptor and/or basic fibroblast growth factor—were decreased in human prostate cancer, SCLC and non-SCLC, and lymphoma models.[21,33,53,59]

GHRH antagonists affect some of the signaling mechanisms involved in cell proliferation, survival, and metastasis, and activate proapoptotic signaling mechanisms. We found that GHRH antagonists inhibit the PKC–MAPK (protein kinase C–mitogen-activated protein kinase) and PI3K–AKT (phosphatidylinositol 3-kinase–protein kinase B) signaling pathways, reduce levels of expression of c-jun and c-fos proto-oncogenes and mutant tumor-suppressor protein p53 in human SCLC, non-SCLC, and prostate cancer models, and decrease the telomerase activity in glioblastomas and other cancers.[19,45,46,60] In addition, GHRH antagonists reduce levels of the apoptosis regulator Bcl-2 (an antiapoptotic protein) and increase levels of the apoptosis regulator BAX (a proapoptotic protein) in non-SCLC models, and in the prostate cancer line LNCaP they trigger a Ca^{2+}-dependent apoptotic mechanism.[33,45,46]

EFFECTS OF GHRH ANTAGONISTS ON EXPERIMENTAL CANCERS

IN VIVO

Prostate cancer

Early GHRH antagonists MZ-4-71 and MZ-5-156, and newer antagonists MZ-J-7-118 and MZ-J-7-138, inhibited the growth of human androgen-independent PC-3 and DU-145 prostate cancers xenografted into nude mice.[28,45,46,53] GHRH antagonist MZ-J-7-118 suppressed the orthotopic growth and metastatic potential, as well as the intraosseous growth, of PC-3 tumors.[54] GHRH antagonists JV-1-38 or MZ-J-7-118 given alone had no effect on the growth of human androgen-sensitive prostate cancers LNCaP and MDA-PCa-2b. These antagonists did, however, enhance the inhibitory effects of androgen deprivation, produced by surgical castration, luteinizing-hormone-releasing hormone (LHRH) agonists or LHRH antagonists, on the growth of subcutaneous and orthotopic prostate tumors.[45,46,52,57,59] These results show that GHRH antagonists greatly potentiate the tumor growth inhibition induced by androgen deprivation. GHRH antagonists might interfere with mechanisms involved in progression of prostate cancer toward androgen independence and could be used clinically as agents preventing relapse in patients who have prostate cancer and receive androgen-deprivation therapies.[45,52] Therapy with GHRH antagonists should be considered for the management of both androgen-dependent and androgen-independent prostate cancers (Box 1).

Breast cancer

The presence of biologically and immunologically active GHRH and mRNA for GHRH in human breast cancers supports the hypothesis that locally produced GHRH might have a role in the proliferation of these tumors. Antagonists of GHRH might, therefore, offer a new approach to the treatment of breast cancer.[45,46] In nude mice bearing subcutaneous xenografts of human estrogen-independent MDA-MB-468 breast cancers, therapy with GHRH antagonists MZ-5-156 or JV-1-36 produced the regression of some tumors, and arrested the growth of others.[45,46] GHRH antagonist JV-1-36 also suppressed the growth of orthotopically implanted MDA-MB-435 human estrogen-independent breast cancers and decreased their metastatic potential.[34] GHRH antagonists JV-1-36 and JV-1-38 also inhibited the growth of MXT estrogen-independent

mouse mammary cancers.[58] GHRH antagonists can be combined with docetaxel chemotherapy to enhance the efficacy of treatment.[51] These investigations indicate that GHRH antagonists could be useful for therapy of breast cancer.

Ovarian cancer

GHRH antagonists JV-1-36, MZ-5-156 and MZ-J-7-138 decreased growth of OV-1063 cancers and reduced the levels of mRNA for IGF-II in tumors.[61] After 22 days of therapy with JV-1-36, the final volume of UCI-107 tumors (which are negative for LHRH receptors) was significantly decreased (by 50%) compared with controls.[45,46] The concentration of IGF-II in tumors was reduced by 66% in the group treated with JV-1-36. Our results suggest that GHRH antagonists inhibit the growth of ovarian cell carcinoma by mechanisms that seem to involve direct effects on the cancer cells.

Endometrial cancer

The GHRH antagonist MZ-J-7-118 dose-dependently inhibited the growth of HEC-1A human endometrial cancers xenografted into nude mice.[17] These results indicate that GHRH antagonists can reduce the growth of human endometrial cancer and could be used as an alternative adjuvant therapy for the management of endometrial cancer.

Renal cell carcinoma

Treatment with GHRH antagonist MZ-4-71 strongly inhibited the proliferation of subcutaneous tumors of Caki-I human renal adenocarcinoma in nude mice and extended tumor doubling time.[45] GHRH antagonist JV-1-38 also suppressed the orthotopic growth of Caki-I tumors and inhibited the development of metastases to lung and lymph nodes.[32]

Brain tumors

Therapeutic modalities for malignant gliomas must be improved.[45] GHRH mRNA was detected in U-87MG tumors, suggesting that GHRH might have a role in the pathogenesis of this tumor.[35] GHRH antagonists MZ-5-156 and JV-1-36 were found to inhibit the proliferation of U-87MG human glioblastomas xenografted subcutaneously into nude mice and extended the survival of animals implanted orthotopically. GHRH antagonists can penetrate the blood–brain barrier and might be considered for therapy of brain tumors.

Box 1 Human cancers in which inhibitory effects by antagonists of growth-hormone-releasing hormone have been demonstrated experimentally.

- Prostate cancer
- Breast cancer
- Ovarian cancer
- Endometrial cancer
- Renal cell carcinoma
- Brain tumors
- Lung cancer
- Pancreatic cancer
- Colorectal cancer
- Non-Hodgkin's lymphomas
- Osteogenic sarcomas
- Gastric cancer[a]
- Carcinoid neuroendocrine tumors[a]

[a]Demonstrated only *in vitro*.

Lung cancer

GHRH could function as an autocrine growth factor in SCLC, because when SCLC cell lines are cultured *in vitro* they express mRNA for GHRH and secrete immunoreactive GHRH.[23] Early GHRH antagonists MZ-4-71 and MZ-5-156 significantly inhibited growth of human H69 SCLC and H157 non-SCLC tumors.[62] GHRH antagonist JV-1-38 also inhibited the growth of H838 human non-SCLC xenografted subcutaneously into nude mice.[25,45,46] Newer and more-potent antagonists including JV-1-65, JV-1-68, MZ-J-7-114, MZ-J-7-118, MZ-J-7-138, and JMR-132 inhibited the subcutaneous or orthotopic growth of H69 and DMS-153 SCLC, as well as H-460 and A-549 non-SCLC tumors in nude mice.[19,33,45,46,60] GHRH antagonists in combination with docetaxel synergistically inhibited growth of H-460 non-SCLC.[60] Treatment with antagonistic analogs of GHRH might offer a new approach to the treatment of SCLC and non-SCLC.

Pancreatic cancer

The expression of GHRH and the SV1 isoform of GHRH receptors in pancreatic cancers indicates the involvement of autocrine GHRH in this malignancy.[14] GHRH antagonists MZ-4-71 and MZ-5-156 inhibited the growth of nitrosamine-induced pancreatic cancers in hamsters and SW-1990 human pancreatic cancers xenografted into nude mice, and reduced IGF-II concentration in tumors.[63]

Box 2 Potential nononcological uses for antagonists of growth-hormone-releasing hormone.[a]

- Diabetic nephropathy (glomerular sclerosis)
- Diabetic retinopathy
- Acromegaly due to growth-hormone hypersecretion
- Prevention of stent re-stenosis and neointimal hyperplasia after angioplasty
- Alzheimer's disease[b]
- Diabetic neuropathy[b]
- Complications of diabetes and cardiovascular disease[b]

[a]Some of these are still hypothetical. [b]By reduction of oxidative stress and levels of reactive oxygen species.

Colorectal cancer

The incidence of colon cancer is increased in patients with acromegaly, suggesting that excessive secretion of GH or IGF-I might be a factor. The expression of GHRH and the SV1 isoform of GHRH receptors also indicates the involvement of autocrine GHRH.[14] The growth of subcutaneous xenografts of HT-29 human colon cancers was inhibited by GHRH antagonists including MZ-5-156 and JV-1-36.[64]

Osteogenic sarcomas

The present therapeutic methods for osteosarcomas are inadequate.[28] Our studies reveal that MNNG/HOS and SK-ES-1 bone sarcomas express SV1 and produce GHRH.[15] These findings suggest that GHRH antagonists could be considered for the treatment of human malignant bone tumors. GHRH antagonists MZ-4-71 and JV-1-38 significantly reduced the tumor growth of human MNNG/HOS osteosarcomas and SK-ES-1 Ewing's sarcomas xenografted subcutaneously into nude mice.[45,46,56] This inhibition was linked to a suppression of IGF-II production in tumors.

Non-Hodgkin's lymphoma

Non-Hodgkin's lymphoma (NHL) is the most frequently diagnosed hematological malignancy. High-affinity binding sites for GHRH and mRNA for pGHRH-R and SV1 were found on RL and HT NHL tumors.[20,21] RL and HT cells contained GHRH, and their growth *in vivo* was significantly inhibited by GHRH antagonists.[21] Our findings suggest that GHRH antagonists inhibit the growth of lymphomas by direct effects mediated by tumoral GHRH receptors. GHRH antagonists could, therefore, offer a new therapeutic modality for the management of advanced NHL.

CLINICAL STUDIES WITH GHRH ANTAGONISTS

In the first clinical study, performed in order to evaluate the potential involvement of GHRH in the generation of GH pulses, the GHRH antagonist of Robberecht[42] (Figure 1) was administered to six healthy young men and compared with placebo.[65] The nocturnal secretion of GH was partially suppressed as shown by the reduction of integrated total and pulsatile GH secretion during antagonist treatment by 40 ± 6% and 75 ± 5%, respectively.[65] The GHRH antagonist was well tolerated and after acute administration of the drug, there were no significant changes in blood pressure, heart rate or body temperature. Most of the participants who received a dose of 400 μg/kg developed hot flushes and erythema.[65] At lower doses (100 μg/kg) no hot flushes or erythema were noted. There were no changes in the hematological and biochemical blood tests, or in plasma concentrations of cortisol, TSH, LH, follicle-stimulating hormone or prolactin.[65] The toxicity profile of this GHRH antagonist was, therefore, considered favorable.

Since then, Robberecht's antagonist (Figure 1) has been tested in numerous clinical studies.[66–75] Some of these studies used the GHRH antagonist as a tool to further elucidate detailed control of the secretion of GH,[66–73] but two investigations involved tests in patients with acromegaly.[74,75]

The dosage range for Robberecht's antagonist used in clinical studies was 33 μg/kg per hour to 400 μg/kg, which indicates that about 1,500 μg/h or about a 20 mg total dose per patient was used.[65,67,72,75] Because our GHRH antagonists are 20–100 times more potent than Robberecht's antagonist,[43,44,47,49] the clinical activity of the existing GHRH antagonists should already be in the practical therapeutic range.

POTENTIAL THERAPEUTIC USES OF GHRH ANTAGONISTS

This subject has been reviewed by Gelato.[7,76] GHRH antagonists could be used in treatment of conditions caused by excess GH, such as acromegaly (Box 2). Another potential target could be diabetic retinopathy, which is the main cause of blindness in patients with diabetes, in whom vascular damage to the eye is thought to be due to GH.[77] Diabetic nephropathy (glomerulosclerosis) is a further example.[78] GH and IGF-I seem to be involved in the pathophysiology of diabetic nephropathy;[78] therefore, this condition might be amenable to therapy with GHRH

antagonists to inhibit glomerular podocytes or mesangial cells. On the basis of the considerations presented in our Review, however, the main applications of GHRH antagonists are likely be in the field of cancer. GHRH antagonists might offer distinctive advantages over other classes of prospective antitumor agents. Therapy with GHRH antagonists should be devoid of the severe adverse effects typical of chemotherapy.

It has been demonstrated that human lymphocytes produce a GHRH-like peptide.[79] Lymphocytes also contain specific receptors for GHRH.[80] In addition, an agonistic analog of GHRH was reported to enhance immune function in aging men and women, in part through stimulation of the GH–IGF-1 axis.[81] Consequently, although no immunosuppressive effects of GHRH antagonists were observed in experimental or clinical studies,[28,45,46,65] the influence of GHRH antagonists on immune function must be monitored.

The advantage of GHRH antagonists over somatostatin analogs would be that a GHRH antagonist could be used for the suppression of tumors that do not express somatostatin receptors; for example, human osteogenic sarcomas or those that contain only low levels of somatostatin receptors. The GH-receptor antagonist pegvisomant blocks the dimerization of GH receptors and induces inhibition of IGF-I production.[76] IGF-II is much less dependent on GH than is IGF-I.[28] Because GHRH antagonists inhibit IGF-II-dependent tumors, they should be superior to GH-receptor antagonists. The oncological activity of pegvisomant is, in any case, very limited.[28]

CONCLUSIONS

Potent antagonists of GHRH receptors have been developed. These GHRH antagonists inhibit the growth, tumorigenicity, and metastases of a wide range of human experimental malignancies. The inhibitory effects could be linked to their multiple mechanisms of action. These mechanisms include suppression of the axis between pituitary GH and hepatic IGF-I, inhibition of autocrine and/or paracrine production of IGF-I and IGF-II in tumors, and the blocking of the stimulatory effect of GHRH produced locally in tumors. The relative importance of these mechanisms seems to vary in different tumors. GHRH antagonists might offer distinctive advantages over other classes of antitumor agents. They lack the toxic adverse effects typically associated with cytotoxic therapies. Because they operate on different pharmacological principles, GHRH antagonists could be combined with standard chemotherapeutic agents for an enhanced antitumor effect.

Inasmuch as GHRH antagonists seem to strongly inhibit a wide range of cancers, they should prove to be clinically effective antitumor agents. GHRH antagonists are also likely to have diverse nononcological applications. Further improvement of the present class of GHRH antagonists should lead to clinically effective antitumor agents that could be used for a wide range of cancers.

KEY POINTS

- The presence of growth-hormone-releasing hormone (GHRH) ligand has been demonstrated in various cancers, suggesting that GHRH could be an autocrine growth factor
- GHRH antagonists inhibit the growth of various human cancer lines xenografted into nude mice; such lines include breast cancers, prostate cancers, small cell lung carcinomas (SCLC) and non-SCLC, malignant gliomas, renal cell carcinomas, pancreatic cancers, colorectal carcinomas and lymphomas
- Splice variants of GHRH receptors and pituitary-type GHRH receptors that might mediate effects of tumoral GHRH and of GHRH antagonists have been demonstrated in many human cancers
- GHRH antagonists seem to be devoid of major adverse effects, which would offer a distinct advantage over other classes of antitumor agents

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Competing interests
The authors declared no competing interests.