Inhibition of growth of human small cell and non-small cell lung carcinomas by antagonists of growth hormone-releasing hormone (GH-RH)

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Abstract. Insulin-like growth factors-I and-II (IGF-I and IGF-II) may be involved in the proliferation of human lung carcinomas. The purpose of this study was to investigate the effects of two potent antagonists of growth hormone-releasing hormone (GH-RH), MZ-4-71 and MZ-5-156 on the growth of the H69 human small cell lung cancer (SCLC) and H157 non-SCLC (NSCLC) lines transplanted into nude mice or cultured *in vitro*. Nude mice bearing H69 and H157 tumors were treated for 3-5 weeks with MZ-4-71 or MZ-5-156 injected s.c. twice a day at a dose of 20 μg/animal. Growth of H69 and H157 tumors in nude mice was significantly inhibited by MZ-4-71 and MZ-5-156 as shown by a reduction in tumor volume and weight. In animals bearing H157 NSCLC, treatment with MZ-4-71 decreased IGF-I and IGF-II levels in tumor tissue. Levels of IGF-I, but not of IGF-II in serum and liver tissue of H157 tumor-bearing nude mice treated with MZ-4-71 were decreased. High affinity binding sites for IGF-I were demonstrated on membranes of H69 and H157 tumors. In cell cultures, the proliferation rate of H69 SCLC cells was suppressed by 10^{-7}-10^{-5} M MZ-4-71, but H157 NSCLC line was only inhibited by 10^{-5} M antagonist. Our findings demonstrate that the GH-RH antagonists MZ-4-71 and MZ-5-156 can inhibit the growth of SCLC and NSCLC. This new approach to the management of lung cancer merits further investigation.

Introduction

Lung carcinoma is the leading cause of cancer-related deaths in the Western world. Surgery, radiation and chemotherapy are of limited effectiveness in the treatment of lung carcinomas and other therapeutic approaches must be explored (1-7). Several human SCLC and NSCLC cell lines secrete and respond to IGF-I and-II (1-7). The presence of receptors for IGF-I and IGF-II on lung carcinomas has been demonstrated (4,6,7). It was also shown that human lung cancer cell lines express IGF-I and-II and IGF-binding protein genes (8). Since IGF-I and to a lesser extent IGF-II are GH-dependent (6,9), the aim of potential new therapies could be to block the GH release from the pituitary in order to lower levels of IGFs. The benefit of this strategy is supported by the inhibitory effect of somatostatin analogs, which suppress GH release, on local proliferation of experimental lung tumors *in vivo* (10-12). However, somatostatin analogs cause only a modest decrease in IGF-I levels in patients with neoplasms potentially dependent on IGF-I (13,14) and there is a clinical need for antagonists of GH-releasing hormone (GH-RH) (13-15). Recently, several potent antagonists of GH-RH were synthesized in our laboratory in an endeavor to develop a new class of antitumor agents (15,16). Among these antagonists [Ibu^0, D-Arg^2, Phe(4-Cl)^6, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm (MZ-4-71) and [PhAc^0, D-Arg^2, Phe(4-Cl)^6, Abu^{15} Nle^{27}]hGH-RH(1-28) Agm (MZ-5-156) were shown to be the most effective for inhibition of GH-RH(1-29)NH_2-induced GH release in rats (15,16). We have shown that antagonist MZ-4-71 significantly inhibited the growth of human osteosarcomas grown in athymic nude mice (17). GH-RH antagonists could selectively block GH-release from the pituitary and suppress the synthesis of IGFs by the liver and other tissues. In this study, we have evaluated the effect of GH-RH antagonists MZ-4-71 and MZ-5-156 on the growth of the human SCLC and NSCLC cell lines H69 and H157 *in vivo*. Some effects of MZ-4-71 were also investigated *in vitro*.

Materials and methods

Peptides. GH-RH antagonists MZ-4-71 ([Ibu^0, D-Arg^2, Phe(4-Cl)^6, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm) and MZ-5-156 ([PhAc^0, D-Arg^2, Phe(4-Cl)^6, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm) were synthesized in our laboratory using solid-phase methods (15,16). For daily injections, the antagonists were dissolved in 0.1% dimethyl sulfoxide (DMSO) in saline and administered in a volume of 0.2 ml.

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Animals. Male athymic (NCr nu/nu) nude mice, approx. 6 weeks old on arrival, were obtained from NCI (Bethesda, MD) and maintained under pathogen limited conditions. Their care was in accord with institutional guidelines.

Cell lines. The human SCLC cell line NCI-H69, was obtained from ATCC, Rockville, MD and the NSCLC cell line NCI-H157, from Dr H. Oie, NCI-Navy Medical Oncology Branch, Bethesda, MD, USA. These cell lines were cultured in RPMI 1640 medium supplemented with 4 mM L-glutamine, 50 units ml^{-1} penicillin G sodium, 50 μg ml^{-1} streptomycin sulphate, 0.125 μg ml^{-1} amphotericin B and 10% fetal bovine serum at 37°C in a humidified 95% air/5% carbon dioxide atmosphere. Cells were passaged weekly and routinely monitored for mycoplasma contamination using a detection kit (Boehringer-Mannheim, Mannheim, Germany). All culture media components were purchased from Gibco (Grand Island, NY, USA). Monolayer tumor cells growing exponentially were harvested by a brief incubation with 0.25% trypsin-EDTA solution (Gibco).

Studies on tumor growth. Xenografts of the NSCLC cell line H157 were initiated by s.c. injection of $1x10^7$ H157 cells into the right flanks of 5 male nude mice. Tumors resulting after 4 weeks were aseptically dissected and mechanically minced; 3-mm^3 pieces of tumor tissue were transplanted s.c. by trocar needle into 40 mice under methoxyflurane anesthesia. One week after transplantation, when tumors had grown to a volume of approximately 20 mm^3, the mice were randomized and divided into 2 experimental groups of 10 animals each, which received the following treatment for 5 weeks: a) injections of saline containing 0.1% DMSO (control) b) MZ-4-71 or MZ-5-156 injected s.c. twice a day at a dose of 20 μg/animal.

Xenografts of the SCLC cell line H69 were initiated by s.c. injection of $1x10^7$ H69 cells into nude mice. The developed tumors were transplanted into 20 mice and the experiment was carried out as described above, except that the treatment with the antagonists lasted 3 weeks.

The tumors were measured with microcalipers, and tumor volume was calculated as length x width x height x 0.5236, as described previously (18). At the end of the treatment period, treated animals and control mice were anesthetized with methoxyflurane and sacrificed by decapitation. Trunk blood was collected, centrifuged and serum was frozen for hormone studies. Tumors were carefully cleaned and weighed, and samples were taken for receptor studies and for measurements of tissue levels of IGF-I and IGF-II. In addition, the livers from animals bearing H157 tumors were removed for measurements of IGF-I and IGF-II levels.

Method of tissue extraction. Tumor and liver tissue concentrations of IGF-I and IGF-II were determined by an adaptation of the methods described previously (19). The tissue was cut in small slices and homogenized in 5 ml of 1 M acetic acid/1 g tissue using Ultra Turrax homogenizer at 4°C. The homogenate was centrifuged at 2000 x g for 20 min at 4°C. The supernatant was collected in vials and the pellet was washed and recentrifuged. The supernatants were combined and lyophilized and reconstituted in 0.1 M phosphate buffer pH 7.6. The BIO-RAD protein assay kit (Hercules, CA) was used for protein determination.

Radioimmunoassays of IGF-I, IGF-II and GH. All serum and reconstituted tissue samples for IGF-I and IGF-II determination were extracted by a modified acid-ethanol cryoprecipitation method described earlier (20,21). This method eliminates most of the IGF binding proteins, which can interfere in the RIA. The extracted IGF-I was measured by RIA using IGF-I (88-G4, from Genentech, San Francisco, CA), as a standard in the range of 2-500 pg/tube and also for iodination by the chloramine-T method. Antibody UB3-189 and UB2-495 (a gift from Dr Underwood and Dr J. van Wyk) obtained from NIDDK was used in the final dilution of 1:10,000 and 1:14,000 in the RIA.

IGF-II was measured using recombinant human IGF-II standard (Bachem Chemical, Torrance, CA) in the range of 2-500 pg/tube. IGF-II was iodinated by lactoperoxidase method and purified by reverse phase HPLC using Vydac C18 column. For the assay, Amano monoclonal antibody generated against rat IGF-II was employed (22). Primary antibody (Amano Enzymes USA, Troy, VA) 10 μg/ml was used at the final dilution of 1:14,285. This antibody cross-reacts 100% with human IGF-II and rat IGF-II and 10% with hIGF-I (22). GH was determined by using materials provided by Dr A.F. Parlow (Pituitary Hormones and Antisera Center, Torrance, CA, mouse GH reference preparation AFP10783B, mouse GH antigen AFP10783B and anti-rat GH-RIA-5/ AFP-411S).

Receptor assay. Receptors for IGF-I on the membranes of H69 and H157 tumors were measured as previously described (23). The LIGAND PC computerized curve-fitting program of Munson and Rodbard (24) was used to determine the types of receptor binding, dissociation constant (K_d) values, and the maximal binding capacity (B_{max}) of receptors.

Growth in serum-free medium. For the MTT assay, cells were seeded into 96-well microplates (Falcon, Lincoln Park, NJ) in HITES medium (25). HITES medium (25) is composed of: RPMI 1640 (Gibco) medium plus 10 nM hydrocortisone, 5 μg/ml insulin, 10 μg/ml transferrin, 10 nM estradiol, 30 nM sodium selenite, 10 mM HEPES buffer and 4 mM L-glutamine (all from Sigma).

MTT assay. This assay is based on a method described by Plumb *et al* (26). Briefly, cells were seeded into 96-well microplates and cultured for 18 h. MZ-4-71 was added to the medium in final concentrations of 10^{-7}-10^{-5} M. Control cultures received HITES medium alone. After 72 h of culture, the medium was removed and 200 μl HITES containing 80 μg MTT [3,(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide; Sigma] was added. The microplates were incubated for 4 h at 37°C in darkness. The medium was removed, cells were washed twice with RPMI and 200 μl dimethyl sulfoxide (DMSO; Sigma) followed by 25 μl of Sorensen's glycine buffer (0.1 M glycine plus 0.1 M NaCl, pH 10.5) was added. After a brief mixing, the plates were read at 540 nm on the plate reader (Beckman, Palo Alto, CA). Results were calculated as % T/C, where T=

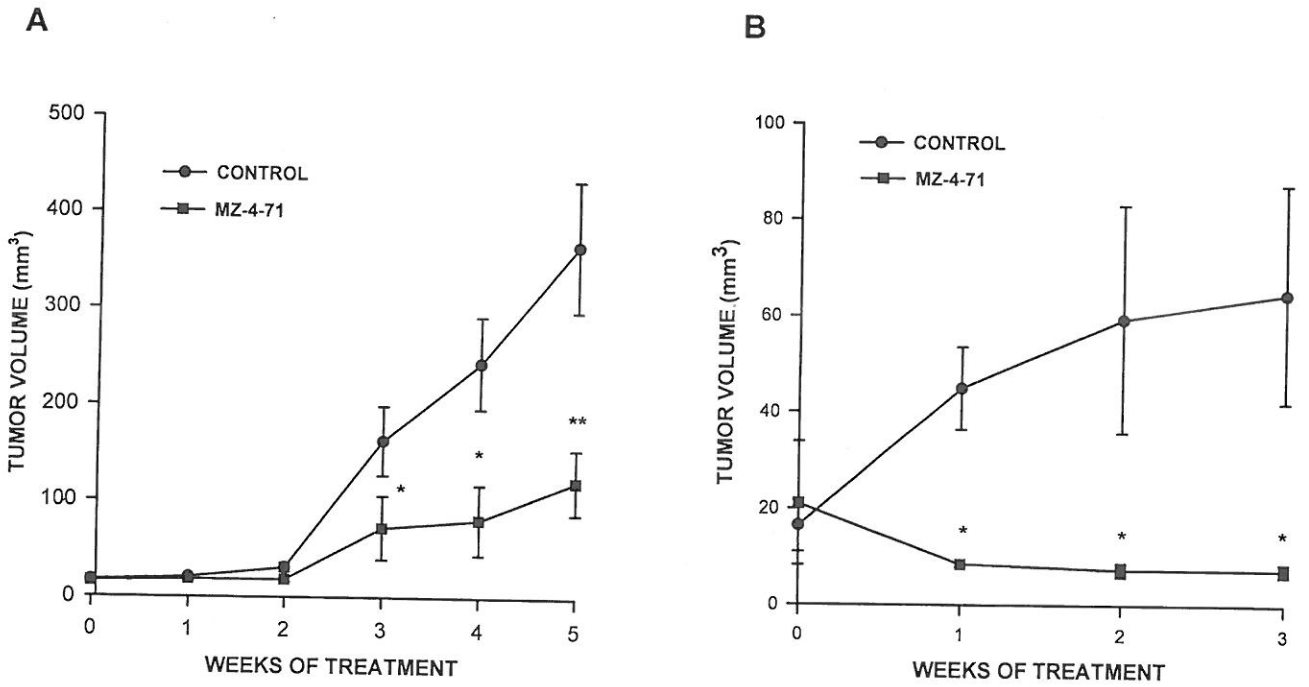

Figure. Tumor volumes in athymic nude mice bearing s.c. transplanted (A) NSCLC H157 and (B) SCLC H69 lung tumors during treatment with GH-RH antagonist MZ-4-71 administrated by daily s.c. injections at a dose of 20 μg/animal twice a day. Treatment was started when the tumors measured approximately 16-21 mm^3 and lasted for 5 and 3 weeks, respectively. Vertical bars represent S.C.; *p<0.05, **p<0.01 vs. control.

optical density ($OD_{540\ nm}$) of treated cultures (HITES medium plus MZ-4-71) and $C=OD_{540\ nm}$ of control cultures (HITES medium alone) X 100.

Statistical methods. Statistical analyses of the data were performed using Duncan's new multiple range test (27) and Student's two-tailed t-test. All p-values are based on two-sided hypothesis testing.

Results

The effect on MZ-4-71 on growth of lung carcinomas in nude mice. At the end of the experiments, there were no significant differences in body weights between groups (Table I). Tumor growth of NSCLC H157 tumors in animals treated with MZ-4-71 (Fig. 1A) or MZ-5-156 (not shown) was significantly inhibited within 21 days from start of the experiment. After 5 weeks, final tumor volume of H157 tumors in groups receiving MZ-4-71 or MZ-5-156 was significantly reduced to 119.6±33.6 mm^3 and 102.4±29.3 mm^3, respectively, as compared with the control groups (364.4±68.2 mm^3 and 487.5±45.2 mm^3), corresponding to 67% and 79% decreases in tumor volumes (Table I). The final tumor weights were significantly ($p<0.01$) diminished in animals treated with MZ-4-71 or MZ-5-156 to 130.3±40.2 mg and 120.6±52.1 mg, respectively, compared with those for controls (340.2±60.0 mg and 501.3±83.0 mg) (Table I).

In the SCLC tumor model, therapy with the GH-RH antagonists was most effective and resulted in regression of H69 tumors (Table I, Fig. 1B). The mean tumor volume and weight were significantly ($p<0.05$) reduced in animals receiving MZ-4-71 for 3 weeks to 7.7±1.3 mm^3 and 8.3±5.2 mg, compared with those in the control group which were 64.6±22.6 mm^3 and 68.3±23.6 mg, respectively (Table I). Final tumor volumes and weights of H69 tumors in the group injected for 3 weeks with MZ-5-156 were significantly ($p<0.01$) decreased to 10.2±1.2 mm^3 and 10.3±6.4 mg, respectively, as compared with those in controls (130.4±38.7 mm^3 and 125.4±43.8 mg), corresponding to decrease in tumor volume and weight of 92% and 91.8%, respectively (Table I).

Serum and tissue IGF-I and IGF-II levels. Serum growth hormone levels in mice bearing SCLC H69 tumors were significantly ($p<0.05$) reduced after treatment with MZ-4-71 to 1.5±0.2 ng/ml, as compared with the controls (5.0±1.7 ng/ml). Serum GH levels in mice with H157 tumors given MZ-4-71 did not show a decrease at the end of the experiment (not shown). Therapy with MZ-4-71 significantly ($p<0.05$) reduced serum IGF-I levels to 117.6±8.6 ng/ml in mice with NSCLC H157 tumors, and to 165.1±19.2 ng/ml in animals with SCLC H69 tumors, as compared with the controls (160.0±14.5 ng/ml and 238.2±13.8 ng/ml, respectively) (Table II). In both tumor models, serum levels of IGF-II at the end of the treatment period with MZ-4-71, were similar to those in controls (Table II). Treatment with MZ-4-71 significantly diminished the IGF-I and IGF-II concentrations in NSCLC H157 tumor tissue to 435.3±175.3 and 147.6±60.0 pg/100 μg protein, respectively, as compared with the corresponding controls which were 1408.2±486.1 and 618.5±97.1 pg/100 μg protein (Table III). After therapy with MZ-4-71, levels of IGF-II in liver tissue of nude mice bearing NSCLC H157 tumors were not decreased, while those of IGF-I were significantly reduced from 118.6±12.5 to 18.4±7.9 pg/100 μg protein ($p<0.01$) (Table III).

Table I. Effect of treatment with GH-RH antagonists MZ-4-71 and MZ-5-156 on body and tumor weight and tumor volume in nude mice bearing xenografts of the human N-SCLC H157 and SCLC cell lines.

Treatment group	Tumor volume (mm^3) Initial	Tumor volume (mm^3) Final	Body weight g	Tumor weight mg
N-SCLC H157				
A Control	17.2±2.6	364.4±68.2	27.2±0.8	340.2±60.0
MZ-4-71	16.3±2.5	119.6±33.6[a]	26.1±0.9	130.3±40.2[a]
B Control	21.0±0.8	487.5±45.2	28.6±1.5	501.3±83.0
MZ-5-156	24.2±2.0	102.4±29.3[a]	25.7±0.9	120.6±52.1[a]
SCLC H69				
A Control	16.5±5.5	64.6±22.6	27.3±1.0	68.3±23.6
MZ-4-71	21.4±12.8	7.7±1.3[a]	27.0±0.9	8.3±5.2[a]
B Control	9.1±1.3	130.4±38.7	26.8±1.2	125.4±43.8
MZ-5-156	12.3±2.8	10.2±1.2[a]	26.1±0.6	10.3±6.4[a]

Values are means ±SE [a]$p<0.01$ vs control.

Table II. Serum insulin-like growth factor-I and -II (IGF-I and -II) levels in nude mice with xenografts of human N-SCLC H157 and SCLC H69 cell lines after treatment with GH-RH antagonists.

Treatment group	IGF-I (ng/ml)	IGF-II (ng/ml)
N-SCLC H157		
Control	160.0±14.5	18.4±0.9
MZ-4-71	117.6±8.6[a]	18.5±0.3
SCLC H69		
Control	238.2±13.8	24.2±1.0
MZ-4-71	165.1±19.2[a]	20.2±0.6

Values are means ±SE [a]$p<0.05$ versus control.

Receptor findings. The binding characteristics of receptors for IGF-I in NSCLC H157 and SCLC H69 tumors were analyzed following treatment with GH-RH antagonists MZ-4-71 and MZ-5-156 and the results are presented in Table IV. Receptor assays on H157 tumor membranes showed high affinity binding sites for IGF-I (K_d: 0.83±0.11 nM and 0.5±0.1 nM). The concentration of receptors of IGF-I was significantly increased by treatment with MZ-4-71 or MZ-5-156 to 248.0±22.0 and 256.0±1.0 fmol/mg of membrane protein, respectively, as compared to the control groups which measured 152.5±23.5 and 144.7±10.6 fmol/mg of membrane protein, respectively, while the affinity of receptors was not changed. High affinity binding sites for IGF-I were also found on SCLC H69 control tumors (K_d: 0.83±0.14 nM) with B_{max} = 225.6±22.2 fmol/mg of membrane protein. No values could be obtained for treated SCLC H69 tumors because of shortage of tumor material.

Table III. Tumor and liver insulin-like growth factor I and II (IGF-I and II) levels in athymic nude mice bearing N-SCLC H157 xenografts after treatment with the GH-RH antagonist MZ-4-71.

Treatment group	Tumor		Liver	
	IGF-I	IGF-II	IGF-I	IGF-II
	pg/100 µg protein			
Control	1408.2±486.1	618.5±97.1	118.6±12.5	28.1±3.1
MZ-4-71	435.3±175.3[a]	147.6±60.0[b]	18.4±7.9[b]	25.0±5.1

All values are means ±SE. [a]$p<0.05$, [b]$p<0.01$ vs controls.

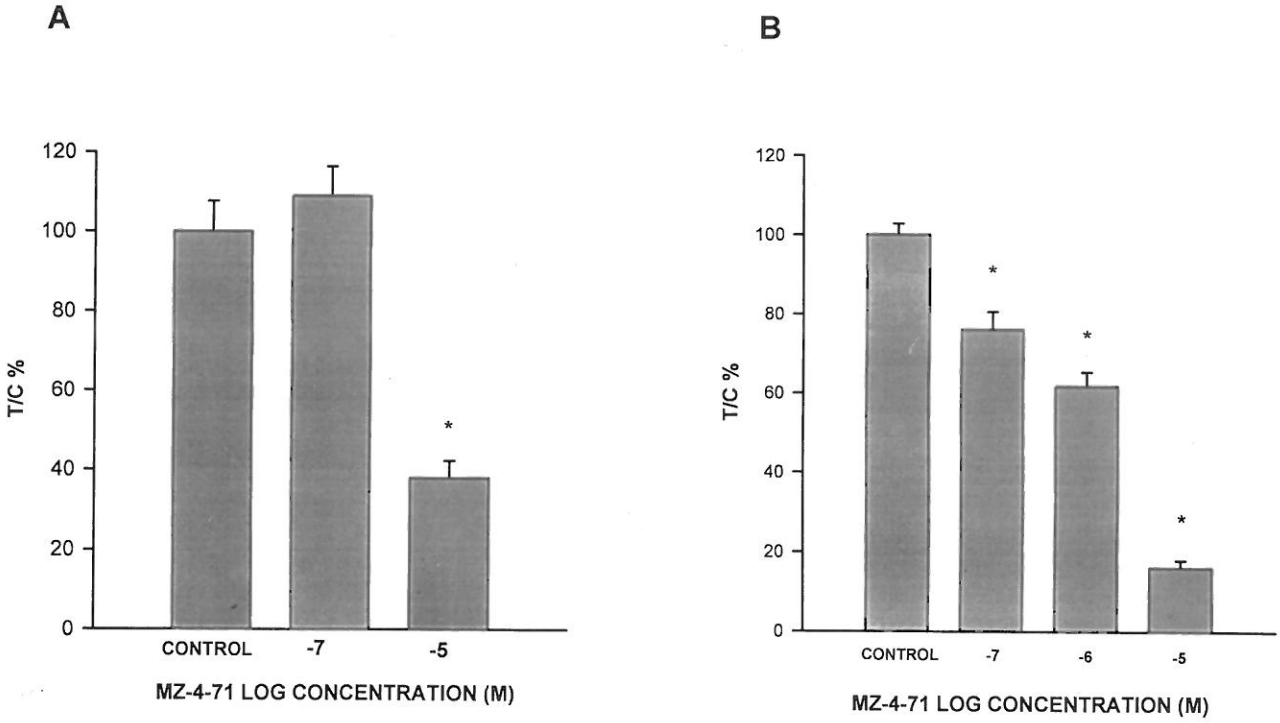

Figure 2. Antiproliferative effect of GH-RH antagonist MZ-4-71 on (A) NSCLC H157 cells and (B) SCLC H69 cells *in vitro* measured by the MTT assay. Cells were cultured in the presence of MZ-4-71 in concentrations of 10^{-7}-10^{-5} M. Results were calculated as %T/C, where T=optical density (OD) of treated cultures and C= OD of control cultures x 100. Vertical bars represent S.E.; *p<0.01 vs. control.

Table IV. Binding characteristics of IGF-I in membranes of NSCLC H157 and SCLC H69 tumors after *in vivo* treatment with GH-RH antagonists.

Groups	K_d (nM)	B_{max} (fmol mg^{-1} of membrane protein)
NSCLC H157		
A Control	0.83±0.11	152.5±23.5
MZ-4-71	0.74±0.2	248.0±22.0[a]
B Control	0.5±0.01	144.7±10.6
MZ-5-156	0.62±0.1	256.0±1.0[b]
SCLC H69		
Control	0.83±0.14	225.6±22.2

Binding characteristics were obtained from 10-point displacement experiments. Significance was calculated with Duncan's new multiple range test. All values represent mean ±SE of 2-3 experiments, each done in triplicate. No values were provided for treated SCLC H69 tumors because of shortage of tumor material. [a]p<0.05, [b]p<0.01 vs control.

Effect of MZ-4-71 on cell proliferation. In order to evaluate antiproliferative activity of MZ-4-71, the MTT assay was used. At a concentration of 10^{-5} M, MZ-4-71 decreased the number of viable NSCLC H157 cells by 62% as compared with the controls (Fig. 2A). The proliferation of H157 cells was not affected by lower concentrations of this antagonist. In contrast, MZ-4-71 significantly (p<0.01) and dose-dependently suppressed the proliferation of SCLC H69 cells in concentrations of 10^{-7}-10^{-5} M (Fig. 2B). In the presence of 10^{-7}, 10^{-6} and 10^{-5} M MZ-4-71, the %T/C values were reduced by 22.3%, 37.0% and 83.6% as compared to cells not exposed to this antagonist.

Discussion

Our findings demonstrate that GH-RH antagonists MZ-4-71 and MZ-5-156 can powerfully inhibit growth of the xenografts of SCLC and NSCLC cell lines in nude mice. SCLC H69 tumors appeared to be inhibited to a greater extent by both GH-RH antagonists than non-SCLC H-157 tumors. Animals bearing SCLC H69 xenografts, given the antagonists, developed small palpable nodules which regressed, being nearly completely inhibited during the 3 weeks of treatment. Antineoplastic actions of GH-RH antagonists may involve multiple mechanisms. A fall in GH levels induced by the GH-RH antagonists could, through mechanisms involving suppression of IGFs and possibly other endogenous growth factors be of major importance for the inhibition of tumor growth.

IGF-I and-II have been shown to play an important role in growth of lung cancer (1-7). Both SCLC and NSCLC cell lines were shown to express IGF-I and IGF-II genes (8). However, it appears that the IGF-II gene is more widely expressed than the IGF-I gene in human lung cancer cell lines (8). Furthermore, IGF-II was demonstrated to be a more potent stimulator of DNA synthesis than IGF-I in NSCLC cells (7).

Measurements of serum IGF levels can be affected by the presence of IGF binding proteins, but our assays were performed after acid-ethanol cryoprecipitation extraction (20,21). Recently, Lee *et al* (28) examined acid-ethanol extraction and acid-chromatography procedure for IGF and reported that the results were nearly identical. Only small amounts of residual small MW IGF binding proteins were found in the acid-ethanol extraction (28). Crawford *et al* (29) also compared various extraction methods for IGF-I in rat serum including acid-ethanol extraction with HPLC method. Their results show that IGF-I levels found after acid ethanol extraction are comparable with those obtained by HPLC (29). They also indicated that acid ethanol extraction method of Daughaday *et al* (21), originally validated for human serum, is also satisfactory for use with rat serum (29).

In our study, levels of IGF-I in tumor and liver tissues and serum were significantly suppressed by MZ-4-71, but the concentration of IGF-II was reduced only in tumor tissue. This suggests that IGF-II production in tumors may be more dependent on GH than in peripheral tissues. Alternatively, the antagonist could exert a direct effect on lung tumor cells, resulting in a decreased autocrine secretion of IGF-II.

Therapy with GH-RH antagonists decreased serum IGF-I levels in all treatment groups, but basal serum GH concentrations was only diminished in animals bearing H69 tumors which were injected daily with MZ-4-71. The lack of relationship between serum IGF-I and GH levels in the other groups might be due to the fact that circulating IGF-I levels are more related to integrated 24-h GH concentrations than to a single sampling of GH levels (30). Prior studies demonstrated positive relationship between basal serum IGF-I and spontaneous GH secretion in young and old men (31,32). Thus, it appears that serum IGF-I levels are related to the magnitude of GH pulses, rather than to basal GH levels between pulses (30).

Our findings, which show the presence of high affinity binding sites for IGF-I in membranes of both tumors, are in agreement with results previously reported by other groups, demonstrating the existence of high affinity receptors for IGF-I in various human lung cancer cell lines (4,6,7). IGF-I and IGF-II bind with different affinities to type-1 IGF receptor, which is thought to mediate the biological effects of both ligands through tyrosine kinase-type activity (33). We observed that chronic treatment of nude mice with the GH-RH antagonists produced an increase in the concentration of IGF-I receptors in both subtypes of lung cancers. This phenomenon might be an indication of a compensatory process caused by suppression of the endocrine or local production of IGF-I or IGF-II.

We have shown that the proliferation of SCLC H69 cells *in vitro* was strongly and dose-dependently inhibited by 10^{-7}-10^{-5} M MZ-4-71. A statistically significant inhibition of proliferation of NSCLC H157 cells was also obtained with 10^{-5} M MZ-4-71, but the growth of this cell line was not decreased at lower concentrations of the antagonist. The lack of a dose-dependent antiproliferative effect of MZ-4-71 on H157 cells *in vitro*, suggests that suppression of the growth of those NSCLC cells seen with high concentrations of the antagonist may not be due to the binding of this compound to a specific GH-RH receptor, but rather to a non-GH-RH-specific phenomenon. GH-RH is a member of the glucagon-secreting family of peptides which demonstrate considerable amino acid sequence homology (34,35). These peptides bind to common receptor proteins, such as that for vasoactive intestinal peptide (VIP), and produce similar biologic responses in most of their target organs (36,37). Thus, GH-RH antagonists might bind with reduced affinity to one of these receptors. GH-RH receptors on NSCLC may also be lost during passages *in vitro*, although they could be re-established and functional *in vivo* as in the case of somatostatin receptors (38).

In view of the observations described above, the inhibitory effect of MZ-4-71 and MZ-5-156 on the growth of NSCLC H157 tumors *in vivo* observed in our study is probably mainly due to suppression of GH and IGF secretion, since the doses of MZ-4-71 used *in vitro* to achieve an inhibition were very high. The concentration of MZ-4-71 in blood of nude mice given a subcutaneous injection of this antagonist at a dose of 20 μg per animal is approximately 20 pM (17), i.e. 500,000 times lower than the dose of MZ-4-71 required to inhibit the growth of NSCLC H157 cells *in vitro* (10 μM). On the other hand, a stronger inhibitory action of the GH-RH antagonists on growth of SCLC H69 tumor might be attributed to direct as well as indirect effects of the antagonists, which might sum up and produce a better suppression of this tumor *in vivo*.

There is a clinical need for antagonists of GH-RH, since somatostatin analogues do not adequately suppress GH and IGF-I levels (12). Compensatory mechanisms, such as increased GH-RH secretion, may attenuate the inhibitory effect of somatostatin analogues on GH secretion (39). GH-RH antagonists could be given alone or together with somatostatin analogues. The use of the combination of both analogues could achieve a more complete suppression of IGF levels (39), which might result in greater tumor growth inhibition.

In conclusion, our results suggest that GH-RH antagonists, exemplified by MZ-4-71 and MZ-5-156, might find applications in the treatment of lung carcinomas and other tumors, like breast cancers, that are influenced by IGFs.

Acknowledgements

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