Drugs of the Future 2001, 26(1): 51-59

CCC: 0377-8282/2001

Review Article

GnRH analog conjugates with high anticancer selectivity

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CONTENTS

Introduction

Gonadotropin-releasing hormone (GnRH) analog agonists, such as buserelin, goserelin, decapeptyl and leuprolide, and antagonists, such as cetrorelix, are used for the treatment of some types of hormone-dependent tumors such as prostate and breast cancer (1, 2). GnRH agonists were shown to inhibit the actions of sex hormones at their target organs and to decrease sex hormone synthesis and secretion with long-term treatment. Continuous exposure of the pituitary gland to long-acting GnRH agonists causes downregulation of GnRH receptors, leading to a profound decrease in gonadotropin secretion and even chemical castration. However, a considerable disadvantage of superactive agonists in the treatment of cancer is that they first promote tumor growth ("flair-up") at the beginning of treatment and, subsequently, after prolonged treatment, decrease tumor progression which parallels inhibition of hormone secretion.

On the other hand, GnRH antagonists compete with GnRH for hypothalamic GnRH receptors and inhibit GnRH secretion. The result is direct inhibition of the hypophyseal-gonadal axis and chemical castration. Unfortunately, these peptide hormone analogs are subject to rapid degradation by proteinases, thus decreasing their activity. To avoid degradation and maintain continuous therapeutic levels, new procedures have been introduced such as encapsulation of GnRH analogs to produce retention preparations (*e.g.*, goserelin). Another option is the coupling of peptide hormone derivatives to carrier molecules resulting in conjugates. Covalent attachment of antitumor drugs to various polymers for retention and/or enhancement of biological activity has been well documented (3-5) and examples of these conjugates include neocarcinostatin (3), doxorubicin (4), daunomycin (6), methotrexate (7) and melphalan (8). Various types of polymers can be used to prepare anticancer drug conjugates. The polymers can be biodegradable, such as the branched chain, polylysine-based polypeptide (*e.g.*, poly[Lys(Ac-Glu_i-DL-Ala_m)]; AcEAK) (9) or nonbiodegradable such as N(2-hydroxypropyl)-methacrylamnide (HPMA) (10), poly(styrene-co-maleic acid/anhydride) (SMA) (11) and poly(N-vinylpyrrolidone-co-maleic acid) (NVP-MA; P) (11, 13).

The aim of our investigations was to find new GnRH agonists and antagonists with enhanced direct antitumor activity by modifying the amino acid sequence in position 6-10 of the GnRH molecule. Such compounds would be more stable with delayed effects and possess biodistribution properties and slower clearance.

We prepared new human (MI-1544) and chicken (MI-1892) GnRH analogs and also tested a new GnRH analog (GnRH-III) from the sea lamprey, *Petromyzon marinus*. All of these analogs showed direct antiproliferative/anticlonogenic activity against GnRH receptor-positive human breast, prostate and endometrium cancer cell lines *in vitro*, and against growth of human breast cancer xenografts *in vivo* (14, 15). We then prepared a conjugate by coupling MI-1544 with the biodegradable branched polypeptide, poly[Lys(Ac-$Glu_{0.96}$-DL-$Ala_{3.1}$)] (AcEAK) (16). Because this conjugate showed some disadvantages (limited water solubility and chemical stability), we chose

another approach for improving therapeutic efficacy by coupling our GnRH analogs to NVP-MA (P) using a tetrapeptide spacer, Gly-Phe-Leu-Gly (X), with the general formula P-X-GnRH analog (17).

The present review summarizes our findings with the new GnRH analog conjugates. The human GnRH antagonist MI-1544 was synthesized as a potential contraceptive compound since it decreased luteinizing hormone (LH) and progesterone release (18). The chicken GnRH analog MI-1892, which resembles the known antagonist in its chemical structure (19, 20), caused low level LH-release inhibition and a weak castration effect. GnRH-III (21) displayed 1000-fold weaker activity in releasing LH as compared to GnRH but exhibited antiproliferative and anticlonogenic potential against GnRH receptor-positive human cancer cells *in vitro* (14, 22).

Synthesis of GnRH analogs

MI-1544

The synthesis of the human GnRH antagonist MI-1544 (Ac-D-Trp-D-Cpa-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH_2) has been previously described (18). Briefly, the peptide was prepared by solid phase synthesis on benzhydrylamine resin. The amino acids were coupled in the presence of N,N'-diisopropylcarbodiimide. The peptide was purified on Sephadex G-50 columns and silica.

MI-1892

Benzhydrylamine resin was used for the synthesis of MI-1892 (Ac-D-Trp-D-Cpa-D-Trp-Ser-D-Lys-β-Asp(N-Et_2)-Leu-Gln-Pro-Ala-NH_2) (19). The peptide was cleaved from the resin using HF and purified Sephadex G-25 and HPLC.

GnRH-III

GnRH-III (Glp-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH_2) was synthesized on a 4-(2,4-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin using an Applied Biosystems 430A peptide synthesizer (21-23). Amino acids were N-α-Fmoc-protected and were coupled as their HOBt active esters. The reactive side-chain functions were protected as follows: His: N-im-trityl; Asp: beta-t-butyl ester; Lys: N-e-t-Boc; Ser: t-butyl ether. The peptide was cleaved from the resin by stirring with a mixture of TFA/EDT/anisole (95:1.6:3.4, v/v/v) for 90 min at room temperature. The crude product was purified to apparent homogeneity by reversed phase (RP) HPLC on a preparative column (50 cm x 1 cm) packed in house with Vydac 218TPB1520 silica and equilibrated with 50 mM NH_4OAc, pH 4.5. Compounds were eluted from the column at a flow rate of 5 ml/min. The concentration of MeCN in the eluting solvent was increased linearly to 33% within 100 min. A280 of the effluent was monitored. Analytical RP HPLC was performed on a Vydac 218TP54 C18 column (250 mm x 4.6 mm). The column was irrigated for 35 min at a flow rate of 1 ml/min with a linear 3-35% gradient of A in B, where A was a solution containing 0.09% TFA and 60% (v/v) MeCN in H_2O, and B was a solution of 0.1% TFA in H_2O. The purity and identity of the peptide was confirmed by analytical RP HPLC, amino acid analysis and low resolution FAB-MS.

Synthesis of copolymers and their GnRH conjugates

Biodegradable polypeptides

The biodegradable branched polypeptides with a poly(L-Lysine) backbone were synthesized (9) to be used as carriers for bioactive molecules (5). Poly[Lys(Glu_i-DL-Ala_m)] (EAK), where i = 0.96 and m = 3.1, was synthesized as previously described (9) with minor modifications (16).

In the present review, we deal with the conjugate composed of the MI-1544 GnRH analog and the biodegradable branched polypeptide, poly[Lys-(X_i-DL-Ala_m)] (XAK) type poly-α-amino acids in which X is an acetylated derivative of glutamic acid (AcEAK) (24). A schematic representation of the conjugate is shown in Figure 1 (16).

The synthesis of the branched polypeptide-MI-1544 conjugate has been described (16). Briefly, AcEAK (49 mg, 95 μmol) was dissolved in 1 ml of deionized water and subsequently diluted with 3 ml of dimethylformamide (DNF). The MI-1544 (0.5 ml calculated for side chains of the polymer) solution was then added to the AcEAK solution. Following addition of 42 mg BOP reagent in 1 ml of DMF, the pH of the reaction mixture was adjusted to approximately 7.5. The mixture was stirred overnight at room temperature after which it was placed in Visking tubing and extensively dialyzed against deionized water for 3 days. The conjugate, MI-1544-AcEAK, was then isolated by lyophilization.

Nonbiodegradable polypeptides

The nonbiodegradable poly(N-vinyl-pyrrolidone-co-maleic acid) (NVP-MA; P) macromolecule, a nontoxic biocompatible polyanionic polymer, was developed and synthesized (13, 25) for use as a drug carrier. The synthesis of GnRH analog conjugates was recently described (17) and the schematic structure is shown in Figure 2. Briefly, polymer-bound oligopeptide nitrophenyl ester (0.3 g) was dissolved in 1 ml of DMF to which 0.5 ml water was added to hydrolyze the intact anhydride moieties (stirring overnight). A sample was taken out and precipitated from the solution and the IR spectrum revealed that no anhydride remained after 1 h (IR: 1740 cm^{-1} COOH, 1540 cm^{-1}, 1350 cm^{-1} NO_2). Next, 30-80 mg of the GnRH analog was dissolved in 0.2 ml of water while stirring and the

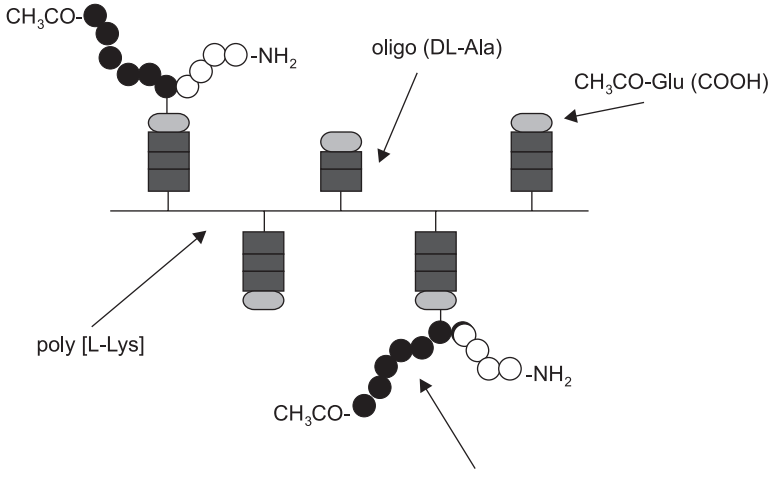

Fig. 1. Schematic representation of the branched polypeptide AcEAK-peptide conjugate.

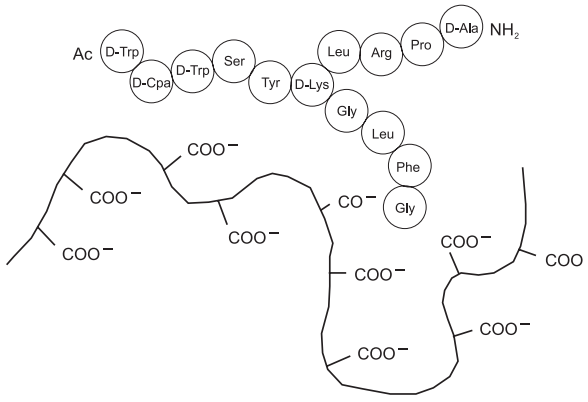

Fig. 2. Schematic structure of the NVP-MA-Gly-Phe-Leu-Gly-MI-1544 conjugate.

pH was adjusted to 8 with TEA. After allowing the mixture to stand overnight, the product was precipitated with ether, filtered, dissolved in 5% $NaHCO_3$ and purified by ultrafiltration. The free GnRH content was less than 3% according to the HPLC analysis. Conjugation of the GnRH analog via Gly-Phe-Leu-Gly spacer group (X) is illustrated in Figure 3 (17). The GnRH content of the conjugates (26) was determined spectrophotometrically. The proton NMR spectra of conjugates in deuterium oxide contained the characteristic peaks for the peptides and the polyacid.

In vitro studies

Cell cultures

The biological activities (*i.e.*, anticlonogenic and antiproliferative effects) of the GnRH analogs and their conjugates were tested on several GnRH receptor-positive human tumor cell lines: MCF-7 and MDA-MB-231 breast, PC3 and LNCaP prostate and Ishikawa endometrium cells.

Clonogenic assay

The anticlonogenic effects of the compounds were tested using a previously described clonogenic assay (14). Briefly, 300 cells were plated onto 35 mm petri dishes in 2.5 ml of medium. Cells were treated the next day with different doses of the compounds (3 plates/treatment) and were cultured in a CO_2 incubator for 8-12 days. The cells were then rinsed and stained with crystal violet. The number of colonies containing a minimum of 50 cells was counted under a dissection microscope. The colony number obtained from treated cultures was expressed as a percentage, using the controls as 100%.

Cell proliferation assay

Cells (2-3 x 10^5) were plated on 90 mm plastic petri dishes and treated on the following day with different doses of the compounds (2 dishes/treatment). In the case of the antagonists, treatment was repeated once or twice, every other day. On the 5th day, the cells were trypsinized and counted using a Neubauer hemocytometer. The decrease in proliferation of the treated culture was expressed in a percentage using the controls as 100% (14).

Sulforhodamine B assay

One or two thousand cells, depending on the rate of proliferation, were added to each well of 96 microwell

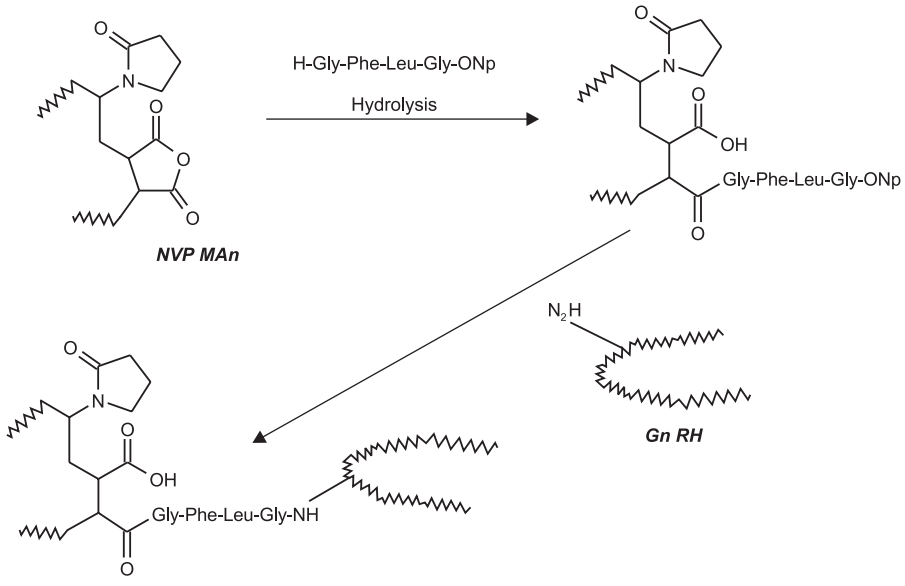

Fig. 3. Conjugation of the GnRH analog to NVP-MA (P) via a Gly-Phe-Leu-Gly (GPLG; X) spacer group.

plates in 200 µl of medium. Cells were treated with the test compounds after 6 h or on the following day. At the end of the experiment, the adherent cells were fixed *in situ* by adding 50 µl cold 50% (w/v) trichloroacetic acid (TCA) and incubated for 60 min at 4 °C. The supernatant was then discarded and the plates were washed 5 times with distilled water and dried at room temperature. TCA-fixed cells were stained for 30 min with 0.4% (w/v) sulforhodamine B (SRB) dissolved in 1% acetic acid. SRB was then removed and the cultures were quickly rinsed 4 times with 1% acetic acid and air dried. Bound dye was solubilized with 10 mM unbuffered Tris base (pH 10.5) for 5 min. Optical density was read at 564 nm using a CLS 962 microplate reader (14).

Apoptosis assay

Changes in cell morphology following treatment of cells were evaluated using an inverted phase contrast microscope (27). DNA strand breaks, characteristic of apoptotic processes, were detected with an ApopTag kit. An ELISA cell death detection kit and a POD *in situ* cell death detection kit were also used for assessing apoptosis following treatment.

cdc25 Phosphatase assay

The New Drug Development Office of the European Organization for Research and Treatment of Cancer recommends this assay as suitable for mechanism-based screening of antimitotic compounds. cdc25 Phosphatase activity is responsible for transition of cells from the G_2 to M phase. The effect of compounds on cdc25 phosphatase activity were measured (27).

Flow cytometry

Alteration of cell cycle phase distribution of control and treated cultures was analyzed by measuring the relative DNA content of individual cells using a fluorescence-activated cell sorter. Samples were prepared (28) and measurements of DNA content were performed using a FACStar Plus flow cytometer.

In vivo studies

The antitumor activity of the compounds was tested on immunosuppressed, xenograft-bearing mice as described previously (15, 27). Briefly, MCF-7 or MDA-MB-231 cells were inoculated into female CBA/Ca HRIJ-T6 and BDF1 mice, which had been previously immunosuppressed (29). Further passages were performed with implanted 2 mm^3 tumor pieces. For growth inhibitory experiments, treatment of tumor-bearing animals was initiated on week 4 following transplantation and lasted for 35 or 42 days. The GnRH analog peptide hormones were given s.c. twice daily while their conjugates were given once a day. Growth of xenografts was assessed each week by measuring with calipers 2 perpendicular diameters of the tumors. Tumor volume was estimated using the following formula: $V = (a^2xb) \times 3.16:6$, where a<b. Student's *t*- test was used for statistical evaluation of the results.

Table I: Effect of GnRH analogs and conjugates on clonogenicity of GnRH receptor-positive human tumor cell lines.

	Dose (μM)	Surviving colonies, % of control MCF-7	MDA-MB-231	PC3	Ishikawa	LNCaP
GnRH-III	50	42 ± 8	34 ± 7	83 ± 5	88 ±3	75 ± 5
P-X-GnRH-III	50	10 ± 10	13 ± 7	70 ± 4	55 ± 6	22 ± 10
MI-1544	50	55 ± 4	76 ± 8	90 ± 6	88 ± 7	70 ± 8
P-X-1544	50	2 ± 1	3 ± 2	4 ± 1	3 ± 1	6 ± 2
MI-1892	50	63 ± 6	67 ± 5	92 ± 4	91 ± 6	81 ± 7
P-X-1892	50	3 ± 1	1 ± 1	2 ± 1	2 ± 1	6 ± 2

Results

In vitro *effects*

In the clonogenic assay, MCF-7 cells were practically insensitive and MDA-MB-231 cells were only slightly sensitive to MI-1544 (50 μM). While the free AcEAK polypeptide exerted no effect on clonogenicity of cells, the MI-1544-AcEAK conjugate considerably reduced cell survival (16).

In antiproliferative experiments, the free AcEAK polymer inhibited cell proliferation by 13-15%. A 6-day treatment with the conjugate (30 μM) resulted in 45-50% inhibition of proliferation. The activity of the free GnRH peptide antagonist was between that of the polymer and the conjugates, suggesting that the effect of the conjugate was the result of additional constituent-induced effects (30).

The effects of MI-1544, MI-1892, GnRH-III, P copolymer and the respective P-X-conjugates on clonogenicity and cell proliferation were examined using 5 human tumor cell lines. Results of the clonogenic assay are summarized in Table I.

A typical dose-response curve is shown in Figure 4. Although neither the copolymer nor the free peptide significantly reduced colony formation, the conjugate achieved nearly 100% inhibition (31).

The effect of MI-1544 and MI-1892 on cell proliferation is summarized in Figure 5. Of the cell lines studied, the Ishikawa cells were the least sensitive to the free GnRH analogs but the most sensitive to their conjugates. It is important to note that cultures were exposed to the free peptides repeatedly (every other day) to maintain drug levels, while conjugates were administered only once during the experimental period (5 days). Figure 6 shows the antiproliferative activity of GnRH-III and its conjugate on MDA-MB-231 cells (14, 31).

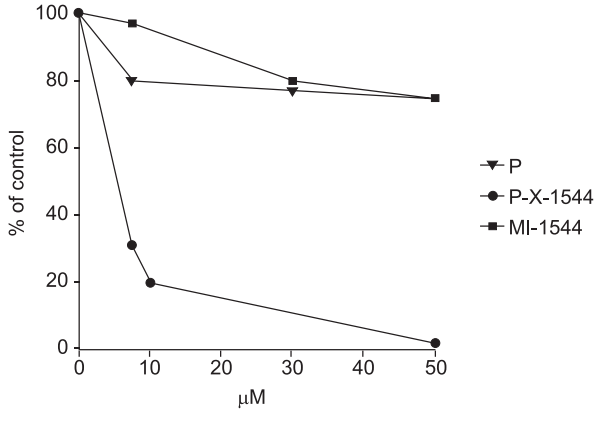

Fig. 4. Dose response curves of MDA-MB-231 cells treated with MI-1544, P-X-1544 conjugate or the P copolymer.

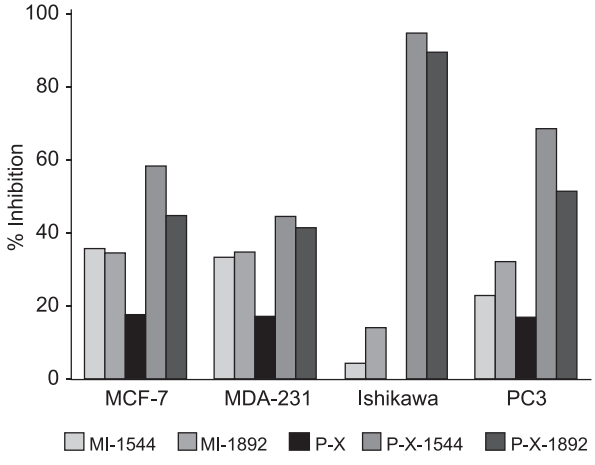

Fig. 5. Results of cell proliferation assays. Cells were treated with GnRH analogs MI-1544 and MI-1892 (30 μM) twice, on days 1 and 3, while the conjugates, P-X-1544 and P-X-1892, were given only once on day 1. The number of cells was counted on day 5.

The direct antitumor activity of GnRH-III surpassed that of the agonist buserelin and the antagonists SB-30 and MI-1544. GnRH-III showed 1000-fold weaker activity than GnRH in releasing LH from superfused rat pituitary cells, indicating selective antitumor activity. The superior antitumor activity of GnRH-III compared with other GnRH analogs may be dependent on the structural features (sequence 5-8) which are different in GnRH-III. Thus, analogs synthesized for improved antitumor activity such as GnRH-III, include changes in 5-8 sequence (32).

The effect of GnRH-III was stronger in the clonogenic assay than in the proliferation assay, although cells were treated only once in the former assay. GnRH-III (50 μM) reduced colony formation to 55% of the control value. Replacement of His^5 with Lys caused a loss of anticlonogenic activity and replacement of Gly^{10} with D-Ala also led

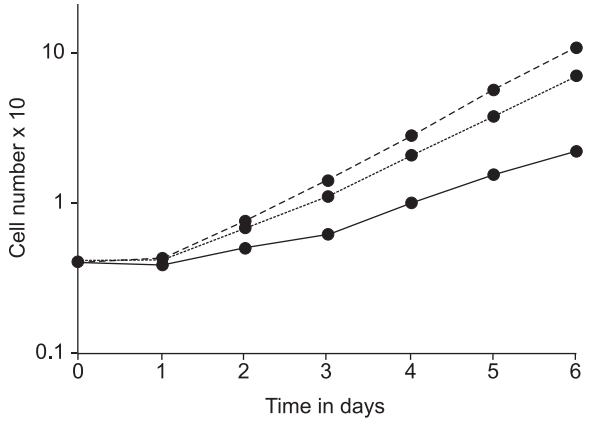

Fig. 6. The effects of GnRH-III (dotted line) and P-X-GnRH-III (solid line) on the proliferation of MDA-MB-231 cells. Control cells (broken line) were incubated in a similar manner.

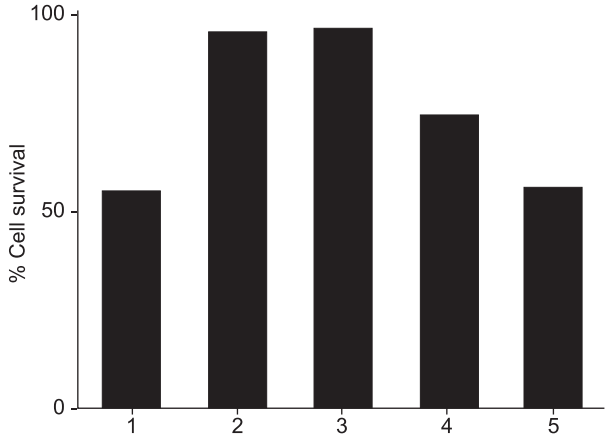

Fig. 7. Percent survival of MCF-7 cells treated for 8 days with 50 μM of GnRH-III (column 1), Lys^5-GnRH-III (column 2), D-Ala^{10}-GnRH-III (column 3), Phe^7-GnRH (column 4) and Lys^5-cyclo-(Asp^6,Lys^8)-GnRH-III (column 5).

to a nearly complete loss of anticlonogenic activity. Introduction of Phe in place of Trp^7 reduced anticlonogenicity to about half that of GnRH-III. [Lys^5]GnRH-III had similar anticlonogenic activity to that of GnRH-III (32-34) (Fig. 7).

In cell cycle studies, the GnRH analog conjugates, similar to the free GnRH peptides, showed specific GnRH receptor binding in GnRH receptor-positive MCF-7 breast and PC3 prostate tumor cell lines (data not shown). This receptor-based mechanism suggested that our GnRH conjugates influenced signal transduction and may inhibit the cell cycle.

Very few studies have been reported the influence of GnRH analogs on the cell cycle (35-37) and those results presented indicate varying effects on different stages of the cycle. For instance, the GnRH antagonist SB-75 induced a slight accumulation of G_2 cells, whereas the GnRH agonist buserelin and leuprolide caused a significant and a slight increase, respectively, in the number of G_0/G_1 cells.

The results of studies on the effect of P-X-MI-1544 and P-X-GnRH-III on cell cycle phase distribution of PC3, Ishikawa and MCF-7 cells are summarized in Table II. As can be seen, the conjugates induced a significant accumulation of G_2/M cells, both at 24 and 48 h following treatment. The effects of these conjugates on phase distribution were much more pronounced than the free peptides mentioned above (26, 33).

The partial premitotic block of the cells suggested that the conjugates exert inhibitory activity on some enzyme function. The effects of the GnRH analogs, the copolymer P and P-X-conjugates on cdc25 phosphatase activity were examined and the results are shown in Table III. P-X-1544 was found to be the most efficient followed by P-X-1892 out of the approximately 18 compounds tested (27).

In vivo *effects*

Results from *in vivo* experiments showed that the AcEAK polypeptide alone had no influence on growth of either MCF-7 or MDA-MB-231 xenografts. However, the GnRH antagonist MI-1544 (12.5 μg b.i.d.) decreased MDA-MB-231 tumor mass by 30% at the end of an 8-week administration period. Treatment with the MI-1544-AcEAK conjugate slowed tumor growth by 70 and 50% when applied daily and every 3 days, respectively. The MCF-7 xenograft was more sensitive to the same treatment. Treatment with MI-1544 (12.5 μg b.i.d.) or the MI-1544-AcEAK conjugate at a similar dose every third day resulted in a 65-70% inhibition of tumor growth, with 28% of the treated animals tumor-free. Untreated controls died within 3-4 months following tumor implantation. The hair of the tumor-free, "cured" MI-1544-AcEAK-treated animals took on a healthy appearance and their body weight increased; these animals died within 12-21 months following treatment (30).

The tumors in the control group grew continuously. Tumor growth was suppressed by treatment with the GnRH antagonist analogs MI-1544 or MI-1892 and their conjugates P-X-1544 and P-X-1892. The macromolecular carrier (P-X-OH) alone (*i.e.*, without the GnRH analogs) did not influence MCF-7 xenograft growth. A significant inhibition of tumor volume was observed after 3 weeks of treatment in the 2 conjugate-treated groups. By the end of the 4th week of administration, a twice-daily dose of human GnRH antagonist MI-1544 (25 μg) decreased tumor volume by 30% (2.455 ± 0.432 cm^3 *vs.* control: 3.507 ± 0.761 cm^3). The chicken GnRH antagonist MI-1892 decreased tumor volume by 22.5% (2.728 ± 0.23 cm^3). Treatment with the P-X-1544 and P-X-1892 conjugates resulted in a 49 and 37% decrease of tumor volume, respectively (Fig. 8). The differences in tumor volumes were statistically significant (p <0.05) by the 5th

Table II: Effect of P-X-1544 on the distribution of cell cycle phases of PC3 and Ishikawa cell lines.

Cell	Conjugated peptide (μM)	Phase distribution (%) 24 h			48 h		
		G_1	S	G_2/M	G_1	S	G_2/M
PC3	0	54	29	17	74	17	9
	50	52	26	22	76	16	8
	100	48	29	23	83	11	6
Ishikawa	0	44	33	23	49	33	18
	50	47	31	22	55	14	31
	100	44	33	23	58	12	30
MCF-7	0	51.9	40.0	8.1	56.1	34.1	9.8
	50	49.3	39.9	10.7	57.6	31.6	10.8
	100	46.7	37.7	15.6	51.6	33.3	15.0

Table III: Effect of GnRH analogs and their conjugates on cdc25 phosphatase activity.

Compound	IC_{50} (μM) Peptide	Conjugate
MI-1544	1000	–
P-X-MI-1544	1.33*	0.86
MI-1892	1000	–
P-X-1892	3.33*	2.07
P-X-OH	–	37.5

*Concentration of peptide conjugated.

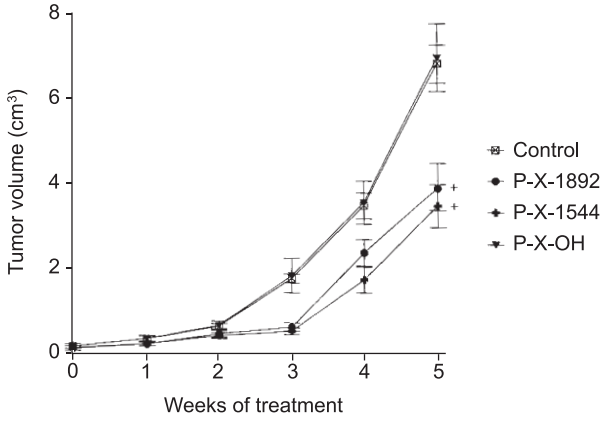

Fig. 8. *In vivo* effects of a daily dose of 50 μg of P-X-1544, P-X-1892 or P-X-OH on the volume of MCF-7 xenografts.

week in the conjugate-treated groups, with 43-49% inhibition observed. At the end of the 6th week, treatment was terminated and tumor weights were measured. Control tumor mass was 7.92 ± 0.83 g (100%) while MI-1544 and MI-1892 decreased tumor mass by 65% (5.14 ± 0.64 g; 35% inhibition) and 74% (5.86 ± 0.76 g; 26% inhibition), respectively. Based on tumor weights, the inhibition achieved by the two conjugates was 39% (P-X-1892, 4.84 ± 0.92 g) and 49% (P-X-1544, 4.01 ± 0.85 g) (15) (Fig. 9).

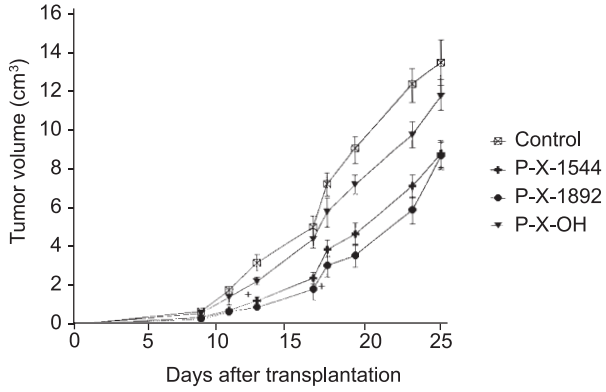

Fig. 9. *In vivo* effects of a daily dose of 50 μg of P-X-1544, P-X-1892 or P-X-OH on the volume of MA-MB-231 xenografts.

Daily doses of GnRH-III (4 mg/kg) had no influence on the growth of MDA-MB-231 xenografts in mice, whereas treatment with P-X-GnRH-III containing 4 mg of conjugated GnRH-III per kg of body weight decreased tumor volume to 0.30 ± 0.15 cm^3 which was 57% of the control volumes (0.70 ± 0.15 cm^3) by the end of the 4th week. Treatment for 56 days caused a 78% decrease in tumor volume and eventually complete regression of tumors (Fig. 10) (27).

Conclusions

In an efforts to prolong the tumor inhibitory effect of new GnRH analog peptides, we looked for polymers to be used as potential carriers. The biodegradable polylysine-based polymer AcEAK and the water-soluble, non-biodegradable copolymer P were found to increase the direct antitumor effects of the GnRH analogs. The carrier molecules appear to have multiple functions. For example, they prolong the effect of GnRH analogs *in vivo* (*i.e.*, delayed effect) while increasing the stability of the GnRH peptide (*i.e.*, increase resistance to proteolysis) and enhance direct antitumor activity. Although the exact

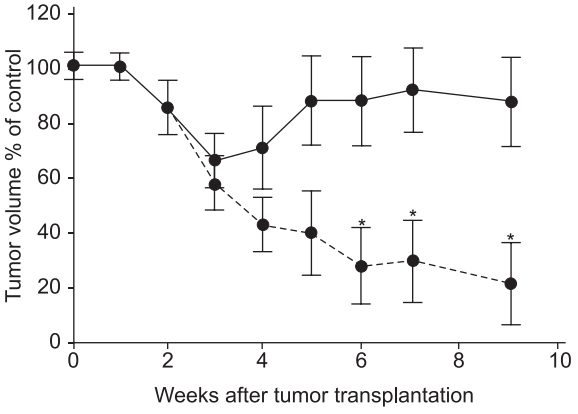

Fig. 10. The effects of GnRH-III (4 mg/kg; solid line) and P-X-GnRH-III (4 mg of conjugated peptide/kg; broken line) on the volume of MDA-MB-231 xenografts.

mechanism of direct action has not been elucidated (38, 39), the biodegradable or nonbiodegradable carrier molecule might contribute to the toxicity of GnRH analog conjugates. The accumulation of cells in the G_2/M phase reflects the inhibition of cdc25 phosphatase by P-X-peptide conjugates; the free peptides were not effective. From the IC_{50} values obtained for the copolymer incorporated in the conjugates it is clear that the peptides contribute to the inhibitory property of the conjugates. The observation (M. Kovács and J. Horváth, personal communication) that the endocrine activity of GnRH analogs in the pituitary was further diminished by conjugation with either AcEAK or P-X indicates an increased selectivity for direct action of peptide conjugates on tumor cells. The potential therapeutic efficacy of the conjugates is based on their specificity in inducing direct antiproliferative effects on cancer cells as compared to the free peptides and not on their interactions at the level of the pituitary.

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