Tumor-specific targeting of an anticancer drug delivery system by LHRH peptide

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The central problem in cancer chemotherapy is the severe toxic side effects of anticancer drugs on healthy tissues. Invariably the side effects impose dose reduction, treatment delay, or discontinuance of therapy. To limit the adverse side effects of cancer chemotherapy on healthy organs, we proposed a drug delivery system (DDS) with specific targeting ligands for cancer cells. The proposed DDS minimizes the uptake of the drug by normal cells and enhances the influx and retention of the drug in cancer cells. This delivery system includes three main components: (i) an apoptosis-inducing agent (anticancer drug), (ii) a targeting moiety–penetration enhancer, and (iii) a carrier. We describe one of the variants of such a system, which utilizes camptothecin as an apoptosis-inducing agent and poly(ethylene glycol) as a carrier. Luteinizing hormone-releasing hormone (LHRH) was used as a targeting moiety (ligand) to LHRH receptors that are overexpressed in the plasma membrane of several types of cancer cells and are not expressed detectably in normal visceral organs. The results showed that the use of LHRH peptide as a targeting moiety in the anticancer DDS substantially enhanced the efficacy of chemotherapy, led to amplified apoptosis induction in the tumor, and minimized the side effects of the anticancer drug on healthy organs. The LHRH receptor targeting DDS did not show *in vivo* pituitary toxicity and did not significantly influence the time course or the plasma concentration of luteinizing hormone and its physiological effects on the reproductive functions of mice.

adverse side effects | apoptosis | cancer | targeted drug delivery

The efficacy of cancer chemotherapy is limited by severe adverse side effects induced by anticancer drugs (1–4). The cytotoxic effect on healthy organs can be significantly diminished by employing special drug delivery systems (DDS) targeted specifically to cancer cells (5, 6). Targeting is especially important in circumstances where a localized tumor is removed surgically, and chemotherapy is prescribed as a follow-up preventive against potential metastases.

Cancer targeting is usually achieved by adding to the DDS a ligand moiety specifically directed to certain types of binding sites on cancer cells. Several different targeting moieties were examined, including sugars (7–11), lectins (12–14), receptor ligands (5, 15–18), and antibodies (19–23) and their fragments (24). Recently, we found that the receptors for luteinizing hormone-releasing hormone (LHRH) are overexpressed in breast, ovarian, and prostate cancer cells (5, 15, 25). LHRH receptors (LHRHRs) are not expressed detectably in most visceral organs. We have taken advantage of this differential receptor expression and used a modified LHRH peptide as a targeting moiety on DDS to enhance drug uptake by the mentioned cancer cells and reduce the relative availability of the toxic drug to normal cells. We constructed and evaluated *in vitro* targeted DDS, which included (i) poly(ethylene glycol) (PEG) polymer as a carrier; (ii) camptothecin (CPT) as an anticancer drug; and (iii) modified LHRH peptide as a targeting moiety (5, 15). *In vitro* evaluations confirmed the high anticancer activity of such conjugates against human ovarian, breast, and prostate

cancer cells (15). Further, it was demonstrated that the cytotoxicity of the LHRH-targeted conjugates in human cancer cells was competitively inhibited by free LHRH peptide (25). The present investigations were aimed at evaluating the antitumor activity and apoptosis-induction capacity of the conjugates in experiments on mice bearing xenografts of human ovarian carcinoma. Tumor and organ distribution profiles of the targeted and nontargeted conjugates were also determined in these mice for cautionary physiological reasons. In normal physiological conditions, LHRH is synthesized in the arcuate nucleus in the hypothalamus. It is secreted in the median eminence of the pituitary, where it enters the capillary plexus and is transferred via the long portal vessel to the gonadotrophic cells of the adenohypophysis. The latter are stimulated by LHRH to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both of which are transferred in the systemic circulation and are essential for the regulation of normal reproductive functions in most mammals, including mice and humans (26). Therefore, it was essential to ascertain whether the drug conjugates with LHRH, when administered into the systemic circulation, could breach the blood–brain and pituitary barriers to target and intoxicate the gonadotrophic cells, affect normal LH/FSH secretion, and result in reproductive malfunctions. Tritium-labeled conjugates, with and without LHRH, were used to determine the distribution profile *in vivo*, and in separate experiments, the reproductive functions of treated and control mice were compared.

Materials and Methods

Synthesis of Conjugates. Camptothecin (CPT) was obtained from Sigma. PEG (MW $\approx$ 5,000) was obtained from Shearwater Polymers (Huntsville, AL). LHRH peptides were synthesized according to our design by American Peptide (Sunnyvale, CA). The sequence of native LHRH peptide, which is similar in human, mouse, and rat, was modified to provide a reactive amino group only on the side chain of a lysine residue, which replaced Gly at position 6 to yield the superactive, degradation-resistant-Lys-6-des-Gly-10-Pro-9-ethylamide LHRH analog (27). The modified sequence of the peptide is presented in Fig. 1. All other chemicals were purchased from Sigma or Fisher Scientific and used as received. The conjugates used in this study were synthesized by using a two-step procedure modified from refs. 28 and 29, as described in ref. 15. Three conjugates were synthesized: nontargeted CTP-PEG, tumor-targeted PEG-LHRH, and CPT-PEG-LHRH. The structures of CPT and CPT-PEG-LHRH conjugate are shown in Fig. 1. CPT was conjugated to

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Abbreviations: DDS, drug delivery system; LH, luteinizing hormone; LHRH, luteinizing hormone-releasing hormone; LHRHR, luteinizing hormone-releasing hormone receptor; CPT, camptothecin; PEG, poly(ethylene glycol); β_2 -m, β_2 -microglobulin.

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Fig. 1. Sequence of the modified LHRH and CPT and LHRH-PEG-CPT conjugate.

cysteine via a biodegradable ester bond to provide intracellular release by hydrolysis and then to PEG by a thioether bond. CPT, CPT-PEG, and CPT-PEG-LHRH conjugates were used to study antitumor activity, gene expression, and apoptosis induction. To study potential pituitary toxicity and reproductive function, we used CPT-PEG-LHRH conjugate because only LHRH targeted CPT-PEG conjugate could potentially be toxic to the pituitary gland and influence reproduction. To study tumor and organ distribution when comparing targeted and nontargeted polymers, we used tritium-labeled PEG and PEG-LHRH. The structural limitation of linear PEG polymer allows us to attach only two components (e.g., LHRH and tritium) simultaneously. Therefore, we did not use CPT-PEG-LHRH conjugate in the distribution study.

Cell Line. The human ovarian carcinoma A2780 cell line was obtained from T. C. Hamilton (Fox Chase Cancer Center, Philadelphia). The human MCF-7 breast and PC-3 prostate cancer cells were purchased from American Type Culture Collection. Cells were cultured in RPMI medium 1640 (Sigma) supplemented with 10% FBS (HyClone). Cells were grown at 37°C in a humidified atmosphere of 5% CO₂ (vol/vol) in air. All experiments were performed on cells in the exponential growth phase.

Animal Tumor Model. A previously developed animal model of human ovarian carcinoma xenografts was used as described in refs. 30 and 31. Briefly, A2780 human ovarian cancer cells (2×10^6) were s.c. transplanted into the flanks of female athymic nude (nu/nu) mice. When the tumors reached a size of ≈ 1 cm³ (15–20 days after transplantation), mice were injected i.p. with PEG, PEG-CPT, PEG-CPT-LHRH conjugates, or saline. The dose of all substances (10 mg/kg for the single injection) corresponded to the maximum tolerated dose of CPT-PEG. The maximum tolerated dose was estimated in separate experiments based on animal weight changes after the injection of increasing doses of the drugs as described in refs. 30 and 31. Tumor size was measured at 6, 12, 18, 24, 36, 48, 72, and 96 h after the treatment. Measurements of tumor size showed that the maximum antitumor activity of CPT-PEG-LHRH conjugate was reached at ≈ 24 h after the treatment when the measured tumor size was at minimum. Therefore, all other parameters were measured at this time point.

Antitumor Activity. Changes in tumor size were used as an overall mark of antitumor activity as described in refs. 30 and 31. Tumor

size was determined at 6, 12, 18, 24, 36, 48, 72, and 96 h after the treatment of mice.

Organ Distribution of PEG and LHRH-PEG. Aliquots of PEG and LHRH-PEG conjugates were radiolabeled with tritium. Radio-labeling was done on LHRH-PEG-NH₂ or PEG-NH₂ with ³H-acetic anhydride in the presence of 1% diisopropylethyl amine and methanol to get LHRH-PEG-NH-CO-CH₃ [³H] and PEG-NH-CO-CH₃ [³H] conjugates, respectively. Tumor and organ (liver, kidney, spleen, heart, lung, brain, and pituitary) distribution of radiolabeled conjugates was studied in nude (nu/nu) mice. Twelve mice were used in this experiment. Six mice were used as a control, whereas the xenografts of human ovarian cancer were transplanted in the rest. When the tumors reached a size of ≈ 1 cm³ (15–20 days after inoculation), mice were treated i.p. with 10 mg/kg desired conjugates. The maximum tolerated dose was detected in separate experiments. Mice without tumor received the same dose of conjugates.

Apoptosis. Two approaches were used to assess apoptosis induction. The first approach was based on the measurement of the enrichment of histone-associated DNA fragments (mono- and oligonucleosomes) in homogenates of the tumor and different organs (liver, kidney, spleen, heart, lung, brain, and ovary) by using anti-histone and anti-DNA antibodies by a cell death detection ELISA^{PLUS} kit (Roche Applied Biosciences) as described in refs. 30 and 32–34. The second approach was based on the detection of single- and double-stranded DNA breaks (nicks) by an *in situ* cell death detection kit (Roche Applied Biosciences) by using terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) method as described in refs. 30 and 32.

Gene Expression. Quantitative RT-PCR was used for the analysis of cDNA and cell and tissue homogenates of expression of genes encoding LHRHRs (*LHRHR*), caspases 3 (*CASP3*), caspase 9 (*CASP9*), and β_2 -microglobulin (β_2 -m) as described in refs. 30 and 32. RNA was isolated by using an RNeasy kit (Qiagen, Valencia, CA). The following pairs of primers were used: *LHRHR*, GAC CTT GTC TGG AAA GAT CC (sense), CAG GCT GAT CAC CAC CAT CA (antisense); *CASP3*, TGG AAT TGA TGC GTG ATG TT (sense), GGC AGG CCT GAA TAA TGA AA (antisense); *CASP9*, TGA CTG CCA AGA AAA TGG TG (sense), CAG CTG GTC CCA TTG AAG AT (antisense); and β_2 -m, ACC CCC ACT GAA AAA GAT GA (sense), ATC TTC AAA CCT CCA TGA TG (antisense). Gene expression was calculated as the ratio of analyzed RT-PCR product to the internal standard (β_2 -m). The expression of *LHRHR* gene was measured in human ovarian, breast, and prostate cancer cells and different healthy visceral human organs (liver, kidney, spleen, heart, lung, brain, thymus, and skeletal muscle). Normal human ovarian tissue and tumor tissue from the

Fig. 2. Expression of genes encoding LHRHRs in ovarian (lane 1), breast (lane 2), prostate (lane 3) human cancer cells; healthy human ovary (lane 4); human ovarian tumor from the same ovary from the patient (lane 5); and in healthy human organs [liver (lane 6), kidney (lane 7), spleen (lane 8), heart (lane 9), lung (lane 10), brain (lane 11), thymus (lane 12), and skeletal muscles (lane 13)]. β_2 -m was used as an internal standard.

Fig. 3. Time course of growth of human ovarian tumor xenografts in mice as depicted by relative tumor size recorded from 6 to 96 h after treatment with saline (●), CPT (■), CPT-PEG (□), and CPT-PEG-LHRH (○). Means $\pm$ SD from four to five independent measurements are shown. *, $P < 0.05$ when compared with tumor treated with saline; †, $P < 0.05$ when compared with tumor treated with CPT; ‡, $P < 0.05$ when compared with tumor treated with CPT-PEG.

same ovary of the patient were also included. To measure the expression of this gene by PCR we used mRNA isolated from cells and cDNA isolated from human organs that was purchased from Clontech.

LH and Reproductive Functions. To further assess for potential pituitary toxicity, we measured serum levels of the LH and reproductive capacity of female mice treated four times (every other day) within 2 weeks with CPT-PEG-LHRH polymer at the maximum tolerated dose level. The maximum tolerated dose of this conjugate was estimated in preliminary experiments based on animal weight changes after the injection of increasing doses of drugs as described in refs. 30 and 31. We found that this dose was equal to 2.5 mg/kg for a four-times treatment sequence. The serum LH concentration was measured four times (once per day) during the average mouse estrus cycle (4 days) by using the LH ELISA kit (Anogen, Mississauga, ON, Canada). To estimate the

influence of the studied targeted CPT-PEG-LHRH conjugate on mice reproductive function, eight female C57BL/6J (The Jackson Laboratory) wild-type mice were treated four times with a maximum tolerated dose of CPT-PEG-LHRH conjugate. After the treatment, mice were placed in one cage with male mice (similarly to control mice). Mice and their offspring were closely monitored for weight changes and behavior during the following 4 weeks.

Results and Discussion

Expression of Targeting Receptors to the LHRH Peptide. Previously, we have found that LHRHRs are overexpressed in several types of human cancer cells including ovarian, breast, and prostate cancers (5, 15, 25). At the same time, these receptors were not detectably expressed in healthy human visceral organs. Similar results were also obtained by other authors (35, 36). This differential expression allowed us to select the LHRH peptide and LHRHR as a targeting moiety and target, respectively, to direct an anticancer drug specifically to cancer cells. A search of the SWISS-PROT database by using the National Center for Biotechnology Information BLAST program (www.ncbi.nlm.nih.gov/blast/) showed that the sequences of this protein are identical in human and mouse. This finding allowed us to use the mouse model in assessing the targeting and toxicity of the proposed LHRH-containing DDS (Fig. 1). The measurement of the expression of genes encoding LHRHRs showed that LHRHRs were overexpressed in cancer cells (Fig. 2). However, these receptors were not expressed detectably in the following healthy human organs: liver, kidney, spleen, heart, lung, brain, thymus, and skeletal muscle. The measurement of LHRHR expression in normal human ovarian tissue and tumor tissue from the same ovary of the patient showed that this receptor was overexpressed in tumor tissue and substantially less expressed in normal ovarian tissue (Fig. 2, lane 4 vs. lane 5). These results demonstrate that the choice of LHRH peptide as a targeting moiety will permit us to selectively direct an anticancer drug specifically to the tumor and, therefore, prevent damage to normal tissues.

Antitumor Activity. To evaluate the antitumor activity of targeted (CPT-PEG-LHRH) and nontargeted (CPT-PEG) conjugates, we treated mice bearing xenografts of human ovarian carcinoma with these conjugates and CPT alone. Control mice received equivalent doses of saline. Tumor size was measured, and its

Fig. 4. Distribution of tritium-labeled PEG and LHRH-PEG conjugates in different tissues of mice without tumor and mice bearing xenografts of A2780 human ovarian carcinoma. Radioactivity is expressed in disintegrations per min (dpm) per g of tissue weight. Means $\pm$ SD from four independent measurements are shown.

Fig. 5. Effect of treatment with saline (1), CPT (2), CPT-PEG (3), and CPT-PEG-LHRH (4) on the expression of the genes encoding caspases 9 (CASP9) and 3 (CASP3) in tumor of mice bearing xenografts of A2780 human ovarian carcinoma. Means $\pm$ SD from four to five independent measurements are shown. *, $P < 0.05$ when compared with tumor treated with saline; †, $P < 0.05$ when compared with tumor treated with CPT; ‡, $P < 0.05$ when compared with tumor treated with CPT-PEG.

relative decrease after the treatment was taken as an indicator of antitumor activity. It was found that conjugation of CPT to PEG led to a significant ($P < 0.05$ at all time points) increase in the antitumor activity of CPT (Fig. 3, plots 2 and 3). Further enhancement was achieved by conjugating CPT-PEG to LHRH peptide as the targeting moiety. Treatment of mice with the targeted CPT-PEG-LHRH conjugate significantly ($P < 0.05$) decreased tumor size (Fig. 3, plot 4). This decrease leveled off 24 h after the treatment. After this time point, tumor size in animals treated with CPT-PEG-LHRH conjugate did not change significantly up to the end of the studied period (96 h). Therefore, targeting of anticancer DDS to ovarian tumor by LHRH peptide substantially enhanced anticancer activity of CPT.

Distribution Profile of Targeted and Nontargeted DDS. To further confirm the specificity of the targeting mechanism and assess its potential involvement in pituitary toxicity, the distribution profiles of tritium-labeled PEG and LHRH-PEG in the tumor and organs of nude mice were determined. The results are shown in Fig. 4. In mice without tumor, nontargeted PEG polymer (bar 1) accumulated predominantly in the liver and at a lower level in the other organs (heart, lung, kidney, and spleen). Only a trace amount of this polymer was found in the brain and the pituitary, indicating that the polymer does not breach the blood–brain and pituitary barriers. The distribution of targeted LHRH-PEG conjugate in control mice was slightly different from the nontargeted PEG polymer for most of the

tissues. There is an appreciable shift from the liver to the ovary with the liver showing lowered accumulation and the ovary showing high accumulation because of the endogenous LHRHR expression in ovaries (Fig. 4, bars 2 and 4). The distribution of both polymers was different in some of the tissues of mice bearing the xenografts of human ovarian carcinoma. Bars 3 and 4 show that both polymers have a low level of accumulation in normal tissues, except for the liver (bar 3) and ovary (bar 4) again demonstrating the shift from the liver to ovary where LHRHRs are expressed (see also Fig. 2). Our previous studies showed that, in contrast to low-molecular-weight drugs, water-soluble polymers are accumulated preferentially in solid tumors (30, 31). This effect is due to the relatively reduced lymphatic clearance of macromolecules from tumors and is called the “enhanced permeability and retention (EPR) effect” (37). EPR functions as a form of “passive” targeting. A consequence of the increase in tumor accumulation of macromolecular DDS is its reduced availability to healthy organs, thereby diminishing adverse side effects. It is important to note the affirmation of the fact that LHRH-PEG polymer does not breach the brain and pituitary barriers from the systemic circulation. The lowest levels recorded (Fig. 4) were in the brain and pituitary, despite the available LHRHRs in the latter. This phenomenon can also be explained by the competitive binding of free physiological endogenous LHRH peptide to pituitary LHRHRs. We previously have shown that such a competition significantly limits the cellular internalization of targeted conjugate (25). Nevertheless, the greater weight of the evidence supports the unbreachable barrier interpretation because tritiated PEG alone was also extremely low in the brain and the pituitary tissues (Fig. 4, bars 1 and 3). The most dramatic accumulation is seen in the case of targeted LHRH-PEG in the tumor (bar 4). The level of accumulation is nearly twice that of PEG alone in the tumor and at least four times higher than that in other tissues. Previously, we have demonstrated that cytotoxicity of LHRH-targeted conjugates in human ovarian cancer cells is competitively inhibited by free LHRH peptide (25). Such competitive inhibition shows that the enhanced tumor accumulation is indeed due to the LHRH peptide/LHRHR binding phenomenon. These data also show that active tumor targeting is more effective when compared with passive targeting of macromolecular drug by the EPR phenomenon. Therefore, the use of LHRH peptide as a targeting moiety in DDS for treatment of tumors, which overexpress LHRHRs, can enhance the specific penetration and cytotoxic action of the anticancer drug to the tumor. Under such circumstances,

Fig. 6. Apoptosis induction after treatment with saline (control), CPT, CPT-PEG, and CPT-PEG-LHRH in tumor and different tissues in mice bearing xenografts of A2780 human ovarian carcinoma. Means $\pm$ SD from four to five independent measurements are shown. *, $P < 0.05$ when compared with tumor treated with saline; †, $P < 0.05$ when compared with tumor treated with CPT; ‡, $P < 0.05$ when compared with tumor treated with CPT-PEG.

Fig. 7. Typical fluorescence microscopy images of tissue slides labeled by TUNEL after treatment of mice bearing xenografts of A2780 human ovarian carcinoma with saline (control), CPT, CPT-PEG, and CPT-PEG-LHRH.

anticancer drugs can be administered at low doses, and by virtue of that, adverse side effects are avoided while treatment efficacy is enhanced.

Induction of Cell Death Signal. To compare the cell death signal triggered by the CPT, CPT-PEG, and CPT-PEG-LHRH, we measured the expression of genes encoding caspases that initiate (caspase 9, CASP9) and execute (caspase 3, CASP3) apoptosis. The results are presented in Fig. 5. Analysis showed that CPT, CPT-PEG, and CPT-PEG-LHRH conjugates induced apoptotic signal by overexpression of caspases 9 and 3 (Fig. 5). Conjugation of CPT to PEG substantially enhanced its ability to induce apoptotic signal (Fig. 5, compare bars 2 and 3). Conjugation of LHRH peptide as a tumor targeting moiety to the DDS significantly induced the expression of caspases (CPT-PEG-LHRH). Therefore, targeting of anticancer DDS to tumor by the LHRH peptide substantially augmented the ability of CPT to induce apoptotic signal when compared with both CPT alone and nontargeted CPT-PEG conjugate. This phenomenon can be explained by (i) an increase in the concentration of CPT in the tumor tissues and (ii) facilitation of the uptake of whole DDS by receptor-mediated endocytosis (5) after the interaction of LHRH peptide (ligand) with the corresponding receptors overexpressed in certain cancer cells.

Apoptosis Induction. The analysis of apoptosis induction in the tumor and the healthy organs confirmed the effectiveness of the tumor-targeting approach in comparison with the drug

Fig. 8. Serum concentration of LH in mice bearing xenografts of A2780 human ovarian carcinoma after treatment (four times) with the maximum tolerated dose of CPT-PEG-LHRH conjugate and in control mice treated with saline. Means $\pm$ SD from five independent measurements are shown.

alone and nontargeted CPT-PEG conjugate (Figs. 6 and 7). The direct measurement of apoptosis induction in the tumor and the healthy organs showed that passive targeting because of the enhanced permeability and retention effect (after the conjugation of low molecular weight CPT with high molecular-weight PEG polymer) led to a decrease in the induction of apoptosis in healthy organs and enhanced the apoptosis induction in the tumor. However, "active" tumor targeting by LHRH peptide dramatically increased apoptosis induction in the tumor and significantly prevented adverse side effects on healthy tissues (Fig. 6). Although targeted DDS induced apoptosis in ovaries more significantly than nontargeted conjugate, this induction was substantially lower when compared with apoptosis induction in tumor. As was shown in *Expression of Targeting Receptors to the LHRH Peptide*, the expression of LHRHRs in normal ovary was substantially lower than in ovarian tumor (see Fig. 2). Therefore, the level of apoptosis in normal ovary and ovarian tumor correlates with the level of the expression of LHRHRs in these tissues. Microscopic analysis of the TUNEL-labeled tissue samples (Fig. 7) supports this conclusion. It is important to note that the positive effects of using LHRH as a targeting moiety were observed by two independent and substantially different methods. These data support the view that targeting of DDS to the tumor by the LHRH peptide is practical and can enhance the antitumor activity of an anticancer drug and prevent its adverse side effects on healthy tissues.

LH and Reproductive Functions. Because LHRHRs are expressed in the pituitary gland, theoretically there is a possibility that DDS targeted to these receptors could be toxic to the pituitary gonadotrophic cells discussed in the introduction. The following *in vivo* experiments were performed to assess this potential toxicity to the pituitary and brain. First, we showed that only trace amounts of targeted LHRH-PEG conjugate accumulated in the brain and pituitary (Fig. 4). In addition, targeted DDS did not induce cell death in the brain tissues (Fig. 5). To further assess for any damage to the secretion of the LH by the pituitary gonadotrophic cells, we measured the serum levels of LH and the

Table 1. Reproductive function of wild-type C57BL/6J female mice (without tumor) after four treatments with saline (control) or CPT-PEG-LHRH conjugate

Series	No. of offspring per mouse								Mean	SD	<i>P</i> (control)
	M1	M2	M3	M4	M5	M6	M7	M8			
Control	7	5	6	8	6	4	7	7	6.25	1.28	
CPT-PEG-LHRH	4	8	7	6	6	5	6	7	6.13	1.25	>0.05

Mn. mouse no.

reproductive capacity of female mice. These mice were treated four times (every other day) within a 2-week period with CPT-PEG-LHRH conjugate at the maximum tolerated dose. We found that this dose was equal to 2.5 mg/kg for a four-times treatment sequence. Serum LH concentration was measured four times (once per day) during the average mouse estrus cycle (4 days). The treatment did not significantly change the profile of LH release (Fig. 8). In another series of experiments, eight wild-type female C57BL/6J mice were treated four times with a maximum tolerated dose of CPT-PEG-LHRH conjugate. After the treatment, female mice were placed in one cage with male mice (similarly to control mice). After 3–4 weeks, all female mice had healthy viable offspring. The number of offspring per mouse (4–8) and their viability, weight change, and behavior during the following 4 weeks were not different from the offspring of the

control mice (Table 1). Taken together, these data indicate that targeted conjugates of PEG polymer and anticancer drug CPT do not breach the pituitary and blood–brain barriers from the systemic circulation (Fig. 4), and the normal reproductive and behavior-related functions of these organs were not detectably impaired.

Summarizing the results of the experiments, one can conclude that the use of the LHRH peptide to target a polymeric anticancer DDS substantially limits adverse side effects on healthy tissues and significantly enhances the antitumor efficacy of the anticancer drug. Therefore, the proposed tumor targeted DDS can be used in the chemotherapy of LHRH-positive tumors.

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