Published in final edited form as:

Reprod Toxicol. 2012 August ; 34(1): 120–124. doi:10.1016/j.reprotox.2012.04.005.

Reproductive Effects of a Pegylated Curcumin

Caitlin J. Murphy, Huadong Tang, Edward A. Van Kirk, Youqing Shen, and William J. Murdoch*

Reproductive Biology Program and Soft Materials Laboratory, University of Wyoming, Laramie, USA

Abstract

Curcumin, a polyphenol derived from the rhizome turmeric, has potential as an anticancer agent. We synthesized an amphipathic/surfactant pegylated curcumin (curcumin-PEG) designed for parenteral administration. Objectives of these investigations were to assess side-effects of a therapeutic regimen of curcumin-PEG in a preclinical model. Intraperitoneal (ip) tumor burdens were reduced in athymic female mice grafted with human SKOV-3 ovarian adenocarcinoma cells and injected intravenously (iv) with curcumin-PEG. There were no gross anatomical or histopathological effects detected in non-reproductive organs. Uteri (luminal fluid imbibition) and ovaries (decreased folliculogenesis) were affected by treatment. Curcumin-PEG ip hastened the onset of puberty in immature female mice. Live births were reduced in mature females housed with males and treated iv with curcumin-PEG; mating (vaginal plugs) was not affected. Accessory gland weights, testicular testosterone concentrations, and spermatogenesis were diminished in mature male mice following iv curcumin-PEG. Estrogenic/antiandrogenic and pregnancy-disrupting effects of a water soluble/bioavailable curcumin were demonstrated.

Keywords

curcumin; polyethylene glycol; cancer; reproduction; mouse

1. Introduction

Curcumin is a polyphenolic yellow pigment of the curry spice turmeric (*Curcuma longa*). Curcumin is effective in vitro against ovarian, breast, cervical, prostate, colon, liver, pancreatic, and bone cancer cells. Anticancer modes of curcumin action have been attributed to cell-cycle arrest and programmed death [1–3]. It appears that curcumin is pharmacologically safe. Notwithstanding, poor gastrointestinal absorption limits plasma curcumin levels and pre/clinical utilities for curcumin have been hampered because it is not readily dispersible in aqueous injection vehicles [4].

Polyethylene glycol (PEG) is a hydrodynamic biocompatible polymer. Conjugation to PEG is a prospective technology for improving the compliancy of lipophilic drugs [5]. We have produced a water soluble curcumin using end-ring thioester linkages to low molecular weight PEG chains (curcumin-PEG; Fig. 1). The conjugate retained inhibitory effects of the

© 2012 Elsevier Inc. All rights reserved.

*Correspondence: wmurdoch@uwyo.edu; 307-766-3293; fax 307-766-2355; Department of Animal Science, 1000 East University Avenue, Laramie, WY, 82071, USA.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

parent curcumin toward human ovarian and breast cancer cell lines and xenografts [6]. Carrier/PEG alone does not produce anticancer or untoward in vivo effects [6,7].

Curcumin can affect a multiplicity of cellular signaling/transduction pathways [1–3]. Therefore, it is reasonable to hypothesize that the curcumin component of curcumin-PEG could cause unintended non-target actions. The purpose of investigations reported herein was to elucidate potential side-reactions of an antitumor dose of curcumin-PEG. Mice were used as the experimental model. Effects on the reproductive systems of females and males were observed.

2. Materials and Methods

Reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless indicated otherwise.

2.1 Pegylated curcumin

Synthesis, molecular characterizations, and pharmacodynamics of curcumin-PEG were described in detail previously [6]. Briefly, PEG454 methyl ether acrylate (9 g), 3-mercaptopropionic acid (2.3 g), and triethylamine (0.1 ml) were mixed in anhydrous tetrahydrofuran (100 ml) and stirred at 22° C for 24 h. PEG oligomer was precipitated with excess anhydrous ether. Oligomer (5.6 g), N, N'-dicyclohexylcarbodiimide (2.2 g), curcumin (1.7 g), and 4-dimethylaminopyridine (0.1 g) were dissolved in tetrahydrofuran (50 ml) and stirred at 22° C for 24 h. Filtered product was precipitated into an excess of anhydrous ether and dried under vacuum at 22° C. Purity (> 99%) was confirmed by high performance liquid chromatography and nuclear magnetic resonance.

Curcumin-PEG for in vivo delivery was sterilized by filtration (22 µm Micron Separations Inc., Westboro, MA). Plasma curcumin concentrations in mice following an intravenous (iv) injection (0.5 mg in 0.1 ml phosphate-buffered saline [PBS] vehicle, tail vein) declined from approximately 7 µg/ml at 5 min to < 1 µg/ml at 2 h. In vivo curcumin is hydrolyzed from its PEG chains [6].

2.2 Animals

After effects of curcumin-PEG on the reproductive system in immunodeficient tumor-bearing mice were noted, further experiments were then conducted in same-strain wild-type/immunocompetent animals (i.e., more analogous to a typical cancer patient). BALB/c strain nu/nu (first experiment) and BALB/c (subsequent experiments) mice were maintained in a pathogen-free environment under controlled temperature (24° C) and lighting (12L:12D) conditions. Sterilized rodent chow and water were supplied ad libitum. Mice were killed for tissue collections by barbiturate overdose. Protocols were approved by the University of Wyoming Animal Care and Use Committee.

2.3 Effects of curcumin-PEG in nude mice bearing intraperitoneal (ip) ovarian tumors

Human SKOV-3 epithelial ovarian cancer cells were obtained from American Type Culture Collection (ATCC, Rockville, MD). Cells were propagated in T-75 flasks at 37° C under an atmosphere of 5% CO₂ in 15 ml RPMI-1640 supplemented with 10% charcoal-stripped/heat-inactivated fetal calf serum, 10 µg/ml insulin, and 1% antibiotic/antimycotic solution (A 9909). Exponentially-growing cells were harvested using 0.25% trypsin/0.03% EDTA.

SKOV-3 cells (5 X 10⁶ suspended in 2.0 ml PBS) were injected into the abdominal cavity of mature females. Curcumin-PEG (0.5 mg) or PBS (0.1 ml) was administered via the tail vein (0.1 ml Hamilton syringe, 30-gauge needle) at five weeks post-inoculation (when tumors

along the mesentery are well established; [8]). Treatments were given four times at 48 h intervals. Six and five mice were included in the curcumin-PEG and control groups, respectively. Animal weights were recorded at necropsy 48 h after the last treatment. Tumors were dissected and cumulative per mouse tumor weights and numbers were determined. Upon dissection it was noted that uteri of animals treated with curcumin-PEG were hyperemic and enlarged; uterine tissue weights were recorded.

Liver, kidneys, spleen, heart, brain, lung, intestines, skeletal muscle, and ovary were processed for light microscopic examination. Tissues were fixed by 48 h immersion in Histochoice (Amresco, Solon, OH), washed in PBS, dehydrated in a graded series of ethanol, cleared in xylene, infiltrated with and embedded in paraffin, and serially-sectioned (6 μ m thickness). Sections were transferred from a water bath (50°C) onto microscope slides, air-dried, deparaffinized in xylene, rehydrated, stained in hematoxylin and eosin, dehydrated, placed in xylene, and coverslipped with mounting medium.

2.4 Effect of curcumin-PEG on onset of puberty

Immature 15-day-old female mice were treated with ip curcumin-PEG (0.5 mg/0.1 ml PBS, n = 18), PBS (0.1 ml, n = 18), or estradiol-17 β (0.2 mg/0.1 ml corn oil, n = 17) for five consecutive days. Vaginal opening was monitored daily until mice were 21-days-old when uteri were dissected and weighed.

2.5 Effects of curcumin-PEG on mating and pregnancy

Mature (five-month-old) primiparous female mice were housed with one proven breeder male and received a daily iv injection of curcumin-PEG (0.5 mg) or PBS (0.1 ml) over a period of twenty days (n = 21 per group). Every five days males were rotated to a different group of females. Vaginal plugs were checked daily to assess mating. Treatments and exposures to males were continued for twenty days regardless of when mating was detected. After twenty days, males were removed and pregnant females were allowed to deliver at term. Litter sizes and sexes were recorded. Additional pairings were conducted to determine if curcumin-PEG had a carry-over effect on previously treated mothers (F0) or pubertal F1 generation pups.

2.6 Effects of curcumin-PEG on male reproductive organs

Mature male mice were treated iv daily with curcumin-PEG (0.5 mg, n = 10) or PBS (0.1 ml, n = 8) for ten days. Body weights were recorded. Seminal vesicles and testes were dissected and weighed 48 h after the last treatment. One testis was processed for light microscopic examination and the other placed in methanol (2 ml) and frozen (–20°C) for testosterone extraction.

An aliquot of methanol (0.2 ml) from each testis (testosterone extraction efficiency = 90%) was dried and resuspended in 1 ml of PBS-gelatin. Testosterone was measured (0.1 ml duplicates) using a specific radioimmunoassay kit according to the instructions of the manufacturer (TKTT1; Siemens USA). The assay was sensitive to 1 pg (100% accuracy) and had a coefficient of variation of 9.5%.

2.7 Statistics

Assignments to treatments and selections of microscopic fields were made at random. Treatment comparisons were made by Student's t-test or Chi-square analysis. Contrasts were considered different at $p < 0.05$ (significant) and $p = 0.1$ (trend). Data are presented as means $\pm$ standard errors (SE).

3. Results

3.1 Antitumor and uterine/ovarian effects

No overt behavioral signs of toxicity (e.g., loss of appetite, lethargy) were observed. Tumors were found in all mice at necropsy. Tumor loads were reduced by curcumin-PEG (Fig. 2) (total weights for controls and curcumin-PEG = 2.4 ± 0.9 vs. 0.6 ± 0.2 g; total numbers for controls and curcumin-PEG = 55 ± 18 vs. 22 ± 7). Body weights were not significantly different for treatment (21.7 ± 2.1 g) and control (25.2 ± 2.1 g) groups.

Uterine hyperemia and luminal fluid imbibition were conspicuous in mice treated with curcumin-PEG. There was a corresponding tendency for an increase in uterine weights (Fig. 3a). These changes are indicators of estrogenic actions [9].

Antral follicles and corpora lutea were counted within a midsagittal section through an ovary of each animal. Populations of follicles were reduced in the curcumin-PEG group (Fig. 3b). Luteal structures were similar between the curcumin-PEG (1.8 ± 0.58) and control (2.0 ± 0.68) group.

There were no gross anatomical or microscopic alterations detected in non-reproductive organs/tissues of curcumin-PEG vs. control mice.

3.2 Puberty

Onset of puberty/vaginal opening (an estrogenic response [10]) was hastened in mice treated with curcumin-PEG. There was no difference between curcumin-PEG and the (positive) estradiol control. A stimulatory effect of curcumin-PEG on uteri of nu/nu mice also was observed in immunocompetent animals. Uterine weights of curcumin-PEG mice were intermediate between the control and estradiol groups (Fig. 4).

3.3 Mating/Pregnancy

There was no effect of curcumin-PEG on incidences of copulations (vaginal plugs) or sex ratios of the litters. However, numbers of live births per dam were reduced ($p < 0.05$) in the curcumin-PEG (5.9 ± 0.72) compared to the control (7.4 ± 0.43) group. The negative effect on pregnancy outcome was temporary; prolificacies after rebreeding of F0 females or after breeding the F1 generation offspring (females and males) were unaffected.

3.4 Male

Body weights were not affected by treatments (27.0 ± 0.8 g control vs. 26.5 ± 0.6 g curcumin-PEG). Seminal vesicle weights were reduced by curcumin-PEG. Testes weights were not altered. However, it appeared that spermatogenic function was disrupted (dissolution of sperm-epithelial cell associations) in a greater proportion of sections of seminiferous tubules of curcumin-PEG treated than control animals. Testicular testosterone tended to be reduced by curcumin-PEG (Fig. 5).

4. Discussion

Results of our first experiment substantiated a regressive effect of curcumin-PEG against ovarian tumors in an immunodeficient mouse model. Ovarian cancer is a deadly insidious disease because it typically remains asymptomatic until it has metastasized beyond the ovaries into the abdominal cavity. Cytoreductive surgery and (toxic) platinum/taxane-based chemotherapy are the mainstays of therapy. Progress in overcoming drug resistance and reducing numbers of mortalities has been painstakingly slow. New treatment options are desperately needed [11, 12].

Curcumin-PEG is a promising new bioavailable agent. Curcumin inhibited proliferation and induced apoptosis in cisplatin-resistant ovarian cancer cell lines [13–16]. Curcumin targets dividing cells by inhibiting genes of cell-cycle progression and survival – preludes to apoptosis and autophagy. Other putative cancer-combating functions of curcumin have been related to its antiinflammatory, antioxidant, and antiangiogenic properties [1–3].

Dietary consumption of curcumin, producing low circulatory concentrations, is not toxic; these results, however, should be viewed with caution [17]. Further studies are needed to determine possible side-effects of therapeutically-relevant doses. Multiple parenteral treatments with curcumin-PEG did not alter behaviors, body weights, or histoarchitectures of non-reproductive organs. Curcumin-PEG increased uterine weights and reduced antral follicle numbers. However, curcumin had no apparent effect on ovulation in rabbits [18] and mice [present study]. Puberty and pregnancy were affected in female mice by curcumin-PEG. Male accessory glands and testes were also affected by curcumin-PEG. The appearance of cavitations within the highly mitotic/meiotic (spermatogenic) seminiferous epithelium induced by curcumin-PEG is analogous to morphological reactions within tumor xenografts indicative of autophagocytosis and resorption [6].

Many of the noted reproductive effects of curcumin-PEG could be attributed to estrogen-mimicking or androgen-antagonizing properties of curcumin. Uterine hypertrophy is the prototype estrogen bioassay [9]. Likewise, vaginal opening in puberal rodents is a classical estrogenic response [10]. Indeed, curcumin displaced estradiol binding to its receptors [19; unpublished observations]. In breast cancer cells, curcumin elicited gene expression profiles typical of estrogen/phytoestrogen actions [20, 21]. Estradiol (akin to a hexane extract of *Curcuma comosa*) reduced seminal vesicle weights in mice [22]. Curcumin down-regulated 17 β -hydroxysteroid dehydrogenase (converts androstenedione to testosterone), 5 α -reductase (converts testosterone to dihydrotestosterone), and androgen receptors/activation in gonads and male accessory organs [23–26]. It also is plausible that curcumin could modulate gonadal functions (e.g., folliculogenesis) indirectly via negative feedback effects on the hypothalamic-pituitary (gonadotropin-releasing hormone/gonadotropin) axis.

There is growing support in the scientific literature for the concept that curcumin can adversely affect reproduction. Motilities and viabilities of human and murine sperm exposed to curcumin were reduced [27–29] - which translated to a failure of fertilization in vitro [29]. Curcumin inhibited mouse blastocyst development/viability in vitro (apoptosis) - embryos which then failed upon transfer to donors [30]. Rates of implantation and litter sizes in mice and rats were decreased by curcumin [18, 29]; the intravaginal route of administration (vs. oral or ip) was most effective [29]. Thus, the reduction in litter sizes of female mice treated with curcumin-PEG during breeding and pregnancy may be due to curcumin toxicities toward sperm and embryos. Litter sizes also were negatively impacted when male mice were fed high concentrations of curcumin and then mated with untreated females [31]. Hazardous reproductive effects of curcumin/curcumin-PEG are transient/reversible [31, 32, present study].

Positive effects of curcumin on ovarian and testicular functions have been reported. Follicular growth and oocyte quality were restored in aged follicle-stimulating hormone receptor +/- mutant mice (a model of early reproductive failure and hyperandrogenemia) treated (subcutaneous implant) with curcumin [33]. Curcumin also protected the ovary against experimentally-induced immune ovarian failure in mice [34]. The addition of curcumin to a bovine semen extender safeguarded sperm cells from freeze-thaw damage [35]. Curcumin protected against toxin-induced damages in rodent testes [36–42].

It is evident that multifaceted/bimodal mechanisms of curcumin actions contribute to a diversity of effects on the reproductive systems of females and males. To our knowledge, this is the first report documenting *in vivo* estrogenic/antiandrogenic and antifertility consequences of antitumor doses of a novel water soluble curcumin-PEG prodrug. An estrogen-mimicking effect of curcumin presumably would be contraindicated in the treatment of certain (steroid-dependent) cancers (e.g., breast, ovary), whereas an antiandrogen action would be advantageous in other anticancer applications (e.g., prostate).

Acknowledgments

Supported by grants from the National Institutes of Health (RR-016474), Department of Defense Medical Research Program (BC-0083821), and National Science Foundation (CBET-0753109).

References

1. Singh S, Khar A. Biological effects of curcumin and its role in cancer chemoprevention and therapy. *Anti Canc Agents in Med Chem.* 2006; 6:259–70.
2. Anand P, Sundaram C, Jhurani S, Kunnumakkara AB, Aggarwal BB. Curcumin and cancer: an “old-age” disease with an “age-old” solution. *Cancer Lett.* 2008; 267:133–64. [PubMed: 18462866]
3. Sa G, Das T. Anti cancer effects of curcumin: cycle of life and death. *Cell Div.* 2008; 3:14. [PubMed: 18834508]
4. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. *Mol Pharm.* 2007; 4:807–18. [PubMed: 17999464]
5. Molineux G. Pegylation: engineering improved biopharmaceuticals for oncology. *Pharmacotherapy.* 2003; 23:3–8S.
6. Tang H, Murphy CJ, Zhang B, Shen Y, Sui M, Van Kirk EA, et al. Amphiphilic curcumin conjugate-forming nanoparticles as anticancer prodrug and drug carriers: *in vitro* and *in vivo* effects. *Nanomedicine.* 2010; 5:855–65. [PubMed: 20735222]
7. Veronese FM, Mero A. The impact of pegylation on biological therapies. *Biodrugs.* 2008; 22:315–29. [PubMed: 18778113]
8. Murdoch WJ, Van Kirk EA, Isaak DD, Shen Y. Progesterone facilitates cisplatin toxicity in epithelial ovarian cancer cells and xenografts. *Gynecol Oncol.* 2008; 110:251–55. [PubMed: 18495224]
9. Owens JW, Ashby J. Critical review and evaluation of the uterotrophic bioassay for the identification of possible estrogen agonists and antagonists: in support of the validation of the OECD uterotrophic protocols for the laboratory rodent. *Crit Rev Toxicol.* 2002; 32:445–520. [PubMed: 12487363]
10. Thigpen JE, Setchell KD, Saunders HE, Haseman JK, Grant MG, Forsythe DB. Selecting the appropriate rodent diet for endocrine disruptor research and testing studies. *ILAR J.* 2004; 45:401–16. [PubMed: 15454679]
11. Agarwal R, Kaye SB. Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nat Rev Cancer.* 2003; 3:502–16. [PubMed: 12835670]
12. Ozols RF. Treatment goals in ovarian cancer. *Int J Gynecol Cancer Suppl.* 2005; 1:3–11.
13. Chan MM, Fong D, Soprano KJ, Holmes WF, Heverling H. Inhibition of growth and sensitization to cisplatin-mediated killing of ovarian cancer cells by polyphenolic chemopreventive agents. *J Cell Physiol.* 2003; 194:63–70. [PubMed: 12447990]
14. Wahl H, Tan L, Griffith K, Choi M, Liu JR. Curcumin enhances Apo2L/TRAIL-induced apoptosis in chemoresistant ovarian cancer cells. *Gynecol Oncol.* 2007; 105:104–12. [PubMed: 17174384]
15. Weir NM, Selvendiran K, Kutala VK, Tong L, Vishwanath S, Rajaram M, et al. Curcumin induces G(2)/M arrest and apoptosis in cisplatin-resistant human ovarian cancer cells by modulating Akt and p38 MAPK. *Cancer Biol Ther.* 2007; 6:178–84. [PubMed: 17218783]
16. Yallapu MM, Maher DM, Sundram V, Bell MC, Jaggi M, Chauhan SC. Curcumin induces chemo/radiation-sensitization in ovarian cancer cells and curcumin nanoparticles inhibit ovarian cancer cell growth. *J Ovarian Res.* 2010; 3:11. [PubMed: 20429876]

17. Burgos-Moron E, Calderon-Montano JM, Salvador J, Robles A, Lopez-Lazaro M. The dark side of curcumin. *Int J Cancer*. 2010; 126:1771–5. [PubMed: 19830693]
18. Garg SK, Mathur VS, Chaudhury RR. Screening of Indian plants for antifertility activity. *Indian J Exp Biol*. 1978; 16:1077–9. [PubMed: 750387]
19. Shenouda NS, Zhou C, Browning JD, Ansell PJ, Sakla MS, Lubahn DB, et al. Phytoestrogens in common herbs regulate prostate cancer cell growth in vitro. *Nutr Cancer*. 2004; 49:200–8. [PubMed: 15489213]
20. Miksicek RJ. Interaction of naturally-occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor. *J Steroid Biochem Mol Biol*. 1994; 49:153–60. [PubMed: 8031711]
21. Bachmeier BE, Mirisola V, Romeo F, Generoso L, Esposito A, Dell'eva R, et al. Reference profile correlation reveals estrogen-like transcriptional activity of curcumin. *Cell Physiol Biochem*. 2010; 26:471–82. [PubMed: 20798532]
22. Piyachaturawat P, Timinkul A, Chuncharunee A, Suksamrarn A. Growth suppressing effect of *Curcuma comosa* extract on male reproductive organs in immature rats. *Pharm Biol*. 1998; 36:44–9.
23. Liao S, Lin J, Dang MT, Zhang H, Kao YH, Fukuchi J, et al. Growth suppression of hamster flank organs by topical application of catechins, alizarin, curcumin, and myristoleic acid. *Arch Dermatol Res*. 2001; 293:200–5. [PubMed: 11380153]
24. Hu GX, Liang G, Chu Y, Li X, Lian QQ, Lin H, et al. Curcumin derivatives inhibit testicular 17 beta-hydroxysteroid dehydrogenase 3. *Bioorg Med Chem Lett*. 2010; 20:2549–51. [PubMed: 20346654]
25. Nakamura K, Yasunaga Y, Segawa T, Ko D, Moul JW, Srivastava S, et al. Curcumin down-regulates AR gene expression and activation in prostate cancer cell lines. *Int J Oncol*. 2002; 21:825–30. [PubMed: 12239622]
26. Aggarwal BB. Prostate cancer and curcumin: add spice to your life. *Cancer Biol Ther*. 2008; 7:1436–40. [PubMed: 18769126]
27. Rithaporn T, Monga M, Rajasekaran M. Curcumin: a potential vaginal contraceptive. *Contraception*. 2003; 68:219–23. [PubMed: 14561543]
28. Ashok P, Meenakshi B. Contraceptive effect of *Curcuma longa* (L.) in male albino rat. *Asian J Androl*. 2004; 6:71–4. [PubMed: 15064838]
29. Naz RK. Can curcumin provide an ideal contraceptive? *Mol Reprod Dev*. 2011; 78:116–23. [PubMed: 21337449]
30. Chen CC, Hsieh MS, Hsuw YD, Huang FJ, Chan WH. Hazardous effects of curcumin on mouse embryonic development through a mitochondria-dependent apoptotic signaling pathway. *Int J Mol Sci*. 2010; 11:2839–55. [PubMed: 21152277]
31. Mishra RK, Singh SK. Reversible antifertility effect of aqueous rhizome extract of *Curcuma longa* L. in male laboratory mice. *Contraception*. 2009; 79:479–87. [PubMed: 19442785]
32. Ganiger S, Malleshappa HN, Krishnappa H, Rajashekhar G, Ramakrishna Rao V, Sullivan F. A two generation reproductive toxicity study with curcumin, turmeric yellow, in Wistar rats. *Food Chem Toxicol*. 2007; 45:64–9. [PubMed: 16987575]
33. Tiwari-Pandey R, Ram Sairam M. Modulation of ovarian structure and abdominal obesity in curcumin- and flutamide-treated aging FSH-R haploinsufficient mice. *Reprod Sci*. 2009; 16:539–50. [PubMed: 19304795]
34. Voznesens'ka T, Bryzhina TM, Sukhina VS, Makohon NV, Aleksieieva IM. Effect of NF-kappaB activation inhibitor curcumin on the oogenesis and follicular cell death in immune ovarian failure in mice. *Fiziol Zh*. 2010; 56:96–101. [PubMed: 20968042]
35. Bucak MN, Baspinar N, Tuncer PB, Coşkun K, Sarıoğlu S, Akalin PP, et al. Effects of curcumin and dithioerythritol on frozen-thawed bovine semen. *Andrologia*. 2011 (Epub ahead of print).
36. Giannessi F, Giambelluca MA, Grasso L, Scavuzzo MC, Ruffoli R. Curcumin protects Leydig cells of mice from damage induced by chronic alcohol administration. *Med Sci Monit*. 2008; 14:BR237–42. [PubMed: 18971866]
37. El-Demerdash FM, Yousef MI, Radwan FM. Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs. *Food Chem Toxicol*. 2009; 47:249–54. [PubMed: 19049818]

38. Ilbey YO, Ozbek E, Cekmen M, Simsek A, Otunctemur A, Somay A. Protective effect of curcumin in cisplatin-induced oxidative injury in rat testis: mitogen-activated protein kinase and nuclear factor-kappa B signaling pathways. *Hum Reprod.* 2009; 24:1717–25. [PubMed: 19279034]
39. Verma RJ, Mathuria N. Effect of curcumin on aflatoxin-induced biochemical changes in testis of mice. *Fertil Steril.* 2009; 91:597–601. [PubMed: 18291380]
40. Sharma P, Singh R. Protective role of curcumin on lindane induced reproductive toxicity in male Wistar rats. *Bull Environ Contam Toxicol.* 2010; 84:378–84. [PubMed: 20182699]
41. Noorafshan A, Karbalay-Doust S, Valizadeh A, Aliabadi E. Ameliorative effects of curcumin on the structural parameters of seminiferous tubules and Leydig cells in metronidazole-treated mice: a stereological approach. *Exp Toxicol Pathol.* 2010 (Epub ahead of print).
42. Aktas C, Kanter M, Erboga M, Ozturk S. Anti-apoptotic effects of curcumin on cadmium-induced apoptosis in rat testes. *Toxicol Ind Health.* 2011 (Epub ahead of print).

A water-soluble conjugate of curcumin and polyethylene glycol with antitumor properties was constructed.

- Parenteral administration of pegylated curcumin affected reproductive functions in mice.
- Estrogenic, antiandrogenic, and antipregnancy effects were demonstrated.

Fig. 1.
Symmetrical structures of curcumin and curcumin-PEG. Curcumin represents the hydrophobic component of the conjugate and the PEG chains impart hydrophilicity.

Fig. 2. Effects of iv curcumin-PEG on ovarian (SKOV-3) tumors in xenografted mice. Tumor masses and total numbers of tumors are shown on the left ($p < 0.05$) and right ($p < 0.05$), respectively. Different superscripts (above SE bars) indicate statistical differences.

Fig. 3.

A, effect of iv curcumin-PEG on uterine weights (a vs. b: $p < 0.1$). Representative photographs of uteri of curcumin-PEG and control groups are shown on the right. B, effect of iv curcumin-PEG on average antral follicles per ovary (a vs. b: $p < 0.05$).

Fig. 4.

Effects of ip curcumin-PEG on the onset of puberty (vaginal opening, left panel) and uterine weights (right panel) in immature mice. Percentages of pubertal mice in the curcumin-PEG and estradiol groups were significantly different from controls (a vs. b: $p < 0.5$). Uterine weights of the curcumin-PEG group tended to be different from the control and estradiol groups (a vs. b: $p < 0.1$); control and estradiol groups were significantly different (c vs. d: $p < 0.05$).

Fig. 5.

A, effects of iv curcumin-PEG on seminal vesicle (a vs. b; $p < 0.05$) and testicular (a vs. a; $p > 0.1$) weights (left panel) and testicular testosterone concentrations (right panel; a vs. b; $p < 0.05$). B, effect of iv curcumin-PEG on spermatogenic function (left panel; a vs. b; $p < 0.05$). Representative photomicrographs of cross-sections of seminiferous tubules are shown on the right.