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Chemotherapeutic Nanoparticles Accumulate in the Female Reproductive System during Ovulation Affecting Fertility and Anticancer Activity

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Introduction

Throughout the female menstrual cycle, physiological changes occur that affect the biodistribution of nanoparticles within the reproductive system. This can have positive or negative effects. We demonstrate a 2-fold increase in nanoparticle accumulation in the ovaries during female mouse ovulation compared to the non-ovulatory stage following intravenous administration. Accumulation in the reproductive system is favored by nanoparticles smaller than 100 nm. Chemotherapeutic nanoparticles administered during ovulation increased ovarian toxicity and decreased short-term and long-term fertility when compared to the free drug. Breast cancer treated with nanomedicines during ovulation results in higher drug accumulation in the reproductive system rather than at the site of the tumor, reducing treatment efficacy. Conversely, ovarian cancer treatment was improved by enhanced nanoparticle accumulation in the ovaries during ovulation. Our findings suggest that the menstrual cycle should be considered when designing and implementing nanotherapeutics for females.

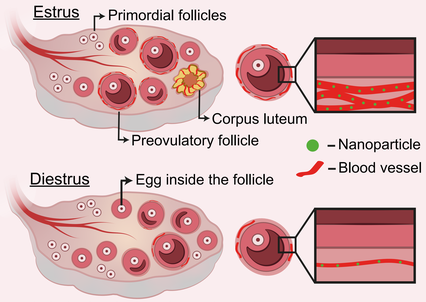
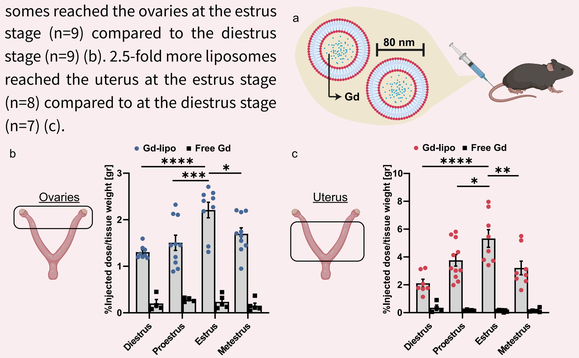


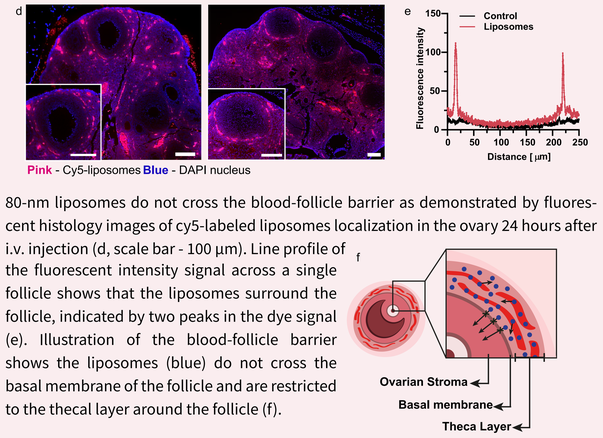
Figure 1. Biodistribution of nanoparticles during the female mouse menstrual cycle. During the estrus stage there is increased blood supply to the ovary to support preovulatory follicles. After ovulation, a dense blood network termed the corpus luteum is observed. Higher density of blood vessels around the follicle result in higher accumulation of nanoparticles (green) in the reproductive system. By contrast, there are fewer blood vessels in the ovary and around the follicles specifically during the diestrus stage

Biodistribution

- **Maximal nanoparticle accumulation in the reproductive system was measured during ovulation**
- **80-nm liposomes are restricted outside the blood-follicle barrier**

80-nm Gadolinuim Gd liposomes (Gd-lipo) or Free Gd were i.v. injected to female mice at different cycle stages (a) and their accumulation after 24 hours was quantified using elemental analysis. Results are shown as the percentage of Gd accumulated out of the total injected dose normalized to the organ's weight. 2-fold more liposomes reached the ovaries at the estrus stage (n=9) compared to the diestrus stage (n=9) (b). 2.5-fold more liposomes reached the uterus at the estrus stage (n=8) compared to at the diestrus stage (n=7) (c).



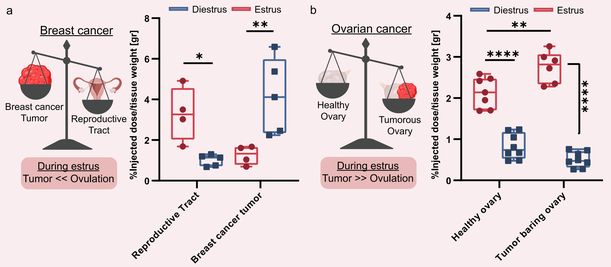


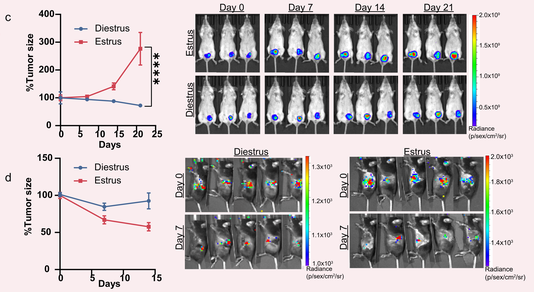
80-nm liposomes do not cross the blood-follicle barrier as demonstrated by fluorescent histology images of cy5-labeled liposomes localization in the ovary 24 hours after i.v. injection (d, scale bar - 100 μm). Line profile of the fluorescent intensity signal across a single follicle shows that the liposomes surround the follicle, indicated by two peaks in the dye signal (e). Illustration of the blood-follicle barrier shows the liposomes (blue) do not cross the basal membrane of the follicle and are restricted to the thecal layer around the follicle (f).

Biodistribution during Cancer

- **The estrous cycle affects tumor biodistribution and nanomedicine therapy**

During breast cancer, Gd-liposomes accumulate in the reproductive system (n=4) instead of at the site of the tumor (n=4) during the estrus stage, while during the diestrus stage they shift towards the tumor (n=5) and away from the reproductive system (n=5) (a). During ovarian cancer, there is increased accumulation of Gd-liposomes both in the tumor-bearing ovary (E. n=6, D. n=8) and in the healthy ovary (E. n=6, D. n=7) during estrus, compared to during the diestrus stage (b).



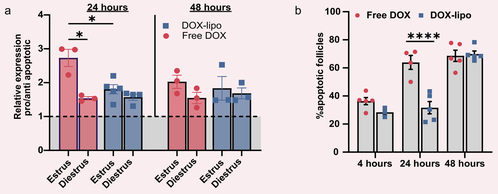


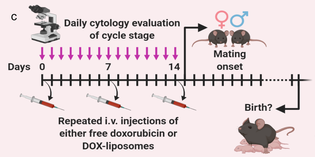
Treatment efficacy of Doxorubicin-liposomes was evaluated in 4T1 mcherry breast cancer model (c) and in an ovarian cancer model expressing luciferase (d) during estrus and diestrus. After three treatments of breast cancer tumor, the average tumor size in the estrus group increased by 276%±58, while in the diestrus-treated group the tumor reduced to 72%±6 of the initial size. During the ovarian cancer treatment, the average tumor size of the diestrus group was ~1.6-fold higher than the average tumor size of the estrus group.

Ovarian Toxicity

- **Doxorubicin-loaded liposomes cause delayed ovarian toxicity**
- **Treatment with doxorubicin-loaded liposomes impairs fertility compared to the free drug**

Healthy female mice received i.v. injection of either free DOX or DOX-lipo and the ovaries were taken to histology and RT-PCR analysis after 24 and 48 hours. RT-PCR of pro/anti apoptotic gene expression during estrus and diestrus (are shown as the relative expression of the ratio between pro (BAX) and anti (bcl2) apoptotic genes (a). Quantification of the total s gnal from immunohistochemistry against active caspase3 in the follicles, demonstrated significantly more damage in the free DOX group compared to DOX-li o 24 hours after .v. njection, however apoptosis levels ecome comparable after 48 hours (n=5 for both groups) (b).





Healthy female mice rece ved repeated .v. njections of either free DOX (n=10) or DOX-lipo (n=10) once a week for 3 treatments and their cycle stage was recorded daily to be compared to that of the control group (n=9) that was not injected (c). After the third injection, females were housed with males and the day of birth, litter size and pups' viability were recorded (d). The time until pregnancy was significantly longer for both the free DOX and the DOX-lipo group, however the pups' viability was lower for the DOX-lipo group.

d

	Birth - Days after mating onset	%Successful births	Average litter size	%Alive pups
Control	26 ± 2	100% (9/9)	5.0 ± 1.4	100%
Free DOX	59 ± 24**	100% (10/10)	5.3 ± 1.9	90%±10%
DOX-lipo	62 ± 10***	70% (7/10)	4.3 ± 2.7	60%±14%*

References - Poley, Maria, et al. "Chemotherapeutic Nanoparticles Accumulate in the Female Reproductive System during Ovulation Affecting Fertility and Anticancer Activity." bioRxiv (2020).

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Results are shown as mean±SEM. Two-way ANOVA and Tukey's t-test were used for statistical analysis of f-h, unpaired two-tail t-test was used for statistical analysis of l. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.