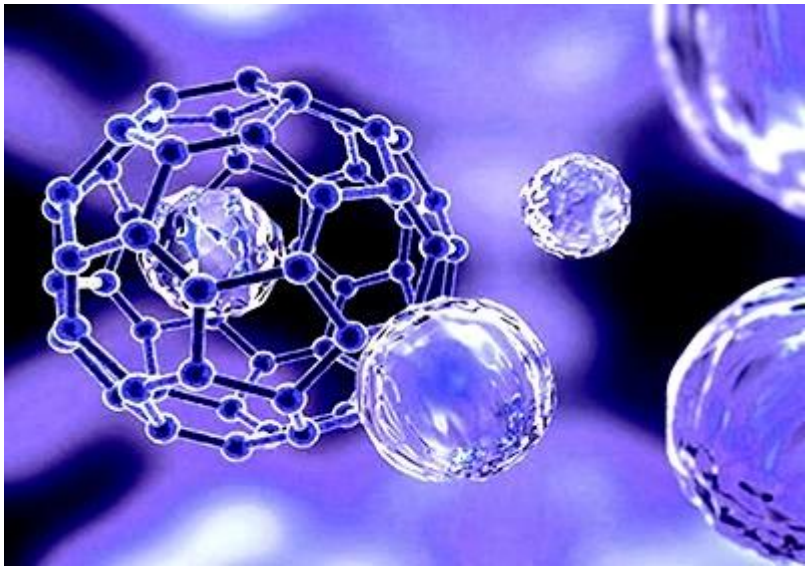
In European legislation, a nanomaterial is defined as “an insoluble or bio-persistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to ≤100 nm”. There is growing concern about the safety of nanoparticles (Nps) in the field of nanomedicine. Due to their small size and unique properties, nanomaterials can overcome biological tissue barriers. In this animal study, the Israeli research team investigated whether hormonal fluctuations during the phases of the female menstrual cycle influence the accumulation and effectiveness of nanoparticles in the female reproductive organs. They also investigated the effects of nanomedicines on the fertility of female mice, and how the phases of the menstrual cycle affect the accumulation of nanoparticles in two cancer models.

NPs are generally classified as natural or synthetic/anthropogenic NPs, depending on their origin. Another classification is their grouping into organic and inorganic nanoparticles. Organic nanoparticles include lipid nanoparticles (LNPs), known as liposomes, dendrimers, micelles, etc. Metallic NPs, such as gold, iron, silver, aluminum, titanium oxide, and zinc oxide are examples of some inorganic NPs. Nanoparticles can exert their harmful effects on cells by increasing the production of reactive oxygen species, damaging DNA and mitochondria, and inducing cell death.

The female reproductive system undergoes cyclic hormonal and physiological changes. The estrous cycle of female mice, which is the equivalent of the human female menstrual cycle, is divided into four stages: diestrus, proestrus, estrus, and metestrus. The ovulation occurs at the end of the proestrus phase. During the pre-ovulatory phase, elevated blood flow, angiogenesis, and perfusion, within the reproductive system support oocyte maturation. Blood-follicle barrier (BFB), formed during the early follicle stage, protects the developing oocyte from toxic and foreign molecules and supplies it with necessary nutrients and growth factors. At ovulation, the BFB ruptures to release the oocyte.

The authors also pointed to some sex-related differences in the pharmacokinetic parameters of nanomedicine. Previous research has demonstrated that the overall clearance rate of PEGylated liposomal doxorubicin (DOX) is slower in females than in males. Also, it was shown that the cellular uptake of nanoparticles differed depending on the cell sex identity.



About the study

At the beginning of this animal study, the research team examined the impact of the menstrual cycle phases on the accumulation and efficacy of 80nm liposomes loaded with gadolinium (Gd-lipo) in developing follicles before, during, and after ovulation. Gd-lipo or free Gd were injected intravenously (i.v.) into the tail vein of the mice at each of the four stages of the menstrual cycle (diestrus, proestrus, estrus, and metestrus). Their accumulation in the ovaries and the uterus was quantified after 24 hours. Vaginal cytology was used to determine the phases of the menstrual cycle. Since the new blood vessels are formed in the thecal layer surrounding developing follicles before ovulation, the increase in new blood vessels during the ovulatory phases was validated by immunohistochemistry analysis of CD31.

To assess whether the ovaries have a nanoscale size cutoff, the authors injected i.v. PEGylated gold nanoparticles (AuNPs) of different sizes (20, 50, 100, and 200 nm) to female mice during the estrus phase. Then, they investigated the toxic effect of liposomal doxorubicin (DOX) on ovaries and fertility. The toxic effects of liposomal DOX on ovaries were assessed after i.v. injection of the free-DOX or DOX-loaded liposomes (DOX-lipo) to healthy mice during estrus and diestrus. After 24 or 48 hours, the pro- and anti-apoptotic genes in the collected ovaries were analyzed by reverse transcription polymerase chain reaction and compared with the results obtained in the untreated control. The toxic effects of DOX-loaded liposomes on fertility were investigated in three groups of healthy female mice: treated with free-DOX, treated with DOX-lipo, and untreated control. The first two

groups were injected once a week with three rounds of either free-DOX or DOX-loaded liposomes. The female mice were then paired with males for mating, and the litter sizes, day of birth, pup weight, and viability were monitored.

Lastly, the authors examined how the menstrual cycle phases influence the accumulation of nanoparticles in two cancer models. The mice with triple-negative breast cancer or epithelial ovarian cancer were i.v. injected with 80nm liposomes loaded with gadolinium (Gd-lipo) during estrus or diestrus, and their biodistribution was quantified 24 hours after the injection.

Results

The biodistribution of nanoparticles in the female reproductive system

The maximal accumulation of 80nm liposomes loaded with gadolinium (Gd-lipo) was significantly higher in the ovaries and uterus during the estrus compared to diestrus. Gd-lipo accumulation was approximately 2-fold higher in the ovaries and 2.5-fold higher in the uterus during estrus compared to diestrus. Similarly, Gd-lipo accumulation during proestrus and metestrus was significantly lower than during estrus.

There were no significant differences in liposomal accumulation in the heart, lungs, kidneys, and spleen, except for the liver, where liposomal accumulation significantly increased during the proestrus compared to the diestrus, estrus, or metestrus. According to the authors, the increased liposomal accumulation in the liver may be related to elevated estrogen levels during proestrus and the effect of estrogens on cytochrome P450 liver metabolism.

Immunohistochemistry analysis, which was used to investigate the formation of new blood vessels in the thecal layer surrounding developing follicles before ovulation, showed a 4-fold increase in the number of CD31-positive cells in the thecal layer during the estrus compared to the diestrus. The authors stated that vascular endothelial growth factor during the proestrus and estrous increases vascularity, capillary leakiness, and permeability, facilitating nanoparticle extravasation through extensive endothelial gaps.

The nanoscale size cutoff of the ovaries was determined by i.v. injection of PEGylated gold nanoparticles (AuNPs) of different sizes during estrus. After 24 hours, larger AuNPs (100 and 200 nm) less accumulated in the ovaries and uterus than smaller AuNPs (20 and 50 nm). AuNPs of 100 nm accumulated approximately 2-fold less in the ovaries and approximately 3.5-fold less in the uterus than smaller AuNPs, while AuNPs of 200 nm

accumulated approximately 5-fold less in the ovaries and approximately 12.5-fold less in the uterus than smaller AuNPs.

The impact of doxorubicin-loaded liposomes on mice ovaries

The toxic impact of liposomal DOX on the ovaries of healthy mice was assessed by i.v. injection of either free-DOX or DOX-loaded liposomes (DOX-lipo) during the estrus and diestrus. 24 hours after the i.v. administration of either free-DOX or DOX-lipo, the level of apoptosis in both small and large follicles was higher in the free-DOX-treated mice than in the DOX-lipo-treated mice, which showed no apoptosis in the smaller follicles. The highest level of apoptosis was identified during estrus. The free-DOX group had approximately 1.5-fold higher levels of apoptosis during the estrus than the DOX-lipo group. The apoptotic effect inside the follicles was confirmed by immunohistochemistry. Previous findings that demonstrated that free-DOX crosses the blood-follicle barrier and damages the developing oocytes are consistent with the results of this study.

Interestingly, after 48 hours, the level of apoptosis did not increase significantly in the ovaries of the free-DOX group, but in the ovaries of DOX-lipo-treated mice, the percentage of apoptotic follicles increased significantly. According to the authors, the delayed toxic effect of DOX-lipo on the ovaries was caused by their initial accumulation.

The impact of doxorubicin-loaded liposomes on mice fertility

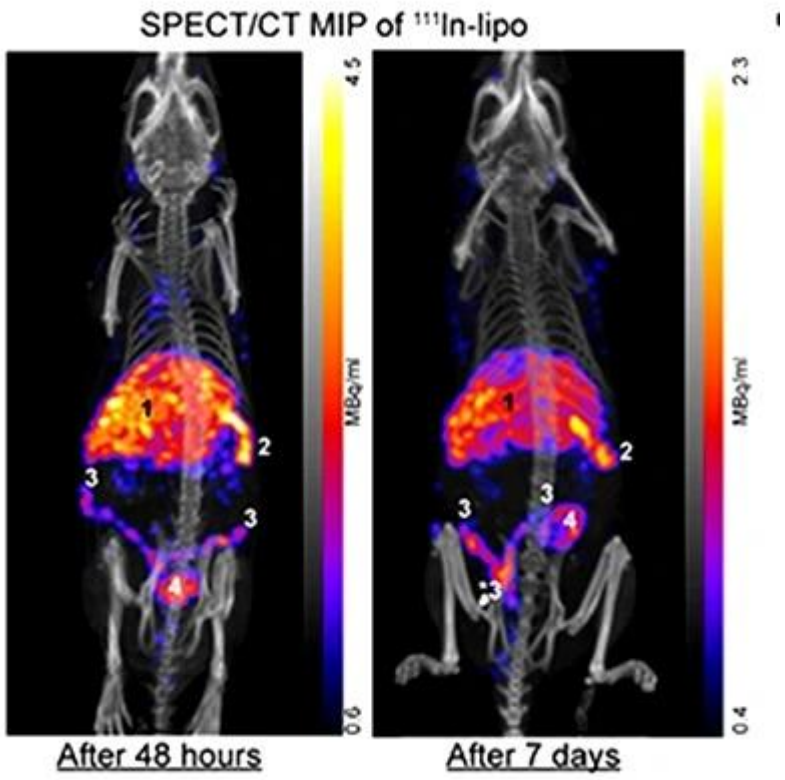
All mice in the control group had successful births 26 ± 2 days after mating. In contrast, free-DOX-treated mice had the first litter after 59 days, whereas DOX-lipo mice had the first litter after 62 days. All mice in the free-DOX group were pregnant and gave birth, showing that the mice in this group regained their ability to ovulate. In contrast, only 70% of the mice within the DOX-lipo group got pregnant.

The average litter size was 5±1.4 pups in the control group, 5.3±1.9 in the free-DOX group, and 4.3±2.7 in the DOX-lipo group. The viability of the pups was 100% in the control group and 90% in the free-DOX group. However, the viability of the pups in the DOX-lipo group significantly decreased to 60%, indicating an elevated ovarian toxicity of DOX-lipo compared to free-DOX. This may be the result of faster clearance of free molecule drugs compared to liposomes.

The biodistribution and nanomedicine therapy in two cancer models

To investigate how the phases of the menstrual cycle impact nanoparticle accumulation in tumors and the reproductive system, the Gd-lipo 80 nm were i.v. injected during estrus or diestrus to mice bearing triple-negative breast cancer or epithelial ovarian cancer. In a breast cancer model, 80 nm liposomes accumulated during the estrus 2.6-fold more in the reproductive system than in the tumor, 24 hours after the injection. In contrast, during the diestrus, the accumulation of liposomes was 4.1-fold higher in the tumor than in the reproductive system.

In mice with cancer in one of the two ovaries, increased Gd-liposome accumulation was found both in the tumor-bearing ovary and healthy ovary during the estrus, compared to the diestrus. However, during the estrus, more liposomes accumulated in the tumor-bearing ovary than in the healthy ovary, implying that cancer vascularization in the tumor-bearing ovary overcomes ovulation angiogenesis in the healthy ovary. These results indicate that in the triple-negative breast cancer model the nanomedicine accumulated during the estrus preferentially in the reproductive system rather than in the tumor. But, when the target of the nanomedicine treatment was the reproductive system, like in ovarian cancer, the outcome was different.



Original illustration from the article of Poley M. ACS Nano 2022. In mice bearing ovarian cancer in one of the two ovaries, there is increased accumulation of Gd-liposomes both in the tumor-bearing ovary and in the healthy ovary during the estrus stage, compared to the diestrus stages.

After 21 days, in mice with triple-negative breast cancer, the average tumor size in the group treated during estrus increased by 276% compared to day 0, whereas in the group treated during diestrus, the tumor decreased to 72% of its initial size. Treatment of ovarian cancer with DOX-lipo was more favorable in the estrus phase than in the diestrus phase. After 14 days, the average tumor size in the group treated during diestrus was approximately 1.6-fold larger than the average tumor size in the group treated during estrus. These results demonstrate that more liposomes reach the tumor during ovulation.

Conclusion

This study has shown that the menstrual cycle phases differently influence the biodistribution of therapeutic nanoparticles in the female reproductive system. During estrus, the maximal accumulation of 80nm liposomes loaded with gadolinium (Gd-lipo) was found in the ovaries and uterus. The results also demonstrated that smaller nanoparticles

were significantly more accumulated in the ovaries and uterus than larger sizes of nanoparticles. The accumulation of nanoparticles in the female reproductive organs impairs oocyte development and can compromise fertility. In addition, cancer treatment with nanomedicines appears to be influenced by the specific biodistribution of nanoparticles in the female reproductive system.

The authors suggest that the menstrual cycle and the potential toxicity to ovaries caused by the accumulation of nanoparticles should be considered in the development and use of nanotherapeutics in women.

This article was published in the scientific journal ACS Nano.

Journal Reference

Poley M. Nanoparticles Accumulate in the Female Reproductive System during Ovulation Affecting Cancer Treatment and Fertility. ACS Nano 2022, 16, 4, 5246–57. http://ACS Nano. 2022 Apr 26; 16(4): 5246–5257.

The article is also available on the pre-print server (Open Access).
https://www.biorxiv.org/content/10.1101/2020.07.22.216168v1.full