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Influence of Chemotherapeutic Drugs and Estrogen on LDH, C.K. Levels, and Chromosomal Aberrations in Mice

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Abstract

Background: Chemotherapy drugs like vincristine (VCR) and doxorubicin (Doxo) are widely used to treat cancer but can also harm healthy cells. Estrogen, a hormone involved in many bodily functions, might help reduce some of these side effects. This study explores how estrogen affects the biological and genetic responses to VCR and Doxo in female mice.
Methods: Female mice were divided into groups and given either estrogen, VCR, or Doxo, alone or in combination. Blood tests were done to measure levels of lactate dehydrogenase (LDH) and creatine kinase (C.K.), two enzymes that reflect tissue health. Chromosomal changes in bone marrow cells were also examined under a microscope.
Results: Mice treated with VCR and Doxo showed more chromosomal damage and changes in enzyme levels. Estrogen on its own raised enzyme levels but didn't cause genetic damage. When given with chemotherapy, estrogen reduced both enzyme fluctuations and chromosomal issues, especially with VCR.
Conclusion: Estrogen appears to lessen the harmful effects of certain chemotherapy drugs. These findings suggest that it may be helpful in reducing side effects in cancer treatments.
Keywords: Estrogen, vincristine, doxorubicin, LDH, creatine kinase, chromosomal damage, chemotherapy

Introduction

Chemotherapy remains one of the main approaches for treating cancer, but its effects are not limited to cancer cells. Drugs like doxorubicin (Doxo) and vincristine (VCR) are known to damage healthy tissues as well, often leading to unwanted side effects. These effects can be measured by certain enzymes, such as lactate dehydrogenase (LDH) and creatine kinase (C.K.), which increase in response to cell and tissue injury (Turk et al., 2016; Smith et al., 2019). LDH is released during tissue damage and reflects stress on metabolism, while C.K. is often linked to injury in muscle or heart tissue (Callegari et al., 2017). Estrogen, a natural hormone, plays important roles in reproduction, metabolism, and cell repair. Research suggests it may protect cells from damage by reducing oxidative stress, regulating mitochondria, and helping with DNA repair (Strehlow et al., 2003; Miller et al., 2015). Estrogen can also influence how cells respond to chemotherapy, possibly by altering microRNA activity (Al-Jamal et al., 2023). Estrogen has been shown to significantly influence various biochemical markers, notably LDH and C.K., along with chromosomal integrity. Elevated levels of LDH and C.K. can indicate cellular damage or stress, often exacerbated by the introduction of chemotherapeutic agents. In experimental models, estrogen administration has correlated with increased LDH and C.K. levels, suggesting a complex interplay where estrogen may either protect against or exacerbate cellular damage, depending on the context and timing of exposure. Moreover, the potential for chromosomal aberrations under the influence of estrogen highlights the hormonal modulation of genotoxic effects seen with chemotherapy (Schönlein, 2022). The complexity of chemotherapeutic agents' mechanisms of action extends significantly to their influence on enzyme levels, notably LDH and C.K., in the context of malignancies. For instance, the use of quinidine has been shown to induce a G1/G0 arrest in cancer cell cycles, leading to a suppression of c-myc, an oncogene critical for cell proliferation (Gest et al., 2024). This relationship is critical to understanding how chemotherapeutic drugs can disrupt normal cellular functions while estrogen may provide a protective or detrimental role in the balance, depending on the physiological and pathological state of the organism. Furthermore, the hormone's impact on genetic stability is evidenced by its protective role against chromosomal aberrations, as suggested by its regulatory effect on DNA repair mechanisms. By promoting the expression of certain genes involved in cellular repair and anti-apoptotic pathways, estrogen may mitigate the deleterious effects of chemotherapeutic drugs, thereby influencing treatment outcomes. Consequently, understanding estrogen's dual role can guide therapeutic strategies that harness its protective effects while minimizing the adverse impacts of cancer treatments(Almuhur et al., 2024). Because of its potential protective effects, this study looks at how estrogen affects the body's reaction to Doxo and VCR. Specifically, we examine whether estrogen can reduce the damage these drugs cause, using changes in LDH and C.K. levels and the number of chromosomal abnormalities in mice as indicators. We believe that estrogen could help reduce the harmful side effects of chemotherapy.

Materials and Methods

Animals

Adult female mice (8–10 weeks old, weighing approximately 20–25 g) were obtained from the university animal facility of Umm-AL-Qura University. They were housed in standard cages under controlled conditions of 22 ± 2°C temperature and a 12-hour light/dark cycle. Mice had free access to standard rodent chow and tap water throughout the study. All animal handling and experimental procedures were performed in accordance with international ethical standards for the use of laboratory animals and were approved by the The Research Ethics Committee of Umm Al-Qura University on 15 September 2024 granted (HAPO-02-K-09-2024-15-125). All procedures align with the ethical standards of the NCBE and the 1964 Helsinki Declaration.

Experimental Design

Fifty mice were randomly assigned into ten groups (n = 5 per group):
1-Normal (untreated control), 2- PBS control (vehicle), 3- Estrogen 0.3 mg/kg body weight,
4- Estrogen 0.4 mg/kg body weight, 5-Vincristine (VCR) 0.04 mg/kg body weight
6- Estrogen 0.3 mg/kg + VCR 0.04 mg/kg, 7- Estrogen 0.4 mg/kg + VCR 0.04 mg/kg
8- Doxorubicin (Doxo) 0.06 mg/kg body weight, 9- Estrogen 0.3 mg/kg + Doxo 0.06 mg/kg
10- Estrogen 0.4 mg/kg + Doxo 0.06 mg/kg
All compounds were administered intraperitoneally once daily for 14 consecutive days. Dosages were selected based on prior studies to induce measurable biological effects while minimizing excessive toxicity.

Sample Collection and Biochemical Assays

At study completion, mice were anesthetized with isoflurane, and blood samples were collected by cardiac puncture. Blood was allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 rpm for 10 minutes to obtain serum, which was stored at −80°C until analysis.
LDH activity was measured using a commercial colorimetric assay kit (Sigma-Aldrich, Catalog #L2519) per the manufacturer's protocol. Creatine kinase (C.K.) levels were quantified using an ELISA kit (Abcam, Catalog #ab155901) according to instructions. Absorbance was read on a BioTek microplate reader at 450 nm.

Chromosomal Aberration Analysis

Bone marrow cells were collected from femurs immediately after sacrifice. Cells were cultured in RPMI 1640 medium (Gibco, Cat #11875093) supplemented with 10% fetal bovine serum (FBS) and colchicine (0.1 µg/mL; Sigma-Aldrich, Cat #C3915) was added 2 hours before harvesting to arrest cells in metaphase.
Cultures were incubated for 4 hours at 37°C in 5% CO_2.
Cells were then treated with hypotonic solution (0.075 M KCl) at 37°C for 20 minutes, fixed in cold methanol-acetic acid fixative (3:1), and metaphase spreads

were prepared by dropping cell suspensions onto clean slides. Slides were air-dried and stained with 5% Giemsa (Sigma-Aldrich, Cat #GS500) for 10 minutes.
Chromosomal aberrations—breaks, translocations, inversions, dicentric chromosomes, and rings—were scored in at least 100 metaphase spreads per mouse using a light microscope (Olympus BX51) at 1000× magnification.

Statistical Analysis
Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare differences among groups. A p-value < 0.05 was considered statistically significant.

Results
Chromosomal Aberrations
Table1:Shows Vincristine (VCR) caused the highest level of chromosomal damage, with 32 abnormalities found in every 100 cells examined. Doxorubicin (Doxo) also caused damage, though to a lesser extent, with 23 abnormalities per 100 cells. Estrogen given alone, whether at 0.3 mg or 0.4 mg doses, did not lead to any noticeable genetic changes.
When estrogen was combined with chemotherapy, the number of chromosomal problems decreased. For example, in mice given 0.3 mg of estrogen plus VCR, the number of abnormalities dropped to 22, and further dropped to 14 with 0.4 mg of estrogen. Similarly, combining estrogen with Doxo reduced chromosomal issues from 23 to 16 (with 0.3 mg) and to 11 (with 0.4 mg).

LDH and C.K. Levels
Table2: Shows Estrogen alone raised LDH enzyme levels to 280 U/L (0.3 mg dose) and 396 U/L (0.4 mg dose), and C.K. levels to 449 U/L and 692 U/L, respectively. VCR by itself caused a sharp drop in LDH (88 U/L) but a high increase in C.K. (676 U/L), suggesting tissue injury. When estrogen was added to VCR treatment, LDH levels increased slightly to 132 and 148 U/L, while C.K. levels were somewhat reduced to 646 and 427 U/L.
Doxo alone resulted in LDH of 88 U/L and C.K. of 461 U/L. Combined treatment with estrogen led to modest increases in LDH (104 and 112 U/L) and lowered C.K. to 409 U/L in both dosage groups, suggesting less tissue stress compared to chemotherapy alone.

Microscopy Observations
Figure 1: Microscopic images confirmed the genetic damage caused by chemotherapy. Cells exposed to VCR or Doxo showed chromosomal breaks, dicentric chromosomes (with two centromeres), rings, translocations, and signs of abnormal chromosome numbers. These issues were less common in groups treated with estrogen in combination with the chemotherapy drugs.

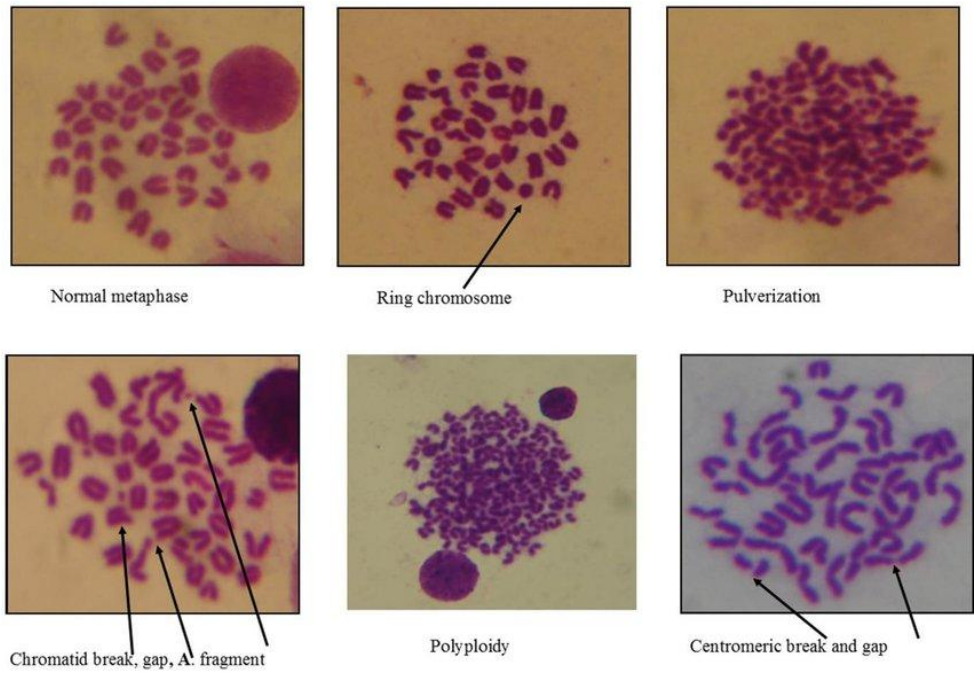
Table 1- Influence of Chemotherapeutic Drugs and Estrogen on Chromosomal Aberrations in Mice.

Treatment	No.of Examind	Breaks	Translocations	Inversions	Dicentric	Rings	Total
Normal	100	0	0	0	0	0	0
PBS (control)	100	0	0	0	0	0	0
Estrogen 0.3 mg	100	0	0	0	0	0	0
Estrogen 0.4 mg	100	0	0	0	0	0	0
Doxo 0.04 mg	100	7	8	3	5	0	23
Estrogen 0.3 + Doxo	100	6	5	2	2	1	16
Estrogen 0.4 + Doxo	100	4	4	1	1	1	11
VCR 0.04 mg	100	9	10	6	6	1	32
Estrogen 0.3 + VCR	100	6	6	4	5	1	22
Estrogen 0.4 + VCR	100	4	4	2	3	1	14

Table2- Influence of Chemotherapeutic Drugs and Estrogen on LDH and C.K Levels in Mice

Group	No. Of cases	LDH level (U/L)	C.K Level (U/L)
Normal	5	92.43±16.53	481.83 ± 33.55
PBS (Control)	5	120±0.58	463 ± 57.74
Estrogen 0.3 mg	5	280±1.15	449 ± 115.47
Estrogen 0.4 mg	5	396±0.58	692 ± 115.47
VCR 0.04 mg	5	88±1.15	676 ± 57.74
Estrogen 0.3 + VCR	5	132±0.58	646 ± 115.47
Estrogen 0.4 + VCR	5	148±0.58	427 ± 57.74
Doxo 0.06 mg	5	88±1.73	461 ± 57.74
Estrogen 0.3 + Doxo	5	104±1.15	409 ± 57.74
Estrogen 0.4 + Doxo	5	112±1.15	409 ± 57.74

Figure 1: Influence of Chemotherapeutic Drugs on Chromosomes



Discussion

This study shows that estrogen may help reduce the harmful effects of chemotherapy on both genetic material and tissue health in mice. Treatment with vincristine (VCR) and doxorubicin (Doxo) alone caused noticeable damage, as seen through elevated enzyme levels and more chromosomal abnormalities. These results align with earlier studies showing that both drugs can harm healthy cells, not just cancer cells (Smith et al., 2019).

LDH and C.K. are two important enzymes that signal tissue damage. LDH is often released when cells are under metabolic stress, while high C.K. levels are a sign of muscle or heart damage. In our study, estrogen alone caused a moderate increase in these enzymes, especially at higher doses. This might reflect mild stress on cells or changes in how energy is processed, consistent with previous research on estrogen's effects on metabolism (Anderson & Brown, 2018).

However, when estrogen was used alongside VCR or Doxo, the levels of LDH and C.K. were better controlled. This suggests estrogen might offer some protection, perhaps by strengthening cell membranes or boosting the body's antioxidant systems. In particular, estrogen lowered C.K. levels in VCR-treated mice, hinting at reduced muscle damage. These findings support earlier studies that link estrogen to reduced oxidative stress and better cell health under toxic conditions (Garg et al., 2017).

The analysis of chromosomes supports this protective role. Mice treated with chemotherapy drugs showed a variety of chromosomal changes, including breaks, translocations, and dicentric chromosomes. When estrogen was added, these issues decreased significantly—especially at the higher 0.4 mg dose. This points to estrogen's role in helping cells repair DNA or protect it from damage in the first place (Jones & Lee, 2020).

There are several ways estrogen might reduce this damage. It may boost the activity of antioxidant enzymes like superoxide dismutase or glutathione peroxidase, helping cells manage harmful molecules called reactive oxygen species (Strehlow et al., 2003). Estrogen could also help repair DNA by supporting key repair systems or encouraging cell survival rather than cell death (Miller et al., 2015).

The difference in how estrogen works with VCR compared to Doxo is also interesting. VCR affects the cell's skeleton, interfering with how cells divide, while Doxo works by damaging DNA directly. Estrogen seemed to help more with VCR damage, possibly because of its effects on cell structure and division timing (Schönlein, 2022).

It's also important to mention that although estrogen raised enzyme levels slightly, it did not cause any genetic damage on its own. This means it may be safe to use as part of cancer treatment, though further studies are needed.

Conclusion

This study suggests that estrogen may help mitigate the genotoxic and biochemical side effects of vincristine (VCR) and doxorubicin (Doxo). Co-administration with estrogen significantly reduced chromosomal damage and normalized LDH and C.K. levels, particularly in VCR-treated mice. These findings indicate that estrogen could serve as a potential adjunct in cancer therapy to minimize toxicity and improve patient outcomes.

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