Exp Oncol 2022
44, 3, 239–242

Experimental ONCOLOGY

OVARIAN TOXICITY OF FAC CHEMOTHERAPY IN RATS AND POSSIBILITY OF ITS CORRECTION WITH PLATELET-RICH PLASMA

V.A. Shamrai[1, 2], *O.I. Misiurko*[1, 2], *D.I. Grebeniuk*[1, *], *I.V. Taran*[1]
[1]*National Pirogov Memorial Medical University, Vinnytsia 21018, Ukraine*
[2]*Communal non-profit enterprise "Podillian Regional Oncology Center of Vinnytsia Regional Council", Vinnytsia 21000, Ukraine*

***Aim*: To compare the dynamics of changes in the hormonal status of female rats in the setting of the FAC (5-fluorouracil, doxorubicin and cyclophosphamide) chemotherapy and after local administration of platelet-rich plasma (PRP). *Materials and Methods*: The study was carried out on female Wistar rats treated according to the FAC chemotherapy scheme (4 courses with a 3-week interval). The ovariotoxic effect of the FAC chemotherapy was assessed by the levels of anti-Mullerian hormone, estradiol (E2) and follicle-stimulating hormone in the proestrus phase. Three weeks after the last course of chemotherapy, 5 rats were administered with local intra- and periovarian injection of PRP (triply with a 1-week interval). *Results*: The dynamics of all investigated hormonal markers of the ovarian reserve in experimental animals was characterized by a progressive decrease in anti-Mullerian hormone and E2 levels and an increase in follicle-stimulating hormone level. The dynamics of the studied parameters after the serial administration of PRP demonstrated an improvement in the hormonal status. *Conclusion*: FAC chemotherapy in the experiment causes premature ovarian failure, and local administration of PRP improves the hormonal parameters of the ovarian reserve.**
***Key Words*: FAC chemotherapy, ovarian toxicity, platelet-rich plasma, anti-Mullerian hormone, estradiol, follicle-stimulating hormone.**

DOI: 10.32471/exp-oncology.2312-8852.vol-44-no-3.18393

Breast cancer (BC) is the most common cancer among women [1]. Even in developed countries, one in eight women is at risk of developing this pathology [2]. The incidence of BC varies in different parts of the world, ranging from 27 per 100,000 in Central Africa and East Asia to 92 per 100,000 in North America [3, 4], and continues to grow despite the progress in its diagnosis and treatment. Given current growth rates, the incidence of BC may reach 3.2 million by 2050 [5].

The combination of 5-fluorouracil, doxorubicin and cyclophosphamide (FAC chemotherapy regimen) is commonly prescribed to the BC patients in many countries [6]. The undeniable advantage of this treatment regimen is its proven effectiveness and low cost of treatment. Usually, the FAC scheme is used in adjuvant, neoadjuvant or palliative settings [7].

Among the toxic effects of the FAC chemotherapy are those manifested by premature chemo-induced ovarian failure and subsequent infertility [8].

Taking into account the fact that the incidence of BC in women of reproductive age is steadily increasing, the question of preserving and/or restoring the ovarian reserve becomes relevant [9].

Platelet-rich plasma (PRP) is a fraction of autologous blood plasma enriched with the platelets that is widely used in the regenerative medicine [10, 11]. In alpha granules of platelets there is stored a complete set of coagulation factors, growth factors, and differentiation factors (insulin like growth factor, platelet-derived growth factor, transforming growth factor β, vascular endothelial growth factor, epidermal growth factor, fibroblast growth factor), which contribute to the regeneration process [12]. The effect of PRP is manifested in the reduction of the inflammatory reactions, and activation of cellular elements, which leads to neoangiogenesis and increases tissue trophicity [13, 14].

The aim of the study is to compare the dynamics of changes in the hormonal status of female rats under the conditions of FAC chemotherapy application and local PRP administration.

MATERIALS AND METHODS

55 female Wistar rats aged less than 1 year and weighing from 160.0 to 216.0 grams (189.60 ± 14.46 g) were studied. The experiment was carried out in accordance with the "Regulations on the Use of Animals in Biomedical Experiments" with the permission of the bioethics committee and in accordance with the provisions of Directive 2010/63/EU of the European Parliament and the Council of the European Union adopted September 22, 2010 "On the Protection of Animals Used for Scientific Purposes".

To study the ovariotoxic effect of the FAC-based chemotherapy, the levels of anti-Mullerian hormone (AMH), estradiol (E2) and follicle-stimulating hormone (FSH) were analyzed. To determine the reference values of the studied indicators, the hormonal status of 5 intact rats was studied. All studies of hormone levels were performed in the proestrus phase. Determination of the menstrual cycle phase was carried out by microscopy of vaginal smears according to the criteria described by Fu *et al.* [15].

Submitted: May 17, 2022.
*Correspondence: E-mail: Doctor.Svo@gmail.com
Abbreviations used: AMH – anti-Mullerian hormone; BC – breast cancer; E2 – estradiol; FAC – 5-fluorouracil, doxorubicin and cyclophosphamide; FSH – follicle-stimulating hormone; PRP – platelet-rich plasma.

Blood sampling was performed by percutaneous puncture of the left ventricular cavity under ketamine anesthesia. The blood levels of sex hormones were assayed by immunoenzyme assay using Rat AMH ELISA kit, Rat E2 ELISA Kit and Rat FSH ELISA Kit (CUSABIO, China).

The rest of the rats underwent chemotherapy according to the FAC scheme. The drugs were administered intraperitoneally. 5-Fluorouracil and cyclophosphamide were administered at a dose of 83.7 mg/kg, doxorubicin — 8.37 mg/kg. Recalculation of human doses of drugs was carried out according to the method proposed by Nair and Jacob [16]. In total, 4 courses of chemotherapy were conducted with an 3-week interval. After each course of chemotherapy, the hormonal status of experimental animals was studied twice: during the first proestrus after the course (to determine the acute ovarian toxicity of the administered drugs) and during the last proestrus before the next course of chemotherapy (to study the recovery of ovarian function). For each study of hormone levels, 5 rats were removed from the experiment.

Three weeks after the last course of chemotherapy, 5 rats were administered with local intra- and periovarian injection of PRP in a volume of 0.1 ml per ovary. After that, this manipulation was repeated two more times with an interval of 1 week between the procedures.

To prepare PRP, 0.5 ml of blood was collected from the tail vein of experimental animals in sterile heparinized tubes, followed by centrifugation at 160 g for 10 min. The upper layer was collected in a separate syringe. Two weeks after the last injection of PRP, sex hormone levels were assayed again.

The obtained data were processed using the statistical software package SPSS 20.0 for Windows. The Student's *t*-test was used to determine the reliability of the differences between the mean values. The differences were considered significant at $p < 0.05$.

RESULTS

The dynamics of changes in the main sex hormones in the rats receiving the FAC chemotherapy is shown in the Table. Levels of AMH in experimental group of animals at all time points of the study were significantly lower than in the intact rats. The dynamics of AMH levels demonstrated a tendency to a progressive decrease. Immediately after each course of chemotherapy, there was a sharp decrease in the AMH levels with their partial recovery before the next course of chemotherapy.

Repeated local PRP administration significantly ($p < 0.05$) increased the levels of AMH in FAC-treated animals by 20% compared with animals not treated with PRP.

The levels of E2 at all time points in FAC-treated animals were also significantly lower than in the intact animals. There was a significant decrease in hormone levels relative to reference values by 16.9% ($p < 0.05$) after the first course of chemotherapy, by 27.3% ($p < 0.01$) after the second, by 37.8% ($p < 0.001$) after the third and by 43.5% ($p < 0.001$) after the fourth. It should be noted that similarly to AMH, the E2 levels sharply decreased immediately after each course of chemotherapy and partially recovered before the next course.

Despite the fact that both AMH and E2 are produced by follicles, there was a varying degree of reduction in their levels. In our opinion, this can be explained by the fact that AMH is produced exclusively by follicles, while E2, in addition to follicles, is produced by the adrenal cortex and adipose tissue.

Local injections of PRP significantly ($p < 0.05$) increased E2 levels in experimental animals by 25.5%.

Changes in FSH levels were characterized by dynamics opposite to the dynamics of E2 levels. The levels of FSH at all time points after chemotherapy were significantly higher than those of intact animals. During the period between the first and second courses of chemotherapy, the levels of FSH significantly increased by 14.9% ($p < 0.05$). In the period between the second and third courses, the increase relative to reference values was 26.9% ($p < 0.01$), between the third and fourth courses — 34.6% ($p < 0.001$), after the fourth course — 85.9% ($p < 0.001$).

Such mutually opposite dynamics of changes in the levels of E2 and FSH could be explained as follows. The decrease in E2 levels is explained by the ovariotoxic and general toxic effect of chemotherapeutic agents. At the same time, the decrease in E2 levels caused an increase in FSH levels by a feedback mechanism. Cessation of the toxic effect of che-

Table. Dynamics of changes in the AMH, FSH and E2 levels after the courses of FAC chemotherapy and local PRP injections

										3 weeks after FAC	
	Control (C)	Course 1, FEC (1)	Course 1, LEC (2)	Course 2, FEC (3)	Course 2, LEC (4)	Course 3, FEC (5)	Course 3, LEC (6)	Course 4, FEC (7)	Course 4, LEC (8)	No treatment	+ PRP (P)
AMH, ng/ml	7.54 ± 0.34	6.74 ± 0.17	6.94 ± 0.15	5.92 ± 0.13	6.04 ± 0.18	4.68 ± 0.19	4.90 ± 0.21	2.88 ± 0.20	3.10 ± 0.21	3.06 ± 0.23	3.72 ± 0.27
p		ΔC; □3, 4, 5, 6, 7, 8, P	ΔC; □3, 4, 5, 6, 7, 8, P	□C, 1, 2, 5, 6, 7, 8, P	□C, 1, 2, 5, 6, 7, 8, P	□C, 1, 2, 3, 4, 7, 8	□C, 1, 2, 3, 4, 7, 8	□C, 1, 2, 3, 4, 5, 6, P	□C, 1, 2, 3, 4, 5, 6, P		□C, 1, 2, 3, 4, 7, 8
FSH, mIU/ml	2.34 ± 0.28	2.73 ± 0.17	2.69 ± 0.19	3.03 ± 0.16	2.97 ± 0.16	3.41 ± 0.18	3.15 ± 0.16	4.47 ± 0.21	4.35 ± 0.18	4.38 ± 0.16	3.86 ± 0.24
p		•C, 3, 4; Δ6; □5, 6, 7, 8, P	•C, 3, 4; Δ6; □5, 7, 8, P	•1, 2; ΔC, 5, P; □7, 8	•1, 2; ΔC, 5, P; □7, 8	•6; Δ3, 4; □C, 1, 2, 7, 8	•5, P; Δ1, 2; □C, 7, 8	□C, 1, 2, 3, 4, 5, 6, P	□C, 1, 2, 3, 4, 5, 6, P		•6; Δ3, 4; □C, 1, 2, 7, 8
E2, pg/ml	280.2 ± 30.39	220.8 ± 34.46	232.8 ± 30.79	198.8 ± 29.07	203.8 ± 30.61	168.8 ± 26.99	174.2 ± 25.59	147.6 ± 25.55	158.2 ± 20.78	157.4 ± 23.56	198.6 ± 23.59
p		•C, 5, 6; Δ7, 8	•C, 6; Δ5, 7, 8	•7, 8; ΔC	•7, 8; ΔC	•1; Δ2; □C	•1, 2; □C	•3, 4, P; Δ1, 2; □C	•3, 4, P; Δ1, 2; □C		•7, 8; ΔC

Notes: FEC – the first estrous cycle after a course of chemotherapy; LEC – the last estrous cycle before the next course of chemotherapy. Statistically significant difference relative to the indicated groups: • – $p < 0.05$; Δ – $p < 0.01$; □ – $p < 0.001$.

motherapy and partial regeneration of tissues made it possible to restore slightly the levels of E2, and accordingly, to reduce slightly the levels of FSH after each course of chemotherapy.

Administration of PRP made it possible to significantly ($p < 0.05$) reduce FSH levels in experimental animals by 11.3%.

DISCUSSION

The FAC chemotherapy is a combination of three chemotherapeutic agents, each of which creates an ovariotoxic effect of varying severity, affecting different pathogenetic links. 5-Fluorouracil is a widely used chemotherapeutic drug from the group of antimetabolites. Apart from reported slight ovarian toxicity of 5-fluorouracil [17, 18], the latest data indicate the reversibility of such changes in the ovaries [19].

Doxorubicin inhibits the topoisomerase II enzyme, causing DNA damage and apoptosis induction. The mechanism of doxorubicin toxicity is complex and not fully understood [17, 20]. A number of studies both *in vitro* and *in vivo* demonstrated the doxorubicin-induced ovarian toxicity [21–24].

The toxic effects of cyclophosphamide, an alkylating agent, are caused by damage to DNA and cell destruction through p53-mediated apoptosis [25]. Reproductive toxicity of cyclophosphamide is manifested by premature chemo-induced ovarian failure, which has been demonstrated in a plethora of studies [26–30].

In our study, immediately after each regular course of chemotherapy, there was a sharp increase in the levels of FSH, as well as a sharp decrease in the levels of AMH and E2. A characteristic feature was that the indicators partially recovered during the period between chemotherapy courses, which, at least in part, can be explained by the combination of an acute ovariotoxic effect and a chronic cumulative toxic effect of the drugs used.

The dynamics of the studied hormones after a series of 3 local intra- and periovarian injections of PRP demonstrated an improvement in ovarian function with a significant increase in AMH and E2 levels, and decrease in FSH levels, although without reaching the levels in the control group. In our opinion, this effect of PRP can probably be explained by stimulation of neoangiogenesis, improvement of ovarian tissue trophism and activation of ovarian follicular apparatus. However, this hypothesis requires further thorough pathomorphological research.

Thus, our study demonstrated that FAC chemotherapy in the experiment causes premature ovarian failure, which is manifested by a progressive and reliable decrease in AMH and E2 levels and an increase in FSH level. Local serial intra- and periovarian administration of PRP to rats with chemo-induced premature ovarian failure considerably improves ovarian function, significantly increasing AMH and E2 levels, and decreasing FSH levels.

FUNDING

The study was carried out as part of the research project of National Pirogov Memorial Medical University, Vinnytsya "Prediction and Prevention of Disorders of the Development and Function of the Female Reproductive System in Different Age Periods and Correction of Detected Disorders" (0116U005804).

REFERENCES

1. **Momenimovahed Z, Salehiniya H.** Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer (Dove Med Press) 2019; **11**: 151–64. doi: 10.2147/BCTT.S176070

2. **DeSantis CE, Ma J, Goding Sauer A, *et al.*** Breast cancer statistics, 2017, racial disparity in mortality by state. CA Cancer J Clin 2017; **67**: 439–48. doi: 10.3322/caac.21412

3. **Ghoncheh M, Mohammadian-Hafshejani A, Salehiniya H.** Incidence and mortality of breast cancer and their relationship to development in Asia. Asian Pac J Cancer Prev 2015; **16**: 6081–7. doi: 10.7314/apjcp.2015.16.14.6081

4. **Ghoncheh M, Momenimovahed Z, Salehiniya H.** Epidemiology, incidence and mortality of breast cancer in Asia. Asian Pac J Cancer Prev 2016; **17**: 47–52. doi: 10.7314/apjcp.2016.17.s3.47

5. **Hortobagyi GN, de la Garza Salazar J, Pritchard K, *et al.*** The global breast cancer burden: variations in epidemiology and survival. Clin Breast Cancer 2005; **6**: 391–401. doi: 10.3816/cbc.2005.n.043

6. **Tecza K, Pamula-Pilat J, Lanuszewska J, *et al.*** Pharmacogenetics of toxicity of 5-fluorouracil, doxorubicin and cyclophosphamide chemotherapy in breast cancer patients. Oncotarget 2018; **9**: 9114–36. doi: 10.18632/oncotarget.24148

7. **Montoya JE, Luna HG, Morelos AB, *et al.*** Association of creatinine clearance with neutropenia in breast cancer patients undergoing chemotherapy with fluorouracil, doxorubicin, and cyclophosphamide (FAC). Med J Malaysia 2013; **68**: 153–6.

8. **Silva C, Caramelo O, Almeida-Santos T, *et al.*** Factors associated with ovarian function recovery after chemotherapy for breast cancer: a systematic review and meta-analysis. Hum Reprod 2016; **31**: 2737–49. doi: 10.1093/humrep/dew224

9. **Cathcart-Rake EJ, Ruddy KJ, Bleyer A, *et al.*** Breast cancer in adolescent and young adult women under the age of 40 years. JCO Oncol Pract 2021; **17** : 305–13. doi: 10.1200/OP.20.00793

10. **Ra Hara G, Basu T.** Platelet-rich plasma in regenerative medicine. BMRAT [Internet] 2014; **1**: 25–31. doi: 10.7603/s40730-014-0005-6

11. **Graiet H, Lokchine A, Francois P, *et al.*** Use of platelet-rich plasma in regenerative medicine: technical tools for correct quality control. BMJ Open Sport Exerc Med 2018; **4**: 1111–5. doi: 10.1136/bmjsem-2018-000442

12. **Pavlovic V, Ciric M, Jovanovic V, *et al.*** Platelet rich plasma: a short overview of certain bioactive components. Open Med (Wars) 2016; **11**: 242–7. doi: 10.1515/med-2016-0048

13. **Everts P, Onishi K, Jayaram P, *et al.*** Platelet-rich plasma: new performance understandings and therapeutic considerations in 2020. Int J Mol Sci 2020; **21**: 7794. doi: 10.3390/ijms21207794

14. **Lichtarska AM, Sokol MM.** The mechanism of action of platelet-rich plasma — composition analysis and safety assessment. Curr Issues Pharm Med Sci 2021; **34**: 212–7. doi: 10.2478/cipms-2021-0038

15. **Fu XY, Chen HH, Zhang N, *et al.*** Effects of chronic unpredictable mild stress on ovarian reserve in female rats: Fea-

sibility analysis of a rat model of premature ovarian failure. Mol Med Rep 2018; **18**: 532–40. doi: 10.3892/mmr.2018.8989

16. **Nair AB, Jacob S.** A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 2016; **7**: 27–31. doi: 10.4103/0976-0105.177703

17. **Ben-Aharon I, Shalgi R.** What lies behind chemotherapy-induced ovarian toxicity? Reproduction 2012; **144**: 153–63. doi: 10.1530/REP-12-0121

18. **Bedoschi G, Navarro PA, Oktay K.** Chemotherapy-induced damage to ovary: mechanisms and clinical impact. Future Oncol 2016; **12**: 2333–44. doi: 10.2217/fon-2016-0176

19. **Naren G, Wang L, Zhang X, *et al.*** The reversible reproductive toxicity of 5-fluorouracil in mice. Reprod Toxicol 2021; **101**: 1–8. doi: 10.1016/j.reprotox.2021.02.002

20. **Sritharan S, Sivalingam N.** A comprehensive review on time-tested anticancer drug doxorubicin. Life Sci 2021; **278**: 119527. doi: 10.1016/j.lfs.2021.119527

21. **Aziz AUR, Yu X, Jiang Q, *et al.*** Doxorubicin-induced toxicity to 3D-cultured rat ovarian follicles on a microfluidic chip. Toxicol In Vitro 2020; **62**: 104677. doi: 10.1016/j.tiv.2019.104677

22. **Ben-Aharon I, Bar-Joseph H, Tzarfaty G, *et al.*** Doxorubicin-induced ovarian toxicity. Reprod Biol Endocrinol 2010; **8**: 20. doi: 10.1186/1477-7827-8-20

23. **Xiao S, Zhang J, Liu M, *et al.*** Doxorubicin has dose-dependent toxicity on mouse ovarian follicle development, hormone secretion, and oocyte maturation. Toxicol Sci 2017; **157**: 320–9. doi: 10.1093/toxsci/kfx047

24. **Zhang T, He WH, Feng LL, *et al.*** Effect of doxorubicin-induced ovarian toxicity on mouse ovarian granulosa cells. Regul Toxicol Pharmacol 2017; **86**: 1–10. doi: 10.1016/j.yrtph.2017.02.012

25. **Voelcker G.** The mechanism of action of cyclophosphamide and Its consequences for the development of a new generation of oxazaphosphorine cytostatics. Sci Pharm 2020; **88**: 42. doi: 10.3390/scipharm88040042

26. **Marcozzi S, Rossi V, Salvatore G, *et al.*** Distinct effects of epirubicin, cisplatin and cyclophosphamide on ovarian somatic cells of prepuberal ovaries. Aging (Albany NY) 2019; **11**: 10532–56. doi: 10.18632/aging.102476

27. **Hao X, Anastácio A, Viñals-Ribé L, *et al.*** Follicle rescue from prepubertal ovaries after recent treatment with cyclophosphamide-An experimental culture system using mice to achieve mature oocytes for fertility preservation. Front Oncol 2021; **11**: 682470. doi: 10.3389/fonc.2021.682470

28. **Pascuali N, Scotti L, Di Pietro M, *et al.*** Ceramide-1-phosphate has protective properties against cyclophosphamide-induced ovarian damage in a mice model of premature ovarian failure. Hum Reprod 2018; **33**: 844–59. doi: 10.1093/humrep/dey045

29. **Elkady MA, Shalaby S, Fathi F, *et al.*** Effects of quercetin and rosuvastatin each alone or in combination on cyclophosphamide-induced premature ovarian failure in female albino mice. Hum Exp Toxicol 2019; **38**: 1283–95. doi: 10.1177/0960327119865588

30. **Singh G, Misra R, Aggarwal A.** Ovarian insufficiency is major short-term toxicity in systemic lupus erythematosus patients treated with cyclophosphamide. J Assoc Physicians India 2016; **64**: 28–31.

ОВАРІАЛЬНА ТОКСИЧНІСТЬ FAC-СХЕМИ ХІМІОТЕРАПІЇ В ЕКСПЕРИМЕНТІ ТА МОЖЛИВОСТІ ЇЇ КОРЕКЦІЇ ПЛАЗМОЮ, ЗБАГАЧЕНОЮ ТРОМБОЦИТАМИ

***В.А. Шамрай*[1, 2], *О.І. Місюрко*[1, 2], *Д.І. Гребенюк*[1], *І.В. Таран*[1]**

[1]Вінницький національний медичний університет ім. М.І. Пирогова, МОЗ України, Вінниця, 21018, Україна

[2]Подільський регіональний центр онкології, МОЗ України, Вінниця, 21000, Україна

Мета: Вивчити динаміку змін гормонального статусу самок щурів в умовах застосування FAC-схеми хіміотерапії та після локального введення плазми, збагаченої тромбоцитами. ***Матеріали та методи***: Дослідження проведено на 55 самках щурів лінії Wistar віком до 1 року та масою тіла 160,0–216,0 г. Проводили хіміотерапію за FAC-схемою — 5-фторурацил (83,7 мг/кг), доксорубіцин (8,37 мг/кг) та циклофосфамід (83,7 мг/кг) у вигляді 4 курсів з інтервалом 3 тиж. Для вивчення оваріотоксичного впливу FAC-схеми хіміотерапії визначали рівні антимюллерового гормону, естрадіолу та фолікулостимулювального гормону у фазі проеструсу. Через 3 тиж після останнього курсу хіміотерапії 5 щурам виконували локальне інтра- та періоваріальне введення плазми, збагаченої тромбоцитами, об'ємом 0,1 мл на кожен яєчник. Після цього таку маніпуляцію повторювали ще двічі з інтервалом в 1 тиж між процедурами. ***Результати***: Динаміка рівнів усіх досліджуваних гормональних маркерів оваріального резерву характеризувалася прогресивним зниженням рівнів антимюллерового гормону та естрадіолу і підвищенням рівня фолікулостимулювального гормону. Динаміка досліджуваних показників після серійного введення плазми, збагаченої тромбоцитами, продемонструвала покращення гормонального статусу. ***Висновок***: Хіміотерапія за FAC-схемою в експерименті викликає хіміоіндуковану передчасну недостатність яєчників. Локальне введення плазми, збагаченої тромбоцитами, дозволяє покращити гормональні показники оваріального резерву в експерименті.

Ключові слова: FAC-схема хіміотерапії; оваріальна токсичність; плазма, збагачена тромбоцитами; антимюллерів гормон; естрадіол; фолікулостимулювальний гормон.

Copyright © Experimental Oncology, 2022