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Reproductive side effects of fluorouracil

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Abstract

Chemotherapy has greatly extended the life span of cancer patients. However, the drugs used in chemotherapy often induce un-ignorable side effects. 5-fluorouracil (5-FU) has been widely used as a chemotherapeutic agent in the clinical treatment of various cancers and many studies showed its adverse effect on reproduction. Reproductive toxicity of 5-FU often associates with developmental block, malformation and ovarian damage in the females. In the males, 5-FU administration alters the morphology of sexual organs, the levels of reproductive endocrine hormones and the progresses of spermatogenesis, ultimately reducing sperm numbers. However, some studies suggested that the toxicity of 5-FU on reproduction is reversible and certain drugs used in combination with 5-FU during chemotherapy could protect reproductive systems from 5-FU both in females and males. Herein, we summarize and discuss the reproductive toxicity of 5-FU and provide a reference for future research and clinical treatment.

Keywords: Fluorouracil; Reproductive toxicity; Embryonic malformation; Ovary; Folliculogenesis; Spermatogenesis; Testis.

Introduction

5-fluorouracil (5-FU) has been widely used as an anti-cancer drug for its tumor suppressor activity since 1957. After administration, 5-FU is catabolized into fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP) in the body. These metabolites disrupt the function of Thymidylate Synthetase (TS) and the RNA synthesis. FdUMP binds with TS and Folate 5, 10-methylenetetrahydrofolate to form a stable Ternary complex named CH2THF, which blocks the binding of normal deoxyuridine monophosphate (dUMP) and inhibits the synthesis of deoxythymidine monophosphate (dTMP). dTMP is a key substrate for the production of deoxythymidine triphosphate (dTTP), and the imbalance of dTMP and dTTP severely impacts DNA synthesis and repair, inhibiting cells growth and killing tumor cells ultimately (Longley et al, 2003). It is currently believed that 5-FU exerts its anti-tumor effect mainly through inhibiting TS (Baba et al, 2003), and TS inhibition is a key component of the mechanism of 5-FU-induced developmental toxicology.

With the continuous development of modern oncology and pharmacology, a series of 5-FU derivatives have been developed, which includes 1-hexylcarbamoyl-5-fluorouracil, uracil tegafur (UFT), capecitabine, tegafur, TAS-102, gimeracil and oteracil porassium etc. These derivatives are widely used in the treatment of various malignant tumors such as digestive system, lung, and breast cancers. Some studies reported that 5-FU and its derivatives used during cancer treatment induce severe adverse effects on reproduction. Therefore, many attempts have been done to alleviate the reproductive toxicity and found that combined administration of 5-FU with FOLFOX, Triptorelin, AGT (06-alkylguanine-DNA alkyltransferase) or IFPL (iridoids-rich containing fraction of Pentas. lanceolata leaves) decrease the reproductive side effect both in females and males. Of note, the treatment plan, type and dosage of chemotherapy drugs are extremely important for future fertility and reproduction, which may interrupt one or more parts of the reproductive function. Several studies showed that 5-FU lead to teratogenicity and the incidence and severity of malformations in embryos and fetuses are related closely to the treatment intensity. However, the underlying mechanism of 5-FU induced reproductive toxicity remains mostly elusive, and currently there is no effective way to avoid the reproductive damage completely.

Adverse effect of 5-FU on the female reproductive system

Embryotoxicity is one of the most common side effects observed after exposure to 5-FU (Table1). Both in vivo and in vitro studies suggested that the adverse effect of 5-FU on embryonic development is related to its dosage. An in vivo study demonstrated that the number of externally malformed fetuses increased in a dose-dependent fashion after administering pregnant rats with a single dose of 5-FU. Similarly, in mice 5-FU exposure reduced embryo implantation rate, increased embryo deformity and mortality proportionally with dose and treatment time. When rat embryos were cultured in vitro using the whole embryo culture system to study developmental toxicity, 5-FU dose-dependently induced embryonic malformations represented by tail and hindlimb bud defects. Of note, when injected into pregnant mice, 5-FU was incorporated by the embryos and accumulated mostly in the RNA. Although the incorporated amount varied between different strains, the amount of 5-FU was positively correlated with the weight of the embryos and the dose administrated.

The mammalian fertility cycle is responsible for the coordination of various cellular events, including ovarian follicle cell DNA synthesis, and of potential importance to the toxicity of 5-FU. Female mice received 5-FU during estrous phase were suffered greater fertility loss compared to those exposed to 5-FU during the metestrus, diestrus, and proestrus stages. This is probably because highest amount of ovarian follicular DNA synthesis happens within the Estrus cycle and 5-FU hinders DNA synthesis. Thus, it is likely that optimizing the fertility cycle timing of 5-FU treatment might be helpful for diminishing the

reproductive damage. Of note, in a clinical report, 3 pregnant women were exposed inadvertantly to 5-FU for the treatment of genital infections before their pregnancies become known, but all the three individuals delivered normal-appearing infants, indicating that short exposure to 5-FU does not always lead to embryotoxicity.

In C. elegans, 5-FU induces germ cell death and inhibits embryonic and larval development, which presumably due to cell-cycle arrest and apoptosis of germline cells. Additionally, 5-FU reduces mitotic nuclei per gonad arm by 30-40% and also down-regulates LIN-29, which is an important transcription factor that affects vulva development and egg laying system. Interestingly, down regulation of the uracil-DNA glycosylase (UNG) could alleviate the 5-FU effects on embryo hatching, suggesting that UNG-mediated removal of the misincorporated 5-FU plays an important role during this process. Indeed, it has been proposed that UNG-1 excise uracil, but the subsequent repair synthesis results in uracil reincorporation, leading to futile cycling of the BER pathway. It is well characterized that the dihydropyrimidine dehydrogenase (DPD) is the first rate-limiting enzyme in the catabolism of 5-FU, whose deficiency leads to impaired decomposition of 5-FU, resulting in severe cytotoxicity. Indeed, overexpression of the homologs of DPD （DPYD-1） and TS （Y110A7A.4） in C. elegans prevented the death of reproductive cells following 5-FU exposure. In contrast, depletion of DPYD-1 increased the 5-FU sensitivity and depletion of 110A7A.4 resulted in severe embryonic lethality. Consistently, biological modeling of the developmental toxicity of 5-FU also indicated that TS inhibition, and the resultant defects in DNA synthesis and disruption of cell cycle represent a crucial mechanistic pathway in the developmental toxicology 5-FU. Furthermore, recent transcriptome analysis revealed that 4 possible pathways, including ECM-receptor interaction pathway, TGF-beta signaling pathway, Focal adhesion and Hypertrophic cardiomyopathy are impaired in C. elegans embryos after 5-FU treatment, of which ECM-receptor interaction pathway and Focal adhesion are important pathways that likely alter the reproduction of C. elegans.

Cytotoxic action of 5-FU has potential gonadotoxic effect to induce ovarian dysfunction, which puts the patients at risk of menopause-related complications and infertility. When young C57BL/6J female mice were injected with 5-FU, all types of follicles were significantly decreased and secondary follicles were lost totally. Furthermore, genes involved in apoptosis and Wnt signaling pathways were significantly increased when ovaries from young mice were cultured in vitro with 5-FU, suggesting that presence of 5-FU deleteriously affect ovarian function. In adult mice, administration of 5-FU induced atresia of secondary and antral follicles, and profoundly reduced corpus luteum counts, leading to decreased ovarian volume. However, primordial or primary follicles were not affected by the 5-FU treatment, whose subsequent growth presumably contributed to the observed recovery of the ovarian function, which in turn suggesting that the reproductive toxicity of 5-FU might be not permanent. In support of this view, we recently reported that multiple intraperitoneal injections of 5-FU in adult female mice resulted in small ovarian size and reduced number of corpus luteum in the ovary, and led to ovulation failure. However, these defects could be recovered and the 5-FU treated adult females gave birth to healthy offspring. Our in vitro experiments further showed that exposure to 5-FU inhibited oocytes maturation and reduced developmental potential of pre-implantation embryos. Therefore, it is likely that 5-FU has negative impact on ovarian function, oocyte and early embryonic development, but the adverse effect could be reversed after withdrawal of 5-FU administration. Interestingly, when used in conjugation with 5-FU, Triptorelin could ameliorate expression of hormones and ovarian index in rats. Triptorelin is a GnRH agonist and often used as a hormone-responsive cancer drug, that probably alleviate the adverse effects of 5-FU via regulating hormone secretion and inhibiting apoptosis.

Effect of 5-FU on the male reproductive system

5-FU also induces male reproductive system injury, especially exposure before prepubertal period or adulthood impacts spermatogenic progression, sperm nuclear quality, and health of offspring (Table2). In male rats, 5-FU cause sloughing of epithelium and giant cell formation. In addition to its direct cytotoxic effect on germ cells, 5-FU also regulates endocrine level of the male reproductive system and significantly decreases the serum levels of testosterone, activin A and prolactin. Within 2 weeks of 5-FU administration, the levels of serum GnRH and pro-alpha C were increased, while the levels of serum inhibin B were decreased, accompanied by morphological changes in sertoli cells. Additionally, weights of reproductive organs including the seminal vesicle and the prostate were significantly decreased and tubular shrinkage, atrophy, and abnormal sperm cell shape were frequently observed after 5-FU administration in rats, eventually reducing sperm count in a dose and time-dependent manner.

Combinatory use of certain agents also relives the side effects of 5-FU on male reproductive system. N-(2-chloroethyl)-N-nitrosourea (CNU) is an alkylating agent often used in combination with 5-FU against a range of cancers. B.4152 is a product of combination of these two drugs and only induces minor damage in spermatogenic tissue when administrated in mice. Another agent, IFPL (iridoids-rich containing fraction of Pentas. lanceolata leaves) may also have a protective role in the 5-FU induced morphological sperm defects.

Conclusion

Numerous studies have demonstrated that 5-FU inevitably induces reproductive toxicity both in female (Table 1).

Year	Dosage and treatment time	Model	Main findings with respect to 5-fluorouracil	Sites of damage
1960	10-40 mg/kg 5-FU	Mice; embryos	Fetal malformations; Tail defects; Skull defects; Limb defects	Fetus; Tail; Limb; Skull
1966	250μg 5-FU	Pregnant rats	Fetal malformation; fetal size and weight reduction	Fetus

1987	1-8μm/ml 5-FU	Mice; embryos	Malformation frequency increased; tail dysmorphogenesis and telencephalic hypoplasia	Tail; Limb; Palate
1990	5-FU inadvertant exposure	Pregnant women	Non-toxic	/
1995	0-40mg/kg 5-FU	Gestation rats	TS inhibition; defects in DNA synthesis; cell cycle arrest; hindlimb defects	Rat
1998	10-30mg/kg 5-FU in vivo; 0.15-0.30 μg/ml 5-FU in vitro	Gestation Rats; Embryos	Fetal malformations; hypoplastic optic vesicles	Fetus
1999	200mg/kg of 5-FU	Mice	Fertility lost; lower weight pups	Fertility; Pup
2008	2, 5 and 400 nM 5-FU	Caenorhabditis elegans	Overexpression of DPD and TS homologs suppressed germ cell death; DPYD-1 and Y110A7A.4 depletion resulted in embryonic death	Germ cell; Embryo
2008	400μM 5-FU soaked for 6h; 400 nM 5-FU plated for 72h	Caenorhabditis elegans	Germ cell death; embryonic development blocked; larvae arrest	Embryo; Larvae; Germ cells
2010	0.15-4.8 g/ml 5-FU	Caenorhabditis elegans	Cell-cycle arrest; germ cell apoptosis; number of mitotic nuclei per gonad arm reduced; LIN-29 expression reduced; vulva development affected; egg laying delayed	Germ cell; Vulva
2015	200 mM 5-FU; 5 μM, 10 μM and 20 μM 5-FU	Caenorhabditis elegans	The embryos decreased in number and defective in development; altered gene expressions	Embryo
2015	80 mg/kg 5-FU; 0.1 mg/kg Triptorelin	Rats; Ovary	Decreased level of AMH, Bcl-2, NF-kB, body and ovarian weight; increased level of E2, FSH and Bax	Ovary; Hormone
2018	150 mg/kg 5-FU	Mice; Ovary	Secondary follicles and antral follicles increased; corpora lutea reduced; atresia rates returned to normal in 7 days	Ovary; Follicle
2018	125 mg/kg 5-FU for 3 times	Mice; Ovary	Growing follicles reduced; ovarian volume decreased; luteum counts reduced	Ovary; Follicle
2021	450 mg/kg 5-FU in vivo; 9.2, 46.1, 92.2 mM of 5-FU in vitro	Mice; Ovary	Follicles number reduced & secondary follicles lost; increased level of Bax/Bcl2, Wnt2, Wnt4; β-catenin immunolabeling in preantral follicles decreased	Ovary; Follicle
2021	50 mg/kg FU for 4 days in vivo; 50, 100 and 500 μM 5-FU in vitro	Mice; Ovary	Small ovarian size; lower number of corpus luteum; ovulation failure; oocytes maturation inhibited; embryos development reduced; all defects recovered after one week	Ovary; Oocyte; Embryo

Table 1: Adverse effects of 5-fluorouracil (5-FU) on female reproduction

And male species investigated to date. The adverse effects could be alleviated by combinatory use of certain agents, but the underlying mechanism needs further systematic investigation. Moreover, long- term reproductive effect of 5-FU treatment is still under debates and further in depth evaluation including systematic analysis of health condition and life span of the descendants should be performed. Particular attention must be paid to the fact that most of the existing

findings were obtained from studies carried out on healthy worm/ animals, and investigation on disease models may provide new, different insights. Despite decades of efforts, the molecular mechanisms of 5-FU induced reproductive toxicities is still mostly elusive and future studies applying metabolic and multi-omics analysis should elucidate further details **(Table 2).**

Year	Dosage and treatment time	Model	Main findings with respect to 5-fluorouracil	Sites of damage
1991	130 mg/kg 5-FU	Rats	Spermatid development arrest; abnormally shaped spermatids; sperm release failure; Increased Sertoli cell lipid; malorientation of spermatids	Testis
1996	25mg/kg B.4152 (CNU+5-FU)	Mice	Spermatocytes and spermatids depletion	Spermatocyte; Spermatid
2000	10, 50 and 100 mg/kg 5-FU	Rats	Epithelium sloughing; giant cell formation	Epithelium
2001	10, 50 and 100 mg/kg 5-FU	Rats	Testes weight, STD and SEH decreased; atrophic tubules; germ cells exfoliated	Testis; Tubule; Germ cells
2002	0, 20 and 30 mg/kg 5-FU for 2-week or 4-week	Rats	Degeneration of seminiferous epithelium; weight of the seminal vesicle and prostate reduced; testosterone level decreased; activin A and prolactin decreased; GnRH and pro-alpha C increased; Serum inhibin B decreased	Testis Plasma hormone
2003	10, 20 and 30mg/kg 5-FU	Rats	Sperm count decreased	Epididymis
2020	75 mg/kg 5-FU +100, 200 and 300mg/kg IFPL	Mice	Percentage of CAs and morphological sperm defects increased; IFPL alleviated the defects	Sperm

Table 2: Adverse effects of 5-fluorouracil (5-FU) on male reproduction

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References:

1. D souza UJ. (2003) Toxic effects of 5-fluorouracil on sperm count in wistar rats. The Malaysian J Med Sci, 10: 1-43.
2. DAGG CP. (1960) Sensitive Stages for the Production of Developmental Abnormalities in Mice with 5-Fluorouracil. J Anat, 106: 89-96.
3. Donnez J, Godin PA, Qu J, Nisolle M. (2000) Gonadal cryopreservation in the young patient with gynaecological malignancy. Curr Opin Obstet Gynecol, 12: 1-9.
4. Hrushesky WJM, Vyzula R, Wood PA. (1999) Fertility maintenance and 5-fluorouracil timing within the mammalian fertility cycle. Reprod Toxicol, 13: 413-420.
5. Kim S, Park DH, Shim J. (2008) Thymidylate synthase and dihydropyrimidine dehydrogenase levels are associated with response to 5-fluorouracil in Caenorhabditis elegans. Mol. Cells, 26: 344-349.