Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 Online ISSN: 2311-6544
University of Kufa / Faculty of Education for Girls
Third International Scientific Conference 5-6/12 /2018

The Protective Effect of Vanadium on Cyclophosphamide-Induced Teratogenesis in Mouse Fetus

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Abstract

Cyclophosphamide(CP) is a chemotherapeutic and immunosuppressive drug used for treatment of neoplastic and some auto-immune diseases, but it has several important adverse effects and can induce external malformations in the fetus. Different materials can use to avoid or prevent its side effects. Trace elements as an antioxidant can prevent oxidative stress by binding to free radicals and reduce adverse effects of chemotherapy drugs. vanadium is present as an ultratrace element has prophylactic effects on teratogenic effects of CP. The aim of our work was to estimate the vanadium effect on CP-induced macroscopic fetal defects in mice. In this study pregnant NMRI mice were divided into four groups. control group received normal saline, CP group received CP (10 mg/kg on 11^{th} GD), V group received vanadium (4mg/kg on 8^{th},10^{th} and 12^{th}) and V/CP group CP (10 mg/kg on 11^{th} GD) with vanadium (4mg/kg on 8^{th},10^{th} and 12^{th}) intraperitoneally. Fetuses were collected on the 19^{th} GD. Then the weight of fetuses, length of crown-rump, limbs, and tail of fetuses were measured. The external teratogenesis were investigated by the stereomicroscope. The results revealed that the CP decreased fetal weight, and also reduced the length, limbs, and tail in fetuses that received only CP ($P \leq 0.05$). In V/CP the reduction in all previous parameters significantly increased compared with CP group. CP also caused some types of external teratogenesis such as a short neck, short limbs, non-separating fingers and toes from each other and malrotated hind limbs, oligodactyly, kinky tail, reduction in the distribution of hair follicles on the body surface, and hemorrhage on body surface, while did not notice in V/CP group. On the basis of results, vanadium has a significant prophylactic effect on preventing teratogenesis that induced via CP.

Keywords: Cyclophosphamide, Vanadium, Teratogenesis, Fetus, Mice.

Introduction

Fetal development abnormalities can be encouraged by drugs, diseases, pollutants, and radiation and that can induce oxidative stress and free radicals [1,2]. In experimental animals, cyclophosphamide (CP) can induce the oxidative stress [3]. CP is a common immunosuppressive and chemotherapy drug used in the treatment of a varied type of neoplastic and some auto-immune diseases [4]. CP considered as an active antimitogenic agent which interferes with mitosis and prevents the growth of cancer cell [5]. Hepatic metabolism of CP take place at mitochondrial level and become active by cytochrome P450 enzymes [6] and, generates two teratogenic metabolic products acrolein (ACR) and phosphoramide mustard (PM) [7, 5, 8]. PM affects DNA, while ACR has the proteins as its main target. PM is toxic to the embryo, and acrolein to the yolk sac [9] and responsible for the cyclophosphamide anticancer effects, but ACR interacts with the antioxidant immune system of the tissues and generates a large number of reactive oxygen species (ROS) that has mutagenic properties and can be easily combined with other molecules such as enzymes, receptors, ion pumps and caused direct oxidation and inactivating or inhibiting their functioning [10], thus causes the apoptosis[11].CP is classified within class D of drugs, therefore known as a pregnancy risk agent. Its use during pregnancy is acceptable only when

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potential maternal benefits greater than the possible risk such as birth defects [12]. Loss weight, stunning size, open eye, polydactyly, syndactyly, synpolydactyly, kinky tail and cleft palate are some of the fetal anomalies that induced by CP treatment during embryonic development [13,14,15].

The use of chemotherapy in cancer patients causes oxidative stress by production of free radicals. The use of supplements and vitamins such as vitamins E and A as well as some of the trace elements as an antioxidant can prevent oxidative stress by binding to free radicals and improve therapeutic methods and reduce its negative effects [16,17]. Metals and its compounds have been used for several years to treat diseases such as bismuth, antimony, arsenic, iron, titanium, gold, rhodium, gallium, platinum and vanadium, as arsenic, bismuth, gold, radium, titanium, gallium, platinum, and vanadium have anticancer activities [18]. Vanadium is one of an essential trace element in body of many species that is widely distributed on earth. Though, the role of vanadium as a micronutrient in man has yet to be established. Man and other species of animals may be received the metal from the atmosphere and food [19]. Foods contain low vanadium concentrations (about 1 ng/g), the dietary intake of vanadium varies between 10-06 µg /day, the plasma concentration is approximately 20 nM, and the total body pool is about 100-200 µg /day [20]. Vanadium can cross the placenta in mice and rats [21,22] and store in certain parts of the embryo. Edel and Sabbioni showed the storage of vanadium in the liver, intestines, kidneys, and bones take place [22]. Vanadium compounds in low concentration did not cause significant incidence of visceral and gross malformations in mouse fetuses [23]. Some biological effects of vanadium are an insulin-like effect [24,25] and decreased fat and blood pressure [26]. Experiments have shown no significant effect when vanadium used at doses lower than 40 mg/kg in adult male and female mice on the mating rate when it was taken before mating, but at higher doses, it reduced fertility rates. In addition, the administration of vanadium at low doses in pregnancy and lactation does not have a direct impact on the development of the embryo and the neonate, which proves that vanadium is a concentration-dependent element that can have different effects [27].

The aim of the current work was to study the effect of vanadium on cyclophosphamide-induced macroscopic fetal defects in mice.

Materials & Methods

Fifty-two NMRI adult female mice, of an average weight of 30±2 gm and an average age of 8-10 weeks were purchased from Pasteur Institute of Iran and used in this study. The animals were housed individually in plastic cages in noise-free, air-conditioned animal house of Biology Department, Science Faculty, Razi University (Kermanshah, Iran). Mice were fed ad libitum by standard laboratory pellet (Pars Company's feed, Iran) with temperature 22±2°C with a relative humidity of 30-40% maintained at and on a light-dark cycle of 12:12 hours. The experiment was conducted according to the norms of the Ethics Committee in Animal Experimentation of Razi university and registered under the protocol number 396-1-022. The female mice in their proestrous were caged overnight with males (Female: Male = 3:1), after which females were examined for evidence a vaginal copulatory plug. Presence of vaginal plug on the following morning established start of pregnancy and the day was numbered as the day 'zero' of gestation. Pregnant mice were randomly divided into four main experimental groups (n=13): control group was received saline for prompting parallel condition (injection and handling) to other groups. CP group received CP (Baxter company Oncology GmbH, Germany) at the dose (10 mg/ kg, intraperitoneally ip) on the 11th day of gestation (GD). This dose was chosen based on a previous dose-response study of Prakash *et al*., (2007) (12). V group received vanadium (Sigma company, USA) at dose (4 mg/kg, ip) at 8th, 10th and 12th GD, this dose was chosen according to a study of Zhang *et al*., (1991) (7), and V/CP group which received vanadium (4 mg / kg, ip) at 8th, 10th and 12th GD plus CP (10 mg / kg, ip) at the 11th GD. Pregnant mice were weighed and sacrificed on the 19th GD by cervical dislocation. Following a large incision in the animal's abdomen and uterus, fetuses were exposed and collected. The length of crown-rump, forelimbs and hind limbs, and tail in each fetus were measured by caliper (pied a coulisse) (Mitutoyo, Kanagawa, Japan). Also, the weight of each fetus was measured by sensitive balance (GR-120 A & D, Japan). All fetuses examined carefully under the stereomicroscope (Olympus IX-71. Japan) coupled with digital camera (D-D72.Olympus, Japan) for external anomalies.

Statistical analysis

All quantitative data derived from our investigation were analyzed by using the SPSS version 20 statistical package and compared by one-way analysis of variance (ANOVA). The results were expressed as the Stand Error of Mean (SEM). $P \leq 0.05$ was considered as statistical significance.

Results

The gross investigation of the fetuses showed a significant decrease in the mean of crown-rump length of fetuses that belong to CP group in comparison to control, V, and V/CP groups ($p \leq 0.05$), while in V/CP group the fetal crown-rump length did not show any significant difference with control and V groups, but showed a significant increase compared to CP group ($p \leq 0.05$) (Fig.1, Table1). The mean of fetal body weight in CP group exhibited a significant reduction compared with control, V, and V/CP groups ($p \leq 0.05$), while it didn't show any significant difference among control, V, and V/CP groups ($p \leq 0.05$) (Table 2).

Also, the macroscopic examination revealed a significant reduction in the mean of forelimbs and hind limbs length in CP group in comparison to control, V, and V/CP groups ($p \leq 0.05$), which in V/CP group did not show any difference with control and V groups, but showed a significant increase compared with CP group ($p \leq 0.05$) (Fig.1, Table 1). The mean of fetal tail length showed a significant decrease in CP group compared with control, V, and V/CP groups ($p \leq 0.05$), while it didn't show any significant difference among control, V, and V/CP groups ($p \leq 0.05$) (Table 1).

The macroscopic examination exhibited various external malformations, of which important were, short limbs, non-separating fingers and toes from each other, elephantiasis of forelimb, malrotated hind limbs, oligodactyly in hind limbs (Fig. 2,3), short neck, kinky tail, reduction in distribution of hair follicles on the body surface, and hemorrhage on body surface, while did not observed in control, V and V/CP groups The skin appearance of fetuses that undergo to CP during pregnancy period was mucoid, which was not observed in control, V, and V/CP groups during current study.

The maternal weight of pregnant mice at the dissecting day (19th GD) in CP group revealed a significant reduction in comparison with control, V and V/CP groups ($p \leq 0.05$), while in V/CP group which showed a significant decrease compared with control and V groups, but it showed a significant increase compared to CP groups ($p \leq 0.05$) (Table 2). No maternal deaths were observed throughout the course of this study, no fetus was absorbed and dead from all studying groups also. In addition, the results showed that some of the pregnant mice of CP group had premature accouchement at different times (day 16th and 17th) of a gestation period, all neonates born on the 16th day of gestation died after a few hours. On the other hand, some of the female offspring from pregnant mice of CP, V and V/CP groups, left them for maturing, at age 60 days mated with other healthy mature male mice, the female mice offspring from V and V/CP groups became pregnant normally, but those of CP group did not become pregnant. Some of these female mice when mated with mature male offspring from same treated groups, showed that the female mice from V and V/CP groups became pregnant normally, but those of CP group did not become pregnant.

Table 1: Mean of crown-rump, forelimbs, hind limbs, and tail in fetuses from pregnant mice who exposed to cyclophosphamide on 11th GD and treated with vanadium on 8th,10th, and 12th GD

Group	Control	CP	V	V/CP
Crown-rump Length (mm)	24.15±0.086[b]	21.10±0.083[a,c,d]	24.11±0.088[b]	23.53±0.085[b]
Forelimbs Length (mm)	7.066±0.023[b]	5.64±0.010[a,c,d]	6.98± 0.030[b]	6.95±0.021[b]
Hind limbs Length (mm)	7.04±0.018[b]	5.68±0.012[a,c,d]	6.93±0.031[b]	6.97±0.024[b]
Tail Length (mm)	11.25±0.150[b]	8.78±0.108[a,c,d]	10.88±0.230[b]	10.81±0.206[b]

[a] Significant compared with Control. [b] Significant compared with CP
[c] Significant compared with V [d] Significant compared with V / CP
Data are expressed as mean ± (SEM). (ANOVA: p≤0.05)
CP: cyclophosphamide, V: vanadium, V / CP: vanadium / cyclophosphamide

Table 2: Mean of maternal and fetal weight in pregnant mice and their fetuses who exposed to cyclophosphamide on 11th GD and treated with vanadium on 8th,10th and 12th GD

Group	Control	CP	V	V/ CP
Maternal weigh (gr)	48.326 ± 0.372[b,d]	39.428 ± 0.331[a,c,d]	47.983 ± 0.367[b,d]	45.978 ± 0.302[a,b,c]
Fetal Weight (gr)	1.37±0.014[b]	1.02±0.010[a,c,d]	1.35±0.015[b]	1.34± 0.012[b]

[a] Significant compared with Control. [b] Significant compared with CP
[c] Significant compared with V [d] Significant compared with V / CP
Data are expressed as mean ± (SEM). (ANOVA: p≤0.05)
CP: cyclophosphamide, V: vanadium, V / CP: vanadium / cyclophosphamide

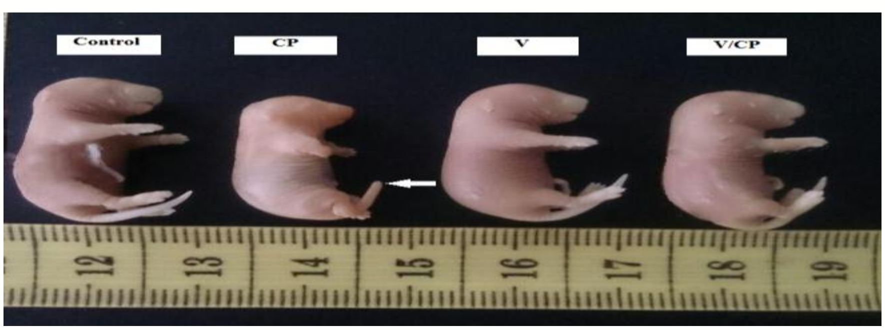

Fig 1. Photograph of crown-rump length in fetuses from pregnant mice who exposed to cyclophosphamide on 11th GD and treated with vanadium on 8th,10th and 12th GD
Short tail (white arrow) induced by CP.
Control: received normal saline, CP: cyclophosphamide, V: vanadium, V/CP: vanadium / cyclophosphamide

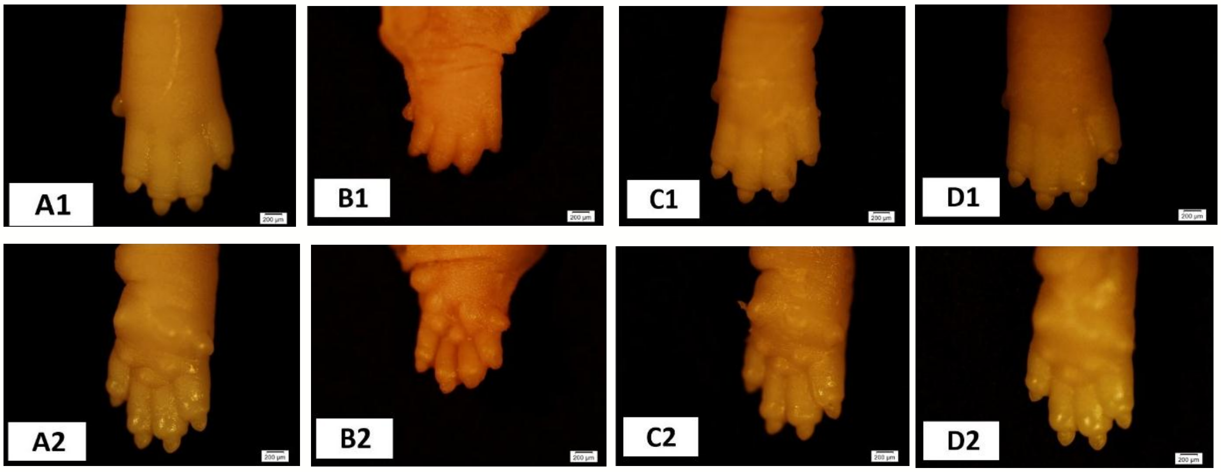

Fig 2. Photographs of dorsal and palmar view of forelimbs in fetuses from pregnant mice who exposed to cyclophosphamide on 11th GD and treated with vanadium on 8^{th},10^{th} and 12^{th} GD. (Scale bar 200 µm). B showed elephantiasis (large size of limb) induced by CP.
A) Control Group, B) CP Group, C) V Group, D) V/CP Group. 1: Dorsal View, 2: Palmar View.

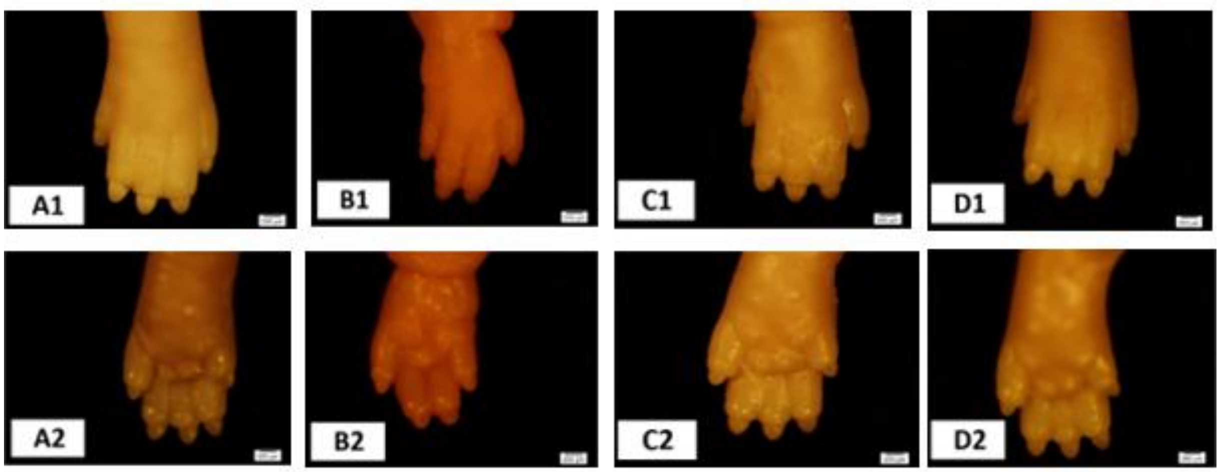

Fig 3. Photographs of dorsal and plantar view of hind limbs in fetuses from pregnant mice who exposed to cyclophosphamide on 11^{th} GD and treated with vanadium on 8^{th},10^{th} and 12^{th} GD. (Scale bar 200 µm). B showed oligodactyly induced by CP.
A) Control Group, B) CP Group, C) V Group, D) V/CP Group. 1: Dorsal View 2: Plantar View

Discussion

As there are no data accessible on vanadium on the teratogenicity of CP in fetuses' mouse. In the current study, for the first time, the protective effect of vanadium on the teratogenicity of CP in fetuses' mouse was estimated. In present investigation CP injected at dose 10 mg/kg, ip at 11^{th} GD induced a reduction in length of fetus, forelimbs, hind limbs, and tail, also it decreased the fetal weight among all fetuses who their mothers received CP only at 11^{th} GD. While the administration of vanadium at dose 4 mg/kg, ip on 8^{th},10^{th} and 12^{th} GD along with 10 mg/kg of CP on 11^{th} GD could improve the reduction in the length of fetus, forelimbs, hind limbs and tail, and fetal weight in fetuses that belong to V/CP group.

It is well known that CP causes fetal defects in different species of animals including mice, rats, hamsters, and rabbits as well as humans [28]. In the current study, the administration of CP 10 mg/kg, ip on 11th GD induced a significant growth retardation and morphological alterations in mouse fetuses. Gibson and Becker, (1986) reported that the body weight and crown-rump length of fetuses from pregnant mice who had been given CP 10 mg/kg, ip at 12th and 13th GD were reduced. Fetuses that treated with CP at doses 10 and 20 mg/kg on 11th dpc had different types of external malformations, of which important were stunting in size, short limbs, malrotated limbs, digits defects, kinking of the tail, and hemorrhage on body surface [29]. Intraperitoneal injection of a single dose of CP 20 mg/kg to pregnant rats at the 10th GD, induced kinky tail, and digital defects [13]. These results agree with our results which showed that the intraperitoneal injection of 10 mg/ kg of CP alone to pregnant mice on the 11th day of pregnancy, reduced a fetal weight, crown-rump length, movement limbs and tail length. Also, it induced digital defects such as oligodactyly and kinking of the tail. This may due to the findings of Mitchell *et al*., (2003) and Alenzi *et al*., (2010) that reported that the CP is one of the most damaging alkylating agents that can cause oxidative stress due to the over-production of reactive oxygen species (ROS) [30, 31]. Also, this drug affects the DNA of replicating cells and rapidly multiplying cells which result in miscoding, cross-linking and DNA breakage by transferring alkyl groups to the guanine compound of the DNA [32]

Mahabady *et al*., (2016) showed that the intraperitoneal administration of CP at dose 15 mg/kg to pregnant rats at 9th day of pregnancy caused significant growth retardation and morphological alterations in rat's fetuses, and also led to reduction the fetal weight and crown-rump length, and limb defects, when they injected CP plus quercetin 75 or 200 mg/kg ip at 9th GD, observed that the quercetin reduced the anomalies that induced via CP [33]. They determined fetal anomalies similar with current investigation including reduction in length and weight of fetuses, and limb defects. These defects were recovered by administration of 4 mg/kg, ip of vanadium on 8th,10th, and 12th GD.

Najafzadeh and Mahabadi evaluated the effect of *Echinacea purpurea* on CP-induced malformations. They injected 15 mg/kg, ip of CP to rats at 13th GD [34]. They determined fetal defects similar with our study including reduction in length and weight of fetuses, and limb defects. These abnormalities improved by using 4 mg/kg, ip of vanadium on 8th,10th, and 12th GD.

Prakash *et al.* [12] revealed that the intraperitoneal administration of CP at doses 10 and 20 mg/kg into pregnant mice on 11th GD caused a significant reduction in maternal weight gain during gestation. Zhao *et al*., [35] reported that after treatment the pregnant mice with 50 mg/kg cyclophosphamide, the maternal body weight was decreased compared with the control group. These results are in agreement with our results that showed that the intraperitoneal injection of a single dose 10mg/kg of CP caused a reduction in maternal weight. The reduction of maternal weight increased significantly by using of vanadium at a dose 4 mg/kg, ip on 8th,10th and 12th GD along with CP at the dose 10 mg/kg, ip at the 11th GD.

The maternal weight reduction may be due to gastrointestinal mucosa damaging that caused by CP in particular in the intestine, therefore food intake becomes difficult as well as the organs dwindle and adipose tissue atrophy [36].

Conclusion

According to our results in present study use CP at single dose of 10 mg/kg during second semester of pregnancy can induce external anomalies in fetuses such stunning size, lose weight, reduction in hair follicle distribution, oligodactyly, and kinky tail. CP can reduce the maternal weight also, while administration of 4 mg/kg of vanadium along with CP during the second semester of pregnancy could recover adverse effect of CP on the body of the fetus and maternal weight.

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