The Effect of β-Carotene Against Adriamycin Toxicity on the Embryo Formation

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Key words: *β-carotene; embryotoxicity; embryogenesis; Adriamycin.*

Summary. *Adriamycin is an anthracycline antibiotic widely used for the treatment of many types of cancer. The cytotoxic effect of Adriamycin occurs by a free radical-mediated mechanism. Thus, to prevent or reduce the toxic effect of Adriamycin, it is possible to use it in combination with antioxidants. The aim of this study was to evaluate a potential effect of β-carotene against Adriamycin-induced toxicity on the embryo formation.*

Materials and Methods. Pregnant rats were treated with Adriamycin, β-carotene, and their combination during the critical stages of embryogenesis. The first group was control group. Adriamycin was administered on day 9 (group 2a) and day 12 (group 2b) of gestation by a single intraperitoneal injection at a dose of 5 mg/kg. β-Carotene was given at a dosage of 0.6 mg/(kg·day) from day 6 to 10 or from day 9 (group 3a) to 13 (group 3b) of gestation 5 times per os; in the case of their combination, β-carotene was given per os 3 times before Adriamycin injection, one time simultaneously with Adriamycin and one time after its injection (groups 4a and 4b). Animals were euthanized on day 21 of gestation. Embryo resorptions and alive fetuses were counted, weighed, and measured. The embryos of each litter were examined macroscopically after the Buen solution fixation for the embryo defects. In order to render the skeleton visible, the soft tissues were macerated using caustic soda, stained with alizarin red, and cleared with glycerin.

Results. Adriamycin induced embryotoxicity; the combination of Adriamycin and β-carotene decreased the number of Adriamycin-induced embryo resorptions about two times. A gavage with Adriamycin alone decreased fetal body weights ($P<0.05$), while giving it in combination, the fetal body weight was similar to that in the control group. Adriamycin induced the retardation of skeletogenesis and external fetal malformations (microphthalmia, hydrocephaly, anencephaly, and others). After an exposure to β-carotene, external malformations (diaphragmatic hernia) of embryos were found only occasionally. β-Carotene in combination with Adriamycin produced no positive effect on Adriamycin-induced skeletodysgenesis or external malformations.

Conclusions. Antioxidant β-carotene in combination with Adriamycin slightly reduced the Adriamycin-induced embryotoxicity, but produced no positive effect on Adriamycin-induced skeletodysgenesis or external malformations.

Introduction

An antioxidant balance and the activity of antioxidative system that regulate the oxidative status are crucial tools for the homeostasis of the organism. Free radicals and other reactive oxygen species (ROS) cause the oxidation of biomolecules, such as proteins, amino acids, unsaturated lipids, and DNA, and ultimately produce molecular alterations related to aging, arteriosclerosis, and cancer. An imbalance between a free radical production and the activity of antioxidant system leads to a phenomenon known as oxidative stress. It is well documented that anticancer cytostatics including Adriamycin (doxorubicin) are associated with oxidative stress (1, 2).

The principal mechanism contributing to a cytostatic activity of Adriamycin is considered to result from its intercalation into the DNA molecule and an inhibition of topoisomerase II activity (3), while the side effects of Adriamycin are mainly mediated by a free radical generation and subsequent oxidative stress (4, 5).

The antioxidants may protect the cytostatics-induced oxidative stress and their provoked negative effects (6, 7).

The role of β-carotene in the prevention of Adriamycin-induced toxicity has not been established yet, especially in the cases of Adriamycin-induced embryotoxicity and teratogenicity. The present study was carried out with the aim to evaluate a potential

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protective effect of β-carotene against Adriamycin-induced toxicity on the embryo formation.

Materials and Methods

Animals and Treatment Schedule. The study of the effect of β-carotene against the Adriamycin-induced toxicity on the embryo formation was carried out on 400 fetuses from 50 rats: 8 rats for the control (group 1) and 7 rats for every experimental group on days 6–10 and 9–13 of embryogenesis (groups 2a, b; 3a, b; and 4a, b). Albino Wistar rats (9–11 weeks old) were obtained from the Animal Facility of the Institute of Immunology, Vilnius University. Animals were housed under conditions of constant temperature, humidity, and standard light/dark cycle (12 h/ 12 h). Food and fresh drinking water were available *ad libitum*. The study was approved by the Lithuanian Animal Care and Use Committee (No. 0019; 2001–2005).

After being acclimated for at least 7 days, female rats were mated overnight with males of the same strain. Vaginal smears from each female rat were collected and subjected to microscopic examination on the following morning in order to determine the estrous cycle and the presence of sperm. The day of sperm detection in vaginal smears was designated as day 0 of gestation.

Critical periods in the embryogenesis, which have to be found to be most sensitive for embryo formation, are defined as follows: days 6–9 of embryogenesis, implantation and early organogenesis; and 10–13 days, placenta formation and active organogenesis. Therefore, Adriamycin was administered by intraperitoneal injection at a dose of 5 mg/kg (a single maximum tolerated dose) on day 9 or day 12. β-Carotene was given at a dosage of 0.6 mg/kg (the dose, which has no toxic effect) 5 times per os from day 6 to 10 and from day 9 to 13 of embryogenesis. In the case of drug combination, β-carotene was given 3 times per os before Adriamycin injection, one time simultaneously with Adriamycin and one time after its injection on day 9 and day 12 of gestation. One group comprised vehicle-treated rats (control group, 1). The treatment schedule is presented in Fig. 1.

The experimental groups were as follows: group 1, control; group 2a, Adriamycin on day 9 of gestation; group 2b, Adriamycin on day 12 of gestation; group 3a, β-carotene on days 6–10 of gestation; group 3b, β-carotene on days 9–13 of gestation; group 4a, combination of Adriamycin and β-carotene on days 6–10 of gestation; group 4b, combination of Adriamycin and β-carotene on days 9–13 of gestation.

Embryotoxicity and Teratogenicity Analysis. All rats were subjected to the Caesarean section in the state of neuroleptanalgesia (Calipsol 0.5 mL per one rat) on day 21 of gestation. The uteruses were removed. After opening the uterus, the number and site of implantations, resorptions, and dead or alive fetuses were recorded in the uterine horn for the determination of the postimplantation mortality indices. The fetuses were weighed, measured, and after the Buen solution fixation examined for detection of external malformations. In order to render the skeleton visible, the soft tissues were macerated using caustic soda, stained with alizarin red, and

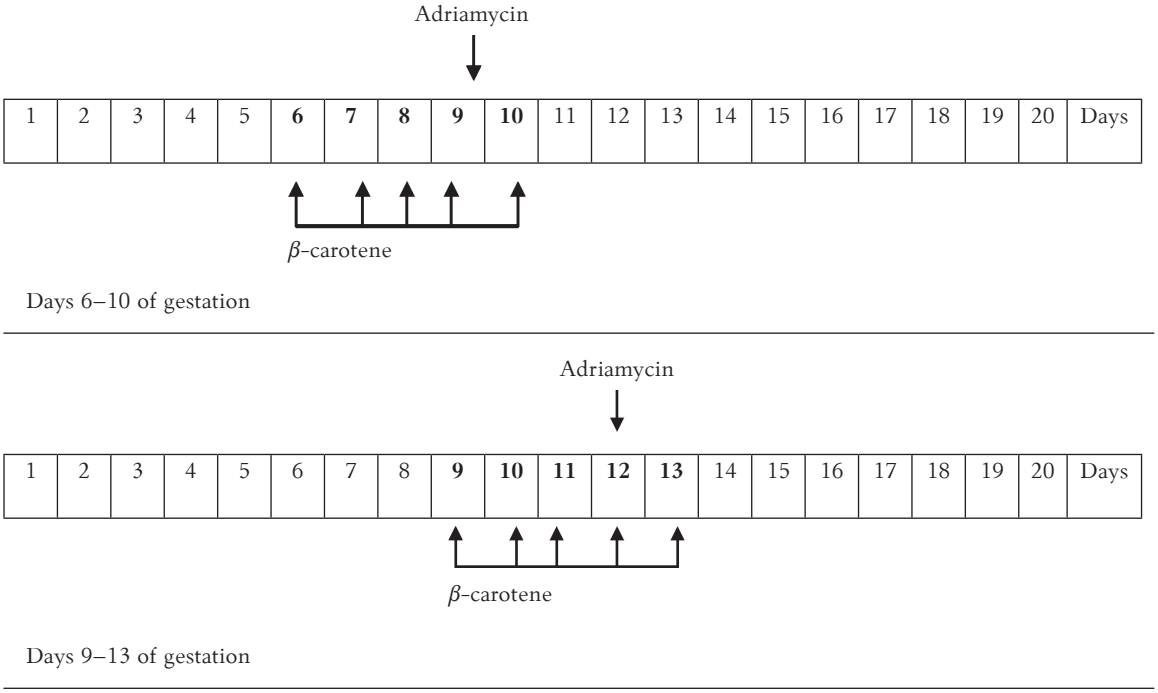

Fig. 1. Experimental groups and the design of the treatment

cleared with glycerin (7).

Comparison was performed using the two-tailed Student *t* test. A *P* value of <0.05 was considered statistically significant.

Results

The study of the effect of β-carotene against Adriamycin-induced toxicity on the embryo formation was carried out on 400 fetuses from 50 albino Wistar rats.

The results of the postimplantation mortality of embryos are presented in Fig. 2. Treatment with Adriamycin resulted in an increase in postimplantation mortality as compared with the control group ($P<0.05$). It must be noted that after an exposure to β-carotene alone, the postimplantation mortality was observed, but it was far less expressed than after an exposure to Adriamycin. The indices of postimplantation mortality were the same when β-carotene was given in combination with Adriamycin.

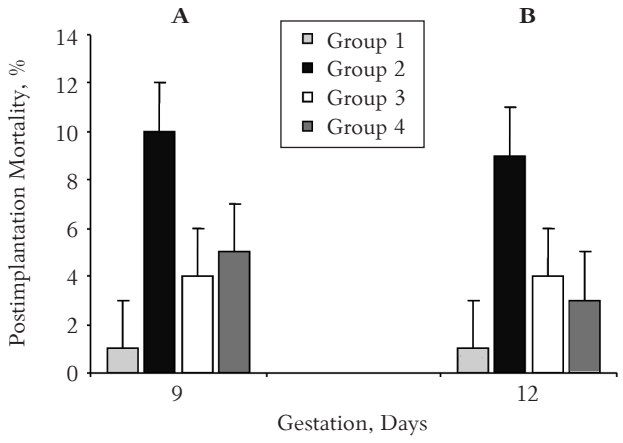

Fig. 2. Postimplantation mortality of embryos after the treatment of pregnant rats on days 9 (A) and 12 (B) of embryogenesis
1, control group; 2, Adriamycin (5 mg/kg); 3, β-carotene (0.6 mg/kg); 4, Adriamycin in combination with β-carotene.

After treatment with Adriamycin, a decrease in fetal body weight was noted in comparison with the control group, while after an exposure to the combination of Adriamycin and β-carotene, the fetal body weight was found to be similar to the fetal body weight in the β-carotene-treated group. On the other hand, the changes in the fetal body length were not considerable in all treated groups and did not differ much from the fetal body length in the control group (Fig. 3).

Adriamycin was found to induce the retardation of skeletodysgenesis and various types of external malformations (such as hydrocephaly, exencephaly, macrognathia, microphthalmia, bradydactyly, oligodactyly, and others) with a frequency of 6.3%, meanwhile after an exposure to β-carotene, external malformations (e.g., diaphragmatic hernia, body edema) were documented very rarely (Figs. 4 and 5). β-Carotene in combination with Adriamycin failed to produce a positive effect on Adriamycin-induced skeletodysgenesis or external malformations.

Discussion

Anticancer agents induce the production of free radicals and other ROS in biological systems. Cytotoxic effects of anticancer drugs depend on rapid proliferation of cancer cells. Oxidative stress occurring during cancer chemotherapy may interfere with those effects through reducing the rate of cell proliferation. ROS may also contribute to chemotherapeutic agent – induced side effects, such as Adriamycin-induced cardiotoxicity, cisplatin-induced nephrotoxicity, bleomycin-induced pulmonary fibrosis induced (9). Several strategies have been employed to help reduce the side effects of these anticancer drugs. Several lines of evidence suggest that antioxidants have a beneficial effect on reducing oxidative damage (10). Therefore, β-carotene, with abilities of antilipid peroxidation and scavenging semiquinone free radicals, possesses effects of

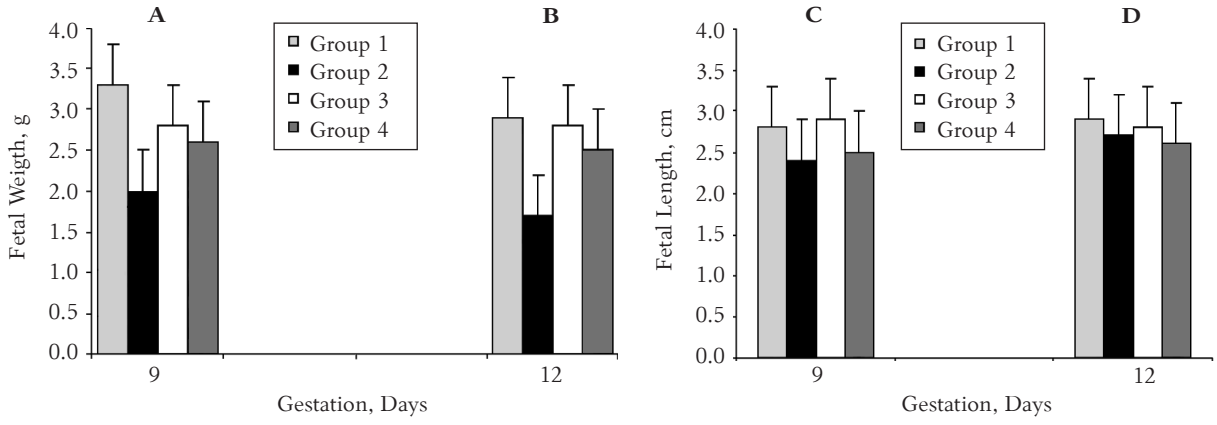

Fig. 3. Fetal weight (A, B) and length (C, D) after the treatment of pregnant rats on days 9 and 12 of embryogenesis
1, control group; 2, Adriamycin (5 mg/kg); 3, β-carotene (0.6 mg/kg); 4, Adriamycin in combination with β-carotene.

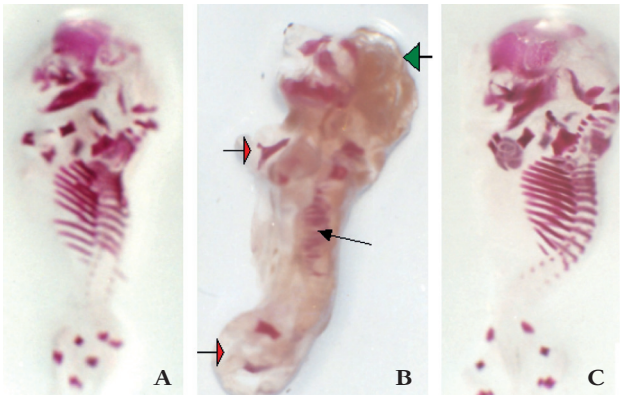

Fig. 4. Embryos after the treatment of pregnant rats on day 9 of skeletodysgenesis

A, control; B, Adriamycin (red arrow, unformed limbs; green arrow, skull bone; black arrow, deformed ribs); C, β-carotene (without retardation of skeletogenesis).

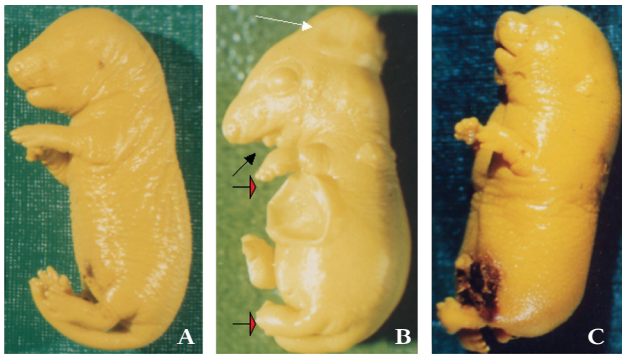

Fig. 5. Embryos after the treatment of pregnant rats on day 9 of embryogenesis

A, control; B, Adriamycin (white arrow, exencephaly; black arrow, micrognathia; red arrow, bradydactyly); C, β-carotene (body edema).

reducing doxorubicin-induced cardiotoxicity (11). Dietary vitamin E decreases doxorubicin-induced oxidative stress, but is not responsible for persistent mitochondrial cardiomyopathy caused by long-term doxorubicin therapy (12).

As many anticancer agents possess a wide toxicity to normal cells in experimental animals and humans, they are known to be embryotoxic and teratogenic (13–16).

Embryotoxic and teratogenic effects of some cytostatic agents were studied by the authors of this study. Embryotoxicity and teratogenicity of alkylating agents – Lophenal, which contains amino acid DL-phenylalanine, Phenalon containing physiologically active amino acid L-phenylalanine, pharanox phosphate containing the cytotoxic group of oxygen, and pharanox selenate containing the antioxidant selenium – were evaluated. The highest death rate of the embryos (from 30% to 100%) due to exposure to the tested agents was observed in the periods of embryogenesis when embryos are most susceptible to adverse factors (days 4 to 6 of embryo development in the stage of blastogenesis). The teratogenic effect of the tested agents was observed only during the period of organogenesis (days 7, 10, and 14 in the stage of organogenesis). Lophenal was found to be the strongest teratogen. Micrognathia was the most frequent malformation caused by exposure to lophenal, cleft lip was mainly caused by exposure to phenalon, and while cleft palate resulted from exposure to pharanoxi (17, 18).

Adriamycin is embryotoxic as well as teratogenic. Adriamycin (2′′R)-4′-O-tetrahydropyranyladriamycin) given at a dose of 0.1 mg/kg produced a delayed ossification of sacrococcygeal vertebra in the second-generation fetuses (F2) (19). In order to evaluate postimplantation embryotoxicity in a study by Giavini et al. (13), CD female rats were injected intraperitoneally on day 3 of pregnancy with 2 and 4 mg/kg of Adriamycin. A significant increase of postimplantation loss was recorded in the group treated with Adriamycin, but no clear signs of teratogenicity were observed. However, other experimental data indicate that Adriamycin is teratogenic for laboratory species, as demonstrated by a number of in vitro and in vivo experiments (20–22). According to the data obtained by Possogel et al., 49% of embryos exposed to Adriamycin (1.75 mg/kg intraperitoneally on days 6 to 9 of gestation) had foregut malformations (20). It seems that the teratogenic activity of Adriamycin is mediated by specific toxic effects directed to the primitive gut (21).

The teratogenicity of many xenobiotics is thought to depend at least in part on their bioactivation by embryonic cytochromes P450, prostaglandin H synthase, and lipoxygenases to electrophilic and/or free radical reactive intermediates that covalently bind to or oxidize cellular macromolecules such as DNA, protein, and lipid, resulting in *in utero* death or teratogenesis (23, 24); therefore, antioxidants reducing oxidative damage might reduce the teratogenic effects of anticancer agents such as Adriamycin.

It has been reported that antioxidant resveratrol, an aryl hydrocarbon receptor antagonist, might bring a beneficial outcome for reducing the incidence and severity of fetal malformations caused exposure to 2,3,7,8-tetrachloridodibenzo-*p*-dioxin in the uterus (25). A protective effect of glycine on cadmium-induced teratogenicity in vitro (26), vitamin C and E on sodium arsenate-induced changes in developing kidneys of albino mice (27), garlic extract and vitamin E on in vivo cypermethrin-induced teratogenic effects in rat offspring (28) has been established as well.

Sodium selenite showing the antimutagenic potential against Adriamycin- and cyclophosphamide-

induced chromosome damage in rat bone marrow cells (29) had no significant protective effect on Adriamycin-induced embryotoxicity and genotoxicity (30). Literature data on the role of antioxidant β-carotene on Adriamycin-induced embryotoxicity and teratogenicity are scarce.

Conclusions

Antioxidant β-carotene in combination with Adriamycin slightly reduces the Adriamycin-induced embryotoxicity, but produces no positive effect on Adriamycin-induced skeletodysgenesis or external malformations.

β-karotino poveikis adriamicino indukuotam toksiškumui formuojantis embrionui

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Raktažodžiai: β-karotinas, embriotoksiškumas, embriogenezė, adriamicinas.

Santrauka. Adriamicinas yra antraciklinų klasės antibiotikas, plačiai vartojamas piktybiniams navikams gydyti. Adriamicino citotoksinis poveikis pasireiškia per laisvųjų radikalų tarpininkaujamąjį mechanizmą. Todėl, siekiant sumažinti šio citostatiko toksinį poveikį, tikslinga šį vaistą derinti su antioksidantais.

Tyrimo tikslas. Nustatyti β-karotino poveikį adriamicino indukuotam toksiškumui embrionui formuojantis.

Medžiaga ir metodai. Vaikingos žiurkės embriogenezės kritiniais laikotarpiais buvo gydomos adriamicinu, β-karotinu ir jų deriniu. 1 grupė kontrolinė. Adriamicino buvo švirkščiama žiurkėms (9 bei 12 nėštumo dienomis, atitinkamai – 2 a ir 2 b grupės) į pilvo ertmę, vienkartinė dozė – 5 mg/kg, β-karotino buvo duodama (6–10 bei 9–13 nėštumo dienomis, atitinkamai – 3 a ir 3 b grupės) penkis kartus gerti, vienkartinė jo dozė – 0,6 mg/kg; derinant β-karotiną su adriamicinu, β-karotino duota tris kartus prieš adriamicino injekciją, vieną kartą kartu su adriamicinu, vieną kartą po adriamicino injekcijos (4 a ir 4 b grupės). 21 embriogenezės dieną žiurkės buvo žudomos, skaičiuotos embrionų rezorbcijos bei embrionai. Embrionai sveriami, matuotas jų ilgis. Išoriniai apsigimimai analizuoti embrionuose, fiksuotuose Bueno tirpalu. Skeleto formavimosi sutrikimai tirti išskaidrintuose natrio šarmu ir alizarinu raudonuoju dažytuose preparatuose.

Rezultatai. Adriamicinas yra embriotoksiškas, β-karotino derinys su adriamicinu beveik du kartus sumažina šio citostatiko toksišką poveikį embrionui. Dėl šio derinio poveikio nenustatyta embrionų kūno masės sumažėjimo, kuris nustatytas duodant vaikingoms žiurkėms adriamicino ($p<0,05$). Dėl adriamicino lėtėja embrionų skeletogenezė, atsiranda išorinių apsigimimų (mikroftalmija, anencefalija, hidrocefalija ir kt.), o dėl β-karotino poveikio konstatuoti tik pavieniai embrionų apsigimimai (pvz., diafragmos išvarža). β-karotino ir adriamicino derinys teigiamos įtakos adriamicino sukeltam skeletogenezės sutrikimui bei embrionų apsigimimams neturi.

Išvada. Antioksidantas β-karotinas nežymiai sušvelnina adriamicino embriotoksinį poveikį, bet neturi teigiamo poveikio adriamicino sukeltiems skeleto ir išoriniams apsigimimams.

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