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Cytological evaluation of the influence of high and low doses of bisphenol A on an erythroblastic cell line of porcine bone marrow

Anna Snarska[1], Dominika Wysocka[1], Liliana Rytel[1], Krystyna Makowska[2], Sławomir Gonkowski[2]

[1]Department and Clinic of Internal Diseases, [2]Department of Physiology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland
anna.snarska@uwm.edu.pl

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Abstract

Introduction: Bisphenol A (BPA) is a substance widely used in industry for the production of polycarbonates and epoxy resins used in packaging and containers for beverages, contact lenses, compact discs (CDs), window panes, and many other elements. This compound belongs to the group of polyphenols and xenoestrogens commonly found in the human environment. What we know about BPA is still insufficient to enable us to protect our health against its adverse effects, and current knowledge of the influence of BPA on erythroblastic cell lines in bone marrow is rather fragmentary. The aim of the experiment was to assess the effect of two doses of BPA (0.05 mg/kg and 0.5 mg/kg b.w. per day) on myeloid haematopoiesis. **Material and Methods:** During this experiment, the number of all types of cells in the erythroblastic cell line was evaluated in porcine bone marrow before and after BPA administration. **Results:** The obtained results clearly indicate changes in haematopoietic activity of the bone marrow, which was demonstrated by a decrease in erythroblastic cell line production in both experimental groups. The haematological effects of the bone marrow changes were anaemia, caused by a number of erythrocytes which was depressed due to their immaturity, and a significant decrease in mean cellular volume in both groups. **Conclusion:** The harmful effect of high and low doses of BPA on haematopoietic processes was proved.

Keywords: pigs, bisphenol A, bone marrow, erythroblasts.

Introduction

Bisphenol A (BPA) is a substance from a group of phenols widely distributed in plastic packaging which can penetrate into food and toxify living organisms. BPA demonstrates activity similar to synthetic non-steroid chemical compound, such as diethylstilboestrol (DES), showing somewhat lower oestrogenic activity (3, 13). It negatively affects proper functioning of tissues, organs, and systems causing health deterioration in humans and animals. Pathological changes most often apply to young individuals and might be evident in every system and tissue (9, 10, 21, 22). In addition, continuous and ever rising environmental pollution with BPA potentially introduces it into water reservoirs and sources of drinking water, increasing the risk of exposure to the substance both for humans and animals. It is believed that BPA plays an important role in initiating changes associated with asthma by interfering with processes that are regulated by steroid hormones (1, 11). Leading to the damage of mast cells in the bone marrow, it causes excessive histamine and cysteinyl secretion, which significantly intensifies the symptoms of respiratory dysfunction, which in turn results in the impairment of physiological gas exchange in individuals with pre-existing asthmatic disease (12). The toxicity of BPA also involves induction of oxidative stress in animal models, which results in significant changes in the functioning and morphology of cells and tissues (7, 15). In numerous studies describing the problem of the influence of BPA on haematopoietic processes, the destructive effects of this compound on bone marrow cells have been proved.

This research was placing particular emphasis on the impairment of lymphocytic and erythroblastic cell line. Variations in the haematopoietic activity of bone marrow include a decrease in the production of cells from the erythroblastic cell line. In turn, it translates into anaemia resulting from a decrease in the number of erythrocytes as a consequence of their immaturity and a significant reduction in erythrocyte volume. These changes are the effect of a significant reduction in the production of erythropoietin, which is an active glycoprotein hormone produced mainly in the kidneys (4, 6). It is worth emphasising that significant changes of a morphological and functional nature in peripheral blood lymphocytes have been observed, which results in weakening of immune processes among other conditions (19).

The aim of this study was to determine the effect of low and high doses of BPA on erythropoietic processes in pigs. Choosing the domestic pig as the experimental animal was not accidental. The similarities between human and pig in the organisms' reactions to pathological states are known (21). For this reason, the authors of this publication decided to use this species for their research. Genetic similarities between these two species determine that the pig is often adopted as an animal experimental model, in many situations being much better suited than rodents which are commonly used. The experiment aimed to determine whether four-week administration of BPA in low doses widely recognised as safe might not cause major disturbances of the erythropoiesis process despite the testimonial of BPA's safety, and whether an increase in BPA dose causes deviations in the process of erythropoiesis in bone marrow, which translate into changes of haematological parameters of peripheral blood.

Material and Methods

The experiment was performed on 15 clinically healthy gilts of White Great Polish breed, about 8 weeks old. Young individuals were the subject of the study because of the particularly toxic effects of BPA on young organisms and attendant detriments to immature animal health. Animals were randomly divided into three equal groups: a control and two experimental groups. Animals from the control group (group 1) received *per os* a placebo with feed in the form of empty gelatine capsules. Group 2 (E1) consisted of animals which received with feed capsules with BPA in a dose permitted under European Union legislation (0.05 mg/kg b.w. per day), and group 3 (E2) received a ten times higher dose (0.5 mg per kg b.w. per day). The placebos and both BPA doses were given for 28 days. All animals were weighed in order to adjust the BPA dose every four days. Peripheral blood for haematological analyses was collected into 2 mL-test tubes with K_2EDTA (Vacuette) before the first BPA administration and at the end of the study after the last BPA administration. Determination of peripheral blood parameters was performed using an ADVIA 2120i haematological analyser (Siemens, Germany). The haematological parameters subjected to analysis included haemoglobin (Hb) concentration, red blood cell count (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW). At the same time, bone marrow samples were obtained, and smears were immediately performed on basic microscope slides (Heinz Herenz, Germany). Bone marrow was sampled from the lateral condyle of the femur under local anaesthesia with xylazine hydrochloride (Rompun, Bayer, Germany, 1.5 mg/kg b.w., intramuscularly), and zolazepam and tiletamine (Zoletil, Virbac, France, 2.2 mg/kg b.w., intramuscularly), using Jamshidi bone marrow biopsy needles (Synthes, Austria). Bone marrow was collected into 2 ml test-tubes without anticoagulant and immediately used to prepare bone marrow smears. Smears were stained with the May-Grünwald-Giemsa method (a May-Grünwald stain for 2 min and a Giemsa stain diluted with nine volumes of phosphate buffer (pH 7.2) for 4 min). Then the smears were evaluated under an Eclipse 80i light microscope (Nikon, Japan) using an SH-96/24D haematological counter (Alchem, Poland). The numbers of particular forms of erythropoietic cells (*i.e.* proerythroblasts, basophilic erythroblasts, polychromatic erythroblasts, and orthochromatic erythroblasts) were ascertained per 1,000 bone marrow cells of all types (mean ±SD). Statistical analysis was performed with an Anova test using Statistica 10 software (StatSoft, now Tibco, USA). The differences were considered statistically significant at $P \leq 0.05$. Normality and variance homogeneity were checked and proved with Student's *t*-test.

Results

During the present investigation some, significant differences in the number of several types of cells from the erythroblastic system were noted between control and experimental animals (Table 1). The influence of BPA on the total number of erythroblastic cells (total erbl) was clearly demonstrated in a decrease in the number of basophilic erythroblasts (baso erbl) (1.4% in the E1 and 2.3% in the E2 groups), orthochromatic erythroblasts (orto erbl) (13.6% in the E1 and 10.4% in the E2 groups), and all normoerythroblasts (total normo) (22.2% in the E1 and 19.4% in the E2 groups). In the E1 group receiving approved doses of BPA, cells such as promegaloblasts and megaloblasts appeared (0.04% in both cases), but the cells were not observed in the group receiving high doses of BPA. This group also had a slight decrease in Hb and MCH (from

8.3 g/dL to 7.58 3 g/dL and from 21.4 pg to 17.1 pg, respectively) (Table 2). The results of haematological analyses in the E2 group showed a significant decrease in RBC (from 6.7×10^6/μL to 5.58×10^6/μL), MCV (from 56.82 f/L to 45.34 f/L), and HCT (from 0.38 l/l to 0.34 l/l) on the 28th day of BPA administration. Also, the percentage of reticulocytes (% retic) in the E2 group was definitely lower (0.74% against 1.14% in control group), which clearly indicates a weakening of the erythropoiesis processes. Haematological analyses elucidated that in both groups receiving BPA a statistically significant decrease in HCT and Hb concentrations was observed in comparison with the control group. There were no statistically significant differences among the parameters determining the mean volume of red blood cells in the E1 group, which clearly indicates the development of normocytic anaemia. However, in the E2 group the value of this parameter significantly decreased, which is evidence of microcytic anaemia. In the group of animals receiving high doses of BPA, an increase in the RDW (an indicator of red blood cell anisocytosis) was noted, which indicates a significant differentiation of red blood cells in terms of size. The obtained results prove the effect of BPA on haematopoietic processes in the E1 and E2 groups.

Table 1. The percentage (mean ±SD) of erythroblastic cell line in porcine bone marrow cells

Parameter	Group of animals		
	Control	Experimental 1	Experimental 2
PROERBL (%)	1.9 ± 1.11	1.8 ± 0.40	1.4 ± 0.61
BASO ERBL (%)	2.6 ± 0.78 [a]	1.4 ± 0.94 [ac]	2.3 ± 0.17 [c]
POLY ERBL (%)	7.84 ± 1.87	5.4 ± 2.74	5.3 ± 0.93
ORTO ERBL (%)	12.0 ± 6.52[b]	13.6 ± 6.96[c]	10.4 ± 4.65[bc]
TOTAL NORMO (%)	24.34 ± 7.56[ab]	22.2 ± 10.28[a]	19.4 ± 4.22[b]
PROMEGALOBL (%)	0	0.04 ± 0.05	0
BASO MEGALO (%)	0	0	0
POLY MEGALO (%)	0	0	0
TOTAL MEGALO (%)	0	0.04 ± 0.05	0
NORMO/MEGALO (%)	0	0.2 ± 0.45	0
PARA ERB (%)	0.02 ± 0.04	0.06 ± 0.13	0.08 ± 0.11
TOTAL ERBL (%)	24.36 ± 7.56 [ab]	22.48 ± 10.39 [a]	19.48 ± 4.29[b]

Statistically significant data ($P \leq 0.05$) in particular animal groups are marked by:
[a]significant difference between control and low dose
[b]significant difference between control and high dose
[c]significant difference between low and high dose
PROERB – proerythroblasts, BASO ERBL – basophilic erythroblasts, POLY ERBL – polychromatic erythroblasts, ORTO ERBL – orthochromatic erythroblasts, TOTAL NORMO – normoerythroblasts, PROMEGALOBL – promegaloblasts, BASO MEGALO – basophilic megaloblasts, POLY MEGALO – polychromatic megaloblasts, TOTAL MEGALO – total number of megaloblasts, NORMO/MEGALO – normoblasts/megaloblasts, PARA ERB – paraerythroblasts, TOTAL ERBL – total number of erythroblastic cells

Table 2. Selected parameters of whole blood

Group	Control group		Experimental 1		Experimental 2	
			Day			
	0	28	0	28	0	28
RBC × 10^6/μL ($\bar{x}$ ± SD)	6.76 ± 0.167	6.7 ± 0.2	6.56 ± 0.336	6.4 ± 0.406	6.7 ± 0.354	5.58 ± 0.286
HGB g/dL ($\bar{x}$ ± SD)	8.26 ± 0.493	8.3 ± 0.4[ab]	8.3 ± 0.430	7.58 ± 0.363[a]	8.3 ± 0.158	7.72 ± 0.409[b]
HCT l/l ($\bar{x}$ ± SD)	0.38 ± 0.192	0.4 ± 0.2[ab]	0.4 ± 0.1	0.36 ± 0.114[a]	0.38 ± 0.130	0.34 ± 0.114[b]
MCV f/L ($\bar{x}$ ± SD)	55.64 ± 8.076	57.32 ± 2.799	54.38 ± 3.655	52.5 ± 3.188[c]	56.82 ± 4.853	45.34 ± 2.783[c]
MCH pg ($\bar{x}$ ± SD)	20.32 ± 2.055	22.24 ± 2.504	21.4 ± 2.187	17.1 ± 1.859	24.04 ± 1.601	22.98 ± 1.268
MCHC g/dL ($\bar{x}$ ± SD)	31.46 ± 2.911	31.38 ± 3.036	30.04 ± 2.056	29.38 ± 2.699	32.22 ± 2.531	30.6 ± 1.528
RDW % ($\bar{x}$ ± SD)	11.88 ± 2.867	12.16 ± 2.070	12.06 ± 1.450	13.62 ± 1.656	12.0 ± 0.784	16.18 ± 0.746[A]
% RETIC ($\bar{x}$ ± SD)	1.14 ± 0.321	1.14 ± 0.532	1.08 ± 0.130	0.94 ± 0.207	1.08 ± 0.148	0.74 ± 0.182
RETIC × 10^9/L ($\bar{x}$ ± SD)	36.8 ± 3.077	38.14 ± 2.454	36.86 ± 3.372	35.7 ± 2.277	36.32 ± 2.957	34.38 ± 2.467

Statistically significant data ($P \leq 0.05$) in particular animal groups are marked by:
[a]significant difference between control and low dose
[b]significant difference between control and high dose
[c]significant difference between low and high dose
RBC – red blood cells count, HGB – haemoglobin concentration, HCT – haematocrit, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, MCHC – mean corpuscular haemoglobin concentration, RDW – red cell distribution width, % RETIC – percentage of reticulocytes, RETIC – number of reticulocytes

Discussion

Due to the wide spectrum of locations of oestrogen receptors, BPA can cause changes and side effects in many tissues and organs, some of which are difficult to predict. The study of O'Brian *et al.* (14), performed on adult mice, proved the great influence of BPA on the release of proinflammatory factors by mast cells in the bone marrow and in a later stage on the susceptibility of animals to developing allergy. The study by Tiwari and Vanage (20) demonstrated the huge impact of BPA on the development of oxidative stress in rat bone marrow, which translates into impairment of normal processes of haematopoiesis due to strong influence on metabolic processes and damage to cellular structures. These changes shorten the life of erythroblastic cells significantly as a result of damage to cell membranes. Tiwari *et al.* (19) demonstrated in a very convincing manner the destructive effects of BPA on myeloid cells and DNA fragmentation in peripheral blood lymphocytes in rats. However, similar studies have not been performed in swine or other farm animals.

The results of the present research clearly indicate that even low doses of BPA disrupt the normal processes of erythropoiesis in swine bone marrow. The authors of this research indicate a significant decrease in the percentage of orthochromatic erythroblasts in bone marrow smears in the group of pigs receiving the higher experimental dose of BPA. The study of Pal *et al.* (16) demonstrated the destructive effect of bisphenol S in rats manifested by a significant decrease in RBC due to haemolysis in the peripheral blood and an increase in the incidence of cardiac disorders. In addition, a decrease in HGB indicating the advancement of anaemia was found in the examined peripheral blood samples. The authors of this publication obtained similar results for the given haematological parameters using BPA, and similar research results have also been described by Horiguchi *et al.* (5). Even small doses of BPA, considered harmless so far, cause slight changes in the erythroblastic cell line of the bone marrow without causing deviations in the results of haematological analyses (8), as evidenced also by the results presented in this study. Only a substantial increase in the BPA dose results in significant differences in the peripheral blood haematological parameters. In the study by Rubin (18) it was demonstrated that BPA shows significantly lower oestrogenic activity than diethylstilboestrol (DES), mediating the magnitude of the decrease in erythropoietin (EPO) concentration and thus the reduction of erythropoietic processes which BPA is capable of causing. The study by Cavalieri and Rogan (2) undoubtedly proved that BPA causes changes including damage to DNA sequences by oxidative stress initiation, which is a very probable mechanism of BPA genotoxic activity. The study by Radzikowska *et al.* (17) in mice proved that BPA promulgates micronuclei in peripheral blood and bone marrow reticulocytes in animals receiving low and high BPA doses. Pal *et al.* (16) demonstrated the harmful effect of BPA on haematopoietic activity in rat bone marrow. The data presented in that study prove that BPA decreases the concentration of HGB, MCH, and RBC leading to anaemia and these changes ultimately lead to hypoxia.

The cited scientific literature mainly describes the effects of BPA in rodents. Due to the lack of data on the influence of BPA on the erythropoiesis in farm mammals, widely dispersed in the environmental though BPA may be, the authors of this study decided to perform this research on pigs. An additional recommendation of this species for the experiment is that the domestic pig is a commonly adopted animal model in science, known for its similarity to humans, and the results of this research can be a valuable source of information about the harmfulness of BPA to the processes of erythropoiesis in humans and animals.

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