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Original article

Tooth development disorders in infants of rat dams exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and protective role of tocopherol and acetylsalicylic acid

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Abstract

Aryl hybrocardon receptor (AhR) activation plays a key role in the pathomechanism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced defective spatial structure of teeth caused by disordered collagen synthesis. The aim of this study was to identify the influence of dioxins present in female Buffalo rats on the dental structure of their offspring's in the neonatal period and the potential of α-tocopherol and acetylsalicylic acid in curbing post-dioxin hard tissue defects. Research material consisted of molar teeth (n=40) of rat pups which had been given a single dose of TCDD and were then treated with tocopherol or acetylsalicylic acid for 3 weeks. In the offspring of rat dams exposed to TCDD, ameloblasts and odontoblasts were less developed in comparison with the control group and less dynamic angiogenesis in the area of dental papilla was observed. In the pups of TCDD-exposed mothers, a smaller number of AhR was found in amelogenic and odontoblastic cells, whereas in the pups of mothers exposed to TCDD followed by tocopherol and acetylsalicylic acid treatment, the expression of AhR in ameloblasts and odontoblasts increased. We conclude that tocopherol and acetylsalicylic acid treatment exerts a protective effect on the TCDD-induced structural defects of tooth tissue.

Key words: dioxin, AhR receptor, rat, offspring, dental structure, tocopherol, acetylsalicylic acid

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Introduction

Despite restrictive obligations imposed by the Stockholm Convention on reducing environmental emission of persistent organic pollutants including dioxins, their production is still significant and results from industrial activity, wide use of plastic and unexpected events i.e. fires, ecological disasters or military action (Fiedler et al. 2007).

On the basis of our earlier experimental studies, it was shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), by inducing inflammatory processes, degrades connective tissue (Całkosiński et al. 2008, Całkosiński et al. 2011, Całkosiński et al. 2014, Całkosiński et al. 2015), inhibits collagen I synthesis, and induces oxidative stress leading to an increased concentration of pro-inflammatory interleukins which activate osteoclastogenesis (Whitlock et al. 1999, Hirai et al. 2002, Całkosiński et al. 2008, Fox et al. 2008, Nishimura et al. 2009, Arslan et al. 2011). Other studies indicate that dioxin-induced inhibition of alkaline phosphatase and some mineralization activators may lead to the formation of poorer quality mineralized tissues (Peters et al. 1999, Thompson et al. 2006, Fox et al. 2008, Nishimura et al. 2009). Therefore, it has been assumed that dioxin may influence the structure of teeth and bone of the alveolar process, especially at development stages. This assumption was proved by changes in the appearance of rat offspring whose mothers were exposed to TCDD (Alaluusua et al. 1996, Gao et al. 2004, Geng et al. 2008). Disturbed developmental of skeleton observed in these studies suggests developmental defects in bones (Hornung et al. 1999, Teraoka et al. 2002). Dioxins, by influencing some hormone activity (estrogens, corticosterone, T_3), may alter calcium deposition in mineralized tissues. Moreover, increased corticosterone concentration stimulates collagen fiber decomposition. By influencing the concentration of the active form of vitamin D_3, dioxin inhibits fibroblast activity (Liang et al. 2011).

Aryl hydrocarbon receptor (AhR) activation plays the key role in the pathomechanism of TCDD which leads to collagen fiber synthesis (Kozak et al. 1997, Jain et al. 1998, Singh et al. 2000 Sahlberg et al. 2002, Andreasen et al. 2007), and, as a consequence, may result in defective spatial structure of osseous tissue and mineralized tissues of teeth (dentin and cement). Therefore, seeking pharmacological products which could bind with AhR, preventing the dioxin from actively binding with it, seems to be justified. Recent experimental studies have shown that two well-known medicines, tocopherol and acetylsalicylic acid, are antagonistic to AhR (Fernandez-Salguero et al. 1996, Hirai et al. 2002, Alsharif et al. 2004, MacDonald et al. 2004, Kloser et al. 2011), which enables us to widen their indications for use. The ability to inhibit an inflammatory reaction caused by dioxins is an additional protective feature of these substances (Fernandez-Salguero et al. 1996).

The aim of this study was to determine the intermediate effects of dioxins in rat females on the dental structure of their offspring at the postnatal stage. We also aimed to identify possibilities of reducing potential post-dioxin defects in mineralized tissue structure in the mothers' offspring by simultaneous application of α-tocopherol or acetylsalicilic acid. Additionally, AhR expression in the teeth of rats whose mothers were exposed to TCDD and treated with acetylsalicylic acid or tocopherol was assessed.

Materials and Methods

Forty offspring from 24 rat females of the *Buffalo* inbreeding strain (body mass: 130-150g, age: 9-11 weeks) were used in these studies. The rats came from breeding in the Department of Pathomorphology in Wroclaw Medical University. All females received human care in compliance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health (NIH publication No. 85-23, revised 1985). All experiments were performed in compliance with guidelines for experimentation on animals. The study was approved by a Local Ethics Council for Animal Experiments (permission number: 38/2009).

All the females were kept under the same conditions: they were in polystyrene cages (60cm x 40cm x 40cm) with metal lids (6 animals to each cage). The experiments were carried out in air-conditioned rooms (ambient temperature 21° ± 1°C, relative humidity $\phi = 55\%$). The females were maintained in a light/dark cycle for 12/12 hours. The rats were fed with the standard "Labofeedh" diet and received water ad libitum.

The animals were divided into 4 groups of six females each from which the infants for investigation were obtained:

1. control group
2. TCDD group – females which, 3 weeks before the examination, were administered (*i.m.*) with 5 μg/kg b.w. of TCDD (Greyhound Chromatography and Allied Chemicals, UK)
3. TCDD+E group – females which, 3 weeks before the examination, were administered with (*i.m.*) 5 μg/kg b.w. of TCDD and every day for 3 weeks with α-tocopherol acetate (Hasco-Lek SA, Poland) at a dose of 30 mg/kg b.w. *s.c.*

4. TCDD+ASA group – females which, 3 weeks before the examination, were administered with (*i.m.*) 5 μg/kg b.w. of TCDD and every day for 3 weeks with acetylsalicylic acid (Bayer, Poland) at a dose of 50 mg/kg b.w. *p.o.*

After 3 weeks from TCDD administration the females of groups 2, 3 and 4 and the females of group 1 were mated with randomly chosen males from the same strain which were not exposed to any chemical substances. After the mating period, pregnant females were kept in separate cages. 2-day old infants (51 days after beginning of the experiment) were euthanatized and the mandibles from 10 pups from each of the 4 groups were chosen randomly and investigated. The pups underwent pharmacological euthanasia with the use of Phenobarbital (Morbital® – Biowet, Poland) administered intraperitoneally at 100 mg/kg b.w., after which tissue material for histological and immunohistochemical tests was taken. Samples with molar teeth were cut out of the mandible and fixed in 4% buffered formalin solution for 48 hours. The material was then rinsed in running water, cut into smaller fragments and decalcified in a mixture of concentrated acids hydrochloric and formic (for 24 hours) dehydrated in alcohol series, cleared in methyl benzoate and embedded in paraffin.

Sections 7-9 μm thick were dried, deparaffinized in xylene and stained with hematoxylin and eosin (Mayer, Germany) in accordance with Delafield's method. For immunocytochemical reaction, after deparaffinization and hydration of the tissue sections, endogenous peroxidase was blocked with Peroxidase Blocking Reagent (DAKO, Poland). The sections were then rinsed twice for 5 minutes in distilled water and were then digested with K proteinase (DAKO, Poland) and rinsed again twice for 5 minutes in distilled water. The sections were incubated with rabbit primary antibody Anti Human AhR (Serotec, UK) for one hour of a dilution 1:80; they were then rinsed twice in PBS solution (pH 7.3) for 5 minutes. A Novolink MinPolymer DS (Novocastra, UK) kit was then used. The next stage included staining with a DAB+ substrate buffer and DAB+ chromagen visualization system (DAKO, Poland) and hematoxylin (Mayer, Germany) was used to stain cell nuclei.

Results

Histological analysis

In group 1, the histological image of developing teeth shows a differentiation process of the enamel organ and dental papilla (Fig. 1). Cell differentiation takes place in the enamel organ. Cells present on the internal surface, i.e. on the dental papilla side, become cylindrical and form the inner enamel epithelium. On the outer surface, cells become flat and form the outer enamel epithelium. In the intermediate stratum there are many of star-shaped cells which are connected to each other by processes and form the pulp of the enamel organ. Simultaneously with differentiation of the enamel organ, changes in mesenchymal dental papilla take place. Numerous blood vessels and cylindrical basophilic cells appear here. They are present at the periphery of the dental papilla and differentiate into odontoblasts. Adjacent to the odontoblastic region there is predentin in the form of canaliculi with peripheral odontoblasts processes. The remaining mesenchymal tissue, remaining after odontoblast differentiation, comprises the pulp bud, whereas connective tissue around tooth bud comprises dental sac. At this stage of tooth development, clearly shaped pulp, forming dentin and ameloblasts which differentiate from the inner enamel epithelium, are visible. Compact, fibrous connective tissue is adjacent to alveolar cavity. The alveolar bone cavity is a type of trabecular osseous tissue. Apart from the already formed alveolar cavity bone there are images of advanced osteogenesis. In group 2 a lower degree of development in both enamel organ and dental papilla can be seen (Fig. 2). In the enamel organ cuboidal cells, rarely cylindrical are present, giving an impression of a partially formed mature organ, are visible. Cells present on the inner enamel epithelium surface become flat. In the area bordering the odontoblastic layer, differentiating mesenchymal cells are accumulated. Mesenchymal cells differentiate into pulp. Moreover, successive dentin formation and ameloblast differentiation from the inner enamel epithelium are observed. The degree of development of the mentioned tissue structures, as well as angiogenesis, is less advanced than in the control group. In the dental bud area, there are large regions of inhibited alveolar bone development. Small areas of immature bone surrounded by only a few osteoblasts are visible. In group 3, the histological image of developing teeth is similar to the image of offspring from mothers exposed to dioxin only. There are some differences, however, that indicate a positive effect of tocopherol. This effect is mostly connected with the dental papillae and enamel organ (Fig. 3). Cells from the inner enamel epithelium surface become mostly cylindrical. There are fewer cells at the differentiation stage (cuboidal). In the mesenchymal papillae, there is clear angiogenesis especially in the odontoblastic layer. In group 4 fewer negative effects of TCDD on dental organ development in comparison with group 2 can be observed (Fig. 4). In the enamel organ there are many more cylindrical than cuboidal cells. In the odontob-

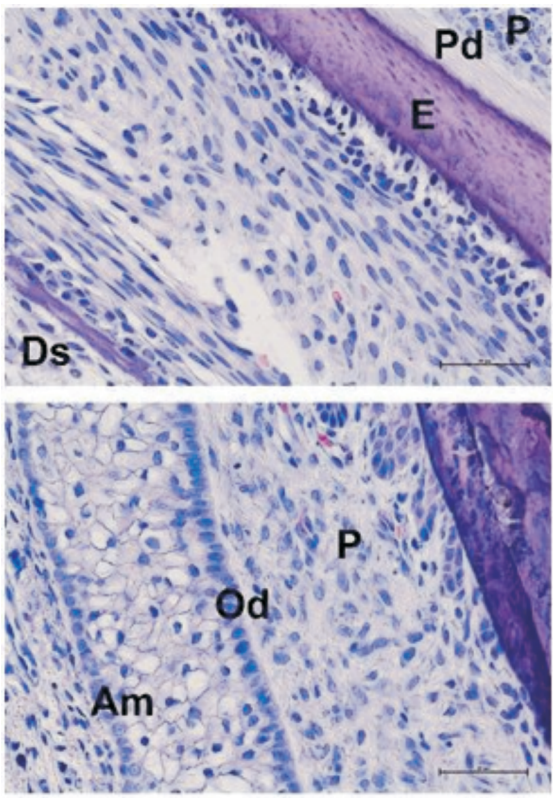

Fig. 1. Tooth bud from group 1, Enamel organ and dental papilla. Process of normal tooth development. HE, x 400. Am – ameloblasts, P – pulp, Od – odontoblasts, Pd – predentin, E – enamel, Ds – dental sac. Scale bar represents 50 μm.

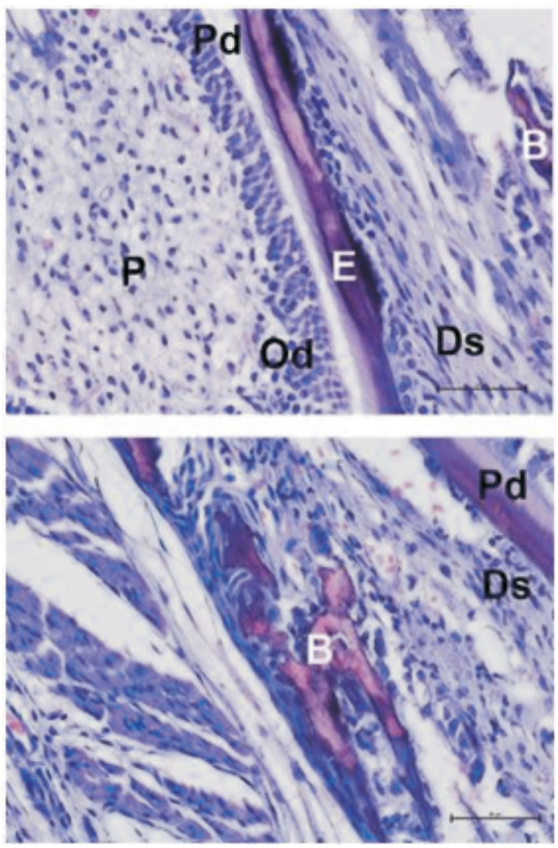

Fig. 2. Tooth bud from group 2, HE, x 400. Retarded development of tooth. B – bone, P – pulp, Od – odontoblasts, Pd – predentin, E – enamel, Ds – dental sac. Scale bar represents 50 μm.

lastic area increased proliferation of odontoblasts takes place. Mainly, they are cylindrical cells with polar variety. In the odontoblastic layer, adjacent to pulp cells, odontoblastic proliferation and further differentiation are visible. In the mesenchymal papillae clear angiogenesis is seen. This process is especially visible in the central part of the papilla, to a lower extent in the odontoblastic area. More advanced development of the alveolar bone present in the peripheral regions of the tooth sac is noted.

Immunohistochemical analysis

In group 1 a positive reaction for AhR was noted in the enamel organ (Fig. 5). Cells of the inner enamel epithelium, on the papilla's side, show a clearly positive immunohistochemical reaction to AhR, whereas on the outer surface this reaction is weaker, which may result from the terminal differentiation of the outer enamel epithelium. In the odontoblastic layer of the papilla, there is reaction but it is much weaker than in the enamel organ. In the area of differentiating mesenchyme, in the future pulp region, AhR is dispersed. In the alveolar bone area, AhR is expressed in the cells of the intermediate layer connecting the alveolar bone with the connective tissue of the dental sac. In group 2 the area of differentiating enamel organ, a much weaker immunohistochemical reaction to AhR receptor was observed (Fig. 6). On the inner enamel epithelium surface, in the area of cuboidal or cylindrical cells, AhR is rarely present. Immunohistochemical reaction in the mesenchymal cell area is also weaker. In the differentiating papilla, in the area of pulp formation and odontoblastic region, there are far fewer receptors. Immunohistochemical reaction to AhR is seen mainly in cells of epithelial origin. In group 3 weak positive reaction in enamel organ was noted, however, a little stronger than in offspring of mothers exposed to TCDD only (group 2) (Fig. 7). On the inner enamel epithelium, in the area of cylindrical cells, immunohistochemical reaction to AhR is very weak. In the area of mesenchymal cells this reaction is also weak, similarly to the area of differentiating odontoblasts. No AhR presence was observed in the area of the dental sac and alveolar cavity bone. In group 4 in the enamel organ, immunohistochemical reaction to AhR is similar to that of group 3 (Fig. 8). A weak immunohistochemical reaction to AhR was observed in the odontoblasts and mesenchymal cells of differentiating pulp.

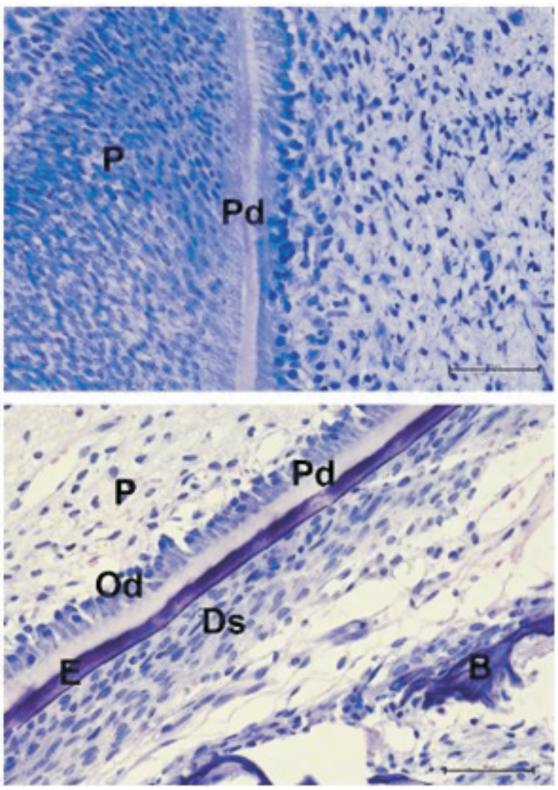

Fig. 3. Tooth bud from group 3. HE, x 400. Am – amelobblasts, B – bone, P – pulp, Od – odontoblasts, Pd – predentin, E – enamel, Ds – dental sac. Scale bar represents 50 μm.

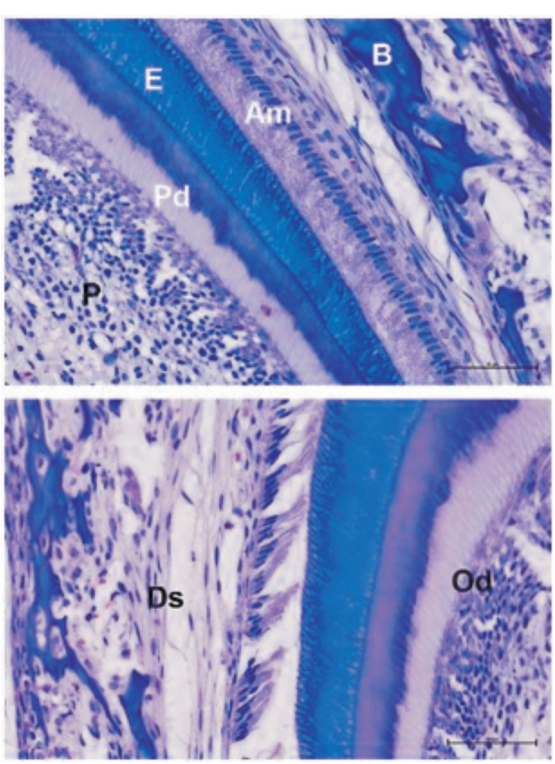

Fig. 4. Tooth bud from group 4, HE, x 400. B – bone, P – pulp, Od – odontoblasts, Pd – predentin, E – enamel, Ds – dental sac. Scale bar represents 50 μm.

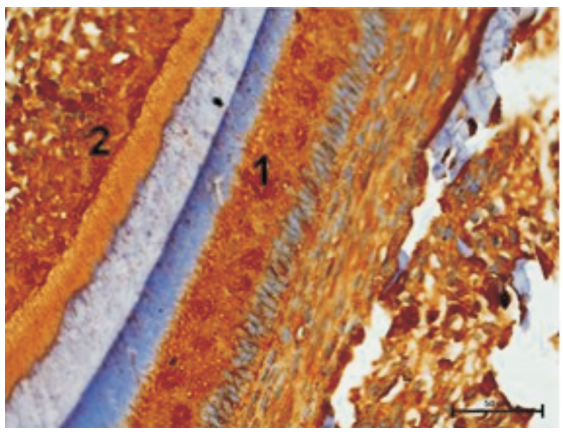

Fig. 5. Tooth bud from group 1. Different reaction to AhR, x 400. 1 – enamel organ, 2 – odontoblastic layer. Scale bar represents 50 μm.

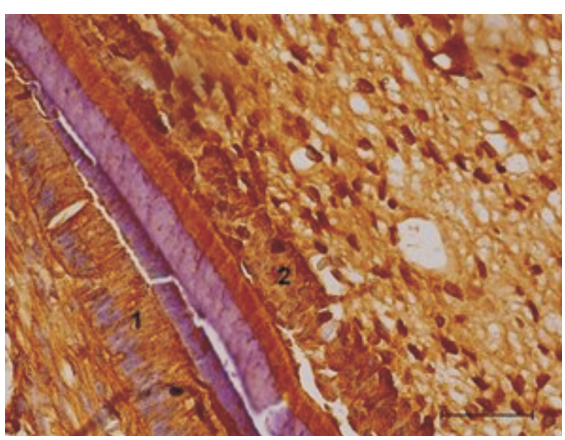

Fig. 6. Tooth bud from group 2. Positive reaction to AhR, x 400. 1 – enamel organ, 2 – odontoblastic layer. Scale bar represents 50 μm.

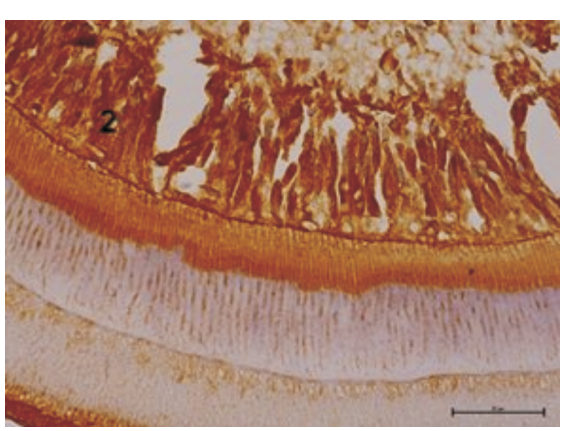

Fig. 7. Tooth bud from group 3. Positive reaction to AhR, x 400. 1 – enamel organ, 2 – odontoblastic layer. Scale bar represents 50 μm.

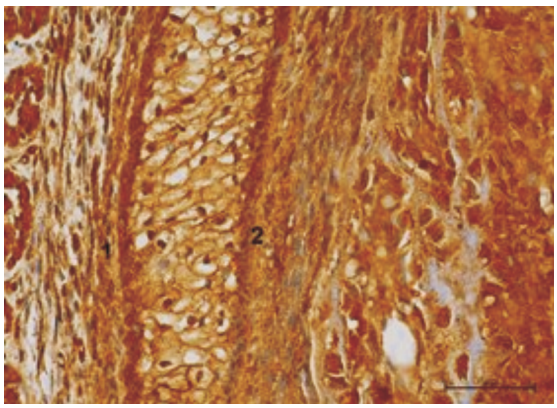

Fig. 8. Tooth bud from group 4 group. Positive reaction to AhR, x 400. 1 – enamel organ, 2 – odontoblastic layer. Scale bar represents 50 μm.

Discussion

In the control group, observed tooth development was typical for rats (Shellis et al. 1981). The experimental study carried out on different types of animals in the developmental age has shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) influences cartilage, bone and tooth structure. Studies by Allen et al. (2001), conducted on pregnant mice that were exposed to TCDD in 0.5 and 1.0 μg/kg b.w. doses, showed defects in their offspring as their mandible body was smaller and of a different shape. Wistar TCDD in doses of 50 and 1000 μg/kg b.w. administered to pregnant rat dams caused a lack of the third molar teeth buds in their offspring, which was more frequent in the group whose mothers received a larger dose of TCDD (Lukinmaa et al. 2001). Premature closing of the apical foramens of roots and arrested dentin formation in the incisors and first molar teeth caused by dentin cell apoptosis were also reported (Lukinmaa et al. 2001).

Studies on rats, in which the influence of administering four different doses of TCDD (0.17; 1.7; 17.0; 170 μg/kg b.w.) for 20 weeks on the structure of developing incisors was assessed, showed that small TCDD doses of – 0.17 and 1.7 μg/kg b.w. did not significantly affect the morphology of these teeth (Kiukkonen et al. 2002). The dose of 17.0 μg/kg b.w. of TCDD caused morphological changes, such as an enlarged pulp chamber and its perforation and apoptosis in odontoblasts and mesenchymal cells of pulp with accompanying pulp vessel enlargement. When the dose of 170 μg/kg b.w. of TCDD was administered, the presence of a non-mineralized, thin layer of predentin, squamous metaplasia of the enamel organ and calcification of pulp vessels were observed (Kiukkonen et al. 2002). By applying a megadose of TCDD of 1000 μg/kg b.w. the authors induced serious lesions in the incisor area, which caused thinning of the dentin and its irregular layout, as well as a lack of odontoblast polarity and pulp cell necrosis (Alaluusua et al. 1993). In the enamel of the molar teeth, defects in structure were observed caused by apoptosis induced by TCDD (Partanen et al. 1998, Partanen et al. 2005). Our own studies have shown that TCDD influence on dental organ structure of offspring whose mothers were exposed to this dioxin at a single dose of 5 μg/kg b.w. leads to less developed ameloblastic cells participating in the enamel formation and odontoblastic cells participating in dentin formation in comparison with the control group. The dynamics of angiogenesis in the mesenchymal papilla of offspring from group 2 was more weakly expressed than in the control group, especially in the mesenchyma adjacent to the odontoblastic layer.

In studies on rats carried out by Gao et al. (2004), it was shown that administration of TCDD at two doses of 500 and 1000 μg/kg b.w. to dams caused enamel mineralization defects in the 22nd day of the lives of their offspring. This was caused by deregulation in enamel matrix derivative removal and correlated with the amount of administered dioxin. Moreover, the dentin layer was much thinner and less mineralized than in offspring from the control group. The enamel of the mandible incisors of rats exposed to TCDD was characterized by hypoplasia, hypomineralization and enamel color defect (Robinson et al. 1998). Tooth hard tissue defects and their decreased thickness caused by dioxin, lead to pathological attrition causing pulp chamber perforation (Alaluusua et al. 1993, Robinson et al. 1998, Dobrzyński et al. 2009). In offspring of rats exposed to TCDD in the lactation period, the alveolar bone process was modified so that it lacked a proper trabecular structure (Geng et al. 2008).

Pro-inflammatory activities of dioxins connected with COX-2 stimulation disturb collagen synthesis and may also influence the development of teeth and skeleton. As a result of IL-1 and TNF activity at the inflammation site, fibroblast activity is inhibited and osteoclasts are activated (Całkosiński et al. 2008, Całkosiński et al. 2011, Całkosiński et al. 2014). A 3-week administration of tocopherol to dams, from the moment of TCDD exposure to the moment of pregnancy, was found to reduce the toxic activity of free radicals produced by the dioxin by inhibiting AhR in a direct and indirect way (Całkosiński et al. 2008, Całkosiński et al. 2011). Positive modifications in the dental organ of offspring from the above mentioned mothers, in comparison with group 2, were connected to a large extent with better angiogenesis in the papilla area (especially in the odontoblastic area) and to lesser extent in the enamel organ (manifested

in a larger number of cylindrical cells over differentiating cuboidal cells). Other authors also showed the positive influence of tocopherol on animals exposed to TCDD (MacDonald et al. 2004). Significant and positive changes were observed in the dental organ of offspring from group 3 that correlated with increased AhR expression after administration of tocopherol, especially in the odontoblastic and amelogenic cell layers in comparison with the offspring of mothers who were exposed to dioxin only (MacDonald et al. 2004).

The histological image of the developing teeth in group 4 is similar to the image of offspring from mothers exposed to dioxin and tocopherol. Acetylsalicylic acid also has a positive influence on the tooth development. This positive influence may be seen especially in the papilla area. It is seen in increased angiogenesis in the papilla's central part in relation to the odontoblastic layer. Increased angiogenesis leads to a better blood supply to the odontoblastic area and, as a result, a larger number of odontoblasts and polarized differentiation of cylindrical cells in this region. Increased odontoblast differentiation, taking part in dentin formation, is observed in the area adjacent to the amelogenic cell area and borders with pulp. As a result, more predentin is produced. Histological images of the dental organ of 2-day old offspring from groups 3 and 4 show that administered tocopherol and acetylsalicylic acid protect odontogenesis from destructive TCDD activity. It is interesting that this activity is indirect. So far, there has been no data on this in the literature. This indirect activity is a result of the cessation of both tocopherol and acetylsalicylic acid administration to dams exposed to dioxin before becoming pregnant. It may be assumed then that in offspring from group 3 and 4 in comparison with group 2 there was a reduction in dioxin transportation via the placenta, as the time of tocopherol and acetylsalicylic acid activity is short and could not last for a pregnancy of about 3-week. The negative effects of dioxins on different tissues and organs result from their ability to induce autometabolic reactions whose toxic indirect products disturb cell homeostasis, alter growth processes and cell differentiation, change proper cell metabolism and induce inflammation. In this process the key role – reaction initiator is performed by cytoplasmatic AhR, enabling dioxin to produce an active complex that can move to the cell nucleus and induce transcription processes (Nebert et al. 2000).

In the studies carried out by Fernandez-Salguero et al. (1996) it has been proved that AhR is the key element in the mechanism of dioxin toxicity. It has been reported that in mice genetically deprived of this receptor, administration of TCDD at a dose of 2000 μg/kg b.w. did not damage organs i.e. liver, thymus, heart, kidneys, pancreas, spleen or lymph nodes, in comparison with the control group. Tooth development in mammals is regulated by interactions between the ectodermal lining cells of the first visceral arch and mesenchymal cells. The presence of AhR in the first visceral arch, where the enamel organ originates, may be important not only for the structure of future enamel in case of dioxin exposure, but may also indirectly influence other tooth cells originating in the mesenchyme. In the studies of Sahlberg et al. (2002) done on mice, a detailed description of AhR expression at different tooth development stages was established. It has been reported that at early development stages it is not possible to detect this receptor in either the epithelial or mesenchymal compartment. On the other hand, in 14-day old embryos (early cap stage) AhR expression in the incisor epithelium of future tooth cusps and osteoblast in the surrounding bone was observed, and lack of it in molar teeth buds. At the bell stage, on the 17th day of embryonic life, the expression of this receptor was visible in the inner enamel epithelium, whereas between the 19th day of embryonic life and the 12th day of ontogenesis – in line with odontoblastic differentiation and dentin formation – AhR expression was observed in preameloblasts and odontoblasts (only after terminal differentiation from mesenchymal tissue). Moreover, it has been found that AhR expression is more intense in ameloblasts and odontoblasts than in the intermediate stratum of the enamel organ or osteoblasts. Also, in the same author's studies on a group of pups of mothers that were not exposed to TCDD, AhR presence on the inner surface of the enamel organ on the side of a cap was reported. Less intense expression of this receptor was reported on the external surface of the cusp in the odontoblastic and mesenchymal area of the forming pulp and also in the intermediate layer of the alveolar bone. This changeable expression of AhR in ameloblasts and odontoblasts depending on the stage of tooth development was observed in animals not exposed to xenobiotics. This phenomenon is a result of the inductive ameloblastic influence on odontoblastic cells differentiating from tooth papilla leading to a varied positive immunohistochemical reaction found in these two types of cells (Liang et al. 2011).

Our own studies showed that in rat newborns whose mothers were exposed to TCDD (group 2) reaction to AhR was present mainly in epithelial cells; however, it was sporadic and of low intensity. This means that AhR present in other areas of the dental organ is blocked by trace amounts of TCDD in newborn organisms. According to Gao et al. (2004), the administration of TCDD to developing rat newborns

reduced reaction to AhR in ameloblasts to a larger extent than in differentiating dental papilla cells. The observed range of AhR expression in ameloblasts and odontoblasts found in our own and cited studies may result from the difference in offspring age ((2-day old newborns – own studies, and 9- and 22-days old in Gao et al. (2004)), as well as the applied TCDD dose (5 μg/kg. b.w. – own studies and 50 μg/kg. b.w. and 1000 μg/kg. b.w. – Gao et al. (2004).

Earlier studies by Całkosiński et al. (2008) carried out on rats exposed to TCDD that were supplemented with tocopherol, proved the effectiveness and purpose of administering this medicine as the concentration of pro-inflammatory interleukins in serum was lower after its administration and reduced its effects, such as apoptotic lesions in other organs, and improved liver functions whose metabolism was disturbed by dioxin activity. Therefore, in these studies tocopherol at an analogical dose and time intervals from the moment of TCDD administration was used and completed with the use of acetylsalicylic acid. The authors' own studies indicate that administration of tocopherol to dams exposed to TCDD might have significantly reduced the amount of dioxin transported to offspring via the placenta, causing increased expression of AhR in some regions of the newborns dental organ (group 3) in comparison with newborns whose mothers received dioxin only (group 2). It may be concluded that administration of tocopherol, which blocks AhR, to dams might have decreased the concentration of TCDD in serum (Całkosiński et al. 2015). Therefore, a smaller amount of this compound was transferred to the fetus via the placenta and resulted in higher expression of AhR in offspring from this group. The latest studies on finding medicine that would reduce the negative effects of dioxins on organisms show that such a pharmacological product with antioxidative, antinflammatory and antagonistic to AhR features is acetylsalicylic acid (MacDonald et al. 2004, Arslan et al. 2011). The administration of acetylsalicylic acid to dams exposed to TCDD in these studies led to an observed immunohistochemical reaction to AhR presence in group 4 that was stronger than in group 2. This means that newborns were less exposed to TCDD that reached their organisms via placenta. Acetylsalicylic acid blocks AhR, similarly to tocopherol, and may have decreased the concentration of dioxin in the mothers' serums. As a result, a reduced amount of this compound reached the newborns' organisms via the placenta. It has been proved that dioxins present in lactating mother's organisms cause developmental disorders in the form of hypoplasia and hypomineralization of primary molar enamel in children (Alaluusua et al. 1996). Therefore, supplementation with tocopherol and/or acetylsalicylic acid administered to mothers exposed to dioxins before pregnancy could to some extent limit the number of developmental tooth defects in children.

Conclusion

In conclusion, the observed increase in AhR expression in some cells of the dental organ of offspring of rat dams exposed to TCDD, which were supplemented with tocopherol or acetylsalicylic acid for 3 weeks, (groups 3 and 4), is probably connected with a decreased concentration of dioxins transported through the placenta to the offspring's organism. On the basis of the studies on mice genetically deprived of AhR, it has been proved that lack of this receptor significantly decreases the effects of dioxins since the TCDD main pathway is through AhR (MacDonald et al. 2004). By using tocopherol and acetylsalicylic acid affinity to AhR, the reduction of the negative biological effects of TCDD may be explained. This fact was observed in earlier studies on tocopherol and in the results of this article.

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