Morphological Effects of non- and mono-ortho PCB on Rat Tubuli Seminiferi *in vitro*

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Objective

To find out whether non-ortho and mono-ortho polychlorinated biphenyls (PCB) could interfere with rat spermatogenesis in vitro, isolated tubuli seminiferi were incubated with medium, containing PCB 77, 126, or 118, and were morphologically evaluated by light microscopy.

Introduction

There is increasing evidence of a substantial fall in average sperm counts in normal men over the past half of this century[1,2]. It is discussed that exposure of humans and animals to inappropriate levels of environ-mentally persistent chlorinated hydrocarbons could be causal. Thus, it is of special interest how these compounds may interfere with spermatogenesis in mammalians and men. Polychlorinated biphenyls (PCB) as well as the related polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) are ubiquitious contaminants with high lipophilicity and accumulation in food chains. They are found in substantial amounts in body fluids and tissues of mammalians and humans. Earlier it was demonstrated that the most toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) interferes with spermatogenesis of marmoset monkeys *(Callithrix jacchus)* and rats in vivo[3,4]. 2,3,7,8-TCDD affected testicular morphology in a dose-dependent manner. The changes found comprised decreased intercellular contact in the germinal epithelium especially in the basic compartment, indicated by enlarged intercellular spaces between Sertoli cells as well as between Sertoli cells and neighbouring germ cells. Furthermore, the dissolution of the germinal epithelium was documented by the occurence of spermatocytes and predominantly premature spermatids in the tubular lumen. Sertoli cells exhibit an increased amount of lipid droplets, phagolysosomes, and vacuoles in the cytoplasm[3,4].

In contrast to 2,3,7,8-TCDD, very little is known about effects of PCB on male reproduction. However, the "dioxin-like" non- and mono-ortho PCB are under particular suspicion of disrupting male reproduction. Since it was shown that 2,3,7,8-TCDD affects spermatids during early spermiogenesis in marmosets, in this study two types of rat tubuli seminiferi i.e. dark (I) and pale (II)

were isolated according to their transillumination pattern[5]. Type (I) included stages II to VII of spermatogenesis and type (II) represented stages VIII to XIV and stage I. This method allows the cultivation of defined stages of the seminiferous cycle. The tubular segments were incubated in PCB-containing medium for 5 or 24 hours and were consequentially morphologically evaluated by light microscopic techniques.

Materials and Methods

Sexually mature Wistar rats (HsdCpd:WU, Harlan Winkelmann, Borchen, Germany) were sacrificed by cervical dislocation and the testes were immediately removed. The tubular segments with a defined transillumination pattern were identified, carefully removed, and transfered either to a four well multidish with PCB-containing supplemented serum-free defined medium (SFDM) or immediately fixed in 5 % glutaraldehyde in s-collidine buffer (0.2 M, pH 7.4)[6]. Sepharose beads (Sephadex G10, Sigma, Taufenkirchen, Germany) covered either with 3,3',4,4'-tetrachlorobiphenyl (PCB 77); 3,3',4,4',5-pentachlorobiphenyl (PCB 126) or 2,3',4,4',5-pentachlorobiphenyl (PCB 118) at 5 µg PCB/g sepharose were used to contaminate the media[7]. Media and PCB-containing sepharose were stirred overnight and finally the sepharose was eliminated through filtration (Sterilfiltration Unit 0.2 µm, Nalge Ltd., Hereford, England). For controls SFDM was either used directly or stirred overnight with uncontaminated G10 sepharose (sepharose-medium). The tubules were cultured 5 or 24 hours at 33°C in a humidified atmosphere containing 5% CO_2. After the incubation period the tubules were fixed as described above and embedded in Epon 812. Serial semi-thin sections (1 µm) were cut and stained with methylene blue/azur II[8].

The PCB were separated from the medium by solid phase extraction on RP-18 columns (Adsorbex, Merck, Germany). The quantitative analysis was performed by high resolution gas chromatography/ low resolution mass spectrometry (HRGC/LRMS) using the corresponding $^{13}C_{12}$-labeled PCB congener as internal standard.

Results

The initial PCB concentrations in the culture media were as follows:

3,3',4,4'-tetrachlorobiphenyl (PCB 77): 4.02 ng/ml
3,3',4,4',5-pentachlorobiphenyl (PCB 126): 6.55 ng/ml
2,3',4,4',5-pentachlorobiphenyl (PCB 118): 4.70 ng/ml

Thus, the molar PCB concentrations were about 10 to 20 nM.

After 5 and 24 hours of incubation tubuli type I (dark) and tubuli type II (pale) of the control group (SFDM or sepharose-medium) revealed no differences when compared with non-cultivated tubules. The tubules showed the characteristic layer arrangement of the germinal epithelium and mostly close intercellular contacts. The effects of all three PCB were clearly time dependent. After an incubation period of 5 hours, PCB 126 and PCB 77 had no effects when compared to the control. PCB 118 treated tubules (I) displayed "empty" appearing areas within the epithelium after 5 hours, whereas in tubules (II) no effect was seen. After 24 hours in PCB 126 and PCB 77 treated tubules (I) loosened intercellular contacts between Sertoli cells and germ cells were found predominantly in the basal compartment. In the adluminal area, especially in the layer of round spermatids, necrotic cells were frequent. The disorganized epithelium of type II tubules revealed multiple forms of cytoplasmatic fragments in the adluminal compartment, mainly originating from affected spermatids. Type I tubules were more sensitive to PCB 118 than type II tubules. After 24 hours destruction of epithelium and necrotic processes were dominant and necrotic spermatids were frequently found.

Discussion

The morphological data clearly demonstrate that the environmentally persistent PCB are able to induce morphological lesions in cultured rat tubuli seminiferi in vitro. It is widely accepted that spermatogenesis depends not only on endocrine mechanisms. This is especially true for the germinal epithelium, where the paracrine interactions between Sertoli cells and germ cells are essential for the development of spermatogonia to mature spermatozoa. Cultivated tubuli seminiferi containing late pachytene and diakinetic primary spermatocytes complete meiosis[9]. Sertoli cells maintain their cyclic function for a certain time in vitro[5]. In the absence of Sertoli cells, the survival of germ cells in culture is reduced, when compared to co-culture of germ cells and Sertoli cells. Additionally, the degree of the morphological differentiation of cultured Sertoli cells is quite different in comparison with Sertoli cells co-cultured with germ cells. Therefore, compared to other culture systems the tubulus-seminiferous-culture appears to be the method of choice for testing the toxicological potency on male reproduction of defined compounds and mixtures.

In a previous study it was demonstrated that 2,3,7,8-TCDD caused identical effects in this in vitro system[10] as were found in vivo[3]. Because of the described stage-dependent morphological lesions in the marmoset monkey in vivo, we isolated tubular segments from rat testis according to their transillumination pattern[5] and thus were able to study the effects of PCB on defined stages of the seminiferous cycle. The effects found in our investigation comprised e.g. loosened intercellular contacts in the basic compartment and affected spermatids in the adluminal area. Under physiological conditions, Sertoli cells closely surround the developing germ cells by long cytoplasmatic evaginations and furthermore are interconnected by tight junctions. These specialized environment is separated from the systemic circulation by tight junctions. Sertoli cell tight junctions are located in the basal region of the cell and form part of the blood-testis barrier[11]. The tight junctions prevent the diffusion of bloodborn substances to the adluminal compartment of the tubuli seminiferi. The enlarged intercellular spaces may indicate that PCB are able to affect this blood testis barrier. Because of the changes within this barrier, paracrine interactions of Sertoli cells and germ cells are disturbed. Furthermore, tubuli type I and tubuli type II revealed that especially spermatids of early spermiogenesis (spermatids 1-14) were affected. Late spermatids (spermatids 15-19 from tubuli type I) exhibited no damage.

PCB 126 and PCB 77 are "dioxin-like" non-ortho chlorinated PCB. Those PCB act by the same mechanism of toxicity as the chlorinated dioxins and furans i.e. via binding to the cytosolic Ah-receptor (Arylhydrocarbon receptor). As well as for the PCDD/PCDF, toxicity equivalency factors (TEF) have been assigned to dioxin-like PCB for risk assessment[12]. The degree of morphological damage of PCB 126 and PCB 77 on rat tubuli seminiferi is consistent with their TEF (PCB126: TEF = 0.1; PCB 77: TEF = 0.0005): the morphological lesions were less obvious in the PCB 77 treated tubuli seminiferi. PCB 118 (TEF = 0.0001) is a mono-ortho chlorinated PCB which is found in significant concentrations in biological samples, e.g. mother's milk. Therefore, toxicological effects of this PCB congener are of particular interest despite its moderate TEF. The degree of damage seen in the tubuli treated with PCB 118 were comparable with those recognized in the tubules treated with the most toxic PCB 126. The initial concentration of PCB 118 in the culture medium was 4.7 ng/ml. This is only about a factor 20 higher than background levels of PCB 118 in blood serum from adult persons in Germany[13].

Conclusion

We conclude that spermatogenesis in vitro is highly susceptible to dioxin-like non- and mono-ortho PCB. The effects found are time-dependent and stage-dependent. There is a selective effect on early spermiogenesis (spermatids 1-14). At present, it is not possible to decide whether the high number of necrotic cells, particulary spermatids, is due to direct toxic influences or is due to disturbed cellular

interactions. The primary target cell for the toxic effects of PCB needs to be discovered. Whether other mono-ortho PCB (e.g. PCB 105, 156) and the environmentally most abundant di-ortho PCB congeners (PCB 101, 138, 153, 180) cause similar effects or not, needs to be investigated.

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