Aroclor-1254 affects mRNA polyadenylation, translational activation, cell morphology, and DNA integrity of rat primary prostate cells

Fabiana Cillo, Magda de Eguileor¹, Fulvio Gandolfi and Tiziana A L Brevini

Laboratory of Biomedical Embriology, School of Veterinary Medicine, UniStem, University of Milan, Milan, Italy
¹Department of Structural and Functional Biology, University of Insubria, Varese, Italy

(Requests for offprints should be addressed to F Cillo Istituto di Anatomia degli Animali Domestici, Via Celoria 10, 20133 Milano, Italy; Email: fabiana.cillo@unimi.it)

Abstract

Environmental xenobiotics have been shown to act as endocrine disruptors and to be implicated in increased cancer susceptibility. In particular, there is a significant concern regarding the impact of these contaminants on prostate cancer development and progression. However, the mechanisms with which these contaminants exert their detrimental effects are yet unclear and need to be further elucidated. In the present study, we investigated the effects of Aroclor-1254, a mixture of more than 60 environmental pollutants belonging to the polychlorinated biphenyl family, on rat prostate primary cultures. The results obtained after 24-h exposure indicated the ability of this contaminant mixture to influence mRNA stability and length of the 3' end poly(A) tail of Connexin-32, Connexin-43, and heat shock protein-70. Consistent with this observation, immunostaining experiments demonstrated the altered availability of the encoded proteins. We also focused our attention on possible effects of Aroclor-1254 on cell morphology and could detect ultrastructural changes with gap junction disruption, fusion of single cells into clusters, and different aspects of apoptosis that became evident when exposure to Aroclor-1254 was extended to 72 h. The effects on the nuclear compartment were confirmed by the results obtained with Comet assay that showed DNA decompression and double-strand breaks already after 24-h exposure. Taken together, these findings show a detrimental effect of Aroclor 1254 on rat prostate cells and indicate a possible association between exposure to polychlorinated biphenyl mixture and induction of transformation process in prostate cells.

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Introduction

Epidemiological studies in the past 20 years suggest a link between male reproductive health disturbances and increasing exposure to environmental xenobiotics, both in wildlife and in humans (Colborn et al. 1993, Sharpe & Skakkebaek 1993). The growing incidence of hormone-dependent pathologies in the male reproductive tract has been associated with exposure to contaminants derived from the polluted environment and capable of affecting and disrupting the endocrine system, although the impact of endocrine disruptors on the male reproductive function remains to be fully appreciated and assessed. Among the many xenobiotic compounds, serious concern is directed at polychlorinated biphenyls (PCBs), because of their ability to interfere with normal reproductive functions and their action as endocrine disruptors. The PCBs are complex mixtures of up to 209 different congeners and isomers formed by the nonspecific chlorination of biphenyl ring that, because of their lipophilicity, preferentially bioaccumulate and biomagnify in higher trophic levels of the food chain (Safe 1990). Despite the fact that PCB commercial production ceased officially in the late 1970s, these compounds continue to enter the food chain as a result of waste disposal, incineration, and industrial leakage. The PCBs were first reported carcinogenic in rodents in Ito et al. (1973). Since then, numerous studies have demonstrated that these chemicals exert a variety of...
toxic effects, such as carcinogenicity, immunotoxicity, teratogenicity, and reproductive toxicity in wild and laboratory animals (Battershill 1994) and have been shown to inhibit intercellular communication in cell culture studies (Krutovskikh et al. 1995, Santomauro et al. 1999). In rodents, the effects of PCBs on male reproduction in vivo comprise reduced fertility, reduced mating, reduced weight of the ventral prostate and seminal vesicles, and alteration of testicular spermatozoa concentration (Ahlborg et al. 1992). Correlation between environmental or occupational exposures to PCBs and human prostate cancer has also been reported in the literature (Hassel et al. 2004, Zeegers et al. 2004). A recent study has provided a strong evidence for the association of prostate cancer with cumulative PCB exposure in an occupational cohort (Prince et al. 2006). Aroclor-1254 (A-1254) is a mixture of more than 60 congeners and is widely used for in vitro studies because its composition is representative of PCB environmental pollution (Stack et al. 1999). Several reports demonstrated that this mixture is able to decrease sperm motility and sperm count and to alter ventral prostate antioxidant system in adult rats (Anbalagan et al. 2003, Sridhar et al. 2004). In the previous studies, we demonstrated that A-1254 interacts with gene expression, affecting mRNA stability by altering the length of the 3'-end poly(A) tail of a number of specific genes in different experimental models (Brevini-Gandolfi & Gandolfi 2001, Pocar et al. 2001, 2006, Brevini et al. 2005a,b). In particular, translational activation of some transcripts, such as Connexin-32 (Cx-32), Connexin-43 (Cx-43), and heat shock protein-70 (Hsp-70), are affected by endocrine disruptors, resulting in altered availability of the encoded proteins. In agreement with this, we recently demonstrated a specific effect of A-1254 on gap junction-mediated communications (Brevini et al. 2004).

Based on these observations, in the present paper, we investigated the effect of A-1254 in rat prostate cells. These cultures were used as an in vitro model for investigating the possible mechanism involved in detrimental effects of PCBs on prostate, which represents one of the primary target for male reproductive tract pathologies. We specifically addressed our attention to the following aspects: (a) A-1254 interaction with mRNA polyadenylation level and protein availability of genes involved in cell–cell communication (Cx-32 and Cx-43) and stress-induced response (Hsp-70); (b) A-1254 ability to cause DNA damage; and (c) A-1254 capability to affect rat prostate cell morphology.

Materials and methods

Animals

Three-month-old male Sprague–Dawley rats (300–350 g weight; Charles River, Italy) were used throughout these experiments. The animals were maintained in animal quarters with controlled temperature and humidity. The light schedule was 14 h light:10 h darkness (lights on at 0630 h). Animals were fed a standard pellet diet and water was provided ad libitum.

Materials

Unless otherwise indicated, all cell culture reagents were obtained from Gibco (Invitrogen S.r.l) and Sigma–Aldrich S.r.l. A-1254 was kindly donated by Dr S Safe, Texas Veterinary College.

Prostate primary cell cultures

Prostates were isolated from male Sprague–Dawley rats in Dulbecco’s PBS and transferred to 6 ml medium RPMI 1640 (Seromed, Biochrom KG, Berlin, Germany), supplemented with l-glutamine (2 mM) and antibiotics (200 UI/ml penicillin G Na, 200 µg/ml streptomycin sulfate, and 50 µg/ml amphotericin B), and minced into small pieces.

Type 2 collagenase was added (150 U/ml), the mixture was incubated at 37 °C for 2 h with constant shaking and then centrifuged at 1000 g for 3 min. Supernatant was removed and cell pellet was resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum and incubated at 37 °C in a humidified atmosphere of 5% CO2 in air.

Cells were plated at a density of 7.5×105 cells/ml and culture media were then changed every 2 days.

Exposure to A-1254

Treatments with A-1254 were performed 8 days after cell plating. A stock solution of A-1254 in neat form was prepared in absolute ethanol at a concentration of 100 µg/ml. This solution was used to obtain the final experimental doses ranging between 0 and 1 µg/ml, which were chosen based on evidences in the literature and previous experiments in our laboratory (Pocar et al. 2001, Endo et al. 2003). Cells were exposed for 24 h with the exception of the ones used in morphological studies, where exposure was extended to 72 h. At the end of the incubation period, cells were removed from culture dishes and processed for different analyses. In control cells, A-1254 was omitted and cells were exposed to vehicle only.
Polyadenylation studies

RNA extraction
RNA was isolated from cells using the acid–phenol method according to Chomczynski & Sacchi (1987). No additional carrier RNA was used as recommended by Salles & Strickland (1995).

PCR poly(A) test
Poly(A) tail length was determined as described by Salles & Strickland (1995), with some minor modifications.

Reverse transcription
RNA was denatured at 65 °C for 5 min in a final volume of 7 µl, saturating the poly(A) tail with 25 ng phosphorylated oligo(dT)(p(dT)12-18; Amersham Pharmacia). Ligation of oligo(dT)[p(dT)12-18] was then carried out at 42 °C in 13 µl pre-warmed mix, consisting of 4 µl sterile water, 10 U T4 DNA ligase (Gibco BRL Life Technologies), 4 µl of 5× Superscript Rnase H reverse transcriptase RT buffer (Gibco BRL Life Technologies), 1 µl of 10 mM dNTPs, and 2 µl of 0.1 M dithiothreitol, and 1 µl of 10 mM ATP. After 30-min incubation, 200 ng specifically designed oligo(dT)-anchor primer were added and temperature was lowered to 12 °C. The oligo(dT)-anchor primer with nucleotide sequence 5′-GCGAGCTCCGGGCCCC(GCGT)12-3′ was ligated with 2-h incubation period to the extreme 3′-end of the poly(A) tail. Samples were then transferred back to 42 °C. Reverse transcription was performed with 500 U Superscript RNase H-RT (Gibco BRL Life Technologies) for 1 h. Enzymes were inactivated with 30 min incubation at 70 °C.

PCR amplification
The PCR Poly(A) test requires the use of primers located close to the 3′-end (within 400 nucleotides) in order to provide the best PCR product size resolution (Salles & Strickland 1995).

PCR runs were carried out with oligo(dT)-anchor and 5′ primers specifically designed for Cx-32 (5′-AGTGGAGGAGGATCTCTG-3′), Cx-43 (5′-ACAGTCTTTTGGAGTACCCGAGCA-3′), and Hsp-70 (5′-GAGAGGTTGCTGGAAGAGTG-3′) respectively. One microliter of the poly(A) cDNA product was amplified in a reaction mix consisting of 3 µl MgCl2 (25 mM), 0.3 µl Taq polymerase (5 U/ml), 2 µl of 10× PCR buffer (Boehringer), 1 µl of 10 mM dNTPs, and 10.2 µl sterile water. To spike the reaction, 5 µCi of 32P-dATP (specific activity 10 mCi/ml) were added to the mix. PCR was carried out in an automated thermal cycler (Perkin–Elmer, Cetus Instruments, USA), using the following conditions: 30 s at 93 °C (double-strand denaturation temperature), 1 min at 61 °C (annealing temperature), and 1 min at 72 °C (double-strand extension temperature), performing 38 cycles. The amplified products were purified using Qiaquick PCR purification spin columns (Qiagen Inc.) and separated on 3.5% TBE polyacrylamide gels (Sambrook et al. 1989) with a labeled DNA ladder (Gibco BRL Life Technologies) in order to allow size determination. Gels were exposed overnight to Amersham Hyperfilm β-max. Size detection of each band in each gel was carried out using Epson GT-8000 Scanner; transcript length of each replicate was then determined with GEL 1.01 as described by Lacroix (1994). Data obtained from three separate sets of experiments with four replicates of cell homogenates per treatment and per gene were subjected to statistical analysis using SuperAnova v. 1.11 (Abacus Concepts Inc.).

The identity of each fragment was confirmed by restriction enzyme analysis, with specific endonucleases known to cut within the region amplified by the PCR primers used.

Protein extraction, gel electrophoresis, western blotting, immunostaining, and densitometric analysis
Cells were homogenized, lysed, and constitutive proteins were extracted (Gandolfi et al. 1989). Protein concentration was assessed by the Coomassie Blu-G Dye-binding methods (Read & Northcote 1981). Aliquots of 50 µg were prepared and resuspended in sample buffer consisting of 10% (w/v) glycerol, 2.3% (w/v) SDS, and 6.25 M Tris–HCl (pH 6.8) and electrophoresed on a 10% SDS-polyacrylamide slab gel (Laemmli 1970). Proteins were then transferred onto nitrocellulose filters according to Towbin et al. (1979), using 0.5 A/cm². Equal sample loading and transfer efficiency were confirmed by staining of the membrane with Ponceau Red. The membrane was probed with specific polyclonal antibodies (diluted in the ratio of 1:500) raised against Cx-32, Cx-43, and Hsp-70 (Santa Cruz Biotechnology). Anti-IgG linked to horseradish peroxidase (Amersham Pharmacia Biothec) were used as secondary antibodies and the presence of immunoreactivity for the molecules of interest was visualized using enhanced chemiluminescence (ECL; Amersham Pharmacia Biothec). Densitometric analysis of the immunostained bands was performed using an Apple One Scanner and processed with Macintosh image 1.33.
Alkaline single cell gel electrophoresis (Comet) assay

The procedure used, followed the method described by Singh et al. (1988) with some modifications. Fully frosted microscope slides were coated with 0.5% normal melting point agarose (NMA) in PBS and covered with a coverslips. Slides were stored at 4 °C in a dark and humid box. Ten microliters of cell suspension were added to 80 μl of 0.5% w/v low melting point agarose (LMPA at 37 °C) in Ca²⁺ and Mg²⁺ free PBS and layered over the NMA. After polymerization of agarose a final layer of 80 μl of 0.5% LMPA was added. Following agarose solidification, the coverslips were removed and slides lowered into freshly made lysis solution (10% DMSO, 1% Triton X-100, 1% N-lauroylsarcosine, 100 mM Na₂-EDTA, 10 mM Tris, and 2.5 M NaCl, pH 10) for at least 1 h at 4 °C. The slides were then placed on a horizontal gel electrophoresis tray and covered with freshly made electrophoresis buffer (0.075 M NaOH, 1 mM Na₂-EDTA, pH > 12) for 20 min to allow the DNA to unwind. Electrophoresis was carried out at 25 V, 300 mA for a further 20 min and the slides were then placed on a staining tray and covered with neutralizing solution (0.4 M Tris base, pH 7.5) for 5 min and repeated three times. Slides were then fixed in 70% ice-cold ethanol for 30 min (Klaude et al. 1996) and stored at room temperature for maximum 1 week, prior to analysis. Before image analysis slides were rehydrated with 200 μl distilled water for 10 min then stained with 50 μl filtered ethidium bromide (20 μg/ml) and overlaid with a coverslip. Analysis for the presence of comets was carried out using a Nikon Eclipse E600 inverted fluorescent microscope (200× magnification). Whole slides were randomly scanned, the amount of damaged DNA was then quantified in terms of increased fluorescence in the tail region. Results were processed with NIH Image 1.63 and are expressed as the percentage of DNA migrated into the comet tail region (tail % DNA), following the criteria suggested by Anderson et al. (1994) (grade of damage: zero or minimal < tail 5%, low-damage tail 5–20%, mid-damage tail 20–40%, high-damage tail 40–75%, and extreme-damage tail >75%).

Electron microscopy

At the end of the incubation period with A-1254 (24 and 72 h), cells were centrifuged at 1000 g for 5 min to remove the medium and the pellet was fixed in a solution of 2% glutaraldehyde in PBS. Cells were then washed in the same buffer and post-fixed for 2 h with 1% osmic acid in 0.1 M Na-cacodylate buffer (pH 7.2). After standard dehydration in ethanol, cells were embedded in an Epon–Araldite 812 mixture. Sections were cut with Reichert Ultracut S ultratome (Leica, Vienna, Austria). Thin sections were stained with uranyl acetate and lead citrate and were observed with a Jeol 1010 EX electron microscope (Jeol, Tokyo, Japan).

Statistical analysis

Data are expressed as the means ± S.D. or S.E.M., according to the set of experiments, and were statistically analyzed following the Dunnett’s test after one-way ANOVA. In experiments on comet assay, statistical differences were assessed by the χ² test with Yates correction for multiple comparisons. All statistical tests with P < 0.05 were considered significant.

Results

A-1254 effect on mRNA polyadenylation

Poly(A) tail length of Cx-32, Hsp-70, and Cx-43 was evaluated in prostate cells treated with 0.1 and 1 μg/ml A-1254 respectively, as well as in untreated cells. Analysis was repeated on three separate experiments with four replicates for each concentration of A-1254. No statistically significant variations between replicates among the three sets of experiments were observed.

The results obtained are shown in Fig. 1 and Table 1. It can be observed that A-1254 affected polyadenylation in all the three genes considered. In particular, exposure to A-1254 caused elongation of the poly(A) tail in Cx-32 and Hsp-70 transcripts at all concentration tested when compared with untreated cells (C). This effect appeared to be dose-dependent, since cells treated with the highest dose of A-1254 considered in the experiment (1 μg/ml) showed a greater increase in poly(A) tail than those exposed to the lower concentration (0.1 μg/ml).

By contrast, A-1254 induced significant shortening of the poly(A) stretch at the 3′-end of Cx-43, though this variation was not dose related and comparable in

![Figure 1 Polyadenylation changes of transcripts coding for Cx-32, Hsp-70, and Cx-43 in rat prostate cells exposed to A-1254 at 0 (C), 0.1 (0.1), and 1 μg/ml (1) respectively. 32P-labeled amplification products were run on 3% TBE polyacrylamide gels after mRNAs were reverse transcribed and amplified using specific primers for each gene (see Materials and Methods for details). The appropriate molecular weight marker is shown on the left of each gel window.](image-url)
cells treated with different concentrations of A-1254 (0.1 and 1 µg/ml).

**A-1254 effect on protein availability**

PAGE, western blotting, and densitometric analysis of Cx-32, Hsp-70, and Cx-43 proteins in cells treated with A-1254 and in untreated cells are illustrated in Fig. 2A and B.

The effect exerted by A-1254 on the availability of these proteins appears to be in agreement with the effect of the contaminant mixture on polyadenylation. Indeed, A-1254 induced an increased availability of Cx-32 and Hsp-70 and a decrease of Cx43. In addition, Cx-32 increment appeared to be dose-related, displaying a significantly raised concentration of Cx-32 in cells treated with the highest dose considered (1 µg/ml). Conversely, no dose-related effect was evident for Hsp-70 and Cx-43 proteins, which showed a comparable increment, regardless to A-1254 concentrations used.

**A-1254 genotoxic effects**

Figure 3 and Table 2 show the results obtained after single cell gel electrophoresis (Comet) assay of rat primary prostate cells exposed to A-1254 at the concentration of 1 µg/ml for 24 h. One hundred cells per slide were analyzed for DNA damage and the analysis was repeated on three separate experiments with three replicates. Based on the criteria reported by Anderson *et al.* (1994; see also Material and Methods), cells were scored for zero-low (l), mid-high (m), and extreme (e) damage (Table 2). Untreated cells (98.2%) had zero-low damage with only 1.8% carrying mid-high damage. By contrast, A-1254 exposed cells demonstrated only 54.4% cells with zero-low damage and a much higher number than control of cells displaying a mid-high damage (45.6%). No extreme damage was detected in either treated or control cells.

**A-1254 effect on cell morphology**

Structural and ultrastructural analysis of control and cells treated for 24 and 72 h with A-1254 (1 µg/ml) is shown in Fig. 4 and demonstrated considerable changes in cellular morphology. In particular, A-1254 exposed cells (A-1254 24 h) appeared round and no microvilles or pseudopodes were observable when compared with control cells (control 24 h, A and B). Following treatment, cells showed an early

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**Table 1** Poly(A) tail differences in mRNA of rat prostate cells exposed to 0.1 and 1 µg/ml A-1254

<table>
<thead>
<tr>
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<th>Control</th>
<th>0.1 µg/ml</th>
<th>1 µg/ml</th>
</tr>
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<tbody>
<tr>
<td>Cx-32</td>
<td>274 ± 4.11a</td>
<td>303 ± 3.26b</td>
<td>344 ± 4.50c</td>
</tr>
<tr>
<td>Hsp-70</td>
<td>612 ± 7.51a</td>
<td>639 ± 3.26b</td>
<td>655 ± 3.68c</td>
</tr>
<tr>
<td>Cx-43</td>
<td>508 ± 6.18a</td>
<td>484 ± 6.12b</td>
<td>479 ± 4.11b</td>
</tr>
</tbody>
</table>

Numbers shown in the table are transcript length (expressed as bps) and represent the mean ± S.D. of the four replicates obtained for each gene considered in the three sets of experiments. Statistical analysis was carried out using Super-Anova 1.11. Different superscript letters indicate significant difference ($P<0.05$).
apoptotic aspect characterized by surface blebs. In addition, it was also possible to observe gap junction disruption and cell fusion (C, dashed arrows). A-1254 effect became even more remarkable in cells treated for 72 h, with the appearance of apoptotic bodies, altered cytoplasm with swollen vacuoles and nucleus characterized by different aspect in chromatin aggregation (D, A-1254 72 h).

**Discussion**

In the present study, we investigated the effects exerted by A-1254 on primary cultures prepared from rat prostate. Our experiments demonstrated that this contaminant mixture has the ability to interfere with polyadenylation levels of all the three genes that were tested. More in details, A-1254 caused elongation of the poly(A) tail of the transcripts coding for Cx-32 and Hsp-70, when compared with untreated cells. This effect appeared to be dose-dependent since cells treated with the highest dose of A-1254 considered in the experiment (1 μg/ml) showed a greater increase in poly(A) tail than those exposed to the lower concentration (0.1 μg/ml). Interestingly, even the highest dose used in this experiment is well under the concentration (0.1 g/ml) of A-1254 that has been suggested to have a role in carcinogenesis in several systems (Oyamada et al. 1990, Hossain et al. 1999). A-1254 induced variations in the availability of Hsp-70 protein that were in agreement with the perturbing effect exerted by A-1254 on polyadenylation of Hsp-70 RNA transcripts.

Hsp-70 proteins are rapidly expressed by individual cells in response to any adverse environmental stress and serves to protect those same cells from the harmful effect of stress conditions. Furthermore, expression of some members of the Hsp family is altered in pathological conditions in prostatic tissues (Alaiya et al. 2000, Tang et al. 2005). Increased concentrations of Hsp-70 in our cultures are thus likely to be related to defense responses activated in the cells following exposure to A-1254. This is also in agreement with data reporting altered levels of Hsp proteins in different experimental models after treatment with PCBs (Fukuda et al. 1999, Yoshioka et al. 2001).

In the present experiments, we found that A-1254 affects the availability of Cx-32 and Cx-43 proteins. Interestingly, though its effect seems to be specific since Cx-32 shows an increased availability, while Cx-43 is decreased to lower concentrations. We have no explanation for this diversity of effect at present, but it is very tempting to hypothesize a possible interregulation among gap junctional proteins. Interestingly, dysregulation of Cx-32 and Cx-43 expression has been suggested to have a role in carcinogenesis in several systems (Oyamada et al. 1990, Hossain et al. 1999) and, more specifically, has been described to cause excessive hyperplastic and neoplastic growth or

**Table 2** Analysis of A-1254 treatment on DNA damage in rat primary prostate cells

<table>
<thead>
<tr>
<th></th>
<th>l</th>
<th>m</th>
<th>e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>884 (98.2%)</td>
<td>6 (1.8%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>A-1254*</td>
<td>490 (54.4%)</td>
<td>410 (45.6%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

Cell distribution for zero-low (l), mid-high (m), and extreme (e) damage in A-1254-treated cells is statistically different from control cells (control). *Indicates significant difference (P<0.05).
excessive cell death (hypoplasia and atrophy) in prostatic tissues (Liu et al. 1997). Consistent with this, Habermann et al. (2002) have shown that Cx-32 and Cx-43 expression and localization are altered in benign prostatic hyperplasia and prostate cancer. Based on the observation that Cx-32 mainly localizes to the luminal cell compartment of the prostatic epithelium, while Cx-43 is restricted to the basal epithelium cells (Habermann et al. 2001); our results support the hypothesis that A-1254 may exerts its detrimental effect by increasing gap junction communication in luminal cells, as shown by the raised availability of Cx-32. Conversely, the decrement of Cx-43 protein concentrations induced by the exposure to the contaminant mixture might reflect a cell downregulation and quiescence of the basal compartment. Furthermore, Chang et al. (1996) demonstrated an inhibitory effect among connexins in testis germ cells, suggesting the possibility that increased concentration of a specific connexin may have, as a final result, the inhibition of other molecules of the same family. In this line, it is possible that Cx-32 protein increase may inhibit Cx-43, which is, at the same time, translationally less active because of poly(A) tail shortening. Gap junction-mediated communication is required for normal cell growth and differentiation. Thus, connexin molecules that form gap junctions are thought to play an important role in maintaining tissue homeostasis by allowing the intercellular exchange of molecules associated with cell growth (Trosko & Chang 2001) and cell signaling (Moorby & Patel 2001). Tumor promoters can inhibit cell–cell communication by interacting with the gap junction structure and/or function (Klauning 1991). Similarly, PCB carcinogenic activity may be associated with the inhibition of intercellular communications. Indeed, PCBs have been previously shown to inhibit capability for intercellular communication in different cell types (Krutovskikh et al. 1995, Santomauro et al. 1999). Therefore, we may hypothesize that A-1254 effect on the availability of connexin molecules in prostate cells might be related to a detrimental action of the contaminant on cell–cell communication. This disturbance of connexin-mediated communications may represent one of the mechanisms through which A-1254 may play a role in tumor induction and promotion (Knerr & Schrenk 2006).

This hypothesis finds support in the results obtained studying A-1254 ability to affect rat prostate cell morphology. This set of experiments demonstrated gap junction disruption, loss of microvilli, and fusion of single cells into clusters. These alterations became even more severe when exposure to the contaminant was extended to 72 h. Many cells show several ultrastructural features typical of apoptotic death and membrane blebs, vacuolization, and nuclear envelope breakdown are some of the most evident morphological changes. At present, it is difficult to say whether the morphological changes described earlier are the result of A-1254 perturbing effects on connexin transcript polyadenylation and encoded protein availability or they represent the primary cause for such
effects and this aspect needs to be further investigated. However, it is interesting to note that A-1254 ability to cause nuclear damage are confirmed by the results obtained with comet assay that demonstrate DNA decompensation and double-strand breaks in a large proportion of prostate cells (45.6%) already after 24-h in vitro exposure to the contaminant mixture. It is important to note that in vivo genotoxic hazard of A-1254 and other PCBs is not limited to this model since it has been shown in leukocytes and erythrocytes, indicating a general harmful effect of these contaminants on DNA structure of different cell types (Taddei et al. 2001, Nigro et al. 2002).

Altogether the results presented in this paper indicate a detrimental effect of A-1254 on rat prostate cells. The contaminant mixture affects gap junction-related molecules, severely disrupts cell morphology and causes DNA damage. Both post-transcriptional and post-translational mechanisms as well as genotoxic effects seem to be involved in the association between A-1254 exposure and its carcinogenic effects in rat prostate cells.

Acknowledgements

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