Effects of low concentrations of a polychlorinated biphenyl, Aroclor 1254 on membrane bound ion dependent ATPases in mice liver

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Aroclor 1254, a polychlorinated biphenyl, is present in the environment in low concentration but references on its toxic effects on liver cell membrane proteins and the mechanism of actions are not abundantly available. Therefore, the present study was undertaken to investigate the low level, sub-acute dose and exposure duration dependent effects of Aroclor 1254 on total, Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+} and Mg\textsuperscript{2+}-ATPases of the mouse liver. The hypotheses tested in the present study were, (a) whether the low, environmentally available dose and the exposure durations of Aroclor 1254 affects the membrane-bound ion dependent ATPases, and (b) if a response was observed, whether it is a direct or indirect effects of the toxicant. Groups of mice were exposed to different doses (0.1 and 1mg kg\textsuperscript{-1} body weight d\textsuperscript{-1}) and exposure durations (4 d, 8 d and 12 d) of Aroclor 1254. The results indicated significant exposure duration dependent changes in the specific activity of the selected membrane bound ATPases. As the observed changes were mostly enzyme stimulation after toxication through oral administration, the effects of the Aroclor were possibly indirect, through complex chain of reactions.

Keywords: Aroclor 1254, ATPases, Liver, Mice

Polychlorinated biphenyls (PCB) are very stable, strongly lipophilic compounds which are persistent in the ecosystem. These compounds are generally enriched in the food chain due to their strong affinity for lipids and are generally resistance to metabolism. Few congeners of PCBs have varieties of toxicological effects in mammals including hepatotoxicity after chronic exposure\textsuperscript{1}. Aroclor, a PCB, is easily absorbed in soil, remains immobile during leaching process and highly mobile in presence of organic solvents\textsuperscript{2}. Oral LD\textsubscript{50} values for Aroclor 1254 are reported to be 1295, 1010 and 4000 mg/kg/day in male Osborne-Mendel, Sherman rats and mink respectively\textsuperscript{3}. PCBs show a higher affinity to liver than other adipose tissues when compared to those PCB congeners which have chlorine atoms in its ortho positions\textsuperscript{4,5}. The high affinity of Aroclor to liver is possibly associated with the induction of binding proteins or various membrane bound proteins like ion channels. These proteins are indicators to follow the toxic effects of a huge variety of compounds as they show early signs of pathological conditions like chronic degenerative diseases\textsuperscript{6}. PCBs are known to generate transient reactive oxygen species which disturbs the function of membrane proteins like ATPases\textsuperscript{7,8}. Some studies showed that the isolated isomers of liver, kidney and brain tissues in fish, rat and other organisms inhibit the activity of total ATPase, Na\textsuperscript{+}, K\textsuperscript{+}-ATPase and Mg\textsuperscript{2+} ATPase after the administration of commercial PCBs\textsuperscript{9,10}. However, most of these studies were conducted using sublethal to near-lethal doses of PCBs. In environment however, PCBs are present in very low amount which may be available to human and other animals through water or food. Reports on toxic effects of the PCB in low concentration on the functions of different key enzyme systems in mammals are rather scanty. Studies on the dose and duration dependent effects of low dose PCB on membrane bound enzyme proteins are also rare. Therefore, the present study has been undertaken to investigate the effect of environmentally available low concentration of PCB in polluted areas. Sub-acute dose and duration dependent effects of Aroclor 1254 on total, Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+} and Mg\textsuperscript{2+}-ATPases of the mouse liver have also been addressed. The study tests two hypotheses, (a) whether the low, environmentally available sublethal dose and the exposure durations of Aroclor 1254 affects the membrane-bound ion dependent ATPases of the hepatic cells and (b) if a response was observed, whether it is a direct or indirect effect of the toxicant.

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Materials and Methods

The PCB, Aroclor 1254, was obtained from Sigma-Aldrich Chemicals Ltd. Adult male Swiss albino mice (63) around 2 months of age and weighing around 30-40 g were used in the study as the mammalian model. The animals kept under prescribed standard conditions were provided commercial rodent diet and water ad libitum. All experiments were conducted according to the ethical norms approved by the CPCSEA, India (CPCSEA/CH/RF/ACK-2003). Different groups of mice were subjected to oral administration of Aroclor 1254 (0.1 and 1 mg/kg body weight /d) dissolved in corn oil (vehicle) for three different exposure durations of 4, 8 and 12 days. After completion of toxic exposure, the whole liver tissue was quickly dissected out and washed in ice cold Sucrose – EDTA - Imidazole buffer (SEI buffer) to remove internal blood and other tissues. The complete liver was homogenized in Elvin-Potter glass homogenizer in chilled SEI buffer at 0 – 4 °C to make a 10% (w/v) tissue concentration. The enzyme extract procedures and the estimation of different membrane bound ion dependent ATPases viz. total-, Na\(^+\),K\(^+\)-, Ca\(^{2+}\)- and Mg\(^{2+}\)-ATPases were done as per the method of Zaugg\(^{11}\) with appropriate modifications\(^{12,13}\). Inorganic phosphate was measured by the method of Fiske and Subbarow\(^{14}\). Total protein content of the tissue extract was estimated as per Lowry et al.\(^{15}\) using crystalline bovine serum albumin as standard. The data were subjected to various statistical analyses for their cumulative acceptability. Two-factor ANOVA, single-factor ANOVA and Student’s t-tests were employed to check the hypotheses designed. All statistical procedures were computed as per Sokal and Rohlf\(^{16}\).

Results and Discussion

In the present study, in vivo dose and duration dependent effects of low concentration Aroclor 1254 on membrane bound ion dependent ATPases were estimated in the liver of adult male mice. The results showed that activity of total ATPase was inhibited in both the doses (0.1 and 1 mg/kg/d) after 4 and 12 days of exposures and higher rate of inhibition was observed in 0.1 mg/kg/d dose of Aroclor 1254. However, after 8 days of exposure, stimulation in the enzyme activity was observed in both the toxicated groups (Fig. 1a). Similar trends were observed in activities of Na\(^+\)-K\(^-\) and Ca\(^{2+}\) ATPases (Fig. 1 b and c). In Mg\(^{2+}\) ATPase, specific activity was inhibited in 0.1 mg/kg/d dose after 4 and 12 days of exposure. But in the higher dose exposed mice at 4 days, the activity was stimulated. However, after 12 days of exposure the enzyme activity was again inhibited. On the other hand, after 8 days of exposure, stimulation was observed in 0.1 mg/kg/day dose group followed by inhibition in 1 mg/kg/d toxicated group (Fig. 1d). The observed effects of Aroclor 1254 was more exposure duration dependent, triggering increased disturbance in the membrane ion channels with increasing time. These alterations could be due to the specific action of Aroclor 1254 on the permeability and general metabolic activity of the hepatocytes\(^{17}\). It is also possible that the imposed stress by the PCB was affecting the cellular transmembrane ion transport which the hepatocytes tried to stabilize by enhancing or inhibiting the specific enzymes involved in ion transport.

Liver, being the largest gland and main detoxifying organ of the body is a principal location for accumulation of absorbed toxicants. Liver damage is one of the clear signs of PCB poisoning in humans. Since the ATPases are responsible for trans-membrane movements of ions, it is possible that the Aroclor affected the transport channels of the liver cells by altering the enzyme activity\(^4\).
The results of two-factor ANOVA showed a predominantly exposure duration dependent effects of Aroclor (Table 1). However, the Student’s t-test performed between the control and toxicated groups showed variations in different enzymes studied in liver. In case of total ATPase, significant variations were observed at 4 and 8 days of exposure between control and the lowest dose, but no significant difference was observed at the higher dose in most of the enzymes studied (Table 2). The results of the single-factor ANOVA showed high significant variations between the exposure durations in most of the enzymes studied (Table 3), possibly governed by a predominantly exposure duration dependent effects. The results of the present study obviously answer the first hypothesis as predominantly exposure duration dependent effects. The use of crude enzyme preparation prohibits unequivocal determination of the mechanism involved in activity. Since Aroclor does not demonstrate specific effect on one type of ATPase the possibility exists that more than one factor might have been involved in the manifestation of toxicity. The results of the present study suggest more complex indirect chain of events answering the second hypothesis. Some studies showed that PCBs-induced toxic manifestations are associated with the production of reactive oxygen species. Hassoun et al. reported that polychlorinated biphenyl 126 induces reactive oxygen species in brain and liver of experimental rats. The reactive oxygen species may propagate the initial attack on lipid membranes of the brain to cause lipid peroxidation. Therefore, one of the possibilities of indirect effect of Aroclor 1254 is oxidative stress which may alter the activity of membrane bound ion dependent ATPases by producing free radicals. In conclusion, the present study revealed a predominant exposure duration dependent effects of the Aroclor 1254 on the membrane bound ion dependent ATPases in mice liver. However, the disturbance in the activity of ATPases activity caused predominantly by exposure durations might be due to indirect effects of PCB in vivo condition driven by oxidative stress.

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References


