FULL PAPER *Toxicology*

Effects and Neuro-toxic Mechanisms of 2, 2', 4, 4', 5, 5'-Hexachlorobiphenyl and Endosulfan in Neuronal Stem Cells

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ABSTRACT. Endocrine disrupters are exogenous compounds thought to mimic the action of estrogen or other hormones and influence endocrine activity in the body (Juberg, 2000). These chemicals have adverse effects not only in the reproductive system but also in the central nervous system during development and throughout life. Polychlorinated biphenyls (PCBs) are a class of environmentally persistent and widespread halogenated hydrocarbons. It has been reported that PCBs are potential neurotoxicants. Endosulfan is an organochlorine insecticide that is extensively used to control pests in vegetables, cotton, and fruits. To determine the effect of 2, 2', 4, 4', 5, 5',-hexachlorobiphenyl(2, 4, 5-HCB) and endosulfan on embryo nervous system, we isolated neural stem cells from rat brain at embryonic day 17. Isolated neural stem cells showed pluripotenty. Stem cells could differentiate into neurons and glia. Neurite formation in endosulfan and 2, 4, 5-HCB treated cells. And it appeared to be decreased as compared with that in untreated cells. In order to know the neuro-toxic mechanisms of 2, 4, 5-HCB and endosulfan in neuronal stem cells, we investigated mitogen-activated protein kinase activity (MAPK) and gap junctional intercellular communication (GJIC). Endosulfan decreased the MAPK activity in dose dependent manner. Endosulfan and 2, 4, 5-HCB inhibited GJIC compared to the untreated cell by scrape loading dye transfer (SL/DT). 2, 4, 5-HCB and endosulfan decreased the expression of connexin 43 in dose dependent manner. These results indicated that 2, 4, 5-HCB and endosulfan may inhibit differentiation and proliferation of neural stem cells and gap junctional intercellular communication which play a crucial role in the maintenance of cellular homeostasis.

KEY WORDS: endosulfan, gap junctional intercellular communication (GJIC), 2, 2', 4, 4', 5, 5',-hexachlorobiphenyl(2, 4, 5-HCB), mitogen-activated protein kinase (MAPK), neural stem cell.

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Endocrine disruptors are chemicals that can disturb the function of the endocrine system [3]. Biochemical compounds, which disrupt normal endocrine function, are numerous and abundant in the environment and in dietary sources and some of them have been used as pharmacological agents. The adverse sequelae of exposure to endocrine disruptors, that mimic or oppose steroid hormone function, include developmental abnormalities, reproductive dysfunction, and proliferative disorders, including malignancies. The mechanism by which endocrine disruptors produce these disorders is poorly understood, despite considerable studies [14]. Endogenous estrogens have adverse effects not only in the female reproductive tract and mammary gland but also in bone, the cardiovascular system, and the central nervous system during development and throughout life [14].

Polychlorinated biphenyls (PCBs) are a class of environmentally persistent and widespread halogenated hydrocarbons with known carcinogenic and hepato- and immuotoxicant activities [2] and these effects may be more pronounced in genetically predisposed species [11, 29, 31]. Recent evidence indicates that PCBs are also potential neurotoxicants. Epidemiological studies have shown that exposure to PCBs and related chemicals in adults is positively correlated with the development of neurological dysfunction such as headaches and decreased nerve conduction velocities [13, 25]. Moreover, children exposed to PCBs and related chemicals *in utero*, or during breastfeeding, are reported to have an increased incidence of headaches, cognitive deficits, and significantly delayed psychomotor development [13, 15, 25].

Endosulfan is an organochlorine insecticide that is extensively used to control pests in vegetables, cotton, and fruits. The accumulation of endosulfan has been reported in various crops in India and other developing countries [5, 8]. Endosulfan also has estrogenic properties and neurotoxicity.

Gap junctions are protein structures that form aqueous pores which connect neighboring cells [26]. They enable direct communication between the cytoplasm of coupled cells, allowing intercellular current flow and the exchange of ions and small molecules [4, 24, 34]. Gap junctions may serve as intercellular pathways for chemical and electrical developmental signals in embryos and for defining the boundaries of developmental compartment [17]. GJIC occurs in specific patterns in embryonic cells and the impairment of GJIC has been related to developmental anomalies and the teratogenic effects of many chemicals [38].

Mitogen activated protein kinases (MAP kinase), also described as extracellular signal-regulated kinases (ERKs), belong to a group of protein-serine/threonine kinases that are activated in response to various stimuli (growth factors, neurotransmitters, differentiating agents, heat shock) in virtually all cell types. Two highly related mammalian MAP kinases, p44mapk and p42mapk, also called ERK1 and ERK2, have been cloned and found to be ubiquitously

expressed in vertebrates [28].

In the present study, we isolated neural stem cells from rat brain at embryonic day 17 to examine the toxic effects of PCB and endosulfan on embryo nervous system.

MATERIALS AND METHODS

Cell culture: Sprague-Dawley rat embryos were removed at embryonic day 17 and placed in a petri dish containing Hanks balanced salt solutions (HBSS, Gibco, U.S.A). Brain was removed and processed for dissociated cell culture. For dissociation and plating cells, the cortex was dissected from the rest of the brain. Isolated cortex were transferred to a 0.5% trypsin solution (Gibco, U.S.A). To obtain small clumps of cells, the solution was gently pipetted up and down about 20 times in 5 m*l* pipette until it attained a milky, homogeneous appearance. The suspension was incubated for 30 min at 37°C. Thereafter 1 m*l* of PBS containing 0.04% deoxyribonuclease (Dnase, type I, 650 KU/mg, Sigma, U.S.A) was added to the tissue. The solution was pipetted up and down several times. Cells were plated in poly-D-lysine 100 mm dishes (Nunc, Denmark) and maintained in a 1:1 mixture of Dulbecoo's Modified Eagle Medium and F12 medium (DMEM/F12, Gibco, U.S.A.) supplemented with 5% fetal bovine serum (Gibco, U.S.A). Dishes were incubated for 7 hr at 37°C in humidified 5% CO_2 : 95% air [23].

Chemicals: Endosulfan (α+β=2+1) and PCB (2, 2, 4, 4, 5, 5,-hexachlorobiphenyl) were purchased from Riedel-deHaën (U.S.A). Stock solutions of these compounds were prepared in DMSO and stored in 4°C. The final concentration of DMSO in working solution was below 0.5%.

Bioassay of cytotoxicity: Cells (1×10^4) were plated in triplicate in 96-well, flat-bottomed microtiter plates. Twenty-four hours later, the medium was changed and fresh medium with the test chemicals was added. Twenty-four hours after treatment, the medium was removed, and cells were washed three times with warm phosphate-buffered saline (PBS) and then neutral red medium (50 μg/m*l*) in growth medium was added to each well. Plates were incubated at 37°C for 2 hr, and then cells were solubilized with 1% acetic acid in 50% ethanol for 20 min. We determined absorbance at 540 nm for each well using 96-well plate reader (Titertek Multiskan, U.S.A).

Immunofluorescence staining: The procedures were slightly modified from the method described previously [37]. Cells were grown on eight well Lab-Tek chamber slide (Nunc, U.S.A). Cells on the slides were fixed in 4% paraformaldehyde (in PBS, pH 7.2) for 30 min at room temperature followed by 2 washes in PBS (pH 7.2). Cells were then permeabilized for 30 sec in pure methanol and washed in PBS and blocked for 1 hr in PBS containing 10% normal goat serum (NGS). After blocking, slides were incubated in anti-nestine mouse monoclonal (IgG) (1:100; PharMingen, U.S.A), anti-glial fibrillary acidic protein (GFAP) rabbit polyclonal (1:200; DAKO, Denmark), anti-neuron specific enolase (NSE) rabbit polyclonal (1:200; Chemicon, U.S.A), anti-connexin 43 rabbit polyclonal (1:100; Zymed Laboratories Inc., U.S.A) antibodies diluted in PBS containig 10% NGS at 4°C overnight. Slides were then washed in PBS three times and incubated in TRITC goat anti-mouse (1:200; Zymed Laboratories Inc., U.S.A) and TRITC goat anti-rabbit (1:200; Zymed Laboratories Inc., U.S.A) secondary antibodies at 37°C for 1 hr. Slides were washed three times in PBS and mounted in Gelvatol (Lab vision, U.S.A).

Hoechst 33258 staining: To detect condensation of nuclei, the cells were fixed for 20 min in freshly prepared 4% paraformaldehyde in PBS, then rinsed with PBS, stained for 15 min in 1 μg/m*l* Hoechst 33258 in PBS. Cultures were then rinsed twice with PBS.

Western blot analysis: Protiens were extracted from cells in 100 mm dishes by treatment with 20% SDS lysis solution containig several protease and phosphatase inhibitior (1 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 1 μM antipain, 0.1 μM aprotitnin, 0.1 μM sodium orthovanadate, 5 mM sodium fluoride). After sonication at three 10 sec pulses from a probe sonicator, the cell lysates were stored at –20°C until use. The protein amounts were determined with the DC protein assay kit (Bio-Rad Co., U.S.A). Proteins were separated on 12% SDS polyacrylamide gels at 200 V for 1 hr and transferred to Nitrocellulose membranes at 100 V, 350 mA for 1 hr. We detected MAPK using anti-MAPK monoclonal antibody (Zymed, Inc., U.S.A) and anti-Connexin 43 monoclonal antibody (Chemicon, Inc., U.S.A), followed by incubation with horseradish peroxidase (HRP) conjugated secondary antibody and detected with the ECL chemiluminiscent detection reagent (Amersham Co., U.S.A). The membranes were exposed to X-ray film for 15-30 sec.

Scrape-loading dye transfer (SL/DT) assay: GJIC was assessed using the SL/DT technique described by el-Fouly [9]. Neural stem cells (3×10^5 cells) in 35 mm cell culture dish were incubated for 24 hr at 37°C. Cells were exposed at various concentrations (consecutively diluted by a factor of 2 from the maximum non-cytotoxic concentration of the test compounds) for 12 hr. The treated neural stem cells were rinsed carefully with PBS and then scraped and incubated with 2 m*l* of 0.05% Lucifer Yellow for 3 min. The cells were then washed with PBS. Junctional permeability was observed with an inverted fluorescent microscope.

RESULTS

Isolation and characterization of neural stem cell: The CNS consists of three major phenotypes: neurons, oligodendrocytes, astrocyte and each of which expresses characteristic antigenic markers. Cells derived from the dissociation of E17 rat brain were suspended in growth medium. Cells were plated in poly-D-lysine coated dishes. Attached cells well differentiate and exhibit outgrowth of process after 3 days of plating (Fig. 1A). When many cells were plated in normal dishes, cells were detached from the plastic and form cell aggregates (Fig. 1B). When the neurosphere attaches to the dish, sphere can differentiate and form neurite (Fig. 1C).

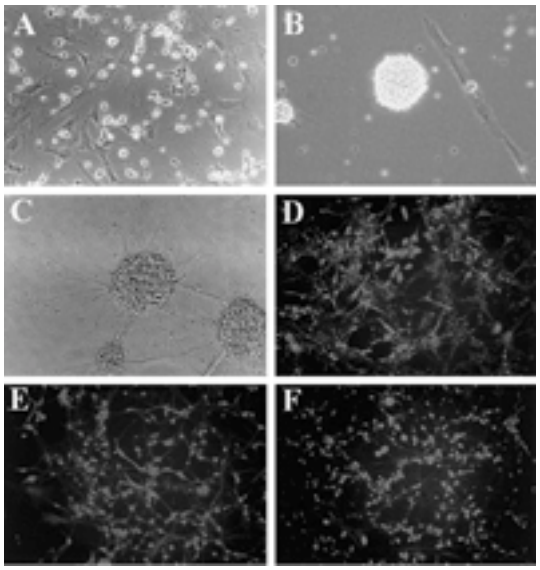

Fig. 1. Characterization of neural stem cell during differentiation *in vitro*. Cells were plated on dish and were fixed and stained on days 2. (A) Neural stem cells were dissociated and plated on poly-D-lysine coated dishes for 3 days. (B) Cells were plated on dishes which were not coated with poly-D-lysine. (C) Stem cells cultured for 3 days on dishes formed neuroshpere. Neural stem cells exhibit immunoreactivity for nestin(D), glial fibrillary acidic protein(E), neuron specific enolase (F).

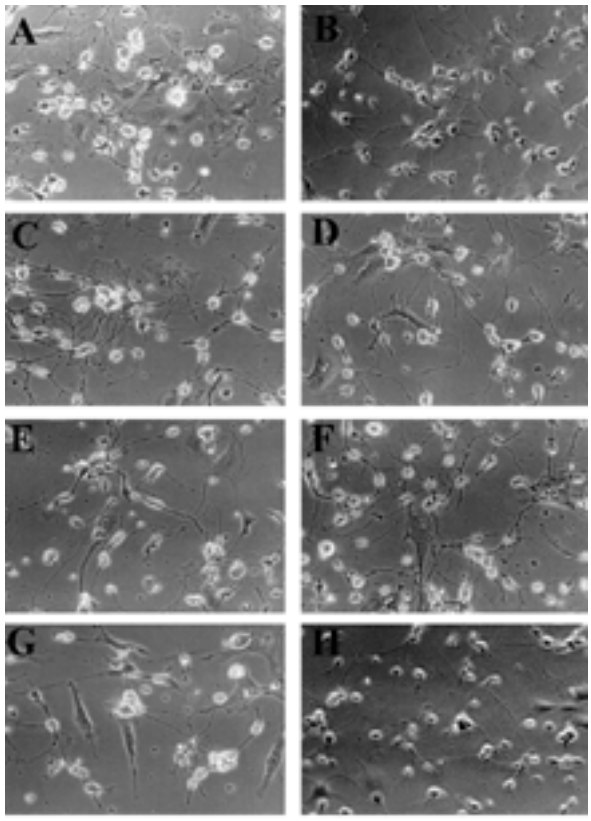

Fig. 2. Effects of endosulfan and 2,4,5 HCB on neurite outgrowth. Cells were plated on poly-D-lysine coated dishes and treated for 3 days. (A) control (B) vehicle control (C) endosulfan 5 μM (E) endosulfan 10 μM (G) endosulfan 20 μM (D) 2,4,5-HCB 10 μM (F) 2,4,5-HCB 20 μM (H) 2,4,5-HCB 40 μM.

To determine if cultured stem cells could differentiate into CNS neurons and glia, cells were plated on cover slides and the processed for immunocytochemistry to detect neuron and astrocyte. Cells were immunoreactive for nestine (Fig. 1D). It means that isolated cells were neural stem cells. Cells, plated on cover slides, have the immunoreactivity for glial fibrillary acidic protein (GFAP) (Fig. 1E), neuron specific endolase (NSE) (Fig. 1F). GFAP is astrocyte marker and NSE is neuronal marker. Thus, neural stem cells from E17 rat embryo were multipotent.

Cytotoxicity test: To determine the cytotoxicity of these chemicals on neural stem cells, the uptake of neutral red dye in viable cells using a microplate reader (OD 540) was examined. Cytotoxicity was observed after 24 hr exposure to endosulfan 20 μM and 2,2',4,4',5,5',-hexachlorobiphenyl (2,4,5-HCB) 40 μM. The concentration of endosulfan and 2,4,5,-HCB did not show any cytoxicity in neural stem cell.

Effects on cell morphology: To determine the effects of endosulfan and 2,4,5-HCB on cell morphology, the cell morphology was observed after exposure to endosulfan and 2,4,5-HCB for 3 days. Neurite formation and length on endosulfan treated cells was decreased dose-dependently (Fig. 2C, E and G). At concentrations of 20 μM, endosulfan showed the significant decrease in the neurite formation (Fig. 2G). The size of sphere on endosulfan treated cells was smaller than that in untreated cells in a dose-dependent manner (Fig. 3C, E G). At concentrations of 10 μM and 20 μM, endosulfan reduced the size of sphere significantly (Fig. 3E, G). Neurite formation and length on 2,4,5-HCB treated cells was decreased at the concentration 40 μM (Fig. 2H). The size of sphere on 2,4,5-HCB treated cells decreased a dose-dependent manner (Fig. 3D, F and E). At concentrations of 20 μM and 40 μM, 2,4,5-HCB decreased the size of sphere significantly (Fig. 3F and E).

Effects on apoptosis: Previous *in vivo* and *in vitro* analyses have shown that PCB and endosulfan induced apoptosis, so we examined apoptosis using nuclear staining with Hoechst 33258. But 2,4,5-HCB and endosulfan treatment groups were not significantly different from untreated control cells (Fig. 4).

Effects on MAPK pathway: The mitogen-activated protein kinase (MAPK) cascade plays a central role in regulating proliferation and differentiation [6]. MAPK activity in neural stem cell after treatment was measured. We treated cells with endosulfan and 2,4,5-HCB for 3 days and extracted protein from cells. MAPK activity was investigated by Western blot. MAPK activity was decreased on

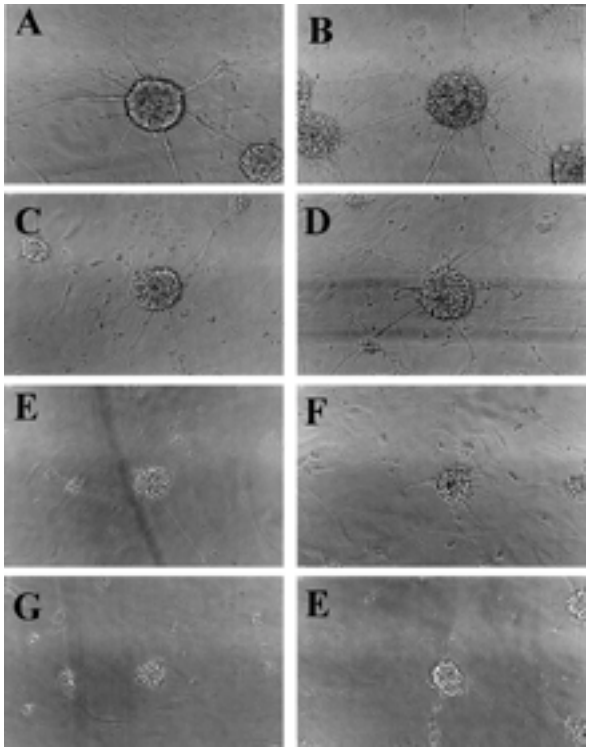

Fig. 3. Effects of endosulfan and 2,4,5-HCB on neurosphere formation. Cells were plated on 96 well plate and treated for 3 days. (A) control (B) vehicle control (C) endosulfan 5 μM (E) endosulfan 10 μM (G) endosulfan 20 μM (D) 2,4,5-HCB 10 μM (F) 2,4,5-HCB 20 μM (H) 2,4,5-HCB 40 μM.

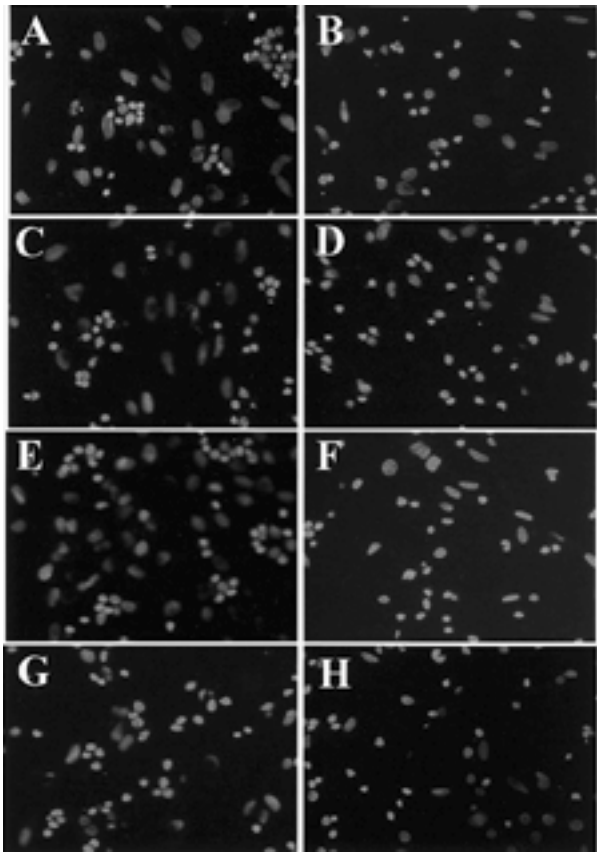

Fig. 4. Effects of endosulfan and 2,4,5-HCB on apoptosis. Cells were plated on poly-D-lysine coated dishes and treated for 24 hr. (A) control (B) vehicle control (C) endosulfan 5 μM (E) endosulfan 10 μM (G) endosulfan 20 μM (D) 2,4,5-HCB 10 μM (F) 2,4,5-HCB 20 μM (H) 2,4,5-HCB 40 μM.

endosulfan treated cells (Fig. 5). ERK2 activity on endosulfan treated cells was decreased in a dose-dependent manner (Fig. 5). At the concentration of 20 μM, endosulfan decreased ERK2 activity significantly (Fig. 5). ERK1 and ERK2 activation on endosulfan treated cells was decreased as compare with untreated cells (Fig. 6A and C). The ratio of ERK2/ERK1 on endosulfan treated cells was decreased a dose-dependent manner (Fig. 6E). But the effect of 2, 4, 5-HCB treated cells was not significant.

Scrape-loading dye transfer (SL/DT) assay: Based on the cytotoxicity results, various concentrations of these test compounds, which were represented by a consecutive dilution with a factor of 2 from the maximum noncytotoxic concentration of the test compounds. Using the scrape loading/dye transfer technique, the effects of test compounds of GJIC were investigated. The inhibition of GJIC on endosulfan 5 μM treated cells was not significant (Fig. 7C). Endosulfan at concentrations of 10 μM and 20 μM inhibited GJIC in a dose-dependent manner (Fig. 7E, G). The reduction of GJIC on 2,4,5-HCB 10 μM treated cells was not significant (Fig. 7D). At concentrations of 20 μM and 40 μM, 2, 4, 5-HCB decreased GJIC a dose-dependent manner (Fig. 7F, H).

Connexin-43 protein expression: We examine the changes in connexin 43 protein levels as well as the degree of phosphorylation following the treatment of the cells with endosulfan and 2, 4, 5-HCB by Western blotting and immunocytochemistry, using antibodies specific to connexin 43. The expression of connexin 43 on endosulfan 20 μM treated cells was decreased as compared with the control group (Fig. 8A). The expression of connexin 43 on 2,4,5-HCB treated cells was not significant (Fig. 8A). In the results of immunocytochemistry, The typical plaques of connexin 43, which are functional when localized on the plasma membrane, were detected in the untreated control (Fig. 9A and B). The membrane connexin 43 proteins were not seen in cells with endosulfan 20 μM, 2,4,5-HCB 20 μM and 40 μM (Fig. 9G, F, H).

DISCUSSION

Many chemicals, both natural and synthetic, can evidently possess estrogenic-like activities and interfere with female reproductive patterns [12]. These estrogenic chemicals have adverse effects not only in the reproductive system but also in the central nervous system during development and throughout life [14]. Chlorinated hydrocarbons like

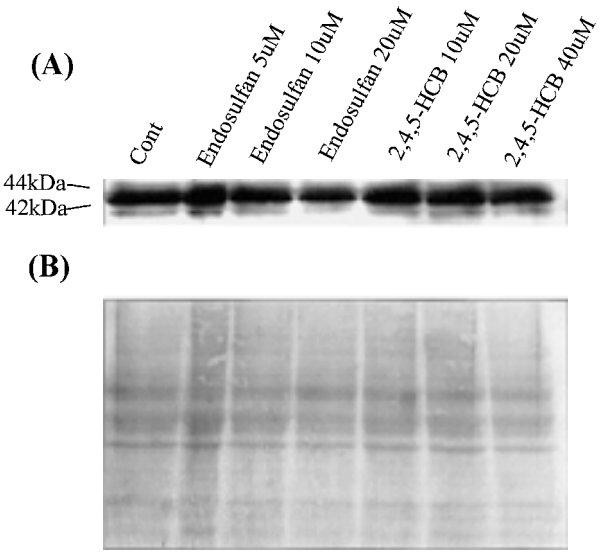

Fig. 5. Effects of endosulfan and 2,4,5-HCB on MAPK activation. (A)The Western blot for the MAPK activity of neural stem cells. (B) Ponsau S Solution stained membrane.

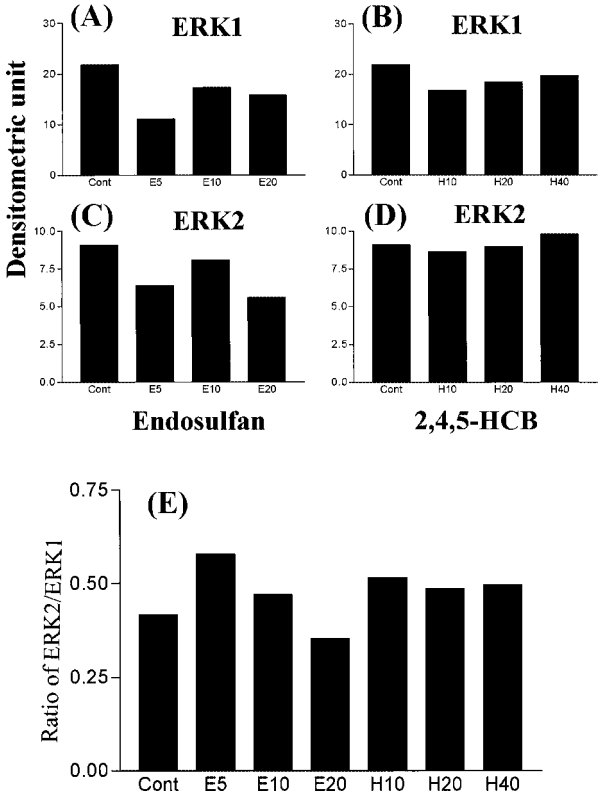

Fig. 6. Effects of endosulfan and 2,4,5-HCB on Erk1 and Erk2. Proportion of Erk1 (A), Erk2 (C) activity on endosulfan treatment group to control group. Proportion of Erk1 (B), Erk2 (D) activity on 2,4,5-HCB treatment group to control group. (E) Ratio of Erk2/Erk1 activity on neural stem cells.

endosulfan and PCB are known as estrogenic chemicals [22, 33].

Rat brain at embryonic day 17 expresses estrogen receptor [30]. To investigate the effects of estrogenic compounds in developing brain, we used isolated neural stem cell from E17 rat brain. Isolated neural stem cells were appeared to be multipotent (Fig. 1). When stem cells cultured on poly-D-lysine coated dishes, stem cell differentiated and formed neurite. But stem cells on untreated dishes floated and formed cell clusters. These cluster called 'neurosphere' because that's shape is shpere [1].

Neurite formation and neurosphere size of endosulfan and 2,4,5-HCB treated cells was decreased as compared with the control group. An important role for various members of the integrin, cadherin, and Ig superfamilies of adhesion molecules in the process of axonal growth and guidance has now been established [7].

In previous study, endosulfan inhibits calmodulin-dependent Ca^{2+}-ATPased in rat brain [35]. *In vivo* studies, when endosulfan administrates in subacute, the plasma calcium level significantly decreased [10]. Previous studies indicated that the uptake of Ca^{2+} by microsomes was decreased in brains of Aroclor-1154-exposed animals [36]. The calcium level is very important for GJIC and neural cell differentiation. Therefore, decrease of the calcium level may inhibit cell differentiation in endosulfan and 2,4,5-HCB treated cells.

Previous studies have shown that PCBs exhibited apoptosis-mediated immunotoxicity [39] and endosulfan can induce apoptosis in a human T-cell leukemic cell line [20]. So, Hoechst 33258 staining for apoptosis was performed. But endosulfan and 2,4,5-HCB did not induce apoptosis. Therefore, it seems that endosulfan and PCB do not induce apoptosis in neural stem cells during development.

The MAPK pathway plays a central role in regulating proliferation and differentiation [6, 7]. MAPK activity on endosulfan treatment group was decreased. Particularly, ratio of ERK2 was decreased in a dose-dependent manner. Among the targets of ERK2 are downstream kinases involved in cellular growth control as well as nuclear transcription factors. ERK2 provides an essential link in transducing the diverse signals from transmembrane growth factor receptors into gene regulatory events [32]. Thus, endosulfan may inhibit the signal from transmembrane to the nucleus and cell proliferation and differentiation.

Gap junctions are membrane channels that permit the transfer of small water-soluble molecules, including cAMP and inositol triphosphate, from the cytoplasm of one cell to that of neighbors [18]. In previous studies, endosulfan and PCB inhibit the cell-to-cell communication in rat liver cells [19, 21]. In neural stem cell, endosulfan and PCB inhibit gap junctional intercellular communication and reduce the expression of connexin 43. Signaling molecules and second messengers such as inositol 1, 4, 5-trisphosphate, cAMP and Ca^{2+} which passing through gap junction are very important

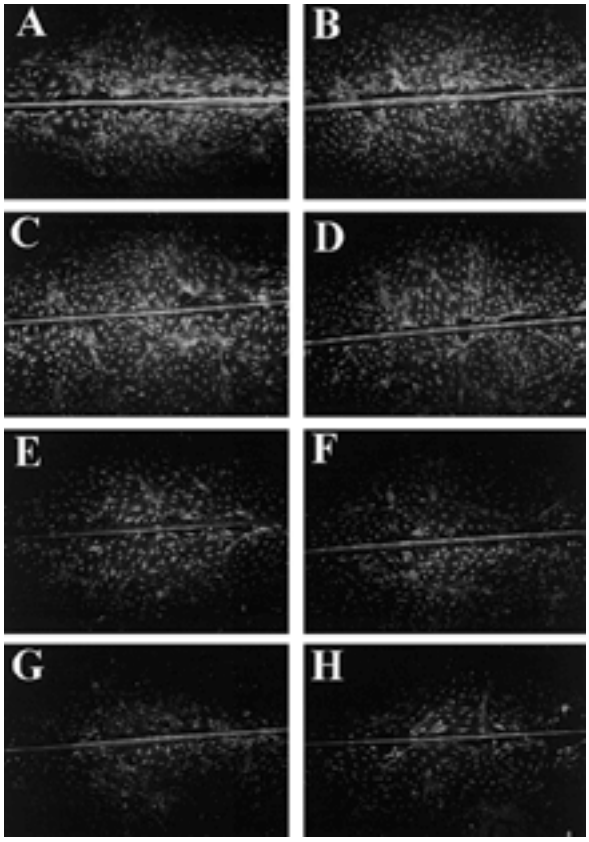

Fig 7. Scrape loading dye transfer images in the neural stem cells treated with endosulfan and 2,4,5-HCB for 12 hr. (A) control (B) vehicle control (C) endosulfan 5 μM (E) endosulfan 10 μM (G) endosulfan 20 μM (D) 2,4,5-HCB 10 μM (F) 2,4,5-HCB 20 μM (H) 2,4,5-HCB 40 μM.

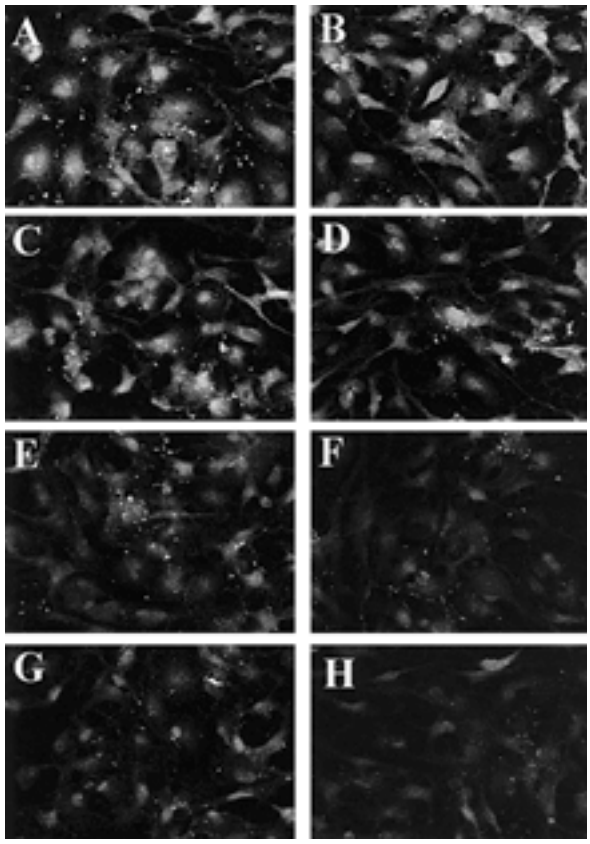

Fig 9. Immunocytochemical staining of connexin 43 on endosulfan and 2,4,5-HCB treatment group for 12 hr. (A) control (B) vehicle control (C) endosulfan 5 μM (E) endosulfan 10 μM (G) endosulfan 20 μM (D) 2,4,5-HCB 10 μM (F) 2,4,5-HCB 20 μM (H) 2,4,5-HCB 40 μM.

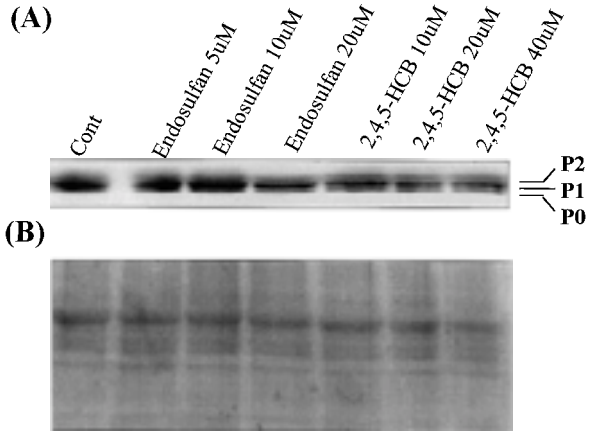

Fig 8. Effects of endosulfan and 2,4,5-HCB on connexin 43. (A)Western blot analysis for connexin 43. (B) Ponsau S Solution stained membrane.

for cell regulation [27]. Thus, the inhibition of gap junction on endosulfan and PCB treatment group may block growth signal from the neighbor cell.

Both Endosulfan and 2,4,5-HCB inhibit neural cell differentiation and proliferation during development. These results suggest that the neurotoxic effects are related to the decrease of ERK2 activity and the inhibition of GJIC.

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REFERENCES

1. Arsenijevic, Y. and Weiss, S. 1998. Insulin-like growth factor-I is a differentiation factor for postmitotic CNS stem cell-derived neuronal precursors: Distinct actions from those of brain-derived neurotrophic factor. *J. Neurosci.* **18**: 2118–2128.
2. Borlakoglu, J.T. and Haegele, K.D. 1991. Comparative aspects

on the bioaccumulation, metabolism, and toxicity with PCBs. *Comp. Biochem. Physiol.* **100**: 327–338.
3. Colborn, T., vom Saal, F.S. and Soto, A.M. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health Perspect.* **101**: 378–384.
4. Dermietzel, R. and Spray, D.C. 1993. Gap junctions in the brain: Where, what type, how may and why? *Trends Neurosci.* **16**: 186–192.,
5. Dethe, M.D., Kale, V.D. and Dharne, P.K. 1990. Gas chromato-graphic studies on residues of endosulfan on brinjal fruits. *J. Natl. Conserv.* **2**: 161–174.
6. Dimitropoulou, A. and Bixby, J.L. 2000. Regulation of retinal neurite growth by alterations in MAPK/ERK kinase (MEK) activity. *Brain Res.* **858**: 205–214.
7. Doherty, P., Williams, G. and Williams, E.J. 2000. CAMs and axonal growth: A critical evaluation of the role of calcium and the MAPK cascade. *Mol. Cell Neurosci.* **16**: 283–295.
8. Dushra, M.S., Hammed, S.F. and Nath, A. 1984. Effect of washing of insecticide residue in califlower curds. *Ind. J. Nutr. Diet.* **21**: 124–128.
9. el-Fouly, M.H., Trosko, J.E. and Chang, C.C. 1987. Scrape-loading and dye transfer: a rapid and simple technique to study gap junctional intercellular communication. *Exp. Cell Res.* **168**: 422–430.
10. Garg, A., Kunwar, K., Das, N. and Gupta, P.K. 1980. Endosulfan intoxication: Blood glucose, electrolytes, Ca levels, ascsorbic acid and glutathione in rats. *Toxicol. Lett.* **5**: 119–123.
11. Gasiewez, T.A., Geiger, L.E., Rucci, G. and Neal, R.A. 1983. Distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in C57BL/6J,DBA/2J and B6D2F1/J mice. *Drug. Metab. Dispos.* **11**: 397–403.
12. Greenman, S.B., Rutten, M.J., Fowler, W.M., Scheffler, L., Shortridge, L.A., Brown, B., Sheppard, B.C., Deveney, K.E., Deveney, C.W. and Trunkey, D.D. 1997. Herbicide/Pesticide effects on intestinal epithelial growth. *Environ. Res.* **75**: 85–93.
13. Hsu, S.T., Ma, C.I., Hsu, S.K., Wu, S.S., Hsu, N.H., Yeh, C.C. and Wu, S.B. 1985. Discovery and epidemiology of PCB poisoning in Taiwan: A four-year followup. *Environ. Health Perspect.* **59**: 5–10
14. Hunter, D.S., Hodges, L.C., Vonier, P.M., Fuchs-Young, R., Gottardis, M.M. and Walker, C.L. 1999. Estrogen receptor activation via activation function 2 predicts agonism of xenoestrogens in normal and neoplastic cells of the uterin myometrium. *Cancer Res.* **59**: 3090–3099.
15. Jacobson, J.L. and Jacobson, S.W. 1996. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *New Engl. J. Med.* **335**: 783–789.
16. Juberg, D.R. 2000. An evaluation of endocrine modulators: implications for human health. *Ecotoxicol. Envirion. Safety.* **45**: 93–105.
17. Kalimi, G.H. and Lo, C.W. 1998. Communication compartments in the gastrulating mouse embryo. *J. Cell Biol.* **107**: 241–255.
18. Kang, K.S., Kang, B.C., Lee, B.J, Che, J.H., Li, G.X., Trosko, J.E. and Lee, Y.S. 2000. Preventive effects of epicatechin and ginsenoside Rb2 on the inhibition of gap junctional intercellular communication by TPA and H_2O_2. *Cancer Letters.* **152**: 97–106.
19. Kang, K.S., Wilson, M.R., Hayashi, T., Chang, C.C. and Trosko, J.E. 1996. Inhibition of gap junctional intercellular communication in normal human breast epithelial cells after treatment with pesticides, PCBs, and PBBs. *Toxicology.* **104**: 192–200.
20. Kannan, K., Holcombe, R.F., Jain, S.K., Alvarez-Hernandez, X., Chervenak, R., Wolf, R.E. and Glass, J. 2000. Evidence for the induction of apoptosis by endosulfan in a human T-cell leukemic line. *Mol. Cell Biochem.* **205**: 53–66.
21. Kenne, K., Fransson-Steen, R., Honkasalo, S. and Warngard, L. 1994. Two inhibitors of gap junctional intercellular communication, TPA and endosulfan: different effects on phosphorylation of connexin 43 in the rat liver epithelial cell line, IAR 20. *Carcinogenesis* **15**: 1161–1165.
22. Kester, M.H., Bulduk, S., Tibboel, D., Meinl, W., Glatt, H., Falany, C.N., Coughtrie, M.W., Bergman, A., Safe, S.H., Kuiper, G.G., Schuur, A.G., Brouwer, A. and Visser, T.J. 2000. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. *Endocrinology* **141**: 1897–1900.
23. Kitani, H., Shiurba, R., Sakakura, T. and Tomooka, Y. 1991. Isolation and characterization of mouse neural precursor cells in primary culture. *In Vitro Cell Dev. Biol.* **27A**: 615–624.
24. Kumar, N.M. and Gilula, N.B. 1996. The gap junction communication channel. *Cell* **84**: 381–388.
25. Kuratsune, M., Yoshimura, T., Matsuzaka, J. and Yamaguchi, A. 1971. Yusho, a poisoning caused by rice oil contaminated with polychlorinated biphenyls. *HSMHA Health Rep.* **86**: 1083–1091.
26. Loewenstein, W.R. 1981. Junctional intercellular communication: the cell to cell membrane channel. *Physiol. Rev.* **61**: 829–913.
27. Naus, C.C., Bechberger, J.F., Zhang Y., Venance L., Yamasaki H., Juneja S.C., Kidder G.M. and Giaume C. 1997. Altered gap junctional communication, intercellular signaling, and growth in cultured astrocytes deficient in connexin43. *J. Neurosci. Res.* **49**: 528–540.
28. Page,s G., Lenormand, P., Allemain, G.L., Chambar, J.C., Meloche, S. and Pouyssegur, J. 1993. Mitogen-activated protein kinases p42mapk and p44mapk are required for fibroblast proliferation. *Proc. Natl. Acad. Sci. U.S.A.* **90**: 8319–8323.
29. Parkinson, A. and Safe, S. 1987. Mammalian biologic and toxic effects of PCBs. pp. 49–79. *In*: Polycholorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology (Safe S. and Hutzinger, eds.), SpringerVerlag. Berlin.
30. Pasterkamp, R.J., Yuri, K., Visser, D.T., Hayashi, S. and Kawata, M. 1996. The perinatal ontogeny of estrogen receptor-immunoreactivity in the developing male and female rat hypothalamus. *Brain Res. Dev. Brain Res.* **91**: 300–303.
31. Poland, A. and Glover, E. 1980. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Segregation of toxicity with the Ah locus. *Mol. Pharmacol.* **17**: 86–94.
32. Prowse, C.N. and Lew, J. 2000. Mechanism of activation of ERK2 by dual-phosphorylation. *J. Biol.Chem.*
33. Soto, A.M., Chung, K.L. and Sonnenschein, C. 1994. The pesticides endosulfan, toxaphene, an dieldrin have estrogenic effects on human estrogen-sensitive cells. *Environ. Health Perspect.* **102**: 380–383.
34. Spray, D.C. and Bennett, M.V.L. 1985. Physiology and pharmacology of gap junctions. *Ann. Rev. Physiol.* **47**: 281–303.
35. Srikanth, N.S., Seth, P.K. and Desaiah, D. 1989. Inhibition of calmodulin-activated Ca2(-)-ATPase by endosulfan in rat brain. *J. Toxicol. Environ. Health.* **28**: 473–481.
36. Tilson, H.A., Kodavanti, R.S., Mundy, W.R. and Bushnel, P.J. 1998. Neurotoxicity of environmental chemicals and their mechanisms of action. 102: 631–635.
37. Tropepe, V., Sibilia, M., Ciruna, B.G., Rossant, J., Wagner, E.F. and van der Kooy, D. 1999. Distinct neural stem cells pro-

liferate in response to EGF and FGF in the developing mouse telencephalon. *Develop. Biol.* **208**: 166–188.
38. Trosko, J.E. and Ruch, R.J. 1998. Cell-cell communication in carcinogenisis. *Front. Biosci.* **3**: 208–236.
39. Yoo, B.S., Jung, K.H., Hana, S.B. and Kim, H.M. 1997. Apoptosis-mediated immunotoxicity of polychlorinated biphenyls (PCBs) in murine splenocytes. *Toxicol. Lett.* **28**: 83–89.