

RESEARCH

Open Access

Inhibition of gap junctional Intercellular communication in WB-F344 rat liver epithelial cells by triphenyltin chloride through MAPK and PI3-kinase pathways

Chung-Hsun Lee^{1,2}, I-Hui Chen², Chia-Rong Lee², Chih-Hsien Chi¹, Ming-Che Tsai¹, Jin-Lian Tsai² and Hsiu-Fen Lin^{*2,3,4}

Abstract

Background: Organotin compounds (OTCs) have been widely used as stabilizers in the production of plastic, agricultural pesticides, antifouling paints and wood preservation. The toxicity of triphenyltin (TPT) compounds was known for their embryotoxic, neurotoxic, genotoxic and immunotoxic effects in mammals. The carcinogenicity of TPT was not well understood and few studies had discussed the effects of OTCs on gap junctional intercellular communication (GJIC) of cells.

Method: In the present study, the effects of triphenyltin chloride (TPTC) on GJIC in WB-F344 rat liver epithelial cells were evaluated, using the scrape-loading dye transfer technique.

Results: TPTC inhibited GJIC after a 30-min exposure in a concentration- and time-dependent manner. Pre-incubation of cells with the protein kinase C (PKC) inhibitor did not modify the response, but the specific MEK 1 inhibitor PD98059 and PI3K inhibitor LY294002 decreased substantially the inhibition of GJIC by TPTC. After WB-F344 cells were exposed to TPTC, phosphorylation of Cx43 increased as seen in Western blot analysis.

Conclusions: These results show that TPTC inhibits GJIC in WB-F344 rat liver epithelial cells by altering the Cx43 protein expression through both MAPK and PI3-kinase pathways.

Background

Organotin compounds have been widely used as agricultural biocides, antifouling agents in boat paint, wood preservatives, and stabilizers for polyvinylchloride polymers (PVC) in industry [1,2]. Triphenyltin (TPT) is an organotin compound which is widely used as fungicides on major food and food-stock crops. It is also used in anti-fouling paints to prevent growth of barnacles and other fouling organisms on boats and ships [3]. Organotin compounds are known to be endocrine disruptors in marine species and may be mahuman beings [4,5]. Tissue concentrations of TPT were correlated with the degree of imposex in rock shells [6,7]. TPT compounds have embryotoxic, myotoxic, genotoxic and immunotoxic effects in mammals [8-11]. The organotin compounds

might be incorporated in the most abundant phospholipid of eukaryotic membrane and caused toxicity [12]. Some toxic effects have been observed in aquatic and terrestrial organisms exposed to TPT, such as increased tumor incidence and immune suppression [13,14]. Some studies have revealed that TPT might inhibit the cytotoxic function of human natural killer cells and triphenyltin hydroxide produced tumors in rats and mice [14-16].

Connexins (Cxs) are a group of at least 20 highly conserved proteins that provide the basis for communication through the direct exchange of ions, nutrients, second messengers, electrical coupling, and small metabolites from one cell to its neighboring cells [17-20]. Cell proliferation, differentiation, apoptosis and adaptive responses of differentiated cells can occur as a consequence of the up- or down-regulation of GJIC [21-23]. Disruption in GJIC may cause loss of homeostatic and cell growth control [18,24-26]. Growing evidence suggests that connexin

* Correspondence: talktofen@gmail.com

² Graduate Institute of Occupational Safety and Health, College of Health Science, Kaohsiung Medical University, Kaohsiung 80708, Taiwan
Full list of author information is available at the end of the article

43 (Cx43), a major gap junction protein, functions as a tumor suppressor gene. Expression of Cx43 is often decreased in human tumor cells and tissues, including those involved in human mammary carcinoma, prostate cancer, human glioblastoma, skin squamous cell carcinoma, lung cancer, esophagus cancer, adrenocortical tumors, ovarian carcinoma, cervical cancer, endometrial carcinoma, and human mesothelioma [27-37]. It has been assumed that using pharmacological stimulation to efficiently restore GJIC in tumor cells might represent a strategy for anti-neoplastic therapies [38-42].

The carcinogenicity of TPTC remained unclear. The present work was undertaken to define the effects of TPTC on GJIC in WB-F344 rat liver epithelial cells.

Materials and methods

Chemicals

Powder of TPTC was supplied by MERCK (Darmstadt, Germany).

Lucifer yellow, DMSO (dimethylsulfoxide), formaldehyde, MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) were supplied by Sigma-Aldrich (St. Louis, MO, USA). D medium and newborn calf serum were from Gibco (Invitrogen cooperation, CA, USA), Trizole was from Invitrogen Life Technologies (Rockville, MD, USA) and 2 X SYBR green PCR master mix was from Applied Biosystems (Foster, CA, USA). The protein kinase C (PKC) inhibitor GF109203X, extracellular signal-regulated protein kinase (ERK) inhibitor PD98059 and PI3 kinase inhibitor LY294002 were from Sigma (St. Louis, MO, USA). Immobilon Western HRP Substrate Peroxide Solution and luminal reagent were supplied by Millipore Corporation (Billerica, MA). All chemicals used in the study were of the highest available purity.

Cell culture and treatment with chemicals

WB-F344 rat liver epithelial cells [43] were cultured in D medium supplemented with 5% fetal bovine serum and 1% [v/v] penicillin/streptomycin antibiotic. The cells were grown at 37°C in a 5% CO₂ incubator before being used in the different experiments. Confluent cells, grown in plates, were exposed to various concentrations of TPTC. To prepare the TPTC stock solution, 0.01 g of TPTC powder was dissolved in 10 ml DMSO and then diluted to a final concentration of 1000 ppm.

Cell toxicity assay of TPTC

The effect of TPTC on the survival of WB F344 cells was assessed using MTT toxicity assay as described previously [44]. In brief, the cells were plated in 100 µl media in 48-well plates (1 × 10⁴/well). On the following day, the experimental medium containing different TPTC concentrations (0, 0.25, 0.5, 1, 2, 3, 4, and 5 ppm) was added,

and then incubated for 30 and 60 minutes. Fifty µl of MTT solution (2 mg/ml in PBS) was added to each well and incubated for 6-8 hours. After careful removal of the medium, 150 µl of DMSO was added to each well, and then after careful shaking, the absorbance was read at 570 nm using an ELISA microplate reader (Zenyth 200rt with ADAP software, Anthos Labtec Instruments, Autria). Cell viability was expressed as a percentage of control cells not treated with TPTC and was designated as 100%.

Colony forming-efficiency assay

Colony forming-efficiency experiments were performed as previously described [45]. In brief, exponentially growing cells were plated at 500 cells/100 mm tissue culture dish in 10 ml D medium, treated with different concentrations of TPTC. Following treatment, the plates were washed two times with the medium. The medium was not replaced, and colonies were fixed and stained after 14 days in culture by water: addition of methanol (1:1) containing crystal violet (1 g/l). Colonies with cell clusters containing more than 50 cells were counted under a dissecting microscope. Data indicate survival as a percentage relative to untreated cells.

GJIC inhibition assay

GJIC assay was carried out in 35 × 10 mm tissue culture dishes with 100% confluent monolayer cells grown in 2 ml D-medium supplemented with 5% newborn calf serum, 100 U/ml penicillin and streptomycin 100 µg/ml. GJIC was detected using the scrape-loading and dye transfer (SL/DT) technique developed by el-Flouly [46]. Assays for different treatments and vehicle control were run in triplicate in cell culture dishes. Monolayer cells with 100% confluence were incubated with target compounds. For dose-dependent inhibition of GJIC, we treated cells with 0.5, 1.0, 1.5 and, 2.0 ppm TPTC for 30 min. For time-dependent inhibition of GJIC, analysis was performed with 1.5 ppm TPTC for 15, 30, 45, and 60 min. After exposure to the target compounds, the cells were rinsed three times with PBS and 1 ml of lucifer yellow solution was then added to the cell cultures and scrape-loaded with several scrapes using a steel surgical blade. The dye solution was left on the cell cultures for 3 min, and then discarded. The cell cultures were carefully rinsed three times with PBS to remove detached cells and background fluorescence. Several drops of 4% formalin in PBS were added to fix the cell cultures. An inverted fluorescence microscope equipped with a digital camera (Nikon Eclipse TE 2000-U system, Nikon ACT-1 version 2.62, Nikon Corporation, Japan) was employed to record the migration of the lucifer yellow dye from the edge cells of the scrape. The migration was measured on the micrograph. An average value of 30 measurements for each treatment (10 measurements per dish) was regarded as

the migration of dye in the cell cultures. The percentage of migration of dye in cell cultures exposed to target compounds to the migration of dye traveling in the vehicle control was employed to evaluate the inhibition of GJIC. For inhibition studies, cultures were pre-incubated for 30 min with various pathway inhibitors prior to treatment with 1.5 ppm TPTC for 30 min.

Western blot analysis

WB F344 liver cells were treated with TPTC of 1.5 ppm for 15 and 30 min. After treatment, the medium was removed and cells were washed twice with PBS and lysed with 0.5% SDS. Lysates were stored at -80°C . Cell lysates were sonicated, and protein levels were determined using a protein detection assay (BioRad). Sample blue buffer (30% sucrose, 10% SDS, 0.1% bromophenol blue, and 0.2% dithiothreitol) was added and the samples were heated for 10 min at 100°C and loaded onto gels (10% SDS-PAGE). SDS-PAGE-separated proteins were blotted onto a PVDF membrane (Immobilon-PSQ, Millipore, Bedford, MA) using a semi-dry blotter (VWR), and the membrane was blocked with 5% milk in PBS-T buffer [1000 ml PBS with 1 ml Tween 20 (pH 7.4)] for more than 1 h at room temperature. The protein was probed with antibodies (Mouse IgG, Zymed) against connexin 43 at 4°C overnight and this was followed by incubation with horseradish peroxidase-conjugated secondary antibodies (Mouse anti-Goat IgG-HRP, Sigma). Protein visualization was carried out using an enhanced chemiluminescence kit (Pierce) according to the manufacturer's protocol.

Immunofluorescence staining

Immunofluorescence staining experiments were performed as previously described [47]. In brief, WB F344 liver cells were plated in 100 μl media in 12 well-plates treated with 1.5 ppm TPTC for 30 min. After treatment, the medium was removed and sections were washed with PBS. 4% paraformaldehyde was added and washed sections with PBS 20 min later. 0.5% triton X-100 (Sigma) was added for 20 min and washed out with PBS. After treatment, diluted primary antibodies mouse IgG against connexin 43 (Santa Cruz Biotechnology, Inc.) with 4% triton X-100 was added and incubated sections for 1 h at room temperature. The sections were washed with PBS, and diluted mouse IgG secondary antibody (Alexa Fluor555 & 488) with 4% triton X-100 was added and incubated sections for 1 h at room temperature. After treatment, 4,6'-diamidino-2-phenylindole (DAPI) (Sigma) was added and incubated sections for 10 min at room temperature. An inverted fluorescence microscope equipped with a digital camera (Nikon Eclipse TE 2000-U system, Nikon ACT-1 version 2.62, Nikon Corporation,

Japan) was employed to record the fluorescent intensity of the cells.

Statistical analysis

Means \pm SEM were calculated and the data are presented as a percentage of control. All data were analyzed by Sigma Plot 8.0 software using repeated measures. ANOVA (SPSS for window version 12.0.1; SPSS, Inc., Chicago, IL) was performed to examine the effect of independent variables (treatment, day, incubation time, time point). Tests for contrasts were carried out to compare the different levels of the independent variables. P values ≤ 0.05 were considered statistically significant.

Results

TPTC dissolved easily in DMSO but not in water. To exclude the toxic effects of DMSO on cell viability and diffusion length of GJIC, tests involving exposure to DMSO were carried out. Results revealed that after exposure to 2% DMSO for 30 minutes, the diffusion length of GJIC did not obviously decrease as compared with that of the control group ($p > 0.05$).

Cytotoxicity of TPTC

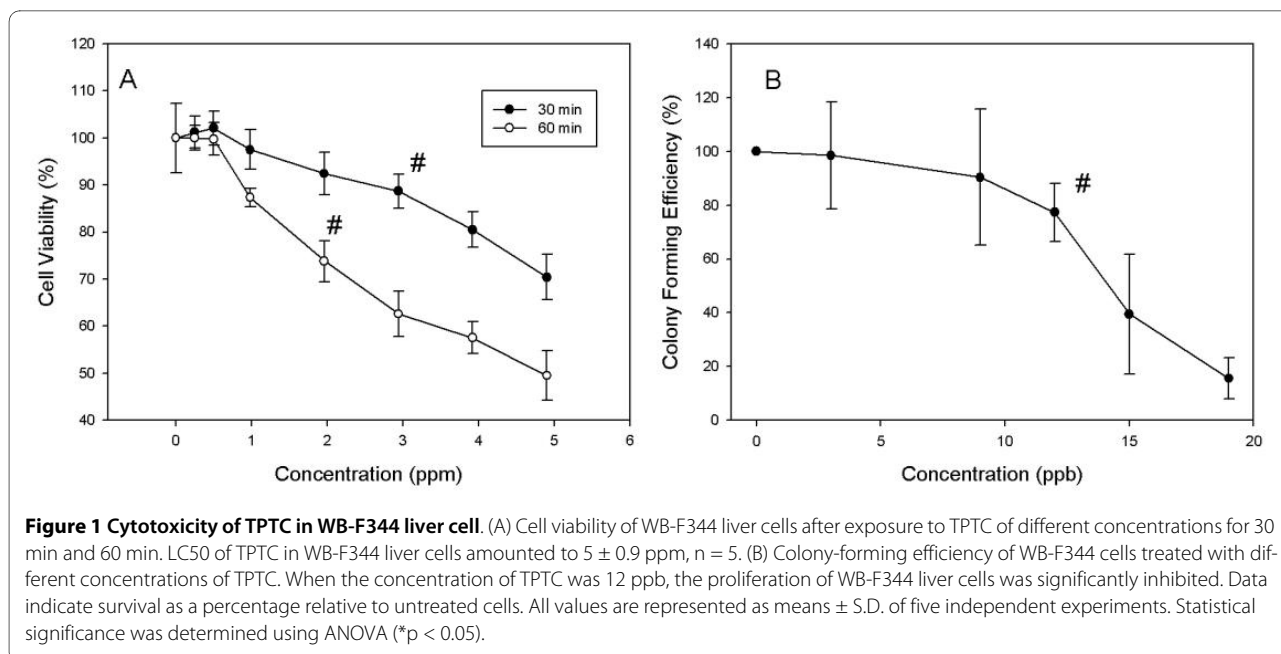
Cytotoxicity evoked by TPTC in WB-F 344 cells was tested with 0, 0.25, 0.5, 1, 2, 3, 4, and 5 ppm of TPTC using the MTT proliferation assay. After 30- and 60-min exposure to TPTC, it was found that cell viability decreased obviously with increasing concentration of TPTC and the lethal concentration 50 (LC 50) in 60 min calculated was 5 ppm (Fig. 1A.)

Colony-forming efficiency in WB-F 344 cells was evaluated using TPTC of 0, 3, 9, 12, 15, 18 ppb. After 14 days of exposure, the colony-forming efficiency decreased significantly when TPTC concentration exceeded 12 ppb (Fig. 1B.)

Dose- and time- dependent inhibition of GJIC by TPTC

Inhibition of GJIC has been suggested to be an important activity of tumor promoters [36]. Therefore, the capacity of TPTC to inhibit GJIC was measured in concentrations with 0.5, 1.0, 1.5 and 2 ppm TPTC after 30 min of exposure. As shown in Figure 2A, TPTC inhibited significantly GJIC in WB-F344 liver cells. The migration of Lucifer yellow dye in scraped WB F344 liver cells treated with TPTC was less than that in untreated cells, when the concentration was 1.0 ppm ($*p < 0.05$).

The effects of TPTC on GJIC were evaluated with cells exposed to TPTC for 15 min, 30 min, 45 min, and 60 min. After 15 min of exposure to 1.5 ppm of TPTC, the diffusion length was significantly decreased as compared with that of the control group ($p < 0.05$) (Fig. 2B). The diffusion length reduced gradually with time and became



almost invisible after 60 min of exposure to 1.5 ppm of TPTC.

Effects of PKC, ERK and PI3 kinase on GJIC response

Organotin compounds showed that inhibition through some kinase pathways is a possible mechanism involved in the apoptotic effects [48]. The mitogen-activated protein kinase (MAPK) pathway has been shown to be involved in the inhibition of GJIC by TPA [49-54]. Its role in the TPTC-induced inhibition of GJIC was studied next. No specific inhibitor of MAPK was available, but PD98059, a MEK1 inhibitor that blocks ERK activation, was used as an inhibitor of the pathway [55-57]. MEK 1 is the direct upstream activator kinase of MAPKs. The cells were pre-exposed to 50 μ M PD98059 for 30 min prior to co-exposure to TPTC (1.5 ppm) for 30 min. The scrape-loading assays were then repeated using the ERK inhibitor PD98059. The data showed that PD98059 restored significantly GJIC in TPTC-treated liver cells ($p < 0.05$) (Fig. 3). Thus, the MAPK signaling pathway was clearly involved in the inhibition of GJIC by TPTC.

Phosphatidylinositol 3'-kinase (PI3K) has been demonstrated to be critical in mediating several aspects of PDGF actions in various cells [23,58-62]. To explore the potential role of PI3K signaling in the signaling processes involved in TPTC-induced disruption of GJIC in liver cells, we measured GJIC in rat liver cells with and without pre-treatment with the PI3K inhibitor LY294002 (100 μ mol/L) before exposure to TPTC (1.5 ppm) for 30 min. As shown in Fig. 4, pre-incubation of rat liver cells with LY294002 (100 μ mol/L) for 30 min almost stopped completely the inhibition of GJIC caused by TPTC, although the inhibitor itself did not exert much influence on GJIC,

as compared with the control. Similar result was also found in the group exposed to TPTC and PD98059 as compared with that exposed to TPTC alone (Fig. 3). Thus, we conclude that TPTC blocked GJIC through MAPK and PI3K pathways.

To study the involvement of protein kinase C (PKC) in the inhibition of GJIC by TPTC, an inhibitor of PKC, GF109203X (bisindolylmaleimide 1) was utilized to block the activity of the enzyme before exposure to TPTC. GF109203X inhibits the isozymes of PKC α , β_1 , β_{1P} , γ , δ , and ϵ [63,64]. The cells were pre-exposed to the PKC inhibitor (10 μ M) for 30 min prior to co-exposure to TPTC (1.5 ppm) and incubated further for 30 min. The diffusion length of GJIC did not obviously decrease when only GF109203X was added. On the other hand, cells were treated with 10 μ M GF109203X for 30 min, followed by addition of TPTC. The diffusion length of GJIC decreased obviously following the addition of TPTC or TPTC with GF109203X (Fig. 5). No change was observed in the inhibition of GJIC by TPTC alone. Thus, the inhibition of GJIC by TPTC was not mediated by PKC.

Neither GF109203X, LY294002 nor PD98059 alone at the indicated concentration had any notable effects on GJIC in these cells.

Effects of TPTC on connexin 43 protein level and phosphorylation

One possible mechanism involved in the inhibition of GJIC is abnormal phosphorylation of connexins [65-67]. WB-F433 cells express Cx43 predominantly as gap junction protein [68]. Western blot analysis was performed to detect the state of Cx43 phosphorylation in WB-F344 cells after treatment with TPTC. In untreated cells, three

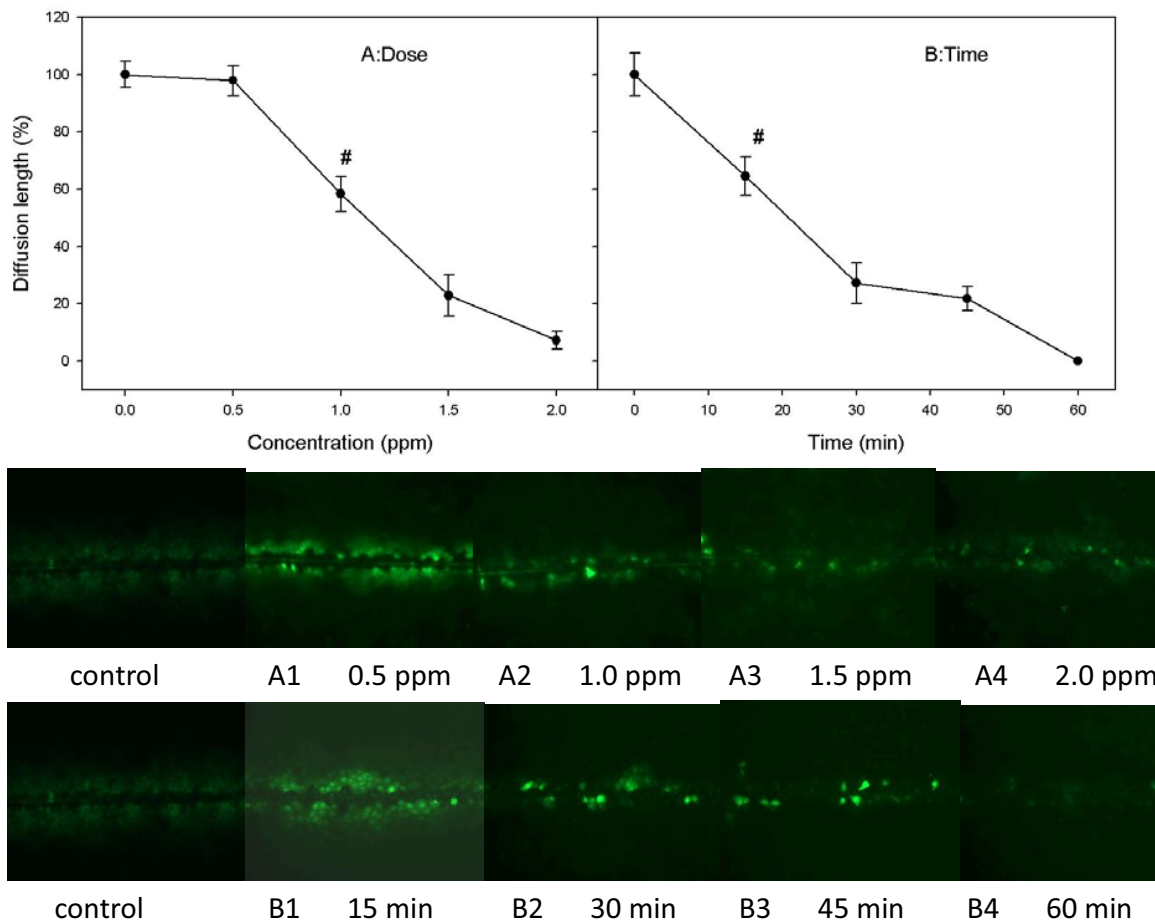


Figure 2 Inhibition of GJIC by TPTC using the modified scrape-loading/dye transfer method with the Lucifer yellow fluorescent dye. (A) Dose-dependent inhibition of GJIC after 30-min TPTC exposure. (B) Time-dependent inhibition of GJIC exposed to TPTC. Cells were treated with 1.5 ppm TPTC. The results are represented as means \pm S.D. of at least three independent experiments. Statistical significance was determined using ANOVA (* $p < 0.05$).

isoforms of Cx43, which correspond to different phosphorylated forms of Cx43, are detectable as P0 (unphosphorylated form), P1 and P2 (phosphorylated forms), respectively [69]. After 15-min and 30-min exposure to TPTC, the P0 band disappeared, and a shift to bands of higher molecular weight occurred (P1) (Fig. 6).

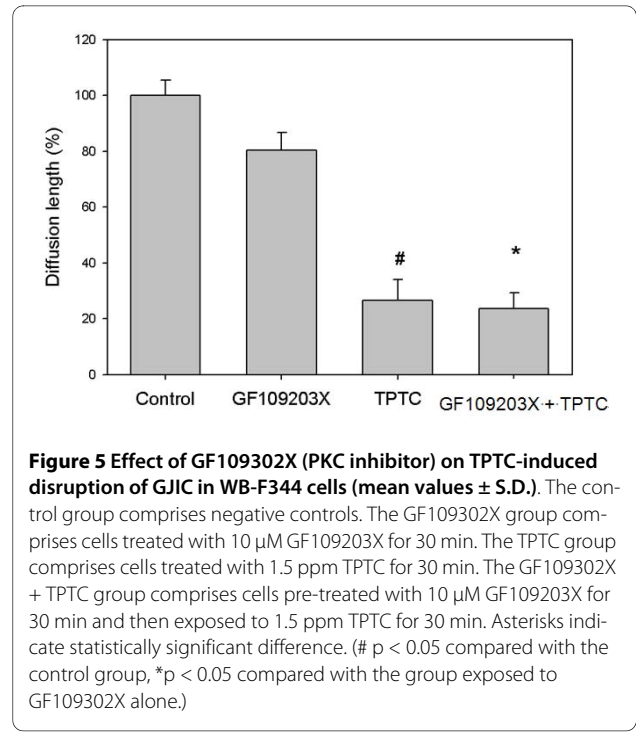
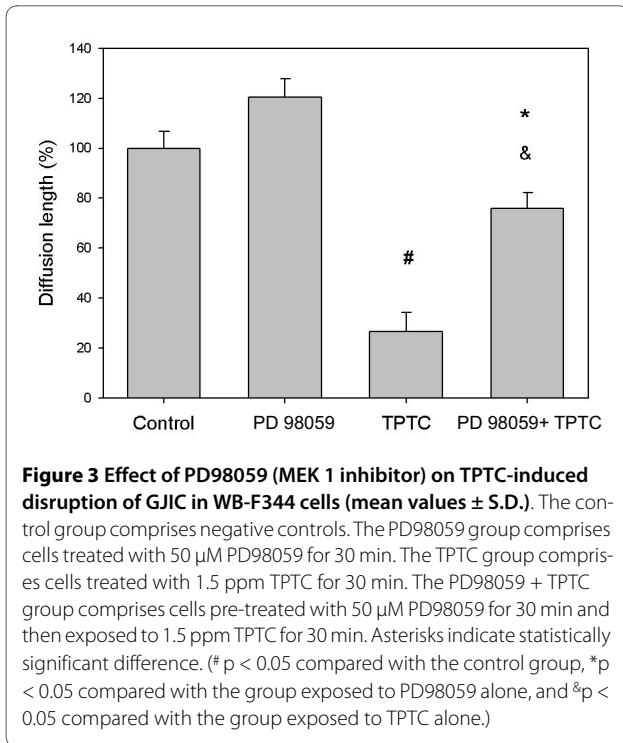
Effects of TPTC on connexin 43 in immunofluorescence staining

The expression of Cx43 in WB-F344 cell under stained with fluorescein isothiocyanate (FITC) and DAPI after 30-min exposure with 1.5 ppm TPTC compared to the control group (A) with 1.5% DMSO was showed (Fig. 7). The fluorescent intensity did decrease in group (B) after exposure with TPTC.

Discussion

Carcinogenesis is a multistep process, including "initiation," "promotion," and "metastasis" ("progression") [70].

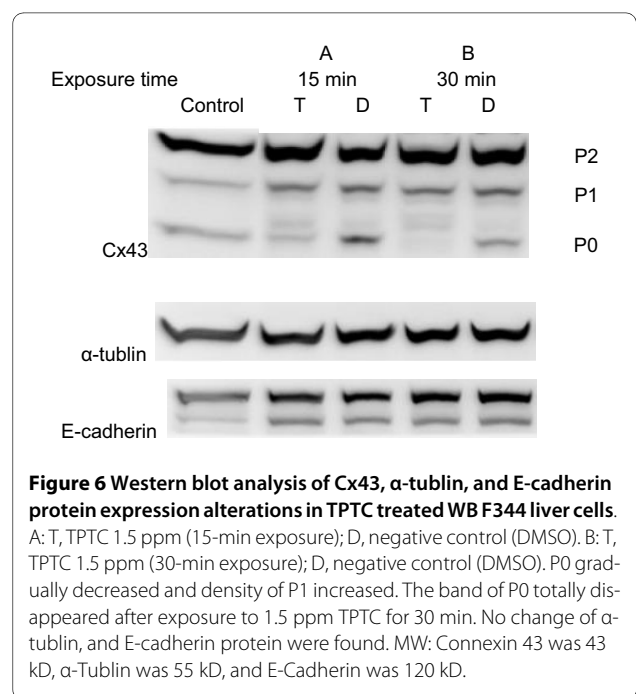
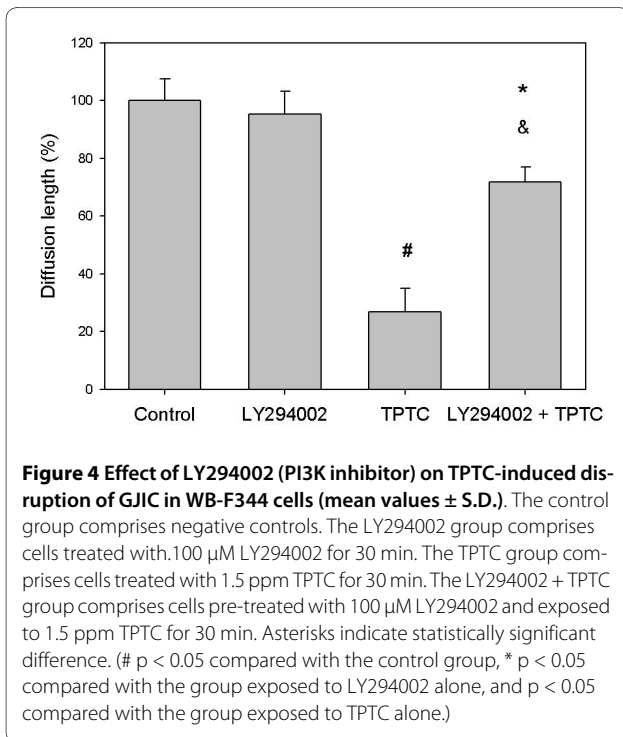
Potter suggested that the initiation process prevents genetically altered stem cells from terminally differentiating [71], and, at the same time, GJIC restricts the growth of these cells. However, when exposed to tumor promoters, which inhibit GJIC, these transformed cells proliferate [37]. The results of this study indicate that the TPTC inhibits GJIC in WB-F344 rat liver epithelial cells in a concentration- and time-dependent manner. In the present study, we demonstrate for the first time that exposure TPTC results in downregulation of Cx43 expression in liver cell cultures. Moreover, we show that TPTC modulates Cx expression predominantly through activation of MAPK and PI3K signaling pathways. Several in vivo and in vitro studies have revealed potential effects of organotins in broad spectrum including immunosuppressive, neurotoxic, endocrinopathic, reproductive, teratogenic, developmental, and possibly carcinogenic activity [3,13,72-75]. Alterations in the phosphorylation status of



connexins are a consequence of the activities of the protein kinase and/or protein phosphatases. GJIC recovered when pre-treated with PD 98059 (ERK inhibitor), and LY294002 (PI3-kinase inhibitor), but did not recover when GF109203X (Protein Kinase C inhibitor) was added. The reactions of fluorescence of Cx43 in WB-

F344 cells after treatment with TPTC did decrease and the phosphorylation of Cx43 was found in Western Blot analysis. Some studies also showed that TPTC could inhibit the phosphorylation and ATP formation in chloroplasts and embryos of marine invertebrate [9,76].

The inhibition of GJIC by TPTC was independent of PKC activity but clearly dependent upon the activation of both MAPK and PI3-kinase pathways. The loss of GJIC



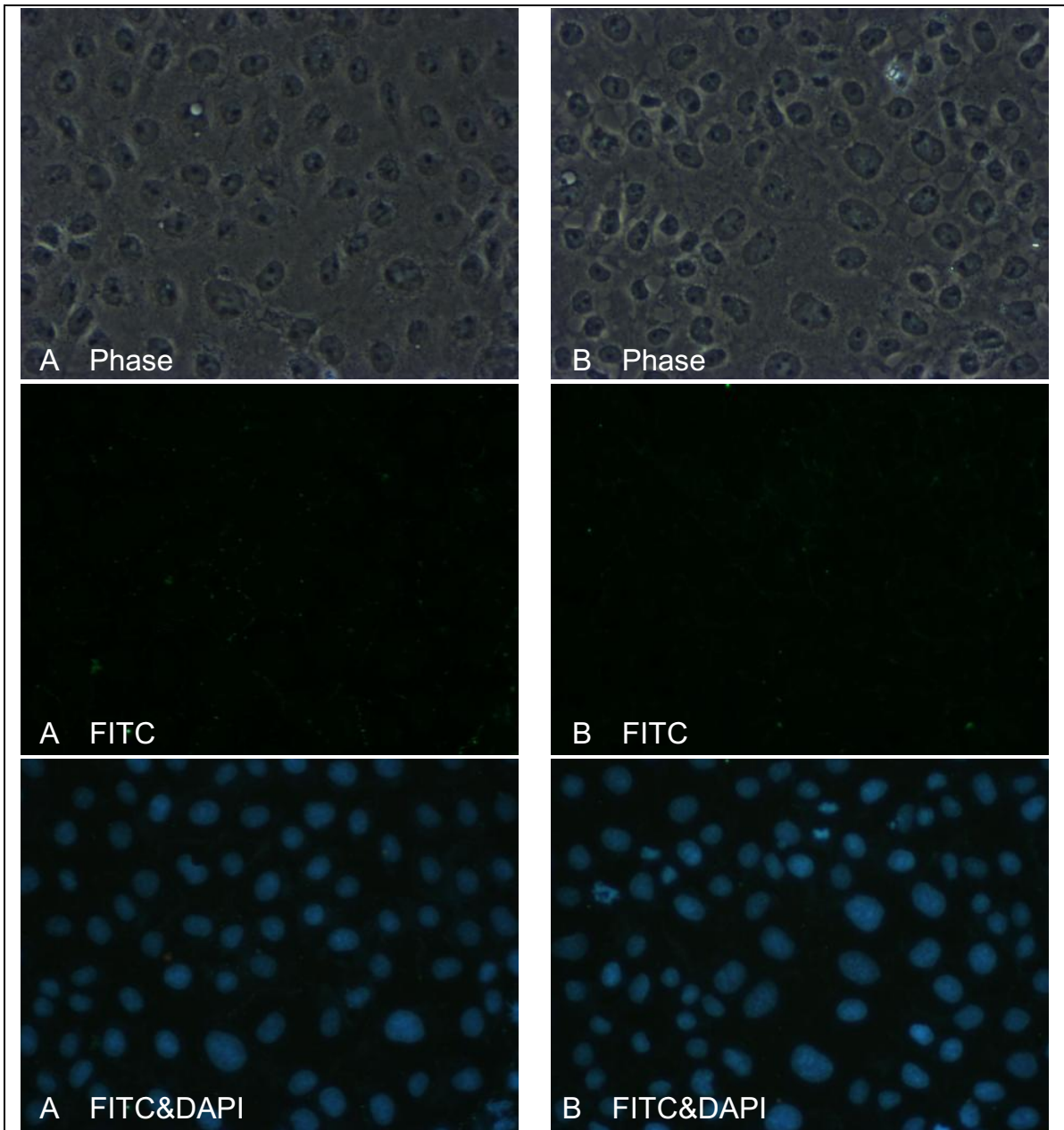


Figure 7 The expression of Cx43 in WB-F344 cell under stained with FITC and DAPI. A. expression of Cx43 in WB-F344 cell with 1.5% DMSO; B: expression of Cx43 in WB-F344 with 1.5 ppm TPTC after 30-min exposure. The fluorescent intensity did decrease in FITC stain after treatment with 1.5 ppm of TPTC for 30 min.

was also described in cancer cells [77,78]. Alteration in expression of connexins may be involved in the expression of neoplastic phenotype [79] and changes in the phosphorylation pattern of connexins are also associated with GJIC inhibition by other tumor-promoting agents and oncogenes [80-82].

Hence, there is no evidence of a causal cross-talk between the two modulatory pathways, MAPK and PI3K. However, both PD58059 and LY294002 abolished com-

pletely the effect of TPTC downregulation of Cx43, implicating both MAPK and PI3K signaling cascades in a common mechanism of Cx regulation. It is possible that MAPK and PI3K act through a common downstream pathway, such as GSK-3 activation [83-86], to control endothelial cellular function through Cxs.

In conclusion, the present study shows that TPTC inhibits GJIC in WB-F344 rat liver epithelial cells by altering the Cx43 protein expression through the MAPK

and PI3-kinase pathways. However, to prove the carcinogenicity of TPTC still needs further study. This preliminary study could provide the possible mechanism for further evaluation of toxicity of TPTC.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CHL participated in the study design, interpretation of results, analysis, and manuscript writing. IHC participated in the study design and analysis. CRL participated in the statistical analysis and manuscript writing. CHC participated in the study design and coordination. MCT participated in the study design and coordination. JLT carried out the immunoassays, the study design, analysis and manuscript writing. HFL participated in the study design, interpretation of results and manuscript preparation. All authors read and approved the final manuscript.

Acknowledgements

This study was supported by a grant (NSC-93-2113-M-037-018) from the National Science Council, Taiwan.

Author Details

¹Department of Emergency Medicine, National Cheng Kung University Hospital, Tainan, Taiwan, ²Graduate Institute of Occupational Safety and Health, College of Health Science, Kaohsiung Medical University, Kaohsiung 80708, Taiwan, ³Department of Ophthalmology, Chang Gung Memorial Hospital, Kaohsiung, Taiwan and ⁴Chang Gung University, College of Medicine, Kaohsiung, Taiwan

Received: 8 March 2010 Accepted: 30 June 2010

Published: 30 June 2010

References

1. Ueno S, Susa N, Furukawa Y, Komatsu Y, Koyama S, Suzuki T: **Butyltin and phenyltin compounds in some marine fishery products on the Japanese market.** *Archives of Environmental Health* 1999, **54**:20.
2. Duncan J: **The toxicology of molluscicides. The organotins.** *Pharmacology & Therapeutics* 1980, **10**:407-429.
3. Kimbrough RG: **Toxicity and health effects of selected organotin compounds: A review.** *Environmental Health Perspectives* 1976, **14**:51-56.
4. Golub M, Doherty J: **Triphenyltin as a potential human endocrine disruptor.** *Journal Of Toxicology And Environmental Health Part B, Critical Reviews* 2004, **7**:281-295.
5. Santos MM, Reis-Henriques Armanda M, Natividade Vieira M, Sole M: **Triphenyltin and tributyltin, single and in combination, promote imposex in the gastropod *Bolinus brandaris*.** *Ecotoxicology and Environmental Safety* 2006, **64**:155-162.
6. Horiguchi T, Shiraishi H, Shimizu M, Morita M: **Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell, *Thais clavigera*.** *Environmental Pollution* 1997, **95**:85-91.
7. Shim WJ, Kahng SH, Hong SH, Kim NS, Kim SK, Shim JH: **Imposex in the rock shell, *Thais clavigera*, as evidence of organotin contamination in the marine environment of Korea.** *Marine Environmental Research* 2000, **49**:435-451.
8. Boyer IJ: **Toxicity of dibutyltin, tributyltin and other organotin compounds to humans and to experimental animals.** *Toxicology* 1989, **55**:253-298.
9. Cima F, Ballarin L, Bressa G, Martinucci G, Burighel P: **Toxicity of Organotin Compounds on Embryos of a Marine Invertebrate (*Styela plicata*; Tunicata).** *Ecotoxicology and Environmental Safety* 1996, **35**:174-182.
10. Ohhira S, Enomoto M, Matsui H: **In vitro metabolism of tributyltin and triphenyltin by human cytochrome P-450 isoforms.** *Toxicology* 2006, **228**:171-177.
11. Sarpa M, De-Carvalho RR, Delgado IF, Paumgartten FJR: **Development toxicity of triphenyltin hydroxide in mice.** *Regulatory Toxicology and Pharmacology* 2007, **49**:43-52.
12. Chicano JJ, Ortiz A, Teruel JA, Aranda FJ: **Organotin compounds alter the physical organization of phosphatidylcholine membranes.** *Biochimica et Biophysica Acta (BBA) - Biomembranes* 2001, **1510**:330-341.
13. Snoeij NJ, Van Iersel AAJ, Penninks AH, Seinen W: **Triorganotin-induced cytotoxicity to rat thymus, bone marrow and red blood cells as determined by several in vitro assays.** *Toxicology* 1986, **39**:71-83.
14. Snoeij NJ, Penninks AH, Seinen W: **Biological activity of organotin compounds--An overview.** *Environmental Research* 1987, **44**:335-353.
15. Whalen MM, Hariharan S, Loganathan BG: **Phenyltin Inhibition of the Cytotoxic Function of Human Natural Killer Cells.** *Environmental Research* 2000, **84**:162-169.
16. Whalen MM, Wilson S, Gleghorn C, Loganathan BG: **Brief exposure to triphenyltin produces irreversible inhibition of the cytotoxic function of human natural killer cells.** *Environmental Research* 2003, **92**:213-220.
17. Chipman JK, Mally A, Edwards GO: **Disruption of gap junctions in toxicity and carcinogenicity.** *Toxicological Sciences: An Official Journal Of The Society Of Toxicology* 2003, **71**:146-153.
18. Kumar NM, Gilula NB: **The Gap Junction Communication Channel.** *Cell* 1996, **84**:381-388.
19. Willecke K, Eiberger J, Degen J, Eckardt D, Romualdi A, Goldenagel M, Deutsch U, Suhl G: **Structural and functional diversity of connexin genes in the mouse and human genome.** *Biological Chemistry* 2002, **383**:725-737.
20. Trosko JE, Ruch RJ: **Cell-cell communication in carcinogenesis.** *Frontiers In Bioscience: A Journal And Virtual Library* 1998, **3**:d208-236.
21. Zahler S, Hoffmann A, Gloe T, Pohl U: **Gap-junctional coupling between neutrophils and endothelial cells: a novel modulator of transendothelial migration.** *Journal Of Leukocyte Biology* 2003, **73**:118-126.
22. Loewenstein WR: **Junctional intercellular communication and the control of growth.** *Biochimica Et Biophysica Acta* 1979, **560**:1-65.
23. Huang R, Liu YG, Lin Y, Fan Y, Boynton A, Yang D, Huang RP: **Enhanced apoptosis under low serum conditions in human glioblastoma cells by connexin 43 (Cx43).** *Molecular Carcinogenesis* 2001, **32**:128-138.
24. Yamasaki H, Naus CCG: **Role of connexin genes in growth control.** *Commentary* 1996, **17**:1199-1213.
25. Krutovskikh V, Yamasaki H: **The role of gap junctional intercellular communication (GJIC) disorders in experimental and human carcinogenesis.** *Histology And Histopathology* 1997, **12**:761-768.
26. Trosko JE, Chang CC, Upham B, Wilson M: **Epigenetic toxicology as toxicant-induced changes in intracellular signalling leading to altered gap junctional intercellular communication.** *Toxicology Letters* 1998, **102-103**:71-78.
27. King TJ, Fukushima LH, Hieber AD, Shimabukuro KA, Sakr WA, Bertram JS: **Reduced levels of connexin43 in cervical dysplasia: inducible expression in a cervical carcinoma cell line decreases neoplastic potential with implications for tumor progression.** *Carcinogenesis* 2000, **21**:1097-1109.
28. Pelin K, Hirvonen A, Linnainmaa K: **Expression of cell adhesion molecules and connexins in gap junctional intercellular communication deficient human mesothelioma tumour cell lines and communication competent primary mesothelial cells.** *Carcinogenesis* 1994, **15**:2673-2675.
29. Lee SW, Tomasetto C, Paul D, Keyomarsi K, Sager R: **Transcriptional downregulation of gap-junction proteins blocks junctional communication in human mammary tumor cell lines.** *The Journal of Cell Biology* 1992, **118**:1213-1221.
30. Garber SA, Fernstrom MJ, Stoner GD, Ruch RJ: **Altered gap junctional intercellular communication in neoplastic rat esophageal epithelial cells.** *Carcinogenesis* 1997, **18**:1149-1153.
31. Tsai H, Werber J, Davia MO, Edelman M, Tanaka KE, Melman A, Christ GJ, Geliebter J: **Reduced connexin 43 expression in high grade, human prostatic adenocarcinoma cells.** *Biochemical And Biophysical Research Communications* 1996, **227**:64-69.
32. Huang RP, Hossain MZ, Sehgal A, Boynton AL: **Reduced connexin43 expression in high-grade human brain glioma cells.** *Journal Of Surgical Oncology* 1999, **70**:21-24.
33. Schlemmer SR, Kaufman DG: **Endometrial stromal cells regulate gap-junction function in normal human endometrial epithelial cells but not in endometrial carcinoma cells.** *Molecular Carcinogenesis* 2000, **28**:70-75.

34. Umhauer S, Ruch RJ, Fanning J: **Gap junctional intercellular communication and connexin 43 expression in ovarian carcinoma.** *American Journal Of Obstetrics And Gynecology* 2000, **182**:999-1000.
35. Murray SA, Davis K, Fishman LM, Bornstein SR: **Alpha1 connexin 43 gap junctions are decreased in human adrenocortical tumors.** *The Journal Of Clinical Endocrinology And Metabolism* 2000, **85**:890-895.
36. Trosko JE, Upham BL: **The emperor wears no clothes in the field of carcinogen risk assessment: ignored concepts in cancer risk assessment.** *Mutagenesis* 2005, **20**:81-92.
37. Yotti LP, Chang CC, Trosko JE: **Elimination of metabolic cooperation in Chinese hamster cells by a tumor promoter.** *Science (New York, NY)* 1979, **206**:1089-1091.
38. Conklin CMJ, Bechberger JF, MacFabe D, Guthrie N, Kurowska EM, Naus CC: **Genistein and quercetin increase connexin43 and suppress growth of breast cancer cells.** *Carcinogenesis* 2007, **28**:93-100.
39. Nojima H, Ohba Y, Kita Y: **Oleamide derivatives are prototypical anti-metastasis drugs that act by inhibiting Connexin 26.** *Current Drug Safety* 2007, **2**:204-211.
40. Sai K, Kanno J, Hasegawa R, Trosko JE, Inoue T: **Prevention of the down-regulation of gap junctional intercellular communication by green tea in the liver of mice fed pentachlorophenol.** *Carcinogenesis* 2000, **21**:1671-1676.
41. Sun H, Liu G-t: **Chemopreventive effect of dimethyl dicarboxylate biphenyl on malignant transformation of WB-F344 rat liver epithelial cells.** *Acta Pharmacologica Sinica* 2005, **26**:1339-1344.
42. Trosko JE, Chang CC: **Mechanism of up-regulated gap junctional intercellular communication during chemoprevention and chemotherapy of cancer.** *Mutation Research* 2001, **480-481**:219-229.
43. Tsao M-S, Smith JD, Nelson KG, Grisham JW: **A diploid epithelial cell line from normal adult rat liver with phenotypic properties of 'oval' cells.** *Experimental Cell Research* 1984, **154**:38-52.
44. Mosmann T: **Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays.** *Journal of Immunological Methods* 1983, **65**:55-63.
45. Shackelford RE, Innes CL, Sieber SO, Heinloth AN, Leadon SA, Paules RS: **The Ataxia telangiectasia Gene Product Is Required for Oxidative Stress-induced G1 and G2 Checkpoint Function in Human Fibroblasts.** *Journal of Biological Chemistry* 2001, **276**:21951-21959.
46. El-Fouly MH, Trosko JE, Chang C-C: **Scrape-loading and dye transfer: A rapid and simple technique to study gap junctional intercellular communication.** *Experimental Cell Research* 1987, **168**:422-430.
47. Coons A, Kaplan M: **Localization of antigen in tissue cells. II. Improvements in a method for the detection of antigen by means of fluorescent antibody.** *J Exptl Med* 1950, **91**:1-13.
48. Wang B-a, Li M, Mu Y-m, Lu Z-h, Li J-y: **Effects of tributyltin chloride (TBT) and triphenyltin chloride (TPT) on rat testicular Leydig cells.** *Zhonghua Nan Ke Xue* 2006, **12**:516-519.
49. Ren P, Mehta PP, Ruch RJ: **Inhibition of gap junctional intercellular communication by tumor promoters in connexin43 and connexin32-expressing liver cells: cell specificity and role of protein kinase C.** *Carcinogenesis* 1998, **19**:169-175.
50. Leithe E, Rivedal E: **Epidermal growth factor regulates ubiquitination, internalization and proteasome-dependent degradation of connexin43.** *Journal Of Cell Science* 2004, **117**:1211-1220.
51. Sirnes S, Leithe E, Rivedal E: **The detergent resistance of Connexin43 is lost upon TPA or EGF treatment and is an early step in gap junction endocytosis.** *Biochemical And Biophysical Research Communications* 2008, **373**:597-601.
52. Vikhamar G, Rivedal E, Mollerup S, Sanner T: **Role of Cx43 phosphorylation and MAP kinase activation in EGF induced enhancement of cell communication in human kidney epithelial cells.** *Cell Adhesion And Communication* 1998, **5**:451-460.
53. Ruch RJ, Trosko JE: **Gap-junction communication in chemical carcinogenesis.** *Drug Metabolism Reviews* 2001, **33**:117-124.
54. Ruch Randall J, Trosko James E, Madhukar BV: **Inhibition of Connexin43 Gap Junctional Intercellular Communication by TPA Requires ERK Activation.** *Journal of Cellular Biochemistry* 2001, **83**:163-169.
55. Dudley DT, Pang L, Decker SJ, Bridges AJ, Saitiel AR: **A Synthetic Inhibitor of the Mitogen-Activated Protein Kinase Cascade.** *Volume 92. Proceedings of the National Academy Sciences of the United States of America*; 1995:7686-7689.
56. Yang S-R, Cho S-D, Ahn N-S, Jung J-W, Park J-S, Jo E-H, Hwang J-W, Jung J-Y, Kim T-Y, Yoon B-S, et al.: **Role of gap junctional intercellular communication (GJIC) through p38 and ERK1/2 pathway in the differentiation of rat neuronal stem cells.** *The Journal Of Veterinary Medical Science/The Japanese Society Of Veterinary Science* 2005, **67**:291-294.
57. Hakulinen P, Rintala E, Maki-Paakkanen J, Komulainen H: **Altered expression of connexin43 in the inhibition of gap junctional intercellular communication by chlorohydroxyfuranones in WB-F344 rat liver epithelial cells.** *Toxicology and Applied Pharmacology* 2006, **212**:146-155.
58. Yao J, Morioka T, Oite T: **PDGF regulates gap junction communication and connexin43 phosphorylation by PI 3-kinase in mesangial cells.** *Kidney International* 2000, **57**:1915-1926.
59. Zhang F, Cheng J, Lam G, Jin DK, Vincent L, Hackett NR, Wang S, Young LM, Hempstead B, Crystal RG, Rafii S: **Adenovirus vector E4 gene regulates connexin 40 and 43 expression in endothelial cells via PKA and PI3K signal pathways.** *Circulation Research* 2005, **96**:950-957.
60. Zhao Y, Rivieccio MA, Lutz S, Scemes E, Brosnan CF: **The TLR3 ligand polyI: C downregulates connexin 43 expression and function in astrocytes by a mechanism involving the NF-kappaB and PI3 kinase pathways.** *Glia* 2006, **54**:775-785.
61. Barac YD, Zeevi-Levin N, Yaniv G, Reiter I, Milman F, Shilkrut M, Coleman R, Abassi Z, Binah O: **The 1,4,5-inositol trisphosphate pathway is a key component in Fas-mediated hypertrophy in neonatal rat ventricular myocytes.** *Cardiovascular Research* 2005, **68**:75-86.
62. Gao Q, Katakowski M, Chen X, Li Y, Chopp M: **Human marrow stromal cells enhance connexin43 gap junction intercellular communication in cultured astrocytes.** *Cell Transplantation* 2005, **14**:109-117.
63. Gekeler V, Boer R, Uberall F, Ise W, Schubert C, Utz I, Hofmann J, Sanders KH, Schachtele C, Klemm K, Grunicke H: **Effects of the selective bisindolylmaleimide protein kinase C inhibitor GF 109203X on P-glycoprotein-mediated multidrug resistance.** *British Journal Of Cancer* 1996, **74**:897-905.
64. Gekeler V, Boer R, Ise W, Sanders KH, Schachtele C, Beck J: **The specific bisindolylmaleimide PKC-inhibitor GF 109203X efficiently modulates MRP-associated multiple drug resistance.** *Biochemical And Biophysical Research Communications* 1995, **206**:119-126.
65. Solan JL, Lampe PD: **Connexin phosphorylation as a regulatory event linked to gap junction channel assembly.** *Biochimica et Biophysica Acta (BBA) - Biomembranes* 2005, **1711**:154-163.
66. Moreno AP, Lau AF: **Gap junction channel gating modulated through protein phosphorylation.** *Progress in Biophysics and Molecular Biology* 2007, **94**:107-119.
67. Lampe PD, Lau AF: **The effects of connexin phosphorylation on gap junctional communication.** *The International Journal of Biochemistry & Cell Biology* 2004, **36**:1171-1186.
68. Ruch RJ, Bonney WJ, Sigler K, Guan X, Matesic D, Schafer LD, Dupont E, Trosko JE: **Loss of gap junctions from DDT-treated rat liver epithelial cells.** *Carcinogenesis* 1994, **15**:301-306.
69. Matesic DF, Rupp HL, Bonney WJ, Ruch RJ, Trosko JE: **Changes in gap-junction permeability, phosphorylation, and number mediated by phorbol ester and non-phorbol-ester tumor promoters in rat liver epithelial cells.** *Molecular Carcinogenesis* 1994, **10**:226-236.
70. Trosko JE, Ruch RJ: **Cell-cell communication in carcinogenesis.** *Front Biosci* 1998, **3**:d208-236.
71. Potter VR: **A new protocol and its rationale for the study of initiation and promotion of carcinogenesis in rat liver.** *Carcinogenesis* 1981, **2**:1375-1379.
72. Matsui H, Wada O, Ushijima Y, Akuzawa T: **Triphenyltin chloride inhibits superoxide production by human neutrophils stimulated with a surface active agent.** *FEBS Letters* 1983, **164**:251-254.
73. McCollister DD, Schober AE: **Assessing toxicological properties of organotin Compounds.** *Environmental Quality And Safety* 1975, **4**:80-95.
74. Snoeij NJ, van Iersel AAJ, Penninks AH, Seinen W: **Toxicity of triorganotin compounds: Comparative in vivo studies with a series of trialkyltin compounds and triphenyltin chloride in male rats.** *Toxicology and Applied Pharmacology* 1985, **81**:274-286.
75. Antizar-Ladislao B: **Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review.** *Environment International* 2008, **34**:292-308.

76. Gould JM: **Inhibition by triphenyltin chloride of a tightly-bound membrane component involved in photophosphorylation.** *European Journal Of Biochemistry/FEBS* 1976, **62**:567-575.
77. Loewenstein WR, Kanno Y: **Intercellular Communication and the Control of Tissue Growth: Lack of Communication between Cancer Cells.** *Nature* 1966, **209**:1248-1249.
78. Vine AL, Bertram JS: **Cancer chemoprevention by connexins.** *Cancer and Metastasis Reviews* 2002, **21**:199-216.
79. Udaka N, Miyagi Y, Ito T: **Connexin expression in mouse lung tumor.** *Cancer Letters* 2007, **246**:224-229.
80. Kamibayashi Y, Oyamada Y, Mori M, Oyamada M: **Aberrant expression of gap junction proteins (connexins) is associated with tumor progression during multistage mouse skin carcinogenesis in vivo.** *Carcinogenesis* 1995, **16**:1287-1297.
81. Asamoto M, Takahashi S, Imaida K, Shirai T, Fukushima S: **Increased gap junctional intercellular communication capacity and connexin 43 and 26 expression in rat bladder carcinogenesis.** *Carcinogenesis* 1995, **15**:2163-2166.
82. Oyamada Y, Oyamada M, Fusco A, Yamasaki H: **Aberrant expression, function and localization of connexins in human esophageal carcinoma cell lines with different degrees of tumorigenicity.** *Journal of Cancer Research and Clinical Oncology* 1995, **120**:445-453.
83. Hideshima T, Nakamura N, Chauhan D, Anderson KC: **Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma.** *Oncogene* 2001, **20**:5991-6000.
84. Hoarau C, Martin L, Faugaret D, Baron C, Dauba A, Aubert-Jacquin C, Velge-Roussel F, Lebranchu Y: **Supernatant from bifidobacterium differentially modulates transduction signaling pathways for biological functions of human dendritic cells.** *Plos ONE* 2008, **3**:e2753-e2753.
85. Risbud MV, Fertala J, Vresilovic EJ, Albert TJ, Shapiro IM: **Nucleus pulposus cells upregulate PI3K/Akt and MEK/ERK signaling pathways under hypoxic conditions and resist apoptosis induced by serum withdrawal.** *Spine* 2005, **30**:882-889.
86. Yu C, Rahmani M, Dai Y, Conrad D, Krystal G, Dent P, Grant S: **The lethal effects of pharmacological cyclin-dependent kinase inhibitors in human leukemia cells proceed through a phosphatidylinositol 3-kinase/Akt-dependent process.** *Cancer Research* 2003, **63**:1822-1833.

doi: 10.1186/1745-6673-5-17

Cite this article as: Lee *et al.*, Inhibition of gap junctional Intercellular communication in WB-F344 rat liver epithelial cells by triphenyltin chloride through MAPK and PI3-kinase pathways *Journal of Occupational Medicine and Toxicology* 2010, **5**:17

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

