Effects of Pentachlorophenol and Tetrachlorohydroquinone on Mitogen-Activated Protein Kinase Pathways in Jurkat T Cells

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When Jurkat human T cells were incubated with 20 µM of pentachlorophenol (PCP) or its metabolite, tetrachlorohydroquinone (TCHQ), for 10 hr, flow cytometric analyses revealed marked increase in the number of apoptotic cells. DNA fragmentation was also observed in these cells. TCHQ was more potent than PCP in causing apoptosis. After incubation with 20 µM TCHQ for 1 hr, all mitogen-activated protein kinases (MAPKs) examined [i.e., extracellular signal-regulated protein kinase (ERK), p38, and c-Jun NH_2-terminal kinase (JNK)] were phosphorylated, whereas no clear phosphorylation was induced by PCP. TCHQ-induced apoptosis was markedly suppressed by treatment with a p38 inhibitor (SB203580) and mildly (but significantly) suppressed by treatment with a MAPK/ERK kinase inhibitor (U0126). When cells were treated with both inhibitors at the same time, TCHQ-induced apoptosis disappeared almost completely. PCP-induced apoptosis was also suppressed by SB203580 and/or U0126. Nevertheless, treatment with LL-Z1640-2, which inhibits JNK phosphorylation, did not suppress the apoptosis caused by either TCHQ or PCP. Thus, p38 and ERK appear to be important signal transduction pathways leading to apoptosis in a human T-cell line exposed to a ubiquitous pollutant or its metabolite in the general and occupational environment. ***Key words*****: apoptosis, Jurkat cells, mitogen-activated protein kinases, pentachlorophenol, tetrachlorohydroquinone.** ***Environ Health Perspect*** **110:139–143 (2002). [Online 10 January 2002]**
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Generally, chlorinated phenols have strong biological effects. Of all chlorinated phenols, pentachlorophenol (PCP) seems to have been produced and used in the largest quantities; annual production of PCP all over the world was estimated to be 25,000–90,000 tons at its peak (*1*). Because PCP is inexpensive and has potent biocidal effects against a broad spectrum of species, PCP and its salts have been used as algicides, bactericides, fungicides, herbicides, insecticides, and molluscides (*2*). Although its use has been reduced greatly and even banned in some countries, PCP is still used widely in the preservation of wood (*1,2*). Hence, PCP is ubiquitous in the environment. For instance, it has been detected in ambient air of rural mountain areas, and PCP levels of 5.7–7.8 ng/m^3 have been found in urban areas (*2*). PCP concentrations in groundwater can be 3–23 µg/L in wood-treatment areas and concentrations in the milligrams per liter range can be found near industrial discharges. As a result, the general population is exposed to PCP through the ingestion of water (0.01–0.1 µg/L) and food (up to 40 µg/kg). People may be exposed to PCP in treated items such as textiles, leather, and paper products, and especially through inhalation of indoor air contaminated with PCP; PCP concentrations up to 25 µg/m^3 have been found in rooms treated 1 to several years earlier (*2*). Workers handling PCP can be exposed to much higher doses of PCP (*1*). Thus, health effects of PCP among workers, as well as among the general population, are of great concern.

Acute exposures to high doses of PCP in accidental or suicidal intoxication are often fatal and are associated with extreme weakness, hyperpyrexia, and profuse sweating (*2*). These signs can be explained by the effects of PCP itself, that is, uncoupling of oxidative phosphorylation in mitochondria (*3*). In contrast, although effects of lower doses of PCP and tetrachlorohydroquinone (TCHQ), the major metabolite of PCP (*4*), on diverse biological systems (including the immune system) have been suggested (*5–9*), neither clinical pictures nor mechanisms of their effects have been clarified. We therefore examined effects of PCP and TCHQ on mitogen-activated protein kinases (MAPKs)—extracellular signal-regulated protein kinase (ERK), p38, and c-Jun NH_2-terminal kinase (JNK)—in a human T-cell line (Jurkat cells) because recent studies have shown that MAPKs play important roles in cellular responses to various harmful signals (*10*).

Materials and Methods

Cell culture and treatments. Jurkat human T cells (RIKEN Cell Bank, Tsukuba, Japan) were grown in RPMI 1640 media supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin (GIBCO BRL, Life Technologies, Inc., Rockville, MD, USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. Exponentially growing Jurkat cells were diluted at 3×10^6 cells in 6-well culture plates or 1×10^5 cells in 96-well culture plates and subsequently used for the experiments.

For each experiment, we prepared fresh solutions of PCP (Sigma Chemical Co., St. Louis, MO, USA) and TCHQ (Sigma Chemical Co.) with dimethyl sulfoxide (DMSO). Jurkat cells were incubated with serum-free medium containing 5–20 µM PCP or TCHQ for 1 or 10 hr at 37°C. Untreated control cells were incubated with serum-free medium containing the same concentration of DMSO (0.1–0.3%) and were treated identically to the cells exposed to PCP or TCHQ.

The MAPK/ERK kinase (MEK) inhibitor U0126 (Promega, Madison, WI, USA) and a specific inhibitor of p38, SB203580 (Calbiochem, La Jolla, CA, USA) were dissolved in DMSO. Cells were preincubated with serum-free medium containing either 0.1–0.2% DMSO, 50 µM U0126, 50 µM SB203580, or 50 µM U0126 plus 50 µM SB203580 for 30 min, and then incubated with 0.1% DMSO, 20 µM PCP, or TCHQ for 10 hr. A radicicol-related macrocyclic nonaketide compound, LL-Z1640-2 (provided by Pharmaceutical Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan), which has been shown to inhibit the activation of JNK (*11,12*), was also dissolved in DMSO. Cells were preincubated with serum-free medium containing either 0.002% DMSO or 50, 100, or 200 ng/mL LL-Z1640-2 for 30 min and then incubated with 0.1% DMSO or 20 µM TCHQ for 10 hr.

WST-8 assay. We determined cell viability using the WST-8 assay. Cell Count Reagent SF (10 µL; Nacalai Tesque, Kyoto, Japan), which consists of 5 mM WST-8 (4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-*2H*-5-tetrazolio]-1,3-benzene disulfonate sodium salt), 0.2 mM

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1-methoxy-5-methylphenazinium methosulfate, and 150 mM NaCl, was added to each well of 96-well culture plates. After incubation for 1 hr at 37°C, the absorbance of each well was measured at 450 nm with a reference wavelength at 655 nm.

Flow cytometric analysis. Jurkat cells (3×10^6) incubated with PCP or TCHQ for 10 hr were washed with phosphate-buffered saline (PBS) and fixed in 70% ethanol on ice for 30 min. The fixed cells were washed twice with PBS, incubated with 250 µg/mL RNase at 37°C for 1 hr, washed again twice, and then stained with 50 µg/mL propidium iodide solution. The DNA content of cells (1×10^4 for each sample) was determined with a Coulter EPICS XL (Coulter Electronics, Miami, FL, USA). Apoptotic cells were estimated by the percentage of cells in the sub-G_1 peak as described previously (*13*).

DNA fragmentation. We performed DNA fragmentation analysis using the method of Herrmann et al. (*14*) with slight modifications. After the incubation with PCP or TCHQ for 10 hr, Jurkat cells were washed with PBS and treated with lysis buffer (1% NP-40, 20 mM EDTA, 50 mM Tris-HCl, pH 7.5) for 30 min on ice. After centrifugation at 1,600 *g* for 5 min, cell lysates were incubated with 1% SDS and 5 mg/mL RNase for 2 hr at 56°C, and then with 2.5 mg/mL proteinase K for 4 hr at 37°C. DNA was precipitated with 1/2 vol. 10 M ammonium acetate and 2.5 vol. ethanol, dissolved in loading buffer, separated by electrophoresis in 1.5% agarose gel containing 0.5 µg/mL ethidium bromide, and visualized under UV light.

Western immunoblotting. After incubation with PCP or TCHQ for 1 hr, Jurkat cells were washed with PBS and lysed with SDS-polyacrylamide gel Laemmli sample buffer. Cell lysates were collected, sonicated, and boiled for 5 min. Aliquots equivalent to 4×10^5 cells were subjected to SDS-PAGE on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane (Hybond-ECL, Amersham Pharmacia Biotech, Buckinghamshire, England). The membrane was blocked with 5% nonfat milk or 1% bovine serum albumin in Tris-buffered saline containing 0.1% Tween-20 for 1 hr at room temperature. We used the following antibodies: phospho-p44/42 MAPK (Thr202/Tyr204) antibody, phosphorylation state-independent p44/42 MAPK antibody, phospho-p38 MAPK (Thr180/Tyr182) antibody, phosphorylation state-independent p38 MAPK antibody, phospho-SAPK/JNK (Thr183/Tyr185) antibody, and phosphorylation state-independent SAPK/JNK antibody (Cell Signaling Technology, Inc., Beverly, MA, USA). The membrane was incubated overnight at 4°C with the primary antibody diluted 1:1000 in 5% bovine serum albumin in Tris-buffered saline containing 0.1% Tween-20. We detected protein using the Phototope-HRP Western blot detection kit (Cell Signaling Technology, Inc.). The bands on the developed film were quantified with NIH Image Version 1.61 (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. Results were expressed as mean ± SEM. We determined statistical significance using one-way analysis of variance followed by the Bonferroni multiple comparison test. When two groups were compared, we used the Student *t*-test. $p < 0.05$ was considered as statistically significant.

Results

The cell viability of Jurkat cells assayed with WST-8 conversion was 92.2 ± 0.9, 79.8 ± 1.7, and 74.9 ± 1.2%, respectively, for cells treated with 5, 10, and 20 µM TCHQ for 10 hr and 99.8 ± 1.5, 97.5 ± 1.1, and 85.5 ± 0.5% ($n = 4$) of untreated control cells for those treated with 5, 10, and 20 µM PCP for 10 hr, respectively. Thus, TCHQ treatment reduced cell viability more than PCP treatment at each concentration ($p < 0.01$).

When Jurkat cells were incubated with 20 µM TCHQ for 10 hr, a marked increase in the number of apoptotic cells was found with flow cytometric analysis (Figure 1). Incubation with 20 µM PCP for 10 hr also increased the number of apoptotic cells, but this increase was less significant than that with the same dose of TCHQ ($p < 0.05$; Figure 1). Consistent with the results of flow cytometric analysis, DNA fragmentation occurred in cells treated with 20 µM TCHQ

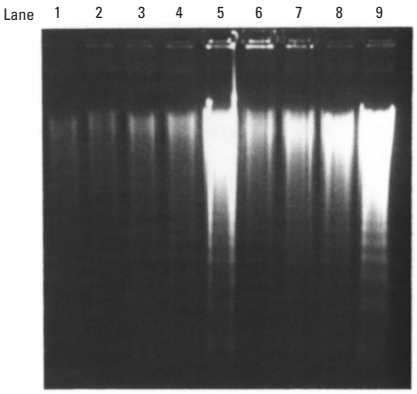

Figure 2. DNA fragmentation in Jurkat cells exposed to TCHQ (lanes 2–5) or PCP (lanes 6–9). Cells were incubated with TCHQ or PCP for 10 hr. Isolated DNA was run on a 1.5% agarose gel containing ethidium bromide. Lane 1, 0.1% DMSO; lane 2, 5 µM TCHQ; lane 3, 7.5 µM TCHQ; lane 4, 10 µM TCHQ; lane 5, 20 µM TCHQ; lane 6, 5 µM PCP; lane 7, 7.5 µM PCP; lane 8, 10 µM PCP; lane 9, 20 µM PCP.

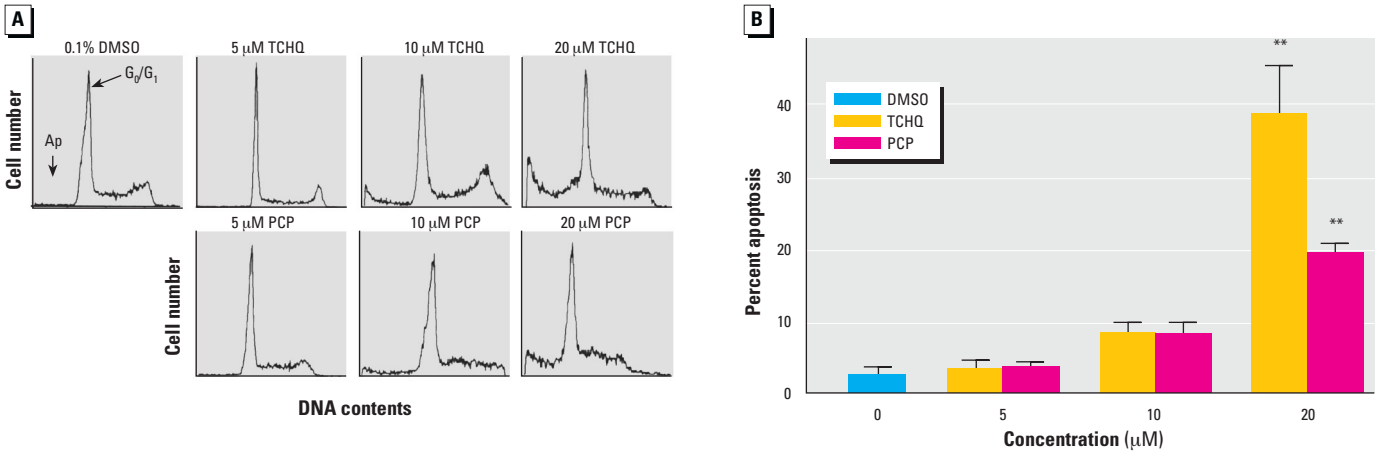

Figure 1. Apoptosis in Jurkat cells exposed to TCHQ or PCP. (*A*) Representative flow cytometric analysis of cells incubated with 0.1% DMSO, 5 µM TCHQ, 10 µM TCHQ, 20 µM TCHQ, 5 µM PCP, 10 µM PCP, or 20 µM PCP for 10 hr at 37°C; apoptotic (Ap) and G_0–G_1 cell populations are indicated by arrows. (*B*) Percentage of apoptosis in control cells and cells exposed to TCHQ or PCP. Apoptotic cells were estimated by the percentage of cells in the sub-G_1 peak. Each bar represents the mean ± SEM of three independent experiments.
**$p < 0.05$ versus control (0.1% DMSO).

(Figure 2, lane 5) and PCP (Figure 2, lane 9). In contrast, incubation with TCHQ or PCP at concentrations higher than 20 µM induced apoptosis less markedly (data not shown).

We determined the phosphorylation status of MAPKs after incubation with TCHQ or PCP for 1 hr, when cell viability of Jurkat cells was not changed as evaluated by the WST-8 assay (data not shown). After incubation with TCHQ for 1 hr, we found significant phosphorylation of ERK (Figure 3A), p38 (Figure 3B), and JNK (Figure 3C) at 20, 10, and 7.5 µM TCHQ, respectively. In contrast, incubation with PCP did not induce clear phosphorylation of MAPKs (Figure 3). The levels of total (phosphorylation state-independent) ERK, p38, and JNK were not changed by the incubation with any concentration of TCHQ or PCP (Figure 3). In the time-course study, the levels of the phosphorylated form of ERK and p38 increased after 15 min and that of JNK increased after 1 hr in response to 20 µM TCHQ exposure (data not shown).

Treatment with an MEK inhibitor, U0126 (50 µM), suppressed the TCHQ-induced increase in the number of apoptotic cells mildly but significantly ($p < 0.01$; Figure 4). In contrast, treatment with a p38 inhibitor, SB203580 (50 µM), protected cells more markedly from TCHQ-induced apoptosis (Figure 4). The difference in the magnitude of suppression of apoptosis between cells treated with U0126 and SB203580 was significant ($p < 0.01$; Figure 4B). When cells were treated with U0126 (50 µM) and SB203580 (50 µM) at the same time, TCHQ-induced apoptosis was abolished almost completely (Figure 5). Treatment with U0126, SB203580, or both compounds together also reduced the number of apoptotic cells in Jurkat cells treated with PCP (25.8 ± 0.7%, $n = 6$) to 21.4 ± 0.6% ($p < 0.05$, $n = 3$), 13.0 ± 0.8% ($p < 0.001$, $n = 3$), and 5.7 ± 0.3% ($p < 0.001$, $n = 3$), respectively. In contrast, treatment with > 50 ng/mL LL-Z1640-2, the concentration that suppressed TCHQ-induced phosphorylation of JNK (data not shown), did not reduce the number of apoptotic cells in cells treated with TCHQ (Figure 6). However, treatment with LL-Z1640-2 (200 ng/mL) alone did not induce apoptosis (Figure 6).

Discussion

Both flow cytometric and DNA fragmentation analyses indicated apoptosis in Jurkat cells treated with 20 µM PCP or 20 µM TCHQ for 10 hr. The flow cytometry showed that apoptosis was more marked when cells were incubated with TCHQ than with PCP. Consistent with this, the WST-8 assay showed that treatment with TCHQ reduced cell viability more markedly than treatment with the same dose of PCP. Wang and colleagues (*15,16*) reported that TCHQ induced apoptosis in mouse fibroblasts and human bladder carcinoma cells, but not in human liver cells, whereas PCP induced no apoptosis in either human cells (*16*). These findings and ours suggest that exposure to PCP or TCHQ induces apoptosis depending on the cell type and that human lymphocytes are sensitive to both PCP and TCHQ, but more so to TCHQ. TCHQ has been detected in urine of humans occupationally exposed to PCP (*17,18*). The concentration of PCP found in blood from workers exposed to PCP (4,730 µg/L) (*19*) was similar to the concentration we used in the present study (20 µM PCP). Thus, lowered lymphocyte count seen in workers with high PCP concentrations in blood (*9*) might be due to apoptotic cell death of lymphocytes. However, it should be noted that we

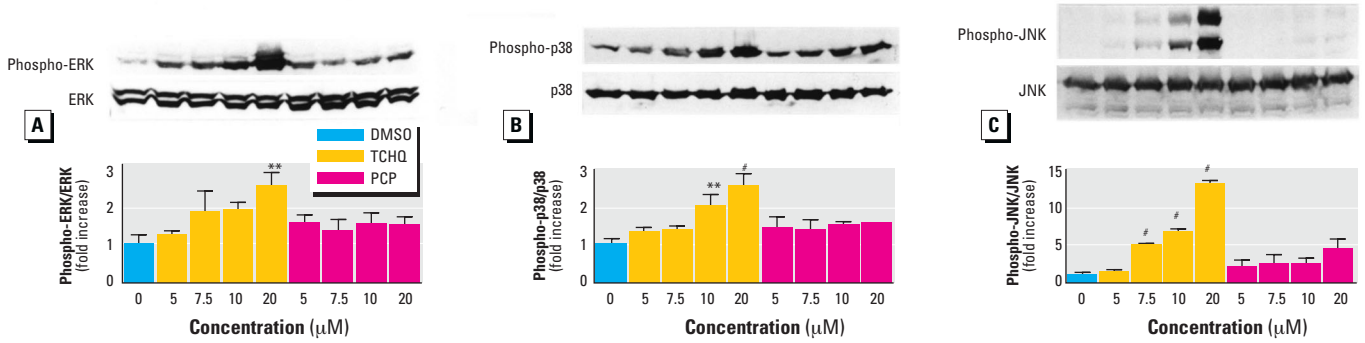

Figure 3. Dose effects of TCHQ and PCP on the accumulation of (*A*) phosphorylated (Phospho) ERK, (*B*) p38, and (*C*) JNK. Jurkat cells were incubated with TCHQ or PCP for 1 hr, and cell lysates were subjected to Western immunoblotting. The levels of phosphorylated ERK, p38, and JNK were determined on the basis of densitometric analyses. Each value is expressed as the ratio of phosphorylated MAPK to the corresponding total MAPK, and the control value was set to 1. Bars indicate the mean ± SEM of three independent experiments.
$^{**}p < 0.05$; $^{\#}p < 0.01$ versus control (0.1% DMSO).

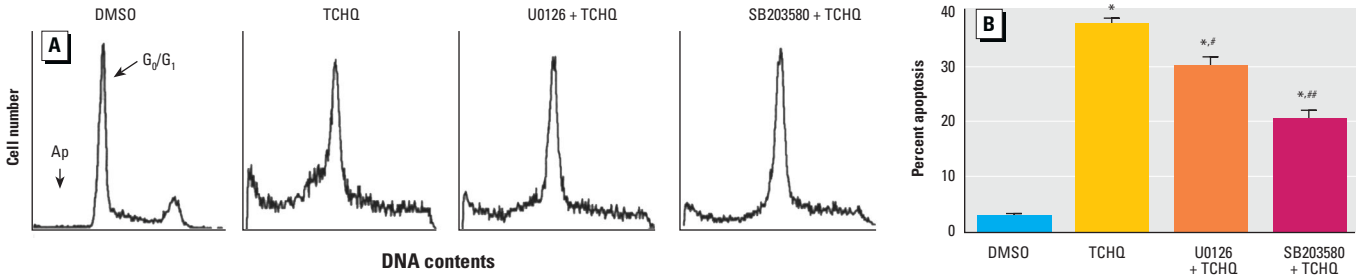

Figure 4. Effects of U0126 or SB203580 on TCHQ-induced apoptosis in Jurkat cells. (*A*) Representative flow cytometric analysis of cells treated with 0.2% DMSO for 10 hr, 20 µM TCHQ for 10 hr, 50 µM U0126 for 30 min plus 20 µM TCHQ for 10 hr, or 50 µM SB203580 for 30 min plus 20 µM TCHQ for 10 hr; apoptotic (Ap) and G_0–G_1 cell populations are indicated by arrows. (*B*) Apoptotic cells estimated by the percentage of cells in the sub-G_1 peak; each bar represents the mean ± SEM of three independent experiments.
$^{*}p < 0.001$ versus control (0.2% DMSO); $^{\#}p < 0.01$; $^{\#\#}p < 0.001$ versus TCHQ.

observed apoptosis in Jurkat cells that were incubated with PCP or TCHQ in the absence of serum, whereas more than 96% PCP may be bound to human plasma protein (*20*). Hence, it remains to be determined whether apoptosis is induced in peripheral lymphocytes following exposures to PCP or TCHQ *in vivo* as well.

MAPKs are a family of serine/threonine protein kinases that transmit extracellular signals into the nucleus (*21*). Three subfamilies of MAPKs have been identified: ERK, p38, and JNK, which is also known as stress-activated protein kinase or SAPK. MAPK cascades have been shown to participate in a diverse array of cellular functions such as cell growth, differentiation, and apoptosis (*10,21*). Previously, we found that heavy metals that can affect the immune system, such as cadmium, inorganic mercury, and organotin compounds, activate MAPKs in CCRF-CEM human lymphoblastoid cells prior to the development of apoptosis (*12,13,22*). We therefore determined the phosphorylation status of ERK, p38, and JNK in Jurkat human T cells treated with PCP or TCHQ. As a result, we found that treatment with TCHQ induced marked phosphorylation of p38 as well as ERK and JNK in Jurkat cells without changing the total protein levels of each MAPK. In contrast to the treatment with TCHQ, treatment with PCP did not induce MAPK phosphorylation clearly at the same doses (5–20 μM) and incubation period (1 hr).

TCHQ has been found to induce DNA single-strand breaks and increase the level of 8-hydroxy-2-deoxyguanosine, whereas these effects were not observed after exposures to PCP (*23–25*). Therefore, TCHQ and PCP may differ in their potentials to damage DNA, and that might account for the differences in MAPK phosphorylation and subsequent development of apoptosis.

MAPKs can be activated in response to other chlorinated compounds. For example, polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and *o,p′*-dichlorodiphenyltrichloroethane (*o,p′*-DDT) have been reported to activate ERK and JNK in various types of cells (*26–32*). Unpurified technical grade PCP is often contaminated with other chlorinated compounds including PCBs, TCDD, and *o,p′*-DDT (*1,2*). It is therefore possible that alterations of lymphocytes seen in workers or in the general population may be partly due to effects of these contaminants. However, our results do not appear to be affected by contaminants because we used purified PCP and TCHQ.

The responses to inhibitors of MAPKs observed in the present experiments are consistent with the involvement of MAPK phosphorylation in TCHQ or PCP-induced apoptosis. SB203580, a p38 inhibitor (*33*), suppressed apoptosis markedly, and U0126, a potent inhibitor of both activated and nonactivated forms of MEK1/2 (*34*), also suppressed apoptosis mildly but significantly. When cells were treated with both inhibitors at the same time, TCHQ-induced apoptosis disappeared almost completely. These observations indicate that p38 and ERK play a major role in TCHQ-induced apoptosis to a different extent and that the two pathways might work synergistically. Because PCP-induced apoptosis was also suppressed by SB203580 and U0126, inhibition of basally activated forms of p38 and ERK might be partially effective. In contrast, treatment with LL-Z1640-2, which suppresses JNK phosphorylation (*11,12*), did not protect cells from apoptosis. This indicates that although JNK phosphorylation was most marked among the MAPKs examined, JNK did not play a major role leading to the apoptosis.

PCP inhibits oxidative phosphorylation (*3*) and also induces morphologic alterations in the mitochondria (*35*). Therefore, to clarify the mechanisms of PCP- and TCHQ-induced apoptosis, mitochondrial functions including membrane potential and cytochrome *c* release remain to be determined.

Thus, the present results indicate that some effects of PCP and its metabolite may be prevented by modifying MAPK pathways. However, more experiments are necessary before such an application is considered because their *in vivo* effects have not been clarified.

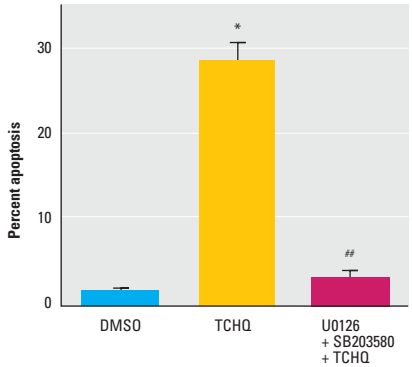

Figure 5. Effects of the combined treatment of U0126 and SB203580 on TCHQ-induced apoptosis in Jurkat cells. Cells were preincubated with 0.2% DMSO or 50 μM U0126 plus SB203580 for 30 min and then incubated with 0.1% DMSO or 20 μM TCHQ for 10 hr. Apoptotic cells were estimated by the percentage of cells in the sub-G_1 peak with flow cytometric analysis. Each bar represents the mean ± SEM of three independent experiments.
*$p < 0.001$ versus control (0.3% DMSO); ##$p < 0.001$ versus TCHQ.

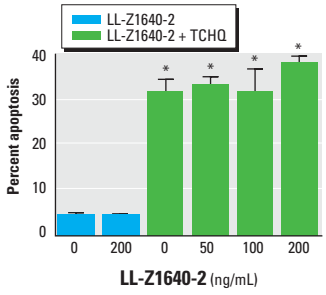

Figure 6. Effects of LL-Z1640-2 on TCHQ-induced apoptosis in Jurkat cells. Cells were preincubated with 0 (0.002% DMSO), 50, 100, or 200 ng/mL LL-Z1640-2 for 30 min and then incubated with 0.1% DMSO or 20 μM TCHQ for 10 hr. Apoptotic cells were estimated by the percentage of cells in the sub-G_1 peak using flow cytometric analysis. Each bar represents the mean ± SEM of three independent experiments.
*$p < 0.001$ versus control (0 ng/mL LL-Z1640-2 plus 0 μM TCHQ).

References and Notes

1. Seiler JP. Pentachlorophenol. Mutat Res 257:27–47 (1991).
2. WHO. Pentachlorophenol. Environmental Health Criteria 71. Geneva:World Health Organization, 1987.
3. Weinbach EC. The effect of pentachlorophenol on oxidative phosphorylation. J Biol Chem 210:545–550 (1954).
4. Juhl U, Witte I, Butte W. Metabolism of pentachlorophenol to tetrachlorohydroquinone by human liver homogenate. Bull Environ Contam Toxicol 35:596–601 (1985).
5. Gerhard I, Derner M, Runnebaum B. Prolonged exposure to wood preservatives induces endocrine and immunologic disorders in women. Am J Obstet Gynecol 165:487–488 (1991).
6. Lang D, Mueller-Ruchholtz W. Human lymphocyte reactivity after in vitro exposure to technical and analytical grade pentachlorophenol. Toxicology 70:271–282 (1991).
7. McConnachie PR, Zahalsky AC. Immunological consequences of exposure to pentachlorophenol. Arch Environ Health 46:249–253 (1991).
8. Daniel V, Huber W, Bauer K, Opelz G. Impaired in-vitro lymphocyte responses in patients with elevated pentachlorophenol (PCP) blood levels. Arch Environ Health 50:287–292 (1995).
9. Daniel V, Huber W, Bauer K, Suesal C, Mytilineos J, Melk A, Conradt C, Opelz G. Association of elevated blood levels of pentachlorophenol (PCP) with cellular and humoral immunodeficiencies. Arch Environ Health 56:77–83 (2001).
10. Robinson MJ, Cobb MH. Mitogen-activated protein kinase pathways. Curr Opin Cell Biol 9:180–186 (1997).
11. Takehana K, Sato S, Kobayasi T, Maeda T. A radicicol-related macrocyclic nonaketide compound, antibiotic LL-Z1640-2, inhibits the JNK/p38 pathways in signal-specific manner. Biochem Biophys Res Commun 257:19–23 (1999).
12. Matsuoka M, Wispriyono B, Iryo Y, Igisu H, Sugiura T. Inhibition of $HgCl_2$-induced mitogen-activated protein kinase activation by LL-Z1640-2 in CCRF-CEM cells. Eur J Pharmacol 409:155–158 (2000).
13. Iryo Y, Matsuoka M, Wispriyono B, Sugiura T, Igisu H. Involvement of the extracellular signal-regulated protein kinase (ERK) pathway in the induction of apoptosis by cadmium chloride in CCRF-CEM cells. Biochem Pharmacol 60:1875–1882 (2000).
14. Herrmann M, Lorenz H-M, Voll R, Grünke M, Woith W, Kalden JR. A rapid and simple method for the isolation of apoptotic DNA fragments. Nucleic Acids Res 22:5506–5507 (1994).

15. Wang Y-J, Ho Y-S, Chu S-W, Lien H-J, Liu T-H, Lin J-K. Induction of glutathione depletion, p53 protein accumulation and cellular transformation by tetrachlorohydroquinone, a toxic metabolite of pentachlorophenol. Chem-Biol Interact 105:1–16 (1997).
16. Wang Y-J, Ho Y-S, Jeng J-H, Su H-J, Lee C-C. Different cell death mechanisms and gene expression in human cells induced by pentachlorophenol and its major metabolite, tetrachlorohydroquinone. Chem-Biol Interact 128:173–188 (2000).
17. Ahlborg UG, Lindgren J-E, Mercier M. Metabolism of pentachlorophenol. Arch Toxicol 32:271–281 (1974).
18. Edgerton TR, Moseman RF, Linder RE, Wright LH. Multiresidue method for the determination of chlorinated phenol metabolites in urine. J Chromatogr 170:331–342 (1979).
19. Bauchinger M, Dresp J, Schmid E, Hauf R. Chromosome changes in lymphocytes after occupational exposure to pentachlorophenol (PCP). Mutat Res 102:83–88 (1982).
20. Uhl S, Schmid P, Schlatter C. Pharmacokinetics of pentachlorophenol in man. Arch Toxicol 58:182–186 (1986).
21. Schaeffer HJ, Weber MJ. Mitogen-activated protein kinases: specific messages from ubiquitous messengers. Mol Cell Biol 19:2435–2444 (1999).
22. Yu Z, Matsuoka M, Wispriyono B, Iryo Y, Igisu H. Activation of mitogen-activated protein kinases by tributyltin in CCRF-CEM cells: role of intracellular Ca^{2+}. Toxicol Appl Pharmacol 168:200–207 (2000).
23. Witte I, Juhl U, Butte W. DNA-damaging properties and cytotoxicity in human fibroblasts of tetrachlorohydroquinone, a pentachlorophenol metabolite. Mutat Res 145:71–75 (1985).
24. Ehrlich W. The effect of pentachlorophenol and its metabolite tetrachlorohydroquinone on cell growth and the induction of DNA damage in Chinese hamster ovary cells. Mutat Res 244:299–302 (1990).
25. Dahlhaus M, Almstadt E, Henschke P, Lüttgert S, Appel KE. Oxidative DNA lesions in V79 cells mediated by pentachlorophenol metabolites. Arch Toxicol 70:457–460 (1996).
26. Fischer LJ, Wagner MA, Madhukar BV. Potential involvement of calcium, CaM kinase II, and MAP kinases in PCB-stimulated insulin release from RINm5F cells. Toxicol Appl Pharmacol 159:194–203 (1999).
27. Olivero J, Ganey PE. Role of protein phosphorylation in activation of phospholipase A_2 by the polychlorinated biphenyl mixture Aroclor 1242. Toxicol Appl Pharmacol 163:9–16 (2000).
28. Slim R, Toborek M, Robertson LW, Lehmler HJ, Hennig B. Cellular glutathione status modulates polychlorinated biphenyl-induced stress response and apoptosis in vascular endothelial cells. Toxicol Appl Pharmacol 166:36–42 (2000).
29. Enan E, El-Sabeawy F, Scott M, Overstreet J, Lasley B. Alterations in the growth factor signal transduction pathways and modulators of the cell cycle in endocervical cells from macaques exposed to TCDD. Toxicol Appl Pharmacol 151:283–293 (1998).
30. Hossain A, Tsuchiya S, Minegishi M, Osada M, Ikawa S, Tezuka F, Kaji M, Konno T, Watanabe M, Kikuchi H. The Ah receptor is not involved in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated apoptosis in human leukemic T cell lines. J Biol Chem 273:19853–19858 (1998).
31. Ikegwuonu FI, Christou M, Jefcoate CR. Regulation of cytochrome P4501B1 (CYP1B1) in mouse embryo fibroblast (C3H10T1/2) cells by protein kinase C (PKC). Biochem Pharmacol 57:619–630 (1999).
32. Tessier DM, Matsumura F. Increased ErbB-2 tyrosine kinase activity, MAPK phosphorylation, and cell proliferation in the prostate cancer cell line LNCaP following treatment by select pesticides. Toxicol Sci 60:38–43 (2001).
33. Cuenda A, Rouse J, Doza YN, Meier R, Cohen P, Gallagher TF, Young PR, Lee JC. SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. FEBS Lett 364:229–233 (1995).
34. Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, Van Dyk DE, Pitts WJ, Earl RA, Hobbs F, et al. Identification of a novel inhibitor of mitogen-activated protein kinase kinase. J Biol Chem 273:18623–18632 (1998).
35. Smith RA, Ord MJ. Morphological alterations in the mitochondria of *Amoeba proteus* induced by uncoupling agents. J Cell Sci 37:217–229 (1979).