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Decreased Human Birth Weights After *In Utero* Exposure to PCBs and PCDFs Are Associated with Decreased Placental EGF-Stimulated Receptor Autophosphorylation Capacity

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SUMMARY

Yucheng (oil disease) is a clinical and metabolic syndrome reported in Taiwanese who consumed rice oil contaminated with large amounts of various polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs), including the 2,3,4,7,8- and 1,2,3,4,7,8-PCDF congeners which are similar in structure and toxicity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. A well known characteristic of Yucheng is the marked decrease in birth weights, although the underlying mechanism of this effect is unclear. Placental epidermal growth factor (EGF) receptor binding and autophosphorylation studies were done using tissue samples taken from Yucheng and unexposed control patients. EGF-stimulated receptor autophosphorylation of the human placental EGF receptor in the Yucheng subjects was decreased more than 60% of control levels, 4–5 years after the exposure had occurred. The decrease in EGF receptor phosphorylation was significantly correlated with decrease in birth weights. Nonlinear regression analysis of the ^{125}I-EGF receptor binding data revealed that there were two distinct EGF receptor binding isotherms representing the high affinity-low capacity (HALC) and the low affinity-high capacity (LAHC) binding sites. In contrast to the placental EGF-stimulated phosphorylation data described above, the binding kinetics of the EGF receptor were not significantly altered in the control [HALC site K_d = 0.10 ± 0.02 (SE) nM, B_{max} = 788 ± 255 fmol/mg of protein; LAHC site K_d = 17.4 ± 8.2 nM, B_{max} = 62 ± 32 pmol/mg) compared to the Yucheng subjects (HALC site K_d = 0.11 ± 0.02 nM, B_{max} = 784 ± 305 fmol/mg; LAHC site K_d = 49.5 ± 24.7 nM, B_{max} = 147 ± 80 pmol/mg). GC-MS analysis of placental specimens showed elevated levels of selected PCB and PCDF congeners in the Yucheng compared to control individuals. Total PCB levels were 0.5 ± 0.2 ppb and 20.0 ± 4.8 ppb for the control and Yucheng subjects, respectively. A significant dose-response relationship was observed between the placental EGF receptor phosphorylation levels and the PCB concentrations (total or concentrations of 2,2′,4,4′,5,5′-hexa- and 2,2′3,3′4,4′,5-heptachlorobiphenyls). In contrast, no significant relationship was found between the EGF receptor phosphorylation activity and the 2,3,4,7,8- or 1,2,3,4,7,8-PCDF congeners, which were at nondetectable levels in the control and between 104 and 374 parts per trillion in the Yucheng subjects. In summary, our data reveal that decreased placental EGF receptor phosphorylation capacity is associated with decreased birth weight. Furthermore, PCB tissue concentrations might be a better predictor of effects than are PCDF concentrations.

In 1968 in the western part of Japan and in 1979 in Taichung province of Taiwan, individuals were heavily exposed to high amounts of halogenated aromatic hydrocarbons via the consumption of contaminated rice oil. Rice oil disease is called "Yusho" in Japanese and "Yucheng" in Chinese. Clinical manifestations of rice oil disease include numerous dermatological, hormonal, and metabolic abnormalities that have been extensively described elsewhere (1–3). Investigations showed that the affected individuals had consumed rice oil contaminated with PCBs and thermal degradative products of PCBs, including PCDFs and quaterphenyls (4). Analyses of PCB levels in rice oil specimens and patterns of oil consumption revealed that the affected individuals consumed an average of 1 g of PCBs over a 3- to 9-month period (5). Although the Yucheng incident occurred many years ago, exposed individuals continued to show high levels of these halogenated aromatics in their blood and tissue samples (6). These results may help to explain why many of the offspring of the Yucheng subjects also display symptoms of rice oil disease such as cola-colored skin, swollen eyelids, ocular discharge, and pigmented and deformed nails. In addition, these offspring were notably small for their gestational age (7). The mechanism by which this toxic exposure lowers birth weight is not clear.

The unfortunate poisoning incident in Taiwan provides a

ABBREVIATIONS: PCB, polychlorinated biphenyl; PCDF, polychlorinated dibenzofuran; EGF, epidermal growth factor; PMSF, phenylmethylsulfonyl fluoride; PIPES, 1,4-piperazinediethanesulfonic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; CB, chlorobiphenyl; CDF, chlorodibenzofuran; GC-MS, gas chromatography-mass spectrometry; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.

unique opportunity to study a human population with high exposure to halogenated aromatics. Our studies have focused on EGF receptor in placentas of individuals exposed to contaminated rice oil. EGF is a polypeptide which is a potent mitogen *in vitro*. Stimulation of the membrane-bound EGF receptor leads to the activation of the receptor-associated tyrosine kinase which results in the autophosphorylation of the EGF receptor, as well as other intracellular sequelae involved in signal transduction (8). Despite being very well characterized *in vitro*, little is known about the exact physiological role of this growth factor and its receptor. Studies of intact human placenta as well as isolated placental cell cultures indicate that EGF receptor binding increases with advancing gestational age (9). These findings and the abundant quantity of EGF receptor in human placenta (10) suggest that changes in EGF receptor actions may be related to fetal development.

In this study, we compared data on EGF receptor properties with the birth weights, and placental and blood analyses of selected PCBs and PCDFs taken from control and Yucheng subjects. We report that exposed subjects had significantly reduced human placental EGF-stimulated receptor autophosphorylation, an effect which was significantly correlated to the decrease in birth weights of the Yucheng victims.

Materials and Methods

Chemicals and reagents. Unlabeled culture and receptor grade EGF, nerve growth factor, fibroblast growth factor, and bovine insulin were obtained from Collaborative Research Inc. (Lexington, MA). ^{125}I-labeled receptor grade EGF (specific activity 100–200 μCi/μg) was prepared at Meloy Laboratories (Springfield, VA) by the chloramine T method (11, 12) and purified by Sephadex G-10 column chromatography. Other reagents were of highest reagent grade available and were purchased from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific Co. (Raleigh, NC).

Human specimens. Term placentas were obtained from eight nonexposed control and eight PCB-exposed (Yucheng) patients. All control and Yucheng subjects in this study were nonsmokers and gave birth to offspring at the same hospital in Taiwan. Control subjects were age-matched within 3 years of the exposed subjects. In collaboration with the National Institute of Preventive Medicine in Taiwan, the placentas were collected, quickly frozen, and transported to our laboratory where they were stored at −70° until analysis. Consistent with an earlier report (7), our medical records of these subjects revealed a significant reduction in the birth weights between the unexposed Taiwanese (3.37 ± 0.13 kg) and the Yucheng (2.86 ± 0.07 kg) subjects (see Table 1; $p < 0.02$).

Preparation of human placental plasma membranes. Crude placental plasma membranes were prepared and measured according to the protocol described by Hock and Hollenberg (13). In brief, tissue (50–100 g) was thawed at 4° in a 0.25 M sucrose 25 mM Tris-HCl buffer (pH 7.4). The placental tissue was dissected free of chorion, amnion, large blood vessels, and umbilicum and then minced with scissors. The minced tissue was repetitively rinsed and homogenized in the same buffer (1.0:1.5, w/v) containing the protease inhibitors leupeptin (10 μg/ml), PMSF (10 μg/ml), and aprotinin (50–100 units/ml). Homogenization was carried out in an ice-chilled Waring blender using six 5-sec bursts followed by six 10-sec bursts with a 15-sec pause separation between each burst. All procedures were carried out at 4°. The homogenate was centrifuged at 600 × *g* for 15 min, and the supernatant was filtered through double-layered gauze to remove debris before recentrifugation at 10,000 × *g* for 30 min. The resulting supernatant was centrifuged at 40,000 × *g* for 40 min. The pellet containing plasma membranes was washed with 50 mM Tris-HCl buffer (pH 7.6), recentrifuged at 40,000 × *g* for 30 min, resuspended in the same buffer, and stored at −70° in 0.2-ml aliquots prior to analysis. Protein was determined by the Bradford method (14) with bovine serum albumin as the standard. Freezing of fresh placentas from the University of North Carolina Hospital had no effect on EGF receptor-binding properties.

EGF radioreceptor binding. In order to control for daily experimental variation, placental plasma membranes of control and Yucheng subjects were prepared and incubated under the same experimental conditions. Aliquots of crude plasma membranes (2.5 μg of protein) were incubated in a phosphate-buffered saline buffer (0.25 mM sodium phosphate; 1 g/l bovine serum albumin, 150 mM NaCl, pH 7.4) in 12 × 75 mm polystyrene tubes containing varying concentrations of ^{125}I-EGF (0.02–2 nM) in the presence (nonspecific bound) or absence (total bound) of 3 μM unlabeled EGF, for 50 min at 22°, unless otherwise stated. These incubation conditions were chosen as a result of preliminary studies which demonstrated that the EGF binding values were linear with respect to protein content (0.5–5 μg/tube) and that there were no significant changes in the binding kinetics with respect to the time of incubation within the range of 25–200 min. Incubation of 2.5-μg membranes in phosphate-buffered saline buffer containing 0.1 nM ^{125}I-EGF, along or in the presence of different molar ratios of various unlabeled competitors such as insulin, nerve growth factor, or fibroblast growth factor for 50 min at 22°, confirmed the results of other workers (9, 10, 15), that the EGF receptor binding in human placental preparations was specific. Each incubation was terminated by dilution with ice-cold phosphate-buffered saline buffer. The bound EGF was separated from free EGF on Whatman GF/D glass filters. Following two 3-ml washes using the same buffer, each filter was analyzed for radioactivity by means of a Packard autogamma counter (80% efficiency). Specific binding was calculated as the difference between the total and the nonspecific binding and was expressed as fmol of ^{125}I-EGF/mg of protein.

EGF-stimulated receptor autophosphorylation. Phosphorylation of the EGF receptor was measured using a modified procedure described by Rubin *et al.* (16). Aliquots containing 200 μg of protein of human placental plasma membranes were individually solubilized in a buffer containing 1% Triton X-100, 10% glycerol, and 30 mM PIPES (pH 7.0), and incubated on ice for 30 min. Following centrifugation at 100,000 × *g* for 30 min, the supernatant was preincubated in a phosphorylation buffer (50 mM PIPES, 1 mM $NaHPO_4/NaH_2PO_4$, 3 mM $MgCl_2$, 0.1 mM $MnCl_2$, 0.24 mM Na_2PO_4, pH 7.0) in the presence or absence of 1 μg/ml of EGF, for 40 min at room temperature. The mixture was then cooled on ice prior to incubation with 5–10 μCi of [^{32}P]ATP (Amersham, 5,000 Ci/mmol) and 1 μM unlabeled ATP, for 1 min on ice. The incubation was terminated by addition of SDS-sample buffer (3.0% SDS, 5% glycerol, 10 mM Tris-HCl, pH 7.8, 0.017% bromophenol blue, 3% 2-mercaptoethanol), followed by boiling the resulting mixture for 3 min. The proteins were separated by means of 8% SDS-PAGE, and the resulting gels were stained and then treated with 1 M NaOH for 2 hr at 50° to remove ^{32}P attached to serine and threonine residues. Following base treatment, the gels were neutralized, dried, and exposed to X-ray film. The EGF-stimulated receptor autophosphorylation was quantitated by cutting the appropriate band from the dried SDS-PAGE gels followed by liquid scintillation counting. ^{32}P-labeled liquid scintillation analysis (80% efficiency) of excised 150- to 170-kDa SDS-PAGE bands permitted quantitative comparison between the samples from Yucheng and unexposed subjects.

Kinetic and statistical analyses. The apparent equilibrium dissociation binding constant (K_d) and the apparent binding capacity (B_{max}), expressed as nM and fmol of ^{125}I-EGF/mg of protein, respectively, were calculated using the LIGAND program, which is an iterative nonlinear regression procedure for the best fit of two independent binding sites (17). Using the *F*-ratio test, preliminary analysis consistently indicated that a two-site model rather than a one-site model yielded a better fit for the EGF binding data. Individual saturation binding data were plotted according to the method of Scatchard (18). Differences were considered significant at $p < 0.05$ by using the nonparametric and parametric statistical tests (19).

Analytical procedures for PCB and PCDF congeners. The analytical procedures used to determine blood and placental tissue concentrations of selected PCB and PCDF congeners were essentially similar to those described previously (20–22). The PCBs analyzed were: 2,3,3′,4,4′-penta-CB; 2,2′,4,4′,5-penta-CB; 2,2′,4,4′,5,5′-hexa-CB; 2,2′,3,4,4′,5-hexa-CB; and 2,3,3′,4,4′,5hexa-CB. The PCDFs analyzed were: 2,3,4,6,7-penta-CDF; 1,2,4,7,8-penta-CDF; 2,3,4,7,8-penta-CDF; and 1,2,3,4,7,8-hexa-CDF. These congeners were selected for analysis because they have been detected in blood and tissues of individuals exposed to PCB-contaminated rice oil (23). Whereas the PCB standards were available at the Brehm Laboratory, only relatively small quantities of the PCDFs were available. Consequently, it was necessary to synthesize and purify the required PCDF standards; this was accomplished by palladium acetate-promoted cyclization of the appropriate chlorinated diphenyl ethers (24). The purity of the synthesized PCDF isomers was 99% as determined by GC-MS and by GC-FID, comparing their retention times with previously reported values (20). The purity of the PCB standards used in this study was also > 98%.

The analytical procedures described in this report utilized stable, isotopically labeled internal standards which were added to each sample prior to extraction. The isotopically labeled standards closely parallel the corresponding native PCDF and PCB analytes in terms of their physical and chemical properties and were, therefore, expected to respond to the extraction and preseparation (or cleanup) procedures in the same manner as the native compounds. Three isotopically labeled PCDF internal standards were used in this study: $^{13}C_{12}$-2,3,7,8-tetra-CDF, $^{13}C_{12}$-1,2,3,7,8-penta-CDF, and $^{13}C_{12}$-1,2,3,4,7,8-hexa-CDF. The PCB internal standard was D6-(3,3′,4,4′)-tetrachlorobiphenyl. The latter compound was obtained for KOR Isotopes, while the labeled CDFs were obtained from Cambridge Isotopes (Cambridge, MA). Extraction and cleanup (including high performance liquid chromatography methods) and analytical procedures (GC-MS) of blood and placental samples were accomplished as described previously (20–22). Concentrations of PCBs and PCDFs in blood (10-ml samples) and placental specimens (50 g) were corrected for recoveries of each congener as determined by samples spiked with internal standards. Limits of detection were approximately 0.3 parts per trillion for the PCDF congeners.

The analytical methodology used in analyzing the blood and placental tissue samples employed a 60-m DB-5 capillary GC column. Although this column is the best choice in terms of performance and lifetime for our analyses, it is known that other PCDF isomers of the same chlorinated congener class can coelute with at least one of the isomers observed in the tissues (2,3,4,7,8-penta CDF), making identification in some cases somewhat ambiguous. In order to resolve this problem, several of the extracts found to contain measurable levels of the PCDFs were reanalyzed at the end of this study using a 60-m SP-2330 capillary GC column, capable of resolving the isomer in question. The results obtained with this column were virtually identical to those obtained with the DB-5 column, and no additional PCDF isomers were observed. Using these methods, identification of PCDFs may be considered to be unequivocal.

Results

The effect of human exposure to contaminated rice oil on the human EGF receptor was examined by studying human placental EGF-stimulated receptor autophosphorylation in the control and the Yucheng subjects. In Yucheng subjects compared to the unexposed controls, there was a decrease in the phosphorylation of the EGF receptor (150- to 170-kDa protein bands representing the intact and the proteolytically cleaved EGF receptor) (Fig. 1). Other than the EGF receptor, EGF-stimulated phosphorylation bands were detected at approximately 190 kDa and just above the front. The identity of these bands is not known, but the 190-kDa band could be phosphorylated PDGF receptor which appears to be present in placental tissue (25). Furthermore, PDGF is known to modify phosphorylation rates of the EGF receptor (26), which raises the possibility that EGF might stimulate PDGF receptor phosphorylation. However, the significance of the unknown phosphorylation bands is not clear. Table 1 shows the average EGF-stimulated receptor phosphorylation values from all of the Yucheng and control subjects involved in this study. There was a significant reduction (>50%; $p < 0.001$) in the average amount of placental EGF receptor autophosphorylation in the Yucheng samples (1229 ± 365 dpm) compared to the controls (2764 ± 420 dpm). It was interesting that this decrease strongly correlated with the birth weight reduction seen in the offspring of the exposed mothers (Fig. 2; Pearson $r^2 = 0.73$; $p = 0.003$). In these studies we examined the possibility that the decrease in EGF-stimulated phosphorylation of receptor occurred *in vitro* as a consequence of release of PCBs during the homogenization/solubilization procedure. However, this does not appear to be the case for the following reason. The maximum concentration of PCBs in the incubation would be 100 pM (calculated by PCB concentrations in placenta and concentration of solubilized membranes in the phosphorylation incubation). Addition of 2,2′,4,4′,5,5′-hexa-CB or 2,2′,3,3′,4,4′,5-hepta-CB to solubilized membranes at a concentration of 1 nM had no effect on EGF-stimulated phosphorylation of receptor.

To determine whether the decreased receptor autophosphorylation seen in the Yucheng subjects may be due to the alteration of the EGF receptor-binding characteristics, ^{125}I-EGF radioreceptor binding studies were done on the same plasma membranes that were used for the EGF receptor phosphorylation studies. Representative saturation binding curves (Fig. 3) and Scatchard plots (Fig. 3, *inset*) of the human placental EGF receptor binding from a control (subject a) and two Yucheng (subjects b and c) individuals are presented. The LIGAND computer procedure was used to estimate iteratively the human placental ^{125}I-EGF receptor binding kinetics, and it revealed two displaceable and saturable ^{125}I-EGF binding sites. Fig. 3 illustrates two examples in which Yucheng individuals had greater (subject b) or lower levels of specific EGF binding (subject c) compared to an unexposed control (subject a). There was considerable inter-individual variability between the binding kinetics within the control and the PCB-exposed groups. When the binding kinetics were analyzed for all of the individuals in each group (Table 1), the average high affinity EGF-receptor binding kinetics were found to be similar between the Yucheng (K_d = 0.11 ± 0.02 nM, B_{max} = 784 ± 305 fmol/mg) and control groups (K_d = 0.10 ± 0.02 nM; B_{max} = 788 ± 225 fmol/mg). However, a slight but not significant increase in both the average dissociation constant, (K_d = 49.5 ± 24.7 nM) and receptor concentration (B_{max} = 147 ± 80 pmol/mg) of the low affinity EGF receptor site was found in the Yucheng placentas when compared to the control subjects (K_d = 17.4 ± 8.2 nM; B_{max} = 62 ± 32 pmol/mg). The inter-individual variation in EGF binding properties or stimulation of receptor phosphorylation did not reflect sex differences in the fetuses.

To determine whether the inter-individual variability in ^{125}I-EGF receptor binding kinetics and EGF-stimulated receptor autophosphorylation levels may be due to the degree of exposure to the contaminated rice oil, analyses for selected PCBs and PCDF congeners in maternal blood and placental tissue of some of the control and Yucheng subjects were also performed. GC-MS analysis of maternal blood and placental tissue samples

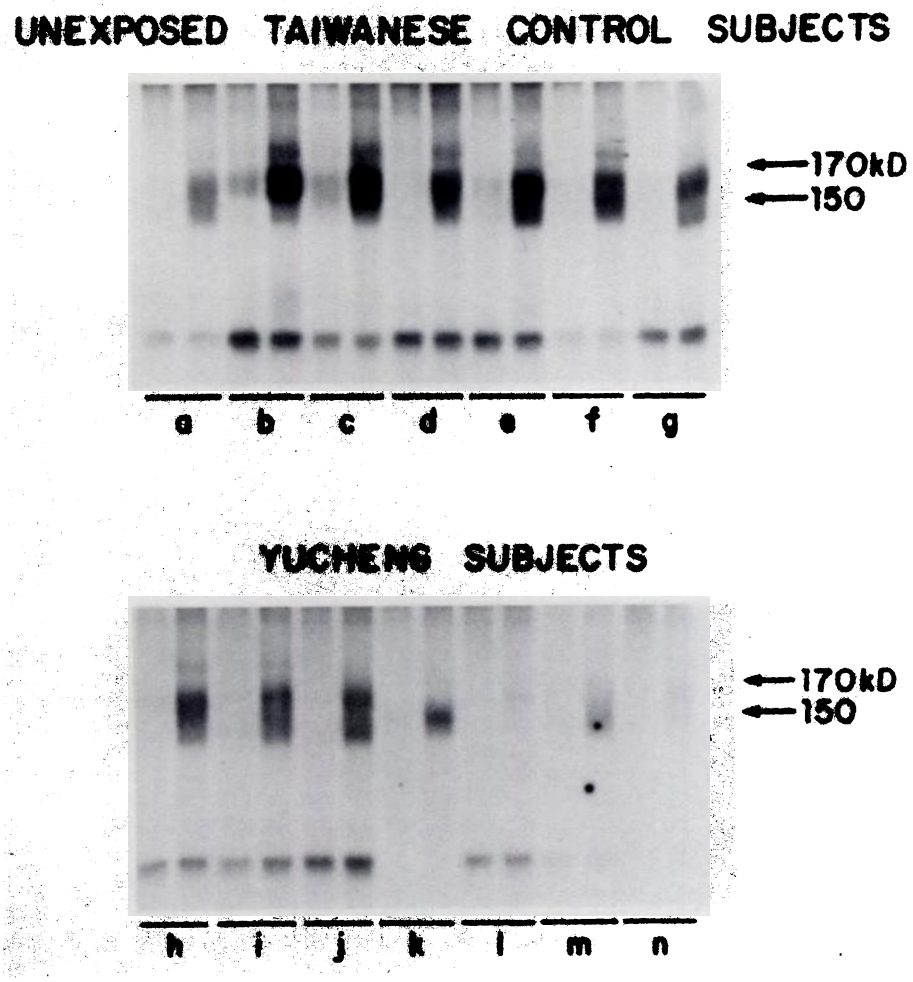

Fig. 1. Autoradiogram illustrating the human placental EGF-stimulated receptor autophosphorylation of seven unexposed control (*top*) and seven Yucheng subjects (*bottom*). EGF-stimulated receptor phosphorylation was measured following the method of Rubin *et al*. (16). Each pair of *lanes* represents a solubilized placental plasma membrane preparation that was incubated with [^{32}P]ATP in the presence (*right lane*) or absence (*left lane*) of EGF and then separated using 8% SDS-PAGE separation. Note the reduction in the phosphorylation of the EGF receptor and its proteolytically cleaved products (150- to 170-kDa bands) between the Yucheng and the control groups. The EGF-stimulated receptor autophosphorylation was quantitated by using liquid scintillation, and it revealed a significant reduction (>60%) in the average amount of placental EGF receptor phosphorylation in all of the Yucheng subjects (control 2764 ± 420 dpm versus Yucheng 1229 ± 365 dpm; $p < 0.03$).

TABLE 1

Summary of birth weights, EGF-stimulated receptor autophosphorylation levels, placental ^{125}I-EGF receptor binding kinetics, and microsomal placental benzo[*a*] pyrene activities of unexposed and Yucheng subjects

Crude placental plasma membranes were prepared and EGF receptor binding kinetics were determined as described under Materials and Methods. The apparent equilibrium dissociation constant, K_d, and the apparent binding capacity, B_{max}, expressed as nM and fmol of ^{125}I-EGF/mg of protein, respectively, were calculated using the LIGAND program (17). Data are presented as the mean ± standard error. The numbers in parentheses denote the number of samples analyzed.

	Birth weight	EGF-stimulated phosphorylation	^{125}I-EGF receptor binding kinetics[a]				Microsomal BPH activities[b]
			High affinity site		Low affinity site		
			K_d	B_{max}	K_d	B_{max}	
	kg	*dpm/150–170-kDa band*	*nM*	*fmol/mg*	*nM*	*pmol/mg*	*pmol/min/mg*
Control	3.37 ± 0.13 (8)	2764 ± 420 (8)	0.10 ± 0.02 (7)	788 ± 225 (8)	17.4 ± 8.2 (8)	62 ± 32 (8)	0.03 ± 0.01 (7)
Yucheng	2.86 ± 0.07[c] (9)	1229 ± 365[c] (7)	0.11 ± 0.02[ns] (5)	784 ± 305[ns] (8)	49.5 ± 24.7[ns] (7)	147 ± 80[ns] (7)	5.09 ± 1.20[c] (9)

[a] In some samples the binding site was nondetectable; in these cases, a B_{max} value of zero was assessed for the samples and the K_d value could not be estimated.
[b] Data taken from Wong *et al*. (7).
[c] Significant difference ($p < 0.05$) compared to unexposed control subjects using the Student's *t*-test and the Wilcoxon rank test (19).
[ns] Not significantly different compared to the control group.

revealed a high concentration of CB and CDF congeners in the Yucheng subjects (Table 2), relative to those present in the tissue samples of the unexposed control subjects. Two toxic PCDFs (2,3,4,7,8-pentachloro- and 1,2,3,4,7,8-hexachloro-) were detected in blood and placental samples of Yucheng subjects but were not detected in controls. In general, placental tissue concentrations of the PCDFs were 25 times greater than those present in the maternal blood.

The total placental PCB quantities were significantly higher in the Yucheng subjects (range of 6.46–30.6 ppb) than in the controls (range of 0.13–1.46 ppb). In the Yucheng group, total blood PCB levels were also analyzed and were found to range from 2.29 to 47.30 ppb, whereas the control values were 0–0.63 ppb (data not shown). Pearson correlation analysis (Table 3) revealed that there was a significant correlation between the placental levels of total PCBs and EGF-stimulated receptor autophosphorylation ($r^2 = 0.80$; $p = 0.01$). Various PCBs were analyzed including (2,3,3′,4,4′-), (2,2′,4,4′,5,5′-), (2,3,3′,4,4′,5-),

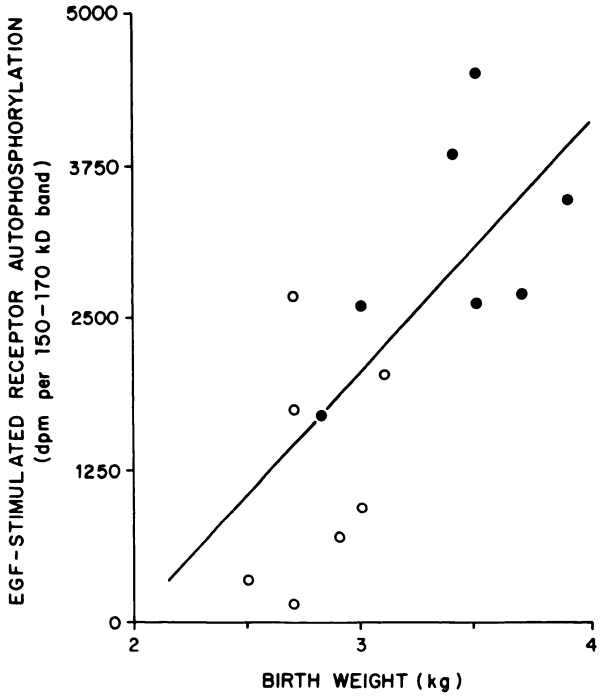

Fig. 2. Association graph between the birth weight and the EGF-stimulated receptor autophosphorylation for the Yucheng (○) and the unexposed control subjects (●). Statistical analysis revealed a significant correlation between the birth weight and the EGF-stimulated receptor autophosphorylation (Pearson $r^2 = 0.73$; $p = 0.003$).

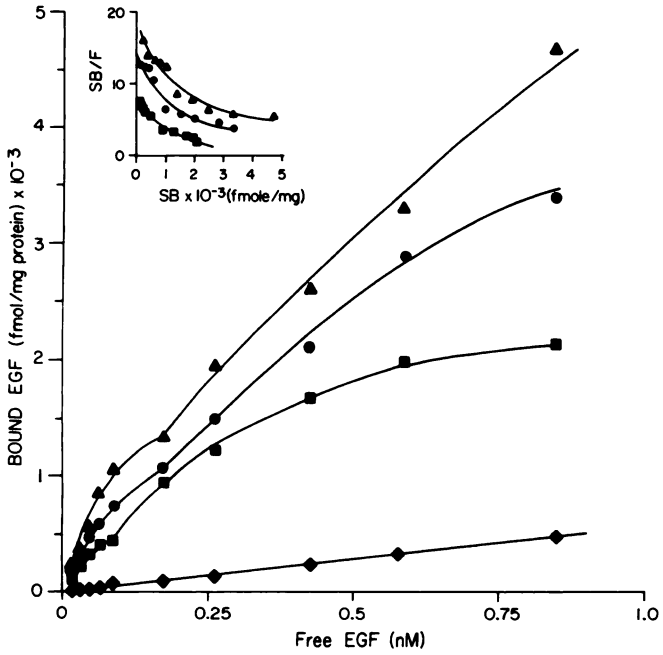

Fig. 3. Representative saturation binding plots of human ^{125}I-EGF binding to placental membranes taken from a control (subject a) unexposed and two Yucheng (subjects b and c) individuals. Membranes were prepared separately and were incubated as described under Materials and Methods. The *inset* shows the Scatchard plot for the binding data. ♦, nonspecific binding for the control subject. Nonlinear regression analysis (17) was used to estimate the EGF receptor binding kinetics. For the unexposed control subject a (●), the EGF receptor binding kinetics were: for the high affinity site, K_d = 0.10 nM, B_{max} = 948 fmol/mg, and for the low affinity site, K_d = 30 nM, B_{max} = 80 pmol/mg. The binding kinetics for Yucheng subject b (▲) were: for the high affinity site, K_d = 0.12 nM, B_{max} = 1472 fmol/mg, and for the low affinity site, K_d = 158 nM; B_{max} = 548 pmol/mg. For Yucheng subject c (■), the binding kinetics for the high affinity site were K_d = 0.02 nM and B_{max} = 100 fmol/mg and for the low affinity site, K_d = 80 nM and B_{max} = 4 pmol/mg.

(2,2′,3,3′,4,4′,5-), and (2,2′,3,4,4′,5,5′-) congeners in placental specimens of the Yucheng and control subjects. GC-MS analysis revealed that, of the different PCB congeners tested, the 2,2′,4,4′5,5′-hexa- and 2,2′3,3′4,4′5-hepta- PCB congeners represented the highest concentrations with respect to total PCBs (37 ± 2% and 10 ± 2%, respectively). It was interesting that the decrease in the placental EGF receptor autophosphorylation levels was significantly associated with placental levels of these two PCB congeners.

Figure 4 illustrates the inverse association between increased levels of total PCB or 2,2′,4,4′,5,5′-PCB and decreased levels of placental EGF-stimulated receptor autophosphorylation. A marginally significant inverse relationship between placental total PCB concentrations and birth weights ($r^2 = 0.58$; $p < 0.08$) was also found and may reflect the association between total PCB and EGF receptor phosphorylation levels.

In contrast, there were no significant correlations between the ^{125}I-EGF receptor binding kinetics and the EGF-stimulated receptor autophosphorylation levels of the Yucheng and the control individuals. No significant relationship ($p > 0.10$) was observed when placental PCDF concentrations were compared to birth weights, microsomal benzo[*a*]pyrene hydroxylase activities (a placental marker of human exposure to toxic polycyclic aromatics; Ref. 7), the ^{125}I-EGF receptor binding kinetics, or EGF receptor phosphorylation levels. Taken together, these data suggest that total PCB concentrations may be a better indicator of effects of exposure to contaminated rice oil on placental EGF-stimulated receptor autophosphorylation and birth weights than PCDF concentrations.

Discussion

In this paper, we report that human exposure to contaminated rice oil containing large amounts of PCBs and PCDFs significantly depressed the autophosphorylation capacity of the EGF receptor in the human placenta. The reduction in EGF-stimulated receptor autophosphorylation was detected in placental samples 4–5 years after the exposure had occurred, which reflects the biological persistence of many of the PCBs and PCDFs present in the rice oil (6). EGF receptor phosphorylation was significantly correlated with birth weights and with the placental concentration of total and two PCB congeners (2,2′,4,4′,5,5′-hexa- and 2,2′,3,3′,4,4′,5-hepta-CBs). However, EFG receptor phosphorylation was not correlated with the ^{125}I-EGF binding kinetics or the placental concentration of PCDF or other PCB congeners.

Our data suggest that PCBs in the contaminated rice oil may be responsible for diminished autophosphorylation of the EGF receptor. Since the physiological role of the EGF receptor in fetal development is not well defined, it is difficult to determine whether the decrease in placental EGF receptor autophosphorylation has a causative role in reduction of birth weight found with Yucheng subjects. The mechanism by which exposure to a complex mixture of chemicals, as found in contaminated rice oil, results in alterations in EGF receptor-kinase activity is also not clear. Numerous structurally diverse chemicals have been shown to modify the EGF receptor. For example, TCDD, a halogenated aromatic hydrocarbon, decreases EGF receptor binding in rat liver (27) and in a cultured human keratinocyte cell line (28). TCDD as well as other polycyclic aromatic

TABLE 2

Human placental EGF-stimulated receptor autophosphorylation, concentrations of PCDFs and PCBs, and microsomal benzo(a)pyrene hydroxylase in some of the Yucheng subjects

The limit of detection of the dibenzofurans and PCBs was approximately 0.3 ppt using the GC-MS analysis described under Materials and Methods.

Subject ID	Tissue	Dibenzofurans congeners		Total PCBs	PCB congeners		EGF-stimulated phosphorylation	Benzo(a)pyrene hydroxylase activities[a]
		2,3,4,7,8-	1,2,3,4,7,8-		2,2′,4,4′,5,5′-	2,2′,3,3′,4,4′,5-		
		ppb		ppb	ppb		dpm/150–170-kDa band	pmol/min/mg
l	Blood	0.004	0.013	47.30	2.0	ND[b]		
	Placenta	0.107	0.312	19.98	5.6	1.5	148	4.90
h	Blood	0.002	0.011	14.86	5.2	2.3		
	Placenta	0.104	0.374	12.38	4.5	1.9	1753	5.70
i	Blood	0.003	0.008	2.29	0.7	0.4		
	Placenta	0.147	0.356	6.48	2.6	1.0	2679	6.80
o	Blood	0.020	0.050	25.41	9.1	3.1		
	Placenta	0.117	0.305	30.60	13.7	3.4	360	1.10

[a] Data taken from Wong *et al.* (7).
[b] ND, not detectable.

TABLE 3

Summary of Pearson rank correlation table[a]

		BW[b]	EGF-R phosphorylation	^{125}I-EGF-R binding kinetics				BPH
				HA site		LA site		
				K_d	B_{max}	K_d	B_{max}	
	BPH	0.05	0.09					
HA	K_d	0.75	0.77					0.92
	B_{max}	0.41	0.55	0.0001				0.52
LA	K_d	0.53	0.09	0.06	0.0001			0.13
	B_{max}	0.34	0.17	0.14	0.03	0.0001		0.15
DBFs	2,3,4,7,8-	0.94	0.33	NA[c]	0.61	0.68	0.80	0.58
	1,2,3,4,7,8-	0.03	0.16	NA	0.10	0.11	0.18	0.73
PCBs	2,2′,4,4′,5,5′-	0.08	0.03	0.41	0.82	0.08	0.06	0.35
	2,3,3′,4,4′,5-	0.16	0.03	0.39	0.58	0.49	0.41	0.19
	Total PCBs	0.08	0.01	0.32	0.92	0.12	0.12	0.23

[a] Data are expressed as p values.
[b] Abbreviations used are: EGF-R, epidermal growth factor receptor; HA and LA, high and low affinity sites, respectively; BW, birth weight; K_d and B_{max}, apparent equilibrium dissociation constant and binding capacity, respectively; BPH, placental microsomal benzo(a)pyrene hydroxylase activities; DBF, dibenzofurans.
[c] NA, Not analyzed due to insufficient number of samples.

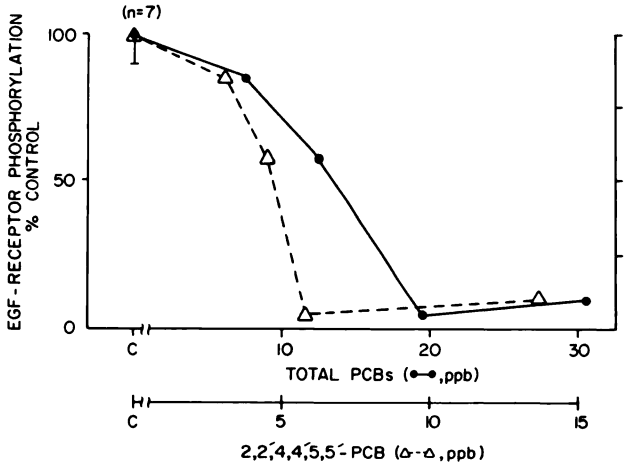

Fig. 4. Inverse dose-response relationship between placental levels of total PCBs (●) or 2,2′,4,4′,5,5′-hexa-CB (Δ) and placental EGF-stimulated receptor autophosphorylation levels in seven control and four Yucheng subjects. EGF receptor phosphorylation data of the Yucheng subjects are expressed as a percentage of the mean for control values (see legend of Fig. 1).

hydrocarbons interact with a specific cellular receptor known as the Ah receptor (29). In fact, like TCDD, some of the PCB congeners and the two PCDF congeners detected in placentas of exposed individuals also interact with the Ah receptor (29). Hudson *et al.* (28) suggest that the reduction in the high affinity EGF receptor concentration by TCDD is mediated by the interaction with the cellular Ah receptor, and a similar mechanism may be involved in our findings. It was interesting that the decrease in EGF-stimulated receptor autophosphorylation levels was correlated with the concentrations of total PCBs that have a low affinity for the Ah receptor, and not with the PCDFs (both 2,3,7,8- and 2,3,4,7,8- congeners), which have much higher binding affinities for the Ah receptor. These data suggest that the reduction in the placental EGF receptor phosphorylation levels in the Yucheng subjects may possibly involve a mechanism other than the Ah receptor system. Another possible explanation could be related to greater daily variations in the tissue-to-blood concentration ratios of PCDFs compared to PCBs. PCBs are sequestered primarily in adipose tissue, whereas PCDFs are sequestered in both liver and adipose tissue. Dietary or physiological factors that influence release of PCDFs from liver may produce transient variation in blood as well as placental PCDF concentrations which may contribute to a lack

of correlation between PCDF concentrations and EGF receptor phosphorylation capacity.

Another possible mechanism may involve the activation of protein kinase C. Lee and Weinstein (30) report that a tumor-promoting agent, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) reduced EGF receptor binding in HeLa cell cultures. This effect of TPA is not mediated through the Ah receptor but is caused by the stimulation of protein kinase C, resulting in the phosphorylation of the EGF receptor and the reduction in EGF binding and tyrosine kinase activity (31). Perhaps one or more of the many compounds in the contaminated rice oil may alter the EGF receptor phosphorylation by a mechanism similar to those of TPA (direct activation of protein kinase C) or TCDD (via the Ah receptor system). However, in contrast to the effects of TPA and TCDD, our data suggest that a decrease in placental EGF-stimulated receptor autophosphorylation in the Yucheng subjects is not accompanied by an alteration in the EGF receptor density or this receptor's binding affinity. Another possibility is that toxic chemical exposure may result in nonspecific changes in membrane structure or in membrane-associated kinases and phosphatases which may modify EGF receptor function. Although the exact mechanism(s) of action is not clear, modification of the placental EGF receptor upon exposure to contaminated rice oil may reflect impaired growth factor-dependent biologic functions that occur in the fetus. Therefore, changes in placental EGF functions may provide a reliable marker for lowered birth weights in individuals exposed to PCBs and PCDFs. A better understanding of the mechanisms responsible for the effects of the halogenated aromatic hydrocarbons on the EGF receptor of placental and fetal tissues may provide greater insight into the susceptibility of the developing fetus to toxic halogenated aromatics.

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