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Attenuation of polychlorinated biphenyl induced uroporphyria by iron deprivation

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Abstract

A toxic sequel to polyhalogenated aromatic hydrocarbon exposure in humans is the development of porphyria cutanea tarda. In a mouse model (experimental uroporphyria) utilizing an environmentally relevant polychlorinated biphenyl (PCB) mixture, we show that the toxicity can be markedly influenced by nutritional status. In mice made susceptible to uroporphyria through a targeted deletion of one allele of uroporphyrinogen decarboxylase (*Uro-D*+/−), an iron deficient diet prevented the development of the uroporphyria and the changes in associated parameters normally seen within three weeks following a single exposure to Aroclor 1254. Iron deprivation also completely prevented PCB-induced uroporphyria in mice wild-type at the *Uro-D* locus (*Uro-D*+/+), a model that requires δ-aminolevulinic acid administration for the development of uroporphyria. In *Uro-D*+/− mice consuming δ-aminolevulinic acid, PCB exposure produced a severe uroporphyria that was attenuated, not prevented, by iron deficiency. This attenuation moderated hepatic uroporphyrin and uroporphyrinogen decarboxylase inhibitor levels, but not the depression of cytosolic uroporphyrinogen decarboxylase activity.

Keywords: Uroporphyria attenuation; Iron deficiency; Polychlorinated biphenyls; Uroporphyrinogen decarboxylase; Mouse model

1. Introduction

An incident over 40 years ago established a definitive link between polychlorinated hydrocarbon exposure and the development of porphyria cutanea tarda (PCT) (Schmid, 1960), a disease characterized by hepatic and skin uroporphyrin accumulation and high uroporphyrin levels in the urine. Over 3000 cases of PCT occurred in Turkey following the ingestion of wheat treated with the fungicide hexachlorobenzene. In some families in which all members had consumed hexachlorobenzene, some family members did not develop PCT. The reason for variability in disease expression can now only be a matter for conjecture, but micronutrient status, particularly iron deficiency, was not explored. A possible link with iron deficiency arises from two key observations. The number of females that developed PCT was much lower than the number of males, and the 1:3 female to male ratio in pre-menache and post-menopause age groups dropped to 1:7 in the 15–39-year-age group (Cam and Nigogosynan, 1963).

Since the original observations with hexachlorobenzene in humans, studies in animals followed that showed that a variety of additional polyhalogenated hydrocarbons, including the environmental contaminants TCDD (2,3,7,8-tetrachlorodibenzo-(*p*)-dioxin) (Goldstein et al., 1973) and other chlorinated dibenzodioxins (Goldstein et al., 1977) and polychlorinated biphenyls (PCBs) (Goldstein et al., 1974) can produce the same toxic manifestation. Considerable evidence from our studies, and those of others, supports the concept that experimental uroporphyria is associated with the generation of an inhibitor of uroporphyrin decarboxylase enzyme in the heme biosynthetic pathway (Rios de Molina et al., 1980; Cantoni et al., 1984; Smith and Francis, 1987; Smith et al., 1990). Animal studies have been used in an attempt to define the factors responsible for the development of the uropor-

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phyria and the generation of the uroporphyrin decarboxylase inhibitor especially since in humans there is a remarkably incomplete association between exposure to recognized risk factors and disease development (Bulaj et al., 2000). Underlying nutritional status is often low on the list of possible modifiers that influence responses to exposure to a drug or toxin across a population.

Effective treatment for PCT once it is clinically recognized is to reduce hepatic iron concentrations by multiple phlebotomies. Iron depletion is thought to reduce the damaging oxidant properties associated with high iron concentrations in tissues. It may be that the initial development of experimental uroporphyria may also depend upon the hepatic iron concentration at the time of exposure to a porphyrinogenic toxin. Only a few animal studies have been performed that directly compare or relate constitutive or subconstitutive hepatic iron concentration to polychlorinated aromatic hydrocarbon-induced experimental uroporphyria. In two rat strains, hexachlorobenzene-induced uroporphyria developed at a slower rate and to a lesser extent in the strain with a constitutively lower hepatic non-heme iron levels (Smith et al., 1979). Tri-weekly treatment of rats with the iron chelator deferoxamine delayed and suppressed hexachlorobenzene-induced uroporphyria such that after 12–14 weeks, the hepatic porphyrin concentration was only 20% of that in animals not co-treated with deferoxamine (Wainstock de Calmanovici et al., 1986). In mice, a low iron, albeit synthetic, diet following multiple phlebotomies prevented the experimental uroporphyria seen after 15 weekly TCDD injections in iron replete mice ingesting a standard laboratory diet (Sweeney et al., 1979; Jones et al., 1981).

According to the WHO (http://www.who.int/nut/ida.htm), iron deficiency is the most common nutritional deficiency worldwide, so influences on toxicity manifestation are likely to be quite common. With the recent development of a genetically modified mouse that models the familial form of PCT and is exquisitely sensitive to the development of experimental uroporphyria (Phillips et al., 2001), we examined the influence of a low iron diet from weaning in a three week model following a single exposure to PCBs.

2. Methods

2.1. Animals and treatment

Mice (C57BL/6J) with the *Uro-D* +/− genotype were generated as previously described (Phillips et al., 2001). Briefly, embryonic stem cells were electroporated with a linearized targeting vector in which the neomycin resistance gene replaced exon 6 of the *Uro-D* gene. Cells that were resistant to G418 were screened for integration at one of the chromosomal *Uro-D* alleles using Southern blotting. A clone containing a correctly targeted integration was injected into a C57BL/6J blastocyst that was subsequently implanted into a foster mother. Chimeric animals were identified by coat color and mated to C57BL/6J mice to produce animals heterozygous at the *Uro-D* locus. Heterozygous mice were mated and the progeny were screened for homozygosity for a null *Uro-D* allele. No homozygous-null animals were identified as early as embryonic day 7.5 (Phillips et al., 2001).

Iron deficient mice were generated by maintenance from weaning (21 days) on a low-iron purified diet (Teklad #80396, 2–5 ppm iron), while control animals were maintained on a standard laboratory rodent diet (3080) containing 300 ppm iron. Experimental treatments were given to animals older than 10 weeks post-weaning. Animal treatment consisted of a single injection of Aroclor 1254 (Monsanto Co., St. Louis, MO) (4 mg in 0.4 ml corn oil i.p.) and when additionally required, drinking water supplemented with δ-aminolevulinic acid (ALA, 2 mg/ml) neutralized to pH 7 was provided. This drinking solution was replaced every 5 days. Analysis indicated that the ALA concentration was stable at neutral pH and room temperature for this time period. All procedures involving animals were approved by the University of Utah Animal Care and Use Committee and were in concordance with NIH guidelines for the humane care of laboratory animals.

2.2. Tissue preparation and assays

After 21 days animals were sacrificed and the livers removed. A small portion of the liver was immediately frozen for subsequent total hepatic porphyrin and iron analysis (Franklin et al., 2001; Phillips et al., 2001). After removal of the gall bladder, the remaining liver was homogenized in four volumes of unbuffered 0.25 M sucrose. A cytosolic fraction was prepared from the homogenate with successive centrifugations of $9000 \times g$ (15 min), $19{,}000 \times g$ (15 min) and $105{,}000 \times g$ (60 min). The cytosol and microsomal protein concentrations were determined using Folin-Ciocalteu's phenol reagent (Lowry et al., 1951). Native uroporphyrinogen decarboxylase (UROD) activity (Phillips and Kushner, 1999) was assayed in the cytosol and ethoxyresorufin O-deethylase activity was determined in the microsomal fraction (Klotz et al., 1984). In addition to determination of native cytosolic UROD activity, an aliquot of $105{,}000 \times g$ supernatant fraction (equivalent to 15 μl of cytoplasm) was denatured in boiling water for 5 min, clarified by centrifugation and assayed for inhibitory activity against 20 ng of recombinant human uroporphyrinogen decarboxylase (rhUROD). Final incubation concentrations in the cytosol inhibitor assay were 90 ng rhUROD protein/ml and 30 μM substrate, uroporphyrinogen I.

2.3. Statistics

Statistical analyses were performed using ANOVA, followed by Fisher's protected least significant difference multiple range test. Differences were considered significant at p-values of <0.05.