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Lead and its Effects on Cytochromes P450

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Abstract

Background: The adverse effects of lead on heme biosynthesis and production of anemia are well known. Lead may disrupt the normal activity of other hemoproteins, such as cytochromes P450 (CYP) which are responsible for drug metabolism. While data in animals suggest a decrease in CYP function with lead exposure, there is little data in humans.

Methods: After IRB approval, consented patients with asymptomatic, low to moderate level (10 mg/dl to 44 mg/dl) lead poisoning were assessed for *CYP1A2* and *CYP2D6* activity at diagnosis, 1 month, 2 months and after lead level was less than 10 mg/dl. Low doses of caffeine (*CYP1A2* activity) and dextromethorphan (DM) (*CYP2D6* activity) were administered and urine collected for 12-24 hours at each visit. Lead levels, free erythrocyte protoporphyrin, and iron studies were performed as well. A mixed linear model was used to analyze the data. *CYP2D6* genotyping was performed using PCR techniques.

Results: Eleven out of the sixteen eligible children (7 males, 4 Caucasian, average age: 33 months) completed the study. Average time to complete the study was 20 months. Seven had lead levels between 10-25 mcg/dl. Eight had elevated EPP. None had low iron levels. A random intercepts mixed linear model was fit to the data. No statistical significance was found for *CYP1A2* or *CYP2D6* metabolic ratio related to the lead level, change in lead level or time.

Discussion: Lead was found to have no effect on the CYP drug metabolizing enzymes at low blood lead levels. Lead has been shown to effect multiple sites on the hemoglobin synthesis pathway resulting in elevated EPP at lead levels >10 mg/dl. However, threshold for decreased hemoglobin levels in children is judged to be approximately 40 µg/dL. Thus, cytochromes may not be affected until higher lead levels are seen. The size of the study limits generalizability.

Conclusion: Based on this study, no changes in drug treatment for children with low blood lead levels are necessary. However, further studies may be needed to assess the effect from higher blood lead levels.

Keywords: Cytochromes P450; Central nervous system; Blood lead levels

Background

Despite the knowledge of clinical effects of lead, there has been a recent resurgence in concerns relative to its biochemical toxicity. Absorption of ingested lead from water, food, or lead-contaminated dust is the major source of exposures in non-occupationally exposed individuals. Following absorption, lead is distributed to two main compartments; the readily exchangeable "pools" represented by blood and soft tissue (including the brain) and a "deep tissue reservoir" represented by bone [1]. Over 95% of blood lead is contained within the erythrocytes [2]. While acute lead exposure (e.g. that seen after ingestion of metallic lead) is limited in distribution to the aforementioned readily exchangeable pools, chronic, persistent, low-level exposure; such as that associated with non-occupational exposure, will result in accumulation of lead. This, in turn, has the potential for producing chronic, subclinical lead poisoning capable of disrupting normal protein synthesis (e.g., heme) and altering cognitive and/or neurobehavioral function.

Only a small fraction of children with chronic lead poisoning are thought to exhibit clinically discernable signs and symptoms suggestive of toxicity. The gastrointestinal tract and the central nervous system (CNS) are the most likely systems to be involved. Children may exhibit abdominal pain, anorexia, nausea, vomiting, and constipation with blood lead levels as low as 20 mcg/dl. CNS disturbances (e.g., hyperactivity, behavioral disorders), hearing loss and cognitive impairment may be seen in association with blood lead levels less than 7.5 mcg/dl [3]. In association with high (i.e., >100 mcg/dl) blood levels, children may exhibit signs of increased intracranial pressure, seizures, and coma (e.g., lead-encephalopathy). The clinical effects are a result of disruption in normal cellular intermediary metabolism including inhibition of mitochondrial oxidative phosphorylation and impaired membrane transport consequent to alterations of sodium, potassium, and calcium ATPases [4]. Lead also alters calcium metabolism by impairing intracellular messenger actions [5].

The cytochromes P450 represent a superfamily of heme-containing proteins that catalyze the biotransformation of many lipophilic endogenous substances (e.g., steroids, fatty acids, fat-soluble vitamins, prostaglandins, leukotrienes, and thromboxanes) and exogenous compounds (e.g., drugs, pesticides, and environmental contaminants). In humans, 57 active *CYP* genes have been found which are divided into 18 families [6]. P450s important in human drug metabolism are

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predominantly found in the *CYP1*, *CYP2*, and *CYP3* gene families. A given compound may be primarily dependent on one particular P450 for its metabolism or be biotransformed to several metabolites by multiple, distinct P450 isoforms. For xenobiotics that are metabolized by *CYP450*s, the rate(s) of a given compound's metabolic clearance is a function of the individual's unique phenotype with respect to the forms and amounts of enzymes expressed [7]. Because the cytochromes P450 are the major hemoproteins contained in the liver, it is reasonable to suspect that inhibition of heme biosynthesis could influence the function of these enzymes.

The potential for lead to modulate the activity of cytochromes P450 is reflected by previous animal studies [8-10]. While data in animals suggest a decrease in *CYP* function with lead exposure, there are little data in humans. Few human studies have evaluated the potential impact of lead on the activity of cytochromes P450. This was indirectly demonstrated in one investigation [11], by determining the elimination half-life of antipyrine (a polyfunctional *CYP450* pharmacologic probe compound) in eight patients with chronic exposure to lead before and after a course of chelation therapy. However, lead removal via chelation therapy did not significantly shorten the average antipyrine half-life. The same study documented P450 activity in mice with acute and chronic lead exposure. The mice with the acute lead exposure were found to have a significant reduction in P450 activity without a demonstrable alteration in the amount of hepatic P450. The effect of chronic lead exposure in mice was not significant. Thus, in our study, we sought to examine the ability of lead to alter the activity of two important human drug metabolizing enzymes, *CYP1A2* and *CYP2D6*.

Methods

After obtaining IRB approval, patients with asymptomatic, low to intermediate level (10-44 mcg/dl) lead poisoning were recruited by informed parent permission and, if appropriate, by patient assent. A complete physical examination and medical history was performed to determine the likely source of lead exposure, presence of physical stigmata of plumbism and determination of general physical condition. On admission to the study and at every study visit, each subject was evaluated for blood lead levels, free erythrocyte protoporphyrin (EPP), and iron stores.

CYP2D6 genotyping

Blood was obtained at admission for isolation of genomic DNA. The DNA samples were subsequently genotyped for *CYP2D6* using previously established PCR-RFLP methods [12]. The following 16 allelic variants were included in the analysis: (*CYP2D6**2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *17, *29, *40, *41, *45, and *46, as well as *1×2, *2×2 and *4×2 gene duplications. Additional information can be found at the *CYP2D6* nomenclature web page (http://www.cypalleles.ki.se/).

CYP1A2 and *CYP2D6 in vivo* phenotyping

Subjects were assessed for *CYP1A2* and *CYP2D6* activity at diagnosis, 1 month and 2 months after enrollment, and after blood lead were less than 10 mg/dl using a protocol previously described [13]. Each subject was given a single oral dose of caffeine (CAF) and dextromethorphan (DM) (3.5 mg/kg and 0.3 mg/kg, respectively) followed by 90 to 120 milliliters of tap water and/or clear fruit juice. Subjects were permitted to consume their normal, age-appropriate diet around the time of the study drug administration and through all sample collection periods. Spontaneously voided urine was collected for 12-24 hr after administration of CAF and DM. For infants who were not toilet trained, a special fiber-based diaper (Tushies®, Tender Care®) proven suitable for urine collection and reliable for the quantitation of DM and CAF was used. Urinary caffeine and its metabolites (*CYP1A2*; Figure 1) and urinary dextromethorphan and its metabolites (*CYP2D6*; Figure 2) were determined by HPLC. *CYP2D6* activity was assessed by the metabolic ratio of DM/DX whereas *CYP1A2* activity was assessed by the metabolic ratio of AFMU+1X+1U/ 1, 7U as previously described [14].

Statistics

A random intercepts mixed linear model was fit to the data. The urinary metabolic ratio for *CYP1A2* and *CYP2D6* was examined in association with change in blood lead level from baseline and longitudinally over time. The significance limit accepted for all statistical analyses was α=0.05.

Results

Thirty children were enrolled in the study. Fourteen failed screening, and 11 out of the 16 remaining eligible children completed the study over an average time of 20 months (SD=12.5 months). Initial blood lead levels ranged from 16-32 mcg/dl (Mean 22 ± 5.2 mcg/dl). Eight of the 11 participants completing the study had elevated EPP and none had a lower serum iron level.

Ten subjects had a *CYP2D6* genotype predictive of an extensive metabolizer phenotype while one had a genotype that predicted ultra-rapid metabolism (Table 1). We also assigned activity scores to each *CYP2D6* genotype (Table 1) which 'facilitates' phenotype prediction from genotype data [15] and provides an additional covariate which to use in the evaluation of lead effects on *CYP2D6* activity.

No statistically significant associations were found for either the *CYP1A2* or *CYP2D6* urinary metabolic ratio examined as a function of either change in blood lead level from baseline (p=0.44 and p=0.69, respectively) or subject age. Likewise, no statistical significance was

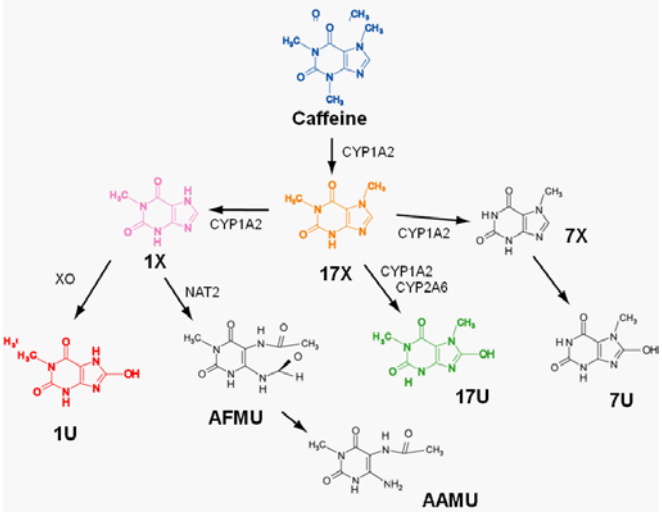

Figure 1: Caffeine biotransformation pathway. Caffeine metabolism is largely dependent on the cytochromes P450 in the adult. However, these enzymes are not fully developed in the infant and young child. *CYP1A2* indicates cytochrome P4501A2; *CYP2A6*, cytochrome P4502A6; XO, xanthine oxidase; NAT-2, N-acetyltransferase 2; 1X, methylxanthine; 7X, 7-methylxanthine; 17X, 1,7-dimethylxanthine, 1U, 1-methyluric acid; AMFU, 5-acetylamino-6-formylamino-3-methyluracil; 17U; 1,7- dimethyluric acid; 7U, 7-methyluric acid; AAMU, 5-acetylamino-6-amino-3-methyluracil.

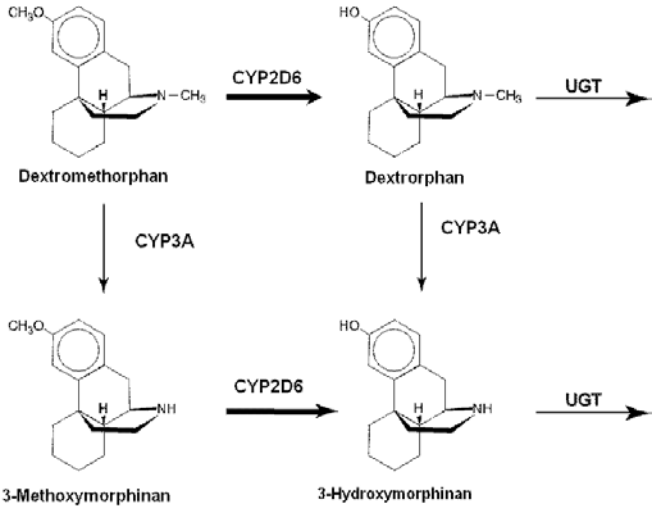

Figure 2: Dextromethorphan biotransformation pathway. Dextromethorphan metabolism is largely dependent on the cytochromes P450 2D6 and 3A4 in children and adults. The cytochromes P450 2D6 is fully developed by one month of age. *CYP2D6* indicates cytochrome P4502D6; *CYP3A4*, cytochrome P4503A4; UGT; glucuronosyltransferase.

found for the association of either *CYP2D6* or *CYP1A2* and time (Figures 3 and 4).

Discussion

The potential for a detrimental interaction between lead and the cytochromes P450 is reflected by the previous work in animal studies [8-10]. Goldberg et al. [8] injected mice intraperitoneally with lead acetate over a period of 14 days. The quantity of P450 and activities of two cytochrome P450 dependent microsomal enzymes were determined. As the lead exposure to the animals increased, progressively decreased microsomal content and activity were documented. A similar study [9] was performed in which mice were, again, injected intraperitoneally with lead acetate, and P450 activity was measured 20 hours after injection. In this study, P450 activity was reduced to 50-60% of control levels within 24 hours after injection. However, the study showed that a decrease in P450 activity may have been, in part, due to inhibition of P450 gene transcription that is unrelated to the effects of lead on heme biosynthesis. Degawa et al. [10] evaluated the effects of lead on the expression of *CYP1A2*. They found that lead had an inhibitory effect on the microsomal activity for *CYP1A2*-dependent biotransformation of aromatic aminies, as well as on the expression of both mRNA and protein of the enzyme in the rat liver.

Few human studies have evaluated the potential impact on the activities of cytochromes P450. In addition to the study previously described [11], only a handful of studies have further attempted to elucidate the effect of lead on *CYP450* activity [16-20]. In two children with clinical as well as biochemical manifestations of acute lead intoxication, antipyrine half-lives were significantly longer than normal, and chelation therapy led to restitution toward normal [16]. Saenger et al. [17] evaluated the inhibitory effects of lead on the metabolism (*CYP3A4* pathway) of cortisol to 6-beta-hydroxycortisol in children with mild to moderate increases in blood lead concentrations and found decreased urinary excretion of 6-beta-hydroxycortisol in those children with elevated urinary lead excretion.

In this study, the activities of *CYP1A2* and *CYP2D6* were not altered from baseline as lead levels decreased in children with low blood lead levels. Evidence of biochemical lead toxicity and disruption of normal heme biosynthesis reflected by elevated EPP values at blood lead levels >10 mg/dl may not be generalized as relates to the function of other critical hemoproteins which are known to be altered in lead intoxication [21].

As reflected by the data illustrated in Figure 4, there was a non-significant trend for *CYP1A2* activity to increase over time in the majority (i.e., 10 of 11 subjects), which was independent of the blood lead concentration. This increase in *CYP1A2* activity was, however, expected based upon the known patterns of ontogeny for the maturation of this particular enzyme in humans. *CYP1A* is not present in fetal and newborn tissues and is the last isoform to be expressed in the human liver [22]. It begins to increase when infants are 3-6 months of age and reaches 50% of adult activity by one year of age [23]. Assessing clearance of the *CYP1A2* substrate theophylline by measuring metabolite ratios over time suggests that full maturation of *CYP1A2* activity occurs by 3 years of age [24]. Other environmental factors, such as cigarette smoking, can increase *CYP1A2* activity [25]. These factors or the potential effects of concomitantly ingested foods or drugs were not evaluated in the present study given their inherent variability with respect to exposure and the relatively small study

Subject	Age (months)	Sex	Race	Duration (months)	Lead Level*	EPP*	Iron*	*CYP2D6* genotype	*CYP2D6* AS**
M01	16	M	C	44	17	13	52	*2×2*2	3
M03	84	F	AA	39	26	126	29	*1*9	1.5
M06	16	F	AA	14	22	38	88	*1*2	2
M08	21	M	H	11	17	38	81	*1*4×2	1
M09	27	M	C	18	26	68	150	*1*5	1
M12	48	F	AA	28	17	18	114	*1*29	1.5
M13	15	M	C	28	32	138	49	*1*2	2
M15	48	F	H	15	27	40	78	*2*41	1.5
M21	17	M	AA	9	16	38	78	*1*41	1.5
M24	18	M	C	11	22	5	113	*2*45 or46	1.5
M28	48	M	AA	8	21	39	114	*1*5	1

Table 1: Demographics of subjects who completed study. *Blood concentrations noted at time of enrollment. Mean lead level 22.1 ± 5.2 mcg/dl. Mean EPP 51 ± 43.5 mcg/dl. Mean iron level 86 ± 35 mcg/dl. EPP indicates erythrocyte protoporphyrin; *CYP2D6*, Cytochromes P450 2D6; C: Caucasian; AA: African American; H: Hispanic; AS: activity score. **AS is a value is assigned to each allele according to its activity. The sum of values for both alleles is the AS. The value of the AS has a predictive phenotype. (AS 0=Poor metabolizer, AS 0.5=Intermediate metabolizer, AS 1-2=Extensive metabolizer, and AS >2=Ultra metabolizer).

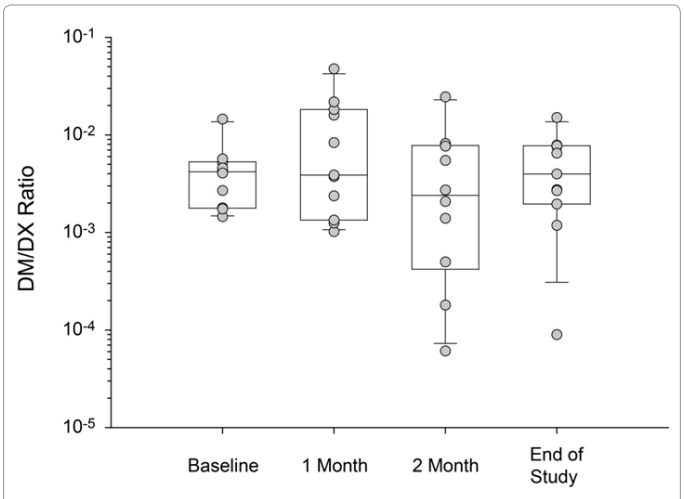

Figure 3: *CYP2D6* activity change for each subject over duration of study.

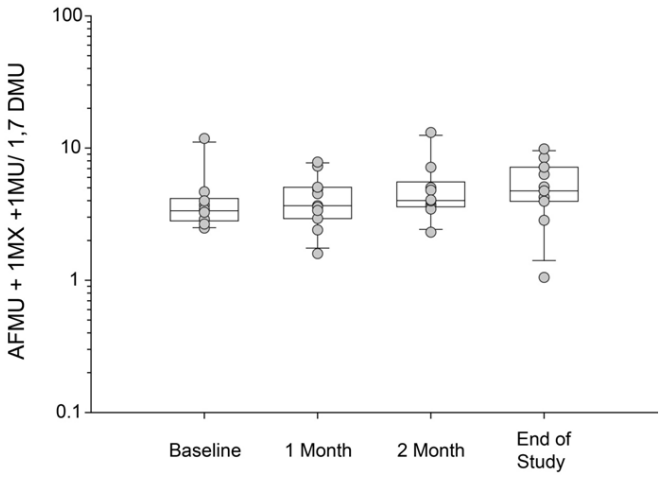

Figure 4: *CYP1A2* activity change for each subject over duration of study. There is a non-statistical trend to increase over time.

cohort. Children in this study showed similar maturation processes as described in the literature, but the study did not reach statistical significance due to interindividual variability. In addition, the children in this study were above the age for which adult activity of the enzyme is expected.

Caveats associated with our preliminary findings reside with the rather small size of our study cohort relative to the inherent variability expected with the biological effects of lead and/or the potential role of polymorphic expression of *CYP2D6* or development on modulating these effects. While our data do support an apparent lack of effect for low level lead exposure on the activity of two specific cytochrome P450 isoforms, these findings cannot be generalized to what might occur with other drug metabolizing enzymes and in patients who may have higher blood lead levels (e.g., acute toxicity). Substantiation of our findings and further exploration of the effects of sub-clinical lead toxicity on drug biotransformation will require larger studies of children with various ages where duration and extent of lead exposure can be more accurately measured. In addition, previous studies have suggested an effect of lead on *CYP3A4* activity [17]. While our study did not assess the activity of this particular P450 isoform, its examination in future studies is warranted.

Conclusion

Our preliminary data suggest that in children with low blood lead levels, lead-associated alteration in biotransformation of therapeutic drugs that are substrates for *CYP1A2* and/or *CYP2D6* would not be expected.

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