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Associations between human liver and kidney cadmium content and immunochemically detected CYP4A11 apoprotein

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Abstract

This present study was undertaken to assess potential effects of cadmium on CYP4A11 apoprotein in human liver and kidney as detected by Western blotting using a highly specific anti-peptide antibody. Liver and kidney cortex samples were autopsy specimens of 37 individuals (26 males and 11 females) whose ages ranged from 3 to 89 years. All were Caucasians who had not been exposed to cadmium in the workplace. Reduced CYP4A11 apoprotein levels were found in chronic hepatitis samples and in liver samples showing fatty changes. In contrast, increased CYP4A11 apoprotein levels were found in liver samples having higher cadmium content compared to the lower cadmium content samples. Increased CYP4A11 levels were also found in liver samples from female donors, compared to male donors; the difference being attributable to higher female liver cadmium burden. In distinction to liver, lowered CYP4A11 levels were seen in the kidney cortex samples which have high cadmium content. It is proposed here that the difference between the absolute cadmium burden of the liver and kidney samples may be responsible for the different patterns of expression of CYP4A11 in these two tissues. Further, since cadmium exposure may be associated with derangement in blood pressure control, it is interesting to note the possible relationship between altered CYP4A11-dependent production of arachidonic acid hydroxy and epoxy metabolites in kidney cortex and altered control of blood pressure. Our findings provide a possible link between these observations.

Keywords: Human autopsy; Cadmium; Cytochrome P450; Fatty acid; Vasoactive metabolites; Blood pressure; Hypertension

1. Introduction

Cadmium is a non-essential heavy metal which is widely distributed in the environment and low levels of human exposure exacerbate diabetes and enhance the risk of renal and bone diseases (for a review of health risks of cadmium exposure see reference [1]). Cadmium is found in all major organs but its accumulation in the proximal tubular cells of the kidney cortex appears to be the most extensive [2]. Cadmium in the kidneys accounts for one-third of the total body cadmium burden, and the most important target tissue for cadmium toxicity is the kidney, and one of the most widely recognised consequences of kidney damage is hypertension [3]. While hypertension of renal origin is well established to be associated with the renin–angiotensin system of blood pressure control more subtle, hitherto unsuspected, earlier changes, associated with mild kidney pathology may also occur. Since evidence has been accumulating for the involvement of a number of kidney cortex CYP isoforms in regulation of blood pressure, we have investigated associations between liver and kidney cadmium content and levels of expression of CYP4A11, one of the CYP isoforms in human kidney microsomes that were implicated in blood pressure control [4].

To do this we used liver and kidney samples from 37 individuals, 3–89 years old, who had died of accidental causes and were not exposed to cadmium in the workplace. The availability of these samples also enabled us to compare inter-individual variation in the effects of non-workplace

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cadmium exposure. CYP4A11 apoprotein in liver and kidney was detected by immunoblotting with an anti-peptide antibody using procedures described previously [5]. Details of peptide selection, antibody preparation and specificity are detailed in Edwards *et al.* [6]. Individual variations in liver and kidney CYP levels were analyzed in relation to variation in tissue cadmium contents. Other factors taken into consideration included donor age, gender, levels of exposure to cigarette smoke and liver histopathology.

2. Materials and methods

All methods used for tissue specimen collection, elemental analysis, microsomal preparation, Western immunoblotting of CYP enzymes and statistical analysis were carried out as described previously [5]. The sample group of this study consisted of 37 individuals, 26 males and 11 females, aged from 3 to 89 years. It was a subset of samples drawn from a wider investigation, designed to quantify human exposure to environmental cadmium [2]. The sample group included those who had died from accidental causes in 1997/1998 and were subject to post-mortem examination at the John Tonge centre for Forensic Sciences, Queensland Health Scientific Services, Brisbane, Australia. None of the cases showed gross pathology in any tissues and histological examination of liver was used to assess any pathological changes in these samples. Age, gender, body weight, organ weight, smoking habits, alcohol use, histology of the lung, liver and kidney of the cases were obtained from autopsy reports.

3. Results

The overall mean age for the sample group was 41 years. The mean age for males (39.5 years) and females (45.5 years) was not significantly different. The overall mean values for liver and kidney cadmium concentrations were 1.04 and 17.39 μg/g wet tissue weight. In females, the mean value for liver cadmium concentrations of 1.63 μg/g wet tissue weight was significantly higher than the mean value of 0.79 μg/g wet tissue weight of males ($P = 0.01$). The difference in mean values for cadmium in kidney cortex samples of females (21.8 μg/g wet tissue weight) and males (15.5 μg/g wet tissue weight) was not significantly different. The level of exposure to cigarette smoke of each subject was assessed as being high, medium or low based on the individual autopsy report, the lung cadmium content relative to liver and kidney levels and lung histology showing evidence of exposure to cigarette smoke such as carbon residues in lung macrophages. Table 1 shows associations between variations in levels of CYP4A11 apoprotein and an individual's age, gender, level of exposure to cigarette smoke, liver and kidney cadmium content and liver histology. It should be noted that both liver and kidney cortex samples were analysed only for 25 of the 37 individuals. Either liver or kidney cortex samples were analysed for three individuals and 9 individuals, respectively. This means that liver samples of 28 subjects were examined whereas the kidney cortex samples were examined for 34 subjects. Lowered CYP4A11 apoprotein levels were found in chronic hepatitis samples and in liver samples showing fatty changes. In contrast, increased CYP4A11 apoprotein levels were found in liver samples having higher cadmium content, compared to the lower cadmium content samples. Increased CYP4A11 levels were also found in liver samples from female donors, compared to male donors; this difference being associated with significantly higher cadmium content of the female liver samples. In distinction to liver, no gender difference was found for CYP4A11 protein levels in kidney, but lowered CYP4A11 levels were seen in the kidney cortex samples which had high cadmium contents. Statistical analysis of liver and kidney CYP4A11 protein abundance and associations with host and environmental factors are detailed in Table 2.

Table 1
Results of tests for associations between variations in levels of CYP4A11 in the liver and kidney cortex microsomal samples with age, gender, cigarette-smoke exposure, tissue cadmium contents and liver histopathology[a]

Tissues	Host factors		Environmental factors		Liver histopathology[e]
	Age[b]	Gender[c]	Cadmium[d]	Cigarette smoke[e]	
Liver	0.30	0.01F[e]	0.02[f]	0.19	0.03[g]
Kidney	0.18	0.44	0.02[g]	0.97	N/A

[a] Numbers shown are *P* values and only the *P* values which are equal to or less than 0.05 were considered to identify statistically significant associations. N/A: not applicable.
[b] Analysis by Spearman's Rank Correlation test.
[c] Analysis by Mann–Whitney U–Wilcoxon Rank Sum *W* test.
[d] Analysis by Kruskal–Wallis one-way ANOVA.
[e] F Indicates increased CYP levels in females.
[f] Indicates increased CYP levels.
[g] Indicates decreased CYP levels.

Table 2
Associations between liver CYP4A11 protein levels and cadmium content, gender and liver histopathology and kidney CYP4A11 protein and cadmium content revealed by Mann–Whitney U–Wilcoxon Rank Sum W test and Kruskal–Wallis one-way ANOVA

	Number of cases	CYP4A11 protein levels				Mean rank values	*P* values
		Trace	+1	+2	+3		
Liver ($N = 28$)							
Cd exposure levels[a]							
Low	15		1	9	5	11.6	0.02
High	13		0	3	10	17.9	0.02
Liver ($N = 28$)							
Gender							
Male	22		1	12	9	12.7	0.01
Female	6		0	0	6	21.0	0.01
Liver ($N = 27$)[b]							
Liver histopathology							
Normal	16		0	4	12	16.7	0.04
Fatty change	7		0	5	2	10.7	0.04
Chronic hepatitis	4		1	2	1	8.7	0.04
Kidney ($N = 34$)							
Cd exposure levels[c]							
Low	20	5	9	3	3	20.6	0.02
High	14	9	4	0	1	13.1	0.02

[a] The lower exposure group had liver Cd concentrations ranging from 0.11 to 0.58 μg/g wet tissue weight whereas in the higher exposure group liver Cd concentration ranged from 0.87 to 3.95 μg/g wet tissue weight.
[b] In this analysis, the case number 36 was excluded due to lack of data on liver histology.
[c] The lower exposure group had kidney Cd concentrations ranging from 2 to 16 μg/g wet tissue weight whereas in the higher exposure group liver Cd concentration ranged from 19 to 63 μg/g wet tissue weight.

4. Discussion

In seeking associations between tissue cadmium burdens and variation in the expression levels of CYP4A11 in human liver and kidney, we elected to analyse samples from people who died of accidental causes. This sample group was likely to include ''apparently healthy'' individuals and is closest to a random sample of the general population since an accidental cause of death may be viewed as a random incident [5]. Since disease and other host factors are known to contribute also to CYP expression, analysis of samples from apparently healthy individuals should minimize confounding effects that may be encountered when samples from hospital patients are used. Analysis of CYP expression in both liver and kidney samples of the same individuals was also possible. An additional advantage of using autopsy specimens is the availability of tissue histology in confirming the health status of the subjects while cigarette smoke exposure status could be verified using lung histology in conjunction with lung cadmium content.

Expression of certain CYP forms in human liver is known to be altered in people with overt liver diseases [7]. In this present study, liver pathology, manifested as fatty change and/or chronic hepatitis, was found to be associated with reduced levels of liver CYP4A11 protein (Table 2), compared to those of histologically normal livers. We know of no other previous studies showing reduction in levels of CYP4A11 in relation to human liver pathology. Since CYP4A11 in human liver microsomes has been shown to catalyse the conversion of arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE) [8]; a highly potent vasoconstrictor [3], our finding indicates the possible importance of liver pathology in local regulation of blood flow.

Interestingly, in the present study, we also found associations between variations in the levels of CYP4A11 protein in liver and kidney and tissue cadmium content. As shown in Table 2, a positive correlation was found between hepatic CYP4A11 abundance and tissue cadmium content when low and high cadmium content groups were compared. This finding has parallels with our earlier demonstration of a positive association between human liver cadmium content and CYP2C9 apoprotein abundance [5]. As indicated previously such correlations could arise from indirect associations and do not necessarily imply induction of CYP enzymes by cadmium. In addition we show here that females had higher liver cadmium content than males (Table 2) and accordingly females also had higher CYP4A11 than males. The molecular mechanisms underlying the positive correlations between liver cadmium, CYP4A11 expression and female gender are not known but our findings suggest the possibility that gender differentiated mono-oxygenase expression may depend on differential liver cadmium accumulation between men and women. The present study has also shown that the relationship between CYP4A11 expression in liver and kidney and tissue cadmium burden is different since the expression of

CYP4A11 in kidney is suppressed in the high cadmium content group (Table 2). The reasons underlying these differences in CYP4A11 expression in liver and kidney are not known but we note that the overall mean value for liver cadmium concentrations (1.04 μg/g wet tissue weight) was 17-fold lower than the value for cadmium in kidney cortex samples (17.39 μg/g wet tissue weight). In light of previous findings showing effects of cadmium treatment on CYP expression in animals [9,10], it is proposed that the difference between the absolute cadmium burden of the liver and kidney samples may be responsible for the different patterns of expression of CYP4A11 in these two tissues.

In several experimental animal species including monkey and dog, hypertension has been induced by chronic exposure to low levels of cadmium in drinking water or the diet [11,12]. Human population studies have not provided unequivocal evidence on this due to different tissue cadmium burdens of the sample groups studied and the complexity of the aetiology of essential hypertension in people [13]. The present results showing an inverse correlation between CYP4A11 protein expression in kidney and kidney cadmium burden may provide a mechanistic link between deranged blood pressure regulation and exposure to cadmium in the range 30–50 μg per day [2]. This follows from observations that CYP4A11 is one of the human CYP isoforms that have been shown to catalyse the conversion of arachidonic acid to 20-HETE [8] whose pressor functions are well established [4]. However, other CYP isoforms in human kidney have also been shown to catalyse production of HETEs with pressor function [4,8] and investigation of the relationships between other CYP isoforms, in particular CYP4F2 and CYP2J2 in kidney, and cadmium burden, are underway in our laboratory.

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