

## Urinary Biomarker Detection of Melamine- and Cyanuric Acid-Induced Kidney Injury in Rats

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**Oral coexposure of rats to melamine (MEL) and cyanuric acid (CYA) results in a dose-dependent increase in the formation of MEL-CYA crystals in the kidney. The aim of this study was to determine if urinary biomarkers of acute kidney injury could be used to non-invasively detect renal damage associated with crystal formation in the kidneys of MEL- and CYA-exposed rats. Urine was obtained on days 0 (predose), 2, 4, 14, and 28 from male and female Fischer 344 rats fed a diet supplemented with 0, 120, 180, or 240 ppm each of MEL and CYA. A number of urinary protein biomarkers (kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, osteopontin, albumin, alpha-GST, GST-Yb1, renal papillary antigen 1 [RPA-1], and clusterin) were measured using a multiplex assay system. The results showed that RPA-1 (distal tubule and collecting duct injury biomarker) was elevated on day 28 at the 120 ppm dose and higher in male rats and at the 180 ppm dose and higher in female rats; however, other urinary protein biomarkers were significantly elevated only at the 240 ppm dose. Significant elevation in blood urea nitrogen and serum creatinine levels, and severe renal damage evidenced by histopathology, were observed after 28 days of exposure to the highest dose, despite the fact that MEL-CYA crystals were observable at the 120 and 180 ppm doses. These data indicate that RPA-1 may serve as a noninvasive urinary biomarker for the detection and monitoring of obstructive nephropathy associated with MEL-CYA exposure.**

**Key Words:** kidney injury; obstructive nephropathy; nephrotoxicity biomarker; melamine; cyanuric acid.

In the spring of 2007, there was an outbreak of acute renal failure associated with food contamination in cats and dogs across North America, which led to the largest pet food recall in U.S. history. The contaminated pet foods were investigated

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and were found to have been adulterated intentionally with melamine (MEL) and MEL-related compounds, including cyanuric acid (CYA) (Brown *et al.*, 2007; Dobson *et al.*, 2008; Filigenzi *et al.*, 2008; Puschner and Reimschuessel, 2011). The following year in China, the adulteration of baby formula with MEL resulted in the illness of approximately 300,000 infants, among which more than 50,000 underwent hospitalization and six deaths were reported (WHO, 2008).

MEL (2,4,6-triamino-1,3,5-triazine) is a commonly used compound in industry for manufacturing laminates, plastics, coating, commercial filters, glues, and some dishes and kitchenware. Given its high nitrogen content (ca. 66% by weight), MEL has been marketed as a fertilizer (Lattupalli *et al.*, 2008; Shelton *et al.*, 1997). Because commonly used methods for food protein analysis are based on total nitrogen content, they cannot distinguish between nitrogen from protein and from non-protein sources. MEL was deliberately added to food to artificially boost its apparent protein content. CYA (1,3,5-triazine-2,4,6-triol), a deaminated derivative of MEL, is commercially used in preparation of herbicides, dyes, resins, and antimicrobial agents, and as a stabilizer and disinfectant in outdoor swimming pools (Downes *et al.*, 1984). As a by-product of MEL synthesis, it was also present in the “scrap MEL” that was used in the adulteration of the pet food. Studies in animal models have shown that MEL or CYA individually have low toxicity. MEL has low direct cytotoxicity *in vitro* when tested in renal cell lines (Choi *et al.*, 2010). In animal studies, MEL exhibited low toxicity when administered to rats, cats, and dogs and did not have an effect on renal function based on blood urea nitrogen (BUN) or creatinine (Cr) concentrations (Choi *et al.*, 2010; Jacob *et al.*, 2011; Puschner *et al.*, 2007). CYA has also been evaluated for acute and chronic toxicity in rats, mice, and dogs. Similar to MEL, CYA has a low toxicity with an oral LD<sub>50</sub> in rats of > 10 g/kg. In a subchronic toxicity study, rats fed a high dose of CYA alone developed bladder calculi, but there was no indication of renal toxicity (Hammond *et al.*, 1986; Puschner *et al.*, 2007). In contrast, the administration

of a combination of MEL and CYA is extremely nephrotoxic and has been recognized as a potent nephrotoxicant in animals (Dalal and Goldfarb, 2011). Combined exposure of MEL and CYA to rats resulted in a 100-fold potentiation of toxicity compared with exposure to each chemical individually (Choi *et al.*, 2010). Recently, Jacob *et al.* (2011) showed that MEL or CYA individually did not induce any significant kidney toxicity after 7 days of exposure in rats. In contrast, coexposure to MEL and CYA mixtures caused severe kidney injury and a significant elevation of serum Cr and BUN. The combination of MEL and CYA can lead to the formation of MEL-CYA crystals in kidney tubules. The crystals physically block the renal tubules that results in the development of obstructive nephropathy and can lead to acute kidney failure (Dobson *et al.*, 2008; Filigenzi *et al.*, 2008; Puschner *et al.*, 2007; Reimschuessel *et al.*, 2010).

Nephrotoxicity has been traditionally evaluated by histopathology and elevated clinical chemistry markers such as BUN and serum Cr; however, these routinely used nephrotoxicity biomarkers often do not indicate that renal injury has occurred until a significant degree of renal function is lost (Schnellmann, 2008). Thus, there is an urgent need for the use of new and sensitive biomarkers to detect renal injury at earlier stages. The Food and Drug Administration's Critical Path Initiative calls for the development of improved methods for a "better product safety toolkit," including more sensitive and predictive biomarkers of toxicity (U.S. Food and Drug Administration [U.S. FDA], 2009). Seven urinary renal safety biomarkers (kidney injury molecule-1 [Kim-1], albumin, clusterin, trefoil factor 3, total protein, cystatin C, and  $\beta_2$ -microglobulin) submitted by the Predictive Safety Testing Consortium's Nephrotoxicity Working Group have been qualified by U.S. FDA and European Medicines Agency for monitoring nephrotoxicity in preclinical testing (Dieterle *et al.*, 2010). Early and sensitive biomarkers of nephrotoxicity are valuable in drug discovery, preclinical safety evaluation of drugs and medical device materials, clinical trials, and evaluation of toxic responses following exposure to environmental toxicants. In our previous studies, we showed that a number of novel biomarkers (Kim-1, neutrophil gelatinase-associated lipocalin (NGAL), alpha-GST, GST-Yb1, renal papillary antigen 1 [RPA-1]) can detect acute kidney injury (AKI) at earlier stages following exposure of rats to nephrotoxicants, such as gentamicin, mercury, and chromium, than the traditionally used markers, BUN and Cr (Zhou *et al.*, 2008). The objective of this study was to determine if these urinary biomarkers of AKI can be used to detect nephrotoxicity associated with crystal formation and obstructive nephropathy in rat kidneys following dietary coexposure to MEL and CYA.

## MATERIALS AND METHODS

Rat urine samples were obtained from animals evaluated in the 28-day study at NCTR-FDA reported by Gamboa da Costa *et al.* (forthcoming), where a detailed description of the animal treatment, histopathological, and clinical chemistry procedures is provided. Briefly, F344 rats (12 males and 12 females

per dose group, 10 weeks old) were fed *ad libitum* for 28 days with NIH-41 irradiated meal containing 0, 120, 180, or 240 ppm each of MEL and CYA. On days -1, 1, 3, 13, and 27, the rats were transferred to individual metabolic cages and a 24-h urine sample was collected on days 0 (pre-exposure sample), 2, 4, 14, and 28. The urine was collected on ice in 50-ml polypropylene tubes containing 1 ml of 1% sodium azide. The volume of the urine was measured and the urine was stored at  $-80^{\circ}\text{C}$  until analysis. Urinary Cr levels were determined using an Alfa Wassermann ALERA analyzer (West Caldwell, NJ). At the end of the 28-day exposure period, the animals were euthanized by carbon dioxide inhalation. Blood was collected by cardiac puncture for biochemical analyses and the kidneys were fixed in formalin for histopathological analysis. The results of these analyses are reported in detail in Gamboa da Costa *et al.* (forthcoming).

**Urinary biomarkers analyses for renal injury.** For assessment of nephrotoxicity biomarkers in urine, a subset of 8 male and 8 female rats from each treatment group were selected at random. Urine Cr was used as an internal control to normalize urinary biomarker concentrations. Urinary protein biomarkers of Kim-1, albumin, osteopontin, alpha-GST, GST-Yb1, NGAL, RPA-1, and clusterin were measured using commercially available rat multiplex assay kits on the SECTOR Imager 2400 electrochemiluminescence detection platform (Meso Scale Discovery, Gaithersburg, MD).

**Statistical analyses.** Data analysis was conducted using GraphPad Prism 4.0 statistical software. A one-way analysis of variance followed by Bonferroni's *post hoc* test was used to compare the samples obtained from different days to those of day 0 within the same dose group. Data are expressed as mean  $\pm$  SD ( $n = 8$ ). A  $p$  value  $< 0.05$  was considered statistically significant.

## RESULTS

As reported in Gamboa da Costa *et al.* (forthcoming), acute kidney toxicity was observed in the 240 ppm dose group and only 9 rats were maintained until the end of the scheduled treatment (28 days). Therefore, in order to exclude rats with advanced renal damage from this study, urinary biomarkers were analyzed only on days 0, 2, 4, and 14 at the 240 ppm dose group.

### Renal Histopathological Changes

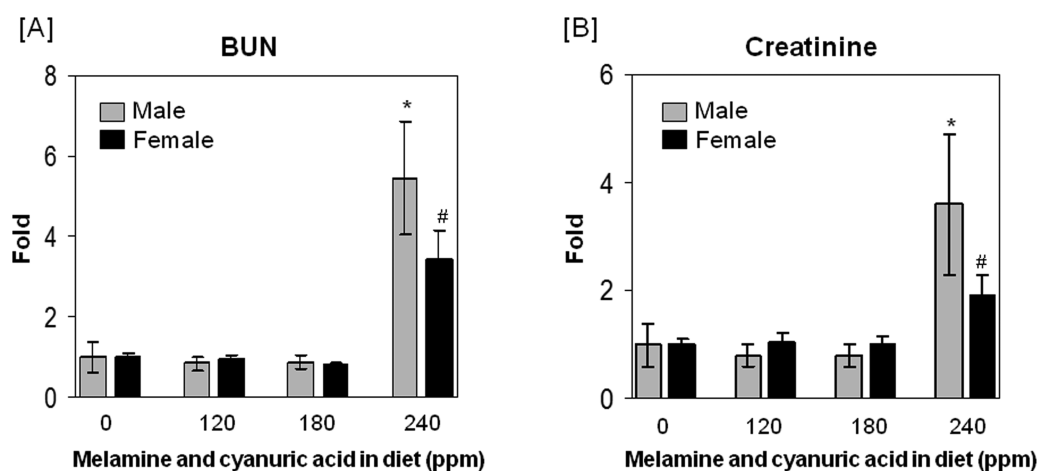
The histopathological findings are reported in detail in Gamboa da Costa *et al.* (forthcoming). The incidence and severity of kidney lesions in rats used for urinary biomarker analysis are summarized in Table 1. The renal lesion severity for individual animals is shown in Supplementary table 2. Renal histopathological changes were prominent in the 240 ppm MEL and CYA treatment groups and, to a lesser extent, in the 180 ppm treatment group. The 120 ppm group did not reveal significant histopathological changes but did have minimal crystal deposits in  $> 50\%$  of the animals. Renal lesions affected both the cortex and medulla with involvement of both proximal and distal tubules along with collecting ducts. Intratubular yellow-brown crystals (presumably MEL cyanurate) were evident in the distal and proximal areas of the kidney, resulting in renal tubular dilation accompanied by epithelial cell degeneration and/or necrosis. Significant tubular cell regeneration involving the proximal and distal convoluted tubules was also present. Intratubular and peritubular inflammatory cell infiltrates along with interstitial fibrosis were noted in many animals, but the severity was minimal to mild. Renal lesions occurred in both sexes, but the

**TABLE 1**  
**Summary of Incidence and Severity of Rat Kidney Lesions after 28-day Exposure to Diet Containing 0 (control group), 120, 180, or 240 ppm of MEL and CYA<sup>a</sup>**

Dose group (ppm of MEL and CYA in feed)	0		120		180		240	
	M	F	M	F	M	F	M	F
Crystals	0 <sup>b</sup>	0	87	37	75	75	100	100
Dilatation, renal tubule	0	0	0	0	75 (2.4)	25 (2.0)	100 (4.0)	100 (3.7)
Fibrosis	0	0	0	0	12 (2.0)	0	50 (3.2)	50 (2.5)
Regeneration, renal tubule	87 (1.0)	37 (1.0)	87 (1.1)	50 (1.0)	100 (2.5)	100 (1.9)	100 (4.0)	100 (3.7)
Necrosis, renal tubule, epithelium	0	0	0	0	25 (1.0)	0	100 (2.2)	100 (1.8)
Infiltration cellular, lymphocyte	0	0	0	0	37 (1.0)	25 (1.0)	37 (2.0)	37 (1.6)
Hyperplasia, pelvis, transitional epithelium	0	0	12 (2)	0	12 (3)	12 (2)	87 (2.0)	100 (1.6)

<sup>a</sup>Data are from Gamboa da Costa *et al.* (forthcoming) and represent 8 rats per dose group used for urinary biomarker analysis. Kidney lesion severity values for individual rats are provided in Supplementary table 2.

<sup>b</sup>% incidence; severity value in parentheses: 0, not observed; 1, minimal; 2, mild; 3, moderate; 4, marked.



**FIG. 1.** BUN and Cr changes in F344 rats after 28-day exposure to diet containing 0 (control group), 120, 180, or 240 ppm of MEL and CYA. At the end of study, blood was collected for BUN (panel A) and Cr (panel B) analysis. The data are expressed as means  $\pm$  SD for each dose group ( $n = 8$ ). BUN (control) =  $17.1 \pm 6.3$  mg/dl (male),  $16.4 \pm 1.92$  mg/dl (female); serum Cr (control) =  $0.5 \pm 0.2$  mg/dl (male),  $0.44 \pm 0.05$  mg/dl (female). Symbol \* and # indicate the statistically significant difference with  $p < 0.05$  between control and treatment group in male and female rats, respectively.

histopathological changes were more severe in male rats at doses of 120 and 180 ppm compared with the females.

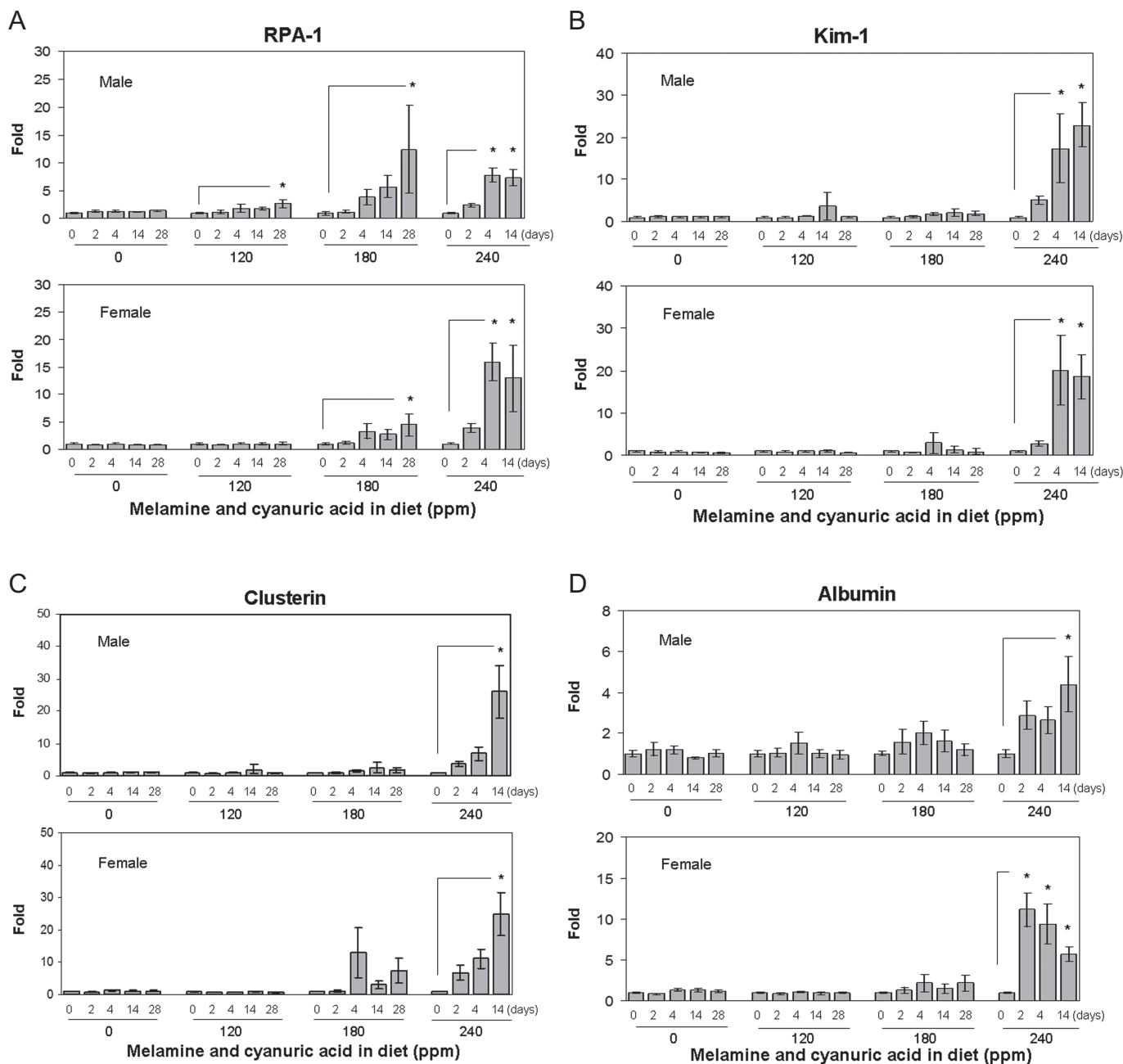
#### Blood Chemistry Findings

Figure 1 depicts the BUN and serum Cr levels for the subset of 8 male and 8 female rats from the Gamboa da Costa *et al.* (forthcoming) study that were evaluated for urinary protein biomarkers of nephrotoxicity in this study. Treatment of the rats with feed containing MEL and CYA caused a significant increase of BUN (Fig. 1A) and Cr (Fig. 1B) levels in the highest dose group (240 ppm) in both male and female animals (see numerical summary data in Supplementary table 1 and data for individual rats in Supplementary table 2). Elevations of BUN and serum Cr were higher in male rats compared with female rats; however, there were no significant differences observed

between responses of male or female rats in the 120 and 180 ppm groups compared with their matching controls.

#### Analysis of Urinary Protein Biomarkers

Twenty-four-hour urine samples of male and female F344 rats fed a diet containing 0, 120, 180 or 240 ppm of MEL and CYA were collected on days 0 (pre-exposure) 2, 4, 14, and 28 to allow monitoring of urinary levels of a number of biomarkers of AKI using a multiplex immunoassay system. Urinary biomarker data with means, SDs, and coefficients of variation are presented in Supplementary table 1. Among these biomarkers, a dose-dependent elevation of RPA-1 levels, starting with the lower doses, was observed. As shown in Figure 2A, in the males, there was a 2.7- and 12.4-fold increase of urinary RPA-1 on day 28 in the two lower dose groups



**FIG. 2.** Urinary biomarkers analysis in F344 rats following a 28-day exposure to diet containing 0 (control group), 120, 180, or 240 ppm of MEL and CYA. (A) RPA-1, (B) Kim-1, (C) clusterin, (D) albumin, (E) osteopontin, (F) alpha-GST, and (G) GST-Yb1. All the above urinary biomarkers are normalized by urinary Cr concentration. Data are expressed as mean  $\pm$  SD for each group ( $n = 8$ ). RPA-1 (control) =  $1.45 \pm 0.36$   $\mu\text{g}/\text{mg Cr}$  (male),  $1.75 \pm 0.31$   $\text{ng}/\text{mg Cr}$  (female); Kim-1 (control) =  $0.82 \pm 0.15$   $\text{ng}/\text{mg Cr}$  (male),  $1.08 \pm 0.24$   $\text{ng}/\text{mg Cr}$  (female); clusterin (control) =  $7.56 \pm 2.48$   $\text{ng}/\text{mg Cr}$  (male),  $4.72 \pm 0.71$   $\text{ng}/\text{mg Cr}$  (female); albumin (control) =  $37.3 \pm 12.7$   $\mu\text{g}/\text{mg Cr}$  (male),  $10.4 \pm 2.7$   $\mu\text{g}/\text{mg Cr}$  (female); osteopontin (control) =  $2.01 \pm 0.79$   $\text{ng}/\text{mg Cr}$  (male),  $2.75 \pm 1.44$   $\text{ng}/\text{mg Cr}$  (female); alpha-GST (control) =  $99.6 \pm 31.8$   $\text{ng}/\text{mg Cr}$  (male); GST-Yb1 (control) =  $20.8 \pm 5.06$   $\text{ng}/\text{mg Cr}$  (male). Urine for day 28 from rats at high dose group (240 ppm) is not analyzed due to advanced renal damage. Asterisks represent statistically significant difference compared with samples obtained on day 0 within each dose group ( $p < 0.05$ ).

(120 and 180 ppm) compared with day 0 values within each dose group ( $1.50 \pm 0.37$  and  $2.17 \pm 1.27$   $\mu\text{g}/\text{mg Cr}$ ), respectively. In the highest dose group (240 ppm), changes in RPA-1 were significantly increased by 7.7- and 7.3-fold compared with day 0 values in the same dose group ( $1.51 \pm 0.53$   $\mu\text{g}/\text{mg Cr}$ ) on day

4 and 14, respectively. In male rats of the low-dose groups (120 and 180 ppm), there was a trend toward time-dependent increases of RPA-1 levels from day 2 to 14; however, there was no statistically significant difference compared with day 0 values within each dose group. In female rats, there was

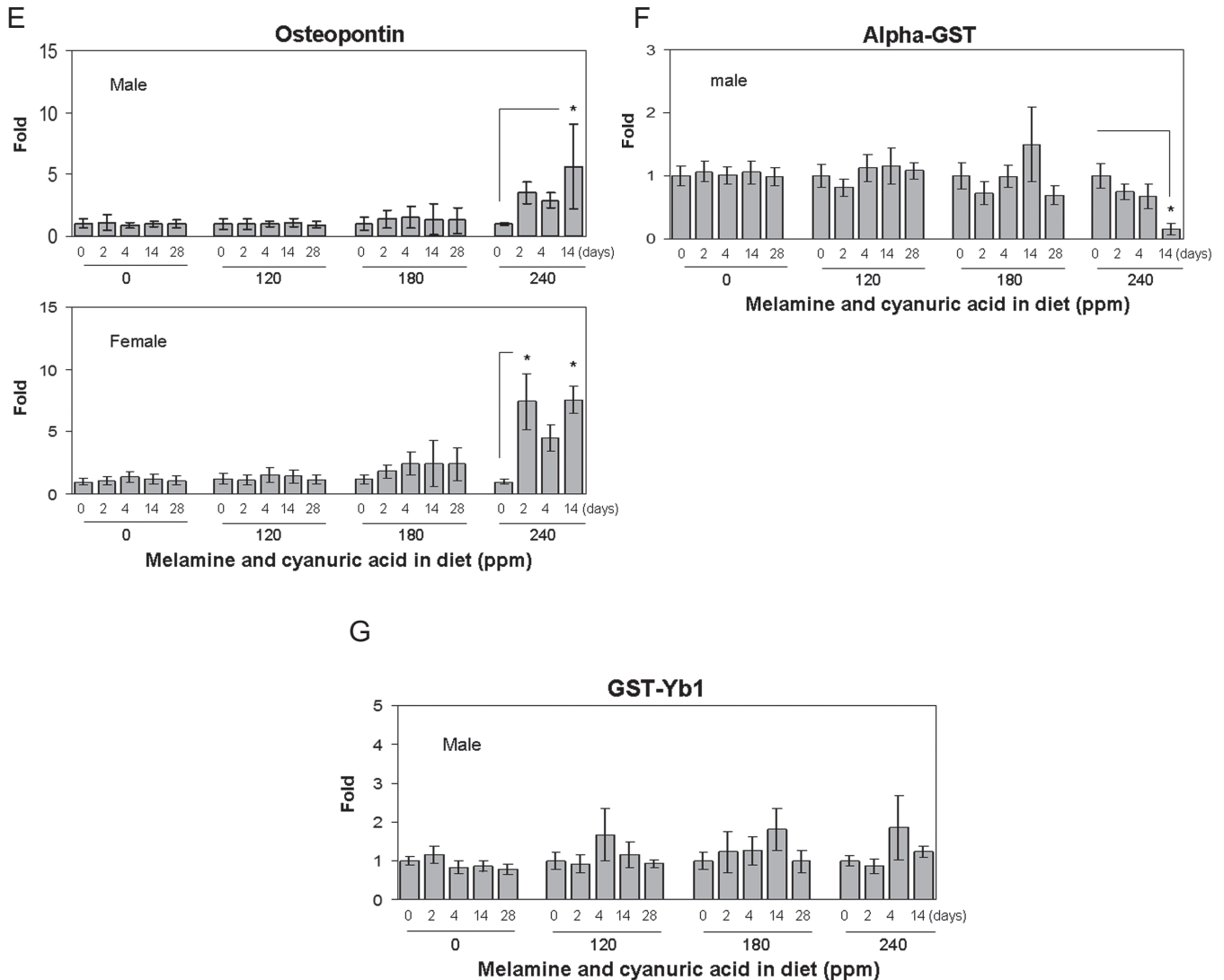


FIG. 2. Continued.

a 4.4-fold increase observed in RPA-1 on day 28 at the 180 ppm dose group compared with the same dose group of day 0 control values ( $1.71 \pm 0.39 \mu\text{g}/\text{mg Cr}$ ). In the highest dose group (240 ppm), RPA-1 was increased by 15.9- and 12.9-fold on day 4 and 14 compared with day 0 values within the dose group ( $1.2 \pm 0.28 \mu\text{g}/\text{mg Cr}$ ), respectively. In contrast, other biomarkers (Kim-1, osteopontin, clusterin, and albumin) were observed to significantly increase only in the highest dose group (240 ppm), corresponding to the dose that produced severe histopathological damage in the kidney and high blood levels of BUN and Cr (Figs. 2B–E). For alpha-GST and GST-Yb1, the levels were an order of magnitude lower in females of both control and treated groups compared with male rats and also highly variable (data not shown). In males, treated rats exhibited no increases in alpha-GST and GST-Yb1 despite evidence of pathological injury at higher doses (Figs. 2F and 2G). Surprisingly, there was a time-dependent decrease of

alpha-GST levels at the highest dose group (240 ppm) in male rats compared with the day 0 values within the dose group. Urinary levels of NGAL were generally undetectable across all groups and time points in both male and female rats (data not shown). Collectively, among the biomarkers tested, the results indicated that RPA-1 appears to be a relatively sensitive urinary protein biomarker to detect AKI following a dietary coexposure to MEL and CYA in rats.

## DISCUSSION

Renal function is routinely monitored by serum Cr and BUN concentrations; however, these biochemical indicators often only show elevation when a significant amount of kidney function is lost. Early detection and diagnosis of kidney injury could prevent progression of the disease to more severe

kidney damage and renal failure. As a result, new biomarkers are needed to aid in the early diagnosis and prediction of kidney injury. In recent years, a number of urinary proteins have been proposed as sensitive biomarkers of renal injury and as indicators of the localization of the kidney lesions. Included among these new biomarkers are Kim-1, NGAL, albumin, clusterin, glutathione-*s*-transferase (alpha-GST and GST-Yb1), and RPA-1 (Cruz *et al.*, 2011; Dieterle *et al.*, 2010; Khan *et al.*, 2010). The goal of this study was to determine if these new biomarkers are able to detect kidney injury at early stages of obstructive nephropathy in rats following a dietary coexposure to MEL and CYA.

In this study, a suite of novel urinary nephrotoxicity biomarkers was investigated using a multiplex assay system. As shown in Figure 2A, RPA-1 was significantly increased starting at the 120 ppm group in male rats and 180 ppm group in female rats; however, other biomarkers were observed to increase only in the highest dose group (240 ppm), a dose group where severe histopathological damage in the kidney and high blood levels of BUN and Cr were observed. Renal histopathological changes were prominent in the 240 ppm group and to a lesser extent in the 180 ppm group, although variable amounts of crystals were detectable by wet-mount analysis in all rats in the 120 ppm group (Gamboa da Costa *et al.*, forthcoming). RPA-1 was observed to increase in urine at this lowest dose level, suggesting that the biomarker was able to detect early crystal-induced kidney damage.

RPA-1 is a urinary biomarker used to detect injury of distal tubules and collecting ducts resulting from nephrotoxicity (Betton *et al.*, 2012; Falkenberg *et al.*, 1996; Hildebrand *et al.*, 1999). Studies have shown that RPA-1 is an early preclinical marker of renal papillary collecting duct injury in Sprague Dawley and Wistar rats following exposure to toxicants such as 2-bromoethanamine and N-phenylanthranilic acid (Price *et al.*, 2010). In addition, immunohistochemical studies demonstrated that RPA-1 was localized in the collecting duct and loop of Henle epithelia in Sprague Dawley rats exposed to gentamicin, mercury, or chromium (Zhang *et al.*, 2008). In this study, detection of elevated RPA-1 levels occurred at lower exposure levels compared with other markers, which indicates that renal injury could initially localize in distal tubules and collecting ducts. This finding is in agreement with the detection of intratubular crystals in the proximal and distal areas of the kidneys of the rats in this study, and with other studies demonstrating the localization and distribution of crystals in renal tubules after coexposures to MEL and CYA in rats, cats, and dogs (Dobson *et al.*, 2008; Kobayashi *et al.*, 2010; Puschner *et al.*, 2007). In our study, at the end of 28 days, renal histopathological changes showed that renal lesions occurred in both the cortex and medulla with involvement of both proximal and distal tubules along with collecting ducts. Intratubular yellow-brown crystals were evident in the distal and proximal areas of the kidney. Puschner *et al.* (2007) found that crystals were primarily present within the lumens of collecting ducts and distal tubules

in cats fed a MEL-CYA mixture for 48 h. Moreover, Kobayashi *et al.* (2010) reported that crystal formation was observed in the renal distal tubular lumens and the collecting ducts of the renal papilla after 2 weeks of exposure to an intermediate dose (12 mg/kg/day of MEL and CYA) in rats. At a dose of 120 mg/kg/day, crystallization was found not only in the collecting duct but also in proximal tubules of the renal cortex. In addition, histopathologic specimens from affected cats and dogs during the 2004 and 2007 outbreaks showed necrosis of distal tubular cells and dilated distal tubules containing crystals (Brown *et al.*, 2007). In infants drinking MEL-contaminated milk, several studies using ultrasonographic imaging have localized stones to the renal collecting system (Lam *et al.*, 2008, 2009). Taken together, the RPA-1 elevation suggests that the site of initial renal injury is the distal tubules and collecting ducts, a finding consistent with the site of injury identified by histopathological changes reported in cats and dogs and by ultrasonography in humans. It is not clear why MEL and CYA form crystals only after localizing in the papillary collecting ducts. A possible explanation is that water absorption generally occurs in this area, which increases the chemical saturation through the gradient of osmotic pressures in the tubules, facilitating the formation of MEL and CYA to insoluble precipitates in the kidney. The crystals physically block the renal tubules and cause acute kidney failure.

Of the other biomarkers examined, urine levels of Kim-1 (proximal tubular injury), clusterin (generalized renal injury), osteopontin, and albumin were found significantly increased only at the highest dose group (240 ppm). The finding in this dose group is consistent with the histopathological observations, i.e., evidence of overt AKI (Table 1). In both control and treated female rats, alpha-GST (proximal tubular injury) and GST-Yb1 (distal tubular injury) levels in urine were an order of magnitude lower than those in male rats and highly variable (data not shown). Recently, NGAL was reported to be a useful noninvasive biomarker of obstructive nephropathy in children (Wasilewska *et al.*, 2011). In our study, NGAL was not detectable in the urine of control or treatment groups in male or female rats (data not shown). This result is consistent with other findings in which NGAL protein was also shown to be undetectable in the urine of untreated male F344 rats compared with urine of male Wistar and Sprague Dawley rats (Rached *et al.*, 2008). The appearance of NGAL may be a strain-specific characteristic. Recently, kidney gene expression changes of these nephrotoxicity urinary biomarkers were evaluated using quantitative real-time reverse transcription PCR in MEL and CYA coexposed rats (Camacho *et al.*, 2011). The F344 rats were exposed to 0, 7, 23, 69, 229, or 694 ppm MEL and CYA for 7 days; these concentrations resulted in a daily MEL and CYA exposure of *ca.* 0, 0.9, 2.8, 8.6, 17.6, or 29.8 mg/kg bw/day, respectively. The results showed significantly increased expression changes of the genes encoding Kim-1, clusterin, osteopontin, and NGAL in both male and female rats at

the higher doses of 229 or 694 ppm MEL-CYA mixtures. The study also reported extremely low basal expression of the gene encoding NGAL in this strain of rats. We reached the same conclusion in the detection of the urine protein NGAL in our current study. In addition, we also observed a sex-specific response in the levels of RPA-1 in the urine, with the male rats presenting a statistically significant elevation of the protein levels at a lower dose group (120 ppm). These observations are consistent with the more pronounced histopathological changes observed in the male rats (see Table 1 with discussion in detail in Gamboa da Costa *et al.*, forthcoming, and Supplementary table 2 with data for individual rats used to analyze renal biomarkers).

In conclusion, a panel of new urinary biomarkers has been used to detect early kidney damage following dietary coexposure to MEL and CYA in F344 rats. We demonstrated that RPA-1 (distal tubule and collecting duct injury biomarker) was elevated at doses lower than those at which severe kidney histopathological changes occurred or commonly used biomarkers of nephrotoxicity (BUN and Cr) were elevated. The results suggest that RPA-1 may serve as a promising noninvasive urinary biomarker for earlier detection and monitoring of AKI associated with crystal obstruction following coexposure to MEL and CYA and possibly other forms of obstructive nephropathy as well.

#### SUPPLEMENTARY DATA

Supplementary data are available online at <http://toxsci.oxfordjournals.org/>.

#### FUNDING

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