Drug Metabolism & Toxicology

Maso et al., J Drug Metabol Toxicol 2011, S7
http://dx.doi.org/10.4172/2157-7609.S7-001

Research Article Open Access

Testosterone and Estradiol Affect Renal Oxidative Metabolism and Glutathione Pathway of Wistar Rats

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Abstract

The impact of sexual hormones on kidney metabolism may be relevant to modulate the effect of nephrotoxic substances. In the present research the effects of castration, testosterone, and estradiol on renal oxidative metabolism [cytochrome P-450 (CYP)], glutathione pathway [glutathione content (GSH), glutathione S-transferases (GST) activities, and cysteine-conjugate ß-lyase [as glutamine transaminase K (GTK) activity] have been studied. Naive male rats have a significantly lower GSH content but show a significantly higher GST activities and CYP content than females, whereas no sex difference was for GTK activity. Castration significantly reduces GSH content and GTK activity in females and CYP content in males, partially but significantly restored by testosterone. Testosterone significantly increases GSH content and GTK activity in males and GTK activity and CYP content in females. Estradiol increases GSH content in males, whereas decreases GST activities in females and CYP content in both sexes. Castration followed by testosterone treatment increases GTK activity in both sexes whereas increases CYP content and reduces GSH content in females. Castration followed by estradiol treatment increases GSH content and reduces GST activities in males and CYP content in both sexes. In conclusion, the results suggest that sex hormones influence metabolic pathways of the kidney and that they are probably responsible of the sex-related differences of chemical-induced nephrotoxicity. An univocal model to define sex-related toxicity of xenobiotic substances is far to be identified.

Keywords: Testosterone; Estradiol; Oxidative metabolism; Glutathione pathway; Glutamine transaminase K

Introduction

Effects of androgens on drug metabolism of the liver are well known and these are attributed to anabolic properties of the hormones [1,2]. On the other hand, the impact of the influence of the sexual hormones on kidney metabolism is relevant in the modulation of nephrotoxic substances effect. Early studies of Smith et al. [3] showed that androgens regulate cytochrome P450 (CYP) expression in renal mouse: infact CYP content of male mice was 3-4 folds higher than female one. Changes of androgen concentration modified enzyme content, confirming that expression of renal CYP gene is inducible or repressible by male hormones [4,5]. Further, level of CYPIIE1 is usually much higher in male than in female mice: testosterone treatment induced female CYPIIE1 content at level similar to that of male [6].

Sexual differences were observed in phase II metabolism also: liver glutathione S-transferases (GST) showed that substrate is determinant for the enzyme activity. For instance, the enzyme had higher activity in male than in female rats with a variety of substrates [7,8], thus underlining significant gender-related differences [9]. On the contrary, renal GST activities were higher with several substrates in female than in male rats [10] and sex differences were found also in isophorms of rat and human enzyme [11]. In addition, the half-life of renal reduced glutathione (GSH) is shorter in male (29 minutes) than in female (57 minutes) mouse [12].

Finally, mouse kidney shows a greater cysteine conjugate ß-lyase (ßL) activity in females than in males [13].

Sexual dimorphism was also recently established for ornithine aminotransferase in the mouse kidney [14] that is naturally down-regulated by testosterone, or renal organic anion transporter 2 in rat and mice kidney that exhibits gender differences such as strong androgen inhibition and weak estrogen and progesterone stimulation [15].

The aim of the present research was to get further insights in the knowledge of the effects of testosterone, estradiol, and castration on renal oxidative metabolism (CYP), glutathione pathway (GSH content and GST activities), and cysteine-conjugate ß-lyase (GTK) activity in rats.

Group N.	males	females	castrated	testosterone	estradiol
1	●				
2	●		●		
3	●			●	
4	●		●	●	
5	●				●
6	●		●		●
7		●			
8		●	●		
9		●		●	
10		●	●	●	
11		●			●
12		●	●		●

Table 1: Distribution of groups and treatments.

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Received October 24, 2011; **Accepted** November 21, 2011; **Published** November 23, 2011

Citation: Maso S, Nicolli A, Gambalunga A, Mongillo M, Chiara F, et al. (2011) Testosterone and Estradiol Affect Renal Oxidative Metabolism and Glutathione Pathway of Wistar Rats. J Drug Metabol Toxicol S7:001. doi:10.4172/2157-7609.S7-001

Materials and Methods

Animals

Albino male and female Wistar rats (Harlan Italy) were purchased at one month of age and maintained in plastic cages about one week for acclimatization. At the end of acclimatization period, an half of males and females were surgically castrated under ketamine (20 mg/kg b.w.) and diazepam (5 mg/kg b.w.) anaesthesia. After three weeks, rats (five animals per group) were subdivided in twelve groups as in Table 1 Eight groups were weighted and treated s.c. on alternate days with 10 mg/kg b.w. of testosterone propionate (Fluka, Buchs, Switzerland) or ß-estradiol-3-benzoate (Fluka, Buchs, Switzerland) dissolved in 0.2 ml of corn oil for a totak of ten treatments. The other groups were treated s.c. with 0.2 ml of corn oil only. Animal husbandry and treatment were performed according to Italian laws on animals for experimental use and the research project was approved by Padua University Laboratory Animal Care Service and by Italian Board of Health.

Methods

Twenty four hours after the last injection, rats were killed with isoflurane overdose. Kidneys were quickly removed and immediately prepared to dose GSH content according to Sedlak and Lindsay [16] as non protein sulfhydryl groups (NPSH), GST activities according to Habig et al. [17] using 1-chloro-2,4-dinitrobenzene (Sigma, St. Louis, MI, USA) as substrate and CYP content according to Omura and Sato [18]. ßL, as GTK, was determined according to Cooper and Meister [19] using L-phenylalanine (Fluka, Buchs, Switzerland) and α-keto-γ-methiolbutyrate (Sigma Chemical Co., St. Louis, USA) as substrates.

Apparatus

Spectrophotometer Perkin-Elmer lambda 5 model was used for spectrophotometric determinations.

Statistics

Two sided Mann-Whitney U test was used for the statistical evaluation of the results. Significance was set at $p<0.05$. Statistical

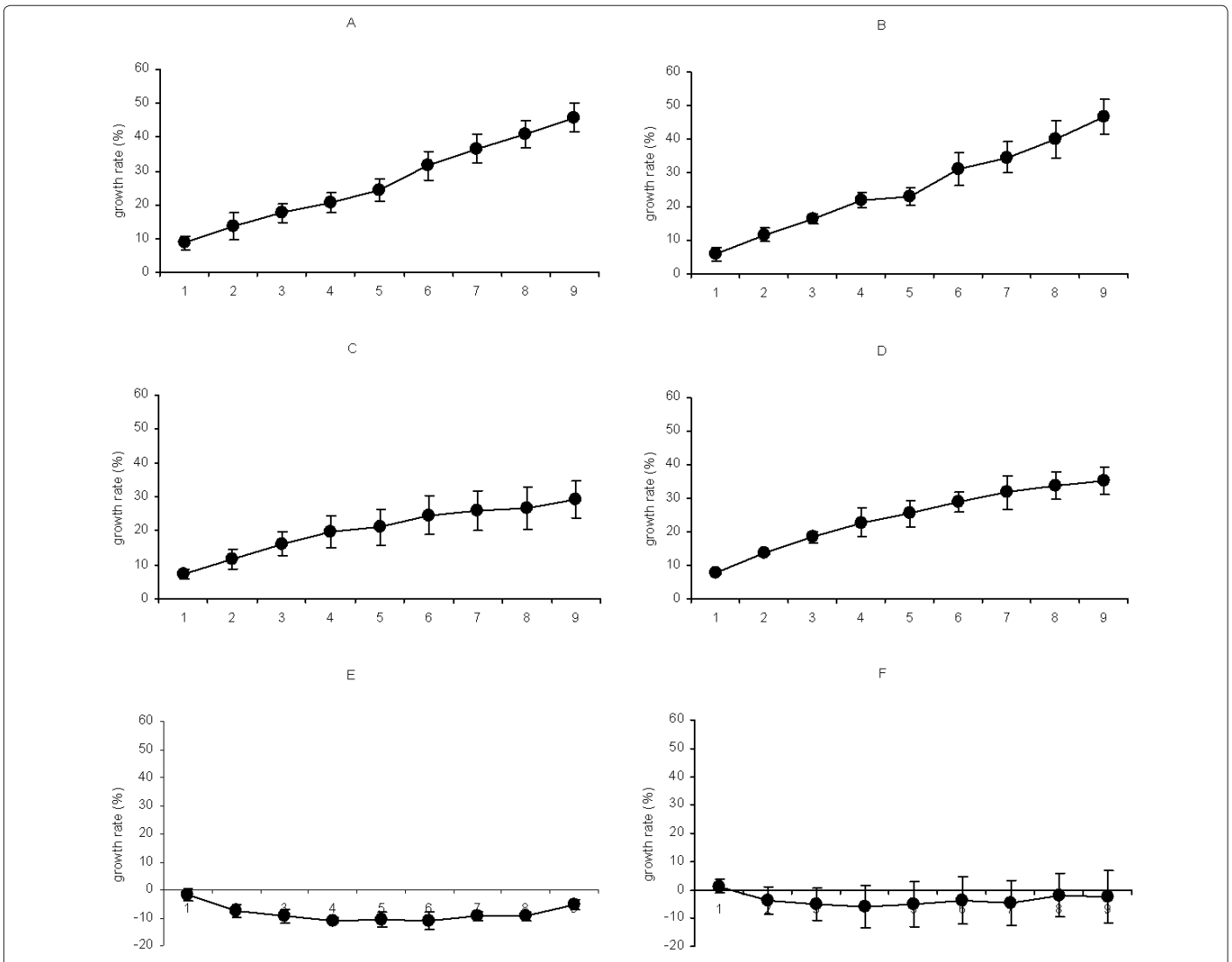

Figure 1: Growth rate of male rats: (A) naive, (B) castrated, (C) treated with testosterone, (D) castrated and then treated with testosterone, (E) treated with estradiol, and (F) castrated and then treated with estradiol.

analysis was carried out using the Stats Direct statistical software (Statsdirect 2.7.7 version, Statsdirect Ltd, UK).

Results

Each group of rats, weighted on alternate days, showed different behaviour in growth rate during hormonal treatment and/or castration. Castration did not influence growth rate of males (Figure 1B), whereas testosterone treatment caused a significant decrease of the growth in naive and castrated males (Figure 1C-D, p=0.0079 and p=0.0159, respectively); estradiol caused a reduction of the weight under the starting values (Figure 1E-F) in naive and castrated males (p=0.0079 for both). Castration (Figure 2B, p=0.0159) and testosterone treatment (Figure 2C, p=0.0317) increase the growth rate in females. As in males, estradiol (Figure 2E-F) treatment causes a decrease of the weight under the starting values in naive (p=0.0079) and castrated (p=0.0043) animals.

GSH content

Naive female rats (Table 2) showed a significantly higher GSH content than male ones (+27%, p=0.0079), whereas castration reduced it in females (-20%, p=0.0079) but not in males. Testosterone significantly increased GSH content in males (+15%, p=0.0079) and decreased it in castrated females (-17%, p=0.0079); on the contrary, estradiol appeared to have a high influence in GSH content of males (+59%, p=0.0079) and castrated males (+42%, p=0.0079), but not in females and castrated females.

GST activities

GST activities showed no difference related to gender (Table 2), and castration or testosterone treatment not influenced it. In contrast, estradiol significantly decreased enzyme activity in castrated males (-33%, p=0.0159), naive females (-15%, p=0.0159) and castrated females (-21%, p=0.0173).

GTK activity

GTK activity also showed no difference related to gender (Table 2). On the contrary, castration caused a slight, but significant decrease in females (-6%, p=0.0397), whereas testosterone caused a high increase

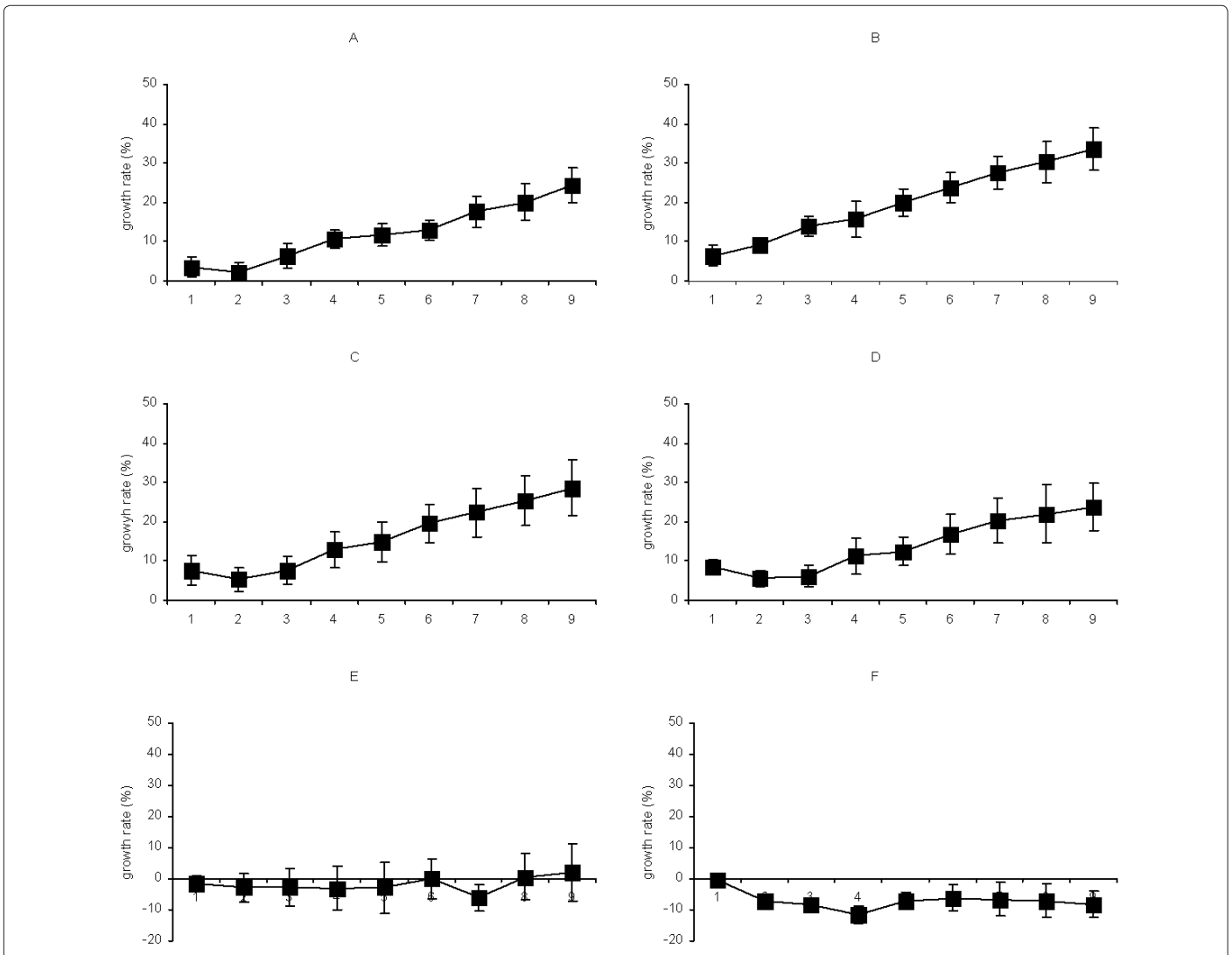

Figure 2: Growth rate of female rats: (A) naive, (B) castrated, (C) treated with testosterone, (D) castrated and then treated with testosterone, (E) treated with estradiol, and (F) castrated and then treated with estradiol.

of the enzyme activity in naive males (+59%, p=0.0079) and castrated males (+48%, p=0.0079), naive (+26%, p=0.0079) and castrated females (+28%, p=0.0079). Finally, estradiol was not found to influence the enzyme activity in both sexes.

CYP content

Male rats displayed a significantly higher CYP content (2.5 folds, p=0.0079) than female ones (Table 2). Castration reduced the enzyme content in males at the similar low level of females (p=0.0079), only partially but significantly (p=0.0079) restored by treatment with testosterone. On the contrary, treatment with estradiol significantly reduced enzyme content in naive and castrated male (-69%, p=0.0079 and -77%, p=0.0079, respectively) and in naive and castrated female rats (-47%, p=0.0159 and -24%, p=0.0346, respectively).

Discussion

The aim of the present research was to better study the influence of sex hormones on renal metabolic steps in both male and female rats. To this aim, the experimental design was performed in basal conditions, in castrated animals, and/or after testosterone or estradiol treatment.

The results show that female rats have significantly higher renal GSH content than males, but lower GST activities and CYP content, whereas GTK is not influenced by gender. Interestingly, testosterone treatment significantly increases GSH content in male but not in female rats, whereas castration decreases it in females at the same level of males. By contrast, estradiol causes a high increase in both sexes suggesting the possibility that GSH content is estradiol-dependent.

Nevertheless male have a higher GST activities than female rats; treatment with testosterone does not affect enzyme activity, whereas estradiol decreases the enzyme activity in both genders. These results suggests a down-regulation determined by female sexual hormones on the enzyme.

GTK appeared highly influenced by testosterone treatment in male and (in minor extent) in female rats, but no differences related to gender are observed; castration or estradiol treatment have no effect. These results suggest the possibility that enzyme activity is strongly regulated by testosterone.

Finally, female have a lower content of CYP than male rats, as just defined in mice [4], suggesting the relevant role of testosterone in enzyme expression. These results are further supported by the fact that castration reduces CYP content in males at level of females or lesser, and testosterone increases CYP content in females and castrated males. Further, these data are consistent with those reported by Sabolic et al. [20] even if some CYP subfamilies (i.e. 1A1) are sexually dimorphic [21].

Sex hormones are highly involved in gender differences in transport of chemicals [22] and in pharmacokinetic variability [23] suggesting that sex-based differences play a role in pharmacokinetic parameters. These differences are related to the fact that females have lower average body weight, higher body fat composition, smaller plasma volume, and lower average organ blood flow than males [23]. All these parameters affect the rate and extent of distribution of chemicals.

The gender-related effects of nephrotoxic chemicals depend on the type of substance which the animal is exposed to. Hexachloro-1:3-butadiene affects kidney female rats earlier and in higher extent than male ones [24]. This difference is related to hepatic and renal enzymes implicated in detoxification and activation of the solvent [25]. On the contrary, nephrotoxicity observed in male rats is probably ascribable to different metabolic pathway [26]. In addition, dichlorovinyl-cysteine-induced nephrotoxicity in adult mice is higher in females with low and in males with high dose [13], whereas trichlorovinyl-glutathione affects prevalently male mice owing to the higher amounts of the metabolite in liver and kidney [27]. Finally, male mice [3] are more susceptible than female ones to nephrotoxic effects of other chemicals such as chloroform.

The sex-related effects on the kidney of the chemicals appear further linked to the fate of the substances. As well summarized by Dekant and Vamvakas [28], kidney damage is related to three different mechanisms, i.e. accumulation of xenobiotics and xenobiotic-induced accumulation of endogenous macromolecules in renal tissue, renal accumulation of toxic metabolites synthesized in other organs or tissues, and intrarenal activation of xenobiotics to reactive metabolites. This implies that both the type of chemical and its metabolic pathway are relevant to induce nephrotoxicity.

For that reason, the results of our research are relevant because they show that hormone status regulates not only the chemical oxidative metabolism but the mercapturic acid pathway also. We demonstrate that estradiol modulates GSH content (increasing) and GST activities

	N.	GSH	GST	GTK	CYP
		nmol mg^{-1} of proteins			
m	5	23.3±0.5	86.9±2.5	17.2±0.4	0.084±0.002
cm	5	23.2±1.8	85.5±2.6	17.6±0.3	0.023±0.005‡‡
m+t	5	26.9±0.6‡‡	99.6±2.2	27.3±0.7‡‡	0.084±0.001
cm+t	5	26.1±1.9	94.5±2.5	25.5±0.7‡‡	0.063±0.006*
m+e	5	37.0±0.7‡‡	59.0±3.4	18.4±0.3	0.026±0.006‡‡
cm+e	5	33.2±1.2‡‡	57.8±3.7‡	18.3±0.5	0.020±0.002‡‡
f	5	29.7±1.5^{a}	64.1± 2.8^{b}	17.8±0.3^{c}	0.034±0.002^{d}
cf	5	23.7±0.8‡‡	67.2±2.8	16.7±0.3***	0.029± 0.003
f+t	5	29.9±2.1	66.9±3.1	22.4±0.7‡‡	0.074±0.002‡‡
cf+t	5	24.7±0.6‡‡	61.5±3.2	22.9±0.7‡‡	0.052±0.005‡‡
f+e	5	34.9±0.6	54.3±1.2‡	18.2±0.3	0.018±0.003‡
cf+e	5	32.2±0.8	50.7±2.3$^{\#}$	18.3±0.3	0.026±0.002**

Legend: m= males, cm= castrated males; m+t= males treated with testosterone; cm+t= castrated males treated with testosterone; m+e= males treated with estradiol; cm+e= castrated males treated with estradiol; the same abbreviations were used for female (f); *p=0.0317; **p=0.0346; ***p=0.0397; $^{\#}$p=0.0173; ‡p=0.0159; ‡‡p=0.0079. Superscript lowercase letters show significance of differences between genders: ^{a}p=0.0079, ^{b}p=0.0556 (not significant), ^{c}p=0.283 (not significant), ^{d}p=0.0079.

Table 2: Kidney metabolism in male and females after castration, treatment with testosterone or estradiol, or castration and treatment with testosterone or estradiol. Results are supplied as mean ± standard error of the mean (SEM).

(decreasing), whereas testosterone regulates GTK activity. As expected, testosterone profoundly influences on CYP content. According to these results, males appears more sensitive to reactive metabolites produced by oxidative metabolism owing to a higher CYP and lesser GSH content, whereas females are more sensitive to chemicals after metabolism via mercapturic acid pathway.

In conclusion, the results confirm that sex hormones influence metabolic pathways of the kidney and that they are probably responsible of the sex-related differences of chemical-induced nephrotoxicity. However, the extent and severity of renal damage in males or females depends on the metabolic pathway of the chemicals and the mechanisms involved in their toxicity. We postulate that an univocal model to define sex-related toxicity of xenobiotic substances is far to be identified.

Conflict of Interest Statement

The authors declare no conflict of interest.

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This article was originally published in a special issue, **Biotransformations & Toxicology** handled by Editor(s). Dr. Eric A.G. Blomme, Abbott Laboratories, USA