Toxicology 341–343 (2016) 47–55

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

The role of biotransformation and oxidative stress in 3,5-dichloroaniline (3,5-DCA) induced nephrotoxicity in isolated renal cortical cells from male Fischer 344 rats

Christopher R. Racine[a], Travis Ferguson[a], Debbie Preston[b], Dakota Ward[a], John Ball[a], Dianne Anestis[a], Monica Valentovic[a], Gary O. Rankin[a,*]

[a] Department of Pharmacology, Physiology, and Toxicology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25755, USA
[b] Department of Pediatrics, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25755, USA

ARTICLE INFO

Article history:
Received 1 September 2015
Received in revised form 15 January 2016
Accepted 20 January 2016
Available online 22 January 2016

Keywords:
Nephrotoxicity
In vitro
Rat
3,5-Dichloroaniline
Oxidative stress

ABSTRACT

Among the mono- and dichloroanilines, 3,5-dichloroaniline (3,5-DCA) is the most potent nephrotoxicant in vivo and in vitro. However, the role of renal biotransformation in 3,5-DCA induced nephrotoxicity is unknown. The current study was designed to determine the in vitro nephrotoxic potential of 3,5-DCA in isolated renal cortical cells (IRCC) obtained from male Fischer 344 rats, and the role of renal bioactivation and oxidative stress in 3,5-DCA nephrotoxicity. IRCC (~4 million cells/ml) from male rats were exposed to 3,5-DCA (0–1.0 mM) for up to 120 min. In IRCC, 3,5-DCA was cytotoxic at 1.0 mM by 60 min as evidenced by the increased release of lactate dehydrogenase (LDH), but 120 min was required for 3,5-DCA 0.5 mM to increase LDH release. In subsequent studies, IRCC were exposed to a pretreatment (antioxidant or enzyme inhibitor) prior to exposure to 3,5-DCA (1.0 mM) for 90 min. Cytotoxicity induced by 3,5-DCA was attenuated by pretreatment with inhibitors of flavin-containing monooxygenase (FMO; methimazole, *N*-octylamine), cytochrome P450 (CYP; piperonyl butoxide, metyrapone), or peroxidase (indomethacin, mercaptosuccinate) enzymes. Use of more selective CYP inhibitors suggested that the CYP 2C family contributed to 3,5-DCA bioactivation. Antioxidants (glutathione, *N*-acetyl-L-cysteine, α-tocopherol, ascorbate, pyruvate) also attenuated 3,5-DCA nephrotoxicity, but oxidized glutathione levels and the oxidized/reduced glutathione ratios were not increased. These results indicate that 3,5-DCA may be activated via several renal enzyme systems to toxic metabolites, and that free radicals, but not oxidative stress, contribute to 3,5-DCA induced nephrotoxicity in vitro.

1. Introduction

Toxicity arising from exposure to anilines, important chemical intermediates used in the production of agricultural, industrial and pharmaceutical products (Lindh et al., 2007; Unger, 1996), has been well established. Exposure to aniline and its chlorinated derivatives has been associated with hematotoxicity (methemoglobinemia, hemolytic anemia; Chhabra et al., 1990; Guilhermino et al., 1998; Pauluhn, 2004; Valentovic et al., 1997), splenotoxicity (splenomegaly, elevated erythropoietic activity, hyperpigmentation, fibrosis; Chhabra et al., 1990; Khan et al., 1999; Ma et al., 2008, 2013), hepatotoxicity (hepatomegaly, elevated serum ALT/GPT levels, centralobular necrosis; Valentovic et al., 1995a,b, 1992), and nephrotoxicity (Hong et al., 2000; Lo et al., 1990, 1991; Racine et al., 2014; Valentovic et al., 1995a). Chloroaniline induced nephrotoxicity in vivo is characterized by oliguria, decreased kidney weight, proteinuria, hematuria, elevated blood urea nitrogen (BUN) concentration, and decreased organic ion transport in the proximal tubule cells (Rankin et al., 1986; Lo et al., 1990; Valentovic et al., 1995a). Morphological changes occur in both the proximal and distal tubules and collecting ducts, with the greatest abnormalities seen in the proximal tubular cells. These morphological changes include blebbing and vacuolization of proximal tubular cells, occluded lumina with sloughed microvilli and enlarged lumina in

Abbreviations: 3,5-DCA, 3,5-dichloroaniline; IRCC, isolated renal cortical cells; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; GPT, glutamic-pyruvic transaminase; GSH, glutathione; GSSG, oxidized glutathione; DMSO, dimethyl sulfoxide; DNP, 2,4-dinitrophenylhydrazone; FMO, flavin-containing monooxygenase; CYP, cytochrome P450; PiBx, piperonyl butoxide; DEDTCA, diethyldithiocarbamate; ASC, ascorbate; NAC, *N*-acetyl-L-cysteine; 3,5-DCAA, 3,5-dichloroacetanilide; 3,5-DCPHA, 3,5-dichlorophenylhydroxylamine; 3,5-DCNB, 3,5-dichloronitrobenzene.

* Corresponding author at: Department of Pharmacology, Physiology & Toxicology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25755, USA. Fax: +1 304 696 7391.
E-mail address: rankin@marshall.edu (G.O. Rankin).

http://dx.doi.org/10.1016/j.tox.2016.01.006
0300-483X/

the distal tubule (Lo et al., 1990). In vitro exposure of rat renal cortical slices to chloroanilines leads to a significant increase in cytotoxicity as seen by a decrease in organic ion accumulation and an increase in lactate dehydrogenase (LDH) release (Valentovic et al., 1992, 1995a,b). Among the mono- and dichloroanilines, 3,5-dichloroaniline (3,5-DCA) was the most potent nephrotoxicant both in vivo and in vitro (Lo et al., 1990; Valentovic et al., 1995a, 1996).

Biotransformation via *N*-oxidation, *N*-acetylation, and phenyl ring oxidation are all known pathways of chloroaniline metabolism (Ehlhardt and Howbert, 1991; Hong and Rankin, 1998; Racine et al., 2014). Based on these studies, a proposed biotransformation pathway of 3,5-DCA can be seen in Fig. 1. Two of these pathways (*N*-oxidation and phenyl ring oxidation) have the potential to lead to the formation of toxic metabolites from aniline compounds. For example, *N*-oxidation of aniline leads to toxic metabolites responsible for hematoxicity (Harrison and Jollow, 1987, 1986) and splenotoxicity (Khan et al., 2000; Ma et al., 2013, 2008), while formation of the 4-aminophenol metabolite can contribute to nephrotoxicity (Harmon et al., 2005; Rankin et al., 1996; Tarloff et al., 1989). A small number of studies have shown that bioactivation can also contribute to the nephrotoxicity associated with some chloroanilines (Racine et al., 2014; Valentovic et al., 1995b). Putative metabolites of chloroanilines arising from *N*-oxidation or phenyl ring oxidation are also toxic to the kidney (Hong et al., 1997, 1996; Rankin et al., 2008a). Nonetheless, the enzyme systems responsible for the bioactivation of chloroanilines studied to date and the ultimate nephrotoxic metabolites formed from these compounds are not clearly defined.

Oxidative stress may contribute to the mechanism of cell death with aniline compounds. Harmon et al. (2005) found that oxidative stress played a role in 4-aminophenol-induced nephrotoxicity in vitro, while Hong et al. (1997) showed that in vivo 4-amino-2,6-dichlorophenol (ADCP), a putative metabolite of 3,5-DCA, increased the oxidized to reduced glutathione ratio in kidney, suggesting that ADCP induced renal oxidative stress. In addition, ADCP nephrotoxicity was prevented by pretreatment with antioxidants. However, it is unclear if oxidative stress plays a role in 3,5-DCA nephrotoxicity.

The purpose of this study was to explore, in more detail, the in vitro nephrotoxicity induced by 3,5-DCA. The in vitro cytotoxicity of 3,5-DCA was determined in isolated rat renal cortical cells (IRCC) from male Fischer 344 rats, and the role of renal metabolizing enzyme systems, including CYP isozymes, in the bioactivation of 3,5-DCA to nephrotoxic metabolites was also examined. Lastly, the role of free radicals and oxidative stress in 3,5-DCA-induced

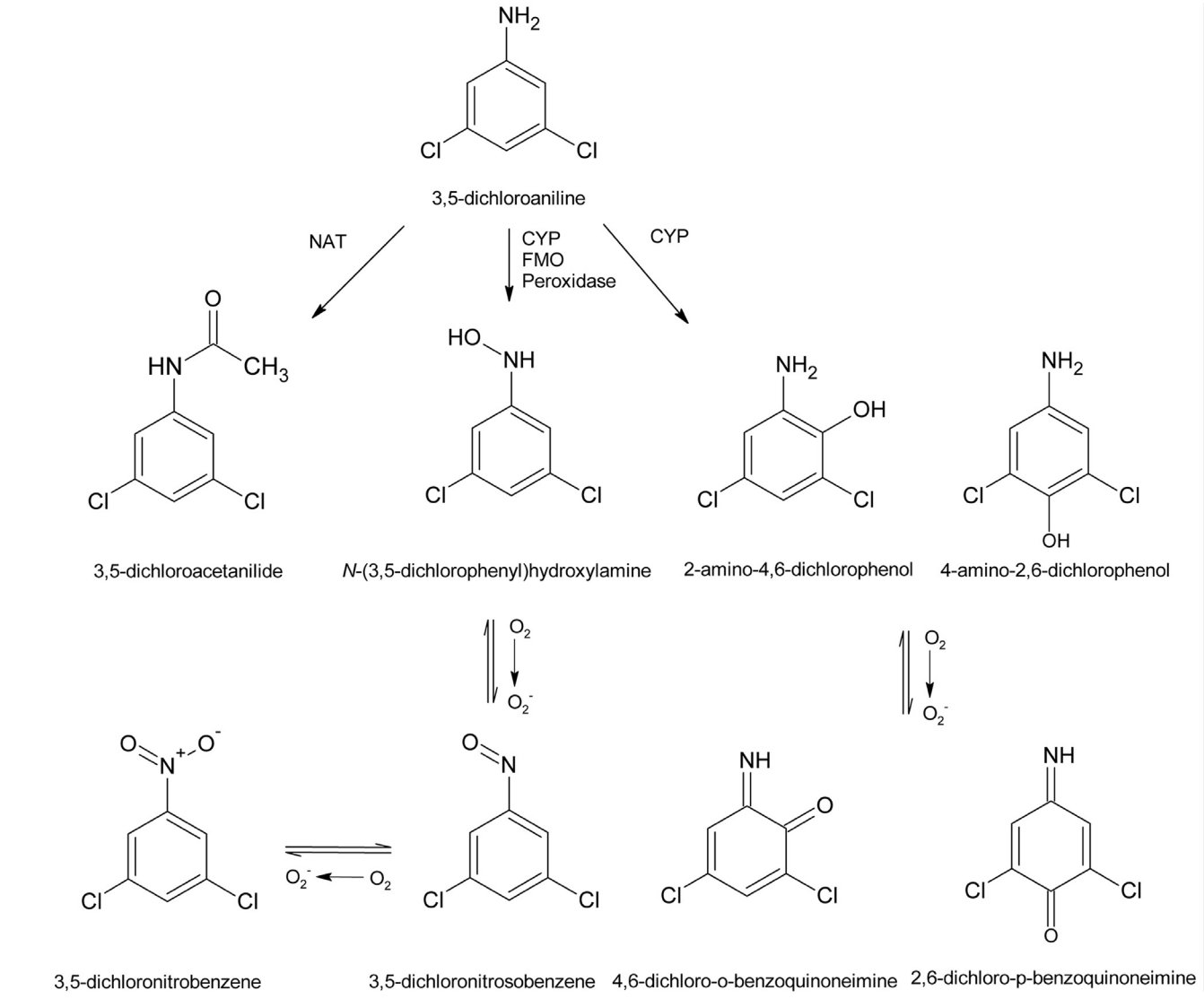

Fig. 1. Proposed renal biotransformation pathway of 3,5-DCA. Abbreviations: CYP = cytochrome P450, FMO = flavin-containing monooxygenase, NAT = *N*-acetyltransferase.