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Abstract

Nitrofurantoin is used in the antibacterial therapy of the urinary tract. This therapy is associated with various adverse effects whose mechanisms remain unclear. Diverse studies show that the nitro reductive metabolism of nitrofurantoin leads to ROS generation. This reaction can be catalyzed by several reductases, including the cytochrome P450 (CYP450) reductase. Oxidative stress arising from this nitro reductive metabolism has been proposed as the mechanism underlying the adverse effects associated with nitrofurantoin. There is, however, an apparent paradox between these findings and the ability of nitrofurantoin to inhibit lipid peroxidation provoked by NADPH in rat liver microsomes. This work was aimed to show the potential contribution of different enzymatic systems to the metabolism of this drug in rat liver microsomes. Our results show that microsomal lipid peroxidation promoted by NADPH is inhibited by nitrofurantoin in a concentration-dependent manner. This suggests that the consumption of NADPH in microsomes can be competitively promoted by lipid peroxidation and nitrofurantoin metabolism. The incubation of microsomes with NADPH and nitrofurantoin generated 1-aminohidantoin. In addition, the biotransformation of a classical substrate of CYP450 oxidative system was competitively inhibited by nitrofurantoin. These results suggest that nitrofurantoin is metabolized through CYP450 system. Data are discussed in terms of the in vitro redox metabolism of nitrofurantoin.

Keywords: nitrofurantoin biotransformation, lipid peroxidation, oxidative stress

Introduction

Nitrofuran drugs are a family of nitro heterocyclic compounds that include nifurtimox and nitrofurantoin [1,2]. These drugs, used in antimicrobial therapies, are often associated to multiple adverse effects [3–5]. Two-electron reductases occurring in bacteria and parasites can catalyze the reduction of the nitro group in nitrofurans to the corresponding hydroxylamine, through generation of a nitroso intermediate [6–9]. The hydroxylamine derivative of nitrofuran drugs is an electrophilic agent that can bind to biomolecules, altering their function [10]. This phenomenon seems to represent the antimicrobial mechanism of these drugs.

In contrast to bacteria and parasites, mammalian cells display a high abundance of one-electron reductases [11,12]. Early enzymatic studies have shown that several of these reductases can catalyze the reduction of nitrofurantoin, including the NADPH-cytochrome P450 (CYP450) reductase [13–17]. This reduction generates a reaction intermediate corresponding to a nitro anion radical. This intermediate can react, in a futile cycle,

with molecular oxygen producing superoxide anion and regenerating the nitro drug [13,14,17–20]. A great deal of research can be found regarding nitrofurantoin nitro reduction by liver microsomes [13,14,18–20]. In fact, all these studies have detected the production of ROS or oxidative consequences of ROS production, such as lipid peroxidation [19–22]. It has been proposed that oxidative stress arising from the one-electron reductive metabolism of nitrofuran drugs would explain the adverse effects observed with these drugs in the human population [3–5].

Nitrofurantoin is widely used in the treatment and prophylaxis of lower urinary tract infections (lower UTIs) [23]. Although the mechanism of action of nitrofurantoin is not yet clear, it has been postulated that it acts by inhibiting several bacterial enzymes [10]. This hypothesis presumes that nitro reductases occurring in sensitive bacteria transform nitrofurantoin in highly reactive electrophiles, which would target ribosomes, effectively inhibiting bacterial protein synthesis [10]. Unfortunately, nitrofurantoin can cause problems associated with its prolonged use: respiratory dysfunction [24–26], hepatic

damage [27,28], peripheral polyneuropathy [29], and hematologic disorders [30]. In humans, lung and liver toxicity has been attributed to the reactive intermediates generated through nitro reduction of nitrofurantoin [14,15,31–34]. Even short-term treatment with nitrofurantoin can cause gastrointestinal adverse reactions, with nausea and abdominal pain being the most reported side effects.

NADPH is known to provoke microsomal lipid peroxidation [35,36] and, paradoxically, nitrofuran drugs inhibit this phenomenon [21,22]. Given that nitro reduction of nitrofuran drugs and NADPH provokes microsomal lipid peroxidation, an additional mechanism must exist to explain this apparent contradiction. The CYP450 system catalyzes the oxidation of lipophilic molecules, including drugs, in the presence of molecular oxygen and NADPH [37–40]. Therefore, a competition for NADPH between NADPH-elicited microsomal lipid peroxidation and the oxidation of a CYP450 system substrate would be expected when microsomes are incubated with NADPH and that substrate. Noteworthy, if the oxidative reaction catalyzed by CYP450 does not generate ROS, it should not lead to microsomal lipid peroxidation. Accordingly, drugs undergoing oxidative demethylation through the CYP450 system have been shown to inhibit microsomal lipid peroxidation elicited by NADPH [36].

In this work, we evaluated reductive and oxidative biotransformation pathways of nitrofurantoin. To this end, we incubated rat liver microsomes with nitrofurantoin under conditions leading to its biotransformation through the CYP450 oxidative system [19]. We found evidences supporting the idea that nitrofurantoin can bind to the substrate-binding site of the CYP450 monooxygenase. Also, nitrofurantoin inhibited the *O*-demethylation of *p*-nitroanisole and the hydroxylation of naphthalene, hallmark activities of the CYP450 oxidative system [37–40]. Given the chemistry of nitrofurantoin, this drug might be oxidized by CYP450 system to 1-aminohydantoin. The finding of this metabolite in the meat and milk from animals treated with this antimicrobial [41,42] supported our postulate. To this end, we developed a fluorescence-based HPLC detection for 1-aminohydantoin. Using this method, we identified this metabolite in the supernatant of microsomes incubated with nitrofurantoin and NADPH, specific cofactor of the CYP450 system. Oxidative stress arising from enzymatic nitro reduction of nitrofurantoin has been proposed as the mechanism underlying the adverse effects associated with this drug. Nitrofurantoin however inhibited microsomal lipid peroxidation elicited by NADPH, in a biphasic and concentration-dependent manner. These findings are an apparent paradox: nitrofurantoin may be reduced and generate oxidative stress; in addition, it may suffer oxidation, phenomenon that consumes NADPH, causal agent of lipid peroxidation in rat liver microsomes. We discuss these results in regards to the contribution of different enzymatic systems to the redox metabolism of nitrofurantoin and the oxidative stress proposed as responsible mechanism of its adverse reactions.

Materials and methods

Chemicals

1-aminohydantoin (HPLC standard), apocynin, bovine serum albumin (BSA, fraction IV), Folin-Ciocalteu's reagent, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, β-NADP, naphthalene, α-naphthol, aminopyrine (4-dimethylaminoantipyrine), *p*-nitrophenol, *p*-nitroanisole, *N*-(5-nitro-2-furfurylidene)-1-aminohydantoin (nitrofurantoin), and SKF-525A (proadifen) were obtained from Sigma-Aldrich Química Limitada (Santiago, Chile). Naphthalene, thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased in Merck (Santiago, Chile). AccQ-Fluor™ reagent kit was obtained from Waters (Massachusetts, USA). All other chemicals used were of analytical grade.

Animals

Adult male Sprague Dawley rats (200–250 g), maintained at the vivarium of the Facultad de Ciencias Químicas y Farmacéuticas (Universidad de Chile, Santiago, Chile) were used. The rats were allowed free access to pellet food, maintained with controlled temperature (22°C) and photoperiod (lights on from 07:00 to 19:00 h). All procedures were performed using protocols approved by the Institutional Ethical Committee of the Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, and according to the guidelines of the Guide for the Care and Use of Laboratory Animals (NRC, USA).

Liver microsomal fraction

Microsomal fractions were prepared as previously reported [19]. Microsomes were stored at -80°C until use. Total microsomal protein was determined according to Lowry et al. [43], using BSA as standard.

Microsomal lipid peroxidation

Assay of thiobarbituric acid reactive substances (TBARS) is an economic and rapid method to evaluate lipid peroxidation that has been often characterized as ancient and/or unspecific due to certain interferents. For this reason we have standardized our assay conditions for the method to be in linear dependence with the concentration of biological membranes and incubation time. Microsomes (1 mg/mL) were incubated with a NADPH-generating system comprising 6 mM glucose-6-phosphate, 0.6 mM NADP, and 0.15 U/mL glucose-6-phosphate dehydrogenase in 50 mM phosphate buffer (pH 7.4) for 30 min at 37°C. Increasing concentrations of nitrofurantoin (up to 3 mM), and/or different concentrations of selected inhibitors, as detailed in the text and table legends were tested. The extent of microsomal lipid peroxidation was estimated assaying TBARS as previously described by Letelier et al. [19]. In brief, lipoperoxidation reaction was stopped with 0.5 mL of 20% TCA and then supernatant was separated by centrifugation. The samples were then incubated with

TBA 1% for 1 h and absorbance was read at 532 nm. The results are expressed as nmol of TBARS per minute per mg of microsomal protein.

In addition, we determined malondialdehyde concentration by high-performance liquid chromatography. In this case, the reaction was stopped adding 20 µL/mL of a tert-butyl-hydroxytoluene solution containing 2.2 mg/mL. MDA quantification was developed through fluorimetric detection as described by Young and Trimble [44]. The results are expressed as µmol of MDA per mg of microsomal protein.

CYP450 monooxygenase spectrum

CYP450 monooxygenase spectrum was obtained as previously described [45]. Microsomes (1 mg protein/mL) were exposed to increasing concentrations (up to 15 µM) of nitrofurantoin in 4 mM $MgCl_2$ and 50 mM phosphate buffer at pH 7.4 during 5 min at 25°C. Afterwards, reaction mixture was supplemented with 5 mM sodium dithionite and then carbon monoxide was bubbled to the sample and 390–500 nm spectrums were generated in a UV3 Unicam UV–VIS spectrophotometer, using the mixture without carbon monoxide as blank.

On the other hand, microsomes (1 mg protein/mL) were treated with 5 mM sodium dithionite. Then, the mixture was exposed to increasing concentrations (up to 15 µM) of nitrofurantoin in 4 mM $MgCl_2$ and 50 mM phosphate buffer at pH 7.4 during 5 min at 25°C. Afterwards, carbon monoxide was bubbled to aliquots of this mixture (sample) and 390–500 nm spectrums were using the mixture without carbon monoxide as blank.

p-Nitroanisole O-demethylation

The *O*-demethylating microsomal activity of the CYP450 system was determined as previously reported [46]. In order to evaluate the inhibitory effect of nitrofurantoin, this activity was assayed in the presence of 3 mM of this compound.

Naphthalene hydroxylation

The hydroxylating activity of the CYP450 system was determined as previously described [47]. In order to evaluate the inhibitory effect of nitrofurantoin, this activity was assayed in the presence of 3 mM of this compound.

Determination of 1-aminohydantoin by a reverse HPLC method

Microsomes (5 mg protein/mL) were incubated with buffer (control), 1 mM nitrofurantoin alone, or 1 mM nitrofurantoin and the NADPH-generating system, in the absence or presence of 50 µM SKF-525A, for 30 min at 37°C. Then, samples were deproteinized by addition of 2 volumes of acetonitrile and centrifuged for 15 min at 10,000 g. Twenty microliters of each supernatant were incubated with the AccQ-Fluor™ reagent in a final 0.1 mL volume, at 55°C for 20 min to ensure formation of the 1-aminohydantoin biderivative. Then, 5 µL of each sample were injected in a C_{18} column (150×3.9 mm) in a Waters 2695 Alliance System with a 2475 Fluorescence Detector. Reverse phase HPLC was run for 10 min in 13% acetonitrile/87% acetate buffer (65 mM sodium acetate, 1 mM Ca Titriplex and 0.11% triethylamine pH 5.45), then 5 min for a linear gradient up to 60% acetonitrile 40% buffer, 1 min isocratically with 60% acetonitrile/40% water, and finally reached the initial condition 13% acetonitrile/87% buffer in 1 min and equilibrated for 9 min before the next injection. Columns were washed with 60% acetonitrile/40% water between runs. All separation procedures were performed at 37°C. The AccQ-Fluor™ tag was detected at 238 nm excitation wavelength and 368 nm emission wavelength. The signal for 1-aminohydantoin AccQ-Fluor™ biderivative was consistently found at a retention time of 9.7–9.95 min. Calibration curve was obtained, with a linear range for quantification from 0.5 to 10 pmol of 1-aminohydantoin. To evaluate oxidative metabolism of nitrofurantoin, supernatants from acetonitrile-treated microsomes incubated in different conditions were derivatized and quantification of the 1-aminohydantoin biderivative was performed.

Statistical analyses

Data are presented as the mean of at least four independent experiments (using different microsomal preparations) ± SD. Analyses of the significance of the differences in
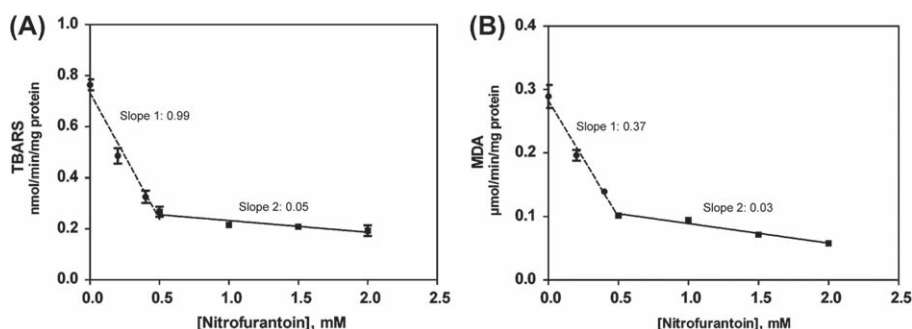

Figure 1. NADPH-elicited microsomal lipid peroxidation in the presence of nitrofurantoin. NADPH-elicited microsomal lipid peroxidation was estimated by TBARS (A) or HPLC (B) in the presence or absence of increasing concentrations of nitrofurantoin, as detailed in Materials and Methods. Data represent the mean of four independent experiments ± SD and lines correspond to linear regression of the data.
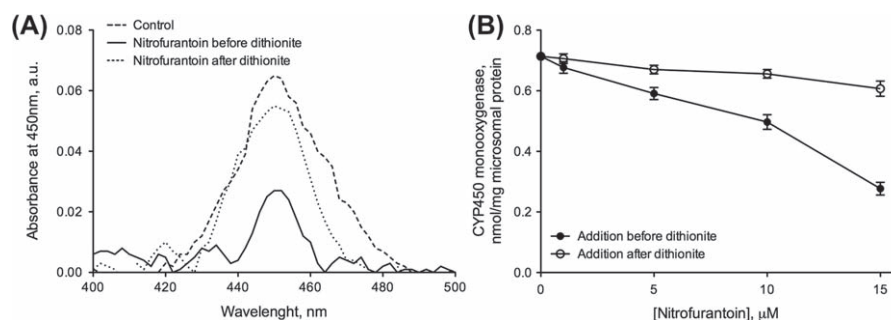
Figure 2. Effect of nitrofurantoin on the detection of the microsomal CYP450 monooxygenase spectrum. (A) Spectra of the CYP450 monooxygenase obtained in the absence or presence of nitrofurantoin—15 µM—before or after adding sodium dithionite. (B) Effect of increasing concentrations of nitrofurantoin on CYP450 monooxygenase content, before or after adding sodium dithionite as detailed in Materials and Methods. Data represent the mean of four independent experiments ± SD.

means were performed using Student's t-test. Data were considered significantly different when $p < 0.05$. All statistical analyses were performed using GraphPad Prism, version 5.0.

Results

NADPH-elicited microsomal lipid peroxidation: effect of nitrofurantoin

Our results showed that nitrofurantoin inhibited microsomal lipid peroxidation provoked by NADPH-generating system. This phenomenon was concentration dependent and showed a biphasic behavior (Figure 1). Thus, two linear regressions with different slopes were observed. The slope values were similar when TBARS or MDA conjugates were quantified (Figure 1A and B).

Effect of nitrofurantoin on the catalytic activity of the CYP450 system

Few data exist on the in vivo metabolism of nitrofurantoin. Lipophilic drugs are biotransformed mainly through CYP450 system. These drugs bind to the CYP450 monooxygenase only in its Fe^{3+} form, in the first and rate-limiting step of the CYP450 system catalytic cycle [38,48]. The CYP450 monooxygenase in its Fe^{2+} form can bind

carbon monoxide, forming a complex with a characteristic absorbance peak at 450 nm [45]. The addition of nitrofurantoin to the mixture decreased the absorbance of this complex in a concentration-dependent manner (Figure 2). When nitrofurantoin is added before sodium dithionite (closed circles) led to a more significant decrease of this absorbance than after the reducing agent (open circles). (Figure 2A and B).

The above experiments show that nitrofurantoin has affinity for CYP450 monooxygenases. Therefore, it is reasonable to expect that the drug is metabolized at least partly through this biotransformation system. Thus, we also evaluated the effect of nitrofurantoin on *p*-nitroanisole *O*-demethylation and naphthalene hydroxylation, reactions catalyzed by the CYP450 system. Both reactions, carried out under similar conditions, were inhibited by 3 mM of nitrofurantoin to different extents (Figure 3). Nitrofurantoin inhibited 83% the *p*-nitroanisole *O*-demethylation (Figure 3A) and 48% the naphthalene hydroxylation (Figure 3B). Inhibition of naphthalene hydroxylation was further characterized in terms of changes in the "apparent kinetic constants". Nitrofurantoin significantly increased the apparent K_m for naphthalene in about 2.7-fold, while eliciting no significant changes in the apparent V_{max} values of this activity (Table I). Nitrofurantoin also decreased in about 64% the catalytic efficiency (V_{max}/K_m) of naphthalene hydroxylation reaction (Table I).

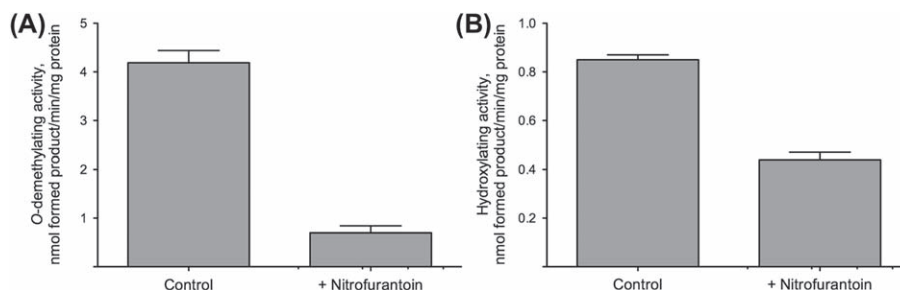Figure 3. Effect of nitrofurantoin on microsomal *p*-nitroanisole *O*-demethylation and naphthalene hydroxylation. Microsomal protein: 2 mg/mL, *p*-nitroanisole: 1 mM, naphthalene: 2.5 mM. Activities were assayed in the absence or presence of 3 mM nitrofurantoin, as detailed in Materials and Methods. Data represent the mean of four independent experiments ± SD. *$p < 0.05$ compared with the control.

Table I. Apparent kinetic constants for microsomal naphthalene hydroxylation: effect of nitrofurantoin.

Condition	V_{max}	K_m	V_{max}/K_m
Control	0.87 ± 0.03	3.9 ± 0.3	0.22
+ 3 mM nitrofurantoin	0.85 ± 0.05	$10.6 \pm 0.6^*$	0.08

Hydroxylation of naphthalene was assayed in the presence or absence of nitrofurantoin, as described in Materials and Methods. Values of V_{max} (nmol α-naphthol/min/mg protein) and K_m for naphthalene (mM) were calculated from Lineweaver–Burk analyses of each saturation curve. Data represent the mean of four independent experiments $\pm$ SD. $^*p < 0.05$ compared with the control.

Generation of 1-aminohydantoin by rat liver microsomes incubated with nitrofurantoin in the presence of NADPH

Figure 4 shows the standardization of our HPLC based method to quantify 1-aminohydantoin generation. Incubation of microsomes with nitrofurantoin in the presence of the NADPH-generating system led to the formation of the biderivative of 1-aminohydantoin a similar quantity to the standard (100 pmol; Figure 5). Addition of the general inhibitor of the CYP450 monooxygenase SKF-525A decrease the presence of
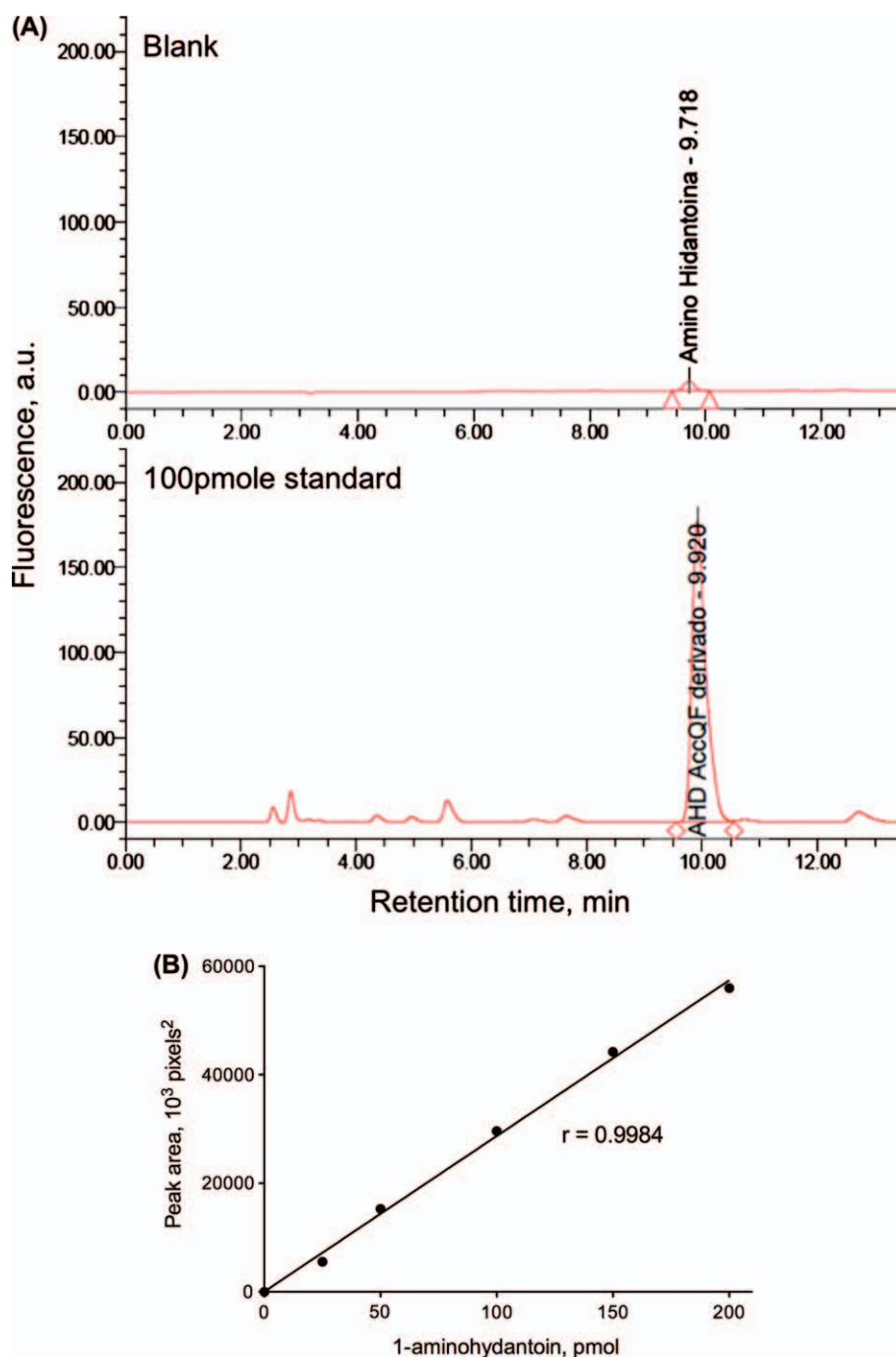
Figure 4. HPLC detection of 1-aminohydantoin by derivatization of AccQ Fluor. 1-Aminohydantoin standards were derivatized with the AccQ-Fluor™ reagent and detected by HPLC as detailed in Materials and Methods. (A) Top and bottom panels depict representative chromatograms from at least five determinations of derivatized blanks and 100 pmole of 1-aminohydantoin standard, respectively. (B) Calibration curve of the biderivative of 1-aminohydantoin. Data represent the mean of three independent experiments $\pm$ SD.

(A) AHD ST (100 pmoles)

AHD AccQF derivado — 9.920

Microsomes (5mg protein/mL)
1mM nitrofurantoin
NADPH-generating system

AHD AccQF derivado — 9.848

Microsomes (5mg protein/mL)
1mM nitrofurantoin
NADPH-generating system
50μM SKF525-A

AHD AccQF derivad

Fluorescence, a.u.

Retention time, min

(B)

1-aminohydantoin, pmoles

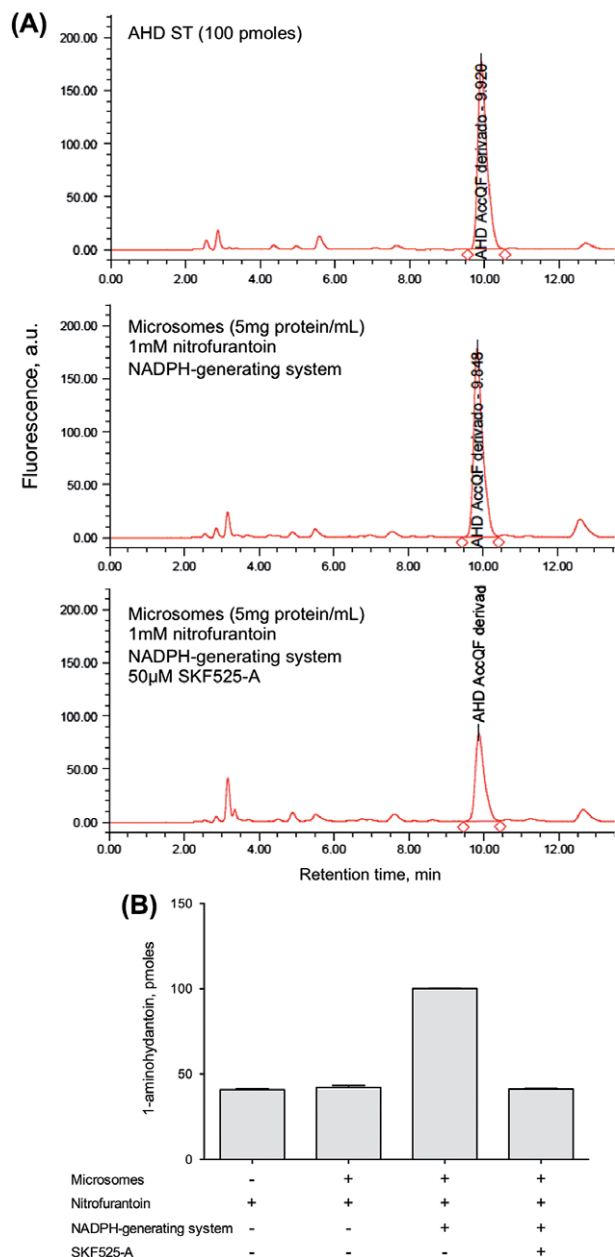| Microsomes	-	+	+	+
Nitrofurantoin	+	+	+	+
NADPH-generating system	-	-	+	+
SKF525-A	-	-	-	+

Figure 5. Quantification of 1-aminohydantoin in microsomes incubated with nitrofurantoin. Microsomes were incubated under different conditions prior to deprotenization and derivatization with AccQ-Fluor™, as detailed in Materials and Methods. (A) Representative chromatograms of 100 pmole of 1-aminohydantoin standard (top panel), supernatant from microsomes incubated with nitrofurantoin and the NADPH-generating system in the absence (middle panel) or presence of SKF525-A (bottom panel). (B) Quantification of 1-aminohydantoin generation under different conditions, as indicated under each column. Data represent the mean of three independent experiments ± SD.

this biderivative in the reaction mixture (Figure 5, bottom). CYP450 monooxygenase content in the rat liver microsomes used in this study was 1.02 nmol/mg of microsomal protein. Considering this data, the specific activity of CYP450 system to catalyze the generation of 1-aminohydatoin is 1.71 nmol/30 min/nmol of monooxygenase.

Discussion

Nitrofurantoin is a drug widely used in the treatment of urinary infections [23,49]. This drug is associated with several adverse reactions in the human population, including gastrointestinal, hepatic, and pulmonary side effects [5,24–27,30,50]. Most of the evidence regarding in vitro toxicological effects of nitrofurantoin is focused in its nitro reductive metabolism, which leads to the generation of ROS and oxidative stress [14]. This drug, however, inhibits microsomal lipid peroxidation elicited by NADPH [21,22]. As for other lipophilic drugs, nitrofurantoin could be a substrate of the CYP450 oxidative system [38,48]. In fact, some evidence of hydroxylation has been reported for nitrofuran drugs [51,52]. Both nitroreduction and putative oxidation of these drugs require NADPH. Our results seem to indicate that only the former reaction leads to ROS generation promoting microsomal lipid peroxidation. If this is correct, inhibition by nitrofurantoin of microsomal lipid peroxidation provoked by NADPH may be explained in terms of the biotransformation of this drug by the CYP450 system. This inhibitory phenomenon was biphasic and concentration dependent, and one order of magnitude was the difference between the two slopes obtained. Such behavior suggests that at least two mechanisms underlie the inhibition by nitrofurantoin of microsomal lipid peroxidation promoted by NADPH. One of these mechanisms may be the oxidative biotransformation of the drug and the other, the nitro reduction of the drug that may compete with NADPH to generate ROS since it is a futile cycle. We are actively testing this postulate. Previous reports postulated that the nitrofurantoin in the presence of NADPH leads to microsomal lipid peroxidation [19]. These results contradict those obtained in this work. This inconsistency seem to be due to experimental differences: the control condition in those experiments contained all reagents except NADPH, whereas the experiments used in this study lacked nitrofurantoin. Lipid peroxidation from the reductive metabolism of nitrofurantoin in the presence of NADPH must be distinguished from that caused by the interaction between NADPH and possible NADPH oxidases present in liver microsomes. The absence of NADPH in the control does not allow us to separate both lipid peroxidation processes.

Very little information is available on the possible catalysis of nitrofurantoin oxidation by the CYP450 system. In this regard, we have previously shown that this drug may bind to the CYP450 monooxygenase and inhibit *p*-nitroanisole *O*-demethylation, an oxidative reaction catalyzed by the CYP450 system [19]. Catalysis of the oxidative biotransformation of drugs by the CYP450 system requires binding of the drug to the substrate-binding site on the CYP450 monooxygenase. Therefore, binding of nitrofurantoin to this monooxygenase could indicate that this drug could be oxidized through the CYP450 system. Nitrofurantoin altered the CYP450 monooxygenase spectrum to a higher extent

when it was added to microsomes before reducing agents. This condition maintains the heme iron in its oxidized state (Fe^{3+}), necessary requirement for the binding of substrates to the enzyme. Thus, these results seem to indicate that specific binding to the monooxygenase occurs. Furthermore, this drug behaved as an inhibitor of *p*-nitroanisole *O*-demethylation and naphthalene hydroxylation. Changes on the "apparent kinetic" for naphthalene hydroxylation elicited by nitrofurantoin are also in agreement with a classic competitive inhibition by the drug.

It should be noted that CYP450 monooxygenases are not specific enzymes, although the extent of the affinity for lipophilic substrates widely differ. Therefore, using liver preparations enriched in endoplasmic reticulum (microsomes) to test CYP450 system activity (including all the isoforms of CYP450 monooxygenases), compared with reconstituted systems using purified enzymes seems to be closer to what would occur in vivo. Altogether, our results suggest that 1) inhibition of NADPH-elicited microsomal lipid peroxidation by nitrofurantoin is likely the consequence of its biotransformation through the CYP450 oxidative system, and 2) nitrofurantoin is a likely substrate of the CYP450 oxidative system. In agreement with this hypothesis, hydroxylation metabolites of nitrofurantoin have been identified [52,53]. In addition, 1-aminohydantoin has been identified as a nitrofurantoin metabolite in meat and milk products from animals treated with nitrofurantoin [41,42]. Here, we show that 1-aminohydantoin is specifically produced in vitro when rat liver microsomes are incubated with nitrofurantoin under conditions that allow its oxidative biotransformation through the CYP450 system. These data strongly suggest that nitrofurantoin can undergo oxidative biotransformation in liver tissue. In light of these findings; several issues need to be addressed to clearly understand oxidative stress associated to the metabolism of nitrofurantoin in humans. These include the actual contribution of the CYP450 reductase to oxidative stress arising from nitrofurantoin nitro reduction. We are actively evaluating these issues.

Declaration of interest

References

[1] D'Arcy PF. Nitrofurantoin. Drug Intell Clin Pharm 1985;19:540–547.
[2] Urbina JA. Specific chemotherapy of Chagas disease: relevance, current limitations and new approaches. Acta Trop 2010;115:55–68.
[3] Gleckman R, Alvarez S, Joubert DW. Drug therapy reviews: nitrofurantoin. Am J Hosp Pharm 1979;36:342–351.
[4] Westphal JF, Vetter D, Brogard JM. Hepatic side-effects of antibiotics. J Antimicrob Chemother 1994;33:387–401.
[5] Triantafyllou K, Vlachogiannakos J, Ladas SD. Gastrointestinal and liver side effects of drugs in elderly patients. Best Pract Res Clin Gastroenterol 2010;24:203–215.
[6] Symons T, Tocher JH, Tocher DA, Edwards DI. Electrochemical studies of nitroheterocyclic compounds of biological interest. VII. Effect of electrode material. Free Radic Res Commun 1991;14:33–40.
[7] Squella JA, Letelier ME, Lindermeyer L, Nuñez-Vergara LJ. Redox behaviour of nifuroxazide: generation of the one-electron reduction product. Chem Biol Interact 1996;99:227–238.
[8] Boiani M, Piacenza L, Hernández P, Boiani L, Cerecetto H, González M, Denicola A. Mode of action of nifurtimox and N-oxide-containing heterocycles against Trypanosoma cruzi: is oxidative stress involved? Biochem Pharmacol 2010;79:1736–1745.
[9] Hall BS, Bot C, Wilkinson SR. Nifurtimox activation by trypanosomal type I nitroreductases generates cytotoxic nitrile metabolites. J Biol Chem 2011;286:13088–13095.
[10] McOsker CC, Fitzpatrick PM. Nitrofurantoin: mechanism of action and implications for resistance development in common uropathogens. J Antimicrob Chemother 1994;33:23–30.
[11] Murataliev MB, Feyereisen R, Walker FA. Electron transfer by diflavin reductases. Biochim Biophys Acta 2004;1698:1–26.
[12] Deller S, Macheroux P, Sollner S. Flavin-dependent quinone reductases. Cell Mol Life Sci 2008;65:141–160.
[13] Zenser TV, Mattammal MB, Palmier MO, Davis BB. Microsomal nitroreductase activity of rabbit kidney and bladder: implications in 5-nitrofuran-induced toxicity. J Pharmacol Exp Ther 1981;219:735–740.
[14] Moreno SN, Mason RP, Docampo R. Reduction of nifurtimox and nitrofurantoin to free radical metabolites by rat liver mitochondria. Evidence of an outer membrane-located nitroreductase. J Biol Chem 1984;259:6298–6305.
[15] Viodé C, Bettache N, Cenas N, Krauth-Siegel RL, Chauvière G, Bakalara N, Périé J. Enzymatic reduction studies of nitroheterocycles. Biochem Pharmacol 1999;57:549–557.
[16] Wang Y, Gray JP, Mishin V, Heck DE, Laskin DL, Laskin JD. Role of cytochrome P450 reductase in nitrofurantoin-induced redox cycling and cytotoxicity. Free Radic Biol Med 2008;44:1169–1179.
[17] Bartel LC, Montalto de Mecca M, Castro JA. Nitroreductive metabolic activation of some carcinogenic nitro heterocyclic food contaminants in rat mammary tissue cellular fractions. Food Chem Toxicol 2009;47:140–144.
[18] Fernandez Villamil SH, Dubin M, Brusa MA, Duran RP, Perissinotti LJ, Stoppani AO. Generation of radical anions of nifurtimox and related nitrofuran compounds by ascorbate. Free Radic Res Commun 1990;10:351–360.
[19] Letelier ME, Izquierdo P, Godoy L, Lepe AM, Faúndez M. Liver microsomal biotransformation of nitro-aryl drugs: mechanism for potential oxidative stress induction. J Appl Toxicol 2004;24:519–525.
[20] Letelier ME, Entrala P, López-Alarcón C, González-Lira V, Molina-Berríos A, Cortés-Troncoso J, et al. Nitroaryl-1,4-dihydropyridines as antioxidants against rat liver microsomes oxidation induced by iron/ascorbate, nitrofurantoin and naphthalene. Toxicol In Vitro 2007;21:1610–1618.
[21] Dubin M, Grinblat L, Fernandez Villamil SH, Stoppani AO. Nitrofuran inhibition of microsomal lipid peroxidation. FEBS Lett 1987;220:197–200.
[22] Dubin M, Fernandez Villamil SH, Paulino de Blumenfeld M, Stoppani AO. Inhibition of microsomal lipid peroxidation and cytochrome P-450-catalyzed reactions by nitrofuran compounds. Free Radic Res Commun 1991;14:419–431.
[23] Guay DRP. Contemporary management of uncomplicated urinary tract infections. Drugs 2008;68:1169–1205.

[24] Witten CM. Pulmonary toxicity of nitrofurantoin. Arch Phys Med Rehabil 1989;70:55–57.

[25] Bialas MC, Shetty HG, Houghton J, Woods F, Routledge PA. Nitrofurantoin rechallenge and recurrent toxicity. Postgrad Med J 1997;73:519–520.

[26] Goemaere NNT, Grijm K, Van Hal PTW, Den Bakker MA. Nitrofurantoin-induced pulmonary fibrosis: a case report. J Med Case Rep 2008;2:169.

[27] Penn RG, Griffin JP. Adverse reactions to nitrofurantoin in the United Kingdom, Sweden, and Holland. Br Med J 1982; 284:1440–1442.

[28] Paiva LA, Wright PJ, Koff RS. Long-term hepatic memory for hypersensitivity to nitrofurantoin. Am J Gastroenterol 1992; 87:891–893.

[29] Jacknowitz AI, Le Frock JL, Prince RA. Nitrofurantoin polyneuropathy: report of two cases. Am J Hosp Pharm 1977;34:759–762.

[30] Gait JE. Hemolytic reactions to nitrofurantoin in patients with glucose-6-phosphate dehydrogenase deficiency: theory and practice. DICP 1990;24:1210–1213.

[31] Martin WJ. Nitrofurantoin: evidence for the oxidant injury of lung parenchymal cells. Am Rev Respir Dis 1983;127:482–486.

[32] Martin WJ. Nitrofurantoin. Potential direct and indirect mechanisms of lung injury. Chest 1983;83:51S–52S.

[33] Minchin RF, Ho PC, Boyd MR. Reductive metabolism of nitrofurantoin by rat lung and liver in vitro. Biochem Pharmacol 1986;35:575–580.

[34] Rossi L, Silva JM, McGirr LG, O'Brien PJ. Nitrofurantoin-mediated oxidative stress cytotoxicity in isolated rat hepatocytes. Biochem Pharmacol 1988;37:3109–3117.

[35] Hochstein P, Ernster L. ADP-activated lipid peroxidation coupled to the TPNH oxidase system of microsomes. Biochem Biophys Res Commun 1963;12:388–394.

[36] Orrenius S, Dallner G, Ernster L. Inhibition of the TPNH-linked lipid peroxidation of liver microsomes by drugs undergoing oxidative demethylation. Biochem Biophys Res Commun 1964;14:329–334.

[37] Danielson PB. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab 2002;3:561–597.

[38] Guengerich FP. Cytochrome p450 and chemical toxicology. Chem Res Toxicol 2008;21:70–83.

[39] Hollenberg PF, Kent UM, Bumpus NN. Mechanism-based inactivation of human cytochromes p450s: experimental characterization, reactive intermediates, and clinical implications. Chem Res Toxicol 2008;21:189–205.

[40] Kramer MA, Tracy TS. Studying cytochrome P450 kinetics in drug metabolism. Expert opin Drug Metab Toxicol 2008;4: 591–603.

[41] Finzi JK, Donato JL, Sucupira M, De Nucci G. Determination of nitrofuran metabolites in poultry muscle and eggs by liquid chromatography-tandem mass spectrometry. J Chromatogr B 2005;824:30–35.

[42] Rodziewicz L. Determination of nitrofuran metabolites in milk by liquid chromatography-electrospray ionization tandem mass spectrometry. J Chromatogr B 2008;864:156–160.

[43] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–275.

[44] Young IS, Trimble ER. Measurement of malondialdehyde in plasma by high performance liquid chromatography with fluorimetric detection. Ann Clin Biochem 1991;28: 504–508.

[45] Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. J Biol Chem 1964;239:2379–2385.

[46] Letelier ME, Faúndez M, Jara-Sandoval J, et al. Mechanisms underlying the inhibition of the cytochrome P450 system by copper ions. J Appl Toxicol 2009;29:695–702.

[47] Letelier ME, Del Villar E, Sanchez E. Drug tolerance and detoxicating enzymes in Octodon degus and Wistar rats. A comparative study. Comp Biochem Physiol C 1985;80: 195–198.

[48] Estabrook RW. A passion for P450s (rememberances of the early history of research on cytochrome P450). Drug Metab Dispos 2003;31:1461–1473.

[49] Schultz HJ, Edson RS. Cystitis treatment in women, circa 2011: new role for an old drug. Mayo Clinic Proc 2011;86: 477–479.

[50] Karpman E, Kurzrock EA. Adverse reactions of nitrofurantoin, trimethoprim and sulfamethoxazole in children. J Urol 2004;172:448–453.

[51] Olivard J, Rose GM, Klein GM, Heotis JP. Metabolic and photochemical hydroxylation of 5-nitro-2-furancarboxaldehyde derivatives. J Med Chem 1976;19:729–731.

[52] Jonen HG. Reductive and oxidative metabolism of nitrofurantoin in rat liver. Naunyn Schmiedebergs Arch Pharmacol 1980;315:167–175.

[53] Jonen HG, Oesch F, Platt KL. 4-Hydroxylation of nitrofurantoin in the rat. A 3-methylcholanthrene-inducible pathway of a relatively nontoxic compound. Drug Metab Dispos 1980; 8:446–451.