Liver Microsomal Biotransformation of Nitro-aryl Drugs: Mechanism for Potential Oxidative Stress Induction

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Toxic effects of several nitro-aryl drugs are attributed to the nitro-reduction that may be suffered *in vivo*, a reaction that may be catalysed by different reductases. One of these enzymes is NADPH–cytochrome P450 reductase, which belongs to the cytochrome P450 oxidative system mainly localized in the endoplasmic reticulum of the hepatic cell. This system is responsible for the biotransformation of oxidative lipophilic compounds, so that oxidative and reductive metabolic pathways of lipophilic nitro-aryl drugs can take place simultaneously. Because of the affinity of nitro-aryl drugs (xenobiotics) for the endoplasmic reticulum, we propose this subcellular organelle as a good biological system for investigating the toxicity induced by the biotransformation of these or another compounds.

In this work we used rat liver microsomes to assess the oxidative stress induced by nitro-aryl drug biotransformation. Incubation of microsomes of rat liver with nifurtimox and nitrofurantoin in the presence of NADPH induced lipoperoxidation, UDP-glucuronyltransferase activation and an increase in the basal microsomal oxygen consumption. Nitro-aryl-1,4-dihydropyridines did not elicit these prooxidant effects; furthermore, they inhibited lipoperoxidation and oxygen consumption induced by Fe^{3+}/ascorbate. Nifurtimox and nitrofurantoin modified the maximum absorption of cytochrome P450 oxidase and inhibited *p*-nitroanisole O-demethylation, an oxidative reaction catalysed by the cytochrome P450 system, signifying that oxidation may proceed in a similar way to that described for nitro-aryl-1,4-dihydropyridines. Thus the balance between lipophilic nitro-aryl drug oxidation and reduction may be involved in the potential oxidative stress induced by biotransformation.

INTRODUCTION

Nitro-aryl compounds have enjoyed widespread use in medicine as antibiotics, radiosensitizers and antibacterial and antiprotozoal agents. Under aerobic conditions, one of the biotransformation pathways of these compounds appears to be reduction of the nitro group, a reaction that leads to a 'futile metabolism'. Nitro-aryl reduction has been described in different subcellular organelles — microsomes, mitochondria and cytosol — and there is direct proof that a one-electron reduction can provoke the formation of a superoxide anion ($O_2^{\cdot-}$) and a hydroxyl radical ($^{\cdot}OH$) (Moreno *et al.*, 1984; Docampo *et al.*, 1988; Iwata *et al.*, 1992). It is widely accepted that the generation of reactive oxygen species (ROS) without their efficient removal can lead to oxidative modification of proteins, lipids and DNA, which may alter their biological functions (Neuzil *et al.*, 1993). The nitro-anion radical ($NO_2^{\cdot-}$), the first intermediate of the nitro-reduction pathway, can react with molecular oxygen to regenerate the original nitro-compound, forming $O_2^{\cdot-}$ and $^{\cdot}OH$ as by-products. Accordingly, it has been suggested that intracellular reduction of nifurtimox explains both its trypanocide action and its toxicity in mammals (Núñez-Vergara *et al.*, 1997; Docampo, 1990). A similar hypothesis has been proposed to explain the toxicity of nitrofurantoin, an antibacterial drug (Hoener *et al.*, 1989; Foth, 1995; Amit *et al.*, 2002). However, the $-NO_2^{\cdot-}$ intermediate may continue in the reductive pathway leading to the nitroso-derivative (Wardman, 1985; Docampo *et al.*, 1988; Orna and Mason, 1989) with no apparent toxicological consequences. Nevertheless, not all nitro-compounds seem to suffer nitro-reduction as an important metabolic pathway. Thus, the adverse effects described for nitro-aryl dihydropyridines (N-1,4-DHPs), which are calcium channel antagonists, have not been associated with oxidative stress (Messerli, 2002); moreover, these drugs are administered to cardiovascular patients over long periods and different authors have demonstrated that N-1,4-DHPs can induce antioxidant protective effects in cardiac and liver membranes and brain slices (Janero and Burghardt, 1988; Engineer and Sridhar, 1989; Díaz-Araya *et al.*, 1998).

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Nitro-aryl drugs are mainly lipophilic xenobiotics, therefore they need to be metabolized primarily by the cytochrome P450 (Cyt-P450) oxidative system prior to their elimination from the organism. One of the enzymes involved in nitro-aryl reduction is NADPH–Cyt-P450 reductase and it belongs to the Cyt-P450 system localized mainly in hepatic endoplasmic reticulum (Karuzina and Archakov, 1994). Oxidation and reduction of nitro-aryl drugs depend on the NADPH conditions under which this oxidative system catalyses both enzymatic reactions. Therefore, oxidative and reductive pathways may occur as competitive and simultaneous processes and the toxicity of lipophilic nitro-aryl drugs associated with oxidative stress may be a consequence of the relative contributions from both metabolic pathways.

It is known that hepatic endoplasmic reticulum is the principal cellular organelle involved in xenobiotic metabolism. Exposure of the microsomal fraction (endoplasmic reticulum-enriched preparation) to ROS induces oxygen consumption, lipid peroxidation and UDP-glucuronyltransferase activation (Bentley *et al.*, 1979; Groot *et al.*, 1985; Docampo *et al.*, 1988). Because xenobiotic biotransformation may lead to ROS generation, liver microsomal preparations are likely to be good *in vitro* models for assessing the oxidative stress induced by nitro-reduction of lipophilic compounds.

In the present work we attempted to establish a correlation between microsomal biotransformation of lipophilic nitro-compounds and modifications induced by oxidative stress on microsomal lipids and UDP-glucuronyltransferase activity. The occurrence of ROS by biotransformation processes was corroborated by measuring the induction of microsomal oxygen consumption and the presence of oxygen free radicals by election paramagnetic resonance (EPR). Our data suggest that both oxidative and reductive metabolic pathways can take place in the biotransformation of drugs and that the relative contribution of these pathways may be determinant for the assurance of a particular nitro-aryl drug to generate oxidative stress. In addition, the chemical structure of nitro-aryl drugs should be considered an important property for predicting the possible toxicological effects.

METHODS

Reagents

(Nitrofurantoin (1-[(5-nitro-2-furaldehyde-*p*-hydroxybenzoyl)-hydrazone]), nifedipine (4-(2-nitrophenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine), nimodipine ((2-methoxy-1-methylethyl)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridin-dicarboxylic acid ester), nisoldipine (3-isobutyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate), nicardipine (4-(2-nitrophenyl)-2,6-dimethoxycarbonyl-1,4-dihydropyridine) were obtained from Chile Laboratories (Santiago, Chile). Nifurtimox (4-(5-nitro-furfuryliden) amine-3-methylthiomorpholine-1,1-dioxide) was obtained from Bayer Laboratories (Buenos Aires, Argentina). Dimethylsulphoxide (DMSO) was used to dissolve these drugs: a 1% v/v final concentration of DMSO in the experimental mixture did not affect the control values for any of the experiments.

Ascorbic acid, thiobarbituric acid (TBA), NADP, glucose-6-phosphate dehydrogenase, glucose-6-phosphate (G-6-P), UDP-glucuronic acid (UDPGA), *p*-nitrophenol, tris-(hydroxymethyl)-aminomethane (TRIS) and 2,4-dithiothreitol (DTT) were obtained from Merck (Chile).

Animals and treatment

Male Wistar rats (200–250 g) were obtained from the Biological Unit of Medicine Faculty, University of Chile, and fed on commercially available rat chow (Champion, Chile) *ad libitum*.

Microsomal preparation

Animals were fasted for 15 h with water *ad libitum* and sacrificed by decapitation. Livers were perfused *in situ* with four 25-ml volumes of 0.9% w/v NaCl, excised and placed on ice. All homogenization and fractionation procedures were performed at 4 °C using either a Suprafuge 22 Heraeus centrifuge or an XL-90 Beckmann ultracentrifuge. Liver tissue devoid of connective and vascular tissue was homogenized with five volumes of 0.154 M KCl, with eight strokes in a Dounce Wheaton B homogenizer. Homogenates were centrifuged at 9000 *g* for 15 min and the sediments were discarded. Supernatants then were centrifuged at 105 000 *g* for 60 min; sediments (microsomes, enriched in endoplasmic reticulum) were stored at −80 °C until use. Protein determinations were performed according to Lowry *et al.* (1951).

Redox conditions

Biotransformation condition. This condition was used to probe the pro-oxidant capacity of nitro-aryl drugs. Microsomes (1.5 mg ml^{-1}) were incubated with 50 mM buffer phosphate (pH 7.4), the NADPH generator system (1 mM NADP, 10 mM G-6-P, 5 U of G-6-P dehydrogenase) and each of the nitro-aryl compounds studied.

Oxidative condition. This condition was used to assay the antioxidant capacity of the nitro-aryl drugs. Microsomes were incubated with 600 µM $FeCl_3$ and 1 mM sodium ascorbate.

Lipoperoxidation

The extent of microsomal lipoperoxidation following both the biotransformation and oxidative conditions was estimated by determining malondialdehyde (MDA) concentrations, essentially according to Díaz-Araya *et al.* (1998). The preincubation periods at 37 °C to induce microsomal lipoperoxidation were 60 min in the presence of drug and NADPH (biotransformation condition) and 10 min in the presence of Fe^{3+}/ascorbate (oxidative condition).

UDP-glucuronyltransferase (UDPGT) activity

p-Nitrophenol (PNP) conjugation was studied essentially using the method described in Letelier *et al.* (1984). The reaction mixture contained 0.5 mM PNP, 2 mM UDPGA, 100 mM TRIS·HCl, (pH 8.5), 4 mM $MgCl_2$ and 1 mg of microsomal protein in a total volume of 0.5 ml. Blanks had no UDPGA; it was only added to the samples to initiate the enzymatic reaction. Blanks and samples were

incubated at 37 °C for 2 h (biotransformation condition) and for 20 min in the presence of Fe^{3+}/ascorbate (oxidative condition). The reaction was stopped by the addition of 0.5 ml of 10% w/v tricarbacaylic acid (TCA). The mixtures were centrifuged at 10 000 *g* for 10 min; 0.4-ml aliquots of the supernatants were alkalinized with 2.75 ml of 0.5 M NaOH. The estimation of PNP-glucuronide formed corresponded to the difference between the initial concentration present in the blanks and the residual concentration of samples.

Microsomal cytochrome P450 oxidase spectrum

Cytochrome P450 (Cyt-P450) oxidase can bind the lipophilic substrates that are going to be oxidized only when haemin-iron of Cyt-P450 oxidase is in the form Fe^{3+}. On the other hand, the reduced form of Cyt-P450 oxidase binds carbon monoxide and the conjugate formed presents a maximal absorption at 450 nm (Omura and Sato, 1964). To investigate if nitro-aryl drugs are oxidized by the Cyt-P450 system, we assayed the changes of the Cyt-P450 oxidase spectrum induced by its interaction with nitro-aryl drugs. Thus, microsomes (1 mg of protein) were incubated with a nitro-aryl drug for 10 min at 25 °C in the absence and presence of NADPH. Then, 5 mM sodium dithionite was added to the mixture (blank and sample) and the spectrum was developed after adding carbon monoxide to the sample. Interaction of the drug with Cyt-P450 oxidase was estimated by the decrease in the control absorption at 450 nm of Cyt-P450 oxidase measured in the absence of the drug.

p-Nitroanisole O-demethylation

p-Nitroanisole (PNA) O-demethylation — a reaction catalysed by the oxidative system of Cyt-P450 — was studied essentially using the method described in Letelier *et al.* (1985). The reaction mixture contained 2 mg of microsomal protein, the drug assayed, 50 mM buffer phosphate (pH 7.4), 1 mM PNA, 0.6 mM G-6-P, 6 mM NADP and 5 U of G-6-P dehydrogenase in a total volume of 1 ml. Blanks and samples were incubated with each nitro-aryl drug for 10 min and the enzymatic reaction was initiated by adding G-6-P deshydrogenase to the samples only. Then, mixtures were incubated for 20 min at 37 °C with constant agitation. The reaction was stopped by the addition of 0.5 ml of 10% w/v TCA. The mixtures were centrifuged at 10 000 *g* for 10 min; 0.5-ml aliquots of the supernatants were alkalinized with 0.75 ml of 1.0 M NaOH and absorbance of the samples at 410 nm was determined. The PNP formed in the enzymatic reaction was quantified using a PNP standard solution.

Oxygen consumption

The extent of oxygen consumption was determined polarographically for 10 min continuously with a Clark electrode 5331 (Yellows Springs instrument) in a Gilson 5/6 oxygraph.

Statistical analysis

Groups of test data (mean ± SD) were compared using Student's *t*-test for paired observations. Values were considered to differ significantly at $P < 0.05$.

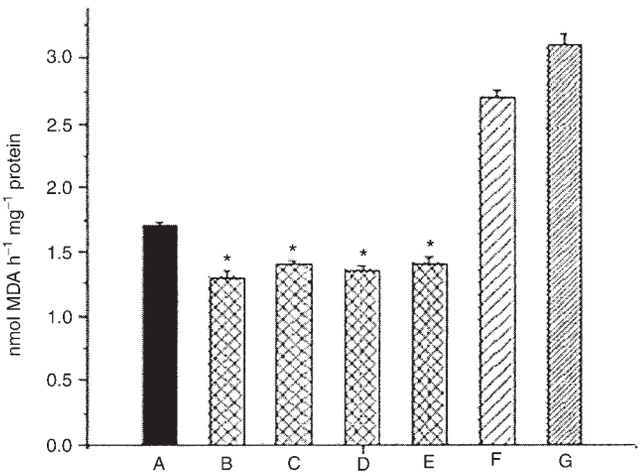

Figure 1. Microsomal lipoperoxidation in the presence of NADPH and nitro-aryl drugs: (A) reaction mixture in the absence of nitro-aryl drugs; (B) nicardipine; (C) nimodipine; (D) nifedipine; (E) nisoldipine; (F) nifurtimox; (G) nitrofurantoin. All drugs were added at 1 mM concentration. Assay conditions are described in the text. All values represent the mean of at least four independent experiments ± SD. *Values not statistically different ($P > 0.05$).

Table 1—Effect of nitro-aryl drugs on microsomal lipoperoxidation induced by Fe^{3+}/ascorbate

Drug	IC_{50}
Nifedipine	68.7
Nimodipine	27.7
Nicardipine	10.4
Nisoldipine	7.2
Nitrofurantoin	0.0
Nifurtimox	0.0

The IC_{50} values were obtained by percentage inhibition vs log[drug] graphics. Each value represents the drug concentration required to inhibit 50% of the microsomal lipoperoxidation induced by Fe^{3+}/ascorbate.

RESULTS

Lipoperoxidation

Only the microsomal preincubation with nifurtimox and nitrofurantoin in the presence of NADPH induced lipoperoxidation; values of nmol MDA formed h^{-1} mg^{-1} microsomal protein were 2.8 and 3.2, respectively, compared with the basal value of 1.7 obtained in the absence of drugs. In contrast, in the same conditions, all of the N-1,4-DHPs assayed (nicardipine, nimodipine, nifedipine and nisoldipine) inhibited basal microsomal lipoperoxidation by ca. 20% (Fig. 1). On the other hand, nitrofurantoin and nifurtimox had no effect on microsomal lipoperoxidation induced by the incubation of microsomes with Fe^{3+}/ascorbate before the addition of drugs. However, all of the N-1,4-DHPs assayed inhibited this lipoperoxidation and the higher effects were reached by nisoldipine and nicardipine; IC_{50} values were 7.2 and 10.4 μM, respectively, compared with 27.7 and 68.7 μM for nimodipine and nifedipine, respectively (Table 1).

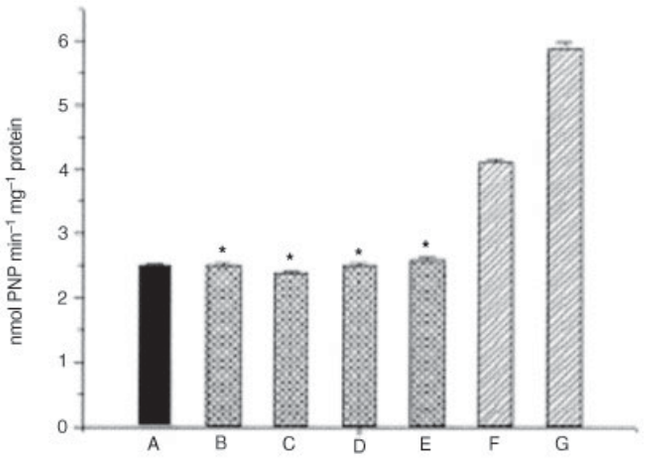

Figure 2. Microsomal UDPGT activity in the biotransformation condition of nitro-aryl drugs: (A) reaction mixture in the absence of nitro-aryl drugs; (B) nicardipine; (C) nimodipine; (D) nifedipine; (E) nisoldipine; (F) nifurtimox; (G) nitrofurantoin. All drugs were added at 1 mM concentration. Assay conditions are described in the text. All values represent the mean of at least four independent experiments ± SD. *Values not statistically different ($P > 0.05$).

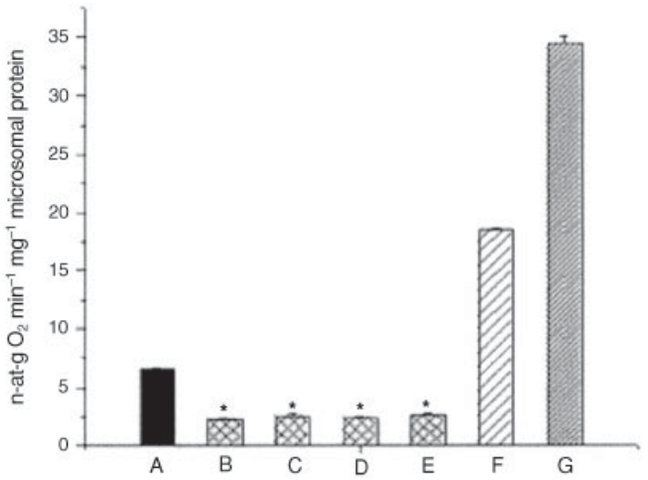

Figure 3. Microsomal oxygen consumption in the biotransformation condition of nitro-aryl drugs: (A) reaction mixture in the absence of nitro-aryl drugs; (B) nicardipine; (C) nimodipine; (D) nifedipine; (E) nisoldipine; (F) nifurtimox; (G) nitrofurantoin. Assay conditions are described in the text. All values represent the mean of at least four independent experiments ± SD. * Values not statistically different ($P > 0.05$).

Table 2—Effect of nitro-aryl drugs on UDPGT activation induced by Fe^{3+}/ascorbate

Drug[a]	UDPGT activities[b]
Control	1.12 ± 0.087
Fe^{3+}/ascorbate	3.79 ± 0.123[c]
Nifedipine	1.74 ± 0.079
Nimodipine	2.03 ± 0.098
Nisoldipine	1.85 ± 0.093
Nicardipine	1.90 ± 0.092
Nifurtimox	3.75 ± 0.134[c]
Nitrofurantoin	3.80 ± 0.126[c]

[a] The control is the reaction mixture without nitro-aryl drug and Fe^{3+}/ascorbate. All drugs were added to 1 mM concentration and assay conditions are described in the text.
[b] UDPGT activities were expressed as nmol PNP min^{-1} mg^{-1} microsomal protein. All values represents the mean of at least four independent experiments ± SD.
[c] Values not statistically different ($P > 0.05$).

UDP-glucuronyltransferase activity

Incubation of microsomes with nifurtimox and nitrofurantoin in the presence of NADPH increased PNP conjugation with UDPGA, a reaction catalysed by UDPGT enzyme; the control value (measured in the absence of drugs) was 2.4 nmol PNP min^{-1} mg^{-1} microsomal protein, compared with values of 4.2 and 6.1 obtained in the presence of nifurtimox and nitrofurantoin, respectively. In the same condition, none of the N-1,4-DHPs assayed modified the control value (Fig. 2).

Microsomal PNP conjugation with UDPGA was increased threefold in the presence of Fe^{3+}/ascorbate. All of the N-1,4-DHPs partially inhibited this activation by ca. 30% (Table 2). However, nifurtimox and nitrofurantoin did not affect the UDPGT activation induced by that oxidant system; the activity values in the presence of these drugs were similar to that obtained in the presence of Fe^{3+}/ascorbate and the absence of drugs (Table 2).

Oxygen consumption

The incubation of microsomes with nifurtimox and nitrofurantoin in the presence of NADPH stimulated the basal microsomal oxygen consumption (measured in the absent of drugs) by 2.5- and fivefold, respectively. However, all of the N-1,4-DHPs assayed inhibited the basal microsomal oxygen consumption by approximately 65% (Fig. 3).

Microsomal cytochrome P450 oxidase spectrum

Preincubation of microsomes with all the nitro-aryl drugs assayed before the addition of dithionite (reducing agent) decreased the maximum absorption of Cyt-P450 oxidase at 450 nm in a concentration-dependent manner (Table 3); the highest decreasing value was reached with 10 μM nifurtimox (100%). However, when dithionite was added after the nitro-aryl drug, this effect was not observed (data not shown).

O-Demethylation of *p*-nitroanisole

This reaction is catalysed by the Cyt-P450 system and can be inhibited by different lipophylic xenobiotics, which are the substrates of this oxidative system. The preincubation of microsomes with nitrofurantoin and nifurtimox inhibited O-demethylation of PNA in a concentration-dependent manner. The maximum concentration assayed was 10 μM and the percentage inhibitions developed by nitrofurantoin and nifurtimox were 100% and 75%, respectively (Fig. 4).

On the other hand, the incubation of microsomes with Fe^{3+}/ascorbate decreased the control value of PNA O-demethylation from 4.64 to 2.38 nmol PNP min^{-1} mg^{-1} microsomal protein. The preincubation of microsomes with all the N-1,4-DHPs assayed prevented (by 100%) inhibition of this enzymatic activity induced by Fe^{3+}/ascorbate (Table 4).

Table 3—Effect of nitro-aryl drugs on the absorption spectrum of cytochrome P450

Condition	Absorption at 450 nm
Control	0.053 ± 0.005
Nitrofurantoin	
1 μM	0.053 ± 0.004
5 μM	0.049 ± 0.005
10 μM	0.044 ± 0.004
Nifurtimox	
1 μM	0.040 ± 0.005
5 μM	0.032 ± 0.005
10 μM	0.000 ± 0.004
Nifedipine	
1 μM	0.049 ± 0.003
5 μM	0.048 ± 0.005
10 μM	0.041 ± 0.005
Nisoldipine	
1 μM	0.043 ± 0.005
5 μM	0.039 ± 0.003
10 μM	0.036 ± 0.002
Nicardipine	
1 μM	0.053 ± 0.003
5 μM	0.048 ± 0.005
10 μM	0.043 ± 0.005
Nimodipine	
1 μM	0.048 ± 0.005
5 μM	0.043 ± 0.003
10 μM	0.035 ± 0.003

Each value represents the mean of four independent experiments ± SD.

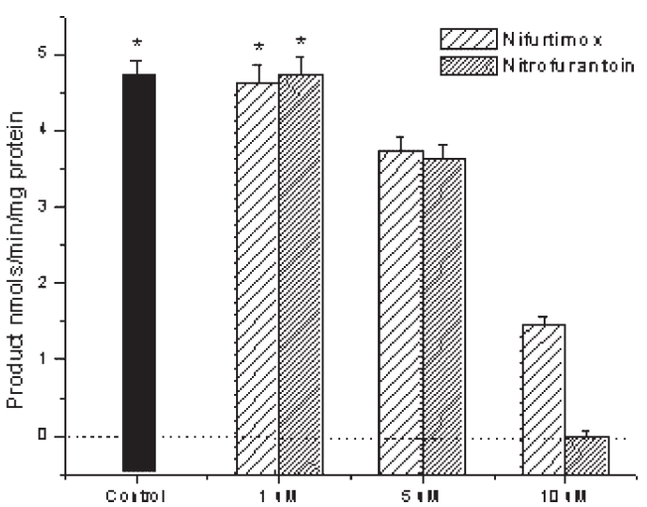

Figure 4. Effect of Nifurtimox and Nitrofurantoin on PNA O-Demethylation
Control: nitro-aryl drug was not added to reaction mixture. Assay conditions are described in method. All values represents the mean of at least four independent experiments ± SD. * Values statistically no different ($p > 0.05$).

DISCUSSION

One of the biotransformation pathways of nitro-aryl drugs is the nitro-reduction that can induce oxidative stress. The toxic effects observed after the administration of nitrofurantoin and nifurtimox have been associated with

Table 4—Effect of nitro-aryl drugs on PNA O-demethylation in the presence of Fe^{3+}/ascrobate

Drug[a]	nmol PNP min^{-1} mg^{-1} microsomal protein[b]
Control	4.64 ± 0.136[c]
Fe^{3+}/ascorbate	2.38 ± 0.074
Nicardipine	4.38 ± 0.143[c]
Nisoldipine	4.49 ± 0.131[c]
Nifedipine	4.44 ± 0.128[c]
Nimodipine	4.58 ± 0.119[c]

[a] The control is the reaction mixture in the absence of nitro-aryl drug and Fe^{3+}/ascorbate. All drugs were added to 1 mM concentration and the assay conditions are described in the text.
[b] All values represents the mean of at least four independent experiments ± SD.
[c] Values not statistically different ($P > 0.05$).

ROS generation induced by the nitro-reduction of these drugs. Nifurtimox is an anti-chagasic drug that provokes toxicity in chronic patients treated for relatively prolonged periods (Docampo, 1990) and nitrofurantoin is a drug administered only for short periods to patients with urine bacterial infection (up to 600 mg daily) because of its secondary effects (Procter and Gamble, 2000). However, N-1,4-DHPs are drugs used extensively in cardiovascular disease for long periods and they seem to provoke oxidative stress across their biotransformation pathways. Our *in vitro* results proved that nitrofurantoin and nifurtimox were able to induce ROS formation in microsomal biotransformation conditions: they provoked microsomal oxygen consumption (Fig. 3) and a characteristic peak of anion superoxide in the EPR spectrum appeared (data not shown). Thus, the microsomal lipoperoxidation and UDPGT activation observed may be associated with ROS generation by nitro-reduction of these drugs. In contrast, the N-1,4-DHPs developed antioxidant effects on microsomal lipoperoxidation and UDPGT activation induced by Fe^{3+}/ascorbate. Moreover, all of the N-1,4-DHPs inhibited microsomal oxygen consumption (Fig. 3) and no signal of oxygen radical species appeared in the EPR spectrum when microsomes were incubated with NADPH, oxygen and these drugs (data not shown). These results would indicate that, although nitro-reduction is one of the biotransformation pathways of these drugs (Scherling, 1988; Scherling, 1991), it would not be implicated in oxidative stress induction (Tables 1 and 2).

It is necessary to note that, in the microsomal biotransformation conditions assayed, oxidation and reduction of nitro-aryl compounds could be occurring as competitive and simultaneous processes. There is an abundance of date on the oxidative metabolism of N-1,4-DHPs in the literature (Auer *et al.*, 1982; Rush *et al.*, 1986; Scherling *et al.*, 1988; Grundy and Foster, 1996) but no antecedents exist for the oxidative metabolism of nitrofurantoin and nifurtimox. Published data indicate only that one-third of the nitrofurantoin dose administered is eliminated without being metabolized (Conklin and Hailey, 1969; Albert *et al.*, 1974). To investigate the possible oxidative metabolism of these drugs, we assayed the spectral changes of Cyt-P450 oxidase in their presence. The first step in the oxidative metabolism catalysed by Cyt-P450 oxidase required the binding of lipophilic drug to Fe^{3+}; in this condition, the spectroscopic properties of this enzyme and its maximum absorbance at 450 nm change. In the absence of

NADPH (not enzymatic nitro-reductive conditions) we observed a decrease of this absorption in a concentration-dependent manner in the presence of all the nitro-aryl drugs assayed. These results may point to all these compounds being substrates of the Cyt-P450 oxidative system (Table 3); the same results were obtained in the presence of NADPH, i.e. enzymatic nitro-reductive/nitro-oxidative conditions (data not shown). It is necessary to note that these effects were not observed when drugs were added to a mixture of the microsomal fraction and dithionite, a reducing agent of Cyt-P450 oxidase (Fe^{3+}) (data not shown). On the other hand, a change in the phospholipid environment seems not to be related to the decrease in maximum absorption of Cyt-P450 oxidase induced by nitrofurantoin and nifurtimox; lipoperoxidation in biotransformation conditions was not detectable at the incubation time of these experiments (10 min). In addition to these data, nitrofurantoin and nifurtimox inhibited the oxidative biotransformation of PNA in a concentration-dependent manner, similar to that observed in the experiments of maximum absorption of Cyt-P450 oxidase (Fig. 4 and Table 3). These data suggest that nitrofurantoin and nifurtimox may suffer nitro-reduction and oxidative metabolism. Additional experiments to identify possible oxidized metabolites of nitrofurantoin and nifurtimox must be realized to confirm this.

Because the nitro-reduction and oxidative metabolic pathways could proceed simultaneously in the organism, the balance between both biotransformation reactions may be one of the variables involved in the biological redox effects of nitro-aryl drugs. *In vivo* studies of the biotransformation of nimodipine in rat, dog and monkey confirm this by showing a large number of metabolites produced by common oxidative biotransformation reactions catalysed by the Cyt-P450 system (glucuronidation as a phase II reaction and reduction of the aromatic nitro group) (Scherling *et al.*, 1991). The administration of nimodipine for a prolonged period did not induce oxidative stress, although an aromatic nitro-reducing metabolic pathway occurred.

It is important to consider also the antioxidant capacity of the biological system analysed *in vivo* or *in vitro* because the generation or sequestering of ROS is regulated by the physiological antioxidant mechanism. In this respect, the presence of 1 mM GSH and GSH-transferase peroxidase activity in liver microsomes has been demonstrated, both of which seem to account for the main antioxidant capacity of the endoplasmic reticulum (Mosialou and Morgenstern, 1989; Hwang *et al.*, 1992). The nitro-reduction metabolic pathway of nitro-aryl drugs and the production of ROS can be occurring but oxidative effects on biological molecules occur only if the antioxidant mechanisms are overloaded.

One more factor to consider is the chemical characteristic of each compound. Additional chemical groups apart from the nitro-aryl group may display enzymatic or non-enzymatic redox properties: in the presence of air and daylight there is rapid oxidation of the dihydropyridine group to a pyridine ring (Díaz-Araya *et al.*, 1998); and the amine group of the dihydropyridine ring may act as an electron donor to the ring and as a proton donor. All inhibitory effects induced by N-1,4-DHPs were similar apart from the IC_{50} inhibition values of lipoperoxidation, which can be explained by the differences in the lipophilicity of these compounds. Such data is appointing because the dihydropyridine group is an important factor involved in the antioxidant effects of N-1,4-DHPs. In addition to the balance in the oxidative and reductive biotransformation pathway, the antioxidant capacity of the biological system investigated and the special chemical structure of dihydropyridines may contribute to the antioxidant effect developed by N-1,4-DHPs; it may explain the antagonist redox effect observed between N-1,4-DHPs and nitrofuran compounds and also the absence of redox-toxic effects observed *in vivo*.

Drugs are mainly lipophilic xenobiotics that must be metabolized across different pathways to water-soluble compounds in order to be eliminated in the urine of the organism. The Cyt-P450 oxidative system of hepatic endoplasmic reticulum oxidizes a large range of lipophilic drugs, from hydrocarbons to hydroxy- and amine-compounds. Several oxidative and reductive processes catalysed by the Cyt-P450 system — which transform drugs to toxic metabolites — are described (Karuzina and Archakov, 1994). Moreover, ROS and radical intermediates are formed across this metabolic pathway, which may activate UDPGT. Because toxic metabolites of the Cyt-P450 system can be eliminated across the UDPGT metabolic pathway and because glucuronides are pharmacology inactive and water-soluble compounds, this activation phenomenon may represent a physiological mechanism of detoxification.

Taking into account that the majority of the described redox toxic effects induced by biotransformation involve the Cyt-P450 oxidative system and that microsomes offer different chances to probe oxidative damage to macromolecules, we propose a liver microsomal preparation as a good *in vivo* approximation to probe the oxidative stress induced by reductive and oxidative metabolic pathways.

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