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Nitrofuran inhibition of microsomal lipid peroxidation

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Two nitrofuran compounds, nifurtimox and nitrofurantoin, inhibited in a concentration-dependent manner the NADPH-, iron-induced lipid peroxidation in rat liver microsomes, as shown by the decreased rate of MDA accumulation. Other nitro compounds (benznidazole and chloramphenicol) were relatively inactive. Nifurtimox inhibition affected polyenoic fatty acids and cytochrome P-450 degradation that follows lipid peroxidation. The ascorbate- or *tert*-butyl hydroperoxide-dependent lipid peroxidations were much less inhibited than the NADPH-dependent one. Nifurtimox and nitrofurantoin, but not benznidazole and chloramphenicol, strongly stimulated the microsomal NADPH-oxidase activity, thus supporting electron diversion, as the main cause of the inhibition of peroxidation initiation.

Microsome; Lipid peroxidation; Nitro compound; Nifurtimox; (Liver)

1. INTRODUCTION

One of the toxic consequences of oxidative stress in cells would be expected to be lipid peroxidation, initiated by active oxygen species [1] but, paradoxically, some oxyradical generators, such as quinones are also powerful antioxidants [2–4]. In the present study we have examined several nitro compounds (nifurtimox, nitrofurantoin, benznidazole and chloramphenicol) for their action on lipid peroxidation in rat liver microsomes. It is known that redox cycling of nitro compounds, especially nifurtimox and benznidazole, generates nitroanions and oxyradicals [5–7]. Nifurtimox and benznidazole are drugs extensively used for the treatment of American trypanosomiasis (Chagas' disease).

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Abbreviations: nifurtimox, 3-methyl-4-(5′-nitrofurfuryl-ideneamino)tetrahydro-4*H*-thiazone-1,1′-dioxide; benznidazole, *N*-benzyl-2-nitro-1-imidazole acetamide; MDA, malondialdehyde; DMFA, dimethylformamide; *t*-BuOOH, *tert*-butyl hydroperoxide; G-6-P, glucose 6-phosphate

2. MATERIALS AND METHODS

2.1. *Microsomal preparations*

Microsomes were obtained from the livers of 20 h fasted, male Wistar rats, 240–280 g, fed a Purine-like rat chow. The homogenate was prepared as in [8] and the microsomal fraction as in [6]. The pellet was resuspended in 150 mM KCl and used immediately for the peroxidation experiments.

2.2. *Assay of lipid peroxidation*

For the assay of NADPH-dependent lipid peroxidation, the incubation mixture consisted of liver microsomes (1.5 mg/ml), the NADPH-generating system (0.55 mM $NADP^+$, 5.5 mM G-6-P, 3.5 U G-6-P dehydrogenase and 5.5 mM $MgCl_2$), 1.7 mM ADP, 0.1 mM $FeCl_3$, 130 mM KCl and 23 mM Na_2HPO_4-NaH_2PO_4; · pH 7.4. The $FeCl_3$-ADP mixture was prepared separately before the beginning of the experiment. After thermal equilibration at 37°C, the reaction was initiated by addition of G-6-P dehydrogenase.

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Aliquots (1.0 ml) were withdrawn at the indicated times and MDA formation was measured. For the ascorbate-Fe-induced lipid peroxidation the incubation mixture consisted of microsomes (1.5 mg/ml) pre-heated at 100°C for 8 min, 2.0 mM ADP, 67 μM $FeCl_3$, 0.5 mM ascorbate(Na), 130 mM KCl and 23 mM Na_2HPO_4-NaH_2PO_4, pH 7.4. The reaction was started by adding ascorbate. The ascorbate solution was prepared immediately before use and kept under a stream of nitrogen. For the *t*-BuOOH-Fe-induced peroxidation, the incubation mixture consisted of 0.11 mM EDTA, 0.1 mM $FeSO_4$, 2.6 mM *t*-BuOOH, 130 mM KCl, microsomes and phosphate buffer as in the NADPH system. The reaction was started by adding *t*-BuOOH. The nitro compounds were added dissolved in DMFA. Controls received the same volume of solvent whose concentration never exceeded 1% (v/v); DMFA failed to affect the rate of lipid peroxidation. The incubation mixture's final volume was always 3.0 ml. Incubations were performed in a New Brunswick gyratory shaker, at 60 cycles/min and 37°C, under air, for the time indicated in section 3.

2.3. *Analytical methods*

MDA and cytochrome P-450 were determined as in [9] and [10], respectively, the corresponding values being expressed in nmol MDA (or P-450)/mg of microsomal protein. Protein and fatty acid content of microsomal membranes was determined as indicated in [11] and [12], respectively.

Oxygen uptake was measured with a Gilson Oxygraph, model 5/6, using the Clark electrode. The experimental conditions are described in table 2 legend.

2.4. *Chemicals*

Nifurtimox and benznidazole were generously provided by Bayer, Leverkusen, FRG and Hoffmann-La Roche, Basle, Switzerland, respectively. Nitrofurantoin, chloramphenicol, $NADP^+$, G-6-P, G-6-P dehydrogenase (from baker's yeast), ADP (grade IV), Na-ascorbate, *t*-BuOOH, DMFA, thiobarbituric acid and bovine serum albumin were purchased from Sigma, St. Louis, MO, USA. CO was purchased from Matheson, East Rutherford, NJ, USA. Other reagents were of analytical grade.

2.5. *Statistical analysis*

This was performed using Student's *t*-test for paired values. The values presented are the average ± SE of three or more independent experiments. When duplicate samples were measured, the experimental values deviated from the mean less than 5%.

3. RESULTS AND DISCUSSION

Fig.1 shows the effect of increasing concentrations of nifurtimox on MDA formation by microsome samples incubated with either the NADPH, the ascorbate or the *t*-BuOOH systems. With the former, nifurtimox effectively inhibited MDA formation in a concentration-dependent manner, but with the ascorbate and the *t*-BuOOH systems, nifurtimox scarcely inhibited lipid peroxidation. In close agreement with the inhibition of MDA accumulation, nifurtimox (i) prevented the destruction of microsomal polyenoic fatty acids (table 1) and (ii) inhibited the peroxidation-related [13] destruction of cytochrome P-450 (fig.2).

Comparison of several nitro compounds for their action on MDA formation revealed the greater activity of the two nitrofurans (fig.3). The calculated 50% inhibitory concentrations were (in

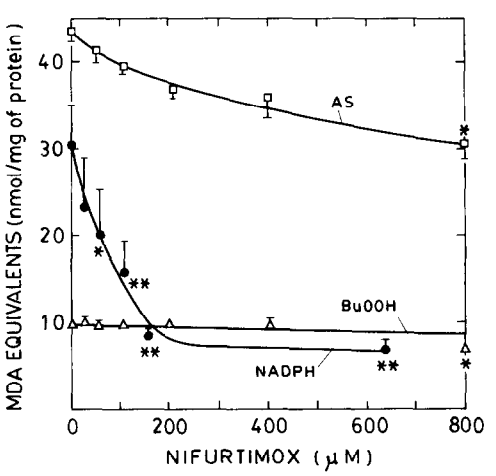

Fig.1. Effect of nifurtimox on MDA formation by microsomes incubated with the NADPH system (NADPH); the ascorbate system (AS) and the *t*-BuOOH system (BuOOH). Experimental conditions were as described in section 2; incubation time (min): 60 (NADPH), 90 (AS); 15 (BuOOH). * $P < 0.02$; ** $P < 0.01$, as compared with the sample without nifurtimox. The bar over the experimental points represents SE.

μM): 85 (nifurtimox), 175 (nitrofurantoin) and >1000 (benznidazole and chloramphenicol), which correlated, at least qualitatively, with the effect of the same nitro compounds on microsomal NADPH-oxidase activity (table 2). Nifurtimox inhibition of the *t*-BuOOH-dependent lipid peroxidation increased significantly when NADPH was added to the incubation mixture (table 3).

Our observations (figs 1–3 and table 1) clearly show the inhibitory effect of nifurtimox on lipid peroxidation. Similar inhibitions of MDA accumulation were obtained with nitrofurantoin but not with benznidazole and chloramphenicol (fig.3), which stresses the importance of the nitrofuran structure for peroxidation inhibition. Since reducing equivalents are consumed by both lipid peroxidation and nitro compound reduction, it seems reasonable to assume that nifurtimox and nitrofurantoin diverted electrons that would otherwise initiate the former process, thus inhibiting it [14–16]. This hypothesis is supported by the absence of extensive effects of nifurtimox on the ascorbate- and *t*-BuOOH-dependent lipid peroxidations (fig.1) and the different effects of nitro compounds on microsomal NADPH-oxidase activity (table 2). Since redox cycling of the assayed nitro compounds generates the corresponding nitro anions [5–7], their action as electron traps must depend on their rate of oxidation by oxygen, which was apparently faster for the nitrofurans

Table 1

Effect of incubation with NADPH and nifurtimox on the fatty acid composition of microsomal lipids

Fatty acid	NADPH-generating system		
	Omitted	Added	
		Nifurtimox omitted	Nifurtimox added
16:0	23.1 ± 2.1	29.9 ± 1.0	26.5 ± 1.1
18:0	26.8 ± 1.7	34.5 ± 3.9	33.1 ± 5.9
18:1*n*9	8.6 ± 0.8	12.1 ± 0.3	10.1 ± 0.6
18:2*n*6	11.6 ± 0.6	10.6 ± 1.1	11.2 ± 1.1
20:4*n*6	20.8 ± 1.3	9.8 ± 1.6	12.3 ± 1.2[a]
20:5*n*3	3.4 ± 0.5	1.5 ± 0.5	2.4 ± 0.9[a]
22:6*n*3	6.0 ± 0.9	2.0 ± 1.2	4.4 ± 2.5[a]
$\frac{20:4n6}{18:1n9}$	2.42 ± 0.27	0.81 ± 0.03	1.23 ± 0.13[a]
$\frac{22:6n3}{18:1n9}$	0.70 ± 0.12	0.16 ± 0.09	0.44 ± 0.02[a]

The experimental conditions were as described in section 2; 53 μM nifurtimox; 1 h incubation; values represent average wt% occurrence ± SE (n = 4 experiments).
[a] $P<0.05$ for samples with nifurtimox vs samples without nifurtimox

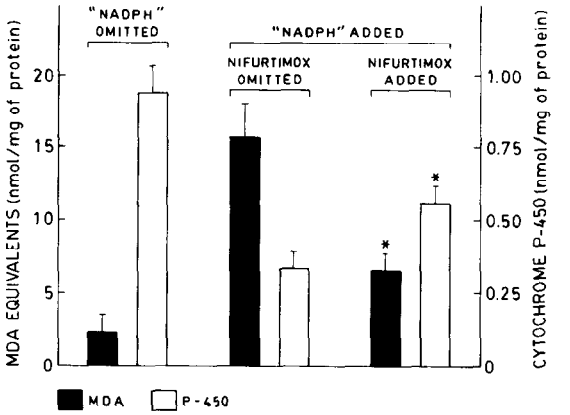

Fig.2. Effect of nifurtimox on cytochrome P-450 content and MDA formation in microsomal samples incubated with the NADPH system (NADPH). Experimental conditions were as described in section 2; 53 μM nifurtimox; 60 min incubation. * $P < 0.05$ for samples with nifurtimox vs samples without nifurtimox.

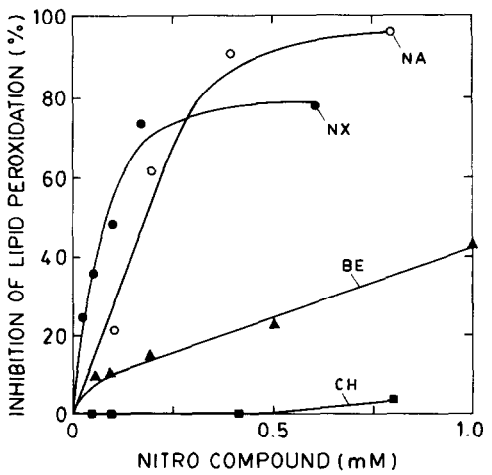

Fig.3. Effect of nifurtimox (NX), nitrofurantoin (NA), benznidazole (BE) and chloramphenicol (CH) on lipid peroxidation in microsomes incubated with the NADPH system. Experimental conditions were as described in section 2. Measurements in duplicate. 60 min incubation.

Table 2

Effect of nitro compounds on microsomal NADPH-oxidase activity

Nitro compound (200 μM)	Oxygen uptake $\left(\frac{\text{nmol } O_2/\text{min}}{\text{mg of protein}}\right)$
None	7.1 ± 0.3
Nifurtimox	20.4 ± 1.7[a]
Nitrofurantoin	37.0 ± 0.7[a]
Benznidazole	8.9 ± 0.4
Chloramphenicol	7.3 ± 0.4

Oxygen uptake measured polarographically, for 4–5 min; the incubation mixture contained NADPH-generating system, 130 mM KCl, 23 mM phosphate buffer, pH 7.4, and microsomes (1.1 mg/ml of protein). The nitro compounds were dissolved in DMFA; all samples contained 64 mM DMFA. Other experimental conditions were as described in section 2. Values represent the average ± SE of 4 measurements. [a] $P < 0.05$

Table 3

Effect of NADPH on the inhibition of *t*-BuOOH-dependent lipid peroxidation by nifurtimox

Nifurtimox (μM)	MDA equivalents (nmol/mg of protein)	
	− NADPH	+ NADPH
0	8.32	9.14
200	6.93(17)	5.77(37)

Experimental conditions were as described in section 2; 0.5 mM NADPH; 45 min incubation. The values are the average of two experiments and do not include the zero time values which were: 1.27 (*t*-BuOOH); 1.23 (*t*-BuOOH + NADPH); 1.37 (*t*-BuOOH + nifurtimox); 0.88 (*t*-BuOOH + NADPH + nifurtimox), in units as above. In parentheses, percentage inhibition of MDA formation

than for the other compounds (table 2). However, the possibility that nifurtimox nitroanion could inhibit the peroxidation propagation also deserves consideration. The effect of NADPH (table 3) lends support to such inhibition, as occurs with menadione [4] and anthracenediones [17], but NADPH was less active with nifurtimox, thus limiting the importance of the latter as an inhibitor of propagation reactions.

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