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THE EFFECT OF NITROFURAZONE AND FURAZOLIDONE ON INDUCTION OF CYTOCHROME P-450 DEPENDENT MONOOXYGENASE SYSTEM*

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Nitrofurazone (5-nitro-2-furaldehyde semicarbazone) and furazolidone 3-[(5-nitrofurfurylidiene)-amino]-2-oxazolidone) (Scheme 1) are antibacterial drugs applied in the therapy of mixed bacterial infections (mainly of the urinary tract and skin). They are in common use in veterinary medicine in the form of composite preparations. Although the genotoxic action of nitrofurazone and furazolidone, as well as of other 5-nitrofuranes, has been well documented [1], its mechanism of action still remains unelucidated. It has been suggested that reductive conversion of 5-nitrofuranes is responsible for genotoxicity of these compounds: the conversion leads to the formation of ammonium radical, and nitroso- and hydroxylaminofurane derivatives [1].

However, studies performed in this laboratory demonstrated only small participation of free oxygen radicals in the nitrofurane genotoxicity and drew attention to a possible role of nitrofurane radicals and hydroxylamine derivatives [2].

The present studies are aimed at determining the effect of nitrofurazone and furazolidone on the cytochrome P-450 dependent monooxygenase

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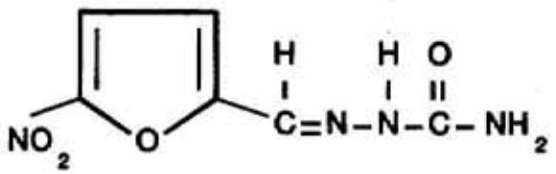
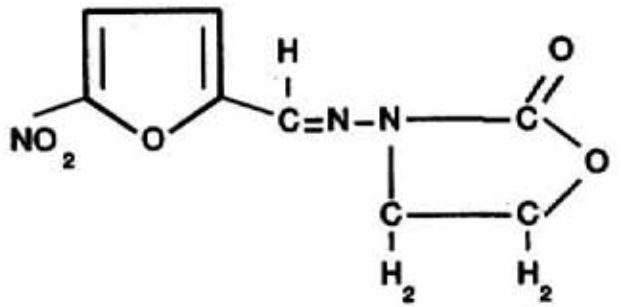


Nitrofurazone → 5-nitro-2-furaldehyde semicarbazone



Furazolidone → 3-[(5-nitrofurfurylidiene)-amino]-2-oxaz

Scheme 1. Structure of 5-nitrofurane derivatives

system. Since most of the toxic substances (drugs, poisons) are physiologically inactive but exhibit toxicity following transformation by several enzymes, especially the mixed function cytochrome P-450 dependent monooxygenase system, various molecular forms of this system are readily induced at the moment of appearance of a proper substrate [3].

Chemical inducers have been classified into three groups according to the type of cytochrome P-450 induced: i.e. the phenobarbital type (inducing cytochrome P-450), the 3-methylcholanthrene type (inducing cytochrome P-448), and those of the pregnenolone 16-γ-carbonitrol type (induction of other cytochrome forms) [3].

For testing induction of the cytochrome P-450 dependent monooxygenase system by nitrofurazone and furazolidone the CYPIA test (cytochrome P-450 induction assay) [4] was applied; this permits to distinguish between the phenobarbital and the 3-methylcholanthrene type of induction of the cytochrome P-450 dependent monooxygenase system.

Nitrofurazone or furazolidone, dissolved in sunflower oil, were injected into male Wistar rats (100 g body weight), either as a single injection (80 mg/kg body weight) and the animals were killed 48 h after the injection; or by injections (80 mg/kg body weight each) on three consecutive days, and the animals were killed 24 h after the last injection.

Phenobarbital dissolved in water was given intraperitoneally (80 mg/kg body weight each time) for three consecutive days. 3-Methylcholanthrene, dissolved in oil, was applied in a single intraperitoneal injection (80 mg/ kg body weight). The control animals received oil alone in a single or repeated intraperitoneal injections.

The S9 liver fraction was prepared and used according to Ames [5] for activation of the two standard promutagens: ethidium bromide (EtBr) and cyclophosphamide (CPA), respectively, by *Salmonella typhimurium* prototrophic strains: TA98 and TA100.

Induction of cytochrome P-450 dependent monooxygenases by the inducers of the phenobarbital or 3-methylcholanthrene type leads to reversion of *his* mutation in *Salmonella typhimurium* strains.

Ethidium bromide was used for testing induction of the 3-methyl--cholanthrene type of the inducer (0.5 - 5 μg/plate) and cyclophosphamide for that of the phenobarbital type of the inducer (200 - 800 μg per plate). S9 liver fraction (100 μl) was used per plate. Protein was determined according to Lowry *et al.* [6] and cytochrome P-450 concentration according to Omura & Saito [7]. The number of spontaneous his^+revertants (29

+ 9 colonies for *S. typhimurium* TA 98 and 164 + 33 for *S. typhimurium* TA 100) and those formed by liver preparation in the absence of promutagens (60 - 80 colonies) were taken into account.

Nitrofurazone at the dose of 1 × 80 mg or 3 × 80 mg/kg body weight did not cause induction of the cytochrome P-450 dependent monooxygenase form(s) of the 3-methylcholanthrene type (Table 1).

Table 1

Mutagenicity of ethidium bromide (EtBr) in S. typhimurium TA98 and cytochrome P-450 concentration in fraction S9 (100 µl) from rat liver after administration of 3-methylcholanthrene, nitrofurazone or furazolidone

EtBr at the concentration used in the test (1 µg) produced 30 ± 12 his^+ revertants. The results are expressed as mean values from 3 separate experiments

Compound tested (mg/kg body weight)		Number of his^+ revertants/mg EtBr	Cytochrome P-450 concentration (nmol/mg protein)
Oil alone (control)	0.2 - 0.4 ml	20	0.146
3-Methylcholantrene	1 × 80	2148	0.664
Nitrofurazone	1 × 80	28	0.111
	3 × 80	17	0.115
Furazolidone	1 × 80	45	0.110
	3 × 80	844	0.171

Induction of this type of the enzyme by furazolidone when applied in a high dose (3 × 80 mg/kg body weight) (Table 1) was very slight. Induction of cytochrome P-450 form(s) of the phenobarbital type was caused neither by nitrofurazone or furazolidone either at the dose of 1 × 80 mg or 3 × 80 mg/kg body weight (Table 2).

No significant changes were observed in the content of cytochrome P-450 in fraction S9 from the liver of rats treated with nitrofurazone or furazolidone, either at the dose of 1 × 80 or 3 × 80 mg/kg body weight while the content of cytochrome P-450 in fraction S9 obtained from the liver of rats injected with 3-methylcholanthrene or phenobarbital was in agreement with the literature data [4, 8, 9].

Table 2

Mutagenicity of cyclophosphamide (CPA) in S. typhimurium TA100 and cytochrome P-450 concentration in fraction S9 (100 µl) from rat liver after administration of phenobarbital, nitrofurazone or furazolidone

CPA at the concentration used in the test (800 µg) produced 227 + 42 revertants of his^+. The results are expressed as mean values from 3 separate experiments

Compound tested (mg/kg body weight)		Number of his^+ revertants/800 mg CPA	Cytochrome P-450 concentration (nmol/mg protein)
Oil alone (control)	0.2 - 0.4 ml	220	0.146
Phenobarbital	1 × 80	1194	0.822
Nitrofurazone	1 × 80	138	0.111
	3 × 80	190	0.115
Furazolidone	1 × 80	158	0.110
	3 × 80	161	0.171

The results obtained indicate that most probably toxicity of nitrofurazone and furazolidone is not based on the induction of the cytochrome P-450 dependent monooxygenase.

REFERENCES

1. Mac Calla, D. R. (1983) *Environ. Mutagen.*, **5**, 745 - 765.
2. Gajewska, J., Szczypka, M., Tudek, B. & Szymczyk-Wasiluk, T. (1990) *Mut. Res.*, **232**, 191 - 197.
3. Miller, E. C. & Miller, J. A. (1976) *ACS Monograph Series BO.*, **173**, American Society, 737 - 762.
4. Lesca, P., Fournier, A., Lecointe, P. & Cresteil, T. (1984) *Mut. Res.*, **129**, 299 - 310.
5. Ames, B. N., Mc Cann, J. & Yamasaki, E. (1975) *Mut. Res.*, **31**, 347 - 364.
6. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265 - 270.
7. Omura, T. & Saito, R. (1964) *J. Biol. Chem.*, **239**, 2370 - 2378.
8. Harada, M. & Omura, T. (1981) *J. Biochem. (Tokyo)*, **93**, 1361 - 1363.
9. Le Prevost, E. T., Gresteil, T., Columelli, S. & Leroux, J. P. (1983) *Biochem. Pharmacol.*, **32**, 1673 - 1682.