Minireview

Characterization of the genotoxic properties of nitrofurans: nitrofurazone and furazolidone

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Nitrofurans are a large group of synthetic nitro compounds widely used for various purposes: as drugs for humans or animals, as additives or preservatives [1, 2]. Nitrofuran derivatives (e.g. 5-nitrofurans and 2-substituted 5-nitrofurans) possess antibacterial, antiprotozoal, antifungal and antihelmintic properties. Some of these compounds show antibiotic activity against a wide variety of gram-positive and gram-negative bacteria, and they find clinical use in the management of infections of the urogenital tract and as typical agents for prevention and control of infections of burns, vaginitis and urethritis [3, 4]. 5-Nitro-2-furaldehyde-semicolonbarazon (nitrofurazone) and 3-[(5-nitrofururyliydiene)-amino]-2 oxazolidone (furazolidone) (Scheme 1) are antibacterial drugs applied in therapy of mixed bacterial infections (mainly of the urinary tract and skin).

The data on the genotoxicity of 5-nitrofurans and related derivatives are fragmentary [5]. The first publications on mutagenic activity of nitrofurans appeared in the late 1970’s [3, 4]. Later various authors [6-9] demonstrated their mutagenicity in tester strains of S. typhimurium with plasmid pKM101 (TA98, TA100). Also, Zampieri & Greenberg [10] reported that nitrofurans had a similar activity on the strains: Escherichia coli trp (susceptible to base-pair substitution) and E. coli lac (susceptible to frameshift mutation). It seems that nitrofurans and their analogs are directly acting mutagens [5-8].

In studies in vivo some nitrofurans caused a slight increase in the percentage of erythrocytes with micronuclei and did not induce chromosomal aberrations in bone marrow of rat [8].

Other 5-nitrofuran derivatives are carcinogenic; they can induce benign and malignant tumors of the mammary glands, adenomas or adenocarcinomas of the kidney, adenocarcinomas of the intestine and carcinomas of the external auditory canal [11]. On the other hand, Hayllar et al. [12] suggested that nitrofurazone as well as related 5-nitrofurans might have some antitumor activity, especially against primary and metastatic testicular tumors.

Recently, nifurtimox [1-((5-nitrofururyliydiene)amino)-3-methylthiomorpholine-1,1-dioxide] and other structurally related 5-nitrofurans with antiparasitic activity were tested for genotoxicity in the wing somatic mutation and recombination test (SMART) in Drosophila melanogaster, and were found to be both mutagenic and recombinogenic [13].

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1Abbreviations: CYFIA, cytochrom P-450 induction assay; 3-MC, 3-methylcholanthrene; PB, phenobarbital.
Nitrofurazone $\rightarrow$ 5-nitro-2-furaldehyde semicarbazone

Furazolidone $\rightarrow$ 3-[(5-nitrofurfurylidene)-amino]-2-oxazolidone

Scheme 1. Chemical structure of 5-nitrofuran tested

The mutagenicity of nitrofurazone and furazolidone

In our laboratory genotoxic properties of 5-nitrofurans were studied [14, 15] using the Ames test, SOS-Chromotest, repair test and CYPIA1 (cytochrome P-450 induction assay) test. Gajewska et al. [14] studied the mutagenic activity of nitrofurazone and furazolidone with tester Salmonella typhimurium strains TA97 and TA102. Both compounds were found to act as strong mutagens. The mutagenic activity was stronger with respect to strain TA97 (susceptible to DNA lesions of the frameshift mutation type) than to TA102 (susceptible to DNA lesions of the base pair substitution type). Both the two nitrofurans and their S9 mediated metabolites have similar mutagenic potency (J. Gajewska and M. Sczypka, unpublished).

The use of DNA repair S. typhimurium mutants in the mutation test often provides an additional source of information on the mode of action of the compounds tested. The results of the repair test showed that nitrofurazone and furazolidone produced an appreciable difference in zones of killing in S. typhimurium TA1538 (uvrB) and TA1978 (uvrB) in the presence and absence of metabolic activation (fraction S9). The obtained results suggested that both nitrofurazone and furazolidone and their metabolites formed in the presence of fraction S9 could be covalently bound to DNA (J. Gajewska and M. Sczypka, unpublished). Gajewska et al. [14] demonstrated also that the two nitrofurans studied were strong inducers of the SOS-repair system in strain PQ37 of E. coli K-12. Their S9 mediated metabolites have also mutagenic activity in the SOS-Chromotest (J. Gajewska and M. Sczypka, unpublished). Furazolidone was a better SOS-system inducer than nitrofurazone. This indicates that the SOS-repair system is involved in the mechanism of mutation induction by nitrofurazone and furazolidone. It seems possible that these compounds induce lesions which block the replicative complex which is able to induce the replication block generating base pair substitution. These study supply evidence for the suggestion of McCalla [5] who proposed that active recA and lexA genes belonging to the SOS-system are involved in the process of mutagenesis induced by nitrofurans. For these 5-nitro derivatives Gajewska et al. [14] observed the correlation between the base pair substitution mutation and SOS induction. Nitrofurazone and furazolidone which induced mutations in the Ames tester strain TA102, are also SOS-inducers in the SOS-Chromotest.

In the Ames test and the SOS-Chromotest, the antioxidants: ascorbic acid and sodium selenite, caused but a very slight decrease in the effect of the two 5-nitrofurans studied [14].

In general, to study the biological potency of unknown chemicals or the mechanism of action of known chemicals (drugs, poisons), it is necessary to combine in vitro target cell system with an appropriate mammalian enzyme system responsible for the biotransformation of individual chemicals to a biologically active state [16].

Metabolic activation of chemical compounds is possible due to the existence of several enzymes, especially the mixed function cytochrome P-450-dependent monoxygenase system. This monooxygenase system functions in the oxidative biotransformation of many drugs converting them to polar metabolites, facilitating on this way both their pharmacological inactivation and their elimination from the body. However, this system is also capable of
transforming some chemicals into highly reactive toxic metabolites that may produce a variety of genotoxic effects.

Drugs and other xenobiotics are able to induce in biotransformation processes synthesis of enzymes and of various molecular forms of cytochrome P-450 [16]. For testing induction of the cytochrome P-450-dependent monoxygenase system by nitrofurazone and furazolidone the CYP1A test [15] was applied; it permits to distinguish between the phenobarbital (PB) and the 3-methylcholanthrene (3-MC) type of induction of the cytochrome P-450-dependent monoxygenase system. The effect of nitrofurazone or furazolidone was studied in male Wistar rats (100 g body weight). Nitrofurazone at the doses of 1 × 80 mg/kg or 3 × 80 mg/kg body weight did not cause any induction of the cytochrome P-450-dependent monoxygenase form of the 3-MC-type. Induction of this type of cytochrome by furazolidone, when applied at a high dose (3 × 80 mg/kg body weight) was weak. Similarly, no induction of cytochrome P-450 form of the PB-type either by nitrofurazone or furazolidone was observed, either at the dose of 1 × 80 mg or 3 × 80 mg/kg body weight [15]. Thus it can be concluded that furazolidone administered to animals can be activated in a chain of metabolic reactions, the final stage of which is always linked with one of two specifically induced form of cytochrome P-450 (in this case of 3-MC-type).

The possible mechanisms of genotoxicity of the nitrofurans

The mutagenicity and possible carcinogenicity of nitrofurans may be due to the presence of two potentially reactive functional groups: the 5-nitro groups and the substituent at the 2-position of furan ring [5]. In bacterial systems the basic requirement for mutagenicity of nitrofurans is reduction of the nitro compound by at least three (I, II, III) nitrofuran reductases; this reduction leads to a chain of reactions resulting in formation of electrophilic species which can react with DNA. Parallely the nitrofuran ring is cleaved to the inactive open chain nitrile [5] (see Scheme 2). The nitroreduction and ring opening occur also in mammalian systems involving a variety of enzyme activities, e.g. xanthine oxidase, NADP-cytochrome c reductase and aldehyde oxidase [5].

The genotoxic effects of nitrofurans are assumed to be due mainly to the products of their reductive metabolism: the nitrofuranoanionic radical and the nitroso- and hydroxylaminofuranone derivatives. The hydroxylamine derivative appears to attack DNA via the nitrenium ion. In addition, the nitrofuranoanionic radical leads by autoxidation to formation of incomplete reduction oxygen species (hydrogen peroxide and hydroxide radicals) and to reconstruction of the original compound (Scheme 2) [5, 17]. It is also known that oxygen radicals can cause various DNA lesions and can show a mutagenic activity in bacterial and mammalian tests [18, 19].

However, studies performed by Gajewska et al. [14] demonstrated only weak participation of free oxygen radicals in the nitrofuran genotoxicity. It seems that nitrosofurane radicals and hydroxylamine derivatives formed both under aerobic and anaerobic conditions play a much greater role than other derivatives in the genotoxicity of nitrofurazone and furazolidone.

It should be added that, so far the best characterized metabolites are aminofuranone derivatives and their isometric open chain nitriles but these do not appear to be mutagenic or toxic [20, 21].

Recently, many authors suggest a hypothetical pathway of DNA-adduct formation of nitrofurans [22, 23]. The obtained evidence indicates that nitrofurans cause mutagenicity via a nitrenium ion which could be formed from hydroxylamine or one of its esters (an acetate or sulfate ester of the hydroxylamine). These products appear to attack DNA (Scheme 2).

Debnath et al. [24] described one of the possible mechanisms of mutagenesis of 2-nitrofurans and mechanisms of opening of nitrofurans ring. They also demonstrated that the electron density determined whether the C-O bond of the furan ring would be cleaved to form the inactive nitriles or would be stable enough to keep the original ring structure intact and help the nitrenium ion to bind covalently to DNA bases and cause mutation (Scheme 2). Studies on hydrophilicity and molecular orbital energies of nitrofurans [24] indicated that low electron density on C2 would weaken the carbon oxygen bond, promoting bond breakage and the elimination reaction. On the other
hand, high electron density on C2 would stabilize the furan ring, increasing the probability for the reaction with DNA. These results may be of importance in explaining genotoxic activities of nitrofurans and nitrofuran derivatives.

At present, it seems that the specific antibacterial and antiparasitic activities of nitrofurans may result from formation of nitrofuran free radicals and of oxygen-derived metabolites, which may lead to direct inhibition of biosynthetic reactions (DNA, RNA, protein synthesis) as well as breakage and degradation of macromolecules in the bacteria and in the parasite [5, 25, 26].

The knowledge of possible mechanisms of genotoxicity and the mode of action of nitrofurans and their oxidative metabolites, both in procaryotic and euucaryotic systems, could be helpful in explaining their biological effects and, eventually in selecting nitrofuran drugs for the therapy of humans.

REFERENCES


