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Enhanced Detection of Nitrofurantoin as a Mutagen by a Mutator R-Plasmid

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Enhanced detection of nitrofurantoin as a chemical mutagen by a novel mutator R-plasmid pEB019 isolated from clinical stool specimen was investigated using Ames reverse mutation assay. Plasmid pEB019 protected *E coli* WP_2 *trp*, WP_2 *trp uvrA*, AB1157, AB1157 *uvrA* tester strains against the bactericidal effects of nitrofurantoin. The mutagenicity assay results showed that there was no significant NFT-induced reversion in *E coli* AB1157 prototrophic strain at all concentrations used. There was however, a significant ($P < 0.01$) dose-related induced reversion mutation with strains possessing R-plasmid pEB019 and pKM101, but with the former showing a greater activity compared with pKM101. Reversions in prototrophic and auxotrophic strains, bearing R-plasmid pEB019 were about 10 fold more than the reversion in the isogenic strains without the plasmid. The possible implications of the superior mutability potential of R-plasmid pEB019 compared with pKM101 in the monitoring and detection of environmental chemical mutagens using bacterial test system are highlighted.

Key words: Mutator R-plasmid, Enhanced detection, Nitrofurantoin.

Introduction

Nitrofurantoin is a widely used antibacterial agent in the therapy of urinary tract infection of bacterial origin, such as *Escherichia coli, Staphylococcus aureus, Streptococci spp* and *Klebsiella aerogenes* (McOsker *et al* 1990; Pelletier *et al* 1992). It is also known that nitrofurans could be metabolically reduced to active products by mammalian enzymes including xanthine oxidase and microsomal NADPH-cytochrome c (Rosenkranz and Speck 1975; Rosenkranz and Speck 1976) resulting in DNA-strand breaks and cytotoxicity (Olive and McCalla 1975; Olive and McCalla 1977; Sesame and Boyd 1979). Nitrofurantoin at low concentrations has previously been reported to inhibit the inducible synthesis of both ß-galactosidase and galactokinase in *Escherichia coli* (Herrlich and Schweiger 1976) and of ß-galactosidase in *Klebsiella aerogenes* (Grant and DeSzoes 1971). At higher concentrations, nitrofurantoin treatment inhibits enzymes of the citric acid cycle as well as DNA,RNA and total protein synthesis in bacteria (McCalla 1979) by a mechanism which involves the reaction of electrophiles generated following bacterial reduction of nitrofurantoin with nucleophilic sites on bacterial macromolecules.

The genotoxicity of nitrofurans has been extensively reported to be based on base-pair substitution reversion mutation (McCalla and Voutsinos 1974; McCalla *et al* 1975a; McCalla *et al* 1975b; Green *et al* 1977; Chessin *et al* 1978; Lu and McCalla 1978) and induction of frame shift mutation (Obaseiki-Ebor and Akerele 1986). Since frame-shift mutation is likely to be more deleterious to the body cells than base pair substitution mutation and mutagenicity could serve as an index of carcinogenicity, we report in this study, enchanced detection of nitrofurantoin as a mutagen by a novel mutator R-plasmid pEBO19 isolated from clinical stool specimens.

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Materials and Methods

The bacterial tester strain used were *Escherichia coli* WP_2 *trp,* WP_2 *trp uvrA* (Green and Muriel 1976). The *E coli* transconjugant strains, WP_2 (pKM101), WP_2 *uvrA* (pKM101), AB1157 (pKM101), AB1157 *uvrA* (pKM101), WP_2 (pEB019), WP_2 *uvrA* (pEB019), AB1157 (pEB019), AB1157 *uvrA* (pEB019) were obtained from the conjugal transfer of the R-plasmids pKM101 and pEB019, the novel mutator R-plasmid (Akerele 1995). Single colonies of the tester strains were subcultured on nutrient agar slants and stored at 4°C.

Media: Nutrient broth No.2 (oxoid); nutrient agar (oxoid); Davis and Mingioli (DM) (Davis and Mingioli 1950) minimal salts solution were used. Supplemented minimal media (SMM) (Wang and Smith 1982) Arg-O was SMM agar without arginine. Arg-1.5 was Arg-O agar containing YENB broth at 1.5% ($v\,v^{-1}$) (Sargentini and Smith 1981).

Determination of Minimum Inhibitory Concentration (MIC) of Nitrofurantoin. The MIC of nitrofurantoin against the test bacterial strains was determined by the agar dilution protocol as previously described (Obaseiki-Ebor and Obasi 1986). Davis and Mingioli minimal agar medium supplemented with the relevant amino acids (50 mgl^{-1}) containing varying concentrations of nitrofurantoin were inoculated with about 10^5 colony - forming units (cfu) of the washed test cultures in sterile saline. The MIC was the lowest concentration of nitrofurantoin that completely inhibited growth after 24 h incubation at 37°C.

Determination of Chemical Mutagenesis. For the *E coli* WP_2 *trp* strains, the method of Brusick *et al* (1980) was suitably modified. Sub-inhibitory concentrations of nitrofurantoin were added to 2.5 ml molten agar overlay (0.7% agar No.1 oxoid) held at 45°C. 0.1 ml undiluted, washed test culture was added into the tubes, the contents of the tubes were gently mixed and immediately poured onto the surface of the Davis and Mingioli plates to a total

Table 1
Minimum inhibitory concentrations (MICs) of nitrofurantion (NFT)

Strain	NFT (Mgl^{-1})
AB1157	2.0
AB1157 (pEB019)	15.0
AB1157 (pKM101)	2.0
AB1157 *uvrA*	1.0
AB1157 *uvrA* (pEB019)	10.0
AB1157 *uvrA* (pKM101)	2.0
WP_2 *trp*	5.0
WP_2 *trp* (pEB019)	15.0
WP_2 *trp* (pKM101)	2.0
WP_2 *trp uvrA*	1.0
WP_2 *trp uvrA* (pEB019)	10.0
WP_2 *trp uvrA* (pKM101)	2.0

Table 2
Effect of plasmids pEB019 and pKM101 on nitrofurantoin mutagenesis in wild-type and *uvrA* strains of *E coli* WP_2*

Strain	Concentration (mgl^{-1})	Number of revertants in test plates	Number of revertants in negative control plates	Number of revertants in positive control plates (x 10^5)	P-level
WP_2 *trp*	0.63	17	12	458	0.01
	1.25	23	13	432	0.01
	2.5	41	12	457	0.01
WP_2 *trp*	0.63	145	13	406	0.01
(pEB019)	1.25	178	23	410	0.01
	1.5	222	32	428	0.01
WP_2 *trp*	0.63	15	3	450	0.01
(pKM101)	1.25	26	3	423	0.01
	1.5	38	2	425	0.01
WP_2 *trp*	0.63	23	3	433	0.01
uvrA	1.25	39	7	458	0.01
	2.5	47	9	447	0.01
WP_2 *trp*	0.25	226	13	418	0.01
uvrA	0.50	268	11	426	0.01
(pEB019)	0.75	385	12	453	0.01
WP_2 *trp*	0.25	40	1	482	0.01
uvrA	0.50	67	4	425	0.01
(pKM101)	0.75	89	3	446	0.01

*Mean of three experiments, each done in duplicate.

volume of 20 ml and allowed to solidify. Control plates, positive plates (not containing tryptophan, 50 mgl^{-1} and no test agent) and negative plates (not containing amino acids and test agent) were included. All plates were incubated at 37°C for 72 h and the mean number of colonies growing per plate in triplicate experiments were recorded.

For *E coli* AB1157, 0.1 ml aliquot of the harvested washed culture suspension was inoculated into 2 ml of soft agar (0.7% agar No. 1 oxoid) kept at 45°C, and containing the sub-inhibitory concentrations of nitrofurantoin and YEN B broth at 1.5% ($v\,v^{-1}$). This mixture was immediately poured on Arg-O plates. The mutant colonies were counted after incubation at 37°C for 72 h and the mean per plate in triplicate experiments were recorded.

Results and Discussion

The minimum inhibitory concentrations (MIC) of nitrofurantoin against the tester strains are shown in (Table 1), where *E coli* WP_2 proficient strain has an MIC of $5mgl^{-1}$ compared with the transconjugant strain possessing pEB019 which has an MIC of $15mgl^{-1}$. *E coli* WP_2 *uvrA* was expectedly very sensitive with MIC value of $1mgl^{-1}$, while the corresponding isogenic strain with plasmid pEB019, had MIC of $10mgl^{-1}$. Plasmid pKM101 did not confer nitro-furantoin resistance on the host recipient strains.

It was observed that the NFT resistance conferred by plasmid pEB019 was better expressed in repair deficient strains when compared with the repair proficient strains and it conferred the same level of resistance to NFT in test strains, *E coli* AB1157 and WP_2. Statistical analysis of the data was carried out using the paired student 't'- test at 99% confidence limit.

The mutagenicity assay results showed that there was no significant NFT-induced reversion in *E coli* AB1157 proficient strain at all concentrations used. There was however a significant ($p<0.01$) dose-related reversion with strains possessing plasmids pEB019 and pKM101. At the concentrations employed, induced reversion was about 10 fold more in strains possessing plasmid pEB019 compared with the isogenic strains containing pKM101 (Tables 2 and 3). It was observed

Table 3

Effect of plasmids pEB019 and pKM101 on nitrofurantoin mutagenesis in wild-type and *uvrA* strains of *E coli* K-12 AB1157*

Strain	Concentration (mgl^{-1})	Number of revertants in test plates	Number of revertants in negative control plates	Number of revertants in positive control plates (x 10^5)	P-level
AB1157	0.63	5	3	473	0.01
	1.25	16	8	445	0.01
	2.5	28	12	438	0.01
AB1157	0.63	132	15	402	0.01
(pEB019)	1.25	168	23	430	0.01
	1.5	115	32	424	0.01
AB1157	0.63	7	2	435	0.01
(pKM101)	1.25	12	5	406	0.01
	1.5	21	8	402	0.01
AB1157	0.63	11	3	426	0.01
uvrA	1.25	34	7	462	0.01
	2.5	63	4	418	0.01
AB1157	0.25	164	12	454	0.01
uvrA	0.50	286	25	447	0.01
(pEB019)	0.75	341	37	453	0.01
AB1157	0.25	18	5	465	0.01
uvrA	0.50	43	9	437	0.01
(pKM101)	0.75	80	12	452	0.01

*Mean of three experiments, each done in duplicate.

that the repair deficient *E coli* AB1157 *uvrA* strain was more sensitive than the isogenic AB1157 proficient strain. There was significant NFT-induced reversion ($p<0.01$) at all concentrations in *E coli* WP_2 *trp* and WP_2 *uvrA* strains.The induced reversion in DNA repair proficient and deficient strains possessing plasmid pEB019 was about 10 fold more than the reversion in the isogenic strains without the plasmid pEB019.

The conferment of resistance by plasmid pEB019 with nitrofurantoin bactericidal effects and the significant enhanced nitrofurantoin induced mutability of *E coli* strains showed that the plasmid possessed DNA damage properties since nitrofurantoin induced mutation in *E coli* was reported to be due to processes involving error-prone-repair (Bryant and McCalla 1980; Obaseiki-Ebor and Akerele 1987; Obaseiki-Ebor and Smith 1992). The enhancement of such mutations by R-plasmid pEB019 further corroborates the fact that pEB019 mediates enhanced error-prone repair activities. Earlier, Tazima *et al* (1975) had suggested that R-plasmid pKM101 enhanced mutagenic detection of mutagens which mediated mutagenesis by error-prone recombination repair activity. The observed comparative and enhanced detection of NFT mutagenesis by the novel R-plasmid pEB019, could have been facilitated by the efficient enhanced error-prone recombination DNA-repair properties of the novel R-plasmid. These *E coli* tester strains with R-plasmid pEB019 (particularly WP_2 *uvrA* pEB019) could be adopted for the *in vitro,* quick and efficient monitoring and detection of environmental mutagens using bacterial tester strains.

References

Akerele J O 1995 Enhanced mutagenesis by a novel mutator R-plasmid pEBO19 in *Escherichia coli* tester strains. Ph D thesis, Department of Microbiology, University of Benin, Benin City, Nigeria.

Brusick D J, Simmon U F, Rosenkranz H S, Ray V A, Stafford R S 1980 An evaluation of the *E coli* WP_2 and WP_2 *uvrA* reverse mutation assay. *Mut Res* **76** 169-190.

Bryant D W, McCalla D R 1980 Nitrofuran induced mutagenesis and error-prone repair in *Escherichia coli. Chem Biol Interact* **31** 151-166.

Chessin H, Mclauchlin T, MrOczkowski Z, Rupp W D, Low K B 1978 Radiosensitisation, mutagenicity and toxicity of *E coli* by several nitrofuran and nitroimidazoles. *Rad Res* **75** 423-431.

Davis B D, Mingioli E S 1950 Mutants of *E coli* requiring Methionine or vitamin B_{12}. *J Bacteriol* **60** 17-28.

Grant D J W, DeSzoes J 1971 Inhibitory effects of some antiinflammatory and other analgesics and nitrofurans on the induction of β-galactosidase synthesis in *Klebsiella aerogenes. Biochem Pharmacol* **20** 625-635.

Green M H L, Muriel W J 1976 Mutagen testing using *trp* reversion in *E coli. Mut Res* **38** 3-32.

Green M H L, Roger Muriel W J, Ward A C, McCalla D R 1977 Use of a simplified fluctuation test to detect and characterise mutagenesis by nitrofurans. *Mut Res* **44** 139-143.

Herrlich P, Schweiger M 1976 Nitrofurans, a group of synthetic antibiotics with a new mode of action: discrimination of specific messenger RNA classes. *Proc Natl Acad Sci (USA)* **73** 3386-3390.

Lu C, McCalla D R 1978 Action of some nitrofuran derivatives on glucose metabolism, ATP levels and macromolecular synthesis in *E coli. Can J Microbiol* **24** 650-675.

McCalla D R, Voutsinos D, 1974 On the mutagenicity of nitrofurans. *Mut Res* **26** 3-16.

McCalla D R, Olive P, Tu Y, Fan M L 1975a Nitrofurazone reducing enzymes in *E coli* and their role in drug activation *(in vivo). Can J Microbiol* **21** 1484-1491.

McCalla D R, Voutsinos D, Olive P L 1975b Mutagen screening with bacteria: nitridazole and nitrofurans. *Mut Res* **31** 31-37.

McCalla D R 1979 Nitrofurans. In: *Mechanism of Action of Antibacterial Agents.* ed Hahn F E. Springer-Verlag, New York, USA.

Obaseiki-Ebor E E, Akerele J O 1986a Nitrofuran mutagenicity: Induction of framshift mutations. *Mut Res* **175** 149-152.

Obaseiki-Ebor E E, Obasi E E 1986b Aspects of chloroquine mutagenicity. *Mut Res* **175** 51-59.

Obaseiki-Ebor E E, Akerele J O 1987 Expression of novel R-plasmid pEBO17 compared to pKM101 in *Escherichia coli* wild-type, *recA* and *uvrA* strains. *Mut Res* **192** 175-180.

Obaseiki-Ebor E E, Smit K 1992 Properties of R-plasmid pEBO17 which confers both enhanced UV-radiation reistance and mutagenicity to wild-type *recA* and *umuC* strains of *Escherichia coli* k-12. *Mut Res* **267** 67-76.

Olive P L, McCalla D R 1975 Damage to mammalian cell DNA by nitrofurans. *Cancer Res* **35** 781-874.

Olive P L, McCalla D R 1977 Cytoxigenicity and DNA damage to mammalian cells by nitrofurans. *Chem Biol Interact* **16** 223-232.

Rosenkranz H S, Speck W T 1975 Mutagenicity of metronidazole: Activation by mammalian liver microsomes. *Biochem Biophys Res Commun* **66** 520-522.

Rosenkranz H S, Speck W T 1976 Activation of nitrofurantoin to a mutagen by rat liver nitro-reductase.

Biochem Pharmacol **25** 1555-1556.

Sargentini N J, Smith K C 1981 Much of spontaneous mutagenesis on *Escherichia coli* is due to error-prone DNA repair: implications for spontaneous carcinogenesis. *Carcinogenesis* **2** 863-872.

Sesame H A, Boyd M R 1979 Superoxide and hydrogen peroxide production and NADPH reduction stimulated by nitrofurantoin in lung microsomes: possible implications for toxicity. *Life Sci* **24** 1091-1096.

Tazima Y, Kada T, Murakami A 1975 Mutagenicity of nitrofuran derivatives including furyl furamide, a food preservative. *Mut Res* **32** 55-80.

Wang T V, Smith K C 1982 Effect of the ssb-1 and ssb-113 mutations on survival and DNA repair in UV-irradiated *UVrB* strains of *Escherichia coli* K-12. *J Bacteriol* **151** 188-192.