SISTER CHROMATID EXCHANGES IN PERIPHERAL LYMPHOCYTES OF URINARY TRACT INFECTION PATIENTS TREATED WITH NITROFURANTOIN

ŞARDAŞ, S., METİN, A., GÖK, S., KARAKAYA, A., E., AKYOL, N.

Department of Toxicology, Gazi University Faculty of Pharmacy and Department of Urology and Pediatry. TCDD Ankara Hospital, Ankara, Turkey.

Nitrofurantoin; a widely used antibacterial agent in the therapy of urinary-tract infections of bacterial origin has been widely discussed in recent years due to its genotoxicity. Sister chromatid exchanges were studied in lympohycte cultures of 15 urinary tract infection patients before and after medication with daily oral dose of 10 mg/kg or 400 mgr nitrofurantoin for 10 days. Both stages exihibited similar average numbers of SCE in circulation lymphocyts. The number of SCE was larger in smoking patients. Results of this study suggests that the short-term exposure to nitrofurantoin does not cause detectable cytogenetic abnormalities.

INTRODUCTION

Nitrofurantoin is one of a group of synthetic antimicrobials called nitrofurans which are effective against infections caused both by gram negative and positive bacteria[5]. Nitrofurantoin is effective against most common urinary tract pathogens including Escherichia coli, enterococci, klebsiella and enterobacter and not effective against serratia, pseudomonas and most strains of proteus. It is bacteriostatic in low concentrations (5-10 μ g/ml) and bactericidial in higher concentrations[1].

The exact mechanism of nitrofurantoin bactericidal action is not fully understood, but it appears to interfere with bacterial carbonhydrate metabolism in the Krebs cycle and perhaps other bacterial cell functions[3]. Nitrofurantoin is rapidly absorbed after oral administration and is completely bound to protein in the blood stream. The carrier protein is splitt of in the kidney, so the free drug can act in the urine. Excretion is both by glomerular filtration and tubular secretion and in renal failure excretion is reduced so that it cannot be used in uremic patients[4]. Nitrofurantoin is widely used in urology practice in the treatment of urinary tract infections with the daily dose of 10 mg/kg or 400 mg for 7-10 days.

Potent mutagenicity of nitrofurans in bacteria and their tumorogenicity in laboratory animals have been shown and widely discussed in recent years that they may also present a genotoxic risk to humans[7, 8, 9]. An extremely sensitive way to evaluate the mutagenicity of chemical compounds is to count the number of DNA exchanges between the chromatids in the chromosomes in somatic cells. A increased number of such sister chromatid exchanges (SCE) in lymphocytes reflects the influence of mutagens[6].

In this study an attemp has been made to evaluate the frequency of sister chromatid exchanges in periferal lymphocytes of patients due to short-term nitrofurantoin intake for treatment of urinary tract infections.

MATERIAL AND METHOD

Urinary tract infection was diagnosed in 15 patients admitted to Urologic and Pediatric Clinics of Turkish State Railways Ankara Hospital with the urinary symptoms such as pain or frequency in urination and the detection of more than 6 leucocytes in one microscopic field (40x) in the urine and culturing the urine by disc diffusion test.

13 (86.7 %) of the patients were male and 2 (13.3 %) were female and the average age of the patients were 33.33[10-66]. The accompanying pathologies in the patients were also identified (Table I).

Tablo I: The distribution of urinary pathologies in patients.

Pathology	n	%
Pure urinary tract infection	8	53.3
Urinary tract infection + urinary stone	3	20.0
Urinary tract infection + benign prostatic hyperplasia	4	26.7

Nitrofurantion is given in daily dose of 10 mg/kg or 400 mg according to the urine culture results and when bacteria is not cultured, nitrofurantoin in given ampirically for 10 days orally.

The subjects did not use other drugs during the period of therapy.

The distribution of urinary pathogens were listed in Table - II.

Tablo 2: The distribution of urinary pathogens.

Urinary pathogen	n	%
E. Coli	4	27.6
Klebsiella	1	6.7
Enterococcus	1	6.7
Bacteria uncultured	9	60.0

It is well established that the frequency of SCE is increased in smokers and some papers report a dose-response relationship both in man and in experimental animals exposed to cigarette smoke[2]. Therefore smoking habits and its duration is also taken into consideration (Table III).

Peripheral blood (heparinized) drawn aseptically from each patient before and after 10 days of nitrofurantion therapy was incubated in the medium containing TC 199 (Gibco), % 19 fetal calf serum (Gibco), antibiotics (penicillin, and streptomycin) and 5-bromodeoxyuridine at a final concentration of 10 μ g/ml. The lymphocytes were induced to divide by phytohaemaglutinin (Sigma) for two cell cycles at 37°C for 72 hours. Two hours before harvesting colcemid (10 μ g/ml) was added to accumulate metaphases. Chromosomal preparations made by the conventional method were stained by the fluorescence plus Giemsa technique of Wolf and Perry[20]. An average of 30 metaphase plates with 46 intact chromosomes were scored to calculate the mean SCE frequency. The SCE's were scored blindly in the sense that slides for each case were coded and the scorer did not know the case under study.

RESULTS

The mean SCE/cell frequencies in patients taking nitrofurantoin before and treatment are listed in Table III. The mean SCE/cell before treatment was 6.00 ± 0.70 and 6.06 ± 0.79 after treatment. The frequency of SCE/cell in patients receiving short term nitrofurantoin was not different to pretreatment ($p > 0.05$).

Smokers in the group did have an increased mean SCE/per cell in peripheral blood lymphocyte chromosomes compared to non-smokers. Indeed, the average number of SCE was found to increase progressionaly with the duration and the daily number of cigarettes smoked.

DISCUSSION

The genotoxicity of nitrofurans has been extensively reported to be based on base-pair substitution reversion mutation from tryptophan-dependence to independence during growth of the E. coli WP_2 uvr trp strain in glucose minimum salts medium[4, 10, 11, 12].

Induction of frameshift mutation by nitrofurans has not been extensively reported except in the E. coli Lac (—) strain (a frameshift mutant induced by proflavin) reverted to the lac (+) strain by nitrofurazone[21]. Invitro experiments demonstrating nitrofuran mutagenicity have shown that nitrofuran concentrations inducing mutagenicity were close to or even overlapped the minimum bactericidal concentration[17]. It is known that nitrofurans could be metabolically reduced to active products by mammalian enzymes including xanthine oxidase and microsomal NADPH-cytochrome c, resulting in DNA-strand breaks and cytotoxicity[4, 15, 18, 19] and even enhanced cytotoxicity under hypoxic conditions[13].

Obasideiki[16] using some of the internationally recognised standart tester strains such as S. thyphimurium TA97, E. coli WP_2 and E. coli EE97 showed that therapeutic nitrofurantoin induces frameshift mutations at concentrations several fold lower than the minimum inhibitory concentrations in glucose minimum salts medium and advocate cautious use of nitrofurans in the management of infections since subbactericidal concentrations could produce undesirable cytogenetic effects and also said that nitrofura-

Table III: The mean SCE/cell frequencies before and after nitrofurantoin treatment

Name	Age	Sex	Amount of Cigarettes smoked per day	duration (yr)	Diagnosis	SCE/cell Pretre.	post tre
1.D.S.	39	M	—	—	UTE	4	4
2.H.S.	55	M	20	30	UTE + BPH	9	9
3.H.K.	35	M	20	15	UTE + Calculi	7	7
4.N.Ç.	12	F	—	—	UTE	4	4
5.Z.P.	45	M	20	15	UTE	7	8
6.M.Y.	12	M	—	—	UTE	4	4
7.R.Ç.	53	M	—	—	UTE + BPH	3	3
8.A.Y.	51	M	40	20	UTE + Calculi	10	10
9.B.D.	27	M	10	5	UTE	6	6
10.M.S.	40	M	20	20	UTE + Calculi	8	8
11.M.A.	63	M	20	40	UTE + BPH	9	9
12.F.E.	58	M	5	25	UTE + BPH	5	5
13.N.Ü.	10	F	—	—	UTE	2	2
14.A.S.	50	M	20	30	UTE	8	8
15.K.G.	10	M	—	—	UTE	4	4

UTE : Urinary tract infection
BPH : Benign prostatic hyperplasia.

zone in more potent than nitrofurantoin as a frameshift mutagen.

Based on above mentioned experimental findings and the current knowledge of nitrofurans; it is presumed that each individual treated with therapeutic concentration of nitrofurantoin is under a risk and at the same time induce undesirable effects in the body alls. Our findings of unchanged SCE rates urinary tract infection patients on short term nitrofurantoin therapy do not indicate a detectable chromose damaging effect on its human users. No comparisons are possible as no information of similar study is published. However, significant increase in the mean number of SCE/per cell of smoker patients compared to non-smokers are in agreement with the observation of other authours.

REFERENCES

1. **Andriole, V.T.:** Urinary tract agents: quinolones, nitrofurantoin and methamine in Mandell G.L., Douglas R.G.Jr., Bennentt, J.E. (Eds). Principles and Practice of Infectious Diseases. John Wiley sons, New York, 244, 1985.
2. **Bala Krishna, M.P.:** Frequency of SCE in cigarette smokers. Hum. Genet., 52:343 (1979).
3. **Chamberlain, R.E.:** Chemotherapeutic properties of prominent nitrofurans. J. Antimicrob. Chemother., 2: 325 (1976).
4. **Chessin, H., McLauchin, Z., Mroczkowski, W.D., Low, K.B.:** Radiosensitisation mutagenicity and toxicity of E. coli by several nitrofurans and nitroimidazoles. Radiation Res., 75: 423 (1978).
5. **Cunha, B.A.:** Nitrofurantoin-current concepts. Urology, 32:67 (1988).
6. **Das, B.C.:** Factors that influence formation of Sister Chromatid Exchanges In Human Blood Lymphocytes. CRC Critical Reviews in Toxicology. 19:43 (1988).
7. **Food and Drug Administration:** Furazolidone (NF-180). Fed. Reg., 49: 34971 (1984).
8. **Klemencic, J.M., Wang, C.Y.:** Mutagenicity of nitrofurans in G.T. Brayan (Ed) Carcinogenesis Vol. 4. Nitrofurans: Chemistry, Metabolism, Mutagenesis and Carcinogenesis, Raven, New York, 99 (1978).
9. **Maranpot, R.R.:** Ovarian toxicity and carcinogenicity in eight recent National Toxicology Program studies, Environ. Health Perspect., 73:125 (1987).
10. **McCalla, D.R., Voutsinos, D.:** One the mutagenicity of nitrofurans. Mutation Res., 26:3 (1974).
11. **McCalla, D.R., Olive, P., Tu Y. Fan, M.L.:** Nitrofurazone-reducing enzymes in E. coli and their role in drug activation in vivo. Can. J. Microbiol., 21:1484 (1975).
12. **McCalla, D.R., Voutsinos, D., Olive, P.L.:** Mutagen screening with bacteria: niridazole and nitrofurans. Mutation Res., 31:31 (1975).
13. **Mohindra, J.K., Rauth, A.M.:** Increased cell killing by metronidazole and nitrofurazone of hypoxic cell compared to acrobic mammalian cells. Cancer Res., 36:930 (1976).
14. **Olive, P.L., McCalla, D.R.:** Damage to mammalian cell DNA by nitrofurans. Cancer Res., 35:781 (1975).
15. **Olive, P.L., McCalla, D.R.: Cytotoxigenicity and DNA damage to mammalian cells by nitrofurans. Chem. Biol. Interact., 16:223 (1977).**
16. **Osabeiki-Ebor, E.E., Akerele, J.O.:** Nitrofuran mutagenicity; induction of frameshift mutations. Mutation Res., 175:149 (1986).
17. **Rosenkranz, H.S., Speck, W.T.:** Activation of nitrofurantoin to a mutagen by rat liver nitro-reductase. Biochem. Pharmacol., 25:1555 (1976).
18. **Rosenkranz, H.S., Speck, W.T.:** Mutagenicity of metronidazole activation by mammalian liver microsomes. Biochem. Biophys. Res. Commun., 66:520 (1975).
19. **Sesame, H.A., Boyd, M.R.:** Superoxide and hydrogen peroxide production and NADPH reduction stimulated by nitrofurantoin in lung microsomes: Possible implications for toxicity. Life Sci., 24:1091 (1979).
20. **Wolf, S., Perry, P.:** Differantial Gimsa staining of SCE without autoradiography. Chromosoma (Berl.) 48:341 (1974).
21. **Zampieri, A., Greenberg, J.:** Nitrofurazone as a mutagen in E. coli. Biochem. Biophys. Res. Commun., 14: 172 (1964).