SHORT COMMUNICATION

CHROMOSOME ABERRATIONS AND DNA REPAIR SYNTHESIS IN CULTURED HUMAN CELLS EXPOSED TO NITROFURANS¹⁾

AKIRA TONOMURA AND MASAO S. SASAKI

Department of Human Cytogenetics, Tokyo Medical and Dental University,
Yushima, Bunkyo-ku, Tokyo 113

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Derivatives of nitrofurans are potent antibacterial agents, and have long been widely used as clinically useful topical antiseptics, antibacterial agents in veterinary medicine, feed additives for domestic animals as well as food preservatives. Certain derivatives of nitrofurans have been demonstrated to induce phage in *E. coli* lysogenic for phage λ (Endo *et al.* 1963; McCalla 1964), induce significant number of mutations in *E. coli* (Zampieri and Greenberg 1964) and induce neoplasms in the rats (Stein *et al.* 1966). Since furoylfuranamide²⁾, which is one of the derivatives of nitrofurans and commercially known as AF2, is now widely used as a food preservative in Japan, its genetic effect is an immediate public concern. In view of the testing of the genetic effects of nitrofurans, we examined the chromosome damaging effect and unscheduled DNA synthesis as an indication of DNA damage and its repair.

Nitrofurans tested in the present experiment were 5-nitro-2-furaldehyde semicarbazone (nitrofurazone), N-(5-nitro-2-furfurylidene)-1-aminohydrantoin (nitrofurantoin), 3-(5-nitrofururylidene amino)-2-oxazolidone (furazolidone), 5-nitro-2-furyl acrylic acid amide (nitrofurylacrylamide), 2-(2-furyl)-3-(5-nitro-2-furyl) acrylic acid amide (furylfuranamide), 6-hydroxymethyl-2-[2-(5-nitro-2-furyl)-vinyl]-pyridine (furopyrinol) and 2-amino-5-[2-(5-nitro-2-furyl)-1-(2-furyl)-vinyl]-1,3,4-oxadiazole (furanizole).

In the first instance, the experiments were concerned with the induction of chromosome aberrations in cultured human lymphocytes. Series of lymphocyte cultures were prepared from the peripheral blood of healthy adult donors by suspending leucocyte-rich plasma in medium NCTC-109 supplemented with 20 % fetal calf serum and 2 % phytohemagglutinin. Plasma vs. medium ratio was approximately 1:15. The test compounds were solved and serially diluted with dimethylsulfoxide (DMSO, Aldrich Chemical Co. Inc., Wis.) and added to the cultures at 48 h since the commencement of culture. Regardless of the concentrations of the test compounds, the final concentration of DMSO was 1 %. The cultures treated with 1 % DMSO alone constituted controls. The

1) This work was supported by the grants from the Ministry of Education and US-Japan cooperative Medical Program.

2) Since 1950, authorized by the Japanese Ministry of Health and Welfare, nitrofurazone was used as a preservative for certain foods, such as soybean curd, fish sausage and fish pastes. In 1954, nitrofurylacrylamide was also permitted to use as a food preservative, and they were replaced by furoylfuranamide in 1965.

cultures were incubated for further 24 hrs in the presence of the test compounds and fixed with 4 h prefixation treatment with colchicine. Chromosome preparations were made according to the standard flame-drying method.

The results are presented in Fig. 1. It is evident that the nitrofurans are potent chromosome damaging agents. In control cultures, the range of the frequency of chromosome aberrations was between 0.07 and 0.14 per cell, an average of the 6 cultures being 0.097 per cell. Aberrations in the control cultures were mostly chromatid gaps, chromatid breaks occurring occasionally. In cultures treated with furamizole, nitrofurylacrylamide, furpyrinol and furylfuramide, a significant number of chromosome structural aberrations were observed. The induced chromosome aberrations were essentially of the chromatid type, constituting gaps, breaks and exchanges at the chromatid level. However, with the dose range tested in the present experiment, nitrofurazone, nitrofurantion and furazolidone produced no significant amount of chromosome aberrations. The testing for the chromosome damaging activity is one of the simple cytogenetic test systems for the genetic hazards of chemicals, but the biological significance is restricted to the

Fig. 1. Dose-response relations of chromatid aberrations in cultured lymphocytes exposed to nitrofurans. Chromatid exchanges and isochromatid aberrations were each scored as one aberration. For each dose, 100 cells were scored except in some of the highest doses in which 20-65 cells were available.

Fig. 2. The levels of unscheduled DNA synthesis elicited in normal cells by nitrofurans. For each point, silver grains were counted in 100 lightly labeled nuclei. The levels of nitrofurantion-stimulated unscheduled DNA synthesis in XP cells and control levels for normal and XP cells were within a range covered with two dashed lines.

chromosome structural change which is only a part of the wide spectrum of all possible genetic changes elicited by mutagens.

In the second experiment, nitrofurans were tested for their ability to induce unscheduled DNA synthesis as an indication of the production of damage to DNA (Rasumssen and Painter 1966). Cultured fibroblasts obtained from the skin biopsies from a normal person and a patient with xeroderma pigmentosum (XP) were grown in tissue culture chamber slides (Division Miles Lab., Inc., Ill.) each having 4 small compartments. The cells were cultured in Leibovitz's medium L-15 supplemented with 10 % NCTC-109 and 15 % fetal calf serum. At an about 100 % confluence, medium was replaced with arginine deprived McCoy 5a medium (ADM) supplemented with 5 % dialysed fetal calf serum and 5 μ g/ml arginase. At 24 h of incubation in ADM, the cells were exposed for 1.5 hrs to the test compounds solved in ADM, washed with Hanks' BSS and incubated for 2.5 hrs in the ADM containing 10 μ Ci/ml 3 H-thymidine (S.A. 24 Ci/mM). The slides were then washed with Hanks' BSS, fixed in methanol-acetic acid (3:1) mixture, air dried, soaked in the cold 5 % TCA for 30 mins and washed in running tap water. The slides were prepared for autoradiography by coating with Kodak NTB3 nuclear track emulsion, exposing for 1 week at 4°C and developing for 3 mins at 10°C in the full strength of Kodak developer Dektol. The level of unscheduled DNA synthesis was evaluated by counting the silver grains over lightly labeled nuclei in the non-S phase of the cell cycle.

All nitrofurans did not induce the unscheduled DNA synthesis in the XP cells. However, when tested on normal cells, furamizole, nitrofurylacrylamide, furpyrinol and furylfuramide were effective to induce unscheduled DNA synthesis as indicated by the significantly increased incorporation of 3 H-thymidine into the nuclei of non-S cells (Fig. 2). The level of the unscheduled DNA synthesis stimulated by an equimolar concentration of these compounds decreased in the following order; furamizole, nitrofurylacrylamide, furpyrinol and furylfuramide. With the doses range tested, those for nitrofurazone, nitrofurantion and furazolidone were within the limits of the negative level. Thus, the capacity to induce unscheduled DNA synthesis was in parallel with the chromosome damaging capacity. Both DNA- and chromosome damaging activities of these compounds were not related with their solubility in water.

The XP cells were demonstrated to show a severe deficiency of unscheduled DNA synthesis following ultraviolet light irradiation (Cleaver 1968), possibly resulting from the inherent defect in recognition step in the excision repair of thymine dimers (Cleaver 1969; Setlow *et al.* 1969). The XP cells are also defective in excising the lesions induced in DNA by several carcinogens including 4-nitroquinoline-1-oxide (4NQO) (Stich and San 1971), whereas they can repair damage induced by X-rays, methyl methanesulfonate and N-methyl-N'-nitro-N-nitrosoguanidine and carry out unscheduled DNA synthesis (Cleaver 1968, 1971). According to these lines of experimental evidence coupled with the results obtained from the present experiment, it is reasonable to assume that the nitrofurans induce the excisable DNA damage similar to that by 4NQO. However, it should be pointed out that the action of nitrofurans seems to be somewhat different from that of 4NQO. As compared with their chromosome breaking activity, 4NQO stimulates a relatively larger amount of unscheduled DNA synthesis than nitrofurans. For instance,

with the dose which produce 1 chromosome aberration per cell in lymphocyte culture system, the unscheduled incorporation of ^3H -thymidine was approximately 50 times higher following exposure to 4NQO than to furamizole. These response characteristics of the nitrofurans rather resemble those of mitomycin C, which induces rather small amount of unscheduled DNA synthesis as compared with its strong chromosome breaking activity.

Biological damage responsible for the production of chromosome aberrations by these nitrofurans seems to be complicated and is remained for further studies. Yet, the results of the present experiment clearly indicate that the nitrofuran derivatives, especially furamizole, nitrofurylacrylamide, furpyrinol, and, to the more important in view of the public concern, a food preservative, furylfuramide, induce damage to DNA and provoke recombination of genetic material as indicated by the DNA repair synthesis and the production of chromatid aberrations of the exchange type.

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