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Brief Communication

Ultra-Wide Band Electromagnetic Radiation Does Not Affect UV-Induced Recombination and Mutagenesis in Yeast

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Cell samples of the yeast *Saccharomyces cerevisiae* were exposed to 100 J/m^2 of 254 nm ultraviolet (UV) radiation followed by a 30 min treatment with ultra-wide band (UWB) electromagnetic pulses. The UWB pulses (101–104 kV/m, 1.0 ns width, 165 ps rise time) were applied at the repetition rates of 0 Hz (sham), 16 Hz, or 600 Hz. The effect of exposures was evaluated from the colony-forming ability of the cells on complete and selective media and the number of aberrant colonies. The experiments established no effect of UWB exposure on the UV-induced reciprocal and non-reciprocal recombination, mutagenesis, or cell survival. *Bioelectromagnetics 19:128–130, 1998.* © 1998 Wiley-Liss, Inc.

Key words: UWB; recombination; mutagenesis; yeast; ultraviolet light

INTRODUCTION

Ultra-wide band (UWB) systems have recently been developed for use as radars and as a means to suppress electronically vulnerable targets. UWB radiation is produced as short high-voltage pulses with an extremely fast rise, providing for the spectral bandwidth from 0 Hz to 2 GHz. The unusual properties of UWB radiation have raised concerns about its bioeffects and possible health hazards to personnel.

At present time, only a few studies of UWB bioeffects have been carried out, and they were focused on behavioral and physiological effects in laboratory animals [Walters et al., 1995; Sherry et al., 1995]. There have been no studies of cellular or genetic UWB effects, though evaluation of genetic risks is necessary for any potentially hazardous environmental factors (radiations, chemicals, etc.). In addition, a number of studies have suggested that cell DNA may be a target for various types of electromagnetic radiations [Blank et al., 1997; Lai et al., 1997; Belyaev et al., 1996].

To address this issue, we used the yeast *Saccharomyces cerevisiae* strain D7 specifically designed for screening of mutagenic and recombinagenic effects [Zimmermann et al., 1975]. Our recent experiments with this cell model demonstrated no UWB effect on spontaneous mutagenesis, recombinagenesis, or colony-forming ability [Pakhomova et al., 1997]. The purpose of the present work was to establish whether UWB exposure alters the induction of heritable chromosome aberrations by ultraviolet light (UV), a standard mutagenic factor.

The employed yeast strain D7 carries specific gene markers *ade2-40*/*ade2-119*, *trp5-12*/*trp5-27*, and *ilv1-92*/*ilv1-92*, which make it possible to detect certain mutagenic and recombinagenic events. Mutation induction by true reverse or allele non-specific suppresser mutation in *ilv1-192* is followed by appearance of iso-

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TABLE 1. Yeast Cell Survival and the Rates of Genetic Aberrations after Sequential UV and UWB Exposures

	Survival %	Crossovers per 10^2 survivors	Segregants per 10^2 survivors	Convertants per 10^5 survivors	Revertants per 10^6 survivors
Exposed (UV + 0 Hz UWB, $n = 6$)[a]	66.8 ± 3.7 (10179)[b]	1.83 ± 0.27 (179)	7.87 ± 0.79 (783)	196 ± 12 (16213)	290 ± 24 (3493)
Control (UV only, $n = 6$)	66.6 ± 4.9 (10019)	1.78 ± 0.22 (176)	7.82 ± 0.45 (776)	200 ± 11 (16310)	294 ± 22 (3652)
Exposed (UV + 16 Hz UWB, $n = 5$)	64.6 ± 5.3 (8385)	1.75 ± 0.23 (150)	7.47 ± 0.74 (618)	201 ± 17 (14362)	285 ± 40 (2915)
Control (UV only, $n = 5$)	62.2 ± 6.5 (8166)	1.71 ± 0.23 (136)	7.61 ± 0.55 (602)	211 ± 25 (14193)	282 ± 38 (2947)
Exposed (UV + 600 Hz UWB, $n = 6$)	62.3 ± 5.9 (9291)	2.01 ± 0.32 (179)	8.06 ± 0.72 (735)	209 ± 14 (15406)	275 ± 26 (3189)
Control (UV only, $n = 6$)	62.0 ± 7.0 (9246)	1.92 ± 0.33 (169)	8.54 ± 0.67 (782)	209 ± 20 (14936)	299 ± 25 (3335)

[a]Shown are the repetition rate of UWB pulses, Hz (0 Hz corresponds to sham exposure), and the number of independent experiments in each series, *n*.

[b]All the data values are given as an average (mean ± SE) for *n* experiments. Numbers in brackets indicate the actual number of colonies scored in all experiments with a particular type of exposure.

leucine non-requiring colonies on isoleucine-free media. Mitotic gene conversion is monitored by appearance of tryptophan non-requiring colonies on selective media. The alleles involved are *trp5-12* and *trp5-27*. Mitotic crossing-over is detected visually as pink and red twin sectored colonies due to formation of homozygous cells *ade2-40*/*ade2-40* (deep red) and *ade2-119*/*ade2-119* (pink) from the originally heteroallelic condition (white). Occurrences such as aneuploidy, point mutations, etc., give rise to more types of colored aberrant colonies, which are regarded below as segregants.

Preliminary experiments established that the threshold UV dose for a noticeable decrease of cell survival is about 50 J/m^2. Production of mutations and recombinations increased with increasing the UV dose up to about 150 J/m^2 and then reached a plateau or decreased. Hence, an intermediate dose of 100 J/m^2 was chosen for the main set of the experiments.

UWB pulses were produced by an exposure system designed at Sandia National Laboratories. UWB irradiation lasted for 30 min at a pulse repetition rate of 0 (sham), 16, or 600 Hz. Within 30 min, the suspension remained virtually uniform, showing no visible signs of sedimentation of cells. Based on measurements of the electric field using an EG&G D-dot sensor (model ACD-1A), the parameters of UWB pulses at the repetition rate of 16 Hz were as following (mean ± SE): 103.7 ± 0.57 kV/m peak amplitude, 1.019 ± 0.004 ns pulse width, and 164.8 ± 0.6 ps rise time. The respective values for the pulse repetition rate of 600 Hz were 101.5 ± 0.3 kV/m, 1.014 ± 0.005 ns, and 165.2 ± 0.9 ps. The exposure system's performance and dosimetry have been described in more detail by Bao et al. [1995]. Possible heating effect of the UWB exposure was evaluated with a Vitek Electrothermia Monitor (model 101) using a non-perturbing probe (#2427). Temperature changes in samples exposed for 30 min at 600 Hz did not exceed ambient temperature fluctuations.

Prior to exposures, yeast cells were grown for three days on YPD agar (1% yeast extract, 2% peptone, 2% dextrose, 2% agar), and suspended in distilled water at a titer of 5×10^6 cells/ml. Samples of 2.5 ml of this suspension in a Petri dish (36 mm diameter) were exposed to 254 nm UV radiation (Cole Parmer lamp model VL-6.LC) at 2.25 J/m^2/s up to the total dose of 100 J/m^2. To prevent possible recovery of genetic lesions by photoreactivation, these and subsequent manipulations were performed in dim red light. Two 1-ml samples of the UV-exposed suspension were transferred into identical polystyrene tubes. One tube was subjected to UWB or sham exposure, and the other one was used as a parallel control. The time interval between the completion of the UV exposure and the onset of the UWB exposure did not exceed 2 min. The sham exposure conditions were equivalent to the actual UWB exposure with zero pulse repetition frequency: All devices were turned on, but no trigger pulses were delivered to cause the high-voltage discharge across the spark gap. To enable a more vigorous experimental data analysis, sham exposures were accompanied with parallel control as well. All the three types of exposure (16 Hz, 600 Hz, and sham) were performed during the same day and using the same original cell suspension. The sequence of these exposures varied randomly from day to day and was not disclosed until the completion of the study to achieve double-blind conditions.

Immediately after the UWB or sham exposure, the samples were appropriately diluted according to

the expected yield of viable clones and plated onto minimal and selective media. The minimal medium was composed of 0.67% Difco yeast nitrogen base without amino acids, solidified with Difco agar (2%) and supplemented with the following growth factors: adenine sulfate (5 mg/l), L-isoleucine (60 mg/l), and L-tryptophan (10 mg/l). The selective media excluded L-isoleucine or L-tryptophan. The plates were incubated at 30 °C in the dark for 6 days. Survivals, mitotic crossovers, and segregants were scored as white or colored colonies formed on the minimal medium (10 plates per sample). Mutants to isoleucine independence and gene convertants to tryptophan independence were scored on the respective selective media (5 plates per sample). The parallel controls were always processed in the same way and simultaneously with respective UWB- and sham-exposed samples.

The experimental results are provided in the Table 1. The UV exposure decreased cell survival to 60–70%, and this level was not altered by the UWB exposure. The frequencies of segregations, mutations to the isoleucine independence, and recombinagenic events (mitotic crossing-over and conversion to tryptophan independence) were remarkably close in the UWB-exposed and parallel control samples, as well as in the UWB-exposed and sham-treated cells. The data showed no statistically significant effects (Student's *t*-test and χ^2 test) or even trends in any of the studied groups.

Our previous study has shown that UWB pulses per se do not provoke any mutagenic or recombinagenic events [Pakhomova et al., 1997]. Current research has supplemented this result, proving that acute exposure to UWB pulses does not affect UV-induced mutagenesis and recombinagenesis.

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