RESEARCH PAPER

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"Caffeine, A Possible Supressor of Ultraviolet Induced Genetic Aberrations"

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In yeast, the low frequency of spontaneous occurrence of mitotic crossing over is increased several fold by ultraviolet irradiation. Since as a consequence of mitotic crossing over, the genes distal to the crossover become homozygous in fifty per cent of the nuclei resulting in the exposure of defective alleles which were otherwise unexpressed due to heterozygous condition of the loci. In the present investigation, inhibition of post- replication repair by 0.1 per cent caffeine greatly reduced (63%) the frequency of UV-induced mitotic crossovers, thereby reducing the chances of occurrence of UV-induced genetic aberrations. Similar studies, if extended to higher systems such as mammalian cells in culture can be of great help in preventing ultraviolet- induced genetic aberrations in humans.

THE phenomenon of mitotic crossing over has been observed in yeast, **Saccharomyces cerevisiae**, although with a very low frequency of spontaneous occurrence.[1,2] But the frequency can be increased several fold by UV-irradiation.[3,4] Mitotic crossing over results in homozygosity of all the genes lying distal to the crossover. This exposes defective alleles thereby resulting in increased genetic aberrations after UV-irradiation. Although, the exact mechanism behind UV-induction of mitotic crossing over is not clearly understood, it has been suggested that a significant portion of DNA-aberrations caused by UV- irradiation is probably repaired by a recombination process called recombinational repair. In the present investigation, it has been observed that the recombinational repair of the UV- lesions is involved in UV-induction of mitotics crossover, as the inhibition of this event by caffeine after UV-exposures greatly reduced the frequency of mitotic crossovers. This implies that caffeine can be of great help in reducing the incidence of ultraviolet-induced genetic aberrations.

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MATERIAL AND METHODS

the investigations were carried out with diploid strain D7 of *S. cerevisiae* kindly provided by Dr. F.K. Zimmermann, Institute Fur Microbiology Technische Hochschule, Dermstadt (G.F.R.) The genotype (a/α, ade 2-40/ade 2-119, trp 5-12/ tro 5-27, ilv 1- 92 and the origin of this strain have been previously described.[5] Mitotic crossing over was studied at ade 2 locus as pink and red twin-sectored colonies due to the formation of homozygous cells of the genotypes ade 2-40/ade 2-40 (deep red) and ade 2-119/ade 2-119 (pink) as a result of reciprocal recombination between the centromere and the ade 2 locus. The two alleles show complementation, so that the heteroallelic diploid ade 2-40/ade 2-119 is white and does not require adenine.

For ultraviolet-irradiation studies, the stationary phase cells of *S. cerevisiae* were exposed to ultraviolet source (30 W germicidal tube with maximum output at 2537°A) for 60 sec (gives 50% survival) in an open petridish at a distance of 45 cm from the source. Irradiated cells were plated on synthetic complete medium with or without 0.1% caffeine, an inhibitor of recombinational repair in bacteria[6] and

Table 1: Effect of caffeine (0.1 per cent) on UV-induced mitotic crossing over.

Treatment	Total number of colonies scored	Survival (%)	Crossover (%)	
control	4692	100	0.03	
Caffeine	4594	98	0.03	
UV	2924	59	2.5350	0.93
UV + Caffeine	2468	50	0.93	

yeast[7], for scoring survivors and mitotic crossovers. All manipulations were done in dim yellow light to prevent photoreactivation. Unirradiated controls were also included.

RESULTS AND DISCUSSION

Plating of unirradiated yeast cells on complete medium containing 0.1% caffeine did not affect survival and frequency of spontaneous mitotic crossovers as compared to control **(Table 1)**. When the yeast cells were irradiated with UV light, there was reduction in survival (from 100 to 59 per cent) but significant increase in the frequency of mitotic crossovers among the survivors (from 0.03 to 2.53 per cent). Upon inhibition of recombinational repair of the UV-damaged DNA by caffeine (0.1%), there was only nine per cent decrease in the survival of UV-irradiated cells, but a significant decrease (63 per cent) in the frequency of UV-induced mitotic crossovers.

Caffeine alone had no effect on the survival of yeast cells and on the spontaneous occurrence of mitotic crossing over. It is expected because in the absence of DNA-damage these will be no induced recombinational repair and hence no effect of caffeine on spontaneous occurrence of mitotic recombination. Upon irradiation of yeast cells the frequency of mitotic crossover was greatly enhanced. Plating of UV-irradiated cells on caffeine medium resulted in three fold reduction in the percentage of ultraviolet-induced mitotic crossovers (Table 1). In support of this, the incorporation of vanillin, an enhancer of post- replication recombinational repair in the post-irradiation media, enhanced recombination between two plasmid DNA PATH4 (Cm^rTc^s) P^{BMx7} (AP^rTc^s) in a uvr A umu C back ground.[8] Therefore the reduction in ultraviolet- induced mitotic crossovers with caffeine is expected in the present investigation because the process of recombination repair starts after DNA replication at four-strand stage to join the gaps present in the daughter strands produced as a result of replication of DNA containing thymine dimers.[9] Therefore, the inhibition of recombinational repair at this stage greatly reduces the frequency of mitotic crossover.

CONCLUSION

In yeast, many-fold enhancement in the expression of the recessive alleles (represented by mitotic crossovers) after UV- irradiation can be checked by caffeine - an inhibitor of recombinational repair. Similar studies, if extended to higher systems like mammalian cells in culture can be of great help in preventing ultraviolet induced genetic aberrations in humans and higher animals.

REFERENCES

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