MITOMYCIN C INDUCED DNA DAMAGE CAN BE REPAIRED BY ENZYMES INVOLVED IN THE ADAPTIVE RESPONSE

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Jeggo et al. (1977) observed that when Escherichia coli were exposed to sub-lethal doses of the mutagen N-methyl-N-nitro-N-nitroso-guanidine (MNNG) and subsequently challenged with a higher dose of that mutagen, both the mutagenic and lethal effects of the challenge were reduced. The degree of reduction was found to be affected by the concentration of mutagen used during the preliminary treatment. This phenomenon was termed the adaptive response. Subsequent studies (Schendel 1981) demonstrated that this response could be obtained with other alkylating agents, not only those which methylated DNA. One factor involved in the adaptive response was the inducible protein O^6 methyl guanine transferase which can dealkylate O^6 alkyl guanine residues of DNA, leading to the restoration of functional guanine residues.

We have investigated the efficacy of the adaptive response with regard to damage produced by the alkylating agent mitomycin C (MMC). MMC also reacts with the O^6 moiety of guanine, producing a bulkier lesion than other mutagens usually associated with this phenomenon. Exponential-phase cultures of E. coli B/r strain WP2 were incubated at 37°C in modified Davis-Mingioli salts medium (Bridges 1972) containing glucose (4 mg ml^{-1}) and tryptophan (20 μg ml^{-1}) together with known concentrations of MMC (0-5ng ml^{-1}) for 90 minutes. Cells were then challenged by incubating 2 ml aliquots of culture with 50 ng ml^{-1} MMC for 30 minutes at 37°C. Challenged cells were washed and both survival and mutation frequency (frequency of cells resistant to β-thienyl-alanine) relative to unchallenged cells determined. In these experiments pretreatment with MMC did not result in any increase in survival. Indeed if anything it appeared to sensitise the cells. Nevertheless despite this effect on viability mutation rates were found to increase significantly with increasing pretreatment doses.

In order to determine whether MMC-induced lesions could be repaired by adaptive response proteins, the effect of preincubation in the presence of MNNG was studied. Exponential E. coli cultures similar to those described above were grown at 37°C in the presence of low concentrations of MNNG (0-1 μg ml^{-1}) and then challenged with either MMC (50 ng ml^{-1}) or MNNG (12 μg ml^{-1}) as described previously. Survival and mutagenesis after challenge were measured and compared with no-challenge controls. Prior incubation in the presence of MNNG resulted in a concomitant decrease in mutagenesis and increase in survival after challenge with either mutagen. The magnitude of the response was to some degree dependent on the size of the preliminary dose.

These results indicate that DNA damage arising from treatment with MMC is not a signal for the induction of the adaptive response. Such damage can however be repaired by the adaptive response if it is induced prior to challenge. Since MMC almost exclusively alkylates DNA by reacting with the O^6 atom of guanine (Waring 1980) it is likely that O^6 alkyl guanine moieties are not the inducing signal.

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