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ENZYMATIC REPAIR OF OXIDATIVE DNA DAMAGE

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Oxidative attack on the methyl group of thymine in DNA results in the formation of 5-hydroxymethyluracil (HmUra).[1-3] This attack may be mediated by ionizing radiation[4] or the active oxygen species generated by activated leukocytes.[5] HmUra is removed from DNA in vitro through the action of HmUra-DNA glycosylase. This enzyme was first identified in murine cells grown in culture[6] and its activity subsequently confirmed in murine tissues[7] where the highest specific activity was in brain and thymus. However, unlike other DNA glycosylases, no activity was detected in extracts of bacteria or yeast.

HmUra is a normal constituent of the DNA of several lytic bacteriophages of B. subtilis where it completely replaces Thy, pairing with Ade.[8] Hence, it is somewhat surprising that it should be subject to enzymatic removal. It is also surprising that enzymatic removal of HmUra does not occur in bacteria. Bacteria contain several repair enzymes directed against oxidatively modified pyrimidines such as thymine glycol[9] indicating that oxidative damage to DNA and its repair is not limited to higher organisms.

We introduced HmUra into the DNA of mammalian cells to determine its genotoxic properties when present in the DNA of cells containing Thy and Cyt as the predominant pyrimidines. This was achieved independently of the introduction of other oxidative DNA modifications by administering the nucleoside 5-hydroxymethyl-2'-deoxyuridine (HmdUrd). This dThd analogue is transported into cells through the action of thymidine kinase and is incorporated into DNA.[10,11] We used ^{3}H-labelled HmdUrd to correlate the physiologic effects of HmUra with the degree of substitution of Thy residues by HmUra residues. This system permitted a study of the repair of HmUra residues in cells.

Using V 79 chinese hamster cells we determined whether the toxicity of HmdUrd in the medium of growing cells was a direct function of the extent of substitution of HmUra in DNA. We found that cells grew normally with levels of substitution of between 1/1000 to 1/3000 HmUra residues/Thy residue. At higher levels of substitution, toxicity was observed. Using double label techniques employing [^{14}C]dThd and [^{3}H]HmdUrd we demonstrated selective loss of ^{3}H radioactivity with time. This was evidence for cellular repair.[12] Using HPLC analysis, we subsequently demonstrated the release of [^{3}H]HmUra into the medium as [^{3}H]HmdUrd disappeared from high molecular weight DNA.[13] This was proof that cellular repair of HmUra was mediated through the action of HmUra-DNA glycosylase. Cells containing HmUra residues in their DNA were exposed to 3-aminobenzamide, an inhibitor of poly(ADP-ribose) synthesis. This treatment proved extremely cytotoxic, resulting in arrest of cells at the G2/M boundary of the cell cycle. These results further confirmed the enzymatic removal of HmUra by showing that interference with the completion of DNA repair via inhibition of synthesis of poly(ADP-ribose) resulted in cell death.[12]

The mutagenicity of HmdUrd was studied at the HGPRT and ouabain resistance

loci in V 79 chinese hamster cells. Mutagenicity was observed at the HGPRT locus using thioguanine resistance as evidence of mutation. No ouabain resistant mutants were obtained. We correlated the frequency of mutation with the degree of substitution and found there was one mutagenic event for each 30,000 HmUra residues in DNA.[14]

On the basis of our determination of the radiogenic yield of HmUra to be about 6 HmUra residues per cellular genome per 0.01 Gy[4] we conclude that the contribution of radiogenically formed HmUra residues in DNA to the toxicity and mutagenicity of ionizing radiation is minimal. Furthermore, we demonstrated that repair of HmUra was not inhibited by doses of radiation of as high as 5 Gy.[14]

These results lead us to conclude that the consequences of oxidation of a Thy residue to an HmUra residue in a preexisting AT pair in DNA are not extremely deleterious to cells in culture. However, it may be that to long lived cells like those of the central nervous system or the immune system, even a rare mutational event is of sufficient consequence to warrant development of a repair enzyme.[7,12]

Another rationale for the repairability of HmUra was suggested by our recent identification of a related repair enzyme activity against 5-hydroxymethylcytosine (HmCyt), HmCyt-DNA glycosylase.[15] We take the existence of this enzyme to indicate that 5-methylcytosine (MeCyt) can be oxidized to HmCyt in a reaction mechanistically similar to that which produces HmUra from Thy.[16] The deamination of MeCyt to Thy has been invoked as the reason for mutational "hot-spots" in bacteria.[17] Mismatch correction systems for the repair of GT mismatch pairs are present in mammalian cells.[18,19] If a HmCyt residue deaminates, the product is HmUra. It may be that the resulting GHmUra pair is not a good substrate for mismatch correction. Alternatively, oxidation of Thy to HmUra in a preexisting GT mismatch would also yield a GHmUra pair which could be repaired by HmUra-DNA glycosylase. Like HmUra-DNA glycosylase, HmCyt-DNA glycosylase was not detectable in bacterial extracts.

In summary, oxidative attack on the methyl group of Thy and probably on the methyl group of MeCyt yields 5-hydroxymethyl derivatives whose formation is deleterious to the organism. In higher organisms this has resulted in the evolutionary development of specific repair enzymes to effect their removal from DNA.

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