Enhanced excision of O^6-alkylguanine in rat liver by pretreatment with acetylaminofluorene

As recently reported by Buckley *et al.*[1], a diet containing 0.06% 2-acetylaminofluorene (AAF) established in rats a condition in which the liver of the animals had an increased capacity for removing O^6-methylguanine from DNA produced by a single dose of dimethylnitrosamine (DMN). These observations are open to many interesting interpretations. However, the authors concentrated exclusively on the concept of induction of specific repair enzymes by the presence of damage from AAF adducts. This interpretation does not pay due attention to the biological state of the cell population in the liver at the time of the investigation.

Chronic ingestion of a liver carcinogen such as AAF will stimulate the liver into hyperplasia. Although the authors specifically excluded animals that had nodular livers, it is likely that livers of all animals at the end of this chronic treatment will contain an increased proliferative population[2,3]. Passage between the non-cycling and proliferative phases of a cell population alters the activity of many enzymes involved in DNA replication and repair. Peripheral lymphocytes stimulated to proliferate by phytohaemagglutinin increase their capacity for repair[4] and synthesise a specific repair enzyme, uracil glycosylase[5]. In the rat liver, DNA polymerase β, which may participate in excision repair, increases during regenerative hyperplasia following partial hepatectomy[6]. The repair systems do not all respond equally, because the system that removes O^6-methylguanine increases during liver hyperplasia after chronic treatment with DMN, whereas that for removing N^3-methyladenine or N^7-methylguanine does not[7]. Excision repair seems to be particularly responsive to the proliferative state of a cell population[4,8-10]. One interpretation of the observations of Buckley *et al.*[1] may therefore simply be that they represent the differences between the repair capacity of a quiescent relative to a proliferating cell population. The increased repair of O^6-methylguanine would not relate specifically to signals from damaged sites inducing the synthesis of new enzymes, but rather to a more generalised consequence of stimulation of proliferation.

Buckley *et al.* imply that exposure to AAF induces the repair of lesions formed by an unrelated carcinogen, DMN. The lesions formed by AAF and the O^6-methylguanine produced by DMN are not, however, as different as might be expected if one considers the pathways by which they are repaired. Both kinds of lesion can be repaired by the nucleotide excision repair pathway[11]. This system removes pyrimidine dimers produced by UV light and is regulated by the genes associated with the human disease xeroderma pigmentosum (XP)[12]. The autosomal recessive mutations in XP eliminate the capacity to remove O^6-methylguanine, O^6-ethylguanine, pyrimidine dimers, and the lesions produced by *N*-acetoxy-AAF[13-16]. Interestingly, O^6-methylguanine is not repaired by the nucleotide excision repair system in *Escherichia coli*, although it is in mammalian cells[15,16], suggesting that prokaryotic systems may be very poor models to use in the interpretation of observations of O^6-methylguanine in mammalian cells.

Buckley *et al.* cite evidence for the induction of post-replication repair by *N*-acetoxy-AAF-treated Chinese hamster cells[17], but that observation only demonstrated alterations in semi-conservative DNA replication confined to the first few hours after an acute dose. Those alterations involved the growth of nascent DNA chains, and did not involve any change in the repair of lesions on the parental DNA; also, they have been interpreted elsewhere in a manner that does not invoke the induction of any system[18].

Therefore, we suggest that a change in the excision capacity of liver by pretreatment with AAF more probably reflects the production of a spectrum of enzymes associated with cell proliferation, especially for DNA replication and repair, than the specific induction of unique repair enzymes. Moreover, we should also point out an interesting implication of observations of Buckley *et al.* in relation to the role of O^6-methylguanine as a critical lesion for carcinogenesis by alkylating agents[19]. When rats were given the same feeding regimen of AAF and DMN, the subsequent yield of hepatocellular carcinomas reached nearly 100%, whereas a single dose of DMN given alone produced no liver cancers[20]. Thus, increased repair of O^6-methylguanine correlates with increased susceptibility to hepatocarcinogenesis, whereas the opposite correlation prevails in the neonatal rat brain, in which decreased repair of O^6-methylguanine correlates with elevated carcinogenesis[21]. These contrasting correlations indicate that any attempt to ascribe a unique, definite role to O^6-alkylguanine in carcinogenesis is premature; the role of this product and its repair are not yet fully understood. At the very least, the balance between DNA repair and DNA replication in each experimental situation needs to be carefully considered.

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BUCKLEY, O'CONNOR AND CRAIG REPLY—Although more detailed information will be available when a fuller report is published on the basis of our recent study[1], two comments should be made on the matters raised by Cleaver and Kaufmann. First, we did not intend to imply that our interpretation rested exclusively on the induction of specific repair enzyme systems. Had that been the case we would not have used the phrase "general repair mechanism". Second, with regard to the final statement on the role of O^6-alkylation of guanine in DNA, we agree that an increased capacity for the repair of O^6-methylguanine in liver DNA correlates with a high susceptibility to hepatocarcinogenesis in the case of rats chronically exposed to dimethylnitrosamine. Indeed, this may also be the case in our experiments with rats exposed to AAF, although the situation has not been evaluated in our own Wistar rats. While recognising that the role of O^6-alkylguanine in carcinogenesis is not fully understood it seems unreasonable to consider just two experimental situations (increased O^6-methylguanine repair after pretreatment and the persistence of O^6-alkylguanine in the brain of neonatal rats with respect to tumour formation) and not to take into account the considerable information now available (reviewed in refs 2–5). In fact, from reports referred to in these reviews it is clear that careful consideration has been given to the relevance of DNA repair and DNA replication.

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