Drug-related killings: a case of mistaken identity

Peter Karran[1] and Margherita Bignami[2]

Abortive attempts at DNA repair can contribute to the effects of DNA damage inflicted by cytotoxic drugs. DNA methylation damage, 6-thioguanine and cisplatin adducts all owe their cytotoxicity in part to the intervention of DNA mismatch repair.

Addresses: [1]Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts., UK and [2]Istituto Superiore di Sanitá, Viale Regina Elena, 299, 00161, Rome, Italy.

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DNA repair is a good thing. It is there to help the cell reverse the damage done to its DNA by a hostile environment and ensure faithful duplication of the genome in each generation. DNA repair prevents the accumulation of mutations that might lead to cellular dysfunction or cell death. There are numerous examples of inherited syndromes in which defective repair is accompanied by increased rates of mutation and sensitivity to killing by DNA damaging agents as diverse as ultraviolet light and water [1]. A major DNA-repair pathway is mismatch correction, whose principal function is to scan newly synthesized DNA and remove errors committed by the DNA polymerases [2]. Mismatch repair has been the focus of a considerable amount of recent attention because of its association with human cancer. In the hereditary nonpolyposis colorectal cancer syndrome, an inherited mutation in one of four known mismatch-repair genes is associated with extremely high rates of early onset colorectal and other malignancies [3]. The message is clear — the mismatch-repair system serves an important function in preventing the cell from becoming malignant. Mismatch repair may not always be so benevolent, however, and a number of recent papers have highlighted the contribution that this important DNA-repair pathway can sometimes make to cell death.

DNA methylation damage

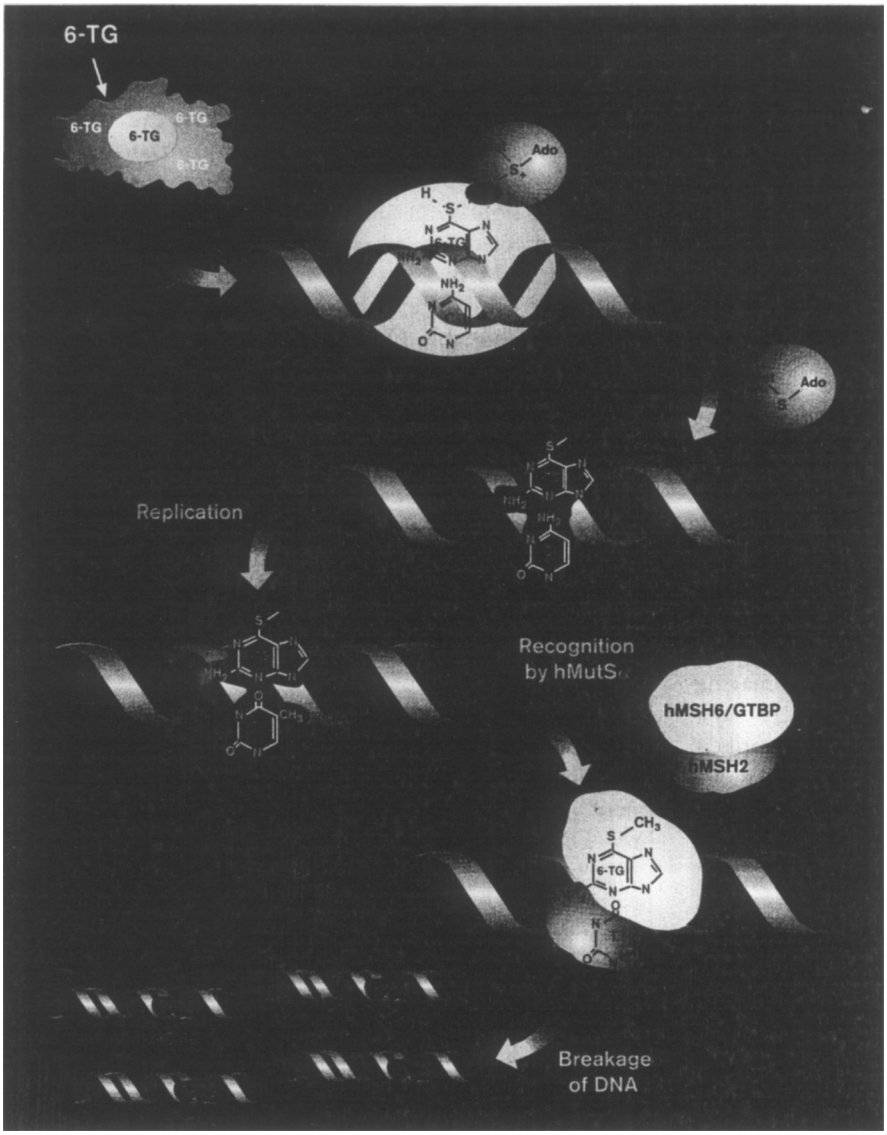
A recent paper [4] suggests that cell death in response to O^6-methylguanine (O^6-meGua) and 6-thioguanine may occur via a common pathway that involves abortive DNA repair, and that S-adenosylmethionine (SAM) may be important in generating the correct substrate from 6-thioguanine for this pathway. Methylating agents kill cells, and the formation of O^6-meGua in DNA is an important part of this cytotoxicity [5]. The apparently ubiquitous distribution of a specific DNA repair enzyme, O^6-methylguanine-DNA methyltransferase (MGMT), which selectively catalyzes the reversion of the modified base to guanine, implies the existence of a prevalent source of 'endogenous' DNA methylation damage. SAM, one of the molecules that shuttle methyl groups around the cell for use in biosynthetic reactions, was identified as a candidate DNA methylating agent that can methylate DNA by a non-enzymatic methyl group transfer [6]. It was realized, however, that this compound was unlikely to generate a significant amount of O^6-meGua, because the reaction mechanism by which SAM transfers its methyl group to DNA dictates that donation to an oxygen will occur only slowly. Peter Swann reasoned that a sensitive indicator of possible O^6-methylation might be generated by replacing the O^6-atom with sulphur, to which methyl-group transfer should be easier. Thus, armed with synthetic oligonucleotides containing 6-thioguanine, he and his colleagues demonstrated [4] that the rate of methyl-group transfer to the S^6-position is several 1000-fold higher than to the O^6-position. Of course, this still leaves SAM as a poor methylator of guanine oxygen atoms, but, in an important insight, Swann and his colleagues realized that the facile methylation of 6-thioguanine might underlie the cytotoxicity of an important class of antitumor chemotherapeutic drugs.

Mercaptopurine and 6-thioguanine

Mercaptopurine and 6-thioguanine are used in the treatment of acute leukaemia. These agents are incorporated into DNA after being metabolized by the pathway that recycles purine bases. Methylation-tolerant cell lines, which have acquired a resistance to the presence of potentially cytotoxic O^6-meGua in their DNA through defects in DNA mismatch repair [7], generally exhibit a cross-resistance to 6-thioguanine, despite having a functional purine recycling pathway. Tolerant cells incorporate 6-thioguanine into DNA as do the normal cells, but only the former can continue to replicate the DNA that contains 6-thioguanine without detrimental effects [8].

Precisely how defects in mismatch repair confer methylation tolerance is not known, but Swann *et al.* [4] modified a current model to explain how 6-thioguanine, with the help of mismatch repair, might kill normal cells and how death is avoided in mismatch-repair-defective cells. They propose the following sequence of events: the base analog is incorporated into DNA where it serves as an acceptor for facile chemical methylation by intracellular SAM. During the next round of DNA replication, the resulting S^6-meThioguanine base pairs with thymine about half of the time. S^6-meThioguanine•T base pairs are recognized by the mismatch-repair system which mistakenly identifies them as *bona fide* replication errors. Engagement of mismatch correction at S^6-meThioguanine•T base pairs kills the cell in the same way that aberrant processing of O^6-meGua-containing base pairs by this pathway results in cell death. Death is thought to be a consequence of

Figure 1



Proposed mechanism of 6-thioguanine cytotoxicity [4]. 6-thioguanine (6-TG) enters the cell and is converted via hypoxanthine-guanine phosphoribosyltransferase, reductase and kinase activities into deoxy-6-thioguanine 5′-triphosphate (dS^{6}GTP). The use of dS^{6}GTP by DNA polymerases during DNA replication introduces dS^{6}GMP (abbreviated to 6-TG here) into DNA. 6-TG in DNA is subjected to infrequent non-enzymatic methylation by cellular S-adenosylmethionine (SAM) to form dS^6-meGMP. During the next round of DNA replication, methylated 6-TG may direct the incorporation of thymine (T) as the complementary base. The imperfect S^6-meGua•T base pair is recognized by the mismatch-binding complex hMutSα which initiates an attempt at mismatch repair. Attempted repair of the mismatch fails, in part, because the removal attempts are directed to the newly synthesized daughter DNA strand, whereas the 'incorrect' (S^6-meThioguanine) base remains in the parental strand. The persistent S^6-meThioguanine is able to provoke further incomplete repair attempts. The long-lived interruptions in the daughter DNA strand opposite S^6-meThioguanine bases are potentially cytotoxic intermediates.

the repair attempts being targeted to the daughter DNA strands, which, by definition, normally contain the replication errors. In the case of damage-provoked mismatch repair, the altered (or 'incorrect') base remains unexcised in the parental DNA strand. This unexcised methylated base will, on completion of the 'repair' of the daughter strand, provoke another repair attempt. These repeated mismatch-repair attempts generate long-lasting interruptions in the daughter DNA strand that eventually lead to cell death [7]. Thus, in cells that are mismatch repair deficient, neither O^6-meGua nor S^6-meThioguanine is particularly cytotoxic.

We have summarized the broad outline of this model in Figure 1. Swann *et al.* [4] present compelling experimental evidence in support of each of the postulated steps, and the suggested sequence of events nicely explains the characteristic delayed cytotoxicity and chromosomal damage produced by 6-thioguanine. Their model also resolves the question of why vastly greater numbers of 6-thioguanine bases than O^6-meGua bases are present in DNA at similar levels of cell killing [8]. From direct analysis of S^6-meThioguanine in cellular DNA, they estimate that only relatively few incorporated 6-thioguanines (estimated at about 2 of every 10^4) become methylated, thus gaining the potential to pair with thymine and become cytotoxic.

Despite similar mechanisms of action, S^6-meThioguanine and O^6-meGua do have demonstrably different effects on cells. Perhaps this is not surprising. 6-thioguanine is used

as a cytotoxic agent, whereas DNA methylating agents are renowned mutagens. The relationship between cytotoxicity and mutagenicity is different for S^6-meThioguanine and O^6-meGua. O^6-meGua-induced mutations can be observed under conditions of relatively high cell survival in both wild-type and mismatch-repair-defective cells. In contrast, 6-thioguanine (and by implication S^6-meThioguanine) is detectably mutagenic only in repair-defective cells because it is too toxic towards wild-type cells [9]. In other words, S^6-meThioguanine is very cytotoxic but not very mutagenic, whereas the converse is true for O^6-meGua.

What is the reason for these differences? Replication of O^6-meGua results in a large preponderance of O^6-meGua•T base pairs over O^6-meGua•C pairs. In contrast, the replication products of S^6-meThioguanine seem likely to be equally divided between S^6-meThioguanine•C and S^6-meThioguanine•T base pairs. The hMutSα mismatch-recognition complex binds more favorably to S^6-meThioguanine•T and to O^6-meGua•T than to the corresponding C-containing base pairs. Because S^6-meThioguanine•C is a more frequent replication product than O^6-meGua•C, this suggests, somewhat paradoxically, that S^6-meThioguanine might not provoke as many potentially cytotoxic repair attempts as O^6-meGua. The equation is not this simple, however; the recently identified hMutSβ mismatch-recognition complex [10] might initiate correction attempts at these C-containing base pairs, and it is known that O^6-meGua•C base pairs can provoke mismatch-repair attempts [11]. A more straightforward explanation for the differences in cytotoxicity and mutagenicity between S^6-meThioguanine and O^6-meGua might lie in the relative affinity of the hMutSα complex for for the different methylated bases paired to T. If recognition by hMutSα of S^6-meThioguanine•T base pairs is more favorable than that of O^6-meGua•T pairs, cells are more likely to mount death-prone repair attempts on the former and to allow the persistence of the promutagenic O^6-meGua•T base pairs.

Azathioprine, a drug that is related to mercaptopurine and 6-thioguanine, has been widely used as an immunosuppressant in renal transplant patients. Azathioprine is metabolised to 6-thioguanine, which is incorporated into DNA. Swann and his collaborators [4] suggest that incorporation of 6-thioguanine and its subsequent methylation might contribute to the development of the cancers that are inevitably associated with organ transplants. They propose that S^6-meThioguanine may either act directly as a mutagen or facilitate the selection of mismatch-repair-defective cells. There is some experimental support for these possibilities in an animal system. 6-thioguanine can induce 'xenogenization', a process by which tumor cells become non-tumorigenic in syngeneic hosts [12]. The effect relies on the generation of mutated cellular proteins as sources of new antigens, which elicit cytotoxic T-cell responses. Methylating agents, either acting as direct mutagens or as selective agents for a mutator phenotype, are good inducers of xenogenization [13]. Treatment *in vitro* with 6-thioguanine can thus apparently lead to the production of mutated proteins. In transplant patients, this could result in neoplastic transformation. Overall, however, it seems likely that the increased incidence of tumors is mainly a consequence of immune suppression *per se*. After all, tumors arise in recipients of immunosuppressive regimes that are not known to induce mutations, and in many cases the tumors appear to have a viral aetiology. In addition, as pointed out above, 6-thioguanine is a rather poor mutagen. Nonetheless, it would seem prudent to explore this potential mechanism of tumorigenesis further.

Cisplatin

The possibility that mismatch repair might be involved in the lethality of another important antitumor agent, cisplatin, was raised some time ago [14]. Recently it has been established that ovarian carcinoma cells selected *in vitro* for resistance to this agent have defects in mismatch repair, as do cells tolerant to methylating agents and 6-thioguanine [15–17]. Cisplatin is one of the success stories of chemotherapy. It is a highly effective treatment of testicular and ovarian tumors although, as with all antitumor therapies, resistance develops. Considerable effort is being expended to investigate the interaction of human mismatch-repair factors with cisplatin-modified DNA.

The first results from Derek Duckett and coworkers in the laboratory of Paul Modrich [18] and Jill Mello in John Essigmann's laboratory in collaboration with Samir Acharya and Richard Fishel [19], indicate that human mismatch recognition proteins can interact with DNA containing cisplatin-1,2-diguanyl intrastrand crosslinks. These are the major DNA adducts of cisplatin and probably contribute most to its cytotoxic effect. Duckett *et al.* [18], using a substrate that contained a single 1,2diguanyl intrastrand cisplatin crosslink, demonstrated that the modified DNA was recognized by an extensively purified mismatch recognition factor, hMutSα. hMutSα is a heterodimer composed of hMSH2 and hMSH6/GTBP (G/T binding protein) subunits [20,21] (see Fig. 1), and it initiates mismatch repair by binding to mismatched DNA. Both subunits are homologues of an *E. coli* mismatch-recognition protein (MutS), and recognition of cisplatin damage could conceivably be initiated by either of them. Mello *et al.* [19] report that cisplatin-damaged DNA can be recognized by the purified hMSH2 subunit acting alone. These biochemical studies use large excesses of binding factor over cisplatin-modified DNA, so it is not clear what happens in a cisplatin-treated cell and whether hMSH2 or hMutSα carries out the recognition of cisplatin-modified DNA. Nevertheless, the findings establish the important

principle that mismatch-recognition factors are likely to initiate mismatch-repair attempts at cisplatin adducts. In an intriguing addendum to their binding data, Mello *et al.* demonstrate that hMSH2 expression is apparently highest in testis and ovary, the two tissues in which tumors are most responsive to cisplatin therapy. If hMSH2 levels reflect the cell's capacity to initiate mismatch-repair attempts at cisplatin adducts, and by implication cell death, these data strongly support the idea that cisplatin is another addition to the list of drugs that kill cells by provoking mismatch repair to misbehave.

Mismatch repair and other drugs

The contribution of the mismatch-repair pathway to the cytotoxicity of O^6-meGua and 6-thioguanine (now S^6-meThioguanine) was consistent with their similarity to unmodified guanine and their abilities to participate in some kind of aberrant base pairing. The inclusion of cisplatin crosslinks into the list seems to have removed the requirement of structural similarity to unmodified guanine for recognition by the mismatch-repair pathway (although the potential effects of these adducts on base-pairing during DNA replication are not yet fully known). Indeed, resistance to the therapeutic agent doxorubicin can also be acquired by loss of mismatch repair [15,17]. This drug may be cytotoxic through its ability to intercalate between the two DNA strands, or it may generate DNA-base-damaging oxygen radicals.

What is required for recognition by the mismatch-repair machinery? One property shared by O^6-meGua and 1,2-diguanyl crosslinks generated by cisplatin is their relative ease of replication. O^6-meGua is not a good replication block and is copied quite well by DNA polymerases. Until recently, 1,2-diguanyl crosslinks seemed refractory to bypass by purified DNA polymerases, but it now appears that these lesions might be bypassed more frequently than previously supposed [22]. As mismatch repair is a post-replicative process, it is perhaps more likely to be provoked by drug-damaged DNA that has undergone replication. In this case, candidates for lethal processing by mismatch repair might be identified by a relative inability to arrest DNA replication. A second, not exclusive, possibility is that mismatch repair becomes involved when the normal excision (or reversal) of DNA damage is inadequate. The mismatch-repair-related cytotoxic effects of O^6-meGua are most obvious in Mex^- cells, which express very low levels of MGMT; this enzyme can be considered the first line of defence against these methylated bases [5]. Cisplatin may be cytotoxic towards wild-type cells at least partly because the 1,2-diguanyl crosslinks are poor substrates for nucleotide excision repair [23].

Perhaps we should look for a contribution by mismatch repair to the cytotoxicity of any drugs that introduce DNA lesions that are not particularly good substrates for the normal protective DNA repair pathways of nucleotide and base excision repair. Might the interaction of mismatch repair proteins with drug-damaged DNA generally have a beneficial outcome after all? Mismatch repair would serve a positive and advantageous function for the tissue (or organism) if it deleted cells that have incurred too much DNA damage.

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