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Review

Role of DNA repair in the mechanisms of cell resistance to alkylating agents and cisplatin

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Introduction

Cellular resistance to antitumor agents represents one of the major drawbacks of therapy involving cytotoxic drugs. Among the numerous chemotherapeutic agents available, this review focuses specifically on alkylating agents and cisplatin.

Various mechanisms are involved in cellular resistance to alkylating drugs or cisplatin, including (1) impaired uptake of the drug [9, 47, 56, 64, 69], (2) increased levels of drug metabolism [23, 35], (3) increased concentrations of secondary nonessential targets such as glutathione (GSH) or metallothioneins [44, 70], and (4) enhanced repair of DNA damage [4–7, 26, 46, 56]. Two additional points are worth mentioning: (1) more than one mechanism is frequently observed in a single cell line resistant by adaptation to increasing drug concentration [50], and (2) most reports on cell lines involve studies based on high levels of resistance acquired in vitro, but very few analyze the mechanisms of in vivo resistance [17, 42].

Among the various processes leading to drug resistance, only DNA repair is considered in the present review. First, the DNA lesions and their repair pathways are presented. Then, the role of DNA repair in resistance to alkylating agents is analyzed. Finally, the use of an in vitro assay [71] for the quantification of repair efficiency in cell extracts to study the links between repair and resistance is discussed.

DNA damage induced by alkylating agents and cisplatin

Alkylating agents are used in the treatment of germ-cell cancer, lymphomas, childhood tumors, multiple myeloma, breast cancer, and small-cell lung cancer. This class of agents includes melphalan, chlorambucil, busulfan, cyclophosphamide, nitrosoureas, and mitomycin C [10]. Most alkylating agents form positively charged carbonium/aziridinium ions that react with the nucleophilic centers on DNA, proteins, and small molecules such as GSH. The N7 position of guanine is a frequent site of alkylation, but many other base modifications are possible and the spectrum of these varies with the compound used [61].

Bifunctional agents (melphalan, chlorambucil, and busulfan) form both monoadducts and, in a second reaction, biadducts that are mainly interstrand DNA cross-links. Cyclophosphamide is activated by metabolism into phosphoramide mustard, which reacts with DNA, and acrolein as active metabolites. Nitrosoureas undergo a decomposition reaction in aqueous solution to produce a chloroethyl carbonium ion and an isocyanate group. The former reacts with DNA and creates a mono- and then a bifunctional DNA cross-link. The isocyanate group reacts with amines in a carbamoylation reaction. Following its activation by a reduction reaction, mitomycin C reacts as an alkylating species and binds to DNA, with cross-links being the main lesion responsible for its toxicity [20].

cis-Diamminedichloroplatinum(II) (CDDP) is widely used in the treatment of solid tumors. After undergoing a hydration step, it reacts within the cell by covalent binding to nucleophiles such as proteins and cysteine residues of GSH and DNA [40, 52]. Its covalent binding to DNA involves the formation of monofunctional adducts, particularly at the N-7 atom of guanine and adenine. In a second step, these monoadducts react with another purine base or nucleophilic center of a protein to form bifunctional adducts. The two major adducts (90% of platinum bound to DNA) are *cis*-$Pt(NH_3)_2d(pGpG)$ and *cis*-$Pt(NH_3)_2d(pApG)$, both corresponding to intrastrand cross-links of CDDP between adjacent bases. Bifunctional interstrand cross-links represent about 5% of the total platinum binding. Bifunctional adducts are more toxic than monofunctional ones, but it is not yet clear whether the inter- or the intrastrand cross-links are relevant for cytotoxicity [40].

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The following events occur after treatment with these various drugs: (1) a decrease in DNA synthesis, (2) a correlation between toxic effects and the extent of DNA damage, (3) an induction of chromosomal damage, and (4) an increased toxicity in DNA-repair-deficient mutants. On the basis of these results, it is generally considered that DNA is the ultimate target responsible for cell toxicity and antitumor activity. However, the precise chemical adduct(s) responsible for cell lethality has not been conclusively established [7, 28, 39, 53, 74].

DNA repair mechanisms

Due to the conversion kinetics of monoadducts into biadducts, a protection effect could take place by a mechanism, superimposed on the repair processes, that allows blockade of the evolution of monofunctional adducts. Such a reaction is dependent on the presence of either GSH or the suicide-repair protein *O*6-alkylguanine alkyltransferase (MGMT) [1, 4, 24, 25, 43, 51, 72].

The tripeptide GSH protects the cell against cisplatin toxicity either by lowering the reactive species capable of binding to DNA or by quenching the relatively poorly toxic monoadducts [2, 3, 25, 49]. Similarly, GSH is involved in cell protection against alkylating agents [1, 2, 32]. Second, the enzyme MGMT is responsible for the removal of *O*6-alkylguanine lesions, which are premutagenic lesions triggered by methylating and chloroethylating alkylating agents. It operates by transferring the alkyl group to one of its cysteine residues in a suicide reaction. This protein can therefore prevent the formation of interstrand cross-links provoked by alkylating agents such as nitrosoureas and dacarbazine [4, 5, 18, 24, 31, 58, 67, 72].

Among the mechanism of DNA repair, three main processes have been described [29]: reversion, excision, and tolerance of the lesion. Reversion and excision mechanisms represent DNA repair *stricto sensu*, which can be defined as the removal of damage from DNA and restoration of the genetic information. However, tolerance of the lesion should also be considered as a mechanism of DNA repair, illustrated in bacteria by the so-called SOS response [68]. The involvement and efficiency of each repair process depends on (1) the chemistry of the lesion, (2) the intrinsic cellular capacity for repair, and (3) the structure and dynamics of the chromatin (active transcription and replication) [8].

In the case of antitumor alkylating agents and cisplatin, the main pathway of repair is the excision-repair process, which leads to the removal of either mono- or bifunctional lesions. The excision-repair mechanism is well known in bacteria [66] but remains under investigation in eukaryotes [36]. This mechanism consists of recognition of the lesion, incision on both the 3′ and 5′ ends of the damage, displacement of the modified oligonucleotide, and gap filling by DNA synthesis. Following the ligation step, the DNA template is restored. This process, which in bacteria and eukaryotes, respectively, requires 3 and at least 7 genes for the incision step, is expressed at a basal level. The DNA excision-repair proficiency of cells is a major factor in the level of cell resistance to many drugs since repair-deficient cells are hypersensitive to mitomycin C, CDDP, or other cross-linking agents [37, 48, 53, 62]. Enzymes are likely to cooperate in the case of interstrand cross-links, excision repair, and recombination [14].

Table 1. Examples of techniques used to study DNA repair

Quantification of adduct removal in DNA:
Radioactively labeled adduct
Antibodies (mono- or polyclonal)
Direct identification of the adducts by HPLC
In vitro enzymatic removal with UVRABC excinuclease
PCR assay (blockade of Taq polymerase)
DNA repair:
Unscheduled DNA-repair synthesis
In vitro assay for DNA-repair synthesis
Transfection with a damaged plasmid

HPLC, High-performance liquid chromatography; PCR, polymerase chain reaction

Is DNA repair involved in cellular resistance?

To circumvent the toxic effects of DNA alkylation, different mechanisms of resistance take place that allow tumor cells to escape the cytotoxic effect of chemotherapy. Among these various mechanisms of resistance, DNA repair has recently been emphasized [4–7, 26, 46, 56].

Different approaches have been undertaken to measure DNA repair, some of which are listed in Table 1. Repair activity has been monitored as enhancement of (1) unscheduled DNA synthesis [73], (2) the rate of adduct removal [6, 26, 46], and (3) the expression and survival of damaged plasmid [11, 13, 16, 38, 41, 60]. However, the extent of plasmid reactivation did not correlate with the level of cell resistance to the compound tested [38, 60]. These results imply that additional events are responsible for the specificity of repair of the damaged chromatin. The higher rate of DNA-adduct removal suggests that the critical step in resistance is likely to be the preincision event rather than the polymerase reaction. Increased excision repair might rely on the enhancement of specific gene expression as with bacterial *uvrA,B,D* genes [30, 66]. Resistant cell lines might also exhibit an overexpression of damaged-recognition proteins [12, 15, 19] that could modulate the efficacy of excision repair. A modification of topoisomerase II activity could also provoke an indirect effect on the excision pathway. Despite contradictory reports [21, 22], inhibitors of topoisomerase II act synergistically with CDDP in clinical experiments. Moreover, in some cases resistance to alkylating agents can be associated with collateral sensitivity to topoisomerase II inhibitors, and vice-versa [27, 45, 63].

As discussed above, increased DNA-repair activity could result from an alteration in reversion, excision, and/or tolerance of the lesion and the different repair mechanisms could act coordinately. A tolerance mechanism is hypothetical and largely stems from our knowledge of the repair processes in bacteria. For instance, the fidelity of the repair process has not yet been measured, and a difference in the quality of repair could account for the difference

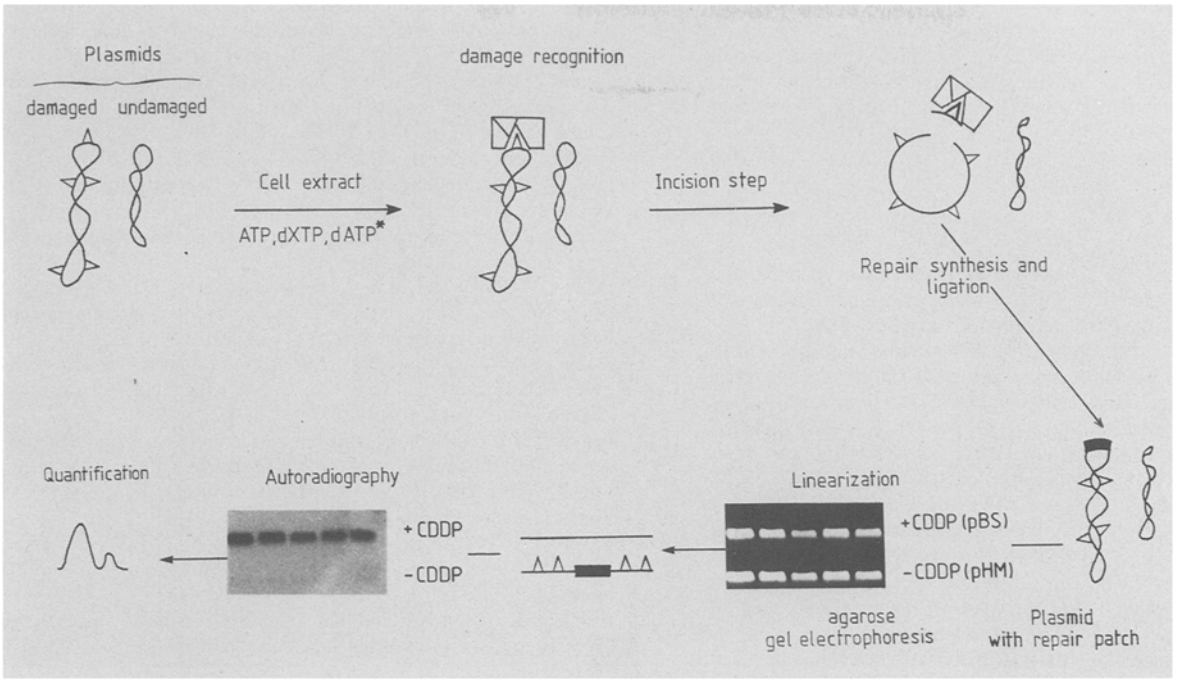

Fig. 1. Scheme for the DNA-repair synthesis in vitro assay (adapted from Wood et al. [71]). The repair activity was obtained by incubation of damaged and undamaged plasmids with cell extracts in the presence of deoxynucleoside triphosphates (dNTPs), adenosine triphosphate (ATP), and [α^{32}P]-deoxyadenosine triphosphate (dATP). The damaged plasmid is pBS (2959 bp) and the control plasmid is a 3738-bp derivative. The latter is a substrate for nonspecific endonucleolytic activity and polymerization activity on nicked plasmid. Plasmid DNA was purified from reaction mixtures, linearized, and then electrophoresed. Data were quantified by autoradiography and densitometry and/or scintillation counting of excised DNA bands

in survival of sensitive and resistant Walker cell lines [54, 57].

The involvement of DNA repair in resistance mechanisms has been studied under the assumption that lesions are chemically similar in resistant and sensitive cell lines. However, alkylating-agent binding to DNA might be modulated by the chromosomal structure. Finally, DNA repair is gene-specific since the efficiency of repair correlates with the transcriptional activity of a specified gene [8]. However, differences exist not only between DNA-damaging agents but also between lesions caused by a specific agent; for example, CDDP intrastrand adducts are rapidly removed from actively transcribed genes, but interstrand cross-links show no preferential repair in active genes [8]. Therefore, additional experiments should be done to look for a correlation between gene-specific repair and resistance.

Assessment of DNA-repair involvement in cell resistance to alkylating agents by an in vitro repair assay

DNA-repair involvement in cell resistance has been demonstrated in resistant cell lines obtained by in vitro stepwise selection leading to 100-fold increases in resistance in some cases [27]. However, the absence of a correlation between the level of resistance and the rescue of a damaged vector appeared to be a limitation of this assay. A molecular biochemical approach was required to investigate this question in tumor cells grown either in vitro or in vivo. We therefore used an in vitro assay previously described by Wood et al. [33, 71], which allows quantification of the excision-repair capacity in cell extracts (Fig. 1). In this assay, the excision process is monitored by the incorporation of [α^{32}P]-deoxyadenosine monophosphate (dAMP) into damaged plasmid DNA during the repair-synthesis step. Some drawbacks in this quantification of excision-repair capacity in cell extracts should be emphasized: (1) this assay deals with global repair as opposed to in vivo preferential repair of transcribed genes: (2) the DNA-repair process takes place on naked plasmid DNA instead of chromosomal DNA, and the efficiency of lesion removal is quite low [71]; and (3) during the preparation of cell extracts, one can lose some specific proteins involved in the repair mechanism. However, excision repair by cell extracts is close enough to genomic repair in cells to allow purification of repair proteins [59]. Moreover, cell extracts can carry out repair synthesis in DNA damaged by ultraviolet light, psoralen, acetylaminofluorene, or platinum(II) agents, and monoadducted DNA can be used as a substrate for in vitro excision repair [33, 34, 55, 65, 71].

The level of DNA repair tested by this assay is dependent on different parameters: (1) the cell-protein extract concentration, (2) the KCl content of the assay, (3) the reaction time, and (4) the level of plasmid modification [33, 71]. After the optimization of these parameters, which are dependent on the extent of damage and the cell line or tumor sample used, this assay should be of interest in investigations of the mechanism of cell resistance to alkylating agents and cisplatin with regard to a possible modulation of DNA-repair capacities.

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