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Review paper

Generation of DNA damage by anti-neoplastic agents

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DNA has been one of the major targets of cancer chemotherapy. A variety of anti-neoplastic agents can cause different types of DNA lesions, including base alterations, single- or double-strand DNA breaks, DNA–DNA cross-links and DNA–protein cross-links. The exact processes by which these DNA lesions lead to cell death remain uncertain. However, pivotal roles of intracellular Ca^{2+} ion mobilization, activation of Ca^{2+}–Mg^{2+}-dependent endonuclease and induction of several oncogenes have been proposed. Understanding the mechanism of DNA damage and subsequent cell death will be important to improve the efficacy of cancer chemotherapy.

Key words: Anti-neoplastic agents, apoptosis, cancer, chemotherapy, DNA damage, endonuclease.

Introduction

Cellular genomes are continuously exposed to endogenous and exogenous insults which induce structural alterations. These are termed spontaneous and environmental DNA damage, respectively.[1] Fortunately, cells have a specific surveillance system to sense and repair these DNA lesions. Thus, observed DNA damage is the consequence of the true DNA damage induced by causative agents and DNA repair activity. Spontaneous DNA damage is now recognized as a causative factor for aging, carcinogenesis and cellular differentiation.[2–4] These intrinsic lesions are byproducts of normal cellular metabolic events such as oxidation or methylation.[1,3]

Among the exogenous processes are various chemical and physical agents including anti-neoplastic drugs. The following types of drug-induced DNA lesions have been described:[5] base alterations, single- or double-strand DNA breaks, DNA–DNA cross-links and DNA–protein cross-links. In addition, a line of recent evidence has suggested that secondary endonucleolytic cleavage of DNA can occur by drugs which do not interact with DNA directly.[6] Chemotherapeutic agents and associated enzymes involved in the generation and repair of DNA damage are listed in Table 1. Notably, most DNA-damaging agents can induce more than one specific type of DNA lesion.

The scope of this review article is to describe the characteristics of DNA damage caused by anti-cancer drugs such as alkylating agents, anti-metabolites, cytidine analogs and topoisomerase inhibitors. The scope is also to discuss the intracellular events following DNA damage which lead to cell death.

DNA damage caused by anti-neoplastic agents

Alkylating agents

Alkylating agents were the first group of drugs identified that cause DNA damage. These agents can be divided into two groups, monofunctional or bifunctional, depending on their number of reactive sites with DNA. The N^7 position of guanine and the N^3 position of adenine are the most reactive sites.[7] Transcriptionally active regions of nucleosomal DNA are more susceptible to alkylation.[8] Consequently, bifunctional alkylating agents produce interstrand or intrastrand DNA–DNA cross-links, which have been considered to be responsible for the cytotoxicity.[8]

O^6-alkylguanine adducts in DNA are removed by O^6-alkylguanine-DNA-alkyltransferase through transfer of the alkyl group to an internal cysteine residue of the enzyme.[9] Although the amount of O^6-alkylguanine DNA lesions does not directly correlate with the cytotoxic effects caused by

Table 1. Type of DNA damage by anti-neoplastic agents

DNA Damage	Drug	Enzyme[a]
Base alteration	alkylating agents	O^6-alklylguanine-DNA-alkyltransferase
Single/double-strand breaks	bleomycin	
	antimetabolites	uracil-DNA glycosylase
	cytidine analogs	DNA ligase
		DNA polymerase
DNA–DNA cross-links	alkylating agents	
	cisplatin	
DNA–protein cross-links	anthracyclines	topoisomerase II
	epipodophyllotoxins	
	aminoacridines	
	camptothecin	topoisomerase I
Nucleosomal DNA cleavages	glucocorticoid	Ca^{2+}–Mg^{2+}-dependent endonuclease
	tumor necrosis factor	

[a] Enzymes denote those which are associated with either generation or repair of DNA lesions by anti-neoplastic agents.

alkylators, the following evidence suggests the role of O^6-alkylguanine in their toxicity: (1) tumor cells deficient in O^6-alkylguanine-DNA-alkyltransferase (this phenotype has been referred as Mer^- cells) were found to be more susceptible to alkylating agents than those with an abundance of the enzyme,[10] (2) transfection of the gene for alkyltransferase to Mer^- cells decreased the sensitivity to alkylating agents[11] and (3) depletion of the enzyme's activity by addition of O^6-benzylguanine significantly enhanced the toxicity.[12]

Antimetabolites

Antimetabolites, such as methotrexate (MTX), 5-fluoropyrimidine or hydroxyurea, are widely used as anti-cancer drugs. The mechanism(s) by which these agents kill cells have been an issue of extensive investigation. Apart from their primary effects on cellular metabolism, attention has recently been focused on DNA lesions caused by these drugs.

The primary target of MTX has been the enzyme dihydrofolate reductase.[13] Thus, MTX treatment results in an inhibition of both *de novo* purine biosynthesis and the conversion of dUMP to TMP by depletion of cellular reduced folates.[14] These metabolic effects lead to DNA synthesis inhibition and this has been regarded as the principal mechanism of MTX cytotoxicity. The ability of MTX to induce DNA strand breaks, which was first postulated by Li and Kaminskas,[15] correlated well with MTX cytotoxicity. These authors attributed such stand breaks to the defective repair of naturally occurring DNA lesions, because they appeared only in mature DNA. This type of defective repair was caused by the limitation of TTP and purine nucleotides. Another hypothesis presented by Goulian *et al.*[16] was the misincorporation of dUMP residues into DNA due to the increased dUTP/TTP ratio as a consequence of inhibition of thymidylate synthetase. Misincorporated uracil is subsequently removed by uracil-DNA glycosylase, which is then recognized as DNA strand breaks. Further evidence that there exists an inverse correlation between dUTPase activity and MTX toxicity favors this hypothesis.[17] However, Fraser and Pearson have not approved such a misincorporation.[18]

Finally, the role of deoxyribonucleoside triphosphate (dNTP) pool imbalance has been documented. dNTP pool imbalance can be achieved either by addition of various deoxyribonucleosides or by inhibition of *de novo* dNTP synthesis. For example, treatment of cells with deoxyadenosine plus an adenosine deaminase inhibitor, deoxycoformycin, causes the accumulation of DNA strand breaks accompanied by elevated dATP levels in both resting[19,20] and proliferating lymphocytes.[21] This is also observed after treatment of mouse FM3A cells with 2-chlorodeoxyadenosine, presumably through depletion of dATP and dGTP.[22] Hydroxyurea, an inhibitor of ribonucleotide reductase, has also been shown to induce DNA lesions.[23] MTX, as mentioned above, causes a profound decrease in TTP and purine nucleotides. The association of such dNTP pool changes was proven when simultaneous additions of thymidine and hypoxanthine were shown to counteract the appearance of DNA lesions.[24] The maintenance of

intracellular dNTP pools is balanced by *de novo* synthesis and the salvage of deoxyribonucleosides.[25] Thus, a mutational loss of the salvage enzyme(s) is expected to result in a decrease of the corresponding dNTP pool through perturbation of the substrate cycle.[25,27] Sano *et al.* have shown that thymidine kinase-deficient cells were more susceptible to MTX toxicity and generated more DNA lesions induced by MTX than those of wild-type or hypoxanthine-guanine phosphoribosyl transferase-deficient cells.[27] In addition, thymidine kinase-deficient cells had only one-fifth of TTP of the wild-type, which decreased quickly after MTX treatment. This phenomenon pointed to an important role for TTP pools during the formation of DNA damages by MTX.

5-fluorouracil (5-FU) is another antimetabolite which has DNA damaging activity.[28,29] 5-FU is a prodrug, whose active metabolite is 5-fluoro-(deoxy)uridine monophosphate. An important element of the toxicity is RNA directed, although the toxic mechanism cannot be fully explained by RNA damage.[30] At present, the induction of DNA lesions by 5-FU can be attributed to two different mechanisms. The first is due to direct incorporation of 5-FU into DNA, albeit to a small degree. Such DNA lesions are prevented by preincubation of cells with aphidicolin, an inhibitor of DNA polymerase α and δ.[29] The second is the inhibition of thymidylate synthase, which is reversed by thymidine.[31] These DNA lesions are augmented by the simultaneous addition of leucovorin, possibly because of the formation of a stable complex between thymidylate synthase and FdUMP.[32] One plausible explanation for the induction of DNA damage by TTP depletion recently put forward is an activation of Ca^{2+}–Mg^{2+}-dependent endonuclease,[33] which will be discussed in a later section.

Cytidine analogs

1-β-D-arabinofuranosylcytosine (ara-C) must be converted to the active form, ara-CTP, to exert its biological activity.[34,35] The interaction of ara-CTP with DNA is 2-fold: one is the direct inhibition of DNA polymerase α and the other is a chain termination after its incorporation into DNA.[35] This results in the inhibition of DNA replication as well as unscheduled DNA synthesis which is necessary for DNA repair. This may explain, at least in part, the mechanism for the accumulation of DNA strand breaks in ara-C-treated cells. Moreover, the exposure of human leukemia cell lines to ara-C decreases DNA ligase activity by 40–92%, presumably due to inhibition of ligase–adenylate complex generation.[36] Considering the activity of ara-C as a DNA repair inhibitor, synergy between ara-C and other DNA-damaging agents can be expected. In fact, DNA lesions induced by cisplatin,[37] mitoxantrone[38] or MTX[24] have been enhanced when they are combined with ara-C, and this correlates with the cytotoxicity. However, these laboratory data do not necessarily correspond to those found in the clinical settings.

Azacytidine (azaCR), a cytidine analog with a nitrogen at the 5 position of the pyrimidine ring, has also been used as an anti-cancer drug.[39] AzaCR is mainly incorporated into RNA.[39] In comparison with its deoxy congener, azadeoxycytidine (azaCdR), the incorporation into DNA is much less in azaCR. In consequence, azaCdR generates significantly more DNA strand breaks than azaCR following a 24 h incubation.[40] However, at lower concentrations of these nucleosides, azaCR is more potent in inhibiting repair synthesis following X-ray irradiation. Interestingly, an increased responsiveness to ara-C, when combined with azaCR, was observed in refractory leukemia of childhood.[41]

Topoisomerase inhibitors

Topoisomerase II has been regarded as the molecular target of various anti-cancer drugs,[42–44] such as anthracyclines (doxorubicin, daunorubicin), epipodophyllotoxins (etoposide, teniposide) and acridines (m-AMSA). The interaction of topoisomerase II with DNA consists of the following:[42,44] (1) reversible cleavage of both DNA strands through covalent binding at the 5′ termini of the broken end, (2) the passing of an intact double-stranded DNA through the break under consumption of ATP and (3) religation of the cleaved DNA. Topoisomerase II targeting drugs are believed to interfere with the topoisomerase II mediated DNA breakage and reunion reaction by stabilizing an intermediate between DNA and the enzyme, termed the 'the cleavable complex'.[42–44] As a cleavable complex, the enzyme is so tightly associated with the break site termini that DNA cleavage can be detected only when lyzed in the presence of a denaturing detergent such as sodium dodecyl sulfate. Thus, this type of DNA damage was defined as protein-associated DNA breaks or DNA–protein cross-links.[42–44] DNA breaks by

topoisomerase II inhibitors are generated rapidly, reaching a plateau after 30–60 min of drug exposure. Such breaks are immediately resealed upon drug removal.[45]

Several lines of evidence have proven that the initial generation of protein-associated DNA breaks is responsible for the cytotoxicity. First, Rowe *et al.* have found a good correlation between cytotoxic effects and DNA–protein cross-links which were induced by a series of closely related acridine compounds.[46] Second, cell lines resistant to topoisomerase II inhibitors have been selected except for overproduction of MDR1 genes. These have either alterations of the catalytic activity in topoisomerase II,[47] decreased protein content of the enzyme[48] or mutation of the drug binding sites.[49] Although the sites of mutation differ from cell to cell, the general outcome was a decreased formation of the cleavable complex induced by the drug used for the selection. Finally, various factors which antagonize topoisomerase II mediated cytotoxicity, such as ethidium bromide,[50] ouabain,[51] polyamines[52] and retinoids,[53] exhibited a parallel decrease of DNA–protein cross-links.

Topoisomerase II levels increase with cell proliferation with their peak at the G_2/M phase of the cell cycle.[54,55] Concomitant with the change, DNA cleavages are most prominent at the G_2/M phase, whereas the cytotoxic effect is at a maximum at the late S/early G_2 phase.[55] This disparity, together with rapid resealing of DNA lesions upon drug removal, has suggested the existence of a new factor triggering cell death. One of the candidates is newly synthetized endonuclease, since cytotoxicity of topoisomerase II inhibitors has been attenuated by cycloheximide.[56,57]

Topoisomerase I creates a single-stranded break to pass the unbroken DNA strand. In contrast to topoisomerase II, the reaction mediated by topoisomerase I does not require either ATP or divalent cations. Topoisomerase I inhibitor, camptothecin, has been thought to stabilize the complex formed by DNA and topoisomerase I.[43] Again, denaturation of the cleavable complex with a denaturant has revealed protein-associated, single-stranded DNA breaks.[43] The levels of topoisomerase I and the generation of camptothecin-mediated DNA lesions are constant throughout the cell cycle. However, the cytotoxic effect of camptothecin specifically appeared in the S phase.[58] To explain this discrepancy, Hsiang *et al.* proposed a collision model.[59] According to this hypothesis, a collision between moving replication forks and the camptothecin-induced cleavable complex causes an irreversible arrest of the replication fork. In fact, transient inhibition of DNA synthesis by pretreatment of cells with aphidicolin almost completely abrogated camptothecin-induced cytotoxicity without changing the formation of any cleavable complex.[60]

From DNA damage to cell death

Although the association of DNA damage initially induced by anti-neoplastic agents with their cytotoxicities is convincing, the precise mechanism by which they lead to cell lethality remains unclear. Transcriptional inhibition of essential genes for cell survival is one possible explanation.[5] Recently, a large body of cellular events have been identified following DNA damage. The roles of these events in the cytotoxicity will be discussed in the following sessions.

Activation of endonuclease

'Apoptosis' or 'programmed cell death' has been recognized as a typical form of physiological cell death during fetal development[61] or in the cellular turnover of normal adult tissues.[62] Apoptosis has two major characteristics:[63,64] one is internucleosomal DNA cleavage which shows a ladder of fragments of approximately 180 base pairs and the other is a morphological change with condensed chromatin. In extensive studies aimed at clarifying the mechanism of apoptosis, the role of activation of Ca^{2+}–Mg^{2+}-dependent endonuclease for the generation of DNA fragmentation has been documented.[63,64] Supporting evidence is that inhibitors of endonuclease, such as Zn^{2+} ions[65] or aurintricarboxylic acid,[66] have prevented DNA fragmentation during apoptosis.

Kaufmann has reported that treatment of human leukemia cells with various anti-cancer agents including etoposide, camptothecin, MTX or ara-C, resulted in internucleosomal DNA fragmentation.[6] Such DNA fragmentation was also induced following exposure to glucocorticoid[67] or tumor necrosis factor,[68] which are not considered to attack DNA directly. Therefore, DNA fragmentation by these drugs is one of the general steps leading to cell death. Notably, etoposide-induced DNA fragmentation was inhibited by Zn^{2+} ions.[69] Initial studies on apoptosis revealed that the occurrence of apoptosis depends to a great extent upon new protein synthesis. Wyllie *et al.* has shown that

apoptosis induced in thymocytes by glucocorticoid or calcium ionophores was prevented by cycloheximide.[70] However, subsequent studies have not necessarily agreed with this notion.[6,65] Instead, inhibition of protein synthesis itself was found to induce apoptosis in other experimental systems.[6,71] Numerous reports have shown that inhibition of protein synthesis has protected cells from the cytotoxic effects of certain anti-neoplastic drugs.[72–74] Since the inhibition of protein synthesis has resulted in the perturbation of several cellular events including the cell cycle,[74] the interpretation of this data should remain cautious. The problem of whether endonuclease operated in apoptosis is constitutive or newly synthetized awaits further investigation.

What is the mechanism involved in an activation of endonuclease? Previous studies emphasized the importance of the alteration of intracellular Ca^{2+} during apoptosis. For example, glucocorticoid-induced DNA fragmentation in thymocytes was dependent on an early sustained increase in intracellular Ca^{2+} levels.[66] Doxorubicin[75] or etoposide (T Shimizu and M Kubota, unpublished observation) also had an ability to increase cytosolic Ca^{2+} concentrations. These changes were rather late effects, which were unlike their rapid induction in mitogen-stimulated lymphocytes,[76] and they seemed to precede the appearance of DNA fragmentation. In addition, removal of Ca^{2+} ions from culture medium prevented cytotoxicity by etoposide.[77] It is important to note that elevation of intracellular Ca^{2+} has also activated several degradative enzymes, which results in loss of cellular integrity.[66,78,79] Another candidate which participates in the generation of apoptosis is protein kinase C (PKC). The role of PKC is rather conflicting, because both positive and negative effects have been postulated. For example, addition of phorbol 12,13-dibutyrate has inhibited interleukin 2 (IL-2) deprived cell death in an IL-2 dependent T cell line.[80] Phorbol esters have been shown to block DNA fragmentation and cell death in thymocytes exposed to glucocorticoid.[81] However, 12-*O*-tetradecanoylphorbol 13-acetate (TPA) itself has induced apoptotic changes in immature thymocytes.[82] With regard to the action of anti-tumor drugs, the following evidence has suggested the association of PKC: (1) doxorubicin has activated PKC,[83] (2) pretreatment of cells with TPA partially protected cells from the cytotoxic effects of etoposide, vincristine, mitoxantrone and MTX,[84–85] and (3) some drug resistant lines have a mutation on PKC.[86,87] Although still speculative, post-translational modification of endonuclease through protein phosphorylation may, in part, explain these results.

Activation of poly(ADP-ribose) polymerase

Poly(ADP-ribose) polymerase is an enzyme ubiquitous in eukaryotic nuclei, which transfers ADP-ribose from NAD to diverse nuclear proteins.[88,89] ADP-ribosylation functions as a post-translational protein modification like phosphorylation and methylation. Activation of poly(ADP-ribose) polymerase by DNA strand breaks has been observed following treatment with alkylating agents.[90,91] Moreover, addition of inhibitors of the enzyme, such as 3-aminobenzamide (3-ABA) or nicotinamide, have retarded the repair process.[92] The role of poly(ADP-ribosy)lation in DNA damaged cells remains unsolved. An early hypothesis presented by Creissen and Shall was the activation of DNA ligase II after ADP-ribosylation.[93] However, subsequent experiments cast doubt on this claim, since the general consequence of ADP-ribosylation of the enzyme has resulted in its inhibition.[94,95] Another proposal is a change in chromatin structure. The alterations of chromatin protein, especially histones, have made it easier for DNA repair enzymes to access DNA.[89] The final hypothesis is the programmed removal of cells with extensively damaged DNA.[96,97] As noted above, massive DNA strand breaks can trigger a severe drop in cellular NAD due to activation of poly(ADP-ribose) polymerase. Concomitantly, ATP levels decrease, since NAD works as an essential cofactor for glycolysis. A cascade of events which conclude in cell mortality following massive DNA damage is termed a 'suicide response'.[96]

DNA damaging anti-cancer drugs including doxorubicin,[98,99] etoposide[100] and MTX[101] were reported to activate poly(ADP-ribose) synthesis. Incubation with glucocorticoid[102] or tumor necrosis factor[103] also stimulated poly(ADP-ribose) polymerase, although it was a late effect. Therefore, it is possible that activation of poly(ADP-ribose) polymerase is a consequence of secondary endonucleolytic DNA cleavage. Tanizawa *et al.* have demonstrated that etoposide-induced interphase cell death in human leukemia cells was accompanied by a profound decrease in NAD and ATP.[100] Notably, such effects were prevented by 3-ABA or nicotinamide. This suggests that suicidal activation of poly(ADP-ribose) synthesis has, at least in part,

a role in cell death mediated by etoposide. However, there have been several reports which demonstrate an increased cell lethality by an anti-cancer drug after co-incubation with inhibitors of poly(ADP-ribose) polymerase.[104,105] One possible explanation for it is an inhibition of repair synthesis. The other is that 3-ABA may allow activation of Ca^{2+}–Mg^{2+}-dependent endonuclease, since this enzyme was shown to be inhibited by poly(ADP-ribosy)lation.[106] These discrepancies may be a reflection of the versatility of the polymer's functions. Recently, Chatterjee *et al.* have selected mutants with defective poly(ADP-ribose) synthesis.[107] These cells have revealed resistance to etoposide without any changes in the initial cleavable complex formation. Whatever the mechanism may be, this also shows the association of poly(ADP-ribose) synthesis with etoposide cytotoxicity.

Other genes activated by DNA damage

Several genes and proteins other than Ca^{2+}–Mg^{2+}-dependent endonuclease or poly(ADP-ribose) polymerase are activated in response to DNA damage. These have been extensively studied in procaryotes and more than 20 genes which are transcriptionally activated have been identified. Although a paucity of data exists in eukaryotes, DNA damage can induce several genes of known functions including *cdc9* (DNA ligase), *pol1* (DNA polymerase 1), *cdc8* (thymidylate kinase), *rnr2* (ribonucleotide reductase), *rad2* (excision repair) and *rad54* (recombinational repair).[108] Most of these genes have been associated with DNA replication. Recently, Fornace *et al.* have reported genes that encode DNA damage (UV irradiation or alkylating agents) inducible transcripts in rodent cells, which were designated gadd (growth arrest and DNA damage inducible).[109] They suggested a role for these genes in the negative control of cell growth. Additionally, Chao *et al.* have demonstrated the induction of DNA damage-recognition protein following treatment with cisplatin. Overexpression of this protein in cisplatin-resistant cells may suggest a crucial role in DNA repair.[110]

Another set of genes induced by DNA damages are early response genes. For example, exposure of human leukemia cell lines to ara-C[111] or etoposide[112] induced expression of c-*jun* or c-*fos* proto-oncogenes. The induction of c-*jun* expression by etoposide reaches a maximum at 3 h during periods of oligonucleosomal DNA fragmentation.[112] Transient activation of c-*fos* transcription was also observed by using UV light.[113] Moreover, elevation of c-*myc* protein by alkylating agents or gamma irradiation was documented.[114] Inhibition of DNA strand rejoining with 3-ABA maintained the c-*myc* protein level in an elevated state. It should be noted that the activation of proto-oncogenes, mainly at transcriptional levels, can be achieved in apoptosis during hormonal deprivation.[115] At present, it is plausible that an increase of proto-oncogene expressions represents one element of the cellular response to alterations in DNA structure. However, whether it works as a promotive or a protective factor for the lethal effects by DNA damaging agents still requires further study.

Cell cycle perturbation

Several DNA damaging agents have been reported to arrest cells in the G_2 phase of the cell cycle.[116,117] It was initially hypothesized that the event was presumably due to inhibition of transcription of the essential genes for passage to mitosis, albeit without any definite evidence.[117] The recent discovery of the *rad9* gene and its mutant in the yeast, however, has provided the idea that G_2 arrest is a positively regulated process.[118-119] The gene product of *rad9* is essential for G_2 arrest following DNA damage. Thus, cells lacking the *rad9* gene are highly susceptible to DNA damage, since they are unable to arrest in the G_2 phase. Another important cell cycle gene related to G_2 arrest by anti-cancer drugs is the *cdc2* gene. Lock and Ross have reported inhibition of *cdc2* kinase within 1 h of addition of etoposide, which had a close association with G_2 arrest induced by the drug.[120,121] One of the biological roles of G_2 arrest seems to allow the cells the opportunity to repair DNA lesions. Therefore, promotion of cell cycle progression without repairing DNA lesions by caffeine potentiates the toxicity of DNA damaging agents.[122]

Conclusion

Although hypothetical, the intracellular events from DNA damage by anti-neoplastic agents to subsequent cell death are summarized in Figure 1. However, we do not know exactly whether each event described here is a cause or a consequence of the cytotoxicity. Whatever the mechanism(s) of cell death may be, it is obvious that the amount of initial DNA lesions introduced by DNA damaging drugs

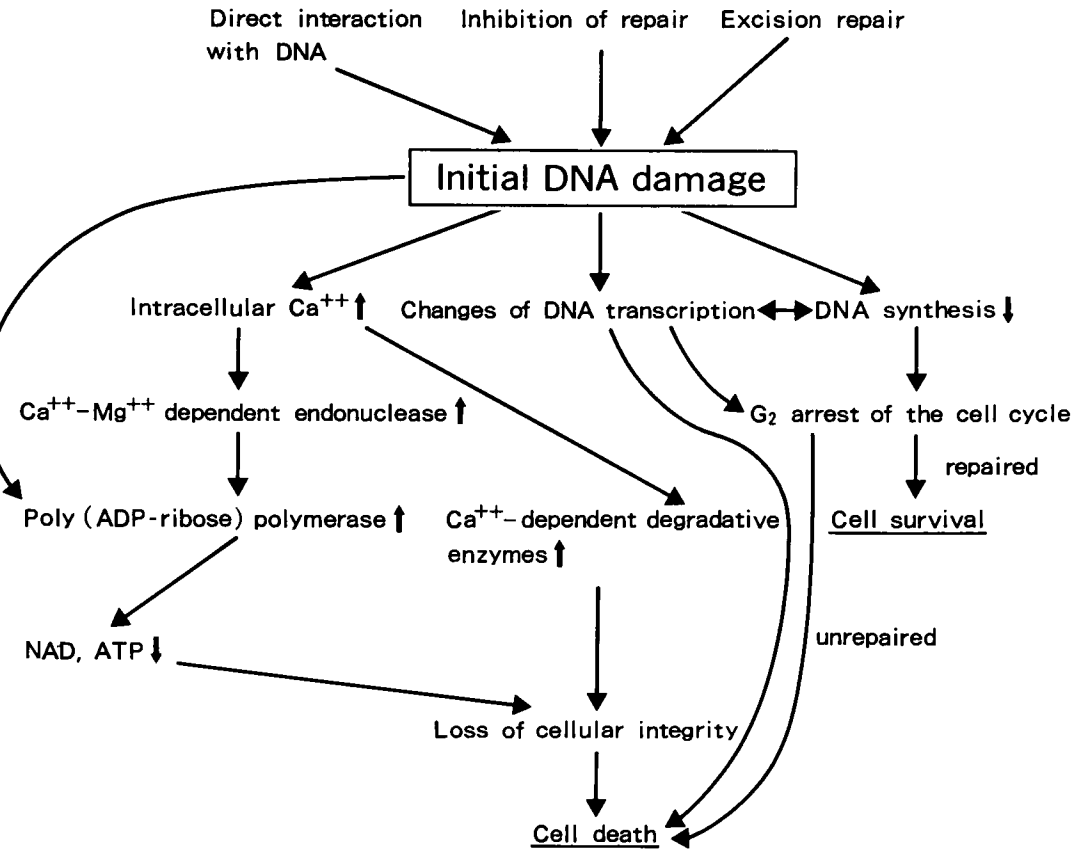

Figure 1. Hypothetical scheme for the intracellular events from DNA damage to cell death by anti-neoplastic agents.

is a crucial determinant of their cytotoxicity. Therefore, measuring DNA damage in cancer cells will provide us with a good prediction of chemosensitivity to either a single drug or a drug combination. Drug interaction is an important issue, because modern cancer chemotherapy is based on multiple drug combinations. Several patterns of drug combinations have been proven to be effective and have correlated well with the generation of DNA lesions. However, some combinations have turned out to work antagonistically from the points of both generation of DNA lesions and cytotoxicity.[74,123]

Aggressive and multiple drug combination chemotherapy has achieved tremendous progress in cancer treatment in recent years. In pediatrics, for example, acute lymphocytic leukemia is considered to be curable at the present time.[124] In turn, several deteriorating effects including secondary cancer have emerged.[125] Thus, clinical oncologists are responsible for establishing more scientific and more individualized cancer chemotherapy based on the knowledge of basic research.

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