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THE RELEVANCE OF DNA REPAIR IN THE CYTOTOXIC RESPONSE OF MAMMALIAN CELLS TO ALKYLATING AGENTS

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Summary. - *Some experiences on the relevance of the DNA repair mechanisms in the sensitivity of mammalian cells to alkylating agents are illustrated. Using mouse tumor cell lines we showed an interesting correlation between O^6-alkylguanine repair, reduced cross-link formation, and resistance to antineoplastic drugs like chloroethylnitrosoureas. The same lines displayed cross-resistance to a methylating monomethyltriazene indicating a possible role of O^6-methylguanine in the toxicity of alkylating agents. In other studies we characterized some modified tissues (nodules) developed during hepatic chemical carcinogenesis in the rat. Such hyperplastic nodules displayed higher DNA repair ability, in keeping with their resistant phenotype against toxic and carcinogenic compounds. Both approaches, using cells in culture or animals, gave interesting hints suggesting future developments which may be useful in the understanding of cell transformation mechanisms as well as in cancer chemiotherapy.*

Riassunto (Considerazioni sull'importanza della riparazione del DNA nella sensibilità ad agenti alchilanti tossici in cellule di mammifero). - *Il presente lavoro illustra alcune esperienze sperimentali che documentano il ruolo dei meccanismi di riparazione del DNA sulla sensibilità di cellule di mammifero nei confronti di agenti alchilanti. Utilizzando linee cellulari tumorali di topo, noi abbiamo rilevato una interessante correlazione fra riparazione dell'O^6-alchilguanina, ridotta formazione di cross-link, e resistenza a farmaci antineoplastici della famiglia delle cloroetilnitrosouree. Le medesime linee cellulari presentavano una cross-resistenza verso un monoalchitriazene metilante indicando un possibile ruolo dell'O^6-metilguanina sulla tossicità del farmaco. In altri studi abbiamo caratterizzato alcuni tessuti modificati (noduli) che si sviluppano nel corso della carcinogenesi chimica nel fegato di ratto. Questi noduli iperplastici presentano una accresciuta capacità riparativa che si accorda col loro fenotipo resistente nei confronti di composti tossici e cancerogeni. Entrambi gli approcci, con cellule in coltura o con animali, hanno fornito interessanti spunti per sviluppi futuri i quali potrebbero essere utili per la comprensione di alcuni meccanismi di trasformazione cellulare o nel campo della terapia chimica dei tumori.*

Introduction

Alkylating agents may induce both cell death and neoplastic transformation in a wide range of animal species. As a consequence of their interaction with DNA, a broad spectrum of toxic and mutagenic DNA lesions is induced [1-3]. However, the biological effects of chemicals can vary greatly among different systems (organs, cells in tissues, culture, etc.) because many independent mechanisms affect the cellular response to DNA damaging agents. In this respect DNA repair processes play an important role in counteracting the presence of produced lesions. This paper summarizes the main results obtained by investigating DNA repair activities in: a) mouse tumor cell lines of varying sensitivity to alkylating agents widely used as antineoplastic drugs; and b) rat liver during carcinogenesis induced by alkylating agents.

Studies using cells in culture

In several cell lines the cytotoxicity of chloroethylnitrosoureas (CNU) appears inversely related to the cellular content of O^6-alkylguanine-DNA alkyltransferase (O^6-AT) [4]. It has been proposed that cells that are deficient in O^6-AT (Mer^-) do not remove the chloroethyl adducts on guanine which, through successive reactions, cause the formation of DNA-interstrand cross links (DNA-ISC) [5-7]. Most studies have been performed using cell lines that posses different biological properties. Therefore it is possible that in addition to the repair mechanisms, other factors are involved in differential

cellular sensitivity to these drugs. We investigated the relationship between CNU-induced DNA damage, repair, and cytotoxicity in the L1210 mouse leukemia cell line. Two cell variants were available: the parental cell line, sensitive to CNU, and a subline resistant to the drug (L1210/BCNU). Table 1 shows that L1210/ BCNU cells are less susceptible than L1210 cells to growth inhibition and DNA-ISC induction following a 1 h treatment with CNU. The two cell lines differ in the O^6-AT content. Thus the lower level of DNA-ISC caused by CNU in L1210/BCNU can be reasonably explained by the more efficient repair of potentially cross-linkable adducts on the O^6 atom of guanine.

These data are in accord with many results recently reported on the involvement of O^6-AT in the sensitivity of cells to CNU [4, 8, 9]. It is noteworthy that L1210 and L1210/BCNU bearing mice are also sensitive and resistant, respectively, to the antitumor activity of CNUs, suggesting that our observations in cells in culture can be extrapolated to the *in vivo* situation.

As a consequence of the observation that L1210/ BCNU cell line was cross-resistant to methyltriazenes, a study of DNA damage and repair induction by temozolomide (or methazolastone) was performed [10]. This experimental anticancer drug spontaneously decomposes producing 5-(3-methyl-1-triazeno)imidazole-4-carboxamide, a potent alkylating agent supposed to be the active intermediate [11]. Temozolomide is able to produce a series of DNA lesions, the most abundant being the 7-methylguanine (7-MeGua), whose biological effect is still unknown. DNA alkali-labile sites (ALS) are also produced after drug treatment probably as a consequence of the repair of 7-MeGua. In Table 2, the number of ALS, as determined by the alkaline elution technique, indicates that there was no difference in ALS repair between the two cell lines. Similarly no significant difference was observed in the enzymatic activity for 7-MeGua repair between the two nuclear extracts.

The lack of correlation between repair of 7-MeGua and cytotoxicity, suggests that 7-MeGua is not involved in the killing events induced by temozolomide treatment. By contrast, the levels of O^6-AT in the two cell lines (Table 1) might explane their differential response to the toxic effect of temozolomide indicating a possible role of O^6-methylguanine (O^6-MeGua) in cell killing. Several lines of evidence indicate that O^6-MeGua besides being mutagenic and carcinogenic could also produce some lethal effects [13-16]. The role of O^6-MeGua in cytotoxicity is still debated [17-19] and needs further studies. In particular, the relationship between repair of O^6-alkylguanine and sensitivity to cell killing is difficult to be evaluated when cell strains derived from different species are compared. We studied the repair activities in V79 Chinese hamster cells, which are rather resistant to the cytotoxic effect of monofunctional alkylating agents like N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), N-methyl or N-ethyl-N-nitrosourea (MNU, ENU) [20, 21] as compared to Mer$^-$ cell lines. The persistence of O^6-ethylguanine (O^6-EtGua), 7-ethylguanine (7-EtGua), and 3-ethyladenine (3-EtAde) in the cellular DNA of V79 cells after ENU administration is reported in Fig. 1. These results indicate that this cell line displays an efficient repair activity towards the two N-alkyl purines, 7-EtGua and 3-EtAde. On the contrary O^6-EtGua is not appreciably repaired over a 48 h period. Previous data [22, 23] showed that this cell line is also unable to remove O^6-MeGua.

Studies using whole liver tissue

To examine whether neoplastic progression may be related *inter alia* to some relevant changes of the DNA repair systems, we started a series of experiments based upon a two-stage model of liver chemical carcinogenesis (initiation-promotion) [24]. In this model the development of hepatoma is preceeded by the appearance of many hepatocyte nodules; the majority of these nodules disappear with time after withdrawal of the inducing chemicals, while the others are persistent and may progress to malignancy.

Hepatocyte nodules of both types are easily distinguishable by gross appearance and can be dissected from the surrounding parenchima; they display a variety of

Table 1. - *The sensitivity to killing and cross-links formation by CNU treatment, and the O^6-alkylguanine-DNA alkyltranferase activity of L1210 murine leukemia cell lines*

	Growth inhibition *(a)* (CNU 10 μM; 1 h) (%)	DNA-ISC *(b)* (CNU 10 μM; 1 h) rad equival.	O^6-AT *(c)* fmol/mg DNA
L1210	94	41	1700
L1210/BCNU	35	n.d.	5700

(a) Growth inhibition was evaluated after 3 replications (*i.e.* 72 h) in both cell lines.

(b) DNA interstrand cross-links (DNA-ISC) have been determined by the method of alkaline elution. Here we report the maximum number of DNA-ISC obtained 9 h after exposure to CNU. n.d. = not detectable.

(c) O^6-alkylguanine-DNA alkyltransferase (O^6-AT) was measured by O^6-MeGua disappearance from alkylated DNA after 1 h incubation according to the method described in [8].

Table 2. - *The alkali-labile sites formation by monomethylating agent temozolomide and 7-methylguanine repair in L1210 cell lines*

	Alkaline elution *(a)* temozolomide 100 μM	400 μM	7-MeGua-DNA *(b)* glycosylase pmol/min/mg DNA
L1210	0.316	0.772	23
L1210/BCNU	0.313	0.777	24

(a) Alkaline elution was performed at pH 12.6 for a total of 15 h (5 fractions of 3 h). Numbers represent -logDNA retention on filter after 12 h elution.
(b) The kinetics of excision of 7-MeGua by DNA glycosylase was measured in 120 min time range according to the protocol described in [12].

changes in many metabolic pathways which could be related to the observed resistance of the nodules to the damaging effects of many toxic and carcinogenic compounds [25]. Since hepatoma induction should require additional genomic changes within some nodular hepatocytes, a significant decrease in both rate and extent of DNA repair could play a relevant role in the conversion of nodules into hepatomas. Alternatively, an increase in DNA repair activities should represent one of the many aspects of the complex resistant phenotype.

The repair of O^6-MeGua by hepatic nodules as a function of the amount of the nuclear homogenate is reported in Table 3. The concentration (activity) of O^6-AT is four times higher in the nodules as compared to normal liver.

The data on the enzymatic removal of 3-methyladenine (3-MeAde) and 7-MeGua from substrate DNA by nuclear extracts of hepatocyte nodules and normal liver are shown in Table 4.

A significant increase in both base excision and O^6-AT activities is evident in hepatocyte nodules, their extent being similar. Even though a small contribution of cell proliferation and/or carcinogen pretreatment cannot be ruled out, the observed increase suggests that the "xenobiotic resistant phenotype" of hepatocyte nodules may also include changes of the DNA repair activities that could provide additional advantage for hepatocyte nodules to cope with a genotoxic evironment. Since many of the persistent hepatocyte nodules do not progress further (*i.e.*, do not develop cancer), experiments are in progress to establish if their phenotype includes changes in DNA repair systems distinguishable from those observed within liver tumors.

Conclusions

We have shown in this manuscript some examples of the importance of DNA repair mechanisms to the biological responses of mammalian cells to alkylating agents. A better knowledge of DNA repair mechanisms may provide useful applications in cancer chemiotherapy. It is conceivable, for example, that inhibition

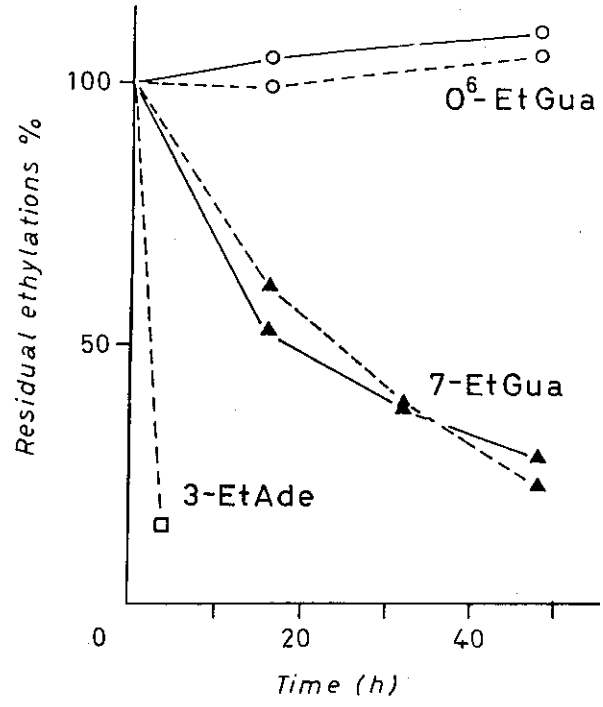

Fig. 1. - Time course of ethylated purines removal from cellular DNA of V79 cultures after ENU treatment. ^{3}H-adduct concentrations were corrected for DNA synthesis monitored by the dilution of ^{14}C-prelabelled DNA according to the method reported in [22].

Table 3. - *The O^6-alkylguanine-DNA alkyntransferase in the liver and hepatocyte nodules from Fischer 344 rats*

Nuclear extracts	O^6-AT fmol/mg DNA
Normal liver	3600
Hepatocyte nodules	15100

For further details see Table 1.

Table 4. - *The 3-methyladenine- and 7-methylguanine-DNA glycosylase activities in the liver and hepatocyte nodules from Fischer 344 rats*

Nuclear extracts	Base excision fmol/min/mg DNA 3-MeAde	7-MeGua
Normal liver	490	210
Hepatocyte nodules	2160	495

For further details see Table 2.

of O^6-AT can increase the effectiveness (and overcome resistance) of cancer cells to some alkylating chemicals.

Furthermore additional studies on repair of O-alkyl-pyrimidines and apurinic-apyrimidinic sites as well as on the effects of the perturbance of deoxynucleotide pools are necessary for a better understanding of the cellular responses to alkylating agents.

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