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Review

Chloroethylating nitrosoureas in cancer therapy: DNA damage, repair and cell death signaling

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ABSTRACT

Chloroethylating nitrosoureas (CNU), such as lomustine, nimustine, semustine, carmustine and fotemustine are used for the treatment of malignant gliomas, brain metastases of different origin, melanomas and Hodgkin disease. They alkylate the DNA bases and give rise to the formation of monoadducts and subsequently interstrand crosslinks (ICL). ICL are critical cytotoxic DNA lesions that link the DNA strands covalently and block DNA replication and transcription. As a result, S phase progression is inhibited and cells are triggered to undergo apoptosis and necrosis, which both contribute to the effectiveness of CNU-based cancer therapy. However, tumor cells resist chemotherapy through the repair of CNU-induced DNA damage. The suicide enzyme O^6-methylguanine-DNA methyltransferase (MGMT) removes the precursor DNA lesion O^6-chloroethylguanine prior to its conversion into ICL. In cells lacking MGMT, the formed ICL evoke complex enzymatic networks to accomplish their removal. Here we discuss the mechanism of ICL repair as a survival strategy of healthy and cancer cells and DNA damage signaling as a mechanism contributing to CNU-induced cell death. We also discuss therapeutic implications and strategies based on sequential and simultaneous treatment with CNU and the methylating drug temozolomide.

Contents

Abbreviations: ACNU, 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)- 3-nitrosourea; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; BRCA, breast cancer protein; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-L-nitrosourea; CHK, checkpoint kinase; CNU, chloroethylnitrosourea; DDR, DNA damage response; dsDNA, double-stranded DNA; DTIC, Dacarbazine; DSB, double-strand break; FA, FANC, Fanconi anemia; Fotemustine, 1-[*N*-(2-chloroethyl)-*N*-nitrosoureido] ethylphosphonic acid diethyl ester; γH2AX, histone 2A.X phosphorylated at serine 139; HR, homologous recombination; ICL, interstrand crosslink; MeCCNU, 1-(2-chloroethyl)-3-(4-methlycyclohexyl)-1-nitrosourea; MGMT, O^{6-}methylguanine DNA-methyltransferase; MMC, mitomycin C; MRE11, meiotic recombination protein 11; NBS1, NBN, nibrin; NER, nucleotide excision repair; N7-ClEtG, N7-chloroethylguanine; N7-OHEtG, N7-hydroxy-ethylguanine; O^6-ClEtG, O^6-chloroethylguanine; O^6-OHEtG, O^6-hydroxy-ethylguanine; O^6-MeG, O^6-methylguanine; PARP, poly (ADP-ribose)-polymerase; RPA, replication protein; SSB, single-strand break; ssDNA, single-stranded DNA; STZ, streptozotocin; TLS, translesion synthesis; TMZ, temozolomide; UV, ultra-violet light; XP, xeroderma pigmentosum protein.

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1. Introduction

Alkylating anticancer drugs induce a plethora of DNA lesions of which DSB and ICL are highly cytotoxic [1,2]. DNA repair mechanisms and DNA damage tolerance ensure normal cell survival. In tumor cells, DNA repair is often limited and out of balance, which makes them either more vulnerable to therapy or, adversely, more resistant to the cytotoxic drug [3]. Understanding and manipulating these repair processes can sensitize cancer cells and hopefully improve cancer therapy and patient outcomes. This review summarizes our understanding of the role of DNA repair in the therapy of cancer with alkylating drugs belonging to the group of chloroethyl-nitrosoureas (CNU).

The nitrosoureas lomustine (CCNU), nimustine (ACNU), carmustine (BCNU), semustine (MeCCNU) and fotemustine are used in cancer therapy akin to the methylating drugs temozolomide (TMZ), dacarbazine (DTIC), procarbazine and streptozotocin (STZ). While for methylating drugs the killing lesions and downstream triggered pathways have been intensively studied [4], CNU are not in the focus of research, although the mechanism of action is of high interest both for therapy and basic research. Similar to the methylating agents, CNU rapidly generate DNA adducts through the transfer of chloroethylene ions to DNA bases (Fig. 1). The N7 position of guanine is the preferred site for substitution, forming N7-chloroethylguanine (N7-ClEtG) and N7-hydroxyethylguanine (N7-OHEtG) in DNA [5]. An important nucleophilic site on DNA is the O^6-position of guanine, where O^6-ClEtG and O^6-hydroxy-ethylguanine (O^6-OHEtG) are formed. O^6-ClEtG is an unstable DNA adduct, which hydrolyses in aqueous solution to N1-hydroxyethylguanine (N1-OHEtG) or, in organic solution, to N2-ethanoguanine [5]. Through an intramolecular rearrangement, the unstable O^6-ClEtG forms N1-O^6-ethanoguanine that binds covalently to a cytosine of the complementary DNA strand and generates a stable DNA lesion, the N1-guanine-N3-cytosine ICL [6–8] (Fig. 2). These ICL represent only a small fraction (1–5%) of all formed adducts, however, they impart a high cytotoxic potency due to inhibition of DNA replication and transcription [9]. It has been estimated that as few as 20 to 40 ICL can be lethal to a mammalian cell if it is defective in ICL repair [9]. CNU differ in their potency of inducing ICL, with ACNU showing the highest potency compared to CCNU, BCNU and fotemustine [10]. The efficiency in the generation of ICL determines both the therapeutic response and the unwished side effects in high-grade glioma patients [11]. The most frequently observed side effects after therapeutic use of CNU are nausea, vomiting, marked and prolonged myelosuppression and - less often, but sometimes severe - hepatotoxicity or lung toxicity [12]. Pulmonary complications, namely interstitial pneumonitis, are observed with BCNU and CCNU [13], while ACNU [14] and fotemustine [13] are not associated with such complications. It seems that the potency to induce ICL does not correlate indispensably with the systemic toxicity of CNU.

2. Repair of CNU-induced DNA damage in replicating cells

2.1. The alkyltransferase MGMT

First line in the defense against CNU is the suicide repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) as O^6-ClEtG is a substrate for MGMT [15]. MGMT transfers the alkyl group from the exocyclic oxygen of the O^6-alkylguanine to the reactive Cys145 buried within a hydrophobic pocket in its active site, thereby restoring guanine [16]. This is an irreversible stoichiometric reaction, which leads to the inactivation of MGMT and its ubiquitin-mediated degradation by proteasomes [17–20] (Fig. 3). It has been suggested that different O^6-alkylguanines bind to the active center in either a reactive or an unreactive orientation, though the small width of the hydrophobic pocket favors the anti-conformation structure for larger alkyl groups [16]. Unlike other O^6-alkylguanines, for O^6-ClEtG it is difficult to study the binding mechanism and to measure the rate of removal by MGMT because of its rapid internal conversion to N1-O^6-ethanoguanine [21].

The capacity of a cell to repair O^6-alkylguanine is dependent on the preexisting amount of MGMT and the rate at which it is synthesized [22,23]. MGMT provokes expression-dependent resistance to alkylating agents [23] and protects hypersensitive homologous recombination (HR) mutants against methylating agents like TMZ [24] and chloroethylating agents like ACNU [25]. There is a high variability in MGMT expression between individuals, between tissues and even between cells of the same tissue [26]. The expression and activity are highly variable in cancer cells. High MGMT expression was reported for lung and breast tumors compared to normal tissues [27–31]. On the other hand, in malignant melanomas and glioblastomas its expression is relatively low [27,30,31]. Consequently, chemotherapy with alkylating agents is anticipated to be effective especially for the latter group of cancers. This is likely true also for brain metastases for which CNU are routinely used as the drug is able to cross the blood-brain barrier. However, therapy-relevant background information regarding the MGMT level in brain metastases of different origin is usually lacking.

Low expression of MGMT is associated with high methylation of CpG islands in the promoter region of the MGMT gene [28] and MGMT promoter methylation in tumor tissue is associated with significantly longer median overall survival after adjuvant chemotherapy with CCNU and TMZ [32]. Other clinical trials provided compelling evidence for a prognostic importance of the MGMT promoter methylation status in radiotherapy and TMZ combination schemes [33–

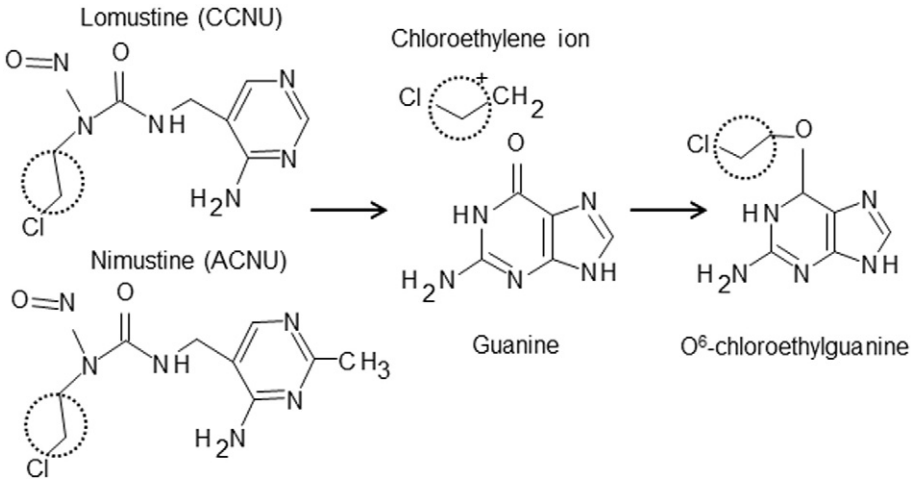

Fig. 1. Alkylation of DNA by CNU. O^6-chlorethylguanine (O^6-ClEtG) is the primary DNA-adduct formed after treatment with CCNU and ACNU.