DNA repair

68

Potentiation of topoisomerase II poison cytotoxicity in the K562 human leukaemia cell line by the novel DNA-dependent protein kinase inhibitor NU7026

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Topoisomerase II (topo II) poisons are widely used in adult leukaemia treatment protocols, particularly acute myelogenous leukaemia (AML). Relapse rates remain high, and current approaches to improve therapeutic efficacy focus on altering drug combinations and schedules, or introducing novel drugs into conventional therapies. Topo II-targeted drugs form drug-stabilised 'cleavable complexes' and production of DNA double strand breaks (DSB) arising from processing of cleavable complexes is considered to be part of the cytotoxic mechanism. DNA-dependent protein kinase (DNA-PK) is essential for the repair of DSB by non-homologous end joining (NHEJ). The Experimental Therapeutics Group at Newcastle, in collaboration with KuDOS Pharmaceuticals, has synthesised and evaluated novel DNA-PK inhibitors. A lead compound, NU7026, has been identified as a potent and specific inhibitor of DNA-PK which potentiates ionising radiation in cell culture at 10uM [1]. We are currently investigating the effect of combining NU7026 with topo II poisons. NU7026 (10uM) potentiated etoposide-induced growth inhibition in K562 cells (XTT assay). The dose enhancement factor (at 50% growth inhibition) was 8.6 (+ 2.0 SD). Data obtained using the TARDIS (trapped in agarose DNA immunostaining) assay [2] showed that NU7026 had no effect on etoposide-induced cleavable complex levels. This suggests that NU7026 does not increase etoposide-induced cleavage per se, but acts downstream of complex formation. We postulate that NU7026 potentiates etoposide cytotoxicity by inhibiting DNA-PK mediated NHEJ. Preliminary data indicates that NU7026 potentiates the cytotoxicity of other topo II poisons (amsacrine and idarubicin), and present studies are aimed at identifying the mechanism(s) by which this enhancement of cytotoxicity occurs.

References

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69

Mechanism of nucleotide excision repair of DNA adducts formed by chemical carcinogens and antitumor drugs

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We have been investigating recognition and repair of a series of bulky DNA adducts formed by well-known carcinogens, such as 1-nitropyrene (NP), 2-aminofluorene (AF), N-acetyl-2-aminofluorene (AAF), and benzo[a]pyrene diol epoxide (BPDE), as well as the antitumor drug, mitomycin C. Our studies suggest that there are at least two steps in the DNA damage recognition by the nucleotide excision repair system, UvrABC nuclease, in Escherichia coli. It includes recognition of helical distortion in duplex DNA followed by recognition of the type of chemical modification in a single stranded region of DNA. The DNA repair intermediates featured with a DNA strand opening structure at the adduct site formed during nucleotide excision repair were critically examined. We noted that the dimension and position of this strand opening depends on the types of DNA adducts. This was demonstrated by the optimal efficiency of incision of the AF- and AAF-DNA adducts located in a bubble formed by three mismatched nucleotides, while the same for NP- and BPDE-DNA adducts was most efficient in a bubble of six mismatched nucleotides. Whether the adduct intercalates in DNA had little influence in causing the dimension and position differences of the bubble, because structural studies have shown that AAF-, NP-, and (+)-cis-BPDE-dG adducts remain intercalated in DNA, while AF- and (+)-trans-BPDE-dG adducts do not intercalate and cause more distortion to the DNA helix. Compared to all these monoadducts, excision repair of mitomycin C-induced DNA-DNA interstrand crosslink is more complex. The excision nuclease system appears to incise one strand preferentially and a notable incision occurred at 8th phosphodiester linkage 5' to the crosslink. A comparison of repair of monoadducts and interstrand crosslinks will be discussed.

70

Motexafin gadolinium inhibits potentially lethal damage repair *in vitro* and *in vivo* following exposure to ionizing radiation

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Purpose: To evaluate the effect of motexafin gadolinium (Xcytrin®, MGd) on potentially lethal damage repair (PLDR) *in vitro* and *in vivo* following exposure to ionizing radiation.
Materials & Methods: For PLDR measurements *in vitro*, A549 human lung adenocarcinoma cells were seeded 10 days prior to 24 hr treatment of plateau phase cultures with 50 uM MGd. Cells were subcultured immediately following irradiation with 10 Gy or after holding for 4, 12, or 24 hours at 37 oC in a CO2 incubator. RNA was isolated from cells in parallel cultures for oligonucleotide microarray-based analysis. This assay measures the expression levels of over 12,000 human genes. Samples from MGd-treated cultures were compared with those from untreated controls at each time point in order to measure the transcriptional effects of MGd treatment. For PLDR measurements *in vivo*, A549 cells were inoculated into the flank of nude mice. When tumor size reached ca. 10 mm diameter, mice (3 per group) received 15 Gy whole body irradiation 2 hours after IV administration of MGd (20 umol/Kg) or control vehicle. Mice were sacrificed immediately or 4 or 24 hours post-irradiation. Tumors were excised, and a single-cell suspension formed by digestion with 0.25% trypsin, DNase I, and collagenase. Colonies were counted after 11 days of subculture.
Results: In the absence of MGd, there was a 35, 64, and 84-fold increase in clonogenic survival when cultures were allowed to recover for 4, 12, or 24 hr, respectively, prior to subculture, relative to cells treated with trypsin immediately after irradiation (recovery factor, RF = 35, 64, 84). However, this recovery was diminished in cultures incubated with MGd (RF = 2.2, 5.2, and 6.2 for 4, 12, or 24 hr holding, respectively). Prominent changes in RNA expression at all time points include metallothionein transcript up-regulation as a consequence of MGd treatment. The recovery of *in vivo*-derived cells was lower than those cultured *in vitro*, with RF = 3.1 and 3.6 for 4 and 24 hr samples from control animals. This recovery was abrogated in MGd treated animals (RF = 0.9 and 1.3 for 4 and 24 hr samples, $p < 0.001$).
Conclusion: Metallothionein transcripts were up-regulated in MGd-treated cultures, possibly as a consequence of oxidative stress generated by this redox cyling agent. MGd enhanced radiation response in A549 adenocarcinoma cells and in the corresponding tumor xenograft by inhibition of potentially lethal damage repair.

71

Triapine, a ribonucleotide reductase inhibitor, enhances incorporation of gemcitabine into DNA and cytotoxicity to KB cells

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Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) is a novel ribonucleotide reductase inhibitor with broad antineoplastic activity. Triapine inhibits ribonucleotide reductase activity by quenching the tyrosyl radical in the R2 subunit of the enzyme resulting in a reduction in the intracellular pool of deoxynucleotides and subsequent inhibition DNA synthesis and repair. Gemcitabine (2',2'-difluorodeoxycytidine) is a deoxycytidine analogue which has been used clinically as an anti-tumor agent alone or in combination with other drugs. In this study, we investigated whether Triapine could potentiate gemcitabine uptake and incorporation into DNA. Human nasopharyngeal carcinoma KB cells were treated with various doses of Triapine followed by the addition of radiolabeled gemcitabine at different time points. Increased gemcitabine uptake and DNA incorporation (2 to 8 fold) were observed in Triapine treated cells as compared to controls. Synergistic cytotoxic effects were also observed in cells treated with a combination of non-toxic doses of Triapine and gemcitabine.
Combinations of Triapine and gemcitabine are currently being evaluated in Phase I clinical trials.