Inhibition of Base Excision Repair Potentiates Iododeoxyuridine-induced Cytotoxicity and Radiosensitization

Pietro Taverna, Hwa-shin Hwang, Jane E. Schupp, Tomas Radivojevitch, Nancy Nguyen Session, Guru Reddy, David A. Zarling, and Timothy J. Kinsella

ABSTRACT

5-Iodo-2′-deoxyuridine (IdUrd) is a halogenated thymidine analogue recognized as an effective in vitro and in vivo radiosensitizer in human cancers. IdUrd-related cytotoxicity and/or radiosensitization are correlated with the extent of IdUrd-DNA incorporation replacing thymidine. IdUrd cytotoxicity and radiosensitization result, in part, from induction of DNA single-strand breaks (SSB) with subsequent enhanced DNA double-strand breaks leading to cell death. Because base excision repair (BER) is a major DNA repair pathway for SSB induced by chemical agents and ionizing radiation, we initially assessed the role of BER in modulating IdUrd cytotoxicity and radiosensitization using genetically matched Chinese hamster ovary cells, with (AA8 cells) and without (EM9 cells) XRCC1 expression. XRCC1 plays a central role in processing and repairing SSBs and DSBs (9). Additionally, these analogues can affect the rate/extent of IR-damage repair (10). On the basis of these observations, a proposed biochemical mechanism of IdUrd-mediated radiosensitization is that the incorporated halogenated deoxyuridine can react with IR-induced hydrated electrons resulting in highly reactive uracil radicals and halide ions (11). DNA SSBs are then produced by these reactive species in the drug-incorporated DNA strand as well as in unsubstituted complementary-strand DNA, which can subsequently result in increased DSBs (9). Unrepaired or misrepaired DNA-DSBs finally result in cell death (9, 12).

New insights on possible molecular events underlying halogenated pyrimidine incorporation came from our recent observations that human tumor and murine embryonic cell lines mutated in either MLH1 or MSH2 genes involved in DNA MMR are increasingly radiosensitized by IdUrd when compared with their genetically matched MMR-proficient cells (13, 14). We found comparable levels of IdUrd-DNA incorporation immediately after short drug exposures (1–2 cell cycle) but up to two to three times higher levels in MMR-deficient cells persist after drug removal suggesting that IdUrd-DNA is more efficiently repaired (removed) in MMR-proficient cells. We speculate that MMR removes IdUrd-DNA (particularly IdUrd-G mismatches) in the daughter strand after replication similar to repair of other single bp mismatches. Interestingly, we found that repair does not result in significant cytotoxicity or cell-cycle perturbations in MMR-proficient cells in contrast to treatment with other nucleoside analogs including the fluoropyrimidines (FdUrd and 5-FU; Ref. 15) and purine analogs such as 6-thioguanine, where MMR-proficient cells show greater cytotoxicity (≥1–2 log cell kill) and a significant G2 cell-cycle delay compared with MMR-deficient cells (13–15).

DNA-SSBs are one of the most frequent and lethal lesions occurring in cellular DNA either spontaneously or as intermediates of enzymatic repair of base damage during BER (16, 17). In this repair pathway, after the removal of a damaged base by a DNA glycosylase, the resulting AP site can be processed by AP endonuclease cleavage leaving a 5′ deoxyribose-phosphate and by an AP lyase activity leaving a 3′ β-elimination product. The subsequent removal of these AP sites by DNA polymerase β (pol β) or by a proliferating cell nuclear antigen-dependent polymerase allows the repair synthesis to fill a single nucleotide (pol B) or a longer repair patch (pol δε), which is then religated (18). When SSBs arising as repair intermediates are not promptly and efficiently processed, the presence of clusters of damaged sites and of stalled replication forks can then result in DSBs with lethal consequences for the cell (19, 20).

The availability of cellular models characterized by deficiencies in specific DNA repair proteins are useful tools to clarify the molecular mechanisms underlying BER and SSB repair. EM9 cells were derived from the AA8 CHO cell line and characterized by hypersensitivity to agents that induce DNA base damage, specifically simple alkylating agents including methylmethane sulfonate and ethylmethane sulfonate (21). EM9 cells also show a 10-fold increased frequency of spontaneous sister chromatid exchanges and are affected by a deficiency in rejoining DNA-SSB induced by exposure to alkylating agents and IR (21). The human gene XRCC1 can fully correct the repair defect of...
EM9 cells. The repair defect has been defined as a frameshift mutation in the endogenous XRCC1 gene resulting in a truncated polypeptide lacking approximately two-thirds of the gene sequence (reviewed in Ref. 22). Recent studies have elucidated the role of XRCC1 protein as that of a “scaffolding” protein, which binds tightly to at least 3 other factors involved in BER and DNA-SSB repair mechanisms: DNA ligase III, DNA polymerase β, and PARP (23–25). More recently, it was demonstrated that the human polynucleotide kinase enzyme also binds XRCC1, and this interaction stimulates SSB repair reactions in vitro (26).

Dillehay et al. (27) first suggested a possible role for BER in the cytotoxicity of halogenated dThd analogues. More recently, BER-mediated 5-chloro-2′-deoxyuridine cytotoxicity was believed to result from the removal of uracil incorporated in DNA secondary to the inhibition of thymidy late synthase by CdUMP, one of the metabolic intermediates of 5-chloro-2′-deoxyuridine (28). Several other studies have also described mismatch-specific enzymes including thymine DNA glycosylase and methyl-CpG binding endonuclease 1 (also known as MBD4), which remove uracil, 5-bromouracil, and 5-fluorouracil residues from DNA (29, 30). In the present study, we questioned whether BER might be responsible, in part, for IdUrd-related cytotoxicity and/or radiosensitization. We initially used genetically matched CHO cells, with (AA8) and without (EM9) XRCC1 expression. In addition, we evaluated the effects of a BER inhibitor, MX, on cytotoxicity induced by IdUrd in cells proficient or deficient in XRCC1 to evaluate the targeting of BER as a possible pharmacologic approach to increase sensitivity to IdUrd alone or in combination with IR. Finally, we also used a human colorectal cancer cell model (HCT116) with and without intact MMR (31) to assess MX modulation of IdUrd cytotoxicity and radiosensitization.

MATERIALS AND METHODS

Cell Lines and Colony Survival Assay. AA8 and EM9 cells were obtained from the American Type Culture Collection (Manassas, VA). H9T3 cells were a gift of Dr. Lawrence H. Thompson (Lawrence Livermore National Laboratory, Livermore, CA). The parental CHO line clone AA8 was isolated as being heterozygous at the aprt locus (32); the mutant EM9 clone was isolated from AA8 cells after mutagenesis with EMS and carried a frameshift mutation in the XRCC1 gene resulting in a truncated polypeptide two-thirds of the normal sequence. Doubling times for AA8 and EM9 are 12 and 16 h, respectively (21). H9T3-7-1 cells (referred to in the text as H9T3) were derived from EM9 after transformation with a cosmid containing XRCC1 cDNA, which corrects the DNA repair defect of EM9 (33). H9T3 cells have a population doubling time of 15 h. HCT116 and HCT116/3-6 human colon carcinoma cells were provided by Dr. Richard Boland (University of California at San Diego, San Diego, CA). HCT116 cells have a hemizygous nonsense mutation in the MMR MLH1 gene located on chromosome 3 and are MMR-deficient, lacking hMLH1 protein expression. The clone 6 (HCT116/6-3c) cell line was created by microcell transfer of a single normal human chromosome 3 into HCT116 cells (31). HCT116/3-6 cells have been shown to express the hMLH1 transcript and protein, and are proficient in MMR (31). The cell doubling times are 22 ± 2 h for both HCT116 and HCT116/3-6 cells (14).

For clonogenic survival assays in CHO cells, cells were serially diluted (range, 2 × 10−2 to 10 × 103 cells/60-mm plate) and plated in complete αMEM medium, allowed to adhere for 18 h, and then treated with IdUrd (Sigma, St. Louis, MO) for 24 h in medium containing 10% dialyzed FBS (−dThd). For clonogenic survival studies in HCT116 and HCT116/3-6 cells, exponentially growing cells were similarly diluted and plated in complete DMEM medium (+400 μg/ml G418 in HCT116/3-6 cells). IdUrd treatment (1, 3, and 10 μM) in HCT116 and HCT116/3-6 cells was for 48 h in αMEM (−G418) containing 10% dialyzed FBS with a replacement of the drug-containing medium after the initial 24 h. IdUrd-containing medium was then removed in both CHO and HCT116 cell populations, and cultures were washed with PBS and incubated at 37°C for 7–10 days in tissue culture medium containing 10% defined FBS (+dThd). Other CHO and HCT116 cell populations were treated simultaneously with IdUrd and 6 mM MX (Sigma) for 24 (CHO cells) or 24–48 h (HCT116 and HCT116/3-6 cells); plates were then washed with PBS, and surviving colonies were counted after incubation at 37°C in drug-free medium for 7–10 days. For radiosensitization experiments, CHO cells were exposed to 1 or 3 μM IdUrd in dialyzed FBS-containing α-MEM; after 24 h the drug-containing medium was removed, and cells were immediately irradiated (0–6 Gy) in medium containing defined serum. HCT116 and HCT116/3-6 cells were exposed to 2.5 μM IdUrd ± 6 mM MX for 48 h in αMEM containing 10% dialyzed FBS after which the drug-containing medium was removed, and cells were incubated for additional 24 h in drug-free medium and irradiated (0–6 Gy). IR was delivered using a model 109 137Cs irradiator at a dose of 4.1 Gy/min (J.L. Shepherd and Associates, San Fernando, CA). Colonies (>50 cells) were stained and counted 7–10 days after plating.

Cell Survival Analysis of Drug and IR Effects. A two-step biostatistical method for detecting multdrug interactions with respect to IR dose-response curves has been described by our group (34). In this method, the first step is to fit the linear-quadratic IR dose-response model:

\[ S = c e^{-(\alpha D + \beta D^2)} \]

where \( D \) = IR dose; \( \alpha \) = linear coefficient of survival curve; and \( \beta \) = quadratic coefficient of survival curve to the raw (not controlled for plating efficiency) log-survival dose-response data. The second step then is to determine whether or not the observed parameter pair (\( \alpha, \beta \)), under drug pretreatment, differs significantly from that expected under the null hypothesis that drug effects are additive in the parameter space. This is accomplished as follows: (a) we form the two parameter random vector \( b = b_{(1,1)} - b_{(1,0)} - b_{(0,1)} + b_{(0,0)} \), where the subscripts indicate the presence or absence of drug pretreatment, and where \( b_{(i,j)} \) equals the least squares estimate of \( (\alpha, \beta) \); (b) we then sum the underlying covariance matrixes, hereafter called \( Z \); and (c) we use a large sample approximation (35).

\[ b^T Z^{-1} b \sim \chi^2 \]

to determine the probability \( P \) that the estimated parameter \( b \) differs significantly from zero.

PFGE. The induction of DNA-DSB in AA8 and EM9 CHO cells by IdUrd (10 μM for 24 h) or IR (6 Gy) was determined by PFGE, as described previously by our group (36).

SCGE. The induction of DNA damage in CHO cell populations by IdUrd alone (10 μM for 24 h) or in combination with 6 mM MX for 24 h was also determined by SCGE (comet assay). This assay was performed according to the manufacturer’s protocol (Trevigen, Gaithersburg, MD). Comet tail moment was determined by measuring the fluorescence intensity using the public domain NIH Image 1.62 software and the comet analysis macro described by Helma and Uhl (37).

HPLC Analysis of IdUrd Incorporated in DNA. Exponentially growing AA8, EM9, and H9T3 CHO cells were exposed to 1–10 μM IdUrd in tissue culture medium supplemented with dialyzed FBS at 37°C for 24 h. Medium containing IdUrd was then removed, and cells were harvested and processed for HPLC analysis, as described previously (13). We also determined IdUrd-DNA incorporation by HPLC in exponentially growing HCT116 and HCT116/3-6 human tumor cell populations after treatment with 2.5 μM IdUrd ± 6 mM MX for 48 h with a replacement of the drug-containing medium after the initial 24 h. After drug treatment, some cells were incubated in drug-free medium for an additional 24 h. Different cultures of HCT116 and HCT116/3-6 cells were then harvested at 24, 48, and 72 h from the beginning of the treatment and processed for HPLC as above.

RESULTS

CHO Cells Mutants for XRCC1 Are Hypersensitive to IdUrd. We initially evaluated the effect of XRCC1 on IdUrd cytotoxicity in CHO cells. EM9 cells, which are mutant for XRCC1, were >3-fold more sensitive to IdUrd cytotoxicity than the parental cell line AA8 (Fig. 1). The sensitivity to IdUrd observed in EM9 cells could be reversed by transfection of a cosm id carrying the cDNA for XRCC1.
as observed in the H9T3 cells. These data suggest that a genetic inhibition of BER results in enhanced IdUrd cytotoxicity in mammalian cells. We then compared the effects of treatment with IdUrd (3 μM for 24 h) or IR (6 Gy) on DNA DSB fragmentation in AA8 and EM9 cells by PFGE. XRCC1 mutant EM9 cells showed an approximately 2–3-fold increased DNA-DSB formation after a 24-h exposure to 3 μM IdUrd compared with parental AA8 cells (Fig. 2). Exposure of EM9 cells to 6 Gy followed by 1 h of recovery was also effective in producing increased DSB fragmentation in EM9 cells compared with AA8 cells (Fig. 2), similar to data published previously (38).

Incorporation of IdUrd in DNA Is Not Altered by Loss of XRCC1 Function. The observed differences in cytotoxic responses to IdUrd in EM9 cells by clonogenic survival (Fig. 1) or by PFGE (Fig. 2) could be possibly explained by a differential incorporation of IdUrd in DNA of cells lacking XRCC1. To evaluate this possibility, we analyzed DNA samples extracted from the 3 CHO cell populations after 24 h treatment with IdUrd (1–10 μM) by HPLC. Fig. 3 shows that AA8 (wild-type) and EM9 (XRCC1 mutant) CHO cells incorporated IdUrd in DNA to similar levels, whereas H9T3 cells (EM9/XRCC1−) showed slightly lower levels of IdUrd-DNA incorporation. These data suggest that the increased IdUrd-induced cytotoxicity observed in EM9 is not related to increased IdUrd-DNA incorporation.

IdUrd-mediated Radiosensitization Is Increased in CHO Cells Mutants for XRCC1. We next evaluated whether IdUrd-induced radiosensitization was increased in XRCC1 mutant EM9 cells. EM9 cells, which were reported previously to be more radiosensitive than the parental AA8 cells (21, 33), were 10 times more radiosensitive after treatment with 1 μM IdUrd and 2 Gy of IR using a comparison of the surviving fraction after 2 Gy (Fig. 4). In contrast, H9T3 cells (EM9/XRCC1−) and AA8 wild-type cells were only slightly radiosensitized by IdUrd. These findings suggest that a genetic impairment of BER through a mutation of XRCCI gene is not only effective in sensitizing mammalian cells to cytotoxicity by IdUrd (Fig. 1) but also is able to enhance the radiosensitizing effect of this compound (Fig. 4).

MX Increases IdUrd cytotoxicity and DNA Damage in CHO Cells. We then tested whether the small molecule MX, a known inhibitor of AP site repair reactions (39), could sensitize CHO cells to IdUrd...
The wild-type AA8 cells and the XRCC1 reconstituted H9T3 cells showed enhanced cytotoxicity to the combination of IdUrd and MX; the IC50 for IdUrd alone was decreased 10 times for both cell lines when MX was combined with IdUrd (Fig. 5). The XRCC1 mutant EM9 cells were already hypersensitive to IdUrd, and the combination of 6 mM MX and IdUrd was able to decrease the IdUrd IC50 from 0.5 μM to ~0.2 μM (Fig. 5). As shown in Fig. 6, AA8 and H9T3 cells exposed for 24 h to the combination of 10 μM IdUrd and 6 mM MX also showed 2–3-fold more DNA breaks (expressed as increase of the comet tail moment) than cells exposed to 10 μM IdUrd alone. DNA damage was assayed by SCGE (Comet assay), which detects SSBs, DSBs, and alkali labile sites (e.g., AP sites). XRCC1 mutant EM9 cells showed significantly increased DNA damage after treatment with IdUrd alone; the damage was not increased after treatment with MX and IdUrd. These clonogenic survival (Fig. 5) and SCGE data (Fig. 6) also suggest that chemical inhibitors of BER (i.e., MX) can be effective in sensitizing XRCC1−/− mammalian cells to IdUrd.

MX Also Sensitizes Human Colon Cancer Cells to IdUrd-induced Cytotoxicity and Radiosensitivity. We have shown previously that IdUrd-DNA incorporation and IdUrd-mediated radiosensitization...
tion of HCT116 human colon cancer cells is mediated by MMR (13). In this study, we evaluated the cytotoxicity and radiosensitization resulting from combined treatment with IdUrd + MX in HCT116, a MMR deficient (MMR−) colon cancer cell line, and in the genetically matched HCT116/3-6 cell line, which was made MMR proficient (MMR+) by an entire single human chromosome 3 transfer (31). Fig. 7 shows that HCT116 cells incorporated slightly more IdUrd in DNA than HCT116/3-6 cells when exposed to a low concentration (2.5 μM) of IdUrd for 48 h (12.02 ± 1.19% versus 10.13 ± 0.81% in HCT116 and HCT116/3-6, respectively), similar to our previous report (13). However, combined treatment with IdUrd (2.5 μM) and MX (6 mM) in HCT116 cells (MMR−) was able to almost double the amount of IdUrd incorporated in DNA after 48 h of continuous exposure (22.53 ± 1.3%), whereas in HCT116/3-6 cells (MMR+), this increase of IdUrd DNA-incorporation was more limited (16.71 ± 0.87%).

We next questioned whether this increase in IdUrd incorporation into DNA correlated with an increased IdUrd cytotoxicity in these colon cancer cells. For this analysis, cells were exposed to 6 mM MX and to different concentrations of IdUrd for 48 h with a replacement of new drugs and medium after the initial 24 h (Fig. 8). Exposure to 6 mM MX for 48 h was able to decrease the survival of HCT116 cells treated simultaneously with IdUrd. The difference in cell kill between 10 μM IdUrd alone and the combination 10 μM IdUrd plus 6 mM MX was 10-fold in HCT116 cells. In HCT116/3-6 cells, the difference in cell kill between IdUrd alone and the combination IdUrd plus MX was less evident, never exceeding an increase of 2.5-fold.

Additionally, we tested whether the potentiating effect of IdUrd on IR cytotoxicity could be additionally increased by MX. Fig. 9 shows that, when HCT116 and HCT116/3-6 cells were treated for 48 h with 2.5 μM IdUrd and 6 mM MX and then irradiated after 24 h of incubation in drug-free medium, the radiosensitization induced by IdUrd alone was additionally significantly increased, whereas MX alone was not effective in changing IR-related cytotoxicity in these cell lines. We have proposed previously (34) that drug-drug interactions with respect to radiosensitization be quantified using the α and β coefficients of the radiation survival data according to Eq. A (see “Material and Methods”). Tables 1 and 2 show the results of fitting Eq. A to the MX and IdUrd drug pretreatment combinations in HCT116 and HCT116/3-6 cells, respectively. In HCT116 cells, the α coefficient, which after radiation alone was 0.156 ± 0.034, increased to 0.569 ± 0.098 after exposure to 2.5 μM IdUrd and to 1.124 ± 0.206 after 2.5 μM IdUrd plus 6 mM MX. The α coefficient for HCT116/3-6 cells exposed to radiation alone was 0.043 ± 0.036, and increased to 0.336 ± 0.069 after exposure to 2.5 μM IdUrd and to 0.601 ± 0.145 after 2.5 μM IdUrd plus 6 mM MX. With respect to the α and β parameters in this model, two agents are defined as interacting if the expected sum of the difference between IdUrd and controls, added to the difference between MX and controls, differs significantly from that observed for the combination of MX and IdUrd. Using Eq. B (see “Materials and Methods”), we determined Ps of <0.0001 for drug-drug interactions on the radiation survival of both HCT116 and HCT116/3-6 cells.

**DISCUSSION**

The halogenated dThd analogue IdUrd has been recognized as a potential radiosensitizing agent since the early 1960s (11, 40).
DNA incorporation is a prerequisite for radiosensitization of human tumors by IdUrd, and the extent of radiosensitization correlates directly with the percentage of dThd replacement in DNA (8, 41). More recently, we have reported that human tumor cells deficient in DNA MMR show significantly greater IdUrd-DNA incorporation and IdUrd-related radiosensitization than genetically matched MMR-proficient cells (13, 14, 42). This set of data suggested that the incorporated IdUrd might be removed by a DNA repair process.

To better clarify which repair pathway could be involved in removing IdUrd from DNA, we determined sensitivity to this drug in CHO cells defective in XRCC1, a scaffolding protein directly associated with DNA polymerase β, DNA ligase III, and PARP during BER or single-strand break repair reactions. The importance of XRCC1 in coordinating the repair of SSBs induced as intermediates of BER is recognized by the finding that XRCC1-deficient cell extracts are significantly impaired in the final steps of BER pathway (25, 43). More recently, XRCC1 has been described also as a modulator of the different activities involved in BER and specifically of APE, the major human AP endonuclease (44).

The importance of XRCC1 in cell responses to DNA damage has been the subject recently of several studies evaluating whether polymorphism of the *XRCC1* gene contributes significantly to increased cancer risk in selected populations. At least three genetic variants have been identified, with the most common variants being *XRCC1* Arg194Trp, Arg399Gln, and Ser326Cys. Each of these variants has been associated with an increased risk of cancer in different populations, with the Arg194Trp variant being the most consistently associated with increased cancer risk across multiple studies.

These findings suggest that XRCC1 polymorphisms may play a role in the response of cells to DNA damage and may influence the efficacy of cancer therapy. Further research is needed to fully understand the mechanisms by which XRCC1 polymorphisms contribute to cancer risk and to develop targeted therapies that take into account individual differences in DNA repair capacity.
been identified in the XRCC1 gene resulting in single amino acid changes at codons 194, 280, and 399, respectively. The 399Gln polymorphism, involving the XRCC1 BRCA1 carboxy terminal domain, which interacts with PARP, has been linked to an increased risk of colorectal (45), gastric (46), lung (47), breast (48), esophageal (49), and pancreatic (50) cancers. Functional analysis of these polymorphisms has suggested that these variants of XRCC1 may contribute to hypersensitivity to IR or may predict for lack of response to oxaliplatin/5-fluorouracil treatment in advanced colorectal cancer patients (51, 52).

Our study presents evidence that cells characterized by altered XRCC1 expression can be sensitized specifically to treatment with IdUrd. The increased susceptibility of XRCC1 mutant cells to IdUrd-induced cytotoxicity and DNA damage also results in an increased radiosensitization after treatment with IdUrd. However, DNA incorporation of IdUrd in XRCC1 mutant cells is equivalent to what we observed in wild-type CHO cells. This suggests that the differences in cytotoxicity and DNA damage evident in these cell lines after IdUrd treatment are the result of a defect in DNA repair rather that a differential incorporation of IdUrd in DNA. Thus, DNA repair deficiencies associated with XRCC1 polymorphisms may predict a more favorable outcome of anticancer therapies using halogenated pyrimidines and IR.

In this study, we also present evidence that, not only are XRCC1-mutant CHO cells hypersensitive to IdUrd cytotoxicity and radiosensitization, but also that cotreatment of CHO and human colon cancer cells with MX can enhance IdUrd cytotoxicity. MX is able to block single nucleotide BER by a reaction with the aldehydic C1 atom of the acyclic sugar left in the DNA abasic AP site after the glycosylase-driven removal of the damaged nucleotide. The MX-adducted AP site is a stable intermediate, refractory to the deoxyRibose Phosphatase activity of polymerase β/H9252 and to the AP endonuclease cleavage. We have shown previously that the chemical inhibition of BER by MX is a valid pharmacological strategy to overcome resistance to the methylating chemotherapeutic agent Temozolomide (53–55). More recently, Tomicic et al. (56) reported that MX sensitized wild-type and Pol β-complemented mouse fibroblasts to the cytotoxicity of Ganciclovir, a nucleoside analogue used as an antiviral agent and used in experimental suicide gene therapy after transduction of tumor cells with the herpes simplex virus thymidine kinase gene. We now present evidence that the increased IdUrd cytotoxicity observed in cells lacking functional single-nucleotide BER can be explained by the in-

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**Table 1** Plating efficiency (c) and linear quadratic parameters (α and β) of HCT116 human colorectal cancer cells treated with IR alone (control cells), 2.5 μM IdUrd and IR, 6 mM MX and IR, and 2.5 μM IdUrd plus 6 mM MX and IR

<table>
<thead>
<tr>
<th></th>
<th>Control cells</th>
<th>IdUrd-treated cells</th>
<th>MX-treated cells</th>
<th>MX + IdUrd-treated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>0.773 (0.753, 0.793)</td>
<td>0.377 (0.354, 0.401)</td>
<td>0.676 (0.654, 0.699)</td>
<td>0.057 (0.051, 0.064)</td>
</tr>
<tr>
<td>α</td>
<td>0.156 (0.122, 0.191)</td>
<td>0.569 (0.471, 0.667)</td>
<td>0.046 (0.007, 0.086)</td>
<td>1.124 (0.918, 1.330)</td>
</tr>
<tr>
<td>β</td>
<td>0.022 (0.015, 0.029)</td>
<td>0.063 (0.037, 0.089)</td>
<td>0.055 (0.047, 0.063)</td>
<td>0.008 (-0.052, 0.069)</td>
</tr>
</tbody>
</table>

*95% confidence interval.

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**Table 2** Plating efficiency (c) and linear quadratic parameters (α and β) of HCT116/3–6 human colorectal cancer cells treated with IR alone (control cells), 2.5 μM IdUrd and IR, 6 mM MX and IR, and 2.5 μM IdUrd plus 6 mM MX and IR

<table>
<thead>
<tr>
<th></th>
<th>Control cells</th>
<th>IdUrd-treated cells</th>
<th>MX-treated cells</th>
<th>MX + IdUrd-treated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>0.694 (0.672, 0.716)</td>
<td>0.462 (0.439, 0.487)</td>
<td>0.665 (0.643, 0.688)</td>
<td>0.070 (0.063, 0.077)</td>
</tr>
<tr>
<td>α</td>
<td>0.043 (0.007, 0.080)</td>
<td>0.336 (0.267, 0.405)</td>
<td>0.061 (0.020, 0.102)</td>
<td>0.601 (0.456, 0.745)</td>
</tr>
<tr>
<td>β</td>
<td>0.034 (0.027, 0.042)</td>
<td>0.068 (0.052, 0.084)</td>
<td>0.041 (0.032, 0.049)</td>
<td>0.062 (0.026, 0.097)</td>
</tr>
</tbody>
</table>

*95% confidence interval.
increased number of DNA breaks left unrepaired after the removal of IdUrd. The presence of these DNA breaks may be explained by the model proposed recently by Wilstemmer and Osheroff (57): when abasic sites are left unrepaired within a topoisomerase II DNA cleavage site, they act as topoisomerase II poisons and significantly increase the enzyme-mediated DNA cleavage. These transient DNA breaks can be converted to permanent double-strand breaks and, therefore, to suicide substrates by the action of DNA tracking systems.

In addition, our study shows for the first time that, in human colon cancer cells, the use of MX significantly increases the DNA incorporation of IdUrd. These results can be explained by the fact that MX-added AP sites, although refractory to the action of the single-nucleotide BER pathway, can still be processed by the long-patch BER pathway (58). Consequently, DNA synthesis associated with long-patch BER events results in a continuous accumulation of IdUrd in DNA engaging the cell in a “suicide” cycle of excision and incorporation of IdUrd with increased cytotoxicity because of the halogenated analogue alone and potential greater radiosensitization. Our linear-quadratic model used to analyze the radiosensitization data leads to the conclusion that the combined treatment with IdUrd and MX results in a greater than additive effect on the IR-induced cell kill in both HCT116 and HCT116/3-6 cells. Because MX alone does not have any effect on the IR-induced cytotoxicity in either HCT116 or HCT116/3-6 cells, we found that the combined treatment with IdUrd and MX is synergistic in this colon cancer model, particularly for MMR- tumors.

In summary, we provide evidence supporting a central role played by XRCC1 and more generally by BER in mammalian cell responses after DNA incorporation of halogenated pyrimidines, specifically IdUrd. Given the presence of XRCC1 polymorphisms in the human population, our results suggest that those variants characterized by a BER inhibition and sensitization to IdUrd. In summary, we provide evidence supporting a central role played by XRCC1 and more generally by BER in mammalian cell responses after DNA incorporation of halogenated pyrimidines, specifically IdUrd. Given the presence of XRCC1 polymorphisms in the human population, our results suggest that those variants characterized by a BER inhibition and sensitization to IdUrd.

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