The Interaction between Two Radiosensitizers: 5-Iododeoxyuridine and Caffeine

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Abstract

5-Iododeoxyuridine (IUdR) and caffeine are recognized as potential radiosensitizers with different mechanisms of interaction with ionizing radiation (IR). To assess the interaction of these two types of radiosensitizers, we compared treatment responses to these drugs alone and in combination with IR in two p53-proficient and p53-deficient pairs of human colon cancer cell lines (HCT116 versus HCT116 p53−/− and RKO versus RKO E6). Based on clonogenic survival, the three single agents (IR, IUdR, and caffeine) as well as IUdR or caffeine combined with IR are less or equally effective in p53-deficient human tumor cells compared with p53-proficient tumor cells. However, using both radiosensitizers, a significantly greater radiosensitization was found in p53-deficient human tumor cells. To better understand the interaction of these two radiosensitizers, additional studies on DNA repair and cell cycle regulation were done. We found that caffeine enhanced IUdR-DNA incorporation and IUdR-mediated radiosensitization by partially inhibiting repair (removal) of IUdR in DNA. The repair of IR-induced DNA double-strand breaks was also inhibited by caffeine. However, these effects of caffeine on IUdR-mediated radiosensitization were not found in p53-proficient cells. Cell cycle analyses also showed a greater abrogation of IR-induced S- and G2-phase arrests by caffeine in p53-deficient cells, particularly when combined with IUdR. Collectively, these data provide the mechanistic bases for combining these two radiosensitizers to enhance tumor cytotoxicity. This differential dual mode of radiosensitization by combining IUdR and caffeine-like drugs (e.g., UCN-01) in p53-deficient human tumors may lead to a greater therapeutic gain. (Cancer Res 2006; 66(1): 490-8)

Introduction

5-Iododeoxyuridine (IUdR) and caffeine are recognized as potential radiosensitizers with different mechanisms of interaction with ionizing radiation (IR). IUdR is a thymidine analogue which is sequentially phosphorylated to 5-iodo-dUTP and is incorporated into DNA in competition with TTP (1). IUdR-DNA incorporation subsequently alters cellular radiosensitivity by enhancing IR-induced DNA damage (1). Targeted radiosensitization by IUdR of certain human cancers has been considered due to the high proliferation rate and greater uptake of IUdR into DNA in some human cancers compared with many normal tissues (1, 2). Recently, DNA repair pathways were found to influence the therapeutic gain in IUdR-mediated radiosensitization. IUdR cytotoxicity and radiosensitization are affected by both mismatch repair (3–6) and base excision repair (7) with both repair systems capable of recognizing IU-G mispairs in DNA and repairing these IUdR-DNA mispairs (6, 8). We have shown that mismatch repair–deficient cells retain higher levels of IUdR-DNA incorporation in vitro and in vivo, which results in differential radiosensitization in mismatch repair–deficient tumors (3, 4, 9). Although the therapeutic dose range of IUdR (1-10 μmol/L), given as a continuous i.v. infusions in humans (1, 2), generally does not elicit a prominent perturbation in cell cycle progression, p53-proficient cells show a moderate G1 arrest (10). In addition, a substantial fraction of cells may undergo senescence-like growth arrest following IUdR and IR (11). G2 arrest may also be found if a higher IUdR dose is administered (9). However, little is known about the association of IUdR-DNA incorporation and other stress response pathways.

Caffeine is a prototype drug that can sensitize cells to IR and some chemotherapy drugs through S and G2 cell cycle checkpoint abrogation and/or direct inhibition of DNA repair (12, 13). The molecular targets of caffeine for radiosensitization/chemosensitization include ataxia telangiectasia mutated (ATM) and ATM and Rad3-related (ATR) kinases (14). Although caffeine is not suitable for clinical use, similar agents such as 7-hydroxyseratoxpurine (UCN-01) are currently under clinical evaluation (13). The rationale of cancer cell/tumor selectivity by these “G2 checkpoint abrogators” is based on the theory that p53-deficient cells rely more on the G2 checkpoint due to disruption of the G1 checkpoint (12, 15). Alternatively, other investigators attribute the differential effect by p53 on the dual pathways involved in regulating the G2 checkpoint (i.e., one pathway is p53 dependent and the other is p53 independent but sensitive to Chk1 inhibition; refs. 12, 16).

In addition to the cell cycle checkpoint abrogation, caffeine and/or UCN-01 has been shown to suppress DNA damage repair processes including homologous recombination repair (17, 18) and nucleotide excision repair (19, 20). To date, the association of base excision repair and DNA damage response modification by caffeine-like drugs remains less clear (21).

In this study, we investigate whether a repair pathway involved in the processing of IUdR-DNA incorporation is caffeine sensitive. In addition, we hypothesize that the combination of IUdR and caffeine (or caffeine-like drugs) can improve the therapeutic gain. Our primary interest is not merely to increase the radiosensitizing effect by combining the two radiosensitizers but to augment the cancer cell/tumor specificity based on the molecular determinants which may affect the drug-IR interactions. To test our hypotheses, we examine the interaction of the two radiosensitizers using p53-proficient and p53-deficient pairs of human colon cancer HCT116 and RKO cell lines, both of which are mismatch repair deficient (hMLH1−; refs. 22, 23).
Materials and Methods

Cell cultures and chemicals. The two pairs of human colon cancer cell lines used were HCT116 versus HCT116 p53−/− cells (24) and RKO versus RKO E6 cells (25). HeLa cells transfected with a short interfering RNA (siRNA) against Chk1 were also used to assess the role of Chk1 in IUdR-DNA processing. All cell lines were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), t-glutamine, and penicillin/streptomycin at 37°C in a humidified 10% CO2 atmosphere. Dialyzed FBS was used during the IUdR treatment period including the IUdR-free control to mimic the thymidine concentration in humans (10−7 mol/L; ref. 26). The chemicals used were purchased from Sigma (St. Louis, MO) unless otherwise specified. UCN-01 was generously supplied by Dr. J. Sarkaria (Mayo Clinic, Rochester, MN). IR was delivered using a 137Cs γ-irradiator at 370 cGy/min.

Survival assay and analysis of multiantigent interactions. Cell survival was determined by a clonogenic survival assay. Cells were plated on 60-mm dishes to yield 100 to 150 colonies per dish and treated as indicated. Fourteen days after plating, colonies were stained and fixed with methanol-acetic acid (3:1). Colonies were counted and the data were expressed as the surviving fraction (SF) or fraction affected (Fu; Fu = 1 − SF), normalized by the plating efficiency of nontreated controls. Interactions were estimated relative to two different definitions of additivity widely accepted in cancer research (27):

(a) Survival probability independence (Bliss independence) exists if the joint agent exposure survival probability equals the product of the single agent survival probabilities, e.g., if the surviving fraction following IR and treatment with drugs A and B is

\[ SF_{IR \times A \times B} = SF_{IR} \times SF_{A} \times SF_{B} \]

or, equivalently in terms of fraction killed (using SF = 1 − Fa), if

\[ Fa_{IR \times A \times B} = Fa_{IR} + (1 - Fa_{IR}) \times Fa_{A} + (1 - Fa_{IR}) \times (1 - Fa_{A}) \times Fa_{B} \]

(b) Loewe additivity exists if the potencies of the agents are related to each other by constant scale factors. Letting these scale factors be the single agent doses needed to achieve a given cell kill, Chou and Talalay (28) proposed a combination index (CI)

\[ CI = \frac{P_{comb}}{D_{comb}^{\text{dose}}} = \frac{D_{A}^{\text{dose}}}{D_{comb}^{\text{dose}}} + \left( \frac{P_{comb}}{D_{comb}^{\text{dose}}} \right)^m \]

as a measure of synergism (CI < 1) or antagonism (CI > 1). A dose-effect curve of each drug alone is fitted by least squares to

\[ Fa = Fu \left( \frac{D}{D_m} \right)^m \]

where Fu = 1 − Fa = SF, D is dose, and Dm is the median effect dose. This equation is then used to determine the single agent doses that would be needed to achieve the cell kill obtained with the drugs in combination; these expected doses (under Loewe additivity) go into the denominator of the combination index expression. Combination indices were obtained only at specific dose points where experiments were conducted and not across a full range of Fa levels (29). Because IR was delivered following 48-hour pretreatments, note that a potential difference of cell growth rates between the untreated control and IUdR −/− caffeine may result in different cell numbers at the time of IR treatment. Therefore, it is possible that the drug-IR interaction may be overestimated to some degree.

Measurement of IUdR-DNA incorporation. The %IUdR-DNA incorporation was determined according to the method of Belanger et al. (30) with minor modifications as previously described (3, 4). Following enzymatic hydrolysis of DNA, solutions containing nucleosides and the analogue were analyzed by high-performance liquid chromatography with a reversed-phase column. The high-performance liquid chromatography apparatus used was a Waters System controller 600E with an Autosampler 717, a Multiwavelength detector 400E, and a 3.9 × 300-mm μBondapak C18 reverse phase column (Waters Co., Milford, MA). The mobile phase consisted of 20 mmol/L sodium acetate (pH 2.0) with 8% acetonitrile at a flow rate of 1.0 mL/min. UV absorption at 250 and 290 nm detected thymidine (TdR) and IUdR peaks, respectively, and quantitation was done against authentic nucleoside standards using Millennium32 software v. 3.05.01 (Waters). The typical retention times of TdR and IUdR were 6 and 9 minutes, respectively.

The %IUdR-DNA incorporation was calculated by the following formula: IUdR / (IUdR + TdR) × 100.

siRNA transfection and Western blotting. Chk1 siRNA and a scrambled control siRNA (Dharmacon, Lafayette, CO) were transfected into HeLa cells using Oligofectamine (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. Following a 24-hour transfection period, cells were harvested and seeded to new dishes at an appropriate cell density and IR treatment was begun. To confirm the effect of siRNA, Western blot analyses were done using standard procedures (SDS-PAGE followed by an electrotransfer onto a membrane). The antibodies used were Chk1 (G-4) and goat anti-mouse immunoglobulin G-heroseddarase peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA).

Comet assay (single-cell gel electrophoresis). Following the indicated treatments, cells were harvested by trypsinization and suspended in PBS (−105 cells). For measurement of the immediate IR-induced DNA damage, IR was delivered and the cells were immediately placed on ice to inhibit repair. In other experiments, irradiated cells were kept in complete media at 37°C for 1 hour to assess repair of IR-induced damages. A cell suspension of 50 μL was embedded into 500 μL of 1% low melting point agarose gel at 37°C and spread onto slides. The slides were incubated in a lysis solution [2.5 mol/L NaCl, 10 mmol/L Tris, 0.1 mol/L EDTA (pH 10), 1% Triton X-100] at 4°C overnight. The slides were then subjected to electrophoresis at 1 V/cm for 20 minutes under either alkaline (300 mmol/L NaOH, 1 mmol/L EDTA, pH > 13) or neutral (1× Tris-borate EDTA, pH = 8.0) conditions. After fixation in 70% ethanol for 5 minutes and drying in air, DNA was stained with SYBR Green I (Molecular Probes, Eugene, OR) and observed using a fluorescence microscope. The data were analyzed as the tail moment (product of tail length and fraction of DNA in the tail as described; refs. 31, 32). The alkaline comet assay was done to detect DNA single-strand breaks as well as alkaline labile apyrimidinic/apurinic sites and the neutral assay was done to detect DNA double-strand breaks (31, 32). The data from three to five independent experiments were pooled and analyzed (−100 cells in each experiment).

Flow cytometry. Following the indicated treatments, cells were harvested by trypsinization and fixed with cold 70% ethanol. The cells were then incubated in 33 μg/mL propidium iodide, 1 mg/mL RNase, 0.5 mmol/L EDTA, and 0.2% NP40 overnight at 4°C. Flow cytometry analysis was done using a Coulter EPICS XL-MCL (Coulter Co., Miami, FL). At least 20,000 events were analyzed using ModFit LT 2.0 (Verity, Inc., Topsham, ME).

Statistical methods. Cell cycle distribution and IUdR incorporation with caffeine or Chk1 knockdown were analyzed using a linear additive ANOVA model with time and treatment as factors. In the data analyses of the comet assay, the distributions of the ratio of mean tail moments were acquired by a bootstrap method.

Results

Survival analyses of the combination of the two radiosensitizers. We first examined the single agent dose-effect relationships of IR, IUdR (48-hour treatment), and caffeine (24-hour treatment; Fig. 1A). The p53-deficient HCT116 and RKO cells were slightly resistant to IR and IUdR compared with...
p53-proficient cells as we previously published (3). Whereas caffeine showed no difference in the survival of the RKO pair of cells, HCT116 p53<sup>−/−</sup> cells were slightly more sensitive to caffeine than its counterpart. Next, the combined cytotoxicity of IR-IUdR and IR-caffeine was tested using various doses of the radiosensitizers and a fixed dose (2 Gy) of IR. IUdR was administered for 48 hours before IR and caffeine was administered for 24 hours after IR. The zero interactions of IR and the radiosensitizers were acquired by the Bliss independence concept. Synergistic interactions were found for both IR-IUdR and IR-caffeine combinations. The IR-IUdR interaction was similar or smaller in p53-deficient cells compared with p53-proficient cells whereas there was no difference in the IR-caffeine interaction in the cell line pairs. These results were further confirmed by the alternative analysis method using the median effect principle. Overall, the clonogenic survival data suggest that the single modality treatments as well as IUdR-IR or caffeine-IR treatments are less or equally effective in p53-deficient cancer cells compared with p53-proficient cells.
combined effects of both drugs and IR. Bliss independence represents a zero interaction between the two drugs and IR (2 Gy). Using both IUdR and caffeine drug treatments, p53-deficient cells showed greater radiosensitization than the p53-proficient cells. The interactions of IUdR-caffeine alone (0-Gy IR; B) and combined with 2-Gy IR (C) were analyzed by the median effect principle. Greater synergistic interactions were found in p53-deficient cells. D, ratio of surviving fractions in p53-proficient cells divided by p53-deficient cells as an index of therapeutic gain [the extent of normal proliferating cell (p53+/+) survival likelihood relative to malignant cell (p53−/−) survival likelihood]. The IUdR-IR treatment was compared with the IUdR-IR-caffeine combination. The addition of caffeine remarkably improved the therapeutic gain.

**The effect of caffeine on IUdR-mediated radiosensitization.** To investigate the mechanisms of the observed synergistic interaction between the two radiosensitizers using clonogenic survival (Fig. 2), the %IUdR-DNA incorporation was measured in cells treated with IUdR (3 μmol/L) continuously with or without caffeine (2 mmol/L; Fig. 3A). Cotreatment with caffeine decreased cell growth rates, corresponding to a modest increase of G0/G1 population (data not shown). The %IUdR-DNA incorporation of p53-deficient cells seemed to be slightly lower than that of p53-proficient cells without caffeine. In the presence of caffeine, the %IUdR-DNA incorporation was enhanced in both p53-proficient and p53-deficient cells. The increased %IUdR-DNA incorporation with caffeine cotreatment was higher at the later time points (days 2–3) rather than at day 1, suggesting that caffeine affects post-incorporation processing of IUdR. To exclude the effect of caffeine on the rate-limiting step of IUdR metabolism and incorporation, thymidine kinase activity was measured (33). Thymidine kinase activity in HCT116 and RKO cells was, however, found unchanged with 2 mmol/L caffeine (data not shown).

Although caffeine has been extensively investigated as an *in vitro* radio/chemosensitizer, the clinical application of caffeine is limited because millimolar levels of caffeine would be required to achieve significant inhibition of ATM and ATR kinases. Therefore, we also tested the effect of UCN-01, a clinically relevant checkpoint abrogator, on IUdR-DNA incorporation in HCT116 cells. Cotreatment with 100 nmol/L of UCN-01 enhanced IUdR-DNA incorporation to a similar level as caffeine (Fig. 3B). Although other targets are also reported, the primary target of UCN-01 has been considered to be Chk1 (34), mainly for its cytotoxic effects rather than checkpoint abrogation. The effect of Chk1 inhibition on the IUdR-DNA incorporation was further confirmed using siRNA for Chk1 in HeLa cells (Fig. 3C). There was no significant change in the cell cycle distribution except for a modest increase of a sub-G1 population with the Chk1 siRNA transfection.

To further investigate possible mechanisms for the observed enhanced IUdR-DNA incorporation by caffeine, we focused on the repair activity of IUdR in DNA. We first compared repair kinetics with or without caffeine following pulse labeling by IUdR in synchronized HCT116 cells (Fig. 3D). For these experiments, the
cells were synchronized at G1-S border by 16-hour incubation with 1 μmol/L aphidicolin and then released in media containing 10 μmol/L IUdR with or without 2 mmol/L caffeine. Eight hours after release (when most cells completed S phase and entered G2 phase), the medium was changed to IUdR-free medium with or without caffeine. The medium was also serum starved from this point to minimize cells from entering a second S phase. Caffeine was found to significantly reduce the repair (elimination rate) of IUdR (Fig. 3D). Because HCT116 cells are mismatch repair deficient (hMLH1−) and IUdR-DNA incorporation decreased following the S phase, the elimination of IUdR is likely to be processed by the base excision repair system as we previously reported (7, 8). To further confirm that caffeine reduces the repair (elimination) of IUdR, DNA single-strand breaks and/or apyrimidinic/apurinic sites generated during base excision repair processing were measured by an alkaline comet assay in HCT116 cells (Fig. 4A). Whereas IUdR + caffeine resulted in greater IUdR-DNA incorporation compared with IUdR alone in these cells (Fig. 3A), IUdR + caffeine showed significantly less DNA single-strand breaks and apyrimidinic/apurinic sites at equi-IUdR incorporation levels, suggesting that caffeine reduces the efficiency of base excision repair of IUdR-DNA.

Because the principal mechanism of IUdR-mediated radiosensitization is an enhancement of IR-induced DNA damage (1, 2), we also assessed whether the enhanced IUdR-DNA incorporation by caffeine augments IR-induced DNA double-strand breaks. The amount of DNA double-strand breaks was measured by a neutral comet assay done immediately after 0- or 5-Gy IR with or without a 48-hour prior exposure to 3 μmol/L IUdR +/− 2 mmol/L caffeine (Fig. 4B). IUdR + caffeine further increased IR-induced DNA double-strand breaks compared with IUdR alone, suggesting that caffeine enhances IUdR-mediated radiosensitization through increased double-strand break formation following IR.

The effect of IUdR on caffeine-mediated radiosensitization. Caffeine is recognized as a G2 checkpoint abrogator. Therefore, we examined cell cycle progression following IR and assessed whether the combination with IUdR alters the effect of caffeine as a checkpoint abrogator (Fig. 5A). In the absence of caffeine, both HCT116 and HCT116 p53−/− cells showed S- and G2-phase arrests following 2-Gy IR although the extent of the arrest was more pronounced in p53-deficient cells. A G1 arrest was also evident in p53-proficient cells. The observed S- and G2-phase arrests were effectively abrogated by caffeine. In combination with IUdR, the G1 arrest became more pronounced in HCT116 but not in HCT116 p53−/− cells, which rendered the differential cell cycle effects of drug-IR treatment more pronounced. The data indicate a greater abrogation of IR-induced cell cycle arrests by caffeine in p53-deficient cells particularly when combined with IUdR (Fig. 5B).

In addition to the cell cycle checkpoint abrogation, an inhibitory effect of caffeine on DNA double-strand break repair...
was measured using a neutral comet assay. HCT116 and HCT116 p53−/− cells were treated with IUdR (0, 1, and 3 μmol/L) with or without caffeine (2 mmol/L) for 48 hours and harvested. The cells were subjected to single-cell gel electrophoresis (comet assay) under alkaline (pH > 13) conditions. IUdR + caffeine showed less tail moments at equi-IUdR-DNA incorporation levels compared with IUdR alone. B, IUdR and IUdR + caffeine increase IR-induced DNA double-strand breaks. HCT116 and HCT116 p53−/− were treated with PBS (IR alone, control), IUdR (3 μmol/L), or IUdR (3 μmol/L) + caffeine (2 mmol/L) for 48 hours and harvested. The cells were irradiated to 0 or 5 Gy on ice and subjected to single-cell gel electrophoresis under neutral (pH ~ 8) conditions to detect DNA double-strand breaks. The values in the plots represent the difference between the mean tail moments with 5-Gy IR and without IR (95% confidence intervals), which are considered to represent the amount of IR-induced DNA double-strand breaks. IUdR pretreatment showed greater IR-induced double-strand breaks than the control in both cell lines (**, P < 0.005). IUdR + caffeine further enhanced IR-induced DNA double-strand breaks compared with IUdR alone (*, P = 0.05, for HCT116; **, P < 0.005, for HCT116 p53−/−).

Discussion

The data in this study show a synergistic interaction between the two radiosensitizers, IUdR and caffeine. Not only did we find a synergistic drug-drug interaction on cytotoxicity but also a synergy was found when the two drugs were combined with IR, leading to significantly greater radiosensitivity in p53-deficient cancer cell lines compared with p53-proficient cell lines (Fig. 2). These results suggest that the combination of both radiosensitizers may further improve the therapeutic index in p53-deficient tumors.

A mechanism by which caffeine enhances IUdR radiosensitization is, in part, related to the observed increase in IUdR-DNA incorporation (Fig. 3A). Although the possibility that caffeine alters the IUdR intracellular metabolism cannot be entirely excluded, our kinetic analysis using synchronized cell populations suggests that the enhanced IUdR level in DNA can be attributed to a reduced post-incorporation repair (removal) process (Fig. 3D). Because HCT116 and RKO cells are mismatch repair deficient (hMLH1−/−), base excision repair is presumed to be the repair system affected by caffeine resulting in the observed relatively slow removal of IUdR in the synchronized HCT116 cells.

It was somewhat surprising that inhibition of the checkpoint kinases with caffeine and UCN-01 drug treatments or Chk1 siRNA transfection affected IUdR-DNA incorporation (Fig. 3A) because IUdR incorporation generally does not induce a prominent cell cycle perturbation. However, we now recognize that checkpoint function is active and essential for normal cell cycle progression and that checkpoint kinases have constitutive activity in a basal nonphosphorylated status without exogenous DNA damage (35). The DNA damage response pathways are operative under normal conditions and are amplified to a detectable level on an increase in DNA damage (36). Furthermore, recent reports support the dual functions of the checkpoint kinase in regulating both cell cycle progression and DNA repair. For example, UCN-01 is reported to affect the
interaction of XPA and ERCC1 and consequently inhibit nucleotide excision repair (19). This inhibition was also found in quiescent lymphocytes (20).

To date, little is known about the DNA damage response pathways and the base excision repair system. Base excision repair does not cause obvious cell cycle perturbations, at least for low levels of DNA damage, and therefore is referred to as silent repair (37). However, more recent reports show the involvement of certain checkpoint proteins and base excision repair proteins (38, 39). p53 is also a regulatory factor of base excision repair. p53 enhances base excision repair activity for repair of oxidative base damage by increasing levels of apurinic/apurinic endonuclease and 8-oxoguanine glycosylase (40). In contrast, another p53-inducible protein, PPM1D, was shown to dephosphorylate uracil-DNA glycosylase, leading to reduced base excision repair activity (41). These findings suggest that there is a tight regulation of base excision repair efficiency by p53.

Our data show lower levels of IUdR-DNA incorporation in p53-deficient cells compared with p53-proficient cells even without caffeine cotreatment (Fig. 3A) although multiple factors including a slightly faster cell doubling time, a higher S-phase fraction, and a higher thymidine kinase activity in p53-deficient cells might have predicted the opposite (data not shown). It remains to be determined whether dysregulation of base excision repair in p53-deficient cells is a sole mechanism of the lower IUdR-DNA incorporation levels. Using cotreatment with caffeine, an enhancement of IUdR-DNA incorporation by caffeine was found in both p53-proficient and p53-deficient cells (Fig. 3A). Although the difference was modest, enhancement of IUdR-DNA incorporation seemed greater in p53-deficient cells, which may be a mechanism for the observed greater interaction between IUdR and caffeine in p53-deficient cells based on our analyses of clonogenic survival (Figs. 2B and 6C).

It has been suggested that following IR, caffeine abrogates S- and G2-phase cell cycle arrests, which lead to an insufficient time for DNA double-strand break repair (12). Furthermore, a recent study showed a key role of the checkpoint kinases in regulating homologous recombination repair following hydroxyurea or camptothecin treatments (18). It is proposed that caffeine or UCN-01 reduces repair of DNA double-strand breaks through disruption of the ATR-Chk1-Rad51 as well as ATR-Chk1-cdc25 pathways (18). Considering the tight coupling of cell cycle checkpoints and DNA repair in intact cells, it is reasonable to suggest that the degree of checkpoint abrogation by caffeine correlates with the extent of repair inhibition of DNA double-strand breaks (Fig. 6A and B) and, consequently, with an increased cellular radiosensitivity.

![Figure 5. Pretreatment with UdR differentially affects the extent of IR-induced cell cycle checkpoints as well as the cell cycle changes with caffeine treatment following IR. A, cells were pretreated with UdR (0 or 3 μmol/L) for 48 hours and then irradiated (2 Gy). Cells were then treated with caffeine (0 or 2 mmol/L) and harvested at the indicated times. Next, cells were fixed, propidium iodide stained, and then subjected to flow cytometry. Greater S- and G2-phase arrests and their abrogation by caffeine were found in HCT116 p53+/−/ compared with HCT116 cells, particularly with UdR pretreatment. B, changes in the %S + G2-M population for HCT116 and HCT116 p53+/−/ cells following the various drug and IR treatment schedules are graphed for the initial 36 hours following IR. UdR followed by IR (IR+/−IR−NT) significantly differed from IR alone (IR−NT) in the changes of the %G1 population in HCT116 cells (P < 0.05, ANOVA).](cancerres.aacrjournals.org)
One of the most noticeable changes in cell cycle progression with IUdR treatment before IR was the G1 arrest in p53-proficient cells (Fig. 5). Therefore, IUdR pretreatment enhances an IR-induced G1 checkpoint activation, resulting in lower S and G2 populations cells (Fig. 5). Therefore, IUdR pretreatment enhances an IR-induced differential radiosensitization when IUdR and caffeine are combined in p53-proficient and p53-deficient cells may also contribute to the antagonize caffeine-mediated radiosensitization by reducing the extent and duration of an IR-induced S and G2 checkpoint activation in p53-proficient cells in contrast to p53-deficient cells (Fig. 6C). Taken together, the differential cell cycle effects of IUdR in p53-proficient and p53-deficient cells may also contribute to the differential radiosensitization when IUdR and caffeine are combined.

In conclusion, these data provide the mechanistic basis for combining the two radiosensitizers. Cancer-selective activity may be further enhanced by the dual modes of radiosensitization using IUdR and caffeine (or caffeine-like drugs) in p53-deficient tumors.

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References

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