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LETTER TO THE EDITOR

Topoisomerase I involvement in schedule-dependent interaction between 5-fluoro-uracil and irinotecan in the treatment of colorectal cancer

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Abstract 5-Fluoro-uracil (5FU), an antimetabolite drug, stimulates expression of topoisomerase I (tpI) in adenocarcinoma cancer cells. When 5FU is given in combination with Irinotecan (IR), a tpI poison, the most effective regimen is represented by IR given before low doses of 5FU. Hence, despite their distinct mechanisms of action, the molecular basis for successful combination and schedule of 5FU and IR in the treatment of colorectal cancer rests on the opposing drug effects on the expression and poisoning of the tpI enzyme.

Dear Editor,

A paper recently published in this journal reported that levels of topoisomerase I (tpI) in recurrent colorectal cancer were higher than those measured in the primary tumor [1]. All patients had been treated with 5-fluoro-uracil (5FU) after primary tumor resection and before recurrence; hence, the authors suggest a possible drug contribution in the enhancement of tpI levels.

By means of a real time RT-PCR technique, we measured tpI expression in the model adenocarcinoma LoVo cell line after treatment with 5FU and irinotecan (IR), a topoisomerase I poison, used alone or in different temporal association. Indeed, we found that 5FU alone was able to stimulate tpI transcripts, which reached maximum levels (3–5 fold over basal levels) after 3 h treatment. Contrary, IR potently inhibited tpI transcripts which decreased to 10% over initial values (100%). When given in association, tpI transcript levels decreased when IR was given 3 h before 5FU, while using the opposite schedule, i.e. 5FU given 3 h before IR, tpI transcript enhancement induced by 5FU was only partially reverted by subsequent treatment with IR.

Based on these results, it would be rational to hypothesize a better effect of a sequential therapy, where 5FU, an antimetabolite drug but also a tpI inducer as just shown, is given before IR, the topoisomerase I poison. Hence, to evaluate the most effective 5FU/IR temporal sequence, we measured cytotoxic effects on three different adenocarcinoma cell lines (LoVo, Caco-2 and HT-29) by MTT assay. Initially, cytotoxic drug concentration capable of killing 50% of a cell population (CC_{50}) was estimated for each cell line and drug. For LoVo, Caco-2 and HT-29 cells, IR CC_{50} values were 10.0 ± 0.9, 50.0 ± 2.0, 60.0 ± 1.1 μM, respectively; 5FU CC_{50} values were 20.0 ± 0.8, 100.0 ± 3.0 μM, 1.00 ± 0.01 mM, respectively. Clearly, LoVo cells are more susceptible to treatment and IR is more cytotoxic than 5FU. Subsequently, drugs were administered according to the following four schedules: (a) IR at its CC_{50} followed by 5FU at variable concentrations (0.01 μM–1 mM); (b) IR at variable concentrations (0.01 μM–1 mM) followed by 5FU at its CC_{50}; (c) 5FU at its CC_{50} followed by IR at variable concentrations (0.01 μM–1 mM); (d) 5FU at variable concentrations (0.01 μM–1 mM) followed by IR at its CC_{50}. Time delay was set at 3 h, according to previous results [2]. Drug combination effects were estimated according to the fractional effect analysis [3], comparing the experimental cytotoxicity to the theoretical value calculated as the product of the cytotoxicity exhibited by each individual drug at the concentration used in the combination experiments. Contrary to what expected, for all the three cell lines

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tested, the most synergistic, hence cytotoxic, effect was consistently found when IR was given at its CC_{50} before low concentrations of 5FU. Conversely, IR at variable concentration before high doses of 5FU, and the two protocols where 5FU was administered before IR induced additive or moderate antagonistic effects. Interestingly, these data measured in vitro perfectly match current clinical regimen; in fact, all present clinical protocols for the treatment of stage IV and recurrent colon cancer adopt IR given before (4–24 h) 5FU. In addition, 5FU is initially given at a low loading dose, which is increased in the following days of treatment [4].

Taken together, these data indicate that the highest drug efficacy is reached when tpI expression is maintained at low level in cancer cells. TpI activity has been shown to be necessary to repair UV-induced DNA damage [5, 6]. We have ourselves found that DNA damaging agents such as 5FU (and also oxaliplatin) potently induce tpI expression. On the contrary, the decreased enzyme expression observed with IR is likely a cellular response to protein accumulation at the nuclear level promoted by the drug, which functions as a tpI poison. When IR is given first, it decreases tpI levels to such an extent that enzyme expression cannot be rescued by subsequent treatment with 5FU, especially at low doses of the antimetabolite; therefore, less repair systems are available to the cell, hence the higher efficacy of this schedule. Vice versa, administration of 5FU first would prove less effective due to activation of tpI-mediated DNA repair systems, which would be efficiently inactivated only by very high doses of IR capable to saturate all cleavage complexes available.

The above data indicate that the rational for combination and schedule of IR and 5FU involves the tpI enzyme. Its complex cellular machinery can augment or decrease drug potency, depending on drug combination and mechanism of action. Importantly, this control is not limited to topoisomerase-targeted drugs alone, but it can be extended to other co-administered DNA interfering agents.

Conflict of interest statement None.

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