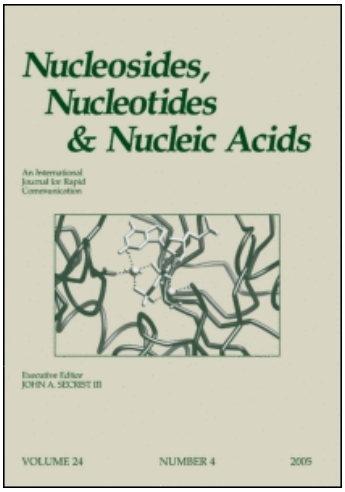

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J. Sigmond[a]; E. M. Comijn[a]; J. A. E. Kamphuis[a]; G. J. Peters[a]
[a] Department of Oncology, VU University Medical Center, Amsterdam, The Netherlands

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Combinations of 5-Fluorouracil with UCN-01 or Staurosporine

J. Sigmond,* E. M. Comijn, J. A. E. Kamphuis, and G. J. Peters

Department of Oncology, VU University Medical Center,
Amsterdam, The Netherlands

ABSTRACT

The action of 5-Fluorouracil (5-FU) is mediated by inhibition of thymidylate synthase (TS), which is regulated by cell cycle proteins controlled by protein phosphorylation. We studied the effects of staurosporine and its analogue UCN-01, inhibitors of protein kinase C (PKC) on 5-FU cytotoxicity in Lovo colon cancer cells. Each drug contributes equally to the cell cycle effects of the 5-FU combinations. In sequential drug administration, the cell cycle distribution was determined by the first drug. Simultaneous 5-FU combinations induced additive effects in induction of apoptosis. When staurosporine was used as the second drug, induction of apoptosis was 2-fold higher than the sum of both drugs alone. Based on induction of apoptosis 5-FU addition prior to the PKC inhibitors seemed preferable.

Key Words: 5-Fluorouracil; UCN-01; Staurosporine.

*Correspondence: J. Sigmond, Department of Oncology, VU University Medical Center, Amsterdam, The Netherlands.

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INTRODUCTION

5-Fluorouracil (5-FU) is an antimetabolite widely used for treatment of various solid tumors.[1] 5-FU has multiple mechanisms of action; its metabolite FdUMP mediates inhibition of thymidylate synthase (TS), which catalyses dTMP synthesis, a precursor for DNA synthesis. 5-FU can also be incorporated into RNA and DNA.[1] Its effects are cell cycle dependent.[2] Cell cycle progress is regulated by phosphorylation/dephosphorylation; e.g. the complex E2F/Rb will dissociate when hyperphosphorylated leading to free E2F, which can enhance the expression of TS.[2] Protein kinase C (PKC) is one of the enzymes responsible for protein phosphorylation, and is also involved in signal transduction pathways that regulate growth factor response, proliferation and apoptosis. PKC can be inhibited by staurosporine and its analogue UCN-01. In addition, UCN-01 is an inhibitor of cell cycle dependent kinase 2. In this study, we investigated several interactions of 5-FU with these two PKC inhibitors with emphasis on cell cycle distribution and induction of apoptosis.

MATERIALS AND METHODS

The colon cancer cell line Lovo B2 (wild-type p53)[3] was cultured in DMEM, containing 5% FCS and 20 mM HEPES at 37°C under an atmosphere of 5% CO_2. Growth inhibition of 5-FU-combinations was determined by IC_{50} values by the SRB assay[4] while additive or synergistic effects were evaluated with median drug effect analysis method (Calcusyn, Elsevier-Biosoft). FACS analysis (PI) was performed to study differences in induction of apoptosis or cell cycle arrest.[3]

RESULTS AND DISCUSSION

The IC50 concentrations for 5-FU, staurosporine and UCN-01 in Lovo cells were 1.86, 0.003 and 0.02 μM, respectively. The combination of 5-FU with staurosporine or

Table 1. Cell cycle distribution after treatment of Lovo B2 cells with drug combinations of 5FU (50 μM) with staurosporine (0.05 μM) or UCN-01 (0.5 μM).

	% of cell cycle distribution		
Treatment	G0/G1 phase	S phase	G2/M phase
Control 48 hr	49.8	13.5	36.7
stau 48 hr	36.7	21.8	41.4
ucn 48 hr	53.0	17.4	29.6
5FU 48 hr	34.0	34.5	31.5
stau + 5FU 48 hr	35.1	31.1	33.8
ucn + 5FU 48 hr	38.4	29.8	31.7
stau 48 hr/5FU 24 hr	35.3	24.7	40.0
ucn 48 hr/5FU 24 hr	51.5	16.0	32.5
5FU 48 hr/stau 24 hr	34.0	33.2	32.8
5FU 48 hr/ucn 24 hr	37.8	32.5	29.7

UCN-01 was synergistic (Combination indices were 0.8 and 0.4, respectively, indicating synergism). To evaluate effects on cell cycle distribution, IC_{100} concentrations were used (Table 1).

Cells were exposed to drugs simultaneously for 48 hr or sequentially (48 hr/24 hr means 48 hr exposure to the first drug of which the last 24 hr exposure to the 2nd drug). Values are means of 4 separate experiments. SEM are less than 30%.

An S phase arrest was induced after exposure to 50 μM 5-FU, but exposure for 24 hr to staurosporine (0.05 μM) or UCN-01 (0.5 μM) induced accumulation of cells in the G_2/M phase or G_0/G_1), respectively, shifting to an S-phase arrest after 48 hr. Simultaneous exposure to 5-FU combinations appears to show an average cell cycle distribution of both drugs when used alone. In sequential drug administration, the cell cycle distribution seemed to be determined by the first drug. Apparently, application of the first drug precluded further progress of the cells in the cell cycle, possibly making them more vulnerable for apoptosis which can then be enhanced by the second drug. Indeed, additive effects in induction of apoptosis were observed in simultaneous and more than additive in sequential 5-FU combinations (Fig. 1).

Cells were exposed to drugs simultaneously for 24 or 48 hr (+ bars with one time point) or sequentially (48 hr/24 hr means 48 hr exposure to the first drug and the last 24 hr exposure to the 2nd drug). Values are means of 4 separate experiments. SEM are less than 30%.

When staurosporine was used as the second drug, induction of apoptosis was 2-fold higher than the sum of both drugs alone. Based on induction of apoptosis 5-FU addition prior to the PKC inhibitors seemed preferable. The results in this study show that PKC inhibitors can enhance the toxicity of 5FU in the Lovo colon cancer cell line. Earlier reports showed that UCN-01 induced apoptosis in human colon carcinoma and leukemia cells independently of p53.[5] These mechanistic studies offer a good basis for evaluation of development of combination therapies of 5-FU with inhibitors of protein phosphorylation and cell cycle dependent kinases.

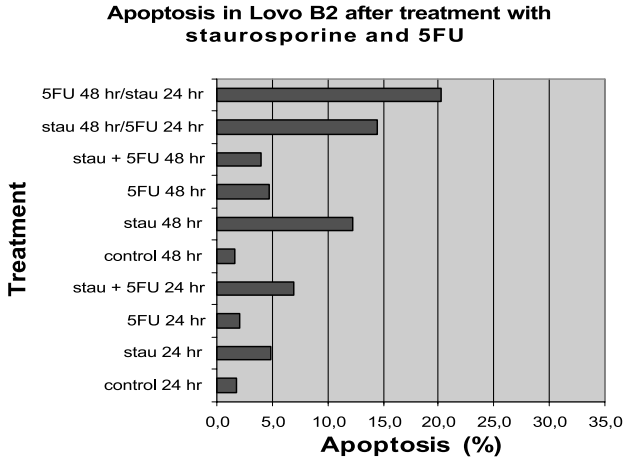

Figure 1. Induction of apoptosis after treatment of Lovo B2 cells with drug combinations of 5-FU (50 μM) with staurosporine (0.05 μM).

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