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Letter to the Editor

Time-dependent Interactions Between 5-Fluorouracil and Mitomycin C on a Human Colon Carcinoma Cell Line, HCT-8, *In Vitro*

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5-FLUOROURACIL (FUra) and mitomycin C (MC) are the most widely employed antineoplastic agents against colorectal cancer. The clinical response rate to either drug is in the order of 20% and several attempts have been made to enhance their activity. Continuous infusions, arterial administration and biochemical modulation of FUra have produced encouraging results, while the attempts to potentiate MC activity involve its combination with a host of other antineoplastic agents and the use of this drug in conditions of low oxygen tension. Despite encouraging preclinical results on the synergistic activity of FUra and MC in mice bearing the P388 leukemia [1], it is disappointing to recognize the poor clinical outcome when these two agents are combined: the response rate to this drug combination in patients with advanced colon carcinoma is lower than either drug alone in a number of clinial studies [2, 3]. Prior to clinical testing of hepatic arterial infusions of FUra and MC, we have investigated the cytotoxicity of these agents, alone and in combination, on a human colon carcinoma cell line, HCT-8, *in vitro,* in search of their best administration schedule. Using a monolayer clonal growth technique, FUra cytotoxicity was markedly time dependent with ED_{50} values after 1 h exposure (200 μM) approx. 200-fold greater than the values observed after a 5 day incubation with this agent (Table 1). On the contrary, MC activity was much less time dependent with only a 20-fold difference in ED_{50} values after the same incubation times.

Table 1. *Sensitivity of HCT-8 cells to FUra and MC as measured by inhibition of colony growth* (ED_{50} *values,* μM ± *S.E. of at least four experiments*)*

	Incubation time		
	1 h	4 h	5 days
FUra	200 ± 7.1	30 ± 5.9	1.3 ± 0.25
MC	0.3 ± 0.05	0.14 ± 0.01	0.015 ± 0.001

*Concentration that inhibits 50% of colony growth as compared to untreated controls.

In general, the doses selected for drug combination experiments were chosen to give a cell kill between 30 and 70%, so that maximum synergy could be observed. The expected survival values for the drug combinations (dashed lines in Fig. 1) were calculated by multiplying the observed survival values of each drug alone for that specific concentration. When the observed survival is lower than the expected survival, the two drugs synergize; in the opposite case they antagonize, and when the two values are similar, they produce additive effects [4].

Since the drugs are usually given to patients as rapid i.v. pushes and have short half lives, we first studied their effects after a 1 h incubation. In these

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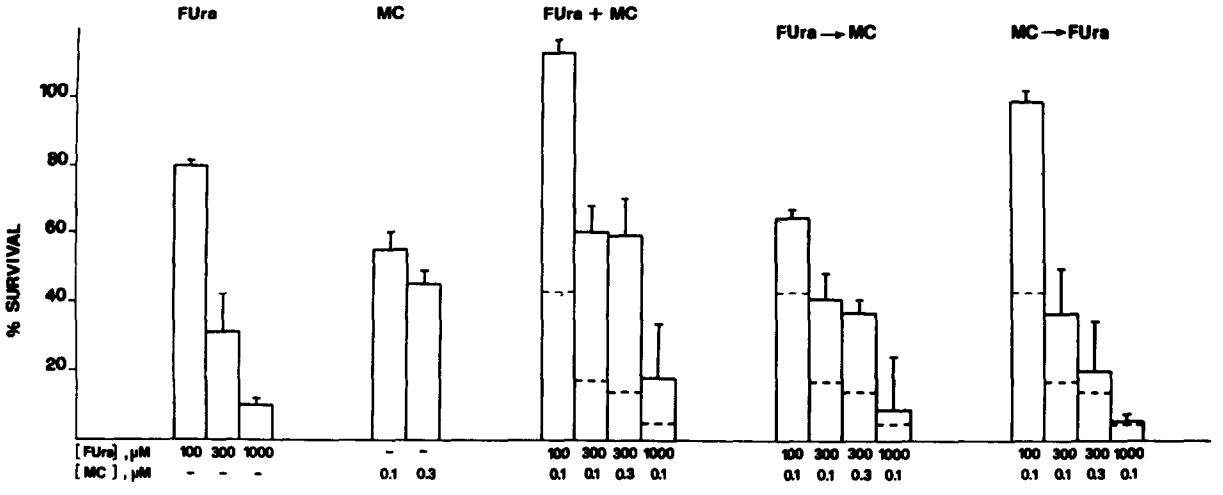

Fig. 1. Effects of FUra–MC combination on the survival of HCT-8 cells: 1 h exposure. Dashed lines indicate expected survival. Bars indicate S.D. in a representative experiment.

studies each agent was administered simultaneously or in the sequences FUra → MC or MC → FUra. The two drugs clearly antagonized, independently of the sequence of administration (Fig. 1); maximal antagonism was observed after the simultaneous administration of MC and FUra. The antagonism observed after a 1 h incubation occurred also when cells were exposed for 4 h to these agents, although the antagonism between MC and FUra was less pronounced then in the 1 h incubation experiments.

The interactions between MC and FUra were then studied under conditions of prolonged exposure to FUra for 5 days, and 1 h pulses of treatment with MC. The pulses were given at the beginning or at the end of the 5-day incubation period. When MC was given before the prolonged exposure to FUra, additive effects were observed; when the antibiotic followed the fluoropyrimidine, only minor antagonism occurred. Side experiments on colony size clarified that this latter finding is due to an artifact of our assay which is scored after 10 days from initial plating: any drug administered half way through the total incubation period will exert its effects on colonies already developed that might not die and might thus be scored as surviving clonogenic cells.

In any case, synergism between MC and FUra was never observed. These data provide experimental support to the antagonism observed in the clinic between FUra and MC. Although our work shows that synergism between the drugs cannot be obtained in HCT-8 cells, no matter the time or sequence of exposure, at least we indicate how the antagonism can be overcome.

Based on these data the EORTC group is now conducting a phase 2 study using prolonged (5 days) hepatic arterial infusion of FUra and boluses of MC on the first day of FUra infusion, with excellent preliminary results: 13 of 18 patients with multiple hepatic metastases from colorectal cancer have responded to this drug schedule so far (Dr. D. Civalleri, personal communication).

REFERENCES

1. Sartorelli AC, Booth BA. The synergistic anti-neoplastic activity of combinations of mitomycin C with either 6-thioguanine or 5-fluorouracil. *Cancer Res* 1965, **25**, 1393–1400.
2. Reitermeier RJ, Moertel CG, Hahn RG. Combination chemotherapy in gastrointestinal cancer. *Cancer Res* 1970, **30**, 1425–1430.
3. Buroker T, Kim PN, Groppe C *et al.* 5-FU infusion with mitomycin-C versus 5-FU infusion with methyl-CCNU in the treatment of advanced colon cancer. *Cancer* 1978, **42**, 1228–1233.
4. Momparler RL. *In vitro* systems for evaluation of combination chemotherapy. *Pharmacol Ther* 1980, **8**, 21–35.