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Perspectives and Commentaries

Sequential MTX and 5-FU: Advance or Myth?

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(A COMMENT ON: Wood CD, Slevin ML, Ponder BAJ, Wrigley PFM. Sequential methotrexate and 5-fluorouracil in the treatment of non-small cell carcinoma of the lung. *Eur J Cancer Clin Oncol* 1985, **21**, 587–589.)

METHOTREXATE (MTX) and 5-fluorouracil (5-FU) are well established cytotoxic agents, each introduced more than a quarter of a century ago and still widely used in the treatment of many types of cancer. The CMF regimen, in which MTX and 5-FU are usually injected at the same time, supplemented with oral cyclophosphamide, is among the most frequently used of all cytotoxic combinations. The interactions between MTX and 5-FU are complex and still incompletely understood, and under some conditions they may be antagonistic. Tattersall *et al.* [1] studied the effects of MTX and 5-FU on L5178Y cells in suspension culture and found less than additive effects when the drugs were used together. Similarly, some dose combinations of MTX and 5-FU were no more effective than single drug therapy against L1210 cells passaged intraperitoneally in BDF_1 mice [1].

The current vogue for sequential administration of MTX and 5-FU stems from the observation of Bertino *et al.* [2] of synergistic antitumour activity in mice bearing S180 sarcomas when MTX was given before 5-FU, an effect confirmed in some other murine tumour systems.

Cadman *et al.* [3] studied logarithmically growing L1210 cells in culture, exposed to varying concentrations of methotrexate followed 3 hr later by 5-FU. Cytotoxicity was increased by prior exposure to MTX, and the effect was dependent on the MTX and 5-FU concentrations used. They found that sequential exposure of cells to MTX and 5-FU was associated with increased accumulation of 5-FU nucleotides, and suggested that this explained the observed synergy. The culture medium used in these experiments included horse serum, which contains low purine levels, and Cadman *et al.* found that addition of hypoxanthine decreased the biochemical effects of sequential MTX and 5-FU in their system, reducing both accumulation of phosphoribosyl pyrophosphate and the formation of 5-FU nucleotides [3]. They did not report the effect of hypoxanthine addition on cytotoxicity.

The importance of the serum source in the L1210 culture system used to demonstrate synergistic cytotoxicity of sequential exposure to MTX and 5-FU was studied by Piper *et al.* [4]. They found that in media supplemented with horse serum, synergy similar to that described by Cadman *et al.* [3] was confirmed, that the effect was not seen if foetal calf serum was used in place of horse serum, but was restored if the foetal calf serum was dialysed. They concluded that the purines present in non-dialysed foetal calf serum might be responsible for the abrogation of synergy, and examined the effects of adding varying concentrations of hypoxanthine to the cultures. These studies demonstrated that synergy was reduced by the addition of hypoxanthine, and that the concentration of hypoxanthine required was dependent on the concentration of MTX used in the experiment, with concentrations in the micromolar range significantly reducing synergistic growth suppression. They also found that thymidine added to the regrowth medium after drug exposure effectively rescued cells exposed to sequential MTX–5-FU [4]. These results cast doubt on the relevance of experiments performed in low-purine media to the clinical use of sequential MTX and 5-FU, since human serum has been shown to contain micromolar concentrations of hypoxanthine and thymidine [4, 5].

At the clinical level, a number of uncontrolled studies have reported encouraging results using

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sequential MTX and 5-FU (e.g. 6, 7]. Before the biochemical rationale described above was proposed, sequential administration of intermediate-dose MTX followed by 5-FU was included in the regimen of Price *et al.* [8], who reported a high response rate of 74% in head and neck cancer. More recently, uncontrolled studies have yielded differing results in several tumour types. In head and neck cancer, Pitman *et al.* [6] reported 35 patients treated with MTX 125–250 mg/m^2 followed 1 hr later by 5-FU 600 mg/m^2. The overall response rate was 71%, including 65.2% of the patients treated for recurrent disease. On the other hand, Jacobs treated 30 patients with recurrent head and neck cancer using an identical regimen, and found a response rate of only 16.7% [9]. A difference as extreme as this is unlikely to be due merely to the small numbers involved: it may reflect a difference in the inherent prognosis of the two groups of patients studied.

This discrepancy in results is also seen in two reported phase II studies of the treatment of colorectal cancer. Wienerman *et al.* [7] reported 10 responders among 29 such patients, while Kaye *et al.* [10] found no responders among 16 patients. The MTX dose of 50 mg/m^2 used by Kaye *et al.* was lower than the 20 mg/kg used by Weinerman *et al.*, and it could be argued that a lower MTX concentration at the time of 5-FU administration would be insufficient to overcome the effects of human serum purine [4].

In advanced breast cancer, Herrmann *et al.* [11] reported a 28% response rate to sequential MTX and 5-FU among patients resistant to the same agents used in other ways, and Kaye *et al.* [10] found a 21% response rate among 29 patients, most of whom were previously untreated.

Wood *et al.* [12] reported a negative phase II study in patients with non-small cell lung cancer. The regimen used was MTX 200 mg/m^2 followed 2 hr later by 5-FU 1000 mg/m^2, repeated every 4 weeks. No responses were observed among 16 previously untreated patients. The dosages used by these authors were similar to those used in other tumour types, but the 4-week interval between cycles of treatment is longer than most groups have found necessary, and is reflected in the low toxicity reported. The low effective dose per unit time detracts from the persuasiveness of their negative results.

Critical evaluation of the contribution of sequential administration of MTX and 5-FU in human cancer therapy will depend on appropriately controlled clinical trials. Only two such trials have yet been reported, neither of which supports the value of sequential administration of MTX and 5-FU. Browman *et al.* [13] reported a trial in 82 patients with head and neck cancer who were randomly assigned to simultaneous or sequential administration of MTX and 5-FU. Dosages were similar to those of Pitman *et al.* [6] and the interval in the sequential group was 1 hr. The response rate for sequential therapy was 38.5% and for simultaneous therapy 61.9%. While evidence for *inferiority* of sequential therapy is marginal ($P = 0.06$), the results clearly are incompatible with superiority for the sequential arm. Coates *et al.* [14] performed a study in which 108 patients including 70 with head and neck cancer were randomized to receive one of two drug sequences: 250 mg/m^2 MTX followed 1 hr later by 600 mg/m^2 5-FU (MF) or the same drugs 1 hr apart but in the opposite order (FM). The response rate for MF in head and neck cancer was 51% and for FM 40%, a non-significant difference. Survival among patients with head and neck cancer was influenced by performance status, and after allowance was made for this, patients randomized to FM survived marginally longer than those randomized to MF ($P < 0.025$).

Clearly, neither of these randomized studies supports the value of the sequential use of MTX followed by 5-FU. Both used a 1-hr interval, which Benz *et al.* [15] have suggested is too short for an optimal synergistic effect in human cells. It is, however, the interval used in most of the reported phase II studies. Controlled trials to test the value of longer intervals may be indicated, but until they are performed the onus of proof rests firmly with those who claim that the clinical use of sequential MTX and 5-FU is an advance rather than a myth.

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