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Letter to the Editor

The Effect of Time of Administration of 5-Fluorouracil on Leucopenia in the Rat

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LEUCOPENIA occurs in over 50% of patients receiving the cancer chemotherapeutic agent 5-fluorouracil (5-FU) [1–3]. Most drug-induced deaths occur when the white blood cell (WBC) count has fallen to below 2000/mm^3, and may be attributable to infection. Thus haematological toxicity, together with gastrointestinal toxicity, can be severely limiting factors in therapy with this drug. One approach to improvement of the selectivity of such drugs is afforded by administration of the drugs at a particular time of day or stage of the cell cycle when the susceptibility of the host tissues to the drugs may be minimal—i.e. the 'chronopharmacological' approach [4, 5].

Cell division in normal tissues of the host, but not in tumour tissue, is synchronized. Hence administration of an antiproliferative agent during the resting phase of the cell cycle may potentially reduce the side-effects on the host-tissues. Previous experiments in this laboratory have shown that there is a marked diurnal variation in the toxicity of 5-FU to the small intestine in the rat [6].

We have therefore now investigated the effect of the time of day of 5-FU administration on the total WBC count in rats.

Male Wistar rats (160–220 g weight) were used. They had been kept caged in groups of four in conditions of controlled day-length [0800–2200 hr British Summer Time (BST)] with free access to water and a standard pelleted diet [Maintenance Diet No. 1 (expanded); Special Diet Services, Ltd., Stebfield, Witham, Essex, U.K.] for at least 1 week before use.

A sterile solution of the sodium salt of 5-FU (0.6 mmol/kg; 192 μmol/ml; Roche Products, Ltd.) was injected between 1200 and 1300 BST or between 1745 and 1830 BST respectively into the sublingual vein of lightly ether-anaesthetized animals [7]. After 10–10.5 days, blood (2 ml) was collected by cardiac puncture while the rat was under ether anaesthesia into a heparinized syringe and mixed with 20 mg of ethylene diamine tetraacetic acid (sodium salt). This combination of anticoagulants prevented clumping of the WBC. These samples were collected between 1200 and 1300 BST on day 10 after the midday (1200–1300) injection and on day 11 after the evening (1745–1830) injection. The WBC were counted by a Coulter counter after 1:50 dilution of the blood in 2% acetic acid. The data were analysed both by Student's t test and by Wilcoxon's non-parametric test.

Figure 1 shows the results. Note that the WBC count after injection of the 5-FU in the evening was not significantly different from that in the control (uninjected) rats ($P > 0.05$ by the t test and by Wilcoxon's test). In contrast, injection at noon was followed by a significant reduction in the WBC count compared with the controls ($P < 0.001$ by the t test; $P < 0.01$ by Wilcoxon's test). In spite of the considerable scatter among the data, the difference in the WBC count in the two groups of injected animals was significant ($P < 0.01$ by the t test and by Wilcoxon's test).

These results indicate clearly that the timing of the administration of 5-FU can be a significant factor in regulating the severity of the subsequent haematological toxicity in the rat. Although it is not yet known whether the optimal time of drug administration for minimal haematological

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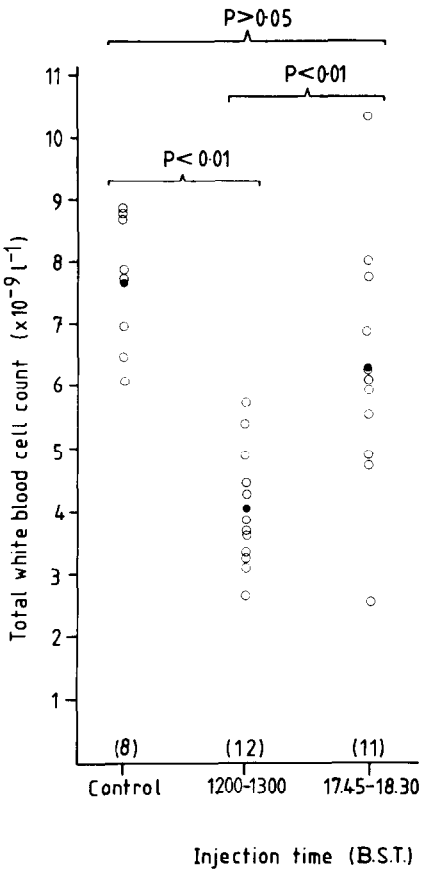

Fig. 1. White blood cell count in control rats and in rats after intravenous injection of 5-FU. The 5-FU was injected either at midday (1200–1300 BST) or in the evening (1745–1830 BST). The open circles show the data for individual animals and the closed circles show the mean value for that group. The numbers of animals are shown in parentheses. The significances (calculated by Wilcoxon's non-paired test) of the differences between groups are shown at the top of the figure.

toxicity coincides with, or is close to, that for minimal intestinal toxicity, these observations supplement the earlier ones on the intestinal side-effects of 5-FU [6] and give further support to the suggestion that manipulation of the time of drug administration may minimize the toxic side-effects. This in itself would be beneficial and additionally could permit the use of higher dosages of the drug. Hence the therapeutic efficacy may be increased, provided, of course, that tumour response is not compromised. The latter aspect requires investigation, although tumours growing asynchronously may be expected to show no diurnal variation in their response to 5-FU. Futher, these observations may provide part of the explanation for the reports [3, 8] that more severe side-effects of 5-FU were observed after rapid administration (at an unspecified time of day) than after slow infusion.

This approach offers a simple, non-invasive and inexpensive means of improving the efficacy of drug regimes in chemotherapy and, possibly, also the efficacy of radiotherapy, although it is recognized that identification of the optimal time of day for administration to humans may be difficult. Nevertheless, we suggest that clinical trials on manipulation of the time of 5-FU administration (as a bolus) whilst monitoring tumour regression and the severity of toxic side-effects including leucopenia are now justified.

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